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Stem Cell

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Stem Cell Technology Research Literatures

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Abstract: Stem cells are derived from embryonic and non-embryonic tissues. Most stem cell studies are for animal stem cells and plants have also stem cell. Stem cells were discovered in 1981 from early mouse embryos. Stem cells have the potential to develop into all different cell types in the living body. Stem cell is a body repair system. When a stem cell divides it can be still a stem cell or become adult cell, such as a brain cell. Stem cells are unspecialized cells and can renew themselves by cell division, and stem cells can also differentiate to adult cells with special functions. Stem cells replace the old cells and repair the damaged tissues. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin. This article introduces recent research reports as references in the related studies.
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Key words: stem cell; technology; life; research; literature

Introduction

The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell. This article introduces recent research reports as references in the related studies.

The following introduces recent reports as references in the related studies.

Abbassi, M. S., et al. (2008). "Genetic characterisation of CTX-M-15-producing Klebsiella pneumoniae and Escherichia coli strains isolated from stem cell transplant patients in Tunisia." Int J Antimicrob Agents **32**(4): 308-314.

Characterisation of extended-spectrum beta-lactamase (ESBL) genes and their genetic environments as well as the presence of integrons were analysed in nine Klebsiella pneumoniae and two Escherichia coli ESBL-positive isolates recovered in the Centre of Bone Marrow Transplantation of Tunisia. All strains harboured the bla(CTX-M-15) gene and presented minimum inhibitory concentrations for cefotaxime and ceftazidime of 256-1024 mg L(-1) and 16-512 mg L(-1), respectively, and eight of them showed different pulsed-field gel electrophoresis patterns. The bla(OXA-1) and bla(TEM-1) genes were detected in eight and ten strains, respectively. In addition, bla(SHV-1), bla(SHV-11) and bla(SHV-27) were found in six, one and one K. pneumoniae strains, respectively. The new variant bla(SHV-103) was characterised in one K. pneumoniae strain. The intI1 gene was detected in eight K. pneumoniae strains and the dfrA5+ereA2 and aadA gene cassettes were found in one and five strains, respectively. All strains harboured a 70 kb plasmid, and its transference in addition to bla(CTX-M-15), bla(TEM-1b) and bla(OXA-1) genes was demonstrated from three K. pneumoniae to E. coli. ISEcp1 and orf477 were located upstream and downstream, respectively, of the bla(CTX-M-15) gene in 10 strains. The occurrence of the bla(CTX-M-15) gene in unrelated strains might have originated from the dissemination of mobile genetic elements in which ISEcp1 may have played an important role.

Abdel-Azim, H., et al. (2015). "Unrelated donor hematopoietic stem cell transplantation for the treatment of non-malignant genetic diseases: An alemtuzumab based regimen is associated with cure of clinical disease; earlier clearance of alemtuzumab may be associated with graft rejection." Am J Hematol **90**(11): 1021-1026.

Hematopoietic stem cell transplantation (HSCT) with matched unrelated donors (MUD), offers potentially curative therapy for patients with non-malignant genetic diseases. In this pilot study conducted from 2006 to 2014, we report the outcomes of 15 patients with non-malignant genetic diseases who

received a myeloablative regimen with a reduced cyclophosphamide dose, adjunctive serotherapy and MUD HSCT [intravenous alemtuzumab (52 mg/m(2)), busulfan (16 mg/kg), fludarabine (140mg/m(2)), and cyclophosphamide (105 mg/kg)]. Graft-versus-host-disease (GVHD) prophylaxis consisted of tacrolimus/cyclosporine and methylprednisolone. Median (range) time to neutrophil engraftment (>500 cells/microL) and platelet engraftment (>20,000/mm(3)) were 15 (12-28) and 25 (17-30) days, respectively. At a median follow-up of 2 (0.2-5.4) years, the overall survival (OS) was 93.3% (95% CI: 0.61-0.99) and disease-free survival (DFS) was 73.3% (95% CI: 0.44-0.89). Among this small sample, earlier alemtuzumab clearance was significantly associated with graft rejection (P = 0.047), earlier PHA response (P = 0.009) and a trend toward earlier recovery of recent thymic emigrants (RTE) (P = 0.06). This regimen was associated with durable donor engraftment and relatively low rates of regimen related toxicity (RRT); future alemtuzumab pharmacokinetic studies may improve outcomes, by allowing targeted alemtuzumab clearance to reduce graft rejection and promote more rapid immune reconstitution.

Adiwinata Pawitan, J. (2012). "Prospect of induced pluripotent stem cell genetic repair to cure genetic diseases." Stem Cells Int **2012**: 498197.

In genetic diseases, where the cells are already damaged, the damaged cells can be replaced by new normal cells, which can be differentiated from iPSC. To avoid immune rejection, iPSC from the patient's own cell can be developed. However, iPSC from the patients's cell harbors the same genetic aberration. Therefore, before differentiating the iPSCs into required cells, genetic repair should be done. This review discusses the various technologies to repair the genetic aberration in patient-derived iPSC, or to prevent the genetic aberration to cause further damage in the iPSC-derived cells, such as Zn finger and TALE nuclease genetic editing, RNA interference technology, exon skipping, and gene transfer method. In addition, the challenges in using the iPSC and the strategies to manage the hurdles are addressed.

Akiyama, M., et al. (2020). "Genetic Profile and Microsatellite Instability in a Case of Secondary Esophageal Squamous Cell Carcinoma 12 Years After Allogeneic Hematopoietic Stem Cell Transplantation for Aplastic Anemia." J Pediatr Hematol Oncol **42**(4): 302-306.

We report on a 16-year-old Japanese boy in whom an esophageal squamous cell carcinoma (ESCC) developed 12 years after allogeneic hematopoietic stem cell transplantation was performed for aplastic anemia. A high frequency of microsatellite instability was detected in samples of ESCC. Moreover, the detection of pathogenic variants, including single nucleotide substitution of TP53 (c.346C>T) and BRCA2 (c.6952C>T) and splicing of KDM6A (c.1194+2T>G), suggest that the development of ESCC in the patient was triggered by impairment of checkpoint and repair for DNA damage and epigenetic modification through accumulation of gene mutations induced by chronic graft-versus-host disease and prolonged administration of tacrolimus.

Altarescu, G., et al. (2012). "Prevention of lysosomal storage diseases and derivation of mutant stem cell lines by preimplantation genetic diagnosis." Mol Biol Int **2012**: 797342.

Preimplantation genetic diagnosis (PGD) allows birth of unaffected children for couples at risk for a genetic disorder. We present the strategy and outcome of PGD for four lysosomal storage disorders (LSD): Tay-Sachs disease (TSD), Gaucher disease (GD), Fabry disease (FD), and Hunter syndrome (HS), and subsequent development of stem cell lines. For each disease, we developed a family-specific fluorescent multiplex single-cell PCR protocol that included the familial mutation and informative markers surrounding the mutation. Embryo biopsy and PGD analysis were performed on either oocytes (polar bodies one and two) or on single blastomeres from a six-cell embryo. We treated twenty families carrying mutations in these lysosomal storage disorders, including 3 couples requiring simultaneous analysis for two disorders (TSD/GD, TSD/balanced Robertsonian translocation 45XYder(21;14), and HS/oculocutaneus albinism). These analyses led to an overall pregnancy rate/embryo transfer of 38% and the birth of 20 unaffected children from 17 families. We have found that PGD for lysosomal disorders is a safe and effective method to prevent birth of affected children. In addition, by using mutant embryos for the derivation of stem cell lines, we have successfully established GD and HS hESC lines for use as valuable models in LSD research.

Aluko, A., et al. (2019). "Prepregnancy Genetic Carrier Screening in Recipients of Hematopoietic Stem Cell Transplants." Obstet Gynecol **134**(4): 756-758.

BACKGROUND: Genetic carrier screening of individuals with a history of hematopoietic stem cell (HSC) transplant is not always straightforward. CASE: We present the case of a couple who underwent prepregnancy genetic screening before fertility treatment. The genetics laboratory flagged the male partner's blood sample because there was a discrepancy between the sex of the patient and the sex chromosome markers, ultimately leading to the discovery of a prior HSC transplant. CONCLUSION: A history of HSC

transplant may confound results of serum-based genetic carrier screening. Hematopoietic stem cell transplant recipients are at risk for misinterpreted genetic carrier screening because the results of such screening may not actually be reflective of their own DNA.

Amar, A., et al. (2022). "Modeling the C. elegans germline stem cell genetic network using automated reasoning." Biosystems **217**: 104672.

Computational methods and tools are a powerful complementary approach to experimental work for studying regulatory interactions in living cells and systems. We demonstrate the use of formal reasoning methods as applied to the Caenorhabditis elegans germ line, which is an accessible system for stem cell research. The dynamics of the underlying genetic networks and their potential regulatory interactions are key for understanding mechanisms that control cellular decision-making between stem cells and differentiation. We model the "stem cell fate" versus entry into the "meiotic development" pathway decision circuit in the young adult germ line based on an extensive study of published experimental data and known/hypothesized genetic interactions. We apply a formal reasoning framework to derive predictive networks for control of differentiation. Using this approach we simultaneously specify many possible scenarios and experiments together with potential genetic interactions, and synthesize genetic networks consistent with all encoded experimental observations. In silico analysis of knock-down and overexpression experiments within our model recapitulate published phenotypes of mutant animals and can be applied to make predictions on cellular decision-making. A methodological contribution of this work is demonstrating how to effectively model within a formal reasoning framework a complex genetic network with a wealth of known experimental data and constraints. We provide a summary of the steps we have found useful for the development and analysis of this model and can potentially be applicable to other genetic networks. This work also lays a foundation for developing realistic whole tissue models of the C. elegans germ line where each cell in the model will execute a synthesized genetic network.

Amberg, N., et al. (2022). "Tissue-wide genetic and cellular landscape shapes the execution of sequential PRC2 functions in neural stem cell lineage progression." Sci Adv **8**(44): eabq1263.

The generation of a correctly sized cerebral cortex with all-embracing neuronal and glial cell-type diversity critically depends on faithful radial glial progenitor (RGP) cell proliferation/differentiation programs. Temporal RGP lineage progression is regulated by Polycomb repressive complex 2 (PRC2), and loss of PRC2 activity results in severe neurogenesis defects and microcephaly. How PRC2-dependent gene expression instructs RGP lineage progression is unknown. Here, we use mosaic analysis with double markers (MADM)-based single-cell technology and demonstrate that PRC2 is not cell-autonomously required in neurogenic RGPs but rather acts at the global tissue-wide level. Conversely, cortical astrocyte production and maturation is cell-autonomously controlled by PRC2-dependent transcriptional regulation. We thus reveal highly distinct and sequential PRC2 functions in RGP lineage progression that are dependent on complex interplays between intrinsic and tissue-wide properties. In a broader context, our results imply a critical role for the genetic and cellular niche environment in neural stem cell behavior.

Anderson, P. D., et al. (2009). "Genetic factors on mouse chromosome 18 affecting susceptibility to testicular germ cell tumors and permissiveness to embryonic stem cell derivation." Cancer Res **69**(23): 9112-9117.

Despite strong heritability, little is known about the genetic control of susceptibility to testicular germ cell tumors (TGCT) in humans or mice. Although the mouse model of spontaneous TGCTs has been extensively studied, conventional linkage analysis has failed to locate the factors that control teratocarcinogenesis in the susceptible 129 family of inbred strains. As an alternative approach, we used both chromosome substitution strains (CSS) to identify individual chromosomes that harbor susceptibility genes and a panel of congenic strains derived from a selected CSS to determine the number and location of susceptibility variants on the substituted chromosome. We showed that 129-Chr 18(MOLF) males are resistant to spontaneous TGCTs and that at least four genetic variants control susceptibility in males with this substituted chromosome. In addition, early embryonic cells from this strain fail to establish embryonic stem cell lines as efficiently as those from the parental 129/Sv strain. For the first time, 129-derived genetic variants that control TGCT susceptibility and fundamental aspects of embryonic stem cell biology have been localized in a genetic context in which the genes can be identified and functionally characterized.

Ansari, M., et al. (2020). "Genetic Susceptibility to Hepatic Sinusoidal Obstruction Syndrome in Pediatric Patients Undergoing Hematopoietic Stem Cell Transplantation." Biol Blood Marrow Transplant **26**(5): 920-927.

Sinusoidal obstruction syndrome (SOS) is a well-recognized and potentially life-threatening complication of hematopoietic stem cell transplantation

(HSCT). SOS arises from endothelial cell damage and hepatocellular injury mostly due to the transplantation conditioning regimens but also to other patient, disease, and treatment-related factors. Understanding risk factors associated with the development of SOS is critical for early initiation of treatment or prophylaxis. The knowledge about genetic contribution is limited; few studies investigated so far selected a set of genes. To get more comprehensive insight in the genetic component, we performed an exome-wide association study using genetic variants derived from whole-exome sequencing. The analyses were performed in a discovery cohort composed of 87 pediatric patients undergoing HSCT following a busulfan-containing conditioning regimen. Eight lead single-nucleotide polymorphisms (SNPs) were identified after correction for multiple testing and subsequently analyzed in a validation cohort (n = 182). Three SNPs were successfully replicated, including rs17146905 (P = .001), rs16931326 (P = .04), and rs2289971 (P = .03), located respectively in the UGT2B10, BHLHE22, and KIAA1715 genes. UGT2B10 and KIAA1715 were retained in a multivariable model while controlling for nongenetic covariates and previously identified risk variants in the GSTA1 promoter. The modulation of associations by conditioning regimens was noted; KIAA1715 was dependent on the intensity of the conditioning regimen, whereas the effect of UGT2B10 was equally applicable to all of them. Combined effect of associated loci was also observed (P = .00006) with a genotype-related SOS risk of 9.8. To our knowledge, this is the first study addressing the genetic component of SOS at an exome-wide level and identifying novel genetic variations conferring a higher risk of SOS, which might be useful for personalized prevention and treatment strategies.

Antonucci, I., et al. (2017). "Amniotic fluid stem cell models: A tool for filling the gaps in knowledge for human genetic diseases." Brain Circ **3**(3): 167-174.

Induced pluripotent stem (iPS) cells have attracted attention in recent years as a model of human genetic diseases. Starting from the diseased somatic cells isolated from an affected patient, iPS cells can be created and subsequently differentiated into various cell types that can be used to gain a better understanding of the disease at a cellular and molecular level. There are limitations of iPS cell generation, however, due to low efficiency, high costs, and lengthy protocols. The use of amniotic fluid stem cells (AFS) presents a worthy alternative as a stem cell source for modeling of human genetic diseases. Prenatal identification of chromosomal or Mendelian diseases may require the collection of amniotic fluid which is not only useful for the sake of diagnosis but also from this, AFS cells can be isolated and cultured. Since AFS cells show some characteristics of pluripotency, having the capacity to differentiate into various cell types derived from all three germ layers in vitro, they are a well-suited model for investigations regarding alterations in the molecular biology of a cell due to a specific genetic disease. This readily accessible source of stem cells can replace the necessity for generating iPS cells. Here, we expand on the applicability and importance of AFS cells as a model for discovery in the field of human genetic disease research. This paper is a review article. Referred literature in this paper has been listed in the references section. The data sets supporting the conclusions of this article are available online by searching various databases, including PubMed. Some original points in this article come from the laboratory practice in our research center and the authors' experiences.

Apewokin, S., et al. (2018). "Host genetic susceptibility to Clostridium difficile infections in patients undergoing autologous stem cell transplantation: a genome-wide association study." Support Care Cancer **26**(9): 3127-3134.

BACKGROUND: Clostridium difficile infection (CDI) is the most common hospital-acquired infection. Unfortunately, genes that identify CDI-susceptible patients have not been well described. We performed a genome-wide association study (GWAS) to determine genetic variants associated with the development of CDI. METHODS: A cohort study of Caucasian patients undergoing autologous stem cell transplantation for multiple myeloma was performed. Patients were genotyped using Illumina(R) Whole Genome Genotyping Infinium chemistry. We then compared CDI-positive to CDI-negative patients using logistic regression for baseline clinical factors and false discovery rate (FDR) for genetic factors [single nucleotide polymorphisms (SNPs)]. SNPs associated with CDI at FDR of $p < 0.01$ were then incorporated into a logistic regression model combining clinical and genetic factors. RESULTS: Of the 646 patients analyzed (59.7% male), 57 patients were tested CDI positive (cases) and were compared to 589 patients who were tested negative (controls). Hemoglobin, albumin, and hematocrit were lower for cases ($p < 0.05$). Eight SNPs on five genes (FLJ16171, GORASP2, RLBP1L1, ASPH, ATP7B) were associated with CDI at FDR $p < 0.01$. In the combined clinical and genetic model, low albumin and three genes RLBP1L1, ASPH, and ATP7B were associated with CDI. CONCLUSION: Low serum albumin and genes RLBP1L1 and ASPH located on chromosome 8 and ATP7B on chromosome 13 were associated with CDI. Of particular interest is ATP7B given its copper modulatory role and the sporicidal properties of copper against Clostridium difficile.

Aran, B., et al. (2012). "Vitrified blastocysts from Preimplantation Genetic Diagnosis (PGD) as a source for human Embryonic Stem Cell (hESC) derivation." J Assist Reprod Genet **29**(10): 1013-1020.

Embryos diagnosed as abnormal in Preimplantation Genetic Diagnosis (PGD) cycles are useful for the establishment of human Embryonic Stem Cells (hESC) lines with genetic disorders. These lines can be helpful for drug screening and for the development of new treatments. Vitrification has proved to be an efficient method to preserve human blastocysts. One hundred and three abnormal or undiagnosed vitrified blastocysts from the PGD programme at Institut Universitari Dexeus were donated for human embryonic stem cell derivation. The overall survival rate after warming was 70.6 %. Our results showed better survival rates when blastocysts have not started the hatching process (initial/expanded 87.8 %, hatching 68.3 % and hatched 27.3 %). Thirty-five blastocysts and 12 partially surviving embryos were seeded. One hESC line with the multiple exostoses type 2 paternal mutation was obtained.

Arnheim, N. and P. Calabrese (2016). "Germline Stem Cell Competition, Mutation Hot Spots, Genetic Disorders, and Older Fathers." Annu Rev Genomics Hum Genet **17**: 219-243.

Some de novo human mutations arise at frequencies far exceeding the genome average mutation rate. Examples include the common mutations at one or a few sites in the genes that cause achondroplasia, Apert syndrome, multiple endocrine neoplasia type 2B, and Noonan syndrome. These mutations are recurrent, provide a gain of function, are paternally derived, and are more likely to be transmitted as the father ages. Recent experiments have tested whether the high mutation frequencies are due to an elevated mutation rate per cell division, as expected, or to an advantage of the mutant spermatogonial stem cells over wild-type stem cells. The evidence, which includes the surprising discovery of testis mutation clusters, rules out the former model but not the latter. We propose how the mutations might alter spermatogonial stem cell function and discuss how germline selection contributes to the paternal age effect, the human mutational load, and adaptive evolution.

Aslan, A. and S. A. Yuka (2023). "Stem Cell-Based Therapeutic Approaches in Genetic Diseases." Adv Exp Med Biol.

Stem cells, which can self-renew and differentiate into different cell types, have become the keystone of regenerative medicine due to these properties. With the achievement of superior clinical results in the therapeutic approaches of different diseases, the applications of these cells in the treatment of genetic diseases have also come to the fore. Foremost, conventional approaches of stem cells to genetic diseases are the first approaches in this manner, and they have brought safety issues due to immune reactions caused by allogeneic transplantation. To eliminate these safety issues and phenotypic abnormalities caused by genetic defects, firstly, basic genetic engineering practices such as vectors or RNA modulators were combined with stem cell-based therapeutic approaches. However, due to challenges such as immune reactions and inability to target cells effectively in these applications, advanced molecular methods have been adopted in ZFN, TALEN, and CRISPR/Cas genome editing nucleases, which allow modular designs in stem cell-based genetic diseases' therapeutic approaches. Current studies in genetic diseases are in the direction of creating permanent treatment regimens by genomic manipulation of stem cells with differentiation potential through genome editing tools. In this chapter, the stem cell-based therapeutic approaches of various vital genetic diseases were addressed wide range from conventional applications to genome editing tools.

Bailey, B., et al. (2009). "Cardiac stem cell genetic engineering using the alphaMHC promoter." Regen Med **4**(6): 823-833.

AIMS: Cardiac stem cells (CSCs) show potential as a cellular therapeutic approach to blunt tissue damage and facilitate reparative and regenerative processes after myocardial infarction. Despite multiple published reports of improvement, functional benefits remain modest using normal stem cells delivered by adoptive transfer into damaged myocardium. The goal of this study is to enhance survival and proliferation of CSCs that have undergone lineage commitment in early phases as evidenced by expression of proteins driven by the alpha-myosin heavy chain (alphaMHC) promoter. The early increased expression of survival kinases augments expansion of the cardiogenic CSC pool and subsequent daughter progeny. MATERIALS & METHODS: Normal CSCs engineered with fluorescent reporter protein constructs under control of the alphaMHC promoter show transgene protein expression, confirming activity of the promoter in CSCs. Cultured CSCs from both nontransgenic and cardiac-specific transgenic mice expressing survival kinases driven by the alphaMHC promoter were analyzed to characterize transgene expression following treatments to promote differentiation in culture. RESULTS & CONCLUSION: Therapeutic genes controlled by the alphaMHC promoter can be engineered into and expressed in CSCs and cardiomyocyte progeny with the goal of improving the efficacy of cardiac stem cell therapy.

Balic, M., et al. (2013). "Genetic and epigenetic analysis of putative breast cancer stem cell models." BMC Cancer **13**: 358.

BACKGROUND: Cancer stem cell model hypothesizes existence of a small proportion of tumor cells capable of sustaining tumor formation, self-renewal and differentiation. In breast cancer, these cells were found to be associated with CD44(+)CD24-low and ALDH(+) phenotype. Our study was performed to evaluate the suitability of current approaches for breast cancer stem cell analyses to evaluate heterogeneity of breast cancer cells through their extensive genetic and epigenetic characterization. METHODS: Breast cancer cell lines MCF7 and SUM159 were cultured in adherent conditions and as mammospheres. Flow cytometry sorting for CD44, CD24 and ALDH was performed. Sorted and unsorted populations, mammospheres and adherent cell cultures were subjected to DNA profiling by array CGH and methylation profiling by Epitect Methyl qPCR array. Methylation status of selected genes was further evaluated by pyrosequencing. Functional impact of methylation was evaluated by mRNA analysis for selected genes. RESULTS: Array CGH did not reveal any genomic differences. In contrast, putative breast cancer stem cells showed altered methylation levels of several genes compared to parental tumor cells. CONCLUSIONS: Our results underpin the hypothesis that epigenetic mechanisms seem to play a major role in the regulation of CSCs. However, it is also clear that more efficient methods for CSC enrichment are needed. This work underscores requirement of additional approaches to reveal heterogeneity within breast cancer.

Balyasnikova, I. V., et al. (2010). "Genetic modification of mesenchymal stem cells to express a single-chain antibody against EGFRvIII on the cell surface." J Tissue Eng Regen Med **4**(4): 247-258.

Human adult mesenchymal stem cells (hMSCs) are under active investigation as cellular carriers for gene therapy. hMSCs possess natural tropism toward tumours; however, the targeting of hMSCs to specific cell populations within tumours is unexplored. In the case of glioblastoma multiforme (GBM), at least half of the tumours express EGFRvIII on the cell surface, an ideal target for antibody-mediated gene/drug delivery. In this study, we investigated the feasibility of genetically modifying hMSCs to express a single-chain antibody (scFv) to EGFRvIII on their surfaces. Nucleofection was used to transfect hMSCs with cDNA encoding scFv EGFRvIII fused with PDGFR or human B7-1 transmembrane domains. The expression of scFv EGFRvIII on the cell surface was assessed by FACS. A stable population of scFv EGFRvIII-expressing hMSCs was selected, based on antibiotic resistance, and enriched using FACS. We found that nucleofection allows the efficient expression of scFv EGFRvIII on the cell surface of hMSCs. hMSCs transfected with the construct encoding scFv EGFRvIII as a fusion with PDGFRtm showed scFv EGFRvIII expression in up to 86% of cells. Most importantly, human MSCs expressing scFv against EGFRvIII demonstrated enhanced binding to U87-EGFRvIII cells in vitro and significantly increased retention in human U87-EGFRvIII-expressing tumours in vivo. In summary, we provide the first conclusive evidence of genetic modification of hMSCs with a single-chain antibody against an antigen expressed on the surface of tumour cells, thereby opening up a new venue for enhanced delivery of gene therapy applications in the context of malignant brain cancer.

Bandara, W., et al. (2021). "Comparative Analysis of the Genetic Variants in Haematopoietic Stem/Progenitor and Mesenchymal Stem Cell Compartments in de novo Myelodysplastic Syndromes." Blood Cells Mol Dis **88**: 102535.

Myelodysplastic Syndromes (MDS) are hematological clonal disorders. Bone marrow (BM) mesenchymal stem cells (MSCs) interact with the haematopoietic stem and progenitor cells (HSPCs) to regulate haematopoiesis. We studied the genetic variation profiles of BM derived CD34(+) HSPCs and MSCs of same patient in a South Asian de novo MDS cohort with 20 patients. A total of 42 genes (variants 471) and 38 genes (variants 232) were mutated in HSPCs and MSCs respectively and majority (97%) were distinct variants. Variants included both known and novel, with variants predicted as pathogenic. In both cell types, most frequently mutated genes were TET2, KDM6A, BCOR, EZH2 and ASXL. DNA methylation and chromatin remodeling were shown to be affected in both cell types with a high frequency. RNA splicing was affected more in HSPCs. Gene variants in the cohesion complex and epigenetic mechanisms were shown to co-exist. We report variant profile of MSCs and CD34(+) HSPCs from a South Asian cohort, with novel variants with potential for further study as biomarkers in MDS. Distinct variants confined to each cellular compartment indicate that the genetic variations occur following lineage commitment.

Barbon, S., et al. (2022). "In Vitro Conditioning of Adipose-Derived Mesenchymal Stem Cells by the Endothelial Microenvironment: Modeling Cell Responsiveness towards Non-Genetic Correction of Haemophilia A." Int J Mol Sci **23**(13).

In recent decades, the use of adult multipotent stem cells has paved the way for the identification of new therapeutic approaches for the treatment of monogenic diseases such as Haemophilia A. Being

already studied for regenerative purposes, adipose-derived mesenchymal stem cells (Ad-MSCs) are still poorly considered for Haemophilia A cell therapy and their capacity to produce coagulation factor VIII (FVIII) after proper stimulation and without resorting to gene transfection. In this work, Ad-MSCs were in vitro conditioned towards the endothelial lineage, considered to be responsible for coagulation factor production. The cells were cultured in an inductive medium enriched with endothelial growth factors for up to 21 days. In addition to significantly responding to the chemotactic endothelial stimuli, the cell populations started to form capillary-like structures and up-regulated the expression of specific endothelial markers (CD34, PDGFRalpha, VEGFR2, VE-cadherin, CD31, and vWF). A dot blot protein study detected the presence of FVIII in culture media collected from both unstimulated and stimulated Ad-MSCs. Remarkably, the activated partial thromboplastin time test demonstrated that the clot formation was accelerated, and FVIII activity was enhanced when FVIII deficient plasma was mixed with culture media from the untreated/stimulated Ad-MSCs. Overall, the collected evidence supported a possible Ad-MSC contribution to HA correction via specific stimulation by the endothelial microenvironment and without any need for gene transfection.

Bartoletti, M., et al. (2012). "Genetic basis for developmental homeostasis of germline stem cell niche number: a network of Tramtrack-Group nuclear BTB factors." PLoS One **7**(11): e49958.

The potential to produce new cells during adult life depends on the number of stem cell niches and the capacity of stem cells to divide, and is therefore under the control of programs ensuring developmental homeostasis. However, it remains generally unknown how the number of stem cell niches is controlled. In the insect ovary, each germline stem cell (GSC) niche is embedded in a functional unit called an ovariole. The number of ovarioles, and thus the number of GSC niches, varies widely among species. In Drosophila, morphogenesis of ovarioles starts in larvae with the formation of terminal filaments (TFs), each made of 8-10 cells that pile up and sort in stacks. TFs constitute organizers of individual germline stem cell niches during larval and early pupal development. In the Drosophila melanogaster subgroup, the number of ovarioles varies interspecifically from 8 to 20. Here we show that pipsqueak, Trithorax-like, batman and the bric-a-brac (bab) locus, all encoding nuclear BTB/POZ factors of the Tramtrack Group, are involved in limiting the number of ovarioles in D. melanogaster. At least two different processes are differentially perturbed by reducing the function of these genes. We found that when the bab dose is reduced, sorting of TF cells into TFs was affected such that each TF contains fewer cells and more TFs are formed. In contrast, psq mutants exhibited a greater number of TF cells per ovary, with a normal number of cells per TF, thereby leading to formation of more TFs per ovary than in the wild type. Our results indicate that two parallel genetic pathways under the control of a network of nuclear BTB factors are combined in order to negatively control the number of germline stem cell niches.

Bartolome, A. (2022). "Stem Cell-Derived beta Cells: A Versatile Research Platform to Interrogate the Genetic Basis of beta Cell Dysfunction." Int J Mol Sci **23**(1).

Pancreatic beta cell dysfunction is a central component of diabetes progression. During the last decades, the genetic basis of several monogenic forms of diabetes has been recognized. Genome-wide association studies (GWAS) have also facilitated the identification of common genetic variants associated with an increased risk of diabetes. These studies highlight the importance of impaired beta cell function in all forms of diabetes. However, how most of these risk variants confer disease risk, remains unanswered. Understanding the specific contribution of genetic variants and the precise role of their molecular effectors is the next step toward developing treatments that target beta cell dysfunction in the era of personalized medicine. Protocols that allow derivation of beta cells from pluripotent stem cells, represent a powerful research tool that allows modeling of human development and versatile experimental designs that can be used to shed some light on diabetes pathophysiology. This article reviews different models to study the genetic basis of beta cell dysfunction, focusing on the recent advances made possible by stem cell applications in the field of diabetes research.

Basci, S., et al. (2021). "Does myeloma genetic have an effect on stem cell mobilization?" Transfus Apher Sci **60**(6): 103249.

BACKGROUND: Autologous stem cell transplantation (ASCT) after induction treatment is the standard of care. Our understanding of myeloma genetics has been very limited and its effect to stem cell mobilization is not widely investigated. We aimed to investigate the effect of genetic abnormalities on stem cell mobilization in myeloma. METHODS: The data of 150 MM patients who underwent stem cell mobilization at our center between 2009-2020 were included and analyzed retrospectively. Pre-treatment bone marrow cytogenetics and fluorescence in situ hybridization tests were performed for each patient. RESULTS: Groups were divided into two as patients with normal cytogenetic and abnormal cytogenetic. No difference observed between groups regarding age,

gender and ECOG (p = 0.4; p = 0.2; p = 0.3). Groups were similar concerning myeloma characteristics, received treatment and treatment response. Median CD34+ cells/kg harvested was 444(2-11.29) in normal cytogenetic group whereas it was 4,8(2.4-8.6) in abnormal cytogenetic group(p = 0.2). Optimal CD34+ cells level achievement was 73 (67 %) in normal cytogenetic group while it was 25(71.4 %) in abnormal cytogenetic group(p = 0.6). Neutrophil and platelet engraftment durations were similar among cytogenetic groups (p = 0.7; p = 0.9). R-ISS based groups were also did not differ regarding harvested CD34+ cells and achievement optimal CD34 level (p = 0.79, p = 0.74). Engraftment durations for neutrophil and platelet were comparable between R-ISS based groups (p = 0.59, p = 0.65) CONCLUSIONS: Here we were not able to find any impact of genetic abnormalities on stem cell mobilization in myeloma patients. Expanded studies can aid to identify the effect of particular genetic anomalies on the stem cell mobilization.

Ben-Yosef, D., et al. (2008). "PGD-derived human embryonic stem cell lines as a powerful tool for the study of human genetic disorders." Mol Cell Endocrinol **282**(1-2): 153-158.

Human embryonic stem (ES) cells are derived from the inner cell mass (ICM) of blastocyst embryos. They are established from spare embryos that have been obtained by in vitro fertilization (IVF) and donated for research purposes. The ICM-derived cell lines have two unique properties, they can be propagated indefinitely in culture and have the potential to develop into practically any cell type in vitro and in vivo. Human embryonic stem (hES) cells carrying specific mutations can be used as a valuable tool for studying genetic disorders in human. One favorable approach to obtain such mutant ES cell lines is their derivation from affected preimplantation genetic diagnosed (PGD) embryos. This review focuses on the importance of deriving human ES cell lines from genetically abnormal embryos, especially in cases where no good cellular and/or animal models exist.

Bertinetto, F. E., et al. (2006). "Role of non-HLA genetic polymorphisms in graft-versus-host disease after haematopoietic stem cell transplantation." Int J Immunogenet **33**(5): 375-384.

Graft-versus-host disease (GvHD) is the main complication after haematopoietic stem cells transplantation (HSCT) and acute forms (aGvHD) occur in 20-40% of cases even after donor (D) and recipient (R) HLA matching, apparently because of D/R minor histocompatibility antigen (mHA) mismatches and cytokine polymorphisms. The genotype of cytokines and mHA of 77 haematological R following HSCT from HLA identical siblings were determined to detect genetic polymorphisms correlated with GvHD. We analysed TNFA (-863 C/A, -857 C/T and G/A at positions -574, -376, -308, -244, -238), IL-10 (-1082 G/A, -819 C/A, -592 C/T), IL-1B (T/C +3953), IL-1RA (VNTR), HA-1 (H/R allele) and CD-31 (C/G at codon 125, A/G at codon 563). Allele frequencies were in Hardy-Weinberg equilibrium and similar to those of 77 healthy controls. We observed positive correlations between a lower risk of clinically significant aGvHD and both the presence of -1082G -819C -592C IL-10 haplotype when both R and D are considered together and the absence of R IL-1RA allele 2. Furthermore, we observed an association between the absence of TNF-A -238 A allele and the risk of extensive chronic GvHD. mHA and cytokines genotyping would thus seem a valid source of information for the prior identification of recipients with a higher risk of aGvHD.

Bogert, N. V., et al. (2020). "A novel approach to genetic engineering of T-cell subsets by hematopoietic stem cell infection with a bicistronic lentivirus." Sci Rep **10**(1): 13740.

Lentiviral modification of hematopoietic stem cells (HSCs) paved the way for in vivo experimentation and therapeutic approaches in patients with genetic disease. A disadvantage of this method is the use of a ubiquitous promoter leads not only to genetic modification of the leukocyte subset of interest e.g. T-cells, but also all other subsequent leukocyte progeny of the parent HSCs. To overcome this limitation we tested a bicistronic lentivirus, enabling subset specific modifications. Designed novel lentiviral constructs harbor a global promoter (mPGK) regulating mCherry for HSCs selection and a T-cell specific promoter upstream of eGFP. Two T-cell specific promoters were assessed: the distal Lck-(dLck) and the CD3delta-promoter. Transduced HSCs were FACS sorted by mCherry expression and transferred into sublethally irradiated C57/BL6 mice. Successful transplantation and T-cell specific expression of eGFP was monitored by peripheral blood assessment. Furthermore, recruitment response of lentiviral engineered leukocytes to the site of inflammation was tested in a peritonitis model without functional impairment. Our constructed lentivirus enables fast generation of subset specific leukocyte transgenesis as shown in T-cells in vivo and opens new opportunities to modify other HSCs derived subsets in the future.

Bolivar, J., et al. (2006). "Genetic dissection of a stem cell niche: the case of the Drosophila ovary." Dev Dyn **235**(11): 2969-2979.

In this work, we demonstrate a powerful new tool for the manipulation of the stromal component of a well-established Drosophila stem cell niche. We have

generated a bric-a-brac 1 (bab1)-Gal4 line that drives UAS expression in many somatic ovary cell types from early larval stages. Using this Gal4 line, we could effectively induce FLP/FRT-mediated recombination in the stromal cells of the ovarian germline stem cell niche. Mutant clones were observed in the developing ovary of larvae and pupae, including in somatic cell types that do not divide in the adult, such as the cap cells and the terminal filament cells. Exploiting the ability of bab1-Gal4 to generate large clones, we demonstrate that bab1-Gal4 is an effective tool for analyzing stem cell niche morphogenesis and cyst formation in the germarium. We have identified a novel requirement for engrailed in the correct organization of the terminal filaments. We also demonstrate an involvement for integrins in cyst formation and follicle cell encapsulation. Finally using bab1-Gal4 in conjunction with the Gal80 system, we show that while ectopic dpp expression from stromal cells is sufficient to induce hyperplastic stem cell growth, neither activation nor inactivation of the BMP pathway within stromal cells affects germline stem cell maintenance.

Borgonovo, T., et al. (2014). "Genetic evaluation of mesenchymal stem cells by G-banded karyotyping in a Cell Technology Center." Rev Bras Hematol Hemoter **36**(3): 202-207.

OBJECTIVE: To present the initial results of first three years of implementation of a genetic evaluation test for bone marrow-derived mesenchymal stem cells in a Cell Technology Center. METHODS: A retrospective study was carried out of 21 candidates for cell therapy. After the isolation of bone marrow mononuclear cells by density gradient, mesenchymal stem cells were cultivated and expanded at least until the second passage. Cytogenetic analyses were performed before and after cell expansion (62 samples) using G-banded karyotyping. RESULTS: All the samples analyzed, before and after cell expansion, had normal karyotypes, showing no clonal chromosomal changes. Signs of chromosomal instability were observed in 11 out of 21 patients (52%). From a total of 910 analyzed metaphases, five chromatid gaps, six chromatid breaks and 14 tetraploid cells were detected giving as total of 25 metaphases with chromosome damage (2.75%). CONCLUSION: The absence of clonal chromosomal aberrations in our results for G-banded karyotyping shows the maintenance of chromosomal stability of bone marrow-derived mesenchymal stem cells until the second passage; however, signs of chromosomal instability such as chromatid gaps, chromosome breaks and tetraploidy indicate that the long-term cultivation of these cells can provide an intermediate step for tumorigenesis.

Brodehl, A., et al. (2019). "Human Induced Pluripotent Stem-Cell-Derived Cardiomyocytes as Models for Genetic Cardiomyopathies." Int J Mol Sci **20**(18).

In the last few decades, many pathogenic or likely pathogenic genetic mutations in over hundred different genes have been described for non-ischemic, genetic cardiomyopathies. However, the functional knowledge about most of these mutations is still limited because the generation of adequate animal models is time-consuming and challenging. Therefore, human induced pluripotent stem cells (iPSCs) carrying specific cardiomyopathy-associated mutations are a promising alternative. Since the original discovery that pluripotency can be artificially induced by the expression of different transcription factors, various patient-specific-induced pluripotent stem cell lines have been generated to model non-ischemic, genetic cardiomyopathies in vitro. In this review, we describe the genetic landscape of non-ischemic, genetic cardiomyopathies and give an overview about different human iPSC lines, which have been developed for the disease modeling of inherited cardiomyopathies. We summarize different methods and protocols for the general differentiation of human iPSCs into cardiomyocytes. In addition, we describe methods and technologies to investigate functionally human iPSC-derived cardiomyocytes. Furthermore, we summarize novel genome editing approaches for the genetic manipulation of human iPSCs. This review provides an overview about the genetic landscape of inherited cardiomyopathies with a focus on iPSC technology, which might be of interest for clinicians and basic scientists interested in genetic cardiomyopathies.

Brodszki, N., et al. (2015). "Novel Genetic Mutations in the First Swedish Patient with Purine Nucleoside Phosphorylase Deficiency and Clinical Outcome After Hematopoietic Stem Cell Transplantation with HLA-Matched Unrelated Donor." JIMD Rep **24**: 83-89.

Purine nucleoside phosphorylase (PNP) is an enzyme active in the purine salvage pathway. PNP deficiency caused by autosomal recessive mutations in the PNP gene leads to severe combined immunodeficiency (SCID) and in two thirds of cases also to neurological effects such as developmental delay, ataxia, and motor impairment.PNP deficiency has a poor outcome, and the only curative treatment is allogenic hematopoietic stem cell transplantation (HSCT). We present the first Swedish patient with PNP deficiency with novel mutations in the PNP gene and the immunological results of the HSCT and evaluate the impact of HSCT on the neurological symptoms. The patient presented early in life with neurological symptoms and suffered later from repeated serious respiratory tract infections. Biochemical tests showed severe reduction in PNP activity (1% residual activity).

Genetic testing revealed two new mutations in the PNP gene: c.729C>G (p.Asn243Lys) and c.746A>C (p.Tyr249Cys). HSCT was performed with an unrelated donor, resulting in prompt and sustained engraftment and complete donor chimerism. There was no further aggravation of the patient's neurological symptoms at 21 months post HSCT, and appropriate developmental milestones were achieved. HSCT is curative for the immunological defect caused by PNP deficiency, and our case strengthens earlier reports that HSCT is effective as a treatment even for neurological symptoms in PNP deficiency.

Bruedigam, C., et al. (2011). "Basic techniques in human mesenchymal stem cell cultures: differentiation into osteogenic and adipogenic lineages, genetic perturbations, and phenotypic analyses." Curr Protoc Stem Cell Biol **Chapter 1**: Unit1H 3.

This unit describes basic techniques in human mesenchymal stem cell (hMSC) cultures. It includes protocols for the differentiation of hMSCs into osteogenic and adipogenic lineages, genetic perturbations, and phenotypic analyses. hMSCs can be differentiated with dexamethasone and beta-glycerophosphate into mineralizing osteoblasts within 2 to 3 weeks, or with dexamethasone, indomethacin, and 3-isobutyl-1-methylxanthine into lipid vesicle-containing adipocytes within 1 to 2 weeks. Phenotypic changes during those highly dynamic differentiation processes can be detected by biochemical and histological assays and gene expression analyses of differentiation markers. In addition, this unit describes an electroporation method that allows the transient genetic perturbation of hMSCs.

Buhler, S., et al. (2020). "Genetic T-cell receptor diversity at 1 year following allogeneic hematopoietic stem cell transplantation." Leukemia **34**(5): 1422-1432.

After allogeneic hematopoietic stem cell transplantation (HSCT), immune reconstitution leads to the development of a new T-cell repertoire. Immune reconstitution could be influenced by events such as conditioning, infections, and graft versus host disease (GVHD). Factors influencing the TCR diversity are of great interest to fine-tune the strategy for donor selection and to optimize standard of care. In this work, immunosequencing of the TCR CDR3beta region was carried out in a large cohort of 116 full chimeric recipients at 1 year post-HSCT and their respective donors prior to transplantation. The repertoire overlap before and after HSCT was minimal, supporting de novo reconstitution as a primary pathway at any age. Among the parameters investigated, increased patient and/or donor age as well as positive CMV serologic status reinforced by CMV infection/reactivation were the ones significantly associated with a reduced diversity at 1 year post-HSCT. CMV-specific T-cell clones were shown to influence the clonality of the repertoire alongside the expansion of limited numbers of non-CMV T-cell populations. Interestingly, at the exception of CMV infection/reactivation, TCR diversity was not predictive of GVHD, relapse, death, or infections post-HSCT.

Buikema, J. W. and S. M. Wu (2017). "Untangling the Biology of Genetic Cardiomyopathies with Pluripotent Stem Cell Disease Models." Curr Cardiol Rep **19**(4): 30.

PURPOSE OF REVIEW: Recently, the discovery of strategies to reprogram somatic cells into induced pluripotent stem (iPS) cells has led to a major paradigm change in developmental and stem cell biology. The application of iPS cells and their cardiac progeny has opened novel directions to study cardiomyopathies at a cellular and molecular level. This review discusses approaches currently undertaken to unravel known inherited cardiomyopathies in a dish. RECENT FINDINGS: With improved efficiency for mutation correction by genome editing, human iPS cells have now provided a platform to untangle the biology of cardiomyopathies. Multiple studies have derived pluripotent stem cells lines from patients with genetic heart diseases. The generation of cardiomyocytes from these cells lines has, for the first time, enable the study of cardiomyopathies using cardiomyocytes harboring patient-specific mutations and their corrected isogenic counterpart. The molecular analyses, functional assays, and drug tests of these lines have led to new molecular insights in the early pathophysiology of left ventricular non-compaction cardiomyopathy (LVNC), hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), and others. The advent of iPS cells offers an exceptional opportunity for creating disease-specific cellular models to investigate their underlying mechanisms and to optimize future therapy through drug and toxicity screening. Thus far, the iPS cell model has improved our understanding of the genetic and molecular pathophysiology of patients with various genetic cardiomyopathies. It is hoped that the new discoveries arising from using these novel platforms for cardiomyopathy research will lead to new diagnostic and therapeutic approaches to prevent and treat these diseases.

Burns, C. E., et al. (2009). "A genetic screen in zebrafish defines a hierarchical network of pathways required for hematopoietic stem cell emergence." Blood **113**(23): 5776-5782.

Defining the genetic pathways essential for hematopoietic stem cell (HSC) development remains a

fundamental goal impacting stem cell biology and regenerative medicine. To genetically dissect HSC emergence in the aorta-gonad-mesonephros (AGM) region, we screened a collection of insertional zebrafish mutant lines for expression of the HSC marker, c-myb. Nine essential genes were identified, which were subsequently binned into categories representing their proximity to HSC induction. Using overexpression and loss-of-function studies in zebrafish, we ordered these signaling pathways with respect to each other and to the Vegf, Notch, and Runx programs. Overexpression of vegf and notch is sufficient to induce HSCs in the tbx16 mutant, despite a lack of axial vascular organization. Although embryos deficient for artery specification, such as the phospholipase C gamma-1 (plcgamma1) mutant, fail to specify HSCs, overexpression of notch or runx1 can rescue their hematopoietic defect. The most proximal HSC mutants, such as hdac1, were found to have no defect in vessel or artery formation. Further analysis demonstrated that hdac1 acts downstream of Notch signaling but upstream or in parallel to runx1 to promote AGM hematopoiesis. Together, our results establish a hierarchy of signaling programs required and sufficient for HSC emergence in the AGM.

Butler Iii, R. R., et al. (2020). "The Genetic Relevance of Human Induced Pluripotent Stem Cell-Derived Microglia to Alzheimer's Disease and Major Neuropsychiatric Disorders." Mol Neuropsychiatry **5**(Suppl 1): 85-96.

Microglia are the primary innate immune cell type in the brain that have been implicated in the pathogenesis of several neurodegenerative and neuropsychiatric disorders, most notably Alzheimer's disease (AD) and schizophrenia. Microglia generated from human induced pluripotent stem cells (hiPSCs) represent a promising in vitro cellular model for studying the neuroimmune interactions involved in these disorders. Among several methods of generating - hiPSC-derived microglia (iMG) - varying in duration and resultant purity - a recent protocol by Brownjohn et al. [Stem Cell Reports. 2018 Apr;10(4):1294-307] is particularly simple and efficient. However, the replicability of this method, transcriptomic similarity of these iMG to primary adult microglia, and their genetic relevance to disease (i.e., enrichment of disease risk loci in genes preferentially expressed in these cells) remains unclear. Using two hiPSC lines, we demonstrated that Brownjohn's protocol can rapidly generate iMG that morphologically and functionally resembled microglia. The iMG cells we generated were found to be transcriptionally similar to previously reported iMG, as well as fetal and adult microglia. Furthermore, by using cell type-specific gene expression to partition disease heritability, we showed that iMG cells are genetically relevant to AD but found no significant enrichments of risk loci of Parkinson's disease, schizophrenia, major depressive disorder, bipolar disorder, autism spectrum disorder, or body mass index. Across a range of neuronal and immune cell types, we found only iMG, primary microglia, and microglia-like cell types exhibited a significant enrichment for AD heritability. Our results thus support the use of iMG as a human cellular model for understanding AD biology and underlying genetic factors, as well as for developing and efficiently screening new therapeutics.

Byun, J. M., et al. (2019). "Combination of Genetic Aberration With International Staging System Classification for Stratification of Asian Multiple Myeloma Patients Undergoing Autologous Stem Cell Transplantation." In Vivo **33**(2): 611-619.

BACKGROUND/AIM: The aim of the study was to contribute to the development of adaptive risk stratification methods specific to Asian multiple myeloma (MM) patients undergoing autologous stem cell transplantation (ASCT). PATIENTS AND METHODS: We conducted this study to evaluate the prognostic impact of genetic abnormalities detected by fluorescent in situ hybridization (FISH) on survival outcomes in combination with the International Staging System (ISS) classification in 161 MM patients. This was a single-center retrospective longitudinal cohort study of newly diagnosed MM patients undergoing ASCT within 12 months from initial diagnosis. A single-center retrospective cohort study of newly diagnosed MM. RESULTS: Patients were divided into 3 groups according to risk stratification: 1) low-risk, patients without del(17p13) nor t(14;16) or t(4;14) and ISS I/II; 2) high-risk, patients with t(4;14), regardless of ISS stage; 3) intermediate-risk, all remaining patients. The median PFS for the low-risk group was 18 months versus 13 months for the intermediate group ($p=0.047$, HR=1.527, 95%CI=1.006-2.316) versus 10 months for the high-risk group ($p<0.001$, HR=2.656, 95%CI=1.572-4.490). CONCLUSION: An ISS/FISH-based prognostication strategy was developed that can predict PFS for Asian MM patients undergoing ASCT.

Cai, X. J., et al. (2012). "[Role of IFN-gamma + 874 genetic polymorphisms in allogeneic hematopoietic stem cell transplantation]." Zhonghua Xue Ye Xue Za Zhi **33**(12): 989-993.

OBJECTIVE: To explore the impact of IFN-gamma + 874 polymorphisms on the outcome in HLA matched sibling HSCT. METHODS: We used PCR-sequence-specific primer analysis (PCR-SSP) to analyze the polymorphisms of IFN-gamma + 874 T/A in 80 recipient and donor pairs from October 2005 to March 2008. RESULTS: Recipients having donors

who possessed IFN-gamma + 874 A/A genotype had significantly earlier neutrophil recovery compared with those having donors with non-A/A genotype (15 (11 - 27) days vs 18 (12 - 30) days, P = 0.029). And IFN-gamma + 874 A/A in both recipients and donors further facilitated neutrophil recovery compared with others (13 (11 - 25) days and 19 (12 - 31) days, P = 0.019). Besides, IFN-gamma + 874 A/A in recipients increased the probability of grade II-IV acute graft versus disease (aGVHD) and cytomegalovirus viraemia compared with IFN-gamma + 874 T/A or T/T genotype (20% vs 4% P = 0.041, 43.6% vs 16.0% P = 0.032), which lead to increased 5-year transplant-related mortality (TRM) (33.7% +/- 6.8% vs 12.0% +/- 6.5%, P = 0.050) and decreased 5-year event free survival (EFS) \[(58.2 +/- 6.7)% vs (84.0 +/- 7.3)%, P = 0.032\] compared with the latter. IFN-gamma + 874 A/A in both recipients and donors also significantly increased the probability of grade II-IV aGVHD and cytomegalovirus viraemia compared with the other (21.7% vs 5.9%, P = 0.050; 45.7% vs 20.6%, P = 0.020), which caused increased 5-year TRM \[(31.6 +/- 7.5)% vs (13.6 +/- 6.5)%, P = 0.048\] and decreased 5-year EFS \[(56.8 +/- 7.3)% vs (79.4 +/- 6.9)%, P = 0.037\] compared with the other. CONCLUSION: In HLA-matched sibling HSCT setting, the presence of IFN-gamma + 874 T allele in recipients or in both recipients and donors significantly decreased the risk of grade II-IV aGVHD and CMV infection and increased EFS. While IFN-gamma + 874 A/A in donors or in both recipients and donors was associated with shorter duration to neutrophil recovery.

Carpinteiro, A., et al. (2002). "Genetic protection of repopulating hematopoietic cells with an improved MDR1-retrovirus allows administration of intensified chemotherapy following stem cell transplantation in mice." Int J Cancer **98**(5): 785-792.

This study was undertaken to analyze the hematotoxicity of paclitaxel (Taxol) and to test whether transduction of repopulating hematopoietic cells with a retroviral vector (SF1m) expressing the human multidrug resistance 1 gene (MDR1) would permit dose intensification following bone marrow transplantation (BMT). While the regimen chosen (8 x 20 mg/kg i.p. within 12 days) produced a non-lethal, reversible hematotoxicity in mice with steady-state hematopoiesis, only 35.3% (6/17) of control mice survived when treated starting 14 days post BMT. In contrast, 83.3% (15/18) of mice transplanted with SF1m-transduced cells survived, owing to a significant protection against severe acute myelotoxicity (as determined by neutrophil counts, white and red blood cell counts and values for hemoglobin and hematocrit). After recovery from chemotherapy, an increase of myeloid cells that were resistant to colchicine and effluxed the fluorochrome Rhodamine 123 was observed in SF1m-mice, but not in controls. These results reveal that the lethal, dose-limiting hematotoxicity of an intensified post-transplantation chemotherapy with paclitaxel can be prevented by retroviral transfer of the MDR1 gene to a minor proportion of repopulating cells. Our mouse model, mimicking clinically achievable gene transfer rates, thus suggests that bone marrow chemoprotection may widen the therapeutic window and permit an earlier onset of post-transplantation chemotherapy.

Carvalho, A., et al. (2010). "Prognostic significance of genetic variants in the IL-23/Th17 pathway for the outcome of T cell-depleted allogeneic stem cell transplantation." Bone Marrow Transplant **45**(11): 1645-1652.

T helper (Th) 17 cells have emerged as important mediators in infectious and inflammatory diseases and, recently, in transplant rejection. We analyzed the associations between five common genetic variants in the IL-23/Th17 signaling pathway, namely in IL17A, IL17F and IL23R genes, and clinical outcome in T cell-depleted allogeneic SCT (allo-SCT). In the multivariate analysis, variants in IL23R and IL17A genes were the most important prognostic factors. Thus, patient GA genotype at rs11209026 in IL23R was associated with improved overall survival (hazard ratio (HR)=0.48; P=0.028) and, in donor, with decreased risk of fungal infections (P=0.05). In contrast, patient TC and CC genotypes at rs8193036 in IL17A gene were associated with increased risk of CMV infection (HR=3.68; P=0.011) and patient acute GVHD (HR=7.08; P=0.008), respectively. These results suggest that genetic variants in the IL-23/Th17 inflammatory pathway are important prognostic factors for the clinical outcome of allo-SCT. Although validation studies are ultimately required, our results would suggest the potential usefulness of IL-23/Th17 genotyping in donor selection and patient evaluation.

Chang, H., et al. (2005). "Genetic risk identifies multiple myeloma patients who do not benefit from autologous stem cell transplantation." Bone Marrow Transplant **36**(9): 793-796.

Genetic aberrations have emerged as major prognostic factors for patients with multiple myeloma (MM). We evaluated 126 MM patients for t(4;14) or t(11;14), 13q or p53 deletions and correlated the number of genetic aberrations with patient's clinical outcome following undergoing autologous stem cell transplantation. We demonstrate the significance of genetic-based risk classification that clearly segregate patients into low (no genetic abnormalities or only t(11;14)), intermediate (any one of the genetic abnormalities other than t(11;14)) and high-risk groups (any two or more of the genetic abnormalities other

than t(11;14)). High-risk patients do not benefit from stem cell transplant and should be offered alternative therapies.

Chang, X., et al. (2022). "Harnessing the Power of Stem Cell Models to Study Shared Genetic Variants in Congenital Heart Diseases and Neurodevelopmental Disorders." Cells **11**(3).

Advances in human pluripotent stem cell (hPSC) technology allow one to deconstruct the human body into specific disease-relevant cell types or create functional units representing various organs. hPSC-based models present a unique opportunity for the study of co-occurring disorders where "cause and effect" can be addressed. Poor neurodevelopmental outcomes have been reported in children with congenital heart diseases (CHD). Intuitively, abnormal cardiac function or surgical intervention may stunt the developing brain, leading to neurodevelopmental disorders (NDD). However, recent work has uncovered several genetic variants within genes associated with the development of both the heart and brain that could also explain this co-occurrence. Given the scalability of hPSCs, straightforward genetic modification, and established differentiation strategies, it is now possible to investigate both CHD and NDD as independent events. We will first overview the potential for shared genetics in both heart and brain development. We will then summarize methods to differentiate both cardiac & neural cells and organoids from hPSCs that represent the developmental process of the heart and forebrain. Finally, we will highlight strategies to rapidly screen several genetic variants together to uncover potential phenotypes and how therapeutic advances could be achieved by hPSC-based models.

Chavali, N. V., et al. (2019). "Patient-independent human induced pluripotent stem cell model: A new tool for rapid determination of genetic variant pathogenicity in long QT syndrome." Heart Rhythm **16**(11): 1686-1695.

BACKGROUND: Commercial genetic testing for long QT syndrome (LQTS) has rapidly expanded, but the inability to accurately predict whether a rare variant is pathogenic has limited its clinical benefit. Novel missense variants are routinely reported as variant of unknown significance (VUS) and cannot be used to screen family members at risk for sudden cardiac death. Better approaches to determine the pathogenicity of VUS are needed. OBJECTIVE: The purpose of this study was to rapidly determine the pathogenicity of a CACNA1C variant reported by commercial genetic testing as a VUS using a patient-independent human induced pluripotent stem cell (hiPSC) model. METHODS: Using CRISPR/Cas9 genome editing, CACNA1C-p.N639T was introduced into a previously established hiPSC from an unrelated healthy volunteer, thereby generating a patient-independent hiPSC model. Three independent heterozygous N639T hiPSC lines were generated and differentiated into cardiomyocytes (CM). Electrophysiological properties of N639T hiPSC-CM were compared to those of isogenic and population control hiPSC-CM by measuring the extracellular field potential (EFP) of 96-well hiPSC-CM monolayers and by patch clamp. RESULTS: Significant EFP prolongation was observed only in optically stimulated but not in spontaneously beating N639T hiPSC-CM. Patch-clamp studies revealed that N639T prolonged the ventricular action potential by slowing voltage-dependent inactivation of Ca(V)1.2 currents. Heterologous expression studies confirmed the effect of N639T on Ca(V)1.2 inactivation. CONCLUSION: The patient-independent hiPSC model enabled rapid generation of functional data to support reclassification of a CACNA1C VUS to likely pathogenic, thereby establishing a novel LQTS type 8 mutation. Furthermore, our results indicate the importance of controlling beating rates to evaluate the functional significance of LQTS VUS in high-throughput hiPSC-CM assays.

Chen, D., et al. (2019). "Genetic alterations and expression of PTEN and its relationship with cancer stem cell markers to investigate pathogenesis and to evaluate prognosis in hepatocellular carcinoma." J Clin Pathol **72**(9): 588-596.

AIMS: To investigate molecular alteration and expression of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) gene in hepatocellular carcinoma (HCC), and to evaluate the correlation between PTEN and cancer stem cell (CSC) markers and the prognostic value of these markers. METHODS: We evaluated changes of PTEN and CSC markers (CD133, epithelial cell adhesion molecule (EpCAM) and CK19) in 183 resection specimens by immunohistochemistry (IHC) and detected PTEN and phosphoinositide-3-kinase catalytic-alpha (PIK3CA) gene by fluorescence in situ hybridisation (FISH) in some specimens. RESULTS: PTEN and CD133, EpCAM and CK19 in 183 resection specimens were studied by IHC, and PTEN and PIK3CA genes were detected by FISH. PTEN expression was reduced in 92 HCC tissues (50.3%). There were 16 HCCs with PTEN deletion (51.6%). Comparison between PTEN IHC and FISH showed that the analysis was highly concordant (54/59, 91.5%). There were 19 HCCs with PIK3CA amplification. Deletion of PTEN was positively correlated with amplification of PIK3CA. Positive expression of CD133, EpCAM and CK19 was correlated with steatosis, moderate to poor differentiation, and so on. Reduction of PTEN

expression was negatively correlated with positive expression of CD133, EpCAM and CK19. Reduced expression of PTEN (p=0.028) was an independent predictor for HCC recurrence and overall survival in HCC. PTEN-/CD133+ group had shorter OS and RFS time. CONCLUSIONS: PTEN plays a key role in hepatocarcinogenesis and reduction of PTEN expression is related to increased expression of CD133, EpCAM and CK19, which is a useful tool to evaluate HCC prognosis and recurrence.

Chen, D. P., et al. (2023). "The association between genetic variants at 3'-UTR and 5'-URR of HLA-G gene and the clinical outcomes of patients with leukemia receiving hematopoietic stem cell transplantation." Front Immunol **14**: 1093514.

In addition to the classical human leukocyte antigen (HLA) genes, the outcomes of post-hematopoietic stem cell transplantation (HSCT) are associated with human leukocyte antigen (HLA)-related genes and non-HLA genes involved in immune regulation. HLA-G gene plays an important role in immune tolerance, assisting immune escape of tumor cells, and decrease of transplant rejection. In this study, we explored the association of genetic variants at the 3'-untranslated region (3'-UTR) and 5'-upstream regulatory region (5'-URR) of HLA-G gene with the adverse outcomes of patients with leukemia receiving HSCT. The genomic DNAs of 164 patients who had acute leukemia and received HSCT were collected for analysis. Nine single nucleotide polymorphisms (SNPs) and six haplotypes in the 3'-UTR and 27 SNPs and 6 haplotypes in the 5'-URR were selected to investigate their relationship with the development of adverse outcomes for patients receiving HSCT, including mortality, relapse, and graft-versus-host disease. Our results revealed that two SNPs (rs371194629 and rs9380142) and one haplotype (UTR-3) located in the 3'-UTR and two SNPs (rs3823321 and rs1736934) and one haplotype (G0104a) located in the 5'-URR of HLA-G were associated with the occurrence of chronic GVHD or development of any forms of GVHD. No SNP was found to associate with the occurrence of mortality and relapse for patients receiving HSCT. These SNPs and haplotypes may play important roles in regulating immune tolerance of allografts post-HSCT that can be used to predict the risk of poor outcomes after receiving HSCT and giving preventive treatment to patients on time.

Chen, J., et al. (2000). "Genetic regulation of primitive hematopoietic stem cell senescence." Exp Hematol **28**(4): 442-450.

OBJECTIVE: To define effects of strain on PHSC (primitive hematopoietic stem cells) senescence (decline in function with age) in vivo, and to map a locus that regulates PHSC senescence. MATERIALS AND METHODS: Long-term function and self-renewal were compared in bone marrow cells (BMC) from old and young mice of three strains: BALB/cBy (BALB), DBA/2 (D2) and C57BL/6 (B6), using competitive repopulation and serial transplantation in vivo. BMC from each old or young donor were mixed with standard doses of congenic, genetically marked BMC and transplanted into lethally recipients. Percentages of donor-type erythrocytes and lymphocytes in the recipients determined the functional ability of donor PHSC relative to the standard, where one repopulating unit (RU) of donor BMC equals the repopulating ability of 100,000 standard competitor BMC. Using similar techniques, repopulating abilities of old and young recombinant inbred (RI) donors of 12 strains derived from BALB and B6 were compared in NK-depleted BALBxB6 Fl recipients to map a locus that appears to have a major role in PHSC senescence. RESULTS: PHSC function declined about 2 fold with age in BALB and D2 BMC, and increased more than 2-fold with age in B6 BMC, with all old/young strain differences significant, p<.01. Ten months after serial transplantation, young B6, BALB, and D2 PHSC had self-renewed 1.6-, 4.2-, and 3.2-fold better than old, with BALB and D2 old/young differences p<.01. Young B6 PHSC self-renewed 1.9- and 2.9-fold better than young BALB and D2 PHSC. The PHSC senescence phenotypes (old/young RU ratios) for 12 CXB RI strains suggested a genetic linkage to D12Nyul7 on Chromosome 12. CONCLUSION: PHSC senescence is genetically regulated, and is much delayed in the B6 strain compared to the BALB and D2 strains. A locus on Chromosome 12 may regulate PHSC senescence.

Chen, K. G., et al. (2014). "Alternative cultures for human pluripotent stem cell production, maintenance, and genetic analysis." J Vis Exp(89).

Human pluripotent stem cells (hPSCs) hold great promise for regenerative medicine and biopharmaceutical applications. Currently, optimal culture and efficient expansion of large amounts of clinical-grade hPSCs are critical issues in hPSC-based therapies. Conventionally, hPSCs are propagated as colonies on both feeder and feeder-free culture systems. However, these methods have several major limitations, including low cell yields and generation of heterogeneously differentiated cells. To improve current hPSC culture methods, we have recently developed a new method, which is based on non-colony type monolayer (NCM) culture of dissociated single cells. Here, we present detailed NCM protocols based on the Rho-associated kinase (ROCK) inhibitor Y-27632. We also provide new information regarding NCM culture with different small molecules such as Y-

39983 (ROCK I inhibitor), phenylbenzodioxane (ROCK II inhibitor), and thiazovivin (a novel ROCK inhibitor). We further extend our basic protocol to cultivate hPSCs on defined extracellular proteins such as the laminin isoform 521 (LN-521) without the use of ROCK inhibitors. Moreover, based on NCM, we have demonstrated efficient transfection or transduction of plasmid DNAs, lentiviral particles, and oligonucleotide-based microRNAs into hPSCs in order to genetically modify these cells for molecular analyses and drug discovery. The NCM-based methods overcome the major shortcomings of colony-type culture, and thus may be suitable for producing large amounts of homogeneous hPSCs for future clinical therapies, stem cell research, and drug discovery.

Chen, K. G., et al. (2017). "Mouse Genetic Analysis of Bone Marrow Stem Cell Niches: Technological Pitfalls, Challenges, and Translational Considerations." Stem Cell Reports **9**(5): 1343-1358.

The development of mouse genetic tools has made a significant contribution to the understanding of skeletal and hematopoietic stem cell niches in bone marrow (BM). However, many experimental designs (e.g., selections of marker genes, target vector constructions, and choices of reporter murine strains) have unavoidable technological limitations and bias, which lead to experimental discrepancies, data reproducibility issues, and frequent data misinterpretation. Consequently, there are a number of conflicting views relating to fundamental biological questions, including origins and locations of skeletal and hematopoietic stem cells in the BM. In this report, we systematically unravel complicated data interpretations via comprehensive analyses of technological benefits, pitfalls, and challenges in frequently used mouse models and discuss their translational relevance to human stem cell biology. Particularly, we emphasize the important roles of using large human genomic data-informatics in facilitating genetic analyses of mouse models and resolving existing controversies in mouse and human BM stem cell biology.

Chen, P. M., et al. (2002). "Detection of reactivation and genetic mutations of the hepatitis B virus in patients with chronic hepatitis B infections receiving hematopoietic stem cell transplantation." Transplantation **74**(2): 182-188.

BACKGROUND: This study elucidates the profiles for hepatitis B virus (HBV) reactivation and genetic mutation of the core promoter and precore regions for HBV-carriers receiving hematopoietic stem cell transplantation (HSCT). METHODS: Sera from 20 HSCT patients diagnosed with hematological diseases, 13 donors and 36 healthy HBV-carriers, were collected regularly for analysis. The hepatic biochemistry profiles, serological HBV markers, and HBV-DNA titers were checked regularly, and primer-amplification of the HBV core promoter or precore region and sequencing were performed once the mutations were identified. RESULTS: Deteriorated liver function was demonstrated for 13 of 20 post-HSCT patients, compared with none of the 36 controls ($P<0.01$). The HBV-DNA was detected more frequently for post-HSCT subjects than for controls ($P=0.001$). Incidence of the HBV precore nucleotide 1896 G-to-A mutation was significantly higher for HSCT patients ($P=0.004$), and a significant association was demonstrated for carriage of core promoter or precore mutations and the development of hepatitis ($P=0.015$). Different HBV genotypes were revealed in post-HSCT patients and the respective donors. CONCLUSIONS: Intensive chemotherapy and immunosuppression may cause HBV reactivation in HBV carriers receiving HSCT, and more frequent core promoter or precore mutations could be detected in HBV carriers receiving HSCT than healthy HBV carriers, with the chemotherapy/immunosuppression-induced immunocompromise possibly contributing to this effect. Donor HBV genotype did not interfere with that of the recipient after HSCT. Core promoter or precore region mutations were associated with a higher incidence of liver dysfunction than wild-type HBV carriers in the HSCT patients.

Chen, X., et al. (2017). "A Chemical-Genetic Approach Reveals the Distinct Roles of GSK3alpha and GSK3beta in Regulating Embryonic Stem Cell Fate." Dev Cell **43**(5): 563-576 e564.

Glycogen synthase kinase 3 (GSK3) plays a central role in diverse cellular processes. GSK3 has two mammalian isozymes, GSK3alpha and GSK3beta, whose functions remain ill-defined because of a lack of inhibitors that can distinguish between the two highly homologous isozymes. Here, we show that GSK3alpha and GSK3beta can be selectively inhibited in mouse embryonic stem cells (ESCs) using a chemical-genetic approach. Selective inhibition of GSK3beta is sufficient to maintain mouse ESC self-renewal, whereas GSK3alpha inhibition promotes mouse ESC differentiation toward neural lineages. Genome-wide transcriptional analysis reveals that GSK3alpha and GSK3beta have distinct sets of downstream targets. Furthermore, selective inhibition of individual GSK3 isozymes yields distinct phenotypes from gene deletion, highlighting the power of the chemical-genetic approach in dissecting kinase catalytic functions from the protein's scaffolding functions. Our study opens new avenues for defining GSK3 isozyme-specific functions in various cellular processes.

Chen, Z., et al. (2018). "Genetic Engineering of Human Embryonic Stem Cells for Precise Cell Fate Tracing during Human Lineage Development." Stem Cell Reports **11**(5): 1257-1271.

It is highly desirable to specify human developmental principles in an appropriate human model with advanced genetic tools. However, genetically engineering human cells with lineage-tracing systems has not been achieved. Here we introduce strategies to construct lineage-tracing systems in human embryonic stem cells (hESCs). The AAVS1 locus was suitable for the integration of the conditional reporter. The Cre-LoxP and Flp-FRT systems were highly sensitive, which may cause inaccurate lineage labeling in human cells. The recombination sensitivity and tracing fidelity could be finely tuned by modification of the LoxP recombination site. Moreover, tamoxifen-controllable Cre(ERT2)-LoxP and Flp(ERT2)-FRT systems showed compelling advantages in tightly tracing human lineages temporally. In proof-of-principle experiments, we traced human PAX6(+) neuroectoderm cells and revealed their full neural lineage differentiation potency both in vitro and in vivo. Devising and optimizing of lineage-tracing systems in hESCs will thus set up a solid foundation for human developmental studies.

Cheung, A. N., et al. (2009). "Pathogenesis of choriocarcinoma: clinical, genetic and stem cell perspectives." Future Oncol **5**(2): 217-231.

Choriocarcinoma is a unique malignant neoplasm composed of mononuclear cytotrophoblasts and multinucleated syncytiotrophoblasts that produce human chorionic gonadotrophin. Choriocarcinoma can occur after a pregnancy, as a component of germ cell tumors, or in association with a poorly differentiated somatic carcinoma, each with distinct clinical features. Cytogenetic and molecular studies, predominantly on gestational choriocarcinoma, revealed the impact of oncogenes, tumor suppressor genes and imprinting genes on its pathogenesis. The role of stem cells in various types of choriocarcinoma has been studied recently. This review will discuss how such knowledge can enhance our understanding of the pathogenesis of choriocarcinoma, enable exploration of novel anti-choriocarcinoma targeted therapy and possibly improve our insight on embryological and placental development.

Cho, I. K., et al. (2014). "Longitudinal monitoring of stem cell grafts in vivo using magnetic resonance imaging with inducible maga as a genetic reporter." Theranostics **4**(10): 972-989.

PURPOSE: The ability to longitudinally monitor cell grafts and assess their condition is critical for the clinical translation of stem cell therapy in regenerative medicine. Developing an inducible genetic magnetic resonance imaging (MRI) reporter will enable non-invasive and longitudinal monitoring of stem cell grafts in vivo. METHODS: MagA, a bacterial gene involved in the formation of iron oxide nanocrystals, was genetically modified for in vivo monitoring of cell grafts by MRI. Inducible expression of MagA was regulated by a Tet-On (Tet) switch. A mouse embryonic stem cell-line carrying Tet-MagA (mESC-MagA) was established by lentivirus transduction. The impact of expressing MagA in mESCs was evaluated via proliferation assay, cytotoxicity assay, teratoma formation, MRI, and inductively coupled plasma atomic emission spectroscopy (ICP-OES). Mice were grafted with mESCs with and without MagA (mESC-MagA and mESC-WT). The condition of cell grafts with induced "ON" and non-induced "OFF" expression of MagA was longitudinally monitored in vivo using a 7T MRI scanner. After imaging, whole brain samples were harvested for histological assessment. RESULTS: Expression of MagA in mESCs resulted in significant changes in the transverse relaxation rate (R2 or 1/T2) and susceptibility weighted MRI contrast. The pluripotency of mESCs carrying MagA was not affected in vitro or in vivo. Intracranial mESC-MagA grafts generated sufficient T2 and susceptibility weighted contrast at 7T. The mESC-MagA grafts can be monitored by MRI longitudinally upon induced expression of MagA by administering doxycycline (Dox) via diet. CONCLUSION: Our results demonstrate MagA could be used to monitor cell grafts noninvasively, longitudinally, and repetitively, enabling the assessment of cell graft conditions in vivo.

Chou, S. C., et al. (2008). "Identification of genetic networks during mesenchymal stem cell transformation into neurons." Chin J Physiol **51**(4): 230-246.

The aim of this experiment is to identify related genes for human umbilical mesenchymal stem cells transformation into nervous cells. After the human umbilical mesenchymal stem cells were treated with neuronal conditioned medium (NCM) for 9 days, the gene expression groups are compared to those only treated with DMEM. The related genes for cell cycles, the human umbilical mesenchymal stem cells treated with DMEM increases the amount of cells that remain in the G2/M phase and S phase, including CAV1, EBF, NRG1, CDH13, MLH1. After treatment, the human umbilical cord mesenchymal stem cells with NCM for 9 days, gene expression related to the G0/G1 phase are also increased, including MYC, CSF3, PETN. Gene expressions related to neural regeneration and neural stem cells also increase significantly, such as CXCL1, BMP2, NRCAM, FGF2, SPG7. This study thereby

provides a foundation for a more detailed understanding of HUMSCs neuronal differentiation.

Chow, M., et al. (2013). "Human pluripotent stem cell-derived cardiomyocytes for heart regeneration, drug discovery and disease modeling: from the genetic, epigenetic, and tissue modeling perspectives." Stem Cell Res Ther **4**(4): 97.

Heart diseases remain a major cause of mortality and morbidity worldwide. However, terminally differentiated human adult cardiomyocytes (CMs) possess a very limited innate ability to regenerate. Directed differentiation of human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) into CMs has enabled clinicians and researchers to pursue the novel therapeutic paradigm of cell-based cardiac regeneration. In addition to tissue engineering and transplantation studies, the need for functional CMs has also prompted researchers to explore molecular pathways and develop strategies to improve the quality, purity and quantity of hESC-derived and iPSC-derived CMs. In this review, we describe various approaches in directed CM differentiation and driven maturation, and discuss potential limitations associated with hESCs and iPSCs, with an emphasis on the role of epigenetic regulation and chromatin remodeling, in the context of the potential and challenges of using hESC-CMs and iPSC-CMs for drug discovery and toxicity screening, disease modeling, and clinical applications.

Christensen, K., et al. (2000). "X-linked genetic factors regulate hematopoietic stem-cell kinetics in females." Blood **95**(7): 2449-2451.

X inactivation makes females mosaics for 2 cell populations, usually with an approximate 1:1 distribution. Skewing of this distribution in peripheral blood cells is more common among elderly women. The depletion of hematopoietic stem cells followed by random differentiation may explain the acquired skewing with age. However, an animal model suggests that selection processes based on X-linked genetic factors are involved. We studied peripheral blood cells from 71 monozygotic twin pairs aged 73 to 93 years and from 33 centenarians, and we found that with age, 1 of the cell populations becomes predominant for most women. We also observed a strong tendency for the same cell line to become predominant in 2 co-twins. This suggests that X-linked genetic factors influence human hematopoietic stem cell kinetics. The fact that females have 2 cell lines with different potentials could be one of the reasons women live longer than men.

Coleman, E. A., et al. (2015). "GWAS of 972 autologous stem cell recipients with multiple myeloma identifies 11 genetic variants associated with chemotherapy-induced oral mucositis." Support Care Cancer **23**(3): 841-849.

PURPOSE: High-dose chemotherapy and autologous stem cell transplant (ASCT) to treat multiple myeloma (MM) and other cancers carries the risk of oral mucositis (OM) with sequelae including impaired nutritional and fluid intake, pain, and infectious complications. As a result of these problems, cancer treatment may have to be interrupted or delayed. In this study, we looked beyond OM's known risk factors of renal function and melphalan dose with a genome-wide association study (GWAS) to evaluate whether genetic variants in conjunction with clinical risk factors influence predisposition for OM. METHODS: Genotyping was performed using Illumina HumanOmni1-Quad v1.0 BeadChip and further assessed for data quality. We tested 892,589 germline single-nucleotide polymorphisms (SNPs) for association with OM among 972 Caucasian patients treated with high-dose melphalan and ASCT in Total Therapy clinical trials (TT2, TT3, TT4) for newly diagnosed MM. Statistical analyses included t tests, stepwise regression modeling, and logistic regression modeling to find baseline clinical factors and genotypes associated with OM. RESULTS: We found that 353 (36.3 %) patients had grades 2-4 OM. Type of treatment protocol, baseline estimated glomerular filtration rate, and melphalan dose along with baseline serum albumin and female gender predicted 43.6 % of grades 2-4 OM cases. Eleven SNPs located in or near matrix metalloproteinase 13, JPH3, DHRS7C, CEP192, CPEB1/LINC00692, FBN2, ALDH1A1, and DMRTA1/FLJ35282 were associated with grades 2-4 OM. The addition of these SNPs increased sensitivity in detecting grades 2-4 OM cases to 52 %. CONCLUSIONS: These SNPs may be important for their roles in inflammatory pathways, epithelial healing, and chemotherapy detoxification.

Collin, J. and M. Lako (2011). "Concise review: putting a finger on stem cell biology: zinc finger nuclease-driven targeted genetic editing in human pluripotent stem cells." Stem Cells **29**(7): 1021-1033.

Human pluripotent stem cells (hPSCs) encompassing human embryonic stem cells and human induced pluripotent stem cells (hiPSCs) have a wide appeal for numerous basic biology studies and for therapeutic applications because of their potential to give rise to almost any cell type in the human body and immense ability to self-renew. Much attention in the stem cell field is focused toward the study of gene-based anomalies relating to the causative affects of human disease and their correction with the potential for patient-specific therapies using gene corrected hiPSCs. Therefore, the genetic manipulation of stem cells is clearly important for the development of future

medicine. Although successful targeted genetic engineering in hPSCs has been reported, these cases are surprisingly few because of inherent technical limitations with the methods used. The development of more robust and efficient means by which to achieve specific genomic modifications in hPSCs has far reaching implications for stem cell research and its applications. Recent proof-of-principle reports have shown that genetic alterations with minimal toxicity are now possible through the use of zinc finger nucleases (ZFNs) and the inherent DNA repair mechanisms within the cell. In light of recent comprehensive reviews that highlight the applications, methodologies, and prospects of ZFNs, this article focuses on the application of ZFNs to stem cell biology, discussing the published work to date, potential problems, and future uses for this technology both experimentally and therapeutically.

Corrales, I., et al. (2017). "IL28B genetic variation and cytomegalovirus-specific T-cell immunity in allogeneic stem cell transplant recipients." J Med Virol **89**(4): 685-695.

A single nucleotide polymorphism (SNP), 3 kbp upstream of the IL28B gene (rs12979860; C/T), has been shown to influence the dynamics of cytomegalovirus (CMV) replication in allogeneic stem cell transplant recipients (Allo-SCT). We investigated whether this SNP had any effect on the dynamics of CMV-specific T-cell immunity in these patients. CMV pp65/IE-1 IFN-gamma CD8(+) and CD4(+) T cells were enumerated by flow cytometry in 85 patients with no prior CMV DNAemia (group A) and in 57 after the onset of CMV DNAemia (group B). Donor IL28B genotype was determined by real-time PCR and plasma levels of IL-28B were quantitated by ELISA. CMV-specific T-cell counts and plasma IL-28B levels in patients in group A were not significantly different among the IL28B genotype groups. Patients harboring the donor IL28B T/T genotype appeared to expand CMV-specific IFN-gamma CD8(+) cells to a higher level in response to viral replication than their C/T and C/C counterparts. Fewer patients in the T/T group received pre-emptive antiviral therapy (P = 0.05). Overall, a significant inverse correlation was observed between median IL-28B levels measured prior to the CMV DNAemia onset and the level of CMV-specific CD8(+) T cells enumerated after detection of CMV DNAemia (sigma = -0.471; P = 0.013). In summary, the data suggested that the protective effect attributed to the rs12979860 SNP minor T allele could be mediated, at least in part, by eliciting robust CMV-specific T-cell responses. J. Med. Virol. 89:685-695, 2017. (c) 2016 Wiley Periodicals, Inc.

Daher-Reyes, G., et al. (2021). "Prognostic impact of the adverse molecular-genetic profile on long-term outcomes following allogeneic hematopoietic stem cell transplantation in acute myeloid leukemia." Bone Marrow Transplant **56**(8): 1908-1918.

The impact of adverse risk genetic profiles on outcomes in acute myeloid leukemia (AML) patients following allogeneic hematopoietic stem cell transplantation (HCT) has not been fully elucidated. Accordingly, we have profiled somatic mutations at diagnosis using next-generation sequencing (NGS) in 178 AML patients who received allogeneic HCT. NGS revealed 598 somatic mutations in 165/178 patients (92.7%). Frequently mutated genes include DNMT3A, TET2, NPM1, RUNX1, IDH2, and FLT3. Commonly detected cytogenetic profiles include normal karyotype, trisomy 8, monosomal karyotype (MK), deletion 5, complex karyotype (CK), and monosomy 7. In univariate analyses, TP53 mutation, MK, CK, and monosomy 7 were associated with decreased overall survival (OS), relapse-free survival (RFS), and a higher relapse incidence (RI). We defined adverse molecular-genetic profile as harboring at least one of the molecular/genetic abnormalities of TP53 mutation, MK, CK, monosomy 7, and deletion 5. The patients harboring adverse molecular-genetic profile (n = 30) showed a lower 2-year OS (24.9% vs. 57.9%; p = 0.003), RFS (23.7% vs. 57.9%; p = 0.002), and higher RI (47.2% and 17.2%; p = 0.001) after HCT when compared to patients without those lesions. Multivariate analysis confirmed adverse molecular-genetic profile as an independent prognostic factor, associated with decreased OS (HR 2.19), RFS (HR 2.23), and higher RI (HR 2.94).

Dai, Q., et al. (2021). "Generation of a human induced pluripotent stem cell line (SIAISi010-A) from a 31-year-old healthy donor with Chinese Han genetic background." Stem Cell Res **53**: 102314.

A healthy 31-year-old Chinese Han female donated peripheral blood mononuclear cells (PBMC). Her PBMCs were reprogrammed with human OKSM (OCT3/4, KLF4 SOX2, and c-MYC) transcription factors by the non-integrating episomal vector system. Immunocytochemistry for pluripotency markers confirmed the pluripotency of transgene-free iPSCs. Their ability to differentiate spontaneously three germ layers in vitro is also confirmed. The iPSC line displayed a normal karyotype. This model can be used as a control in pathological mechanism studies.

Dai, Q., et al. (2020). "Generation of an induced pluripotent stem cell line (SIAISi003-A) from a 79-year-old patient with Alzheimer's disease having APOE3/4 genetic background." Stem Cell Res **48**: 101949.

The genotype of apolipoprotein E (APOE) is closely associated with susceptibility to Alzheimer's disease. Here, we described the generation and characterization of human induced pluripotent stem cells from peripheral blood mononuclear cells (PBMCs) of a 79-year-old female patient with Alzheimer's disease having APOE3/4 genotype. The generated iPSCs expressed pluripotent stem cell markers that were observed using immunocytochemistry. Moreover, they displayed a normal karyotype and had the potential to differentiate spontaneously into three germ layers in vitro. Our model could provide valuable insights into pathological mechanisms, and offer a unique opportunity for developing drugs against specific phenotypes for Alzheimer's disease therapy.

Deregibus, M. C., et al. (2010). "The dynamic stem cell microenvironment is orchestrated by microvesicle-mediated transfer of genetic information." Histol Histopathol **25**(3): 397-404.

It has been commonly supposed that adult stem cells co-localize with supporting cells within specific regions or specialized microenvironment in each tissue/organ, called stem cell niche. This concept was based on the assumption that stem cells are intrinsically hierarchical in nature. However, recent data indicate that stem cells may represent a continuum with reversible alterations in phenotype taking place during the transit through cell cycle. Based on this dynamic interpretation it has been suggested that the so-called niche is represented by a single or only few cell types continually adjusting their phenotype and function to individual circumstances. A critical component in the regulation of the continuum of stem cell phenotypes is the microenvironment. In this context, microvesicles (MVs) account for the transfer of genetic information between cells. Originally considered inert cellular debris, MVs are increasingly recognized to be important mediators of cell-to-cell communication. MVs may transfer receptors, proteins, mRNA and microRNA to target cells via specific receptor-mediated interaction. In stem cell biology the exchange of genetic information may be bidirectional from stromal to stem cells. In the context of tissue injury the MV-mediated transfer of genetic information may reprogram the phenotype of stem cells to acquire features of the injured tissue cells. In addition, MVs derived from stem cells may induce de-differentiation of cells which have survived injury with a cell cycle re-entry that may allow tissue regeneration. In the present review we discuss the possibility of a continuous genetic modulation of stem cells by a MV-mediated transfer of information between cells.

Desai, R., et al. (2020). "Unexpected variation in leukemia stem cell frequency and genetic heterogeneity in two murine leukemia models initiated by AML1/ETO9a and CALM/AF10." Leukemia **34**(6): 1706-1710.

Di Fiore, R., et al. (2013). "Genetic and molecular characterization of the human osteosarcoma 3AB-OS cancer stem cell line: a possible model for studying osteosarcoma origin and stemness." J Cell Physiol **228**(6): 1189-1201.

Finding new treatments targeting cancer stem cells (CSCs) within a tumor seems to be critical to halt cancer and improve patient survival. Osteosarcoma is an aggressive tumor affecting adolescents, for which there is no second-line chemotherapy. Uncovering new molecular mechanisms underlying the development of osteosarcoma and origin of CSCs is crucial to identify new possible therapeutic strategies. Here, we aimed to characterize genetically and molecularly the human osteosarcoma 3AB-OS CSC line, previously selected from MG63 cells and which proved to have both in vitro and in vivo features of CSCs. Classic cytogenetic studies demonstrated that 3AB-OS cells have hypertriploid karyotype with 71-82 chromosomes. By comparing 3AB-OS CSCs to the parental cells, array CGH, Affymetrix microarray, and TaqMan(R) Human MicroRNA array analyses identified 49 copy number variations (CNV), 3,512 dysregulated genes and 189 differentially expressed miRNAs. Some of the chromosomal abnormalities and mRNA/miRNA expression profiles appeared to be congruent with those reported in human osteosarcomas. Bioinformatic analyses selected 196 genes and 46 anticorrelated miRNAs involved in carcinogenesis and stemness. For the first time, a predictive network is also described for two miRNA family (let-7/98 and miR-29a,b,c) and their anticorrelated mRNAs (MSTN, CCND2, Lin28B, MEST, HMGA2, and GHR), which may represent new biomarkers for osteosarcoma and may pave the way for the identification of new potential therapeutic targets.

Ebert, A. D., et al. (2014). "Characterization of the molecular mechanisms underlying increased ischemic damage in the aldehyde dehydrogenase 2 genetic polymorphism using a human induced pluripotent stem cell model system." Sci Transl Med **6**(255): 255ra130.

Nearly 8% of the human population carries an inactivating point mutation in the gene that encodes the cardioprotective enzyme aldehyde dehydrogenase 2 (ALDH2). This genetic polymorphism (ALDH2*2) is linked to more severe outcomes from ischemic heart damage and an increased risk of coronary artery disease (CAD), but the underlying molecular bases are unknown. We investigated the ALDH2*2 mechanisms in a human model system of induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) generated from individuals carrying the most common

heterozygous form of the ALDH2*2 genotype. We showed that the ALDH2*2 mutation gave rise to elevated amounts of reactive oxygen species and toxic aldehydes, thereby inducing cell cycle arrest and activation of apoptotic signaling pathways, especially during ischemic injury. We established that ALDH2 controls cell survival decisions by modulating oxidative stress levels and that this regulatory circuitry was dysfunctional in the loss-of-function ALDH2*2 genotype, causing up-regulation of apoptosis in cardiomyocytes after ischemic insult. These results reveal a new function for the metabolic enzyme ALDH2 in modulation of cell survival decisions. Insight into the molecular mechanisms that mediate ALDH2*2-related increased ischemic damage is important for the development of specific diagnostic methods and improved risk management of CAD and may lead to patient-specific cardiac therapies.

Ebrahim, N., et al. (2020). "Genetic Modification of Mesenchymal Stem Cells for Neurological Disease Therapy: What Effects Does it Have on Phenotype/Cell Behavior, Determining Their Effectiveness?" Mol Diagn Ther **24**(6): 683-702.

Mesenchymal stem cells are a promising tool in regenerative medicine, and their functions can be enhanced through genetic modification. Recent advances in genetic engineering provide several methods that enable gene delivery to mesenchymal stem cells. However, it remains to be decided whether genetic modification of mesenchymal stem cells by vectors carrying reporter or therapeutic genes leads to adverse effects on morphology, phenotypic profiles, and viability of transplanted cells. In this regard, we focus on the description of genetic modification methods of mesenchymal stem cells, their effectiveness, and the impact on phenotype/cell behavior/proliferation and the differentiation ability of these cells in vitro and in vivo. Furthermore, we compare the main effects of genetically modified mesenchymal stem cells with native mesenchymal stem cells when applied in the therapy of neurological diseases.

Ebrahimi, R., et al. (2021). "Effects of melatonin on the Bisphenol-A- induced cytotoxicity and genetic toxicity in colon cancer cell lines, normal gingival cell lines, and bone marrow stem cell lines." Cancer Inform **20**: 11769351211056295.

Bisphenol-A (BPA) is a synthetic chemical that has widely been used in the production of polycarbonate plastic and epoxy resins in the manufacture of consumer products. The most common path of human exposure to BPA is by oral intake that involves genotoxicity, oxidative stress, endocrine disruption, mutagenicity, and carcinogenicity in both in vitro and in vivo models. Melatonin is known as a free-radical scavenger and a powerful antioxidant agent. This study aimed to investigate the effects of melatonin on viability and genetic disorders of normal Human Gingival Fibroblasts (HGF), colon cancer (MKN45), and bone marrow stem cell (MSC) lines exposed to BPA. For this purpose, MTT and Comet assays were performed to evaluate the cytotoxicity and genotoxicity properties of BPA and the role of melatonin. The results showed that BPA exposure resulted in increased oxidative stress parameters including MDA and ROS, and decreased GSH content. The current study demonstrated the cytotoxicity and genotoxicity effects of BPA and the protective role of melatonin in preventing cytotoxicity and DNA damage are induced by BPA.

Forlanini, F., et al. (2023). "Impact of Genetic Diagnosis on the Outcome of Hematopoietic Stem Cell Transplant in Primary Immunodeficiency Disorders." J Clin Immunol **43**(3): 636-646.

To evaluate the relationship between knowledge of genetic diagnosis before HSCT and outcome, we reviewed all HSCTs for primary immune deficiencies (PID) performed at UCSF from 2007 through 2018. SCID, a distinct entity identified since 2010 in California by newborn screening and treated early, was considered separately. The underlying genetic condition was known at the time of HSCT in 85% of cases. Graft failure was less frequent in patients with a genetic diagnosis (19% with a genetic diagnosis versus 47% without, p = 0.020). Furthermore, event-free survival and overall survival (OS) at 5 years were better for those with a genetic diagnosis (78% with versus 44% without, p = 0.006; and 93% versus 60% without, p = 0.0002, respectively). OS at 5 years was superior for known-genotype patients with both SCID (p = 0.010) and non-SCID PID (p = 0.010). There was no difference in OS between HSCT done in 2007-2010 compared to more recently (p = 0.19). These data suggest that outcomes of HSCT for PID with known genotype may reflect specific experience and literature, or that a substantial proportion of patients with PID of undetermined genotype may have had underlying conditions for which HSCT may carry greater risk. The higher rate of graft failure in PID with unknown genotype may be in part explained by insufficient conditioning, which in turn could be dictated by compromised organ function in patients undergoing HSCT late in the course. Widespread availability of PID gene sequencing as standard care can provide genetic diagnoses for most patients with PID prior to HSCT, permitting optimization of transplant approach.

Forte, A., et al. (2014). "Genetic, epigenetic and stem cell alterations in endometriosis: new insights and

potential therapeutic perspectives." Clin Sci (Lond) **126**(2): 123-138.

Human endometrium is a highly dynamic tissue, undergoing periodic growth and regression at each menstrual cycle. Endometriosis is a frequent chronic pathological status characterized by endometrial tissue with an ectopic localization, causing pelvic pain and infertility and a variable clinical presentation. In addition, there is well-established evidence that, although endometriosis is considered benign, it is associated with an increased risk of malignant transformation in approximately 1.0% of affected women, with the involvement of multiple pathways of development. Increasing evidence supports a key contribution of different stem/progenitor cell populations not only in the cyclic regeneration of eutopic endometrium, but also in the pathogenesis of at least some types of endometriosis. Evidence has arisen from experiments in animal models of disease through different kinds of assays (including clonogenicity, the label-retaining cell approach, the analysis of undifferentiation markers), as well as from descriptive studies on ectopic and eutopic tissue samples harvested from affected women. Changes in stem cell populations in endometriotic lesions are associated with genetic and epigenetic alterations, including imbalance of miRNA expression, histone and DNA modifications and chromosomal aberrations. The present short review mainly summarizes the latest observations contributing to the current knowledge regarding the presence and the potential contribution of stem/progenitor cells in eutopic endometrium and the aetiology of endometriosis, together with a report of the most recently identified genetic and epigenetic alterations in endometriosis. We also describe the potential advantages of single cell molecular profiling in endometrium and in endometriotic lesions. All these data can have clinical implications and provide a basis for new potential therapeutic applications.

Foster, G. A. and B. M. Stringer (1999). "Genetic regulatory elements introduced into neural stem and progenitor cell populations." Brain Pathol **9**(3): 547-567.

The genetic manipulation of neural cells has advantage in both basic biology and medicine. Its utility has provided a clearer understanding of how the survival, connectivity, and chemical phenotype of neurones is regulated during, and after, embryogenesis. Much of this achievement has come from the recent generation by genetic means of reproducible and representative supplies of precursor cells which can then be analyzed in a variety of paradigms. Furthermore, advances made in the clinical use of transplantation for neurodegenerative disease have created a demand for an abundant, efficacious and safe supply of neural cells for grafting. This review describes how genetic methods, in juxtaposition to epigenetic means, have been used advantageously to achieve this goal. In particular, we detail how gene transfer techniques have been developed to enable cell immortalization, manipulation of cell differentiation and commitment, and the controlled selection of cells for purification or safety purposes. In addition, it is now also possible to genetically modify antigen presentation on cell surfaces. Finally, there is detailed the transfer of therapeutic products to discrete parts of the central nervous system (CNS), using neural cells as elegant and sophisticated delivery vehicles. In conclusion, once the epigenetic and genetic controls over neural cell production, differentiation and death have been more fully determined, providing a mixture of hard-wired elements and more flexibly expressed characteristics becomes feasible. Optimization of the contributions and interactions of these two controlling systems should lead to improved cell supplies for neurotransplantation.

Franek, R., et al. (2019). "Preservation of female genetic resources of common carp through oogonial stem cell manipulation." Cryobiology **87**: 78-85.

Several experiments were conducted in order to develop an optimal protocol for slow-rate freezing (-1 degrees C/min) and short-term storage (-80 or 4 degrees C) of common carp ovarian tissue fragments with an emphasis on oogonial stem cells (OSCs). Dimethyl sulfoxide (Me(2)SO) with concentration of 1.5 M was identified as the best cryoprotectant in comparison to propylene glycol and methanol. When comparing supplementation of sugars (glucose, trehalose, sucrose) in different concentrations (0.1, 0.3, 0.5 M), glucose and trehalose in 0.3 M were identified as optimal. Short-term storage options for ovarian tissue pieces at -80 degrees C and 4 degrees C were tested as alternatives to cryopreservation and storage in liquid nitrogen. The presence of OSCs was confirmed by immunocytochemistry and viability after storage was determined by the trypan blue exclusion test. This study identified the optimal protocol for OSC cryopreservation using slow rate freezing resulting in approximately 65% viability. The frozen/thawed OSCs were labelled by PKH-26 and transplanted into goldfish recipients. The success of the transplantation was confirmed by presence of fluorescent cells in the recipient gonad and later on by RT-PCR with carp dnd1 specific primers. The results of this study can facilitate long-term preservation of common carp germplasm which can be recovered in a surrogate recipient through interspecific germ cell transplantation.

Gam, R., et al. (2017). "Genetic Association of Hematopoietic Stem Cell Transplantation Outcome

beyond Histocompatibility Genes." Front Immunol **8**: 380.

The outcome of hematopoietic stem cell transplantation (HSCT) is controlled by genetic factors among which the leukocyte antigen human leukocyte antigen (HLA) matching is most important. In addition, minor histocompatibility antigens and non-HLA gene polymorphisms in genes controlling immune responses are known to contribute to the risks associated with HSCT. Besides single-nucleotide polymorphisms (SNPs) in protein coding genes, SNPs in regulatory elements such as microRNAs (miRNAs) contribute to these genetic risks. However, genetic risks require for their realization the expression of the respective gene or miRNA. Thus, gene and miRNA expression studies may help to identify genes and SNPs that indeed affect the outcome of HSCT. In this review, we summarize gene expression profiling studies that were performed in recent years in both patients and animal models to identify genes regulated during HSCT. We discuss SNP-mRNA-miRNA regulatory networks and their contribution to the risks associated with HSCT in specific examples, including forkheadbox protein 3 and regulatory T cells, the role of the miR-155 and miR-146a regulatory network for graft-versus-host disease, and the function of MICA and its receptor NKG2D for the outcome of HSCT. These examples demonstrate how SNPs affect expression or function of proteins that modulate the alloimmune response and influence the outcome of HSCT. Specific miRNAs targeting these genes and directly affecting expression of mRNAs are identified. It might be valuable in the future to determine SNPs and to analyze miRNA and mRNA expression in parallel in cohorts of HSCT patients to further elucidate genetic risks of HSCT.

Gan, G. G., et al. (2016). "Influence of genetic polymorphisms of cytokine genes in the outcome of HLA-matched allogeneic stem cell transplantation in a South East Asian population." Cytokine **78**: 55-61.

Non-HLA gene polymorphisms have been shown to be associated with the risk of graft-versus-host disease (GVHD) and outcome of allogeneic haematopoietic stem cell transplantation (AHSCT). This study aims to investigate the role of IL6, TNFalpha, IL10, IL2 and IL12 gene polymorphisms in the outcome of AHSCT in a South East Asian population. A total of 67 patients and 59 donors who underwent HLA-identical matched sibling AHSCT were available for analysis. There was no significant association between the different cytokine genotypes of patients with the incidence and severity of acute GVHD. Patients with IL2 166 *T allele and patients who received donor stem cells who had IL2 166 *G allele appeared to have reduced incidence of cGVHD. Patients who received donor stem cells with IL12 1188 *C allele are found to be associated with better disease free survival. These results suggest a possible role of IL2 and IL12 gene polymorphisms in the outcome of AHSCT in a South East Asian population.

Ganuza, M., et al. (2012). "Genetic inactivation of Cdk7 leads to cell cycle arrest and induces premature aging due to adult stem cell exhaustion." EMBO J **31**(11): 2498-2510.

Cyclin-dependent kinase (Cdk)7, the catalytic subunit of the Cdk-activating kinase (CAK) complex has been implicated in the control of cell cycle progression and of RNA polymerase II (RNA pol II)-mediated transcription. Genetic inactivation of the Cdk7 locus revealed that whereas Cdk7 is completely dispensable for global transcription, is essential for the cell cycle via phosphorylation of Cdk1 and Cdk2. In vivo, Cdk7 is also indispensable for cell proliferation except during the initial stages of embryonic development. Interestingly, widespread elimination of Cdk7 in adult tissues with low proliferative indexes had no phenotypic consequences. However, ablation of conditional Cdk7 alleles in tissues with elevated cellular turnover led to the efficient repopulation of these tissues with Cdk7-expressing cells most likely derived from adult stem cells that may have escaped the inactivation of their targeted Cdk7 alleles. This process, a physiological attempt to maintain tissue homeostasis, led to the attrition of adult stem cell pools and to the appearance of age-related phenotypes, including telomere shortening and early death.

Garcia, I., et al. (2012). "Genetic strategies to investigate neuronal circuit properties using stem cell-derived neurons." Front Cell Neurosci **6**: 59.

The mammalian brain is anatomically and functionally complex, and prone to diverse forms of injury and neuropathology. Scientists have long strived to develop cell replacement therapies to repair damaged and diseased nervous tissue. However, this goal has remained unrealized for various reasons, including nascent knowledge of neuronal development, the inability to track and manipulate transplanted cells within complex neuronal networks, and host graft rejection. Recent advances in embryonic stem cell (ESC) and induced pluripotent stem cell (iPSC) technology, alongside novel genetic strategies to mark and manipulate stem cell-derived neurons, now provide unprecedented opportunities to investigate complex neuronal circuits in both healthy and diseased brains. Here, we review current technologies aimed at generating and manipulating neurons derived from ESCs and iPSCs toward investigation and manipulation of complex neuronal circuits, ultimately leading to the design and development of novel cell-based therapeutic approaches.

Garcia-Ramirez, I., et al. (2013). "Genetic background affects susceptibility to tumoral stem cell reprogramming." Cell Cycle **12**(15): 2505-2509.

The latest studies of the interactions between oncogenes and its target cell have shown that certain oncogenes may act as passengers to reprogram tissue-specific stem/progenitor cell into a malignant cancer stem cell state. In this study, we show that the genetic background influences this tumoral stem cell reprogramming capacity of the oncogenes using as a model the Sca1-BCRABLp210 mice, where the type of tumor they develop, chronic myeloid leukemia (CML), is a function of tumoral stem cell reprogramming. Sca1-BCRABLp210 mice containing FVB genetic components were significantly more resistant to CML. However, pure Sca1-BCRABLp210 FVB mice developed thymomas that were not seen in the Sca1-BCRABLp210 mice into the B6 background. Collectively, our results demonstrate for the first time that tumoral stem cell reprogramming fate is subject to polymorphic genetic control.

Gassas, A., et al. (2011). "Long-term adaptive functioning outcomes of children with inherited metabolic and genetic diseases treated with hematopoietic stem cell transplantation in a single large pediatric center: parents' perspective." J Pediatr Hematol Oncol **33**(3): 216-220.

Over the past 2 decades, hematopoietic stem cell transplantation (HSCT) has been used as therapy for selected inherited metabolic and genetic diseases (IMGDs). The primary objective of HSCT for these disorders has been to promote long-term survival, optimize quality of life, and improve neurocognitive performance. We performed 45 HSCTs for 44 children with IMGDs (13 related and 32 unrelated); 24 HSCTs for 23 children with Hurler syndrome, 8 for malignant infantile osteopetrosis, 6 for X-linked adrenoleukodystrophy, 2 for metachromatic leukodystrophy, 2 for Gaucher disease, 1 for Ganglioside Monosialic Acid (GM) gangliosidosis, 1 for sialiosis (type 2), and 1 HSCT for Niemann-Pick type A. At a median follow-up of 7.2 years (range: 2.2 to 17.6 y) 18 of 23 patients with Hurler syndrome are alive, 15 attended regular school. Thirteen of 18 were ambulatory, 2 had mobility difficulties, and 1 uses wheelchair. For non-Hurler patients, 5 children suffered secondary graft failure and 4 of them died from progressive disease. The remaining children with osteopetrosis are alive and most children attended regular school. One out of the 4 survivors with adrenoleukodystrophy has been transferred to the adult follow-up clinic and he is in full-time employment. Parents' perspectives and expectations of HSCT in these IMGDs were positive and supportive to continue to offer HSCT for these disorders.

Ge, L., et al. (2021). "A phosphoproteomics study reveals a defined genetic program for neural lineage commitment of neural stem cells induced by olfactory ensheathing cell-conditioned medium." Pharmacol Res **172**: 105797.

Since both Olfactory ensheathing cells (OECs) and neural stem cells (NSCs) have shown certain efficacy in the cellular therapy of nerve injury and disease, there have been a series of investigations in recent years looking at the co-culture of NSCs and OECs. Protein phosphorylation forms the basis for identifying a variety of cellular signaling pathways responsible for regulating the self-renewal and differentiation of NSCs induced by OECs. To better understand the signaling cascades in the early phases of OEC-induced NSC differentiation, changes in the NSC proteome and phosphoproteome during the first 24 h were determined using dimethyl labeling and TiO(2) phosphorylation enrichment coupled with Liquid chromatography-tandem mass spectrometry (LC-MS/MS). A total of 565 proteins and 2511 phosphorylation sites were identified. According to quantitative phosphoproteomics analyses of NSC differentiation induced by OECs during the first 12 and 24 h, it was speculated that there were at least two different signal waves: one peaking within 12 h after stimulation and the second upsurge after 24 h. In addition to understanding the dynamics of the proteome and phosphoproteome in the early stages of NSC differentiation, our analyses identified a key role of the TGF-beta3 protein secreted by OECs, which may be an initiating factor that promotes differentiation of NSCs into neurons induced by OECs. These findings not only redemonstrated a OECs-based therapeutic strategy in cell therapy, but also added a node to the regulatory network for the neural lineage commitment of NSCs induced by OECs.

Geiger, H., et al. (2005). "Regulation of hematopoietic stem cell aging in vivo by a distinct genetic element." Proc Natl Acad Sci U S A **102**(14): 5102-5107.

Until recently, stem cells were thought to be endowed with unlimited self-renewal capacity and, thus, assumed exempt from aging. But accumulating evidence over the past decade compellingly argues that a measurable and progressive replicative impairment in the hematopoietic, intestinal, and muscle stem cell activity exists from adulthood to old age, resulting in a decline in stem cell function and rendering stem cell aging as the possible link between cellular aging and organismal aging. By using a previously uncharacterized congenic animal model to study genetic regulation of hematopoietic stem cell aging, we

have demonstrated definitively that a locus on murine chromosome 2 regulates hematopoietic stem cell aging. In addition to demonstrating that hematopoietic stem cell aging is regulated by a distinct genetic element, experimental evidence links the response of hematopoietic stem cells to DNA double-strand breaks to cellular aging, suggesting DNA integrity influences stem cell aging.

George, M. N., et al. (2021). "Genome Editing Human Pluripotent Stem Cells to Model beta-Cell Disease and Unmask Novel Genetic Modifiers." Front Endocrinol (Lausanne) **12**: 682625.

A mechanistic understanding of the genetic basis of complex diseases such as diabetes mellitus remain elusive due in large part to the activity of genetic disease modifiers that impact the penetrance and/or presentation of disease phenotypes. In the face of such complexity, rare forms of diabetes that result from single-gene mutations (monogenic diabetes) can be used to model the contribution of individual genetic factors to pancreatic beta-cell dysfunction and the breakdown of glucose homeostasis. Here we review the contribution of protein coding and non-protein coding genetic disease modifiers to the pathogenesis of diabetes subtypes, as well as how recent technological advances in the generation, differentiation, and genome editing of human pluripotent stem cells (hPSC) enable the development of cell-based disease models. Finally, we describe a disease modifier discovery platform that utilizes these technologies to identify novel genetic modifiers using induced pluripotent stem cells (iPSC) derived from patients with monogenic diabetes caused by heterozygous mutations.

Hansen, D. K., et al. (2021). "ELN 2017 Genetic Risk Stratification Predicts Survival of Acute Myeloid Leukemia Patients Receiving Allogeneic Hematopoietic Stem Cell Transplantation." Transplant Cell Ther **27**(3): 256 e251-256 e257.

European LeukemiaNet (ELN) 2017 risk stratification by genetics is prognostic of outcomes in patients with acute myeloid leukemia (AML). However, the prognostic impact of the 2017 ELN genetic risk stratification after allogeneic hematopoietic cell transplantation (alloHCT) is not well established. We examined the effect of 2017 ELN genetic risk stratification on alloHCT outcomes of AML. We included 500 adult (>/=18 years) AML patients in first (n = 370) or second (n = 130) complete remission receiving alloHCT from 2005 to 2016. Patients were classified into favorable (12%), intermediate (57%), and adverse (32%) 2017 ELN risk groups. The Cox proportional hazard model was used to conduct the multivariable analyses of leukemia-free survival (LFS) and overall survival (OS). Relapse and nonrelapse mortality were analyzed by the Fine-Gray regression model. OS at 2 years was 72% in the favorable versus 60% in the intermediate versus 45% in the adverse risk groups (P < .001). In multivariable analyses, the 2017 ELN classifier was an independent predictor of OS after alloHCT with significantly higher overall mortality in the intermediate (hazard ratio [HR] = 1.68, 95% confidence interval [CI], 1.06-2.68; P = .03) and adverse (HR = 2.50, 95% CI, 1.54-4.06; P < .001) risk groups compared to the favorable risk group. Similarly, LFS was worse in the intermediate (HR = 1.63, 95%, CI 1.06-2.53; P = .03) and adverse (HR 2.23, 95% CI, 1.41-3.54; P < .001) risk groups while relapse was higher in the adverse risk group (HR = 2.36, 95% CI, 1.28-4.35; P = .006) as compared to the favorable risk group. These data highlight the prognostic impact of the 2017 ELN genetic risk stratification on the survival of AML patients after alloHCT. Patients in the adverse risk group had the highest risk of relapse and worst survival. Thus the 2017 ELN prognostic system can help identify AML patients who may benefit from clinical trials offering relapse mitigation strategies to improve transplant outcomes.

Hansen, J. A., et al. (2010). "Defining genetic risk for graft-versus-host disease and mortality following allogeneic hematopoietic stem cell transplantation." Curr Opin Hematol **17**(6): 483-492.

PURPOSE OF REVIEW: This review explores what is known about the genetics of hematopoietic stem cell transplantation (HCT) and how genetic polymorphism affects risk of graft-versus-host disease (GVHD) and mortality. RECENT FINDINGS: Genetic variation found across the human genome can impact HCT outcome by causing genetic disparity between patient and donor and modifying gene function. Single nucleotide polymorphism (SNP) and structural variation can result in mismatching for cellular peptides known as histocompatibility antigens. At least 25-30 polymorphic genes are known to encode functional histocompatibility antigens in mismatched individuals, but their individual contribution to clinical GVHD is unclear. HCT outcome may also be affected by polymorphism in donor or recipient. Association studies have implicated several genes associated with GVHD and mortality, however results have been inconsistent most likely due to limited sample size, and differences in racial diversity and clinical covariates. New technologies using DNA arrays genotyping for a million or more SNPs promise genome-wide discovery of HCT-associated genes, however adequate statistical power requires study populations of several thousand patient-donor pairs. SUMMARY: Available data offers strong preliminary support for the impact that genetic variation has on risk of GVHD and mortality following

HCT. Definitive results however await future genome-wide studies of large multicenter HCT cohorts.

Hashem, S. I. and W. C. Claycomb (2013). "Genetic isolation of stem cell-derived pacemaker-nodal cardiac myocytes." Mol Cell Biochem **383**(1-2): 161-171.

Dysfunction of the cardiac pacemaker tissues due to genetic defects, acquired diseases, or aging results in arrhythmias. When arrhythmias occur, artificial pacemaker implants are used for treatment. However, the numerous limitations of electronic implants have prompted studies of biological pacemakers that can integrate into the myocardium providing a permanent cure. Embryonic stem (ES) cells cultured as three-dimensional (3D) spheroid aggregates termed embryoid bodies possess the ability to generate all cardiac myocyte subtypes. Here, we report the use of a SHOX2 promoter and a Cx30.2 enhancer to genetically identify and isolate ES cell-derived sinoatrial node (SAN) and atrioventricular node (AVN) cells, respectively. The ES cell-derived Shox2 and Cx30.2 cardiac myocytes exhibit a spider cell morphology and high intracellular calcium loading characteristic of pacemaker-nodal myocytes. These cells express abundant levels of pacemaker genes such as endogenous HCN4, Cx45, Cx30.2, Tbx2, and Tbx3. These cells were passaged, frozen, and thawed multiple times while maintaining their pacemaker-nodal phenotype. When cultured as 3D aggregates in an attempt to create a critical mass that simulates in vivo architecture, these cell lines exhibited an increase in the expression level of key regulators of cardiovascular development, such as GATA4 and GATA6 transcription factors. In addition, the aggregate culture system resulted in an increase in the expression level of several ion channels that play a major role in the spontaneous diastolic depolarization characteristic of pacemaker cells. We have isolated pure populations of SAN and AVN cells that will be useful tools for generating biological pacemakers.

Hatani, T., et al. (2018). "Nano-structural analysis of engrafted human induced pluripotent stem cell-derived cardiomyocytes in mouse hearts using a genetic-probe APEX2." Biochem Biophys Res Commun **505**(4): 1251-1256.

Many studies have shown the feasibility of in vivo cardiac transplantation of human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) in animal experiments. However, nano-structural confirmation of the successful incorporation of the engrafted iPSC-CMs including electron microscopy (EM) has not been accomplished, partly because identification of graft cells in EM has proven to be difficult. Using APEX2, an engineered ascorbate peroxidase imaging tag, we successfully localized and analyzed the fine structure of sarcomeres and the excitation contraction machinery of iPSC-CMs 6 months after their engraftment in infarcted mouse hearts. APEX2 made iPSC-CMs visible in multiple imaging modalities including light microscopy, X-ray microscopic tomography, transmission EM, and scanning EM. EM tomography allowed assessment of the differentiation state of APEX2-positive iPSC-CMs and analysis of the fine structure of the sarcomeres including T-tubules and dyads.

Healy, L., et al. (2011). "Stem cell banks: preserving cell lines, maintaining genetic integrity, and advancing research." Methods Mol Biol **767**: 15-27.

The ability to cryopreserve and successfully recover cell lines has been critical to the conservation of all cell lines, especially the preservation of pristine early-stage cultures and the preparation of well-characterized cell banks. Indeed, the systematic storage and establishment of cryopreserved banks of cells for the stem cell research community is fundamental to the promotion of standardisation in stem cell research and their use in clinical applications. In spite of the significant potential for the use of stem cells in research and therapy, they are challenging to maintain and have been shown to be unstable after prolonged culture that often results in permanent alterations in their genetic make-up, which ultimately alters the phenotype of the culture. This chapter will review the principles of cell bank production, techniques for the scale-up of human pluripotent stem cells, quality control, and characterisation methods for banked cell lines.

Heanue, T. A. and V. Pachnis (2007). "Enteric nervous system development and Hirschsprung's disease: advances in genetic and stem cell studies." Nat Rev Neurosci **8**(6): 466-479.

The enteric nervous system (ENS) has been explored by developmental neurobiologists and medical researchers for decades. Whereas developmental biologists have been unravelling the molecular mechanisms underlying the migration, proliferation and differentiation of the neural crest derivatives that give rise to the ENS, human geneticists have been uncovering the genetic basis for diseases of the ENS, notably Hirschsprung's disease. Here we discuss the exciting recent advances, including novel transgenic and genetic tools, a broadening range of model organisms, and the pursuit of ENS stem cells as a therapeutic tool, that are bringing these fields closer together.

Hedges, E. C., et al. (2021). "Generation of six induced pluripotent stem cell lines from patients with amyotrophic lateral sclerosis with associated genetic

mutations in either FUS or ANXA11." Stem Cell Res **52**: 102246.

Amyotrophic lateral sclerosis (ALS) is characterized by degeneration of upper and lower motor neurons, causing gradual paralysis, and resulting in death 3-5 years from diagnosis. ALS causative mutations have been identified in multiple genes, including Fused in sarcoma (FUS), and recently characterized Annexin A11 (ANXA11). We have derived induced pluripotent stem cell (iPSC) lines from six ALS patient lymphoblastoid cell lines, three with mutations in FUS (Q519E, R521H, R522G), and three with mutations in ANXA11 (G38R, D40G, R235Q). These lines have been characterized and provide a novel resource for investigation into ALS pathology.

Hemmati, P. G., et al. (2017). "Predictive significance of the European LeukemiaNet classification of genetic aberrations in patients with acute myeloid leukaemia undergoing allogeneic stem cell transplantation." Eur J Haematol **98**(2): 160-168.

OBJECTIVES: The purpose of this study was to evaluate the predictive capacity of the European LeukemiaNet (ELN) classification of genetic risk in patients with acute myeloid leukaemia (AML) undergoing allogeneic stem cell transplantation (alloSCT). METHODS: We retrospectively analysed 274 patients transplanted at our centre between 2004 and 2014. RESULTS: The ELN grouping is comparable to the Southwest Oncology Group/Eastern Cooperative Oncology Group (SWOG/ECOG) stratification in predicting the outcome after alloSCT [overall P = 0.0064 for disease-free survival (DFS), overall P = 0.003 for relapse]. Patients with an intermediate-1 profile have a significantly elevated 5-yr relapse incidence as compared to favourable risk patients, that is 40% vs. 15%, [hazard ratio (HR) 2.58, P = 0.048]. An intermediate-1 risk profile is an independent predictor for relapse as determined by multivariate Cox regression analysis (HR 3.05, P = 0.023). In intermediate-1 patients, the presence of an FLT3 internal tandem duplication (FLT3-ITD) is associated with a significantly increased relapse incidence (P = 0.0323), and a lower DFS (P = 0.0465). FLT3-ITD is an independent predictor for overall survival, DFS and relapse incidence in the intermediate-1 subgroup. CONCLUSIONS: The ELN stratification of genetic risk predicts the outcome of patients with AML undergoing alloSCT. Patients with an intermediate-1 profile have a high risk for treatment failure due to relapse, which prompts the development of alternative treatment strategies.

Henckaerts, E., et al. (2004). "Quantitative genetic variation in the hematopoietic stem cell and progenitor cell compartment and in lifespan are closely linked at multiple loci in BXD recombinant inbred mice." Blood **104**(2): 374-379.

The number of bone marrow hematopoietic stem and progenitor cells as defined by the lineage(-), Sca1(++), c-kit(+) (LSK) phenotype and their proliferative capacity in vitro are subject to quantitative genetic variation, and several quantitative trait loci (QTL) have been identified in young mice. Because some traits affecting hematopoiesis also change with age in a mouse strain-dependent fashion, we performed quantitative trait analysis in aged BXD recombinant inbred (RI) mice for the number and frequency of LSK cells, and for their proliferative capacity in vitro. Several novel QTL were identified. The number and frequency of LSK cells in old mice correlated inversely with lifespan. Furthermore, 4 of 7 lifespan QTL overlap with QTL contributing to the number, frequency, or proliferative capacity of LSK cells in young or old mice. Taken together, these data establish a close genetic, and perhaps functional, link between genetic variation in lifespan and characteristics of stem and progenitor cells.

Hillel-Karniel, C., et al. (2020). "Multi-lineage Lung Regeneration by Stem Cell Transplantation across Major Genetic Barriers." Cell Rep **30**(3): 807-819 e804.

Induction of lung regeneration by transplantation of lung progenitor cells is a critical preclinical challenge. Recently, we demonstrated that robust lung regeneration can be achieved if the endogenous stem cell niches in the recipient's lung are vacated by sub-lethal pre-conditioning. However, overcoming MHC barriers is an additional requirement for clinical application of this attractive approach. We demonstrate here that durable tolerance toward mis-matched lung progenitors and their derivatives can be achieved without any chronic immune suppression, by virtue of co-transplantation with hematopoietic progenitors from the same donor. Initial proof of concept of this approach was attained by transplantation of fetal lung cells comprising both hematopoietic and non-hematopoietic progenitors. Furthermore, an even higher rate of blood and epithelial lung chimerism was attained by using adult lung cells supplemented with bone marrow hematopoietic progenitors. These results lay the foundation for repair of lung injury through a procedure akin to bone marrow transplantation.

Hinson, J. T., et al. (2012). "Induced pluripotent stem cell modeling of complex genetic diseases." Drug Discov Today Dis Models **9**(4): e147-e152.

The study of complex disease genetics by genome-wide association studies (GWAS) has led to hundreds of genomic loci associated with disease traits in humans. However, the functional consequences of

most loci are largely undefined. We discuss here the potential for human induced pluripotent stem (iPS) cells to bridge the gap between genetic variant and mechanisms of complex disease. We also highlight specific diseases and the roadblocks that must be overcome before iPS cell technology can be widely adopted for complex disease modeling.

Hmadcha, A., et al. (2016). "Derivation of HVR1, HVR2 and HVR3 human embryonic stem cell lines from IVF embryos after preimplantation genetic diagnosis (PGD) for monogenic disorder." Stem Cell Res **16**(3): 635-639.

From 106 human blastocyts donate for research after in vitro fertilization (IVF) and preimplantation genetic diagnosis (PGD) for monogenetic disorder, 3 human embryonic stem cells (hESCs) HVR1, HVR2 and HVR3 were successfully derived. HVR1 was assumed to be genetically normal, HVR2 carrying Becker muscular dystrophy and HVR3 Hemophilia B. Despite the translocation t(9;15)(q34.3;q14) detected in HVR2, all the 3 cell lines were characterised in vitro and in vivo as normal hESCs lines and were registered in the Spanish Stem Cell Bank.

Hollands, P. (1988). "Embryonic haemopoietic stem cell grafts in the treatment of murine genetic anaemia." Br J Haematol **70**(2): 157-163.

Embryonic haemopoietic stem cells obtained from early post-implantation mouse embryos can be successfully used in the treatment of murine genetic anaemia. Anaemic recipients had either chronic macrocytic anaemia, which was lethal without treatment, or mild anaemia without increased mortality. All successfully grafted recipients developed donor haemoglobin and glucose phosphate isomerase electrophoretic markers, indicating the presence of donor erythrocytes and lymphocytes. The minimum number of embryonic cells resulting in a successful graft in chronic macrocytic anaemia recipients was 0.8 x 10(6) nucleated cells. Athymic (nude) mice were also colonized by embryonic haemopoietic stem cells. Donor electrophoretic markers were seen but all recipients soon died, possibly of pneumonia. Bone marrow grafts into recipients with chronic macrocytic anaemia were not successful. Bone marrow grafts into recipients with mild anaemia resulted in some recipients showing donor markers. These recipients later died, showing symptoms of graft-versus-host disease.

Holler, E., et al. (2008). "The role of genetic variants of NOD2/CARD15, a receptor of the innate immune system, in GvHD and complications following related and unrelated donor haematopoietic stem cell transplantation." Int J Immunogenet **35**(4-5): 381-384.

Previous studies from our group indicated a role of SNPs within the innate immunity receptor NOD2/CARD15 as a risk factor for GvHD and treatment-related mortality allogeneic stem cell transplantation from HLA-identical siblings. We now extended these studies to assess the role of NOD2/CARD15 SNPs in 342 unrelated donor transplants. Overall, presence of any SNPs in patients or donor resulted in an increased risk of severe GvHD (25% in wildtype versus 38% in recipients and donors with variants, P= 0.01), which did not translate in increased mortality. When the analysis was broken down to individual SNPs, the presence of a SNP13 in the donor turned out to be the only highly significant risk factor (GvHD III/IV 22% wt, 42% SNP13 donor, P < 0.004; TRM 33% wt versus 59% SNP13 donor, P= 0.01; overall survival 49% wt versus 26% SNP13 donor, P= 0.007). This association was confirmed in multivariate analysis. Analysis of clinical risk factors suggested that this effect was most prominent in patients receiving any form of T cell depletion. Thus our observation indicates that the presence of a defect in innate immunity signalling in donor monocytes and possibly antigen presenting cells is most prominent in patients having additional T cell deficiency.

Hong, N., et al. (2010). "Accessibility of host cell lineages to medaka stem cells depends on genetic background and irradiation of recipient embryos." Cell Mol Life Sci **67**(7): 1189-1202.

Chimera formation is a powerful tool for analyzing pluripotency in vivo. It has been widely accepted that host cell lineages are generally accessible to embryonic stem (ES) cells with the actual contribution depending solely on the intrinsic pluripotency of transplanted donor cells. Here, we show in the fish medaka (Oryzias latipes) that the host accessibility to ES cell contribution exhibits dramatic differences. Specifically, of three albino host strains tested (i (1) , i (3) and af), only strain i (1) generated pigmented chimeras. Strikingly, this accessibility is completely lost in i (1) but acquired in i (3) after host gamma-irradiation. Host irradiation also differentially affected ES cell contribution to somatic organs and gonad. Therefore, the accessibility of various host cell lineages can vary considerably depending on host strains and cell lineages as well as on irradiation. Our findings underscore the importance of host genotypes for interpreting donor cell pluripotency and for improving ES-derived chimera production.

Horie, K., et al. (2011). "A homozygous mutant embryonic stem cell bank applicable for phenotype-

driven genetic screening." Nat Methods **8**(12): 1071-1077.

Genome-wide mutagenesis in mouse embryonic stem cells (ESCs) is a powerful tool, but the diploid nature of the mammalian genome hampers its application for recessive genetic screening. We have previously reported a method to induce homozygous mutant ESCs from heterozygous mutants by tetracycline-dependent transient disruption of the Bloom's syndrome gene. However, we could not purify homozygous mutants from a large population of heterozygous mutant cells, limiting the applications. Here we developed a strategy for rapid enrichment of homozygous mutant mouse ESCs and demonstrated its feasibility for cell-based phenotypic analysis. The method uses G418-plus-puromycin double selection to enrich for homozygotes and single-nucleotide polymorphism analysis for identification of homozygosity. We combined this simple approach with gene-trap mutagenesis to construct a homozygous mutant ESC bank with 138 mutant lines and demonstrate its use in phenotype-driven genetic screening.

Hu, W., et al. (2019). "Patient Adipose Stem Cell-Derived Adipocytes Reveal Genetic Variation that Predicts Antidiabetic Drug Response." Cell Stem Cell **24**(2): 299-308 e296.

Thiazolidinedione drugs (TZDs) target the transcriptional activity of peroxisome proliferator activated receptor gamma (PPARgamma) to reverse insulin resistance in type 2 diabetes, but side effects limit their clinical use. Here, using human adipose stem cell-derived adipocytes, we demonstrate that SNPs were enriched at sites of patient-specific PPARgamma binding, which correlated with the individual-specific effects of the TZD rosiglitazone (rosi) on gene expression. Rosi induction of ABCA1, which regulates cholesterol metabolism, was dependent upon SNP rs4743771, which modulated PPARgamma binding by influencing the genomic occupancy of its cooperating factor, NFIA. Conversion of rs4743771 from the inactive SNP allele to the active one by clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9-mediated editing rescued PPARgamma binding and rosi induction of ABCA1 expression. Moreover, rs4743771 is a major determinant of undesired serum cholesterol increases in rosi-treated diabetics. These data highlight human genetic variation that impacts PPARgamma genomic occupancy and patient responses to antidiabetic drugs, with implications for developing personalized therapies for metabolic disorders.

Huang, J., et al. (2010). "Genetic modification of mesenchymal stem cells overexpressing CCR1 increases cell viability, migration, engraftment, and capillary density in the injured myocardium." Circ Res **106**(11): 1753-1762.

RATIONALE: Although mesenchymal stem cell (MSC) transplantation has been shown to promote cardiac repair in acute myocardial injury in vivo, its overall restorative capacity appears to be restricted mainly because of poor cell viability and low engraftment in the ischemic myocardium. Specific chemokines are upregulated in the infarcted myocardium. However the expression levels of the corresponding chemokine receptors (eg, CCR1, CXCR2) in MSCs are very low. We hypothesized that this discordance may account for the poor MSC engraftment and survival. OBJECTIVE: To determine whether overexpression of CCR1 or CXCR2 chemokine receptors in MSCs augments their cell survival, migration and engraftment after injection in the infarcted myocardium. METHODS AND RESULTS: Overexpression of CCR1, but not CXCR2, dramatically increased chemokine-induced murine MSC migration and protected MSC from apoptosis in vitro. Moreover, when MSCs were injected intramyocardially one hour after coronary artery ligation, CCR1-MSCs accumulated in the infarcted myocardium at significantly higher levels than control-MSCs or CXCR2-MSCs 3 days postmyocardial infarction (MI). CCR1-MSC-injected hearts exhibited a significant reduction in infarct size, reduced cardiomyocytes apoptosis and increased capillary density in injured myocardium 3 days after MI. Furthermore, intramyocardial injection of CCR1-MSCs prevented cardiac remodeling and restored cardiac function 4 weeks after MI. CONCLUSIONS: Our results demonstrate the in vitro and in vivo salutary effects of genetic modification of stem cells. Specifically, overexpression of chemokine receptor enhances the migration, survival and engraftment of MSCs, and may provide a new therapeutic strategy for the injured myocardium.

Ioffe, E., et al. (1995). "WW6: an embryonic stem cell line with an inert genetic marker that can be traced in chimeras." Proc Natl Acad Sci U S A **92**(16): 7357-7361.

Mutant mice produced by gene targeting in embryonic stem (ES) cells often have a complex or embryonic lethal phenotype. In these cases, it would be helpful to identify tissues and cell types first affected in mutant embryos by following the contribution to chimeras of ES cells homozygous for the mutant allele. Although a number of strategies for following ES cell development in vivo have been reported, each has limitations that preclude its general application. In this paper, we describe ES cell lines that can be tracked to every nucleated cell type in chimeras at all

developmental stages. These lines were derived from blastocysts of mice that carry an 11-Mb beta-globin transgene on chromosome 3. The transgene is readily detected by DNA in situ hybridization, providing an inert, nuclear-localized marker whose presence is not affected by transcriptional or translational controls. The "WW" series of ES lines possess the essential features of previously described ES lines, including giving rise to a preponderance of male chimeras, all of which have to date exhibited germ-line transmission. In addition, clones selected for single or double targeting events form strong chimeras, demonstrating the feasibility of using WW6 cells to identify phenotypes associated with the creation of a null mutant.

Iwamoto, T., et al. (2015). "Effect of Genetic Polymorphism of CYP3A5 and CYP2C19 and Concomitant Use of Voriconazole on Blood Tacrolimus Concentration in Patients Receiving Hematopoietic Stem Cell Transplantation." Ther Drug Monit **37**(5): 581-588.

BACKGROUND: Blood tacrolimus (TAC) concentration delivered via intravenous administration is known to be influenced by genetic polymorphism of CYP3A5 and interaction with triazole antifungal agents. However, interindividual variability of blood TAC concentration is as of yet still difficult to predict during the early stages of hematopoietic stem cell transplantation (HSCT). This study was conducted to assess the wide variability of blood TAC concentrations because of the hepatic metabolic activities of CYP3A and CYP2C19 in HSCT recipients. METHODS: This study is a single-institute prospective study that includes 21 adult patients who underwent HSCT and received 24 hours continuous intravenous administration of TAC at the Mie University Hospital between January 2009 and March 2014. After HSCT, the changes in blood TAC concentration/dose (C/D) ratio and TAC dose reduction from initial dose were investigated. RESULTS: Significant differences between HSCT recipients with CYP3A5*1 allele and CYP3A5*3/*3 genotype were observed with respect to the median TAC C/D ratio on day 14 (563 versus 742 ng/mL per mg/kg, $P < 0.01$) and day 21 (672 versus 777 ng/mL per mg/kg, $P < 0.05$) after HSCT. Concomitant administration of voriconazole (VRCZ), but not of lansoprazole, was found to significantly increase the median TAC C/D ratio on day 14 (557 versus 723 ng/mL per mg/kg, $P < 0.01$). Possession of the CYP3A5*3/*3 genotype (day 14: odds ratio, 32.2; day 21: odds ratio, 33.0; $P < 0.05$) and concomitant administration of VRCZ (day 14: odds ratio, 37.8; $P < 0.05$) were found to be independent risk factors, which significantly contributed to an increased TAC C/D ratio. In HSCT recipients with CYP3A5*3/*3 genotype (78.0%), the median TAC dose ratio (day 21/day -1) was significantly lower compared with HSCT recipients with the CYP3A5*1 allele (94.1%), whereas VRCZ administration itself had no significant influence. Interestingly, in HSCT recipients with CYP2C19*1/*1, we found that the influence of VRCZ on the TAC dose ratio (85.7%) was relatively mild, even in a recipient with CYP3A5*3/*3. CONCLUSIONS: In HSCT recipients, the variability of intravenous TAC concentration in the blood could be explained in part by the genetic variation of CYP3A5. The study results also strongly imply that the magnitude of hepatic interaction between TAC and VRCZ is affected by the genetic polymorphism of both CYP3A5 and CYP2C19 genes.

Jacewicz, R., et al. (2015). "Dangers resulting from DNA profiling of biological materials derived from patients after allogeneic hematopoietic stem cell transplantation (allo-HSCT) with regard to forensic genetic analysis." Arch Med Sadowej Kryminol **65**(4): 225-247.

The study documents the risk that comes with DNA analysis of materials derived from patients after allogeneic hematopoietic stem cell transplantation (allo-HSCT) in forensic genetics. DNA chimerism was studied in 30 patients after allo-HSCT, based on techniques applied in contemporary forensic genetics, i.e. real-time PCR and multiplex PCR-STR with the use of autosomal DNA as well as Y-DNA markers. The results revealed that the DNA profile of the recipient's blood was identical with the donor's in the majority of cases. Therefore, blood analysis can lead to false conclusions in personal identification as well as kinship analysis. An investigation of buccal swabs revealed a mixture of DNA in the majority of recipients. Consequently, personal identification on the basis of stain analysis of the same origin may be impossible. The safest (but not ideal) material turned out to be the hair root. Its analysis based on autosomal DNA revealed 100% of the recipient's profile. However, an analysis based on Y-chromosome markers performed in female allo-HSCT recipients with male donors demonstrated the presence of donor DNA in hair cells - similarly to the blood and buccal swabs. In the light of potential risks arising from DNA profiling of biological materials derived from persons after allotransplantation in judicial aspects, certain procedures were proposed to eliminate such dangers. The basic procedures include abandoning the approach based exclusively on blood collection, both for kinship analysis and personal identification; asking persons who are to be tested about their history of allo-HSCT before sample collection and profile entry in the DNA database, and verification of DNA profiling based on hair follicles in uncertain cases.

Jacewicz, R., et al. (2013). "Genetic investigation of biological materials from patients after stem cell transplantation based on autosomal as well as Y-chromosomal markers." Int J Legal Med **127**(2): 359-362.

The authors presented the results of DNA polymorphism investigation of blood, buccal swabs and hair follicles originating from patients after allogeneic hematopoietic stem cell transplantation. The real-time and multiplex assays based on polymerase chain reaction within the range of autosomal as well as Y-chromosomal markers were applied to assess the possible dangers arising from investigation of these materials in forensic genetics. The results revealed that not only post-transplant blood and buccal swab, but also recipient hair, up to now regarded as devoid of any donor's cells, do not constitute entirely safe material for forensic purposes. Their analysis can lead to the false identification of gender or male haplotype. The investigation of sex-determining region Y and Y-chromosome short tandem repeats performed in female recipients with male donors resulted in the designation of donor's DNA in hair cells as well as in blood and buccal swabs. Therefore, biological stains gathered from crime scenes should not be analysed exclusively based on the investigation of male-specific markers.

Jacques, T. S., et al. (2010). "Combinations of genetic mutations in the adult neural stem cell compartment determine brain tumour phenotypes." EMBO J **29**(1): 222-235.

It has been suggested that intrinsic brain tumours originate from a neural stem/progenitor cell population in the subventricular zone of the post-natal brain. However, the influence of the initial genetic mutation on the phenotype as well as the contribution of mature astrocytes to the formation of brain tumours is still not understood. We deleted Rb/p53, Rb/p53/PTEN or PTEN/p53 in adult subventricular stem cells; in ectopically neurografted stem cells; in mature parenchymal astrocytes and in transplanted astrocytes. We found that only stem cells, but not astrocytes, gave rise to brain tumours, independent of their location. This suggests a cell autonomous mechanism that enables stem cells to generate brain tumours, whereas mature astrocytes do not form brain tumours in adults. Recombination of PTEN/p53 gave rise to gliomas whereas deletion of Rb/p53 or Rb/p53/PTEN generated primitive neuroectodermal tumours (PNET), indicating an important role of an initial Rb loss in driving the PNET phenotype. Our study underlines an important role of stem cells and the relevance of initial genetic mutations in the pathogenesis and phenotype of brain tumours.

Jyoti, S. and S. Tandon (2015). "Genetic basis for developmental toxicity due to statin intake using embryonic stem cell differentiation model." Hum Exp Toxicol **34**(10): 965-984.

The in utero environment is a key factor controlling the fate of the growing embryo. The deleterious effects of statins during the fetal development are still not very well understood. Data from animal studies and retrospective studies performed in pregnant women give conflicting reports. In this study, using in vitro differentiation model of embryonic stem cells, which mimic the differentiation process of the embryo, we have systematically exposed the cells to lipophilic statins, simvastatin, and atorvastatin at various doses and at critical times during differentiation. The analysis of key genes controlling the differentiation into ecto-, meso- and endodermal lineages was assessed by quantitative polymerase chain reaction. Our results show that genes of the mesodermal lineage were most sensitive to statins, leading to changes in the transcript levels of brachyury, Flk-1, Nkx2.5, and alpha/beta-myosin heavy chain. In addition, changes to endodermal marker alpha-fetoprotein, along with ectodermal Nes and Neurofilament 200 kDa, imply that during early differentiation exposure to these drugs leads to altered signaling, which could translate to the congenital abnormalities seen in the heart and limbs.

Kammers, K., et al. (2017). "Integrity of Induced Pluripotent Stem Cell (iPSC) Derived Megakaryocytes as Assessed by Genetic and Transcriptomic Analysis." PLoS One **12**(1): e0167794.

Previously, we have described our feeder-free, xeno-free approach to generate megakaryocytes (MKs) in culture from human induced pluripotent stem cells (iPSCs). Here, we focus specifically on the integrity of these MKs using: (1) genotype discordance between parent cell DNA to iPSC cell DNA and onward to the differentiated MK DNA; (2) genomic structural integrity using copy number variation (CNV); and (3) transcriptomic signatures of the derived MK lines compared to the iPSC lines. We detected a very low rate of genotype discordance; estimates were 0.0001%-0.01%, well below the genotyping error rate for our assay (0.37%). No CNVs were generated in the iPSCs that were subsequently passed on to the MKs. Finally, we observed highly biologically relevant gene sets as being upregulated in MKs relative to the iPSCs: platelet activation, blood coagulation, megakaryocyte development, platelet formation, platelet degranulation, and platelet aggregation. These data strongly support the integrity of the derived MK lines.

Kanatsu-Shinohara, M., et al. (2010). "Genetic influences in mouse spermatogonial stem cell self-renewal." J Reprod Dev **56**(1): 145-153.

Spermatogonial stem cells (SSCs) are slowly dividing cells that undergo self-renewal division to support spermatogenesis. Although the effects of genetic background in stem cell self-renewal have been well studied in hematopoietic stem cells, little is known about its effect on stem cells in other self-renewing tissues, including SSCs. To examine whether genetic factors are involved in regulation of SSC self-renewal, we first studied spermatogenesis in different inbred mouse strains (C57BL/6, DBA/2, AKR, BALB/C and C3H) after chemical damage caused by busulfan. Spermatogenesis in the DBA/2 and AKR strains was relatively resistant to busulfan treatment, whereas spermatogenesis was diminished in C57BL/6 mice and nearly ablated in C3H and BALB/C mice. Serial germ cell transplantation experiments provided functional evidence that SSCs with the DBA/2 background expanded more rapidly than those with the B6 background. Finally, we also employed the Germline Stem (GS) cell culture technique to examine the self-renewal activity in vitro. Although genetic manipulation of GS cells has been limited to those from the DBA/2 background, we produced transgenic offspring of the C3H background by electroporation of GS cells with a plasmid vector. Our results underscore the importance of genetic factors in SSC self-renewal. Furthermore, application of genetic modification techniques to GS cells with non-DBA/2 backgrounds extends the potential of a SSC-based approach in male germline modification.

Kang, H. and D. Shibata (2013). "Direct measurements of human colon crypt stem cell niche genetic fidelity: the role of chance in non-darwinian mutation selection." Front Oncol **3**: 264.

Perfect human stem cell genetic fidelity would prevent aging and cancer. However, perfection would be difficult to achieve, and aging is universal and cancers common. A hypothesis is that because mutations are inevitable over a human lifetime, downstream mechanisms have evolved to manage the deleterious effects of beneficial and lethal mutations. In the colon, a crypt stem cell architecture reduces the number of mitotic cells at risk for mutation accumulation, and multiple niche stem cells ensure that a lethal mutation within any single stem cell does not lead to crypt death. In addition, the architecture of the colon crypt stem cell niche may harness probability or chance to randomly discard many beneficial mutations that might lead to cancer. An analysis of somatic chromosome copy number alterations (CNAs) reveals a lack of perfect fidelity in individual normal human crypts, with age-related increases and higher frequencies in ulcerative colitis, a proliferative, inflammatory disease. The age-related increase in somatic CNAs appears consistent with relatively normal replication error and cell division rates. Surprisingly, and similar to point mutations in cancer genomes, the types of crypt mutations were more consistent with random fixation rather than selection. In theory, a simple "non-Darwinian" way to nullify selection is to reduce the size of the reproducing population. Fates are more determined by chance rather than selection in very small populations, and therefore selection may be minimized within small crypt niches. The desired effect is that many beneficial mutations that might lead to cancer are randomly lost by drift rather than fixed by selection. The subdivision of the colon into multiple very small stem cell niches may trade Darwinian evolution for non-Darwinian somatic cell evolution, capitulating to aging but reducing cancer risks.

Kapur, R., et al. (1998). "Signaling through the interaction of membrane-restricted stem cell factor and c-kit receptor tyrosine kinase: genetic evidence for a differential role in erythropoiesis." Blood **91**(3): 879-889.

Mutations of the receptor tyrosine kinase c-kit or its ligand stem cell factor (SCF), which is encoded as a soluble and membrane-associated protein by the Steel gene in mice, lead to deficiencies of germ cells, melanocytes, and hematopoiesis, including the erythroid lineage. In the present study, we have used genetic methods to study the role of membrane or soluble presentation of SCF in hematopoiesis. Bone marrow-derived stromal cells expressing only a membrane-restricted (MR) isoform of SCF induced an elevated and sustained tyrosine phosphorylation of both c-kit and erythropoietin receptor (EPO-R) and significantly greater proliferation of an erythrocytic progenitor cell line compared with stromal cells expressing soluble SCF. Transgene expression of MR-SCF in Steel-dickie (Sld) mutants resulted in a significant improvement in the production of red blood cells, bone marrow hypoplasia, and runting. In contrast, overexpression of the full-length soluble form of SCF transgene had no effect on either red blood cell production or runting but corrected the myeloid progenitor cell deficiency seen in these mutants. These data provide the first evidence of differential functions of SCF isoforms in vivo and suggest an abnormal signaling mechanism as the cause of the severe anemia seen in mutants of the Sl gene.

Karabon, L., et al. (2018). "The Influence of Genetic Variations in the CD86 Gene on the Outcome after Allogeneic Hematopoietic Stem Cell Transplantation." J Immunol Res **2018**: 3826989.

CD86 molecule is the ligand for both costimulatory (CD28) and coinhibitory (CTLA-4) molecules, and it regulates immune response after allogeneic hematopoietic stem cell transplantation (alloHSCT). Therefore, we postulate that CD86 gene variations might influence the outcome after alloHSCT. Altogether, 295 adult patients (pts) undergoing related (105 pts) and unrelated (190 pts) donor-matched HSCT were genotyped for the following CD86 gene polymorphisms: rs1129055, rs9831894, and rs2715267. Moreover, the donors' rs1129055 polymorphism was determined. None of the investigated SNPs alone were associated with aGvHD and rate of relapse. However, we showed that rs2715267 SNP influenced overall survival (OS) after alloHSCT. The 24-month OS for the rs271526GG recipients was worse than that for the recipients possessing T allelle (TT or GT genotypes) ($p = 0.009$). Moreover, analysis of gene-gene interaction between CD86 and CTLA-4 showed that having both the A allele for CD86 rs1129055 and the CTLA-4 CT60GG genotype in recipients increased the risk of aGvHD about 3.5 times. Interestingly, the donors' rs1129055GG genotype and the recipients' CT60GG genotype also increased the risk of aGvHD about 2.7-fold. We postulate that recipients' CD86 gene polymorphisms influence the overall survival after alloHSCT and, together with CTLA-4 polymorphisms, might be considered a risk factor for aGvHD.

Kargaran, P. K., et al. (2020). "Mitochondrial Medicine: Genetic Underpinnings and Disease Modeling Using Induced Pluripotent Stem Cell Technology." Front Cardiovasc Med **7**: 604581.

Mitochondrial medicine is an exciting and rapidly evolving field. While the mitochondrial genome is small and differs from the nuclear genome in that it is circular and free of histones, it has been implicated in neurodegenerative diseases, type 2 diabetes, aging and cardiovascular disorders. Currently, there is a lack of efficient treatments for mitochondrial diseases. This has promoted the need for developing an appropriate platform to investigate and target the mitochondrial genome. However, developing these therapeutics requires a model system that enables rapid and effective studying of potential candidate therapeutics. In the past decade, induced pluripotent stem cells (iPSCs) have become a promising technology for applications in basic science and clinical trials, and have the potential to be transformative for mitochondrial drug development. Engineered iPSC-derived cardiomyocytes (iPSC-CM) offer a unique tool to model mitochondrial disorders. Additionally, these cellular models enable the discovery and testing of novel therapeutics and their impact on pathogenic mtDNA variants and dysfunctional mitochondria. Herein, we review recent advances in iPSC-CM models focused on mitochondrial dysfunction often causing cardiovascular diseases. The importance of mitochondrial disease systems biology coupled with genetically encoded NAD(+)/NADH sensors is addressed toward developing an in vitro translational approach to establish effective therapies.

Katoh, M. (2007). "Dysregulation of stem cell signaling network due to germline mutation, SNP, Helicobacter pylori infection, epigenetic change and genetic alteration in gastric cancer." Cancer Biol Ther **6**(6): 832-839.

Genetic factors, Helicobacter pylori infection, salt over-uptake, decreased vegetable/fruit consumption, smoking, and metabolic syndrome are risk factors of human gastric cancer. Germline mutations of CDH1 gene, and SNPs of PTPN11 (SHP2), TLR4, IL1B, TNFA, BMP6, GDF15 and RUNX3 genes are associated with gastric cancer. Helicobacter pylori activates CagA-SHP2-ERK and peptidoglycan-NOD1-NFkappaB signaling cascades in gastric epithelial cells using type IV secretion system, and also TRAF6-MAP3K7-NFkappaB and TRAF6-MAP3K7-AP-1 signaling cascades in epithelial and immune cells through lipopolysaccharide recognition by TLR2 or TLR4. IL-1beta, IL-6, IL-8, TNFalpha and IFNgamma are elevated in gastric mucosa with Helicobacter pylori infection. IL-6 and TNFalpha induce upregulation of WNT5A and WNT10B, respectively. WNT signals are transduced to beta-catenin-TCF/LEF, RhoA, JNK, PKC, NFAT, and NLK signaling cascades. WNT-beta-catenin-TCF/LEF signaling induces upregulation of MYC, CCND1, WISP1, FGF20, JAG1 and DKK1 genes. Notch signals are transduced to CSL-NICD-MAML and NFkappaB signaling cascades. FGF signals are transduced to ERK, PI3K-AKT, PKC, and NFAT signaling cascades. Helicobacter pylori infection induces SHH upregulation in parietal cell lineage, while BMP signals induce IHH upregulation in pit cell lineage. Hedgehog signals induce upregulation of GLI1, PTCH1, CCND2, FOXL1, JAG2 and SFRP1 genes. JAG1 and JAG2 activate Notch signaling, while DKK1 and SFRP1 inhibit WNT signaling. Stem cell signaling network, consisting of WNT, Notch, FGF, Hedgehog and BMP signaling pathways, is activated during chronic Helicobacter pylori infection. Epigenetic silencing of SFRP1 gene occurs in the earlier stage of carcinogenesis in the stomach, while amplification and overexpression of FGFR2 gene in the later stage. Dysregulation of the stem cell signaling network due to the accumulation of germline mutation, SNP, Helicobacter pylori infection, epigenetic change and genetic alteration gives rise to gastric cancer. SNP typing and custom-made microarray analyses on genes

encoding stem cell signaling molecules could be utilized for the personalized medicine.

Kernik, D. C., et al. (2020). "A computational model of induced pluripotent stem-cell derived cardiomyocytes for high throughput risk stratification of KCNQ1 genetic variants." PLoS Comput Biol **16**(8): e1008109.

In the last decade, there has been tremendous progress in identifying genetic anomalies linked to clinical disease. New experimental platforms have connected genetic variants to mechanisms underlying disruption of cellular and organ behavior and the emergence of proarrhythmic cardiac phenotypes. The development of induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) signifies an important advance in the study of genetic disease in a patient-specific context. However, considerable limitations of iPSC-CM technologies have not been addressed: 1) phenotypic variability in apparently identical genotype perturbations, 2) low-throughput electrophysiological measurements, and 3) an immature phenotype which may impact translation to adult cardiac response. We have developed a computational approach intended to address these problems. We applied our recent iPSC-CM computational model to predict the proarrhythmic risk of 40 KCNQ1 genetic variants. An IKs computational model was fit to experimental data for each mutation, and the impact of each mutation was simulated in a population of iPSC-CM models. Using a test set of 15 KCNQ1 mutations with known clinical long QT phenotypes, we developed a method to stratify the effects of KCNQ1 mutations based on proarrhythmic markers. We utilized this method to predict the severity of the remaining 25 KCNQ1 mutations with unknown clinical significance. Tremendous phenotypic variability was observed in the iPSC-CM model population following mutant perturbations. A key novelty is our reporting of the impact of individual KCNQ1 mutant models on adult ventricular cardiomyocyte electrophysiology, allowing for prediction of mutant impact across the continuum of aging. This serves as a first step toward translating predicted response in the iPSC-CM model to predicted response of the adult ventricular myocyte given the same genetic mutation. As a whole, this study presents a new computational framework that serves as a high throughput method to evaluate risk of genetic mutations based-on proarrhythmic behavior in phenotypically variable populations.

Khouri, R., et al. (2018). "A genetic IFN/STAT1/FAS axis determines CD4 T stem cell memory levels and apoptosis in healthy controls and Adult T-cell Leukemia patients." Oncoimmunology **7**(5): e1426423.

Adult T-cell leukemia (ATL) is an aggressive, chemotherapy-resistant CD4(+)CD25(+) leukemia caused by HTLV-1 infection, which usually develops in a minority of patients several decades after infection. IFN + AZT combination therapy has shown clinical benefit in ATL, although its mechanism of action remains unclear. We have previously shown that an IFN-responsive FAS promoter polymorphism in a STAT1 binding site (rs1800682) is associated to ATL susceptibility and survival. Recently, CD4 T stem cell memory (T(SCM)) Fas(hi) cells have been identified as the hierarchical cellular apex of ATL, but a possible link between FAS, apoptosis, proliferation and IFN response in ATL has not been studied. In this study, we found significant ex vivo antiproliferative, antiviral and immunomodulatory effects of IFN-alpha treatment in short-term culture of primary mononuclear cells from ATL patients (n = 25). Bayesian Network analysis allowed us to integrate ex vivo IFN-alpha response with clinical, genetic and immunological data from ATL patients, thereby revealing a central role for FAS -670 polymorphism and apoptosis in the coordinated mechanism of action of IFN-alpha. FAS genotype-dependence of IFN-induced apoptosis was experimentally validated in an independent cohort of healthy controls (n = 20). The same FAS -670 polymorphism also determined CD4 T(SCM) levels in a genome-wide twin study (p = 7 x 10(-11), n = 460), confirming a genetic link between apoptosis and T(SCM) levels. Transcriptomic analysis and cell type deconvolution confirmed the FAS genotype/T(SCM) link and IFN-alpha-induced downregulation of CD4 T(SCM)-specific genes in ATL patient cells. In conclusion, ex vivo IFN-alpha treatment exerts a pleiotropic effect on primary ATL cells, with a genetic IFN/STAT1/Fas axis determining apoptosis vs. proliferation and underscoring the CD4 T(SCM) model of ATL leukemogenesis.

Kim, D. W. and F. Hirth (2009). "Genetic mechanisms regulating stem cell self-renewal and differentiation in the central nervous system of Drosophila." Cell Adh Migr **3**(4): 402-411.

Recent studies using the Drosophila central nervous system as a model have identified key molecules and mechanisms underlying stem cell self-renewal and differentiation. These studies suggest that proteins like Aurora-A, atypical protein kinase C, Prospero and Brain tumor act as key regulators in a tightly coordinated interplay between mitotic spindle orientation and asymmetric protein localization. These data also provide initial evidence that both processes are coupled to cell cycle progression and growth control, thereby regulating a binary switch between proliferative stem self-renewal and differentiative progenitor cell specification. Considering the evolutionary conservation of some of the mechanisms and molecules involved, these data provide a rationale

and genetic model for understanding stem cell self-renewal and differentiation in general. The new data gained in Drosophila may therefore lead to conceptual advancements in understanding the aetiology and treatment of human neurological disorders such as brain tumor formation and neurodegenerative diseases.

Kim, I. W., et al. (2013). "Population pharmacokinetics analysis of cyclophosphamide with genetic effects in patients undergoing hematopoietic stem cell transplantation." Eur J Clin Pharmacol **69**(8): 1543-1551.

PURPOSE: To build a population pharmacokinetic (PK) model of cyclophosphamide (CY) and its metabolite, 4-hydroxycyclophosphamide (HCY), in patients undergoing allogeneic haematopoietic stem cell transplantation (HSCT) and to identify covariates, including genetic polymorphisms, which affect CY and HCY PK parameters. METHOD: The study cohort comprised 21 patients undergoing HSCT who received CY intravenously between 2009 and 2011. Clinical characteristics and CY and HCY concentration data were collected for all patients, and ABCB1, ABCC2, GSTA1, GSTM1, GSTP1, GSTT1, CYP2B6, CYP2C19, and CYP3A5 genotyping was performed. A hypothetical enzyme compartment was conducted using the NONMEM program. RESULTS: A population PK analysis showed that the ABCC2 1249 genotype and aspartate aminotransferase levels significantly affected non-induced clearance (CL UI) and induced clearance (CL I) of CY, respectively. The final estimate of the mean CL UI and CL I of CY was 15.5 and 0.683 L/h, respectively, and the mean volume of distribution (V 1) of CY was 88.0 L. The inter-individual variability for CL UI, CL I, and V 1 of CY was 52.8, 200, and 18.0 %, respectively. Additionally, the CL UI of CY was significantly decreased to approximately 51 % in patients with the 1249 GA heterozygous genotype compared to those with the 1249 GG wild-type genotype ($p < 0.05$). There were only three heterozygous GA variants of ABCC2 1249 in the study patients. CONCLUSIONS: The population PK model developed in this study implies an influence of genetic factors on the clearance of CY. Clearance was moderately reduced in patients with the ABCC2 1249GA heterozygous genotype.

Kim, I. W., et al. (2012). "ABCB1 C3435T genetic polymorphism on population pharmacokinetics of methotrexate after hematopoietic stem cell transplantation in Korean patients: a prospective analysis." Clin Ther **34**(8): 1816-1826.

BACKGROUND: Methotrexate (MTX) is often used to prevent graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT). However, MTX has great pharmacokinetic variability and its use can result in fatal complications and/or infections after HSCT. OBJECTIVES: The purposes of this study were to build a population pharmacokinetic model of MTX treatment in Korean patients who have undergone HSCT and to identify covariates, including genetic polymorphisms, that affect the pharmacokinetic properties of MTX. METHODS: Clinical characteristics and MTX concentration data for 20 post-HSCT patients were collected. For each patient, ABCB1, ABCC2, ATIC, GGH, MTHFR, and TYMS genotyping was performed. Population pharmacokinetic analysis was performed using the NONMEM program. Analysis of MTX pharmacokinetic properties was accomplished using a 2-compartment pharmacokinetic model that incorporated first-order conditional estimation methods with interaction. The effects of a variety of demographic and genetic factors on MTX disposition were investigated. RESULTS: The study population consisted of 12 men (60%) and 8 women (40%). Median age and body weight were 28 years (range, 18-49 years) and 55.6 kg (range, 44.8-80.8 kg), respectively. Within the study population, the estimated mean MTX clearance (CL) was 7.08 L/h, whereas the mean central compartment volume (V(1)) of MTX distribution was 19.4 L. MTX CL was significantly affected by glomerular filtration rate (GFR), penicillin use, and the ABCB1 3435 genotype. Interindividual variabilities for CL and V(1) were 21.6% and 73.3%. A 10-mL/min GFR increase was associated with a 32% increase in mean MTX CL, whereas penicillin use was associated with a decrease in MTX CL of 61%. MTX CL was significantly greater (by approximately 21%) in patients with the ABCB1 3435 CC and CT genotype than in those with the ABCB1 3435 TT genotype ($P < 0.001$). CONCLUSIONS: There was great interindividual variation in MTX pharmacokinetic properties in patients who had undergone HSCT. GFR, concurrent penicillin use, and the presence of the ABCB1 3435 C<T genotypes significantly affected MTX CL. The MTX population pharmacokinetic model developed here may provide useful information for individualizing MTX therapy after HSCT.

Kim, M. G., et al. (2015). "Population pharmacokinetics of cyclosporine in hematopoietic stem cell transplant patients: consideration of genetic polymorphisms." Ann Pharmacother **49**(6): 622-630.

BACKGROUND: Cyclosporine (CsA), which is used for graft-versus-host disease prophylaxis in allogeneic hematopoietic stem cell transplant (allo-HSCT), has a narrow therapeutic range and large interindividual and intraindividual pharmacokinetic variability. Nevertheless, population pharmacokinetic (PopPK) studies of CsA in allo-HSCT are scarce. OBJECTIVE: The goal of our study was to build a

PopPK model of CsA in allo-HSCT in consideration of demographic, clinical, and genetic polymorphisms data. METHODS: A total of 34 adult allo-HSCT patients who received CsA were enrolled prospectively. Demographic, clinical, and CYP3A5 *1/*3, CYP2C19 *1/*2/*3, ABCB1 3435C>T, 1236C>T, 2677G>T/A, ABCC2 -24C>T, 1249G>A, VDR Bsml, Apal polymorphisms data were collected. A PopPK modeling was conducted with NONMEM program. RESULTS: A 1-compartment model with a 2-transit absorption compartment model was developed. After the stepwise covariate model building process, weight was incorporated into clearance (CL) as a power function model with the exponent value of 0.419. The final typical estimate of CL was 21.2 L/h; volume of distribution was 430 L; logit-transformed bioavailability was 1.49 (bioavailability: 81%); and transit compartment rate was 2.87/h. None of the genetic polymorphisms in CYP3A5, CYP2C19, ABCB1, ABCC2, and VDR were significant covariates in the pharmacokinetics of CsA. CONCLUSIONS: In our study, it was observed that weight had a significant effect on CL. Genetic polymorphisms did not affect CsA pharmacokinetics. Prospective studies with a larger number of participants is needed to validate the results of this study.

Kim, M. G., et al. (2019). "Effect of glutathione S-transferase genetic polymorphisms on busulfan pharmacokinetics and veno-occlusive disease in hematopoietic stem cell transplantation: A meta-analysis." Basic Clin Pharmacol Toxicol **124**(6): 691-703.

This meta-analysis was conducted to derive an integrated conclusion about the influence of glutathione S-transferase (GST) genetic polymorphisms on busulfan pharmacokinetic (PK) parameters and veno-occlusive disease (VOD). Studies which analysed the effect of GST genetic polymorphisms on area under the curve (AUC), clearance (CL) or VOD were searched for and selected. A pooled analysis was conducted using Comprehensive Meta-Analysis programme. Nineteen studies were included in this meta-analysis. GSTA1*B and GSTM1 null genotypes significantly decreased CL(IV) of busulfan (standardized difference in means (SDM) = -1.103; P = 0.019 and SDM = -0.418; P = 0.002, respectively). GSTA1*B significantly increased AUC(IV) of busulfan (SDM = 0.832; P = 0.046), whereas GSTM1 did not (SDM = 0.155; P = 0.478). The PK parameters of oral busulfan did not differ according to GST genotype. GSTA1, GSTM1 and GSTP1 were not significantly associated with VOD occurrence. GSTA1 and GSTM1 genotypes affected CL(IV) of busulfan, but only GSTA1 affected AUC(IV) . There was no significant difference in the PK parameters of oral busulfan (CL(PO) and AUC(PO)) and VOD when only GST genotypes were considered.

Kim, M. Y., et al. (2018). "Genetic Inactivation of CD33 in Hematopoietic Stem Cells to Enable CAR T Cell Immunotherapy for Acute Myeloid Leukemia." Cell **173**(6): 1439-1453 e1419.

The absence of cancer-restricted surface markers is a major impediment to antigen-specific immunotherapy using chimeric antigen receptor (CAR) T cells. For example, targeting the canonical myeloid marker CD33 in acute myeloid leukemia (AML) results in toxicity from destruction of normal myeloid cells. We hypothesized that a leukemia-specific antigen could be created by deleting CD33 from normal hematopoietic stem and progenitor cells (HSPCs), thereby generating a hematopoietic system resistant to CD33-targeted therapy and enabling specific targeting of AML with CAR T cells. We generated CD33-deficient human HSPCs and demonstrated normal engraftment and differentiation in immunodeficient mice. Autologous CD33 KO HSPC transplantation in rhesus macaques demonstrated long-term multilineage engraftment of gene-edited cells with normal myeloid function. CD33-deficient cells were impervious to CD33-targeting CAR T cells, allowing for efficient elimination of leukemia without myelotoxicity. These studies illuminate a novel approach to antigen-specific immunotherapy by genetically engineering the host to avoid on-target, off-tumor toxicity.

Koneru, S. L., et al. (2021). "Cryptic genetic variation in a heat shock protein modifies the outcome of a mutation affecting epidermal stem cell development in C. elegans." Nat Commun **12**(1): 3263.

A fundamental question in medical genetics is how the genetic background modifies the phenotypic outcome of mutations. We address this question by focusing on the seam cells, which display stem cell properties in the epidermis of Caenorhabditis elegans. We demonstrate that a putative null mutation in the GATA transcription factor egl-18, which is involved in seam cell fate maintenance, is more tolerated in the CB4856 isolate from Hawaii than the lab reference strain N2 from Bristol. We identify multiple quantitative trait loci (QTLs) underlying the difference in phenotype expressivity between the two isolates. These QTLs reveal cryptic genetic variation that reinforces seam cell fate through potentiating Wnt signalling. Within one QTL region, a single amino acid deletion in the heat shock protein HSP-110 in CB4856 is sufficient to modify Wnt signalling and seam cell development, highlighting that natural variation in conserved heat shock proteins can shape phenotype expressivity.

Korody, M. L., et al. (2021). "Rewinding Extinction in the Northern White Rhinoceros: Genetically Diverse Induced Pluripotent Stem Cell Bank for Genetic Rescue." Stem Cells Dev **30**(4): 177-189.

Extinction rates are rising, and current conservation technologies may not be adequate for reducing species losses. Future conservation efforts may be aided by the generation of induced pluripotent stem cells (iPSCs) from highly endangered species. Generation of a set of iPSCs from multiple members of a species can capture some of the dwindling genetic diversity of a disappearing species. We generated iPSCs from fibroblasts cryopreserved in the Frozen Zoo((R)): nine genetically diverse individuals of the functionally extinct northern white rhinoceros (Ceratotherium simum cottoni) and two from the closely related southern white rhinoceros (Ceratotherium simum simum). We used a nonintegrating Sendai virus reprogramming method and developed analyses to confirm the cells' pluripotency and differentiation potential. This work is the first step of a long-term interdisciplinary plan to apply assisted reproduction techniques to the conservation of this highly endangered species. Advances in iPSC differentiation may enable generation of gametes in vitro from deceased and nonreproductive individuals that could be used to repopulate the species.

Kosan, C. and M. Godmann (2016). "Genetic and Epigenetic Mechanisms That Maintain Hematopoietic Stem Cell Function." Stem Cells Int **2016**: 5178965.

All hematopoiesis cells develop from multipotent progenitor cells. Hematopoietic stem cells (HSC) have the ability to develop into all blood lineages but also maintain their stemness. Different molecular mechanisms have been identified that are crucial for regulating quiescence and self-renewal to maintain the stem cell pool and for inducing proliferation and lineage differentiation. The stem cell niche provides the microenvironment to keep HSC in a quiescent state. Furthermore, several transcription factors and epigenetic modifiers are involved in this process. These create modifications that regulate the cell fate in a more or less reversible and dynamic way and contribute to HSC homeostasis. In addition, HSC respond in a unique way to DNA damage. These mechanisms also contribute to the regulation of HSC function and are essential to ensure viability after DNA damage. How HSC maintain their quiescent stage during the entire life is still matter of ongoing research. Here we will focus on the molecular mechanisms that regulate HSC function.

Lackner, A., et al. (2021). "Cooperative genetic networks drive embryonic stem cell transition from naive to formative pluripotency." EMBO J **40**(8): e105776.

In the mammalian embryo, epiblast cells must exit the naive state and acquire formative pluripotency. This cell state transition is recapitulated by mouse embryonic stem cells (ESCs), which undergo pluripotency progression in defined conditions in vitro. However, our understanding of the molecular cascades and gene networks involved in the exit from naive pluripotency remains fragmentary. Here, we employed a combination of genetic screens in haploid ESCs, CRISPR/Cas9 gene disruption, large-scale transcriptomics and computational systems biology to delineate the regulatory circuits governing naive state exit. Transcriptome profiles for 73 ESC lines deficient for regulators of the exit from naive pluripotency predominantly manifest delays on the trajectory from naive to formative epiblast. We find that gene networks operative in ESCs are also active during transition from pre- to post-implantation epiblast in utero. We identified 496 naive state-associated genes tightly connected to the in vivo epiblast state transition and largely conserved in primate embryos. Integrated analysis of mutant transcriptomes revealed funnelling of multiple gene activities into discrete regulatory modules. Finally, we delineate how intersections with signalling pathways direct this pivotal mammalian cell state transition.

Laing, O., et al. (2019). "Rapid PCR Assay for Detecting Common Genetic Variants Arising in Human Pluripotent Stem Cell Cultures." Curr Protoc Stem Cell Biol **49**(1): e83.

Human pluripotent stem cells (hPSCs) are prone to acquiring genetic changes upon prolonged culture. Particularly common are copy number changes, including gains of chromosomes 1q, 12p, 17q, and 20q, and/or loss of chromosomes 10p and 18q. The variant cells harboring common genetic changes display altered behaviors compared to their diploid counterparts, thus potentially impacting upon the validity of experimental results and safety of hPSC-derived cellular therapies. Hence, a critical quality attribute in hPSC maintenance should include frequent monitoring for genetic changes arising in cultures. This in turn places large demands on the genotyping assays for detection of genetic changes. Traditional methods for screening cells entail specialized cytogenetic analyses, but their high costs and a lengthy turnaround time make them impractical for high-throughput analyses and routine laboratory use. Here, we detail a protocol for a rapid, accessible, and affordable PCR-based method for detection of frequently occurring copy number changes in hPSCs. (c) 2019 by John Wiley & Sons, Inc.

Lakatos, A., et al. (2017). "Integrated analysis of genetic, behavioral, and biochemical data implicates neural stem cell-induced changes in immunity, neurotransmission and mitochondrial function in Dementia with Lewy Body mice." Acta Neuropathol Commun **5**(1): 21.

We previously demonstrated that transplantation of murine neural stem cells (NSCs) can improve motor and cognitive function in a transgenic model of Dementia with Lewy Bodies (DLB). These benefits occurred without changes in human alpha-synuclein pathology and were mediated in part by stem cell-induced elevation of brain-derived neurotrophic factor (BDNF). However, instrastriatal NSC transplantation likely alters the brain microenvironment via multiple mechanisms that may synergize to promote cognitive and motor recovery. The underlying neurobiology that mediates such restoration no doubt involves numerous genes acting in concert to modulate signaling within and between host brain cells and transplanted NSCs. In order to identify functionally connected gene networks and additional mechanisms that may contribute to stem cell-induced benefits, we performed weighted gene co-expression network analysis (WGCNA) on striatal tissue isolated from NSC- and vehicle-injected wild-type and DLB mice. Combining continuous behavioral and biochemical data with genome wide expression via network analysis proved to be a powerful approach; revealing significant alterations in immune response, neurotransmission, and mitochondria function. Taken together, these data shed further light on the gene network and biological processes that underlie the therapeutic effects of NSC transplantation on alpha-synuclein induced cognitive and motor impairments, thereby highlighting additional therapeutic targets for synucleinopathies.

Lange, A. (2008). "Genetic factors predicting IFN-gamma generation potential in patients with sarcoidosis and after haematopoietic stem cell transplantation." Int J Immunogenet **35**(4-5): 385-388.

HLA B8, which is known to associate with a poor proliferative response, constitutes a risk factor of antinuclear antibodies formation in asbestos workers. B8 and DR3 are in a linkage disequilibrium with TNF*A2 allele which characterizes individuals as high TNF-alpha producers. Therefore, haplotype having B8, DR3, TNF*A2 characterizes individuals with rather low proliferative response of lymphocytes and low IFN-gamma generation potential. The above genetical features (B8, DR3 and TNF*A2), associated with a characteristic TNF alpha and IFN gamma generation profile, make sarcoidosis patients prone to develop Lofgren syndrome symptoms. Indeed, low IFN gamma producers genotype (3/3 homozygotes) and HLA DRB1*03 constitute risk factors of Lofgren syndrome in a combined fashion. In patients receiving hematopoietic stem cell transplantation, low IFN gamma producer genotype make people more susceptible to CMV and EBV reactivation as well as to acute and chronic GvHD. Therefore, genetical factors associated with the magnitude of immune response are of a value in predicting the natural history of some diseases thus helping in diagnosis and in tailoring of the treatment.

Laws, K. M. and D. Drummond-Barbosa (2015). "Genetic Mosaic Analysis of Stem Cell Lineages in the Drosophila Ovary." Methods Mol Biol **1328**: 57-72.

Genetic mosaic analyses represent an invaluable approach for the study of stem cell lineages in the Drosophila ovary. The generation of readily identifiable, homozygous mutant cells in the context of wild-type ovarian tissues within intact organisms allows the pinpointing of cellular requirements for gene function, which is particularly important for understanding the physiological control of stem cells and their progeny. Here, we provide a step-by-step guide to the generation and analysis of genetically mosaic ovaries using flippase (FLP)/FLP recognition target (FRT)-mediated recombination in adult Drosophila melanogaster, with a focus on the processes of oogenesis that are controlled by diet-dependent factors.

Lazow, S. P., et al. (2021). "Enhancement of transamniotic stem cell therapy for spina bifida by genetic engineering of donor mesenchymal stem cells with an Fgf2 transgene." J Pediatr Surg **56**(6): 1226-1232.

BACKGROUND/PURPOSE: We examined whether engineered overexpression of fibroblast growth factor-2 (Fgf2) in donor mesenchymal stem cells (MSCs) could enhance spina bifida coverage induced by transamniotic stem cell therapy (TRASCET). METHODS: Pregnant Sprague-Dawley dams (n = 24) exposed to retinoic acid for induction of fetal spina bifida were divided in three groups. An untreated group had no further manipulations. Two groups received volume-matched intra-amniotic injections into all fetuses (n = 157) of either amniotic fluid-derived MSCs (afMSC; n = 85) or afMSCs transduced with an Fgf2 transgene (Fgf2-afMSC; n = 72) on gestational day 17 (term=21-22 days). Defect coverage was categorized at term by histology and pan-cytokeratin immunohistochemistry. Statistical coverage comparisons were by logistic regression. RESULTS: Among 84 survivors with isolated spina bifida, 71 had definitive histology. Defect coverage rates in both the afMSC (38.5%) and Fgf2-afMSC (73.3%) groups were statistically significantly higher than in the untreated group (10%; $p<0.001$ for both). There was a

significantly higher coverage rate in the Fgf2-afMSC group compared with the afMSC group (p = 0.025). CONCLUSIONS: Fgf2 overexpression in donor mesenchymal stem cells increases defect coverage rates in a rodent model of transamniotic stem cell therapy for spina bifida. Genetic engineering of donor cells is a promising strategy for the enhancement of this emerging therapy.

Lee, C. W., et al. (2018). "Improvement of Cell Cycle Lifespan and Genetic Damage Susceptibility of Human Mesenchymal Stem Cells by Hypoxic Priming." Int J Stem Cells **11**(1): 61-67.

Hypoxic culture is widely recognized as a method to efficiently expand human mesenchymal stem cells (MSCs) without loss of stem cell properties. However, the molecular basis of how hypoxia priming benefits MSC expansion remains unclear. We report that hypoxic priming markedly extends the cell cycle lifespan rather than augmenting the multipotency of MSC differentiation lineage. Hypoxic priming does not affect to chromosome damage but significantly attenuates the susceptibility of chromosome damage. Our results provide important evidence that multipotency of human MSCs by hypoxic priming is determined by cell cycle lifespan.

Lee, D. F., et al. (2012). "Combining competition assays with genetic complementation strategies to dissect mouse embryonic stem cell self-renewal and pluripotency." Nat Protoc **7**(4): 729-748.

Substantial scientific interest has been dedicated recently to the crucial factors that control the pluripotent state of stem cells. To gain a comprehensive understanding of the molecular mechanisms regulating mouse embryonic stem cell (mESC) self-renewal and lineage differentiation, we have developed a robust method for studying the role of a particular gene in these processes. This protocol describes detailed procedures for the design and generation of the complementation rescue system and its application in dissecting the network of pluripotency-associated factors, using mESCs as a model. Specifically, three main procedures are described: (i) screening pluripotency-associated factors by competition assay; (ii) setting up an inducible complementation rescue system; and (iii) dynamically studying the pluripotency network response to target depletion. Completion of the competition assay and complementation rescue system takes 35 and 30 d, respectively, and an additional 16 d to study the dynamic molecular effects of a gene of interest in the pluripotency network.

Lee, J., et al. (2009). "Genetic reconstruction of mouse spermatogonial stem cell self-renewal in vitro by Ras-cyclin D2 activation." Cell Stem Cell **5**(1): 76-86.

Spermatogonial stem cells (SSCs) undergo self-renewal division and support spermatogenesis. Although several cytokines coordinate to drive SSC self-renewal, little is known about the mechanisms underlying this process. We investigated the molecular mechanism by reconstructing SSC self-renewal in vitro without exogenous cytokines. Activation of Ras or overexpression of cyclins D2 and E1, both of which were induced by Ras, enabled long-term self-renewal of cultured spermatogonia. SSCs with activated Ras responded properly to differentiation signals and underwent spermatogenesis, whereas differentiation was abrogated in cyclin transfectants after spermatogonial transplantation. Both Ras- and cyclin-transfected cells produced seminomatous tumors, suggesting that excessive self-renewing stimulus induces oncogenic transformation. In contrast, cells that overexpressed cyclin D1 or D3 failed to make germ cell colonies after transplantation, which indicated that cyclin expression pattern is an important determinant to long-term SSC recolonization. Thus, the Ras-cyclin D2 pathway regulates the balance between tissue maintenance and tumorigenesis in the SSC population.

Lei, J., et al. (2014). "Mathematical model of adult stem cell regeneration with cross-talk between genetic and epigenetic regulation." Proc Natl Acad Sci U S A **111**(10): E880-887.

Adult stem cells, which exist throughout the body, multiply by cell division to replenish dying cells or to promote regeneration to repair damaged tissues. To perform these functions during the lifetime of organs or tissues, stem cells need to maintain their populations in a faithful distribution of their epigenetic states, which are susceptible to stochastic fluctuations during each cell division, unexpected injury, and potential genetic mutations that occur during many cell divisions. However, it remains unclear how the three processes of differentiation, proliferation, and apoptosis in regulating stem cells collectively manage these challenging tasks. Here, without considering molecular details, we propose a genetic optimal control model for adult stem cell regeneration that includes the three fundamental processes, along with cell division and adaptation based on differential fitnesses of phenotypes. In the model, stem cells with a distribution of epigenetic states are required to maximize expected performance after each cell division. We show that heterogeneous proliferation that depends on the epigenetic states of stem cells can improve the maintenance of stem cell distributions to create balanced populations. A control strategy during each

cell division leads to a feedback mechanism involving heterogeneous proliferation that can accelerate regeneration with less fluctuation in the stem cell population. When mutation is allowed, apoptosis evolves to maximize the performance during homeostasis after multiple cell divisions. The overall results highlight the importance of cross-talk between genetic and epigenetic regulation and the performance objectives during homeostasis in shaping a desirable heterogeneous distribution of stem cells in epigenetic states.

Leite, N. C., et al. (2022). "Genetic manipulation of stress pathways can protect stem-cell-derived islets from apoptosis in vitro." Stem Cell Reports **17**(4): 766-774.

The in vitro production of stem-cell-derived islets (SC-islets) has brought forth the potential of transplanting these cells to restore glycemic control in people with diabetes. Nonetheless, alloimmune and autoimmune responses remain considerable challenges for a broad clinical implementation of beta-cell replacement therapies. beta-cell stress has been implicated in the onset of beta-cell immunogenicity and death and is likely to contribute to beta-cell failure following transplantation. We show that inducing stress and/or administering cytokines causes SC-islet apoptosis, cellular dysfunction, and an increased expression of beta-cell stress- and immune-interaction-related genes. We then demonstrate that manipulating some of these genes results in enhanced protection of SC-islets from apoptosis in vitro.

Li, P., et al. (2021). "Single-cell analysis of Schistosoma mansoni identifies a conserved genetic program controlling germline stem cell fate." Nat Commun **12**(1): 485.

Schistosomes are parasitic flatworms causing one of the most prevalent infectious diseases from which millions of people are currently suffering. These parasites have high fecundity and their eggs are both the transmissible agents and the cause of the infection-associated pathology. Given its biomedical significance, the schistosome germline has been a research focus for more than a century. Nonetheless, molecular mechanisms that regulate its development are only now being understood. In particular, it is unknown what balances the fate of germline stem cells (GSCs) in producing daughter stem cells through mitotic divisions versus gametes through meiosis. Here, we perform single-cell RNA sequencing on juvenile schistosomes and capture GSCs during de novo gonadal development. We identify a genetic program that controls the proliferation and differentiation of GSCs. This program centers around onecut, a homeobox transcription factor, and boule, an mRNA binding protein. Their expressions are mutually dependent in the schistosome male germline, and knocking down either of them causes over-proliferation of GSCs and blocks germ cell differentiation. We further show that this germline-specific regulatory program is conserved in the planarian, schistosome's free-living evolutionary cousin, but the function of onecut has changed during evolution to support GSC maintenance.

Li, X., et al. (2001). "[Genetic polymorphism of polyacrylamide gel electrophoresis loci in patients after non-myeloablative allogeneic peripheral blood stem cell transplantation]." Zhonghua Nei Ke Za Zhi **40**(10): 651-653.

OBJECTIVE: To understand the information of donor and recipient in the mixed hematopoietic chimerism after non-myeloablative allogeneic peripheral blood stem cell transplantation(NM-APBSCT). METHODS: DNA samples were extracted with phenol/chloroform method and were amplified by PCR technique in heparin-blood or heparin-bone-marrow. The PCR products were analyzed using polyacrylamide gel electrophoresis and silver staining means. RESULTS: The amplified fragment length polymorphism was found in the short tandem repeat loci of 10 healthy persons and 8 leukemia patients who were not treated with hematopoietic stem cells transplantation; the bands of silver staining originated from donors and recipients were found in 3 patients after NM-APBSCT, but the brightness in the bands of donor and recipient was different. CONCLUSION: Polymorphism for mixed hematopoietic chimerism can be estimated timely, sensitively and exactly; and the results may be used to guide adoptive immunotherapy for patients after NM-APBSCT. The silver means were simple without contamination of isotopes and without using special equipment. The methods may benefit common hospitals to develop work in this respect.

Liu, S., et al. (2018). "Genetic enhancement of lodging resistance in rice due to the key cell wall polymer lignin, which affects stem characteristics." Breed Sci **68**(5): 508-515.

Lodging in crops seriously restricts plant growth and grain production. The genetic modification of cell walls to enhance plant mechanical strength has been suggested as a promising approach toward improving lodging resistance. However, because of the complexity of the plant cell wall, the exact effects of its polymers on plant lodging resistance remain elusive. To address this issue, we performed large-scale analyses of a total of 56 rice (Oryza sativa L.) varieties that displayed distinct cell wall component and lodging index. Lignin was identified as the key cell wall polymer that positively determines lodging resistance in rice. Correlation analysis between cell wall

composition and plant morphological characteristics revealed that lignin enhanced rice lodging resistance by largely increasing the mechanical strength of the basal stem and reducing plant height. Further characterization of four representative rice varieties, ShenNong9903, YanJian218, KongYu131, and ShenNongK33, displaying varied levels of lodging resistance, revealed the multiple candidate genes (PAL, CoMT, 4CL3, CAD2, CAD7 and CCR20) responsible for increasing lignin level. Hence, our results demonstrate that the high lignin level in the cell wall predominately improves lodging resistance and suggest target genes for the genetic modification of lignin towards breeding rice with high lodging resistance.

Liu, W. and S. X. Hou (2008). "Genetic tools used for cell lineage tracing and gene manipulation in Drosophila germline stem cells." Methods Mol Biol **450**: 61-70.

The advancement of Drosophila germline stem cell research accompanies the development of powerful new tools for genetic analysis. These include the techniques of stem cell labeling, cell lineage tracing, mosaic mutant analysis, and gene manipulation in targeted cell populations, which together constitute the critical methodologies in stem cell research. We discuss four such techniques: the tubulin-lacZ positive-labeling system; the positively marked mosaic lineage (PMML) method; the flipase/flipase recombination target (FLP/FRT)-based mosaic mutant analysis; and the GAL80-based mosaic analysis with a repressible cell marker (MARCM) system.

Liu, W., et al. (2011). "Genetic and epigenetic X-chromosome variations in a parthenogenetic human embryonic stem cell line." J Assist Reprod Genet **28**(4): 303-313.

PURPOSE: To assess the genetic and epigenetic status of parthenogenetic human embryonic stem cells (phESCs). METHODS: Cytogenetics, X chromosome inactivation (XCI) and gene expression patterns were analyzed in one phESC line (FY-phES-018) that was derived from our laboratory. RESULTS: FY-phES-018 cells displayed the classical characteristics of normal hESCs. These cells had a 46, XX karyotype, and no inactive X chromosomes were observed before passage 20. After being cultured long term in vitro, some cells lost one X, and the proportion of cells with only one X gradually increased. At passage 35, almost all the cells displayed a 45, XO karyotype. Interestingly, at passage 45, the recovery of the X-chromosome was observed, and XCI became detectable; the mosaic ratio of 46, XX to 45, XO was 67:33. After passage 60, most cells displayed the 46, XX karyotype again with a mosaic ratio of 97:3. Some aberrant genomic imprinting was also observed in these cells. CONCLUSIONS: The phESCs line FY-phES-018 is both genetically and epigenetically unstable; therefore, further research is needed before using these cells.

Liu, X., et al. (2022). "Quantifying substantial carcinogenesis of genetic and environmental factors from measurement error in the number of stem cell divisions." BMC Cancer **22**(1): 1194.

BACKGROUND: The relative contributions of genetic and environmental factors versus unavoidable stochastic risk factors to the variation in cancer risk among tissues have become a widely-discussed topic. Some claim that the stochastic effects of DNA replication are mainly responsible, others believe that cancer risk is heavily affected by environmental and hereditary factors. Some of these studies made evidence from the correlation analysis between the lifetime number of stem cell divisions within each tissue and tissue-specific lifetime cancer risk. However, they did not consider the measurement error in the estimated number of stem cell divisions, which is caused by the exposure to different levels of genetic and environmental factors. This will obscure the authentic contribution of environmental or inherited factors. METHODS: In this study, we proposed two distinct modeling strategies, which integrate the measurement error model with the prevailing model of carcinogenesis to quantitatively evaluate the contribution of hereditary and environmental factors to cancer development. Then, we applied the proposed strategies to cancer data from 423 registries in 68 different countries (global-wide), 125 registries across China (national-wide of China), and 139 counties in Shandong province (Shandong provincial, China), respectively. RESULTS: The results suggest that the contribution of genetic and environmental factors is at least 92% to the variation in cancer risk among 17 tissues. Moreover, mutations occurring in progenitor cells and differentiated cells are less likely to be accumulated enough for cancer to occur, and the carcinogenesis is more likely to originate from stem cells. Except for medulloblastoma, the contribution of genetic and environmental factors to the risk of other 16 organ-specific cancers are all more than 60%. CONCLUSIONS: This work provides additional evidence that genetic and environmental factors play leading roles in cancer development. Therefore, the identification of modifiable environmental and hereditary risk factors for each cancer is highly recommended, and primary prevention in early life-course should be the major focus of cancer prevention.

Lochhead, P., et al. (2011). "Genetic variation in the prostate stem cell antigen gene and upper

gastrointestinal cancer in white individuals." Gastroenterology **140**(2): 435-441.

BACKGROUND & AIMS: An association between gastric cancer and the rs2294008 (C>T) polymorphism in the prostate stem cell antigen (PSCA) gene has been reported for several Asian populations. We set out to determine whether such an association exists in white individuals. METHODS: We genotyped 166 relatives of gastric cancer patients, including 43 Helicobacter pylori-infected subjects with hypochlorhydria and gastric atrophy, 65 infected subjects without these abnormalities, 58 H pylori-negative relatives, and 100 population controls. Additionally, a population-based study of chronic atrophic gastritis provided 533 cases and 1054 controls. We then genotyped 2 population-based, case-control studies of upper gastrointestinal cancer: the first included 312 gastric cancer cases and 383 controls; the second included 309 gastric cancer cases, 159 esophageal cancer cases, and 211 controls. Odds ratios were computed from logistic models and adjusted for confounding variables. RESULTS: Carriage of the risk allele (T) of rs2294008 in PSCA was associated with chronic atrophic gastritis (adjusted odds ratio [OR], 1.5; 95% confidence interval [CI]: 1.1-1.9) and noncardia gastric cancer (OR, 1.9; 95% CI: 1.3-2.8). The association was strongest for the diffuse histologic type (OR, 3.2; 95% CI: 1.2-10.7). An inverse association was observed between carriage of the risk allele and gastric cardia cancer (OR, 0.5; 95% CI: 0.3-0.9), esophageal adenocarcinoma (OR, 0.5; 95% CI: 0.3-0.9), and esophageal squamous cell carcinoma (OR, 0.4; 95% CI: 0.2-0.9). CONCLUSIONS: The rs2294008 polymorphism in PSCA increases the risk of noncardia gastric cancer and its precursors in white individuals but protects against proximal cancers.

Lodha, M., et al. (2008). "Genetic and epigenetic regulation of stem cell homeostasis in plants." Cold Spring Harb Symp Quant Biol **73**: 243-251.

Plants generate new organs through the activity of small populations of stem cells present in specialized niches called meristems. Stem cell homeostasis is attained by dynamic regulatory networks involving transcriptional regulators, hormones, and other intercellular signals that specify cell fate and convey positional information to the apical stem cells and the organizing center located immediately below. The balance between stem cell maintenance within the shoot apical meristem (SAM) and differentiation of cells that are displaced from the niche to form new organs involves the epigenetic silencing of stem cell regulatory genes. Recent advances have identified highly conserved chromatin remodeling factors as epigenetic regulators of stem cell fate that confer plasticity in plant development and ensure the stable inheritance of repressed expression states during organogenesis. These advances reveal that common mechanisms contribute to stem cell homeostasis in plants and animals.

Loeffler, J., et al. (2010). "Genetic polymorphisms in the cytokine and chemokine system: their possible importance in allogeneic stem cell transplantation." Curr Top Microbiol Immunol **341**: 83-96.

Chemokines represent central players of the innate and adaptive immunity and are involved in the regulation of inflammatory events occurring during infectious complications or during graft vs. host disease (GvHD). Patients after allogeneic stem cell transplantation (alloSCT) are at a high risk for the development of acute GvHD or to suffer from fungal infections. Susceptibility to fungal infections and the course of GvHD can be genetically influenced by single nucleotide polymorphisms (SNPs), which regulate expression or biological activity of chemokines, and therefore have an impact on the outcome of invasive aspergillosis and GvHD. High lightened studies of abetting factors for GvHD revealed SNPs in TNFA, IL-6, IL-10, INF-gamma, CCL2, CCL5 (RANTES), IL-1Ra, IL-23R, IL-7Ralpha, IL-10RB, and CCR9 genes as prevalent considerable. Furthermore, additional SNPs were described to be significantly associated with fungal infections (Aspergillus fumigatus, Candida albicans), including markers in CCL3, CCL4, CCL20, CXCL2, CXCL8, CXCL10, CCR1, and CCR2. This review summarizes the current knowledge about the growing number of genetic markers in chemokine genes and their relevance for patients after alloSCT.

Lopez-Granados, L., et al. (2018). "[Reduced-intensity conditioning haematopoietic stem cell transplantation in genetic diseases: Experience of the Spanish Working Group for Bone Marrow Transplantation in Children]." An Pediatr (Engl Ed) **88**(4): 196-203.

INTRODUCTION: Haematopoietic stem cell transplantation (HSCT) involves implanting cellular elements capable of generating a new and healthy haematopoietic system. Reduced intensity conditioning (RIC) consists of an immunosuppressive treatment to facilitate a progressive implant with lower morbidity. This type of conditioning can also lead to myelosuppression, which is potentially reversible over time. Reduced intensity conditioning enables HSCT to be performed on patients with genetic diseases for whom added comorbidity is undesirable due to the high doses of chemotherapy that accompanies conventional myeloablative regimens. PATIENTS AND METHODS: An analysis was performed on the outcomes of 68 paediatric patients with genetic diseases who underwent HSCT with RIC between 2005 and 2013 in

the of Paediatric Haematopoietic Stem Cell Transplantation Units that are part of the Spanish Working Group for Bone Marrow Transplantation in Children. A multicentre study was conducted including 68 patients, of whom 43 had Primary Immunodeficiency, 21 with congenital haematological diseases, and 4 with metabolic diseases. RESULTS: Fifty (73.5%) of the 68 patients were still alive. The Overall Survival (OS) at nine years was 0.74. Twenty-three (33.8%) had some event during the course of the HSCT, with an event-free survival rate of 0.66. The OS in patients with haematological diseases was 0.81, being 0.7 in primary immunodeficiencies, and 0.4 in metabolic diseases. No significant difference was observed between the 3 groups of diseases. As regards the source of haematopoietic progenitors, there was an OS rate of 0.74 in patients transplanted with peripheral blood, 0.70 with bone marrow, and 0.70 and with cord blood, with no statistically significant differences. CONCLUSIONS: Favourable results have been obtained in HSCT with reduced intensity conditioning in genetic diseases. It should be noted that the risks and benefits of the RIC in patients with metabolic diseases need to be assessed on an individual basis.

McWhir, J., et al. (1996). "Selective ablation of differentiated cells permits isolation of embryonic stem cell lines from murine embryos with a non-permissive genetic background." Nat Genet **14**(2): 223-226.

Embryonic stem (ES) cells enable the engineering of precise modifications to the mouse genome by gene targeting. Although there are reports of cultured cell contributions to chimaeras in golden hamster, rat and pig, definitive ES cell lines which contribute to the germline have not been demonstrated in any species but mouse. Among mouse strains, genetic background strongly affects the efficiency of ES isolation, and almost all ES lines in use are derived from strain 129 (refs 1,4,5) or, less commonly, C57BL/6 (refs 6-8). The CBA strain is refractory to ES isolation and there are no published reports of CBA-derived ES lines. Hence, CBA mice may provide a convenient model of ES isolation in other species. In ES derivation it is critical that the primary explant be cultured for a sufficient time to allow multiplication of ES cell progenitors, yet without allowing extensive differentiation. Thus, differences in ES derivation between mouse strains may reflect differences in the control of ES progenitor cells by other lineages within the embryo. Here we describe a strategy to continuously remove differentiated cells by drug selection, which generates germline competent ES lines from genotypes that are non-permissive in the absence of selection.

Mead, B., et al. (2018). "Mesenchymal Stem Cell-Derived Small Extracellular Vesicles Promote Neuroprotection in a Genetic DBA/2J Mouse Model of Glaucoma." Invest Ophthalmol Vis Sci **59**(13): 5473-5480.

PURPOSE: To determine if bone marrow-derived stem cell (BMSC) small extracellular vesicles (sEV) promote retinal ganglion cell (RGC) neuroprotection in the genetic DBA/2J mouse model of glaucoma for 12 months. METHODS: BMSC sEV and control fibroblast-derived sEV were intravitreally injected into 3-month-old DBA/2J mice once a month for 9 months. IOP and positive scotopic threshold responses were measured from 3 months: IOP was measured monthly and positive scotopic threshold responses were measured every 3 months. RGC neuroprotection was determined in wholemounts stained with RNA binding protein with multiple splicing (RBPMS), whereas axonal damage was assessed using paraphenylenediamine staining. RESULTS: As expected, DBA/2J mice developed chronic ocular hypertension beginning at 6 months. The delivery of BMSC sEV, but not fibroblast sEV, provided significant neuroprotective effects for RBPMS+ RGC while significantly reducing the number of degenerating axons seen in the optic nerve. BMSC sEV significantly preserved RGC function in 6-month-old mice, but provided no benefit at 9 and 12 months. CONCLUSIONS: BMSC sEV are an effective neuroprotective treatment in a chronic model of ocular hypertension for 1 year, preserving RGC numbers and protecting against axonal degeneration.

Mehta, V., et al. (2017). "Genetic Modulation Therapy Through Stem Cell Transplantation for Human Immunodeficiency Virus 1 Infection." Cureus **9**(3): e1093.

Highly active anti-retroviral treatment has changed the dimensions of the outcomes for patients suffering from human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS). However, HIV infection is still an ailment which is spreading throughout the world extensively. Given the confinements of the present restorative methodologies and the non-availability of any strategic vaccination against HIV, there is a squeezing need to build a therapeutic treatment. Viral tropism for HIV includes CD4+ cells, macrophages, and microglial cells, and it is through binding with co-receptors C-C chemokine receptor type 5 (CCR5) and C-X-C chemokine receptor type 4 (CXCR4). While these cell types are present in all individuals, there are rare cases that stayed uninfected even after getting exposed to an overwhelming load of HIV. Research revealed a homozygous 32-base pair deletion (Delta32/Delta32) in CCR5. After careful consideration, a hypothesis was

proposed a few years back that a cure for HIV disease is possible, through hematopoietic stem cells transplantation from a donor homozygous for the CCR5-Delta32 deletion. Hematopoietic stem cell (HSC) based quality treatment may serve as a promising tool as these perpetual, self-renewing progenitor cells could be modified to oppose HIV infection. If done properly, the changed HSCs would offer the permanent creation of genetically modified cells that are resistant to HIV infection and/or have improved hostility to viral action which will eventually clear the contaminated cells. The purpose of this review is to concentrate on two facets of HSC genetic treatment for potentially life-threatening HIV infection: building HIV-resistant cells and designing cells that can target HIV disease. These two strategic approaches can be the frontline of a quality treatment plan against HIV infection and, as an individual treatment or a combination thereof, has been proposed to possibly destroy HIV altogether.

Mellough, C. B., et al. (2009). "Genetic basis of inherited macular dystrophies and implications for stem cell therapy." Stem Cells **27**(11): 2833-2845.

Untreatable hereditary macular dystrophy (HMD) presents a major burden to society in terms of the resulting patient disability and the cost to the healthcare provision system. HMD results in central vision loss in humans sufficiently severe for blind registration, and key issues in the development of therapeutic strategies to target these conditions are greater understanding of the causes of photoreceptor loss and the development of restorative procedures. More effective and precise analytical techniques coupled to the development of transgenic models of disease have led to a prolific growth in the identification and our understanding of the genetic mutations that underly HMD. Recent successes in driving differentiation of pluripotent cells towards specific somatic lineages have led to the development of more efficient protocols that can yield enriched populations of a desired phenotype. Retinal pigmented epithelial cells and photoreceptors derived from these are some of the most promising cells that may soon be used in the treatment of specific HMD, especially since rapid developments in the field of induced pluripotency have now set the stage for the production of patient-derived stem cells that overcome the ethical and methodological issues surrounding the use of embryonic derivatives. In this review we highlight a selection of HMD which appear suitable candidates for combinatorial restorative therapy, focusing specifically on where those photoreceptor loss occurs. This technology, along with increased genetic screening, opens up an entirely new pathway to restore vision in patients affected by HMD.

Menche, C. and H. F. Farin (2021). "Strategies for genetic manipulation of adult stem cell-derived organoids." Exp Mol Med **53**(10): 1483-1494.

Organoid technology allows the expansion of primary epithelial cells from normal and diseased tissues, providing a unique model for human (patho)biology. In a three-dimensional environment, adult stem cells self-organize and differentiate to gain tissue-specific features. Accessibility to genetic manipulation enables the investigation of the molecular mechanisms underlying cell fate regulation, cell differentiation and cell interactions. In recent years, powerful methodologies using lentiviral transgenesis, CRISPR/Cas9 gene editing, and single-cell readouts have been developed to study gene function and carry out genetic screens in organoids. However, the multicellularity and dynamic nature of stem cell-derived organoids also present challenges for genetic experimentation. In this review, we focus on adult gastrointestinal organoids and summarize the state-of-the-art protocols for successful transgenesis. We provide an outlook on emerging genetic techniques that could further increase the applicability of organoids and enhance the potential of organoid-based techniques to deepen our understanding of gene function in tissue biology.

Moore, J. C., et al. (2005). "Human embryonic stem cells: genetic manipulation on the way to cardiac cell therapies." Reprod Toxicol **20**(3): 377-391.

Almost 7 years after their first derivation from human embryos, a pressing urgency to deliver the promises of therapies based on human embryonic stem cells (hESC) has arisen. Protocols have been developed to support long-term growth of undifferentiated cells and partially direct differentiation to specific cell lineages. The stage has almost been set for the next step: transplantation in animal models of human disease. Here, we review the state-of-the-art with respect to the transplantation of embryonic stem cell-derived heart cells in animals. One problem affecting progress in this area and functional analysis in vivo in general, is the availability of genetically marked hESC. There are only a few cell lines that express reporter genes ubiquitously, and none is associated with particular lineages; a major hurdle has been the resistance of hESC to established infection and chemical transfection methodologies to introduce ectopic genes. The methods that have been successful are reviewed. We also describe the processes for generating a new, genetically-modified hESC line that constitutively expresses GFP as well as some of its characteristics, including its ability to form cardiomyocytes with electrophysiological properties of ventricular-like cells.

Moore, K. J. and M. W. Freeman (1999). "Embryonal Stem (ES) Cell-Derived Macrophages : A Cellular System that Facilitates the Genetic Dissection of Macrophage Function." Methods Mol Med **30**: 343-355.

The monocyte/macrophage (Mo) contributes to atherosclerotic lesion initiation and progression through a variety of interactions with cells of the artery wall that depend on the elucidation of a host of cytokines and growth factors by cells residing in the intima. The number and complexity of these interactions make it difficult to determine which cellular functions are contributing to the progression of atherosclerosis and which might be exploited to interrupt that progression. Studies of macrophage functions in atherosclerosis have been hindered by the limitations of available macrophage cell lines and primary cultures, including poor transfectability and the transformed state of imMortal cell lines. Recent studies have demonstrated that pluripotential mouse embryonic stem cells can be differentiated down specific hematopoietic lineages in vitro, including lines that give rise to macrophages (1). This technique provides a genetically tractable cellular system for studying myeloid cell function. Macrophages arising from this differentiation system demonstrate cell surface presentation of classic macrophage markers and macrophage functions including phagocytosis and responses to inflammatory stimuli. There are several important advantages inherent in using embryonic stem (ES) cell derived macrophages as a cell culture system for studying Mo function. As the cells are not transformed, and the progenitor cells arising from ES cells are capable of reconstituting the entire hematopoietic compartment of a mouse, they represent a cell culture system that appears to retain the physiologic regulation on growth and differentiation that is absent from transformed myelomonocytic cell lines.

Muller-Sieburg, C. E., et al. (2000). "Genetic control of hematopoietic stem cell frequency in mice is mostly cell autonomous." Blood **95**(7): 2446-2448.

Previously we reported that the size of the stem cell compartment (measured as LTC-IC) is 11-fold greater in DBA/2 than in C57BL/6 mice, and we identified genes that regulate the size of the stem cell pool. To determine whether stem cell intrinsic or extrinsic events account for these differences, we created chimeras by aggregating morulae from the strains C57BL/6 and DBA/2. In these chimeras stem cells of both genotypes are exposed to a common mixed environment. Thus, an equalization of stem cell frequencies is expected if stem cell extrinsic effects dominate. Conversely, the parental ratio of LTC-IC should be preserved if the regulation is stem cell autonomous. For each chimera, individual LTC-IC were genotyped on the clonal levels by analyzing their progeny. We found that most of the difference that regulates the size of the stem cell compartment was intrinsic.

Nair, V., et al. (2009). "Hematopoietic stem cell transplantation in children with genetic defects." Indian Pediatr **46**(3): 241-243.

Seventeen children (mean age: 7.2 years) with genetic defects involving hematopoietic cell production or function, underwent 19 allogeneic stem cell transplantations from HLA identical siblings. Twelve children were suffering from thalassemia major; 2 from Diamond Blackfan anemia; 2 from Fanconi anemia and 1 from congenital dyserythropoietic anemia. The disease free survival was 77% with a mean follow up of 36 months. The major complications were graft versus host disease, veno-occlusive disease, CMV infection and hemorrhage. One case each of thalassaemia major and Fanconi anemia rejected the graft after 1 year and 11 months, respectively. Both patients were successfully transplanted second time from the same donor with some modification in the conditioning regimen and stem cell source.

Najafi, A., et al. (2021). "Genetic Polymorphisms of Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) and clinical outcomes post-allogeneic hematopoietic stem cell transplantation: A systematic review and meta-analysis." Clin Transplant **35**(8): e14364.

BACKGROUND AND OBJECTIVE: Although HLA matching is considered as a key genetic predictor of allo-HSCT outcomes, genetic polymorphisms in non-HLA genes, especially in genes encoding immunoregulatory proteins, have also been proposed as additional risk factors linked to the occurrence of transplant complications. This study aimed to carry out a systematic review and meta-analysis from all eligible cohort studies to determine the effect of CTLA-4 gene polymorphisms, including rs231775, rs3087243, rs4553808, rs5742909, and rs733618, on clinical outcomes in patients receiving an allo-HSCT. METHODS: A systematic literature search in PubMed, Web of Science, and Scopus was performed to identify the relevant studies, and related information was extracted. The effect size (ES) and corresponding 95% confidence intervals (CIs) were calculated to estimate the association. RESULTS: 16 studies were eligible and included in the meta-analysis. The pooled results showed that only the dominant models of rs3087243 were significantly associated with chronic GVHD (cGVHD), while other SNPs were not significantly associated with overall survival, disease-free survival, relapse, and GVHD. CONCLUSIONS: Our study represents, for the first time, a comprehensive meta-analysis on the role of CTLA-4

polymorphisms on outcomes after allo-HSCT. The results indicate that the CT60 CTLA-4 polymorphism could be a significant risk factor for cGVHD.

Narula, J., et al. (2013). "Mathematical model of a gene regulatory network reconciles effects of genetic perturbations on hematopoietic stem cell emergence." Dev Biol **379**(2): 258-269.

Interlinked gene regulatory networks (GRNs) are vital for the spatial and temporal control of gene expression during development. The hematopoietic transcription factors (TFs) Scl, Gata2 and Fli1 form one such densely connected GRN which acts as a master regulator of embryonic hematopoiesis. This triad has been shown to direct the specification of the hemogenic endothelium and emergence of hematopoietic stem cells (HSCs) in response to Notch1 and Bmp4-Smad signaling. Here we employ previously published data to construct a mathematical model of this GRN network and use this model to systematically investigate the network dynamical properties. Our model uses a statistical-thermodynamic framework to describe the combinatorial regulation of gene expression and reconciles, mechanistically, several previously published but unexplained results from different genetic perturbation experiments. In particular, our results demonstrate how the interactions of Runx1, an essential hematopoietic TF, with components of the Bmp4 signaling pathway allow it to affect triad activation and acts as a key regulator of HSC emergence. We also explain why heterozygous deletion of this essential TF, Runx1, speeds up the network dynamics leading to accelerated HSC emergence. Taken together our results demonstrate that the triad, a master-level controller of definitive hematopoiesis, is an irreversible bistable switch whose dynamical properties are modulated by Runx1 and components of the Bmp4 signaling pathway.

Nava, T., et al. (2018). "Incorporation of GSTA1 genetic variations into a population pharmacokinetic model for IV busulfan in paediatric hematopoietic stem cell transplantation." Br J Clin Pharmacol **84**(7): 1494-1504.

AIMS: The aim of this study is to develop a population pharmacokinetic (PopPK) model for intravenous busulfan in children that incorporates variants of GSTA1, gene coding for the main enzyme in busulfan metabolism. METHODS: Busulfan concentration-time data was collected from 112 children and adolescents (median 5.4 years old, range: 0.1-20) who received intravenous busulfan during the conditioning regimen prior to stem cell transplantation. Weight, sex, baseline disease (malignant vs. non-malignant), age, conditioning regimen and GSTA1 diplotypes were evaluated as covariates of pharmacokinetic parameters by using nonlinear mixed effects analysis. The ability to achieve the target AUC(24h) (3600-6000 muM min(-1)) was assessed by estimating the first dose based on the present PopPK model and by comparing the results with other available models in children. RESULTS: A one-compartment model with first-order elimination best described the data. Allometric scaling of weight and a factor of busulfan metabolism maturation were included in the base model. GSTA1 diplotypes were found to be a significant covariate of busulfan clearance, which was 7% faster in rapid metabolizers and 12% slower in poor metabolizers, in comparison with normal ones. Busulfan doses calculated using the parameters of the proposed PopPK model were estimated to achieve the target AUC in 85.2% of the cases (95% CI 78.7-91.7%). CONCLUSION: This is the first PopPK for busulfan that successfully incorporated GSTA1 genotype in a paediatric population. Its use may contribute to better prediction of busulfan exposure in children and adolescents since the first dose, by tailoring the dose according to the individual metabolic capacity.

Nava, T., et al. (2017). "GSTA1 Genetic Variants and Conditioning Regimen: Missing Key Factors in Dosing Guidelines of Busulfan in Pediatric Hematopoietic Stem Cell Transplantation." Biol Blood Marrow Transplant **23**(11): 1918-1924.

Busulfan (Bu) is a key component of conditioning regimens used before hematopoietic stem cell transplantation (SCT) in children. Different predictive methods have been used to calculate the first dose of Bu. To evaluate the necessity of further improvements, we retrospectively analyzed the currently available weight- and age-based guidelines to calculate the first doses in 101 children who underwent allogenic SCT in CHU Sainte-Justine, Montreal, after an intravenous Bu-containing conditioning regimen according to genetic and clinical factors. The measured areas under the curve (AUCs) were within target (900 to 1500 microM/min) in 38.7% of patients after the administration of the first dose calculated based on age and weight, as locally recommended. GSTA1 diplotypes linked to poor Bu metabolism (G3) and fludarabine-containing regimens were the only factors associated with AUC within target (OR, 4.7 [95% CI, 1.1 to 19.8, P = .04]; and OR, 9.9 [95% CI, 1.6 to 61.7, P = .01], respectively). From the 11 methods selected for dose calculation, the percentage of AUCs within the target varied between 16% and 74%. In some models G3 was associated with AUCs within the therapeutic and the toxic range, whereas rapid metabolizers (G1) were correlated with subtherapeutic AUCs when different methods were used. These associations were confirmed by clearance-prediction analysis, in which

GSTA1 diplotypes consistently influenced the prediction errors of the methods. These findings suggest that these factors should be considered in Bu dose prediction in addition to the anthropometric data from patients. Furthermore, our data indicated that GSTA1 diplotypes was a factor that should be included in future population pharmacokinetic models, including similar conditioning regiments, to improve the prediction of Bu exposure after its initial dose.

Neavin, D., et al. (2021). "Single cell eQTL analysis identifies cell type-specific genetic control of gene expression in fibroblasts and reprogrammed induced pluripotent stem cells." Genome Biol **22**(1): 76.

BACKGROUND: The discovery that somatic cells can be reprogrammed to induced pluripotent stem cells (iPSCs) has provided a foundation for in vitro human disease modelling, drug development and population genetics studies. Gene expression plays a critical role in complex disease risk and therapeutic response. However, while the genetic background of reprogrammed cell lines has been shown to strongly influence gene expression, the effect has not been evaluated at the level of individual cells which would provide significant resolution. By integrating single cell RNA-sequencing (scRNA-seq) and population genetics, we apply a framework in which to evaluate cell type-specific effects of genetic variation on gene expression. RESULTS: Here, we perform scRNA-seq on 64,018 fibroblasts from 79 donors and map expression quantitative trait loci (eQTLs) at the level of individual cell types. We demonstrate that the majority of eQTLs detected in fibroblasts are specific to an individual cell subtype. To address if the allelic effects on gene expression are maintained following cell reprogramming, we generate scRNA-seq data in 19,967 iPSCs from 31 reprogramed donor lines. We again identify highly cell type-specific eQTLs in iPSCs and show that the eQTLs in fibroblasts almost entirely disappear during reprogramming. CONCLUSIONS: This work provides an atlas of how genetic variation influences gene expression across cell subtypes and provides evidence for patterns of genetic architecture that lead to cell type-specific eQTL effects.

Nikitina, T. V. and I. N. Lebedev (2022). "Stem Cell-Based Trophoblast Models to Unravel the Genetic Causes of Human Miscarriages." Cells **11**(12).

Miscarriage affects approximately 15% of clinically recognized pregnancies, and 1-3% of couples experience pregnancy loss recurrently. Approximately 50-60% of miscarriages result from chromosomal abnormalities, whereas up to 60% of euploid recurrent abortions harbor variants in candidate genes. The growing number of detected genetic variants requires an investigation into their role in adverse pregnancy outcomes. Since placental defects are the main cause of first-trimester miscarriages, the purpose of this review is to provide a survey of state-of-the-art human in vitro trophoblast models that can be used for the functional assessment of specific abnormalities/variants implicated in pregnancy loss. Since 2018, when primary human trophoblast stem cells were first derived, there has been rapid growth in models of trophoblast lineage. It has been found that a proper balance between self-renewal and differentiation in trophoblast progenitors is crucial for the maintenance of pregnancy. Different responses to aneuploidy have been shown in human embryonic and extra-embryonic lineages. Stem cell-based models provide a powerful tool to explore the effect of a specific aneuploidy/variant on the fetus through placental development, which is important, from a clinical point of view, for deciding on the suitability of embryos for transfer after preimplantation genetic testing for aneuploidy.

Overeem, A. W., et al. (2019). "Pluripotent stem cell-derived bile canaliculi-forming hepatocytes to study genetic liver diseases involving hepatocyte polarity." J Hepatol **71**(2): 344-356.

BACKGROUND & AIMS: Hepatocyte polarity is essential for the development of bile canaliculi and for safely transporting bile and waste products from the liver. Functional studies of autologous mutated proteins in the context of the polarized hepatocyte have been challenging because of the lack of appropriate cell models. The aims of this study were to obtain a patient-specific hepatocyte model that recapitulated hepatocyte polarity and to employ this model to study endogenous mutant proteins in liver diseases that involve hepatocyte polarity. METHODS: Urine cell-derived pluripotent stem cells, taken from a patient with a homozygous mutation in ATP7B and a patient with a heterozygous mutation, were differentiated towards hepatocyte-like cells (hiHeps). HiHeps were also derived from a patient with MEDNIK syndrome. RESULTS: Polarized hiHeps that formed in vivo-like bile canaliculi could be generated from embryonic and patient urine cell-derived pluripotent stem cells. HiHeps recapitulated polarized protein trafficking processes, exemplified by the Cu(2+)-induced redistribution of the copper transporter protein ATP7B to the bile canalicular domain. We demonstrated that, in contrast to the current dogma, the most frequent yet enigmatic Wilson disease-causing ATP7B-H1069Q mutation per se did not preclude trafficking of ATP7B to the trans-Golgi Network. Instead, it prevented its Cu(2+)-induced polarized redistribution to the bile canalicular domain, which could not be reversed by pharmacological folding chaperones. Finally, we demonstrate that

hiHeps from a patient with MEDNIK syndrome, suffering from liver copper overload of unclear etiology, showed no defect in the Cu(2+)-induced redistribution of ATP7B to the bile canaliculi. CONCLUSIONS: Functional cell polarity can be achieved in patient pluripotent stem cell-derived hiHeps, enabling, for the first time, the study of the endogenous mutant proteins, patient-specific pathogenesis and drug responses for diseases where hepatocyte polarity is a key factor. LAY SUMMARY: This study demonstrates that cells that are isolated from urine can be reprogrammed in a dish towards hepatocytes that display architectural characteristics similar to those seen in the intact liver. The application of this methodology to cells from patients diagnosed with inherited copper metabolism-related liver diseases (that is, Wilson disease and MEDNIK syndrome) revealed unexpected and novel insights into patient mutation-specific disease mechanisms and drug responses.

Partanen, J., et al. (2020). "Review of Genetic Variation as a Predictive Biomarker for Chronic Graft-Versus-Host-Disease After Allogeneic Stem Cell Transplantation." Front Immunol **11**: 575492.

Chronic graft-versus-host disease (cGvHD) is one of the major complications of allogeneic stem cell transplantation (HSCT). cGvHD is an autoimmune-like disorder affecting multiple organs and involves a dermatological rash, tissue inflammation and fibrosis. The incidence of cGvHD has been reported to be as high as 30% to 60% and there are currently no reliable tools for predicting the occurrence of cGvHD. There is therefore an important unmet clinical need for predictive biomarkers. The present review summarizes the state of the art for genetic variation as a predictive biomarker for cGvHD. We discuss three different modes of action for genetic variation in transplantation: genetic associations, genetic matching, and pharmacogenetics. The results indicate that currently, there are no genetic polymorphisms or genetic tools that can be reliably used as validated biomarkers for predicting cGvHD. A number of recommendations for future studies can be drawn. The majority of studies to date have been under-powered and included too few patients and genetic markers. Like in all complex multifactorial diseases, large collaborative genome-level studies are now needed to achieve reliable and unbiased results. Some of the candidate genes, in particular, CTLA4, HSPE, IL1R1, CCR6, FGFR1OP, and IL10, and some non-HLA variants in the HLA gene region have been replicated to be associated with cGvHD risk in independent studies. These associations should now be confirmed in large well-characterized cohorts with fine mapping. Some patients develop cGvHD despite very extensive immunosuppression and other treatments, indicating that the current therapeutic regimens may not always be effective enough. Hence, more studies on pharmacogenetics are also required. Moreover, all of these studies should be adjusted for diagnostic and clinical features of cGvHD. We conclude that future studies should focus on modern genome-level tools, such as machine learning, polygenic risk scores and genome-wide association study-transcription meta-analyses, instead of focusing on just single variants. The risk of cGvHD may be related to the summary level of immunogenetic differences, or whole genome histocompatibility between each donor-recipient pair. As the number of genome-wide analyses in HSCT is increasing, we are approaching an era where there will be sufficient data to incorporate these approaches in the near future.

Phillips, R. L., et al. (1992). "Genetic control of murine hematopoietic stem cell pool sizes and cycling kinetics." Proc Natl Acad Sci U S A **89**(23): 11607-11611.

Bone marrow from each of two inbred mouse strains, C57BL/6J and DBA/2J, was highly enriched for stem cells using flow cytometry and was divided into two stem cell subpopulations using the mitochondrial dye rhodamine 123 (Rh-123). The Rh-123lo population was determined to be more primitive than Rh-123hi based on the expression of stem cell markers such as the c-kit protooncogene (stem cell factor receptor) and the Ly-6A/E stem cell antigen (Sca-1) as well as the lack of in vitro colony-forming ability. Compared to DBA/2J mice, marrow from the C57BL/6J strain consistently showed a higher proportion of "very primitive" (Rh-123lo) cells, suggesting that the sizes of functionally distinct stem cell subpopulations are maintained under precise genetic control. Marrow from both strains exposed to the cytotoxic drug 5-fluorouracil showed a dramatic increase in the proportion of Rh-123lo cells within 2 days as repopulation began. Marrow subpopulations returned to pretreatment proportions by the eighth day in DBA/2J mice but not until 14 days in C57BL/6J mice. This intrinsic difference in 5-fluorouracil recovery time was attributed to an increase rate of stem cell cycling in DBA/2J relative to C57BL/6J mice. When stem cell factor was injected into a C57BL/6J<-->DBA/2J allophenic mouse, blood cell chimerism shifted markedly but transiently toward the DBA/2J genotype, suggesting that the DBA/2J target population, because of an inherent kinetic advantage, was able to respond faster to the cytokine. A model is proposed that is based on these and our earlier observations to explain this strain-specific stem cell behavior and offer new insights into the genetic control of stem cell cycling and population dynamics.

Pickering, S. J., et al. (2003). "Preimplantation genetic diagnosis as a novel source of embryos for stem cell research." Reprod Biomed Online **7**(3): 353-364.

The generation of human embryonic stem (hES) cells has captured the public and professional imagination, largely due their potential as a means of overcoming many debilitating and degenerative diseases by cell replacement therapy. Despite this potential, few well-characterized hES cell lines have been derived. Indeed, in the UK, despite several centres having been active in this area for more than 2 years, there are as yet no published reports of human embryonic stem cells having been generated. Part of the reason for this lack of progress may relate to the quality of embryos available for research. Embryos surplus to therapeutic requirements following routine assisted reproduction treatment are often of poor quality and a large proportion may be aneuploid. This study reports a new approach to hES cell derivation. Embryos surplus to therapeutic requirements following preimplantation genetic diagnosis were used. Although unsuitable for embryo transfer due to the high risk of genetic disease, these embryos are from fertile couples and thus may be of better quality than fresh embryos surplus to assisted reproduction treatment cycles. Embryos donated after cryopreservation were also used, and putative hES lines were derived from both sources of embryos. The cell lines described here are thought to be the first reported hES cell lines to have been derived in the UK.

Pickering, S. J., et al. (2005). "Generation of a human embryonic stem cell line encoding the cystic fibrosis mutation deltaF508, using preimplantation genetic diagnosis." Reprod Biomed Online **10**(3): 390-397.

Human embryonic stem (hES) cells are pluripotent cells isolated from early human embryos. They can be grown in vitro and made to differentiate into many different cell types. These properties have suggested that they may be useful in cell replacement therapy for many degenerative diseases. However, if hES cells could also be manufactured with mutations significant in human disease, they could provide a powerful in-vitro tool for modelling disease processes and progression in a number of different cell types, as well as providing an ideal system for studying in-vitro toxicity and efficacy of drugs and other therapeutic systems such as gene therapy. Embryos with such mutations are generated as part of routine genetic testing during preimplantation genetic diagnosis, providing the opportunity to generate cell lines with significant mutations. A human embryonic stem cell line homozygous for the most common mutation leading to cystic fibrosis in humans (delta F508) has been generated and characterized. This cell line has the same morphology and expresses proteins typical of other unaffected hES cell lines. This cell line represents an important in-vitro tool for understanding the pathophysiology of cystic fibrosis, and presents exciting opportunities to test the efficacy and toxicity of new therapies relevant to CF.

Pollard, S. M., et al. (2009). "Glioma stem cell lines expanded in adherent culture have tumor-specific phenotypes and are suitable for chemical and genetic screens." Cell Stem Cell **4**(6): 568-580.

Human brain tumors appear to have a hierarchical cellular organization suggestive of a stem cell foundation. In vitro expansion of the putative cancer stem cells as stable cell lines would provide a powerful model system to study their biology. Here, we demonstrate routine and efficient derivation of adherent cell lines from malignant glioma that display stem cell properties and initiate high-grade gliomas following xenotransplantation. Significantly, glioma neural stem (GNS) cell lines from different tumors exhibit divergent gene expression signatures and differentiation behavior that correlate with specific neural progenitor subtypes. The diversity of gliomas may, therefore, reflect distinct cancer stem cell phenotypes. The purity and stability of adherent GNS cell lines offer significant advantages compared to "sphere" cultures, enabling refined studies of cancer stem cell behavior. A proof-of-principle live cell imaging-based chemical screen (450 FDA-approved drugs) identifies both differential sensitivities of GNS cells and a common susceptibility to perturbation of serotonin signaling.

Popova, N. V. and R. J. Morris (2004). "Genetic regulation of mouse stem cells: identification of two keratinocyte stem cell regulatory loci." Curr Top Microbiol Immunol **280**: 111-137.

It is well documented that the bulge of hair follicle is a 'niche' for a significant population of mouse keratinocyte stem cells, and 95% of rodent clonogenic keratinocytes originate from the bulge region. The ability to form colonies in vitro is a well recognized test for keratinocyte stem cells. We analyzed the epidermis of seven mouse strains and their segregating crosses [(BALB/c x C57BL/6)F1; (BALB/c x CB6F1); (C57BL/ 6 x CB6F1); (CBF1 x CBF1)F2] for their clonogenic activity in vitro. We found that keratinocyte colony (KC) number is a new quantitative multigenic trait. The analysis of KC size in two parental strains (C57BL/6 and BALB/c), the F1 generation and the segregating crosses demonstrated that the size of KC is a quantitative complex trait also. We determined that mouse epidermis has at least two subpopulations of keratinocytes that gave small (< 2 mm2) and large (> 2 mm2) colonies. The differences in the number of small and large colonies between parental strains (C57BL/6,

BALB/c) were significant (P < 0.01). A genome-wide scan of the intercross and the two backcrosses maps the number of small KC to the central region of mouse Chromosome 9 (genomewide P value = 0.01). We define this locus as Ksc1. The proximal region of chromosome 4 is associated with the high number of large KC. We defined this locus as Ksc2. We found that Ksc1 and minor loci on chromosomes 6 and 7 map close, if not equal to, loci associated with mouse skin carcinogenesis. We conclude that mouse epidermis has at least two subpopulations of clonogenic keratinocyte stem cells that are regulated by different genes. We suggest that keratinocyte stem cells responsible for small colonies may play a major role in the regulation of resistance or sensitivity to skin carcinogenesis. Investigation of the genes regulating the stem cell number should provide new insight into the mechanisms of skin carcinogenesis, and should help to develop new approaches for therapies not only against active proliferating tumor cells but also quiescent tumor stem cells.

Porlan, E., et al. (2016). "Stable and Efficient Genetic Modification of Cells in the Adult Mouse V-SVZ for the Analysis of Neural Stem Cell Autonomous and Non-autonomous Effects." J Vis Exp(108): 53282.

Relatively quiescent somatic stem cells support life-long cell renewal in most adult tissues. Neural stem cells in the adult mammalian brain are restricted to two specific neurogenic niches: the subgranular zone of the dentate gyrus in the hippocampus and the ventricular-subventricular zone (V-SVZ; also called subependymal zone or SEZ) in the walls of the lateral ventricles. The development of in vivo gene transfer strategies for adult stem cell populations (i.e. those of the mammalian brain) resulting in long-term expression of desired transgenes in the stem cells and their derived progeny is a crucial tool in current biomedical and biotechnological research. Here, a direct in vivo method is presented for the stable genetic modification of adult mouse V-SVZ cells that takes advantage of the cell cycle-independent infection by LVs and the highly specialized cytoarchitecture of the V-SVZ niche. Specifically, the current protocol involves the injection of empty LVs (control) or LVs encoding specific transgene expression cassettes into either the V-SVZ itself, for the in vivo targeting of all types of cells in the niche, or into the lateral ventricle lumen, for the targeting of ependymal cells only. Expression cassettes are then integrated into the genome of the transduced cells and fluorescent proteins, also encoded by the LVs, allow the detection of the transduced cells for the analysis of cell autonomous and non-autonomous, niche-dependent effects in the labeled cells and their progeny.

Qiao, L. and Y. Feng (2012). "Genetic variations of prostate stem cell antigen (PSCA) contribute to the risk of gastric cancer for Eastern Asians: a meta-analysis based on 16792 individuals." Gene **493**(1): 83-91.

The associations between polymorphisms of prostate stem cell antigen (PSCA-rs2294008C>T and -rs2976392G>A) and gastric cancer (GC) risk for Eastern Asians have been commonly studied, but the results were conflicting. The aim of the present study was to further assess the associations by the method of meta-analysis. The databases of Medline, Embase and CNKI (up to May 25th, 2011) were retrieved to identify eligible case-control studies. Odds ratio (OR) and 95% confidence interval (95%CI) were used to present the strength of the associations. In total, eight case-control studies in seven articles with 16792 individuals (9738 cases of GC and 7054 controls) were included in this meta-analysis. Through quantitative analyses, we found that T allele of rs2294008C>T and A allele of rs2976392G>A were significantly associated with increased GC risk [rs2294008C>T: OR (95%CI)=1.31 (1.22-1.42), P(z-test)<0.001, P(heterogeneity)=0.166 for TT vs. C carriers; rs2976392G>A: OR (95%CI)=1.36(1.24-1.50), P(z-test)=0.015, P(heterogeneity)=0.111 for AA vs. G carriers]. The results of subgroup analyses (according to histopathology, countries and sources of controls) indicated that T allele of rs2294008C>T and A allele rs2976392G>A were associated with increased risk of both intestinal- and diffuse-type GC, and associated with increased risk of GC for Chinese, Japanese, Koreans, PCC and HCC/PHCC. Furthermore, T allele of rs2294008C>T was also associated with increased risk of cardia and non-cardia GC, and associated with increased risk of GC for males and females. Besides those, this meta-analysis also indicated that the interactions between T allele of rs2294008C>T and A allele of rs2976392G>A was associated with increased risk of GC (A-T vs. G-T: OR=1.16, 95%CI=1.06-1.27, P(z-test)=0.001, P(heterogeneity)=0.835). Although modest limitations and potential bias cannot be eliminated, this meta-analysis suggests that PSCA -rs2294008C>T and -rs2976392G>A are potential factors of GC development for Eastern Asians, and future work may incorporate these findings and evaluate these variants as potential markers for screening and early diagnosis of GC.

Ralston, A. and J. Rossant (2005). "Genetic regulation of stem cell origins in the mouse embryo." Clin Genet **68**(2): 106-112.

'Stem cell' has practically become a household term, but what is a stem cell and where does it come from? Insight into these questions has come from the early mouse embryo, or blastocyst, from which three kinds of stem cells have been derived: embryonic stem

(ES) cells, trophoblast stem (TS) cells, and extraembryonic endoderm (XEN) cells. These stem cells appear to derive from three distinct tissue lineages within the blastocyst: the epiblast, the trophectoderm, and the extraembryonic endoderm. Understanding how these lineages arise during development will illuminate efforts to understand the establishment and maintenance of the stem cell state and the mechanisms that restrict stem cell potency. Genetic analysis has enabled the identification of several genes important for lineage decisions in the mouse blastocyst. Among these, Oct4, Nanog, Cdx2, and Gata6 encode transcription factors required for the three lineages of the blastocyst and for the maintenance their respective stem cell types. Interestingly, genetic manipulation of several of these factors can cause lineage switching among these stem cells, suggesting that knowledge of key lineage-determining genes could help control differentiation of stem cells more generally. Pluripotent stem cells have also been isolated from the human blastocyst, but the relationship between these cells and stem cells of the mouse blastocyst remains to be explored. This review describes the genetic regulation of lineage allocation during blastocyst formation and discusses similarities and differences between mouse and human ES cells.

Ramzi, M., et al. (2019). "Genetic variation of TNF-alpha and IL-10, IL-12, IL-17 genes and association with torque teno virus infection post hematopoietic stem cell transplantation." Acta Virol **63**(2): 186-194.

Little is known about the role of genetic variation in the genes for cytokines and susceptibility to viral infection especially torque teno virus (TTV) following allogeneic hematopoietic stem cell transplantation. In this study, the association between interleukin-12, interleukin-17, interleukin-10 (IL-12,-17,-10) and tumor necrosis factor-alpha (TNF-alpha) polymorphisms was evaluated in patients with TTV infection who underwent allogeneic hematopoietic stem cell transplantation from South of Iran. The single nucleotide polymorphisms in the cytokine genes including IL-12 (-1188A/C), IL-17 (-197G/A), IL-10 (-1082G/A, -819C/T and -592C/A) and TNF-alpha (-308 G/A) were analyzed by PCR-RFLP methods. While our results did not show any association between IL-17, IL-12 and IL-10 (-819C/T and -1082G/A) polymorphisms and TTV infection status, heterozygote genotype of IL-10 (-592C/A) had direct correlation with TTV infection and A allele of TNF-alpha (-308G/A) showed a protective effect against TTV infection (P = 0.05 and P = 0.025, respectively). Within the group of patients who experienced acute graft-versus-host disease, the AA genotype and the A allele of IL-17 (-197 G/A) were significantly higher in non-infected patients compared to infected ones (P = 0.024 and P = 0.057, respectively). It was also observed that among infected patients, the GG genotype of IL-17 and AA genotype of TNF-alpha were significantly increased in hematopoietic stem cell transplanted patients with low grade (grade I+II) acute graft-versus-host disease compared to high grade (grade III and IV) disease (P = 0.056 and P = 0.056, respectively). Taken together, genetic variation of IL-10 (-592C/A) and TNF-alpha (-308G/A) genes might be associated with susceptibility to TTV infection post hematopoietic stem cell transplantation. Keywords: TNF-alpha; interleukins; torque teno virus (TTV); hematopoietic stem cell transplantation (HSCT); graft versus host disease (GvHD).

Rayes, A., et al. (2016). "A Genetic Modifier of the Gut Microbiome Influences the Risk of Graft-versus-Host Disease and Bacteremia After Hematopoietic Stem Cell Transplantation." Biol Blood Marrow Transplant **22**(3): 418-422.

The human gut microbiome is involved in vital biological functions, such as maintenance of immune homeostasis and modulation of intestinal development and enhanced metabolic capabilities. Disturbances of the intestinal microbiota have been associated with development and progression of inflammatory conditions, including graft-versus-host disease (GVHD). The fucosyltransferase 2 (FUT2) gene produces an enzyme that is responsible for the synthesis of the H antigen in body fluids and on the intestinal mucosa. FUT2 genotype has been shown to modify the gut microbiome. We hypothesized that FUT2 genotype influences risk of GVHD and bacterial translocation after allogeneic hematopoietic stem cell transplantation (HSCT). FUT2 genotype was determined in 150 consecutive patients receiving allogeneic HSCT at our center. We abstracted clinical characteristics and outcomes from the transplantation database. Cumulative risk of any acute GVHD varied by FUT2 genotype, with decreased risk in those with A/A genotype and increased risk in those with G/G genotype. In contrast, the cumulative incidence of bacteremia was increased in those with A/A genotype. We conclude that the FUT2 genotype influences risk of acute GVHD and bacteremia after HSCT. We hypothesize that the mechanisms involve altered intestinal surface glycosylation and microbial composition but this requires additional study.

Reisner, Y. (2001). "Stem cell transplantation across major genetic barriers." Ann N Y Acad Sci **938**: 322-326; discussion 326-327.

Megadose haploidentical transplants, mismatched at three HLA loci, engraft rapidly and durably without induction of graft-versus-host disease (GVHD). In vitro studies suggest that veto cells,

contained in the population of hematopoietic progenitors, facilitate this favorable outcome. Cytotoxic T cells, not reactive against the recipient but reactive against a third party, are potent veto cells and can synergize with the stem cells and facilitate allogeneic bone marrow engraftment without GVHD. Experiments with mice deficient in FasL and Fas, with transfer of FasL gene and with anti-CD8 antibody, suggest that the veto activity associated with cytotoxic T lymphocytes (CTLs) requires simultaneous expression of FasL and CD8.

Reisner, Y. (2007). "Hematopoietic stem cell transplantation across major genetic barriers." Immunol Res **38**(1-3): 174-190.

The first successful demonstration that effective T cell depletion can enable immune reconstitution without causing graft versus host disease (GVHD) was achieved in 1980 using lectin-separated hematopoietic stem cells. In leukemia patients undergoing supralethal radio- and chemotherapy, T cell-depleted transplants are vigorously rejected by residual host T cells; this barrier was first overcome in 1993 by the use of megadose stem cell transplants. This clinical observation can be explained, in part, by the demonstration that cells within the CD34 compartments, as well as their immediate early myeloid progeny, are endowed with veto activity. Engraftment of mismatched hematopoietic stem cells following reduced intensity conditioning, still represents a major challenge. Progress has been made recently by using anti-3rd party veto CTLs and T regulatory cells.

Reisner, Y., et al. (2003). "Hematopoietic stem cell transplantation across major genetic barriers: tolerance induction by megadose CD34 cells and other veto cells." Ann N Y Acad Sci **996**: 72-79.

Studies in mice and humans demonstrate that transplantation of hematopoietic progenitors in numbers larger than commonly used ("megadose" transplants) overcomes major genetic barriers. In vitro studies suggest that veto cells, within the population of hematopoietic progenitors, facilitate this favorable outcome. Thus, when purified CD34(+) cells were added to bulk mixed-lymphocyte reactions (MLRs) they suppressed CTLs against the donor's stimulators, but not against stimulators from a third party. This tolerizing activity depends on cell contact and can be blocked by the caspase inhibitor BD-FMK, suggesting that the effector host T cells are deleted by apoptosis upon interaction with the CD34(+) cells. Early myeloid CD33(+) cells generated by short-term ex vivo expansion of CD34(+) cells also exhibit veto activity, and these cells can be grown in large numbers. Tolerance induction can be further enhanced by other veto cells. Perhaps the most potent veto cell is the CD8+ CTL. However, this cell is also associated with marked GVHD (graft-versus-host disease. GVHD can be separated from the veto activity by generating anti-third party CTLs under IL2 deprivation. Under such selective pressure only the stimulated clones which make IL2 can survive, while anti-host clones die. In vivo studies show that such anti-third party veto CTLs can be used safely for tolerance induction without GVHD.

Reisner, Y., et al. (2005). "Hematopoietic stem cell transplantation across major genetic barriers: tolerance induction by megadose CD34 cells and other veto cells." Ann N Y Acad Sci **1044**: 70-83.

Studies in mice and humans demonstrate that transplantation of hematopoietic progenitors in numbers larger than commonly used overcomes major genetic barriers. In vitro studies suggest that veto cells, within the population of hematopoietic progenitors, facilitate this favorable outcome. Tolerance induction can be further enhanced by other veto cells. Perhaps the most potent veto cell is the CD8(+) CTL. However, this cell is also associated with marked GVHD, which can be separated from the veto activity by generating anti-third party CTLs under IL-2 deprivation.

Rieger, A. C., et al. (2019). "Genetic determinants of responsiveness to mesenchymal stem cell injections in non-ischemic dilated cardiomyopathy." EBioMedicine **48**: 377-385.

BACKGROUND: Non-ischemic dilated cardiomyopathy (NIDCM) responds variably to intramyocardial injection of mesenchymal stem cells (MSCs). We hypothesized that NIDCM genotype may influence responsiveness to MSC therapy and performed genotyping on all patients in the POSEIDON-DCM trial. METHODS: POSEIDON-DCM patients (n = 34) underwent genetic sequence analysis and deletion/duplication testing. The results were classified as positive for pathological variants (PV+; n = 8), negative for any variants (V-; n = 6), or as variants of uncertain significance (VUS; n = 20). All outcomes of therapy were analysed for each category of genetic results. FINDINGS: The 3 groups were indistinguishable at baseline with regard to ejection fraction (EF), demographics, medication use, or functional parameters. V- patients had an increase in EF at 12 months: +13.6% (IQR = +7.8%; +20.5%; p = 0.002), compared with VUS (+6.5%; IQR = +0.9%, +11.1%; p = 0.005) and PV+(-5.9%; IQR = -12.7%, +1.0; p = 0.2; p = 0.01 between groups). Six-minute walk distance improved in V- patients, but not in VUS and PV+. V- patients improved MLHFQ, compared to the other 2 groups, which did not improve over time. EPCCFUs increased by 9.7 +/- 1.9 in V- (p = 0.009)

compared to VUS and PV+ patients. V- patients had one-year survival (100%) compared with VUS (85%) and PV+ (40%; p = 0.015 log-rank). Similarly, MACE rates were lower in V- (0%) than PV+ (61.9%) or VUS (42.2%; p = 0.021 log-rank). INTERPRETATION: Our findings support the concept that the genetic profile of NIDCM patients plays a role in responsiveness to MSC therapy, with V- patients more likely to benefit and the converse for PV+. This observation emphasizes the need for further genetic studies, because of important implications for the management of NIDCM syndromes.

Riemens, R. J. M., et al. (2017). "Stem Cell Technology for (Epi)genetic Brain Disorders." Adv Exp Med Biol **978**: 443-475.

Despite the enormous efforts of the scientific community over the years, effective therapeutics for many (epi)genetic brain disorders remain unidentified. The common and persistent failures to translate preclinical findings into clinical success are partially attributed to the limited efficiency of current disease models. Although animal and cellular models have substantially improved our knowledge of the pathological processes involved in these disorders, human brain research has generally been hampered by a lack of satisfactory humanized model systems. This, together with our incomplete knowledge of the multifactorial causes in the majority of these disorders, as well as a thorough understanding of associated (epi)genetic alterations, has been impeding progress in gaining more mechanistic insights from translational studies. Over the last years, however, stem cell technology has been offering an alternative approach to study and treat human brain disorders. Owing to this technology, we are now able to obtain a theoretically inexhaustible source of human neural cells and precursors in vitro that offer a platform for disease modeling and the establishment of therapeutic interventions. In addition to the potential to increase our general understanding of how (epi)genetic alterations contribute to the pathology of brain disorders, stem cells and derivatives allow for high-throughput drugs and toxicity testing, and provide a cell source for transplant therapies in regenerative medicine. In the current chapter, we will demonstrate the validity of human stem cell-based models and address the utility of other stem cell-based applications for several human brain disorders with multifactorial and (epi)genetic bases, including Parkinson's disease (PD), Alzheimer's disease (AD), fragile X syndrome (FXS), Angelman syndrome (AS), Prader-Willi syndrome (PWS), and Rett syndrome (RTT).

Roach, M. L., et al. (1995). "A new embryonic stem cell line from DBA/1lacJ mice allows genetic modification in a murine model of human inflammation." Exp Cell Res **221**(2): 520-525.

The development of embryonic stem (ES) cells and their capacity to generate mice with mutations at specific loci has provided a powerful resource for functional analysis of genes in pathological processes. However, the ability to combine this technology with the large number of existing murine models of human genetic disease has been complicated by the inability to routinely generate ES cell lines from strains other than 129. Here, we report the production of a novel ES cell line derived from an inbred mouse, DBA/1lacJ. This new ES cell line undergoes homologous recombination and efficient colonization of the germline of male chimeric offspring with ES cell microinjection into C57B1/6 embryos. The DBA/1lacJ mouse is a murine model of human inflammation, therefore genetic modifications in the DBA ES cells will allow evaluation of the target gene's role in the inflammatory process.

Robertson, A., et al. (2017). "Genetic ablation of the mammalian sterile-20 like kinase 1 (Mst1) improves cell reprogramming efficiency and increases induced pluripotent stem cell proliferation and survival." Stem Cell Res **20**: 42-49.

Adult fibroblasts can be reprogrammed into induced pluripotent stem cells (iPSC) for use in various applications. However, there are challenges in iPSC generation including low reprogramming efficiency, yield, cell survival and viability. Since the Hippo signalling pathway is a key pathway involved in regulating cell proliferation and survival, we here test whether modification of the Hippo pathway will enhance the efficiency of iPSC generation and improve their survival. The Hippo pathway was modified by genetic ablation of the mammalian sterile-20 like kinase 1 (Mst1), a major component of the pathway. Using adult skin fibroblasts isolated from Mst1 knockout mice (Mst1(-/-)) as a source of iPSC we found that genetic ablation of Mst1 leads to significantly increased reprogramming efficiency by 43.8%. Moreover, Mst1(-/-) iPSC displayed increase proliferation by 12% as well as an increase in cell viability by 20% when treated with a chemical hypoxic inducer. Mechanistically, we found higher activity of YAP, the main downstream effector of the Hippo pathway, in iPSC lacking Mst1. In conclusion, our data suggests that Mst1 can be targeted to improve the efficiency of adult somatic cell reprogramming as well as to enhance iPSC proliferation and survival.

Rodriguez-Leal, D., et al. (2019). "Evolution of buffering in a genetic circuit controlling plant stem cell proliferation." Nat Genet **51**(5): 786-792.

Precise control of plant stem cell proliferation is necessary for the continuous and reproducible development of plant organs(1,2). The peptide ligand CLAVATA3 (CLV3) and its receptor protein kinase CLAVATA1 (CLV1) maintain stem cell homeostasis within a deeply conserved negative feedback circuit(1,2). In Arabidopsis, CLV1 paralogs also contribute to homeostasis, by compensating for the loss of CLV1 through transcriptional upregulation(3). Here, we show that compensation(4,5) operates in diverse lineages for both ligands and receptors, but while the core CLV signaling module is conserved, compensation mechanisms have diversified. Transcriptional compensation between ligand paralogs operates in tomato, facilitated by an ancient gene duplication that impacted the domestication of fruit size. In contrast, we found little evidence for transcriptional compensation between ligands in Arabidopsis and maize, and receptor compensation differs between tomato and Arabidopsis. Our findings show that compensation among ligand and receptor paralogs is critical for stem cell homeostasis, but that diverse genetic mechanisms buffer conserved developmental programs.

Rojek, K., et al. (2016). "Identifying Inherited and Acquired Genetic Factors Involved in Poor Stem Cell Mobilization and Donor-Derived Malignancy." Biol Blood Marrow Transplant **22**(11): 2100-2103.

Analysis of the clinical characteristics of hematopoietic stem cell transplant (HSCT) donors has proven beneficial for identifying cases of heritable hematopoietic disorders. This study examines poor peripheral blood hematopoietic stem cell mobilization after granulocyte colony-stimulating factor administration among 328 donors as a potential marker for suspected familial predisposition to myeloid malignancies. Here, we present data comparing the clinical characteristics of poor-mobilizing versus nonpoor-mobilizing donors and the results of panel-based sequencing of hematopoietic genes in poor-mobilizing donors. From this analysis, we identified a novel case of a donor-derived myelodysplastic syndrome in an HSCT recipient that is consistent with clonal evolution of TET2-mutated clonal hematopoiesis of indeterminate potential (CHIP) within the donor. This study demonstrates the potential risk of using hematopoietic stem cells from a donor with CHIP and raises the question of whether there should be increased screening measures to identify such donors.

Roos-Weil, D., et al. (2011). "Impact of genetic abnormalities after allogeneic stem cell transplantation in multiple myeloma: a report of the Societe Francaise de Greffe de Moelle et de Therapie Cellulaire." Haematologica **96**(10): 1504-1511.

BACKGROUND: The impact of cytogenetic abnormalities in multiple myeloma after allogeneic stem cell transplantation has not been clearly defined. This study examines whether allogeneic stem cell transplantation could be of benefit for myeloma patients with high-risk cytogenetic abnormalities. DESIGN AND METHODS: This is a retrospective multicenter analysis of the registry of the Societe Francaise de Greffe de Moelle et de Therapie Cellulaire, including 143 myeloma patients transplanted between 1999 and 2008. RESULTS: The incidences of cytogenetic abnormalities were 59% for del(13q), 25% for t(4;14), 25% for del(17p) and 4% for t(14;16). When comparing the population carrying an abnormality to that without the same abnormality, no significant difference was found in progression-free survival, overall survival or progression rate. Patients were grouped according to the presence of any of the poor prognosis cytogenetic abnormalities t(4;14), del(17p) or t(14;16) (n=53) or their absence (n=32). No difference in outcomes was observed between these two groups: the 3-year progression-free survival, overall survival and progression rates were 30% versus 17% (P=0.9), 45% versus 39% (P=0.8) and 53% versus 75% (P=0.9), respectively. CONCLUSIONS: These data indicate that allogeneic stem cell transplantation could potentially be of benefit to high-risk myeloma patients.

Roubalova, K., et al. (2011). "Genetic variability of cytomegalovirus glycoprotein O in hematopoietic stem cell transplant recipients." Transpl Infect Dis **13**(3): 237-243.

Genetic variation of cytomegalovirus (CMV) strains can correlate with their pathogenicity for immunocompromised patients. Glycoprotein O (gO), together with glycoprotein L and glycoprotein H, mediate the fusion of the viral envelope with the cell membrane and promotes virus penetration, envelopment, and release. The variability of gO might play a role in CMV cell tropism. The goal was a retrospective analysis of gO variability in a cohort of hematopoietic stem cell transplant (HSCT) recipients to determine the distribution of gO genotypes and to investigate their impact on clinical outcome and manifestation of CMV infection. METHODS: In archived blood samples from 51 adult allogeneic HSCT recipients with active CMV infection, gO was analyzed by sequencing the N-terminal domain of the UL74 gene using the dye deoxy termination method. RESULTS: The gO1 and gO2 clades were most common (39% and 20%, respectively, and gO3 was associated with higher risk of symptomatic infection (P = 0.026 in multivariant analysis). Despite being associated with higher antigenemia levels (P = 0.02), gO4 had the best survival and lower rate of CMV

recurrence. No significant differences were found in clinical manifestation and outcome of CMV disease between patients with various gO clades. Because CMV strains sharing an identical gO sequence differed in glycoprotein B genotypes, sequencing the N-terminal part of the gO gene does not seem to be optimal for the identification of strains. CONCLUSIONS: gO genotyping may contribute to the biological characterization of CMV strains in HSCT recipients.

Routledge, D., et al. (2007). "Quantitative assessment of mixed chimerism in allogeneic stem cell transplant patients: a comparison of molecular genetic and cytogenetic approaches." J Pediatr Hematol Oncol **29**(6): 428-431.

After allogeneic stem cell transplantation, dual donor and recipient populations may be present. Donor/recipient ratio changes over time may predict clinical outcome: accurate measurement of these changes are needed. Chimerism may be measured by XY-fluorescence in situ hybridization for donor/recipient sex mismatch, or polymerase chain reaction amplification of short tandem repeat loci with donor/recipient sex match. Patients were monitored by each method. Additionally, mononuclear cells from 2 sex-mismatched individuals were mixed and analyzed using both methods. Each gave concordant estimates of patient chimerism and discriminated cell population ratios in mixed blood. We conclude that cytogenetic and molecular methods give accurate donor chimerism estimates.

Roybal, J. L., et al. (2010). "Stem cell and genetic therapies for the fetus." Semin Fetal Neonatal Med **15**(1): 46-51.

Advances in prenatal diagnosis have led to the prenatal management of a variety of congenital diseases. Although prenatal stem cell and gene therapy await clinical application, they offer tremendous potential for the treatment of many genetic disorders. Normal developmental events in the fetus offer unique biologic advantages for the engraftment of hematopoietic stem cells and efficient gene transfer that are not present after birth. Although barriers to hematopoietic stem cell engraftment exist, progress has been made and preclinical studies are now underway for strategies based on prenatal tolerance induction to facilitate postnatal cellular transplantation. Similarly, in-utero gene therapy shows experimental promise for a host of diseases and proof-in-principle has been demonstrated in murine models, but ethical and safety issues still need to be addressed. Here we review the current status and future potential of prenatal cellular and genetic therapy.

Russell, L. B., et al. (2007). "Comparison of the genetic effects of equimolar doses of ENU and MNU: while the chemicals differ dramatically in their mutagenicity in stem-cell spermatogonia, both elicit very high mutation rates in differentiating spermatogonia." Mutat Res **616**(1-2): 181-195.

Mutagenic, reproductive, and toxicity effects of two closely related chemicals, ethylnitrosourea (ENU) and methylnitrosourea (MNU), were compared at equimolar and near-equimolar doses in the mouse specific-locus test in a screen of all stages of spermatogenesis and spermiogenesis. In stem-cell spermatogonia (SG), ENU is more than an order of magnitude more mutagenic than MNU. During post-SG stages, both chemicals exhibit high peaks in mutation yield when differentiating spermatogonia (DG) and preleptotene spermatocytes are exposed. The mutation frequency induced by 75mgMNU/kg during this peak interval is, to date, the highest induced by any single-exposure mutagenic treatment - chemical or radiation - that allows survival of the exposed animal and its germ cells, producing an estimated 10 new mutations per genome. There is thus a vast difference between stem cell and differentiating spermatogonia in their sensitivity to MNU, but little difference between these stages in their sensitivity to ENU. During stages following meiotic metaphase, the highest mutation yield is obtained from exposed spermatids, but for both chemicals, that yield is less than one-quarter that obtained from the peak interval. Large-lesion (LL) mutations were induced only in spermatids. Although only a few of the remaining mutations were analyzed molecularly, there is considerable evidence from recent molecular characterizations of the marker genes and their flanking chromosomal regions that most, if not all, mutations induced during the peak-sensitive period did not involve lesions outside the marked loci. Both ENU and MNU treatments of post-SG stages yielded significant numbers of mutants that were recovered as mosaics, with the proportion being higher for ENU than for MNU. Comparing the chemicals for the endpoints studied and additional ones (e.g., chromosome aberrations, toxicity to germ cells and to animals, teratogenicity) revealed that while MNU is generally more effective, the opposite is true when the target cells are SG.

Russo, E., et al. (2014). "The mTOR signaling pathway and neuronal stem/progenitor cell proliferation in the hippocampus are altered during the development of absence epilepsy in a genetic animal model." Neurol Sci **35**(11): 1793-1799.

Hyperactivation of mammalian target of rapamycin (mTOR) signaling pathway occurs after an epileptogenic insult and, its inhibition prevents the development of spontaneous seizures. We have

recently demonstrated that mTOR's inhibition by rapamycin (started before seizure onset), permanently reduces the development of spontaneous absence seizures in WAG/Rij rats, an animal model of absence epilepsy; furthermore, mTOR phosphorylation was increased in adult WAG/Rij rats' cortex, but not other brain areas. However, it was not clear whether this hyperphosphorylation was a cause or a consequence of absence seizure. Here, we have addressed this issue by analyzing immunohistochemically: (1) the brain levels of total and phosphorylated mTOR in young (before seizures) and adult WAG/Rij rats; (2) the proliferation of hippocampal neuronal stem/progenitor cells assessed by BrdU analysis at different ages. WAG/Rij rats have higher levels of total mTOR in several brain areas than Wistar rats; phospho-mTOR staining is higher in young WAG/Rij rats than control and adult WAG/Rij rats. Finally, the age-related decline in hippocampal neural progenitor cell proliferation rate was slower in WAG/Rij than Wistar rats. Our results support a role for persistent mTOR activation and consequent change in hippocampal progenitor cell proliferation during the epileptogenic process leading to the development of absence seizures in WAG/Rij rats.

Sabatino, M., et al. (2008). "Conservation of genetic alterations in recurrent melanoma supports the melanoma stem cell hypothesis." Cancer Res **68**(1): 122-131.

It is generally accepted that human cancers derive from a mutated single cell. However, the genetic steps characterizing various stages of progression remain unclear. Studying a unique case of metastatic melanoma, we observed that cell lines derived from metachronous metastases arising over a decade retained a central core of genetic stability in spite of divergent phenotypes. In the present study, we expanded our previous observations comparing these autologous cell lines of clonal derivation with allogeneic ones and correlated array comparative genomic hybridization (aCGH) with gene expression profiling to determine their relative contribution to the dynamics of disease progression. aCGH and gene expression profiling were performed on autologous cell lines and allogeneic melanoma cell lines originating from other patients. A striking correlation existed between total extent of genetic imbalances, global transcriptional patterns, and cellular phenotypes. They did not follow a strict temporal progression but stemmed independently at various time points from a central core of genetic stability best explained according to the cancer stem cell hypothesis. Although their contribution was intertwined, genomic imbalances detectable by aCGH contributed only 25% of the transcriptional traits determining autologous tumor distinctiveness. Our study provides important insights about the dynamics of cancer progression and supports the development of targeted anticancer therapies aimed against stable genetic factors that are maintained throughout the end stage of disease.

Sackett, S. D., et al. (2022). "Genetic Engineering of Immune Evasive Stem Cell-Derived Islets." Transpl Int **35**: 10817.

Genome editing has the potential to revolutionize many investigative and therapeutic strategies in biology and medicine. In the field of regenerative medicine, one of the leading applications of genome engineering technology is the generation of immune evasive pluripotent stem cell-derived somatic cells for transplantation. In particular, as more functional and therapeutically relevant human pluripotent stem cell-derived islets (SCDI) are produced in many labs and studied in clinical trials, there is keen interest in studying the immunogenicity of these cells and modulating allogeneic and autoimmune immune responses for therapeutic benefit. Significant experimental work has already suggested that elimination of Human Leukocytes Antigen (HLA) expression and overexpression of immunomodulatory genes can impact survival of a variety of pluripotent stem cell-derived somatic cell types. Limited work published to date focuses on stem cell-derived islets and work in a number of labs is ongoing. Rapid progress is occurring in the genome editing of human pluripotent stem cells and their progeny focused on evading destruction by the immune system in transplantation models, and while much research is still needed, there is no doubt the combined technologies of genome editing and stem cell therapy will profoundly impact transplantation medicine in the future.

Saito-Diaz, K. and N. Zeltner (2019). "Induced pluripotent stem cells for disease modeling, cell therapy and drug discovery in genetic autonomic disorders: a review." Clin Auton Res **29**(4): 367-384.

The autonomic nervous system (ANS) regulates all organs in the body independent of consciousness, and is thus essential for maintaining homeostasis of the entire organism. Diseases of the ANS can arise due to environmental insults such as injury, toxins/drugs and infections or due to genetic lesions. Human studies and animal models have been instrumental to understanding connectivity and regulation of the ANS and its disorders. However, research into cellular pathologies and molecular mechanisms of ANS disorders has been hampered by the difficulties in accessing human patient-derived ANS cells in large numbers to conduct meaningful research, mainly because patient neurons cannot be easily biopsied and primary human neuronal cultures cannot be expanded.Human-induced pluripotent stem

cell (hiPSC) technology can elegantly bridge these issues, allowing unlimited access of patient-derived ANS cell types for cellular, molecular and biochemical analysis, facilitating the discovery of novel therapeutic targets, and eventually leading to drug discovery. Additionally, such cells may provide a source for cell replacement therapy to replenish lost or injured ANS tissue in patients.Here, we first review the anatomy and embryonic development of the ANS, as this knowledge is crucial for understanding disease modeling approaches. We then review the current advances in human stem cell technology for modeling diseases of the ANS, recent strides toward cell replacement therapy and drug discovery initiatives.

Samplaski, M. K., et al. (2014). "Genetic and Epigenetic Changes After Spermatogonial Stem Cell Culture and Transplantation." EJIFCC **25**(1): 27-41.

Men with testicular failure, either primary or secondary, have been shown to be interested in fertility preservation. Spermatogonial stem cell (SSC) transplantation is currently being investigated as a treatment for this. Currently this experimental technique consists of cryopreservation of a testicular biopsy prior to cancer treatment, followed by optional in vitro expansion of SSCs and auto transplantation after cancer treatment. This technique may restore the pool of SSCs resulting in restoration of spermatogenesis. While this technique has not been applied to humans due to its highly experimental nature and concerns of malignant contamination, animal studies have been successful. While the offspring obtained from SSCs appear to be healthy in rodent models, there is relatively little data on any genetic and epigenetic changes that occur in either the transplanted SSCs or offspring. In humans, male germ cells undergo unique and extensive chromatin and epigenetic remodeling soon after their destiny as a spermatocyte has been secured. Errors in this remodeling may cause altered genetic information to be transmitted to offspring, resulting in abnormalities. This is particularly pertinent for cancer patients as SSCs obtained from these men may have a predisposition for genetic instability even prior to starting gonadotoxic therapies. In this article, landmarks in the evolution of SSC transplantation are reviewed, along with presently known genetic, epigenetic, and imprinting abnormalities that may occur after in vitro propagation and transplantation.

Santurtun, A., et al. (2017). "Genetic DNA profile in urine and hair follicles from patients who have undergone allogeneic hematopoietic stem cell transplantation." Sci Justice **57**(5): 336-340.

Biological samples from patients who have undergone allogeneic hematopoietic stem cell transplantation (HSCT) constitute a challenge for individual identification. In this study we analyzed the genetic profiles (by the amplification of 15 autosomic STRs) of HSCT patients found in different types of samples (blood, hair and urine) that may be the source of DNA in civil or criminal forensic cases. Our results show that while in hair follicles the donor component was not detected in any patient, thus being a reliable source of biological material for forensic identification, mixed chimerism was detected in urine samples from all patient, and no correlation was found between the time elapsed from the transplant and the percentage of chimerism. These results certainly have practical implications if the urine is being considered as a source of DNA for identification purposes in HSTC patients. Moreover, taking into consideration that chimerism was found not only in patients with leukocyturia (given the hematopoietic origin of leukocytes, this was expected), but also in those without observable leukocytes in the sediment, we conclude that an alternative source or sources of donor DNA must be implicated.

Sauvageot, C., et al. (2005). "Distinct temporal genetic signatures of neurogenic and gliogenic cues in cortical stem cell cultures." J Neurobiol **62**(1): 121-133.

Cortical progenitor cells from rat embryos give rise to neurons or glia following exposure to platelet derived growth factor (PDGF) or ciliary neurotrophic factor (CNTF), respectively. Both growth factors impart their developmental cues quickly through a transcription-dependent mechanism. Do the alternate developmental responses to PDGF and CNTF reflect induction of qualitatively distinct genes? Alternatively, do the same genes respond to each growth factor, but with quantitatively distinct kinetics? Using differential library screening and custom cDNA microarrays we show that a common set of genes responds to either growth factor. However, quantitative differences in the onset and duration of gene induction equate to the expression of factor-specific gene signatures. Multitissue cluster analysis also reveals tissue-specific gene signatures that may play important roles in the developing brain.

Savage, S. A. and S. Chanock (2003). "Genetic variation and hematopoietic stem cell transplantation: expansion of the paradigm." Pediatr Transplant **7 Suppl 3**: 32-39.

Genetic variation has been the mainstay of hematopoietic stem cell transplantation since the first transplants were attempted. A significant expansion of genetic knowledge is under way, as the draft sequence of the human genome is annotated. Hematopoietic stem cell transplantation is a field that will greatly benefit from this new knowledge, but the manner in which it is

applied is daunting. Variation within key molecules related to hematopoietic stem cell transplant in combination with the current knowledge of human leukocyte antigen variation will serve to improve donor-recipient matches and clinical outcome.

Schilling, G., et al. (2008). "Impact of genetic abnormalities on survival after allogeneic hematopoietic stem cell transplantation in multiple myeloma." Leukemia **22**(6): 1250-1255.

We analyzed the prognostic impact of the most frequent genetic abnormalities detected by fluorescence in situ hybridization in 101 patients with multiple myeloma, who underwent allogeneic hematopoietic stem cell transplantation (HSCT) after melphalan/fludarabine-based reduced conditioning. The incidences of abnormalities in the present analysis were as follows: del(13q14) (61%), t(11;14)(q13;q32) (14%), t(4;14)(p16.3;q32) (19%), MYC-gain gains (8q24) (21%), del(17p13) (16%) and t(14;16)(q32;q23) (5%). None of the patients had t(6;14)(p25;q32). The overall complete remission (CR) rate was 50% with no differences between the genetic abnormalities except for patients with del(17p13) who achieved less CR (7 vs 56%; P=0.001). Univariate analysis revealed a higher relapse rate in patients aged >50 years (P=0.002), patients with del(13q14) (P=0.006) and patients with del(17p13) (P=0.003). In multivariate analyses, only del(13q14) (HR: 2.34, P=0.03) and del(17p13) (HR: 2.24; P=0.04) significantly influenced the incidence of relapse, whereas for event-free survival, only age (HR 2.8; P=0.01) and del(17p13) (HR: 2.05; P=0.03) retained their negative prognostic value. These data show that del(17p13) is a negative prognostic factor for achieving CR as well as for event-free survival after HSCT. Translocation t(4;14) might be overcome by allogeneic HSCT, which will have implication for risk-adapted strategies.

Schnabel, L. V., et al. (2012). "Genetic background affects induced pluripotent stem cell generation." Stem Cell Res Ther **3**(4): 30.

INTRODUCTION: The influence of genetic background on the ability to generate induced pluripotent stem cells (iPSCs) has the potential to impact future applications, but has yet to be examined in detail. The purpose of this study was to determine if genetic background affects the efficiency of generating iPSCs during early reprograming as well as the pluripotent stability of the iPSCs during later stages of reprograming. METHODS: Mouse embryonic fibroblasts (MEFs) were isolated from six strains of mice (NON/LtJ; C57BL/6J; DBA/2J; BALB/cJ; 129S1/SvlmJ; CAST/EiJ) that were selected based on genetic diversity and differences in ability to produce embryonic stem cell (ESC) lines. MEFs were reprogramed via doxycycline-inducible lentiviral transduction of murine Oct4, Klf4, Sox2, and c-Myc. Differences in efficiency to generate iPSCs were assessed by comparing the total number of colonies, the percentage of colonies positive for alkaline phosphatase staining and the percentage of cells positive for SSEA1. iPSC colonies were expanded to establish doxycycline-independent cell lines whose pluripotency was then evaluated via ability to form teratomas in NOD.CB17-Prkdcscid/J mice. Proliferation of non-transduced parent MEFs from each strain was also examined over ten days under conditions that simulated reprograming. RESULTS: NON/LtJ and CAST/EiJ strains were more efficient than other strains in generating iPSCs for all parameters measured and parent MEFs from these strains were more proliferative than those from other strains. Doxycycline-independent iPSC lines were established using standard conditions for all strains except BALB/cJ, which required a higher concentration (5x) of leukemia inhibitory factor (LIF). iPSCs from all strains were capable of producing teratomas in NOD.CB17-Prkdcscid/J mice. CONCLUSIONS: The results of this study suggest that genetic background does affect iPSC generation and pluripotent stability. In addition, our results demonstrate that strain differences in efficiency to generate iPSCs during the early stages of reprograming are correlated with those observed in proliferation of parent MEFs. These findings have important implications both for future iPSC applications as well as for future investigation into determining the genes responsible for reprograming efficiency and stability.

Sebastiano, V., et al. (2011). "In situ genetic correction of the sickle cell anemia mutation in human induced pluripotent stem cells using engineered zinc finger nucleases." Stem Cells **29**(11): 1717-1726.

The combination of induced pluripotent stem cell (iPSC) technology and targeted gene modification by homologous recombination (HR) represents a promising new approach to generate genetically corrected, patient-derived cells that could be used for autologous transplantation therapies. This strategy has several potential advantages over conventional gene therapy including eliminating the need for immunosuppression, avoiding the risk of insertional mutagenesis by therapeutic vectors, and maintaining expression of the corrected gene by endogenous control elements rather than a constitutive promoter. However, gene targeting in human pluripotent cells has remained challenging and inefficient. Recently, engineered zinc finger nucleases (ZFNs) have been shown to substantially increase HR frequencies in human iPSCs, raising the prospect of using this technology to correct disease causing mutations. Here, we describe the

generation of iPSC lines from sickle cell anemia patients and in situ correction of the disease causing mutation using three ZFN pairs made by the publicly available oligomerized pool engineering method (OPEN). Gene-corrected cells retained full pluripotency and a normal karyotype following removal of reprogramming factor and drug-resistance genes. By testing various conditions, we also demonstrated that HR events in human iPSCs can occur as far as 82 bps from a ZFN-induced break. Our approach delineates a roadmap for using ZFNs made by an open-source method to achieve efficient, transgene-free correction of monogenic disease mutations in patient-derived iPSCs. Our results provide an important proof of principle that ZFNs can be used to produce gene-corrected human iPSCs that could be used for therapeutic applications.

Shamim, Z., et al. (2006). "Genetic polymorphisms in the genes encoding human interleukin-7 receptor-alpha: prognostic significance in allogeneic stem cell transplantation." Bone Marrow Transplant **37**(5): 485-491.

Interleukin-7 (IL-7) is essential for T-cell development in the thymus and for the maintenance of peripheral T cells. IL-7 signals through IL-7R, that consists of the gammac-chain and an alpha-chain. Sequencing of IL-7Ralpha has revealed the existence of four single nucleotide polymorphisms (SNPs) (+510C/T, +1237 A/G, 2087T/C and +3110A/G), which all give rise to amino-acid substitutions. The aim of the present investigation was to evaluate the significance of IL-7Ralpha SNPs for the outcome in allogeneic stem cell transplantation (SCT). IL-7Ralpha polymorphisms were determined in 195 recipient and donor pairs from either matched sibling donors or matched unrelated donors (MUD). Genotyping of 173 normal controls was performed in parallel. In MUD transplants, the +1237 genotype of the donor was associated with survival after SCT, the mortality being highest and intermediate for the GG and AG genotypes, respectively (P = 0.023). This pattern was more pronounced with respect to treatment-related mortality (P = 0.003), while IL-7Ralpha genotypes were unrelated to the risk of relapse of leukaemia. The IL-7Ralpha +1237 genotype of the recipient and the genotypes of the other three polymorphisms, were not significantly associated with the outcome of SCT. These findings suggest that the IL-7Ralpha polymorphisms may be of importance for treatment-related mortality after SCT.

Skoczen, S., et al. (2016). "Genetic Background of Immune Complications after Allogeneic Hematopoietic Stem Cell Transplantation in Children." Stem Cells Int **2016**: 2626081.

Immune reactions are among the most serious complications observed after hematopoietic stem cell transplantation (HSCT) in children. Microarray technique allows for simultaneous assessment of expression of nearly all human genes. The objective of the study was to compare the whole genome expression in children before and after HSCT. A total of 33 children referred for HSCT were enrolled in the study. In 70% of the patients HSCT was performed for the treatment of neoplasms. Blood samples were obtained before HSCT and six months after the procedure. Subsequently, the whole genome expression was assessed in leukocytes using GeneChip Human Gene 1.0 ST microarray. The analysis of genomic profiles before and after HSCT revealed altered expression of 124 genes. Pathway enrichment analysis revealed upregulation of five pathways after HSCT: allograft rejection, graft-versus-host disease, type I diabetes mellitus, autoimmune thyroid disease, and viral myocarditis. The activation of those pathways seems to be related to immune reactions commonly observed after HSCT. Our results contribute to better understanding of the genomic background of the immunologic complications of HSCT.

Socie, G., et al. (2001). "Both genetic and clinical factors predict the development of graft-versus-host disease after allogeneic hematopoietic stem cell transplantation." Transplantation **72**(4): 699-706.

BACKGROUND: Graft-versus-host disease is the main complication of hematopoietic stem cell transplantation. Recently, pro- and anti-inflammatory cytokines and mismatches of minor histocompatibility antigens between HLA-identical sibling donor/recipient pairs have been implicated in the development of acute graft-versus-host disease. It is not known, however, whether these factors are independent of other clinically recognized risk factors such as age and disease stage. METHODS: In this study, we searched for risk factors of acute graft-versus-host disease using multivariate Cox regression analysis in 100 consecutive patients who underwent allogeneic stem cell transplantation from an HLA-identical sibling donor. Eight polymorphisms from five different cytokine genes were studied (tumor necrosis factor alpha, tumor necrosis factor beta, interleukin (IL) 6, IL-10, and interferon gamma). Mismatches for the minor histocompatibility antigen HA-1 were searched in HLA-A*0201 individuals. In addition to these new risk factors, patient, donor, disease, and transplant risk factors were analyzed by multivariate analysis using the Cox proportional hazards model. RESULTS: Acute graft-versus-host disease was independently associated with IL-10 gene polymorphisms both from the recipient (relative risk=7.9, P<0.0001) and the donor (relative risk=3.5, P=0.02), a donor's positive serology

for cytomegalovirus, and HA-1 mismatches in HLA-A*0201 individuals (relative risk=2.8, P=0.05). Chronic graft-versus-host disease was independently associated with IL-6 gene polymorphism from the recipient (relative risk=4.2, P=0.02), older age (relative risk=2.5, P=0.0009), and previous acute graft-versus-host disease (relative risk=9.7, P=0.003). CONCLUSION: In addition to previously described clinical risk factors, genetic risk factors are independently associated with the risk of developing graft-versus-host disease and may, thus, be considered for the selection of the donor.

Song, H. R., et al. (2011). "Association of a common genetic variant in prostate stem-cell antigen with gastric cancer susceptibility in a Korean population." Mol Carcinog **50**(11): 871-875.

A recent genome wide association study (GWAS) indentified a significant association between rs2294008 (C > T) polymorphism in prostate stem-cell antigen (PSCA) and increased risk of gastric cancer in Japanese and Korean populations. The aim of this study was to determine whether rs2294008 polymorphism is associated with risk of gastric cancer in a Korean population. We conducted a large-scale case-control study of 3,245 gastric cancer patients and 1,700 controls. The frequencies of the CC, CT, and TT genotypes of rs2294008 polymorphism were 17.8%, 49.9%, and 32.3% in the gastric cancer patients; and 24.4%, 48.1%, and 27.5% in the controls, respectively. We found that the CT and TT genotypes were associated with a significantly increased risk of gastric cancer (OR(CT) = 1.50, 95% confidence intervals, 95% CI: 1.28-1.76; OR(TT) = 1.71, 95% CI: 1.43-2.04), compared with the CC genotype. Further, stratified by tumor location and histological type, the effect of the rs2294008 T allele was larger in cardia (OR(TT) = 2.62, 95% CI = 1.42-4.85) than non-cardia (OR(TT) = 1.67, 95% CI = 1.40-2.00), in diffuse-type (OR(TT) = 2.00, 95% CI: 1.55-2.59) than in intestinal-type (OR(TT) = 1.51, 95% CI: 1.22-1.86). Our study showed that rs2294008 in the PSCA gene was associated with increased risks of gastric cancer in a Korean population, suggests that rs2294008 might play an important role in gastric carcinogenesis.

Song, J. H. T., et al. (2021). "Genetic studies of human-chimpanzee divergence using stem cell fusions." Proc Natl Acad Sci U S A **118**(51).

Complete genome sequencing has identified millions of DNA changes that differ between humans and chimpanzees. Although a subset of these changes likely underlies important phenotypic differences between humans and chimpanzees, it is currently difficult to distinguish causal from incidental changes and to map specific phenotypes to particular genome locations. To facilitate further genetic study of human-chimpanzee divergence, we have generated human and chimpanzee autotetraploids and allotetraploids by fusing induced pluripotent stem cells (iPSCs) of each species. The resulting tetraploid iPSCs can be stably maintained and retain the ability to differentiate along ectoderm, mesoderm, and endoderm lineages. RNA sequencing identifies thousands of genes whose expression differs between humans and chimpanzees when assessed in single-species diploid or autotetraploid iPSCs. Analysis of gene expression patterns in interspecific allotetraploid iPSCs shows that human-chimpanzee expression differences arise from substantial contributions of both cis-acting changes linked to the genes themselves and trans-acting changes elsewhere in the genome. To enable further genetic mapping of species differences, we tested chemical treatments for stimulating genome-wide mitotic recombination between human and chimpanzee chromosomes, and CRISPR methods for inducing species-specific changes on particular chromosomes in allotetraploid cells. We successfully generated derivative cells with nested deletions or interspecific recombination on the X chromosome. These studies confirm an important role for the X chromosome in trans regulation of expression differences between species and illustrate the potential of this system for more detailed cis and trans mapping of the molecular basis of human and chimpanzee evolution.

Soni, S. (2007). "Allogeneic stem cell transplantation for genetic disorders." J Ky Med Assoc **105**(1): 12-16.

Thalassemia is the only genetic condition for which stem cell transplantation (SCT) has been considered the standard of care for achieving cure. With the advances in SCT, many new disorders have been added to the list of genetic diseases amenable to transplantation, especially the lysosomal storage disorders. The timing of the transplant is crucial in these conditions as the results are better when it is done before the onset of severe neuro-cognitive damage. Early diagnosis and referral, expedited donor searches, and newer techniques of SCT will improve the future results. This is especially important in the current era of expanded newborn screens now available in many states, including Kentucky.

St Clair, D. and M. Johnstone (2018). "Using mouse transgenic and human stem cell technologies to model genetic mutations associated with schizophrenia and autism." Philos Trans R Soc Lond B Biol Sci **373**(1742).

Solid progress has occurred over the last decade in our understanding of the molecular genetic basis of neurodevelopmental disorders, and of schizophrenia and autism in particular. Although the

genetic architecture of both disorders is far more complex than previously imagined, many key loci have at last been identified. This has allowed in vivo and in vitro technologies to be refined to model specific high-penetrant genetic loci involved in both disorders. Using the DISC1/NDE1 and CYFIP1/EIF4E loci as exemplars, we explore the opportunities and challenges of using animal models and human-induced pluripotent stem cell technologies to further understand/treat and potentially reverse the worst consequences of these debilitating disorders.This article is part of a discussion meeting issue 'Of mice and mental health: facilitating dialogue between basic and clinical neuroscientists'.

Sterlini, B., et al. (2020). "Progress of Induced Pluripotent Stem Cell Technologies to Understand Genetic Epilepsy." Int J Mol Sci **21**(2).

The study of the pathomechanisms by which gene mutations lead to neurological diseases has benefit from several cellular and animal models. Recently, induced Pluripotent Stem Cell (iPSC) technologies have made possible the access to human neurons to study nervous system disease-related mechanisms, and are at the forefront of the research into neurological diseases. In this review, we will focalize upon genetic epilepsy, and summarize the most recent studies in which iPSC-based technologies were used to gain insight on the molecular bases of epilepsies. Moreover, we discuss the latest advancements in epilepsy cell modeling. At the two dimensional (2D) level, single-cell models of iPSC-derived neurons lead to a mature neuronal phenotype, and now allow a reliable investigation of synaptic transmission and plasticity. In addition, functional characterization of cerebral organoids enlightens neuronal network dynamics in a three-dimensional (3D) structure. Finally, we discuss the use of iPSCs as the cutting-edge technology for cell therapy in epilepsy.

Steventon-Jones, V., et al. (2022). "Single Nucleotide Polymorphism (SNP) Arrays and Their Sensitivity for Detection of Genetic Changes in Human Pluripotent Stem Cell Cultures." Curr Protoc **2**(11): e606.

Human pluripotent stem cells (hPSCs) can be grown in culture indefinitely, making them a valuable tool for use in basic biology, disease modeling, and regenerative medicine. However, over prolonged periods in culture, hPSCs tend to acquire genomic aberrations that confer growth advantages, similar to those seen in some cancers. Monitoring the genomic stability of cultured hPSCs is critical to ensuring their efficacy and safety as a therapeutic tool. Most commonly employed methods for monitoring of hPSC genomes are cytogenetic methods, such as G-banding. Nonetheless, such methods have limited resolution and sensitivity for detecting mosaicism. Single nucleotide polymorphism (SNP) array platforms are a potential alternative that could improve detection of abnormalities. Here, we outline protocols for SNP array whole-genome screening of hPSCs. Moreover, we detail the procedure for assessing the SNP array's sensitivity in detecting low-level mosaic copy-number changes. We show that mosaicism can be confidently identified in samples only once they contain 20% variants, although samples containing 10% variants typically display enough variation to warrant further investigation and confirmation, for example by using a more sensitive targeted method. Finally, we highlight the advantages and limitations of SNP arrays, including a cost comparison of SNP arrays versus other commonly employed methods for detection of genetic changes in hPSC cultures. (c) 2022 The Authors. Current Protocols published by Wiley Periodicals LLC. Basic Protocol 1: DNA sample preparation for SNP arrays Basic Protocol 2: SNP array hybridization, washing, and scanning Basic Protocol 3: SNP array data analysis Support Protocol: Assessment of SNP array sensitivity for detection of mosaicism.

Steward, C. G. and A. Jarisch (2005). "Haemopoietic stem cell transplantation for genetic disorders." Arch Dis Child **90**(12): 1259-1263.

Stem cell transplantation (SCT) is used to cure or greatly ameliorate a wide variety of genetic diseases, ranging from inherent defects of haemopoietic cell production or function to metabolic diseases mostly affecting solid organs. It ranks as one of the most remarkable therapeutic advances of the past 40 years. Despite rapid technological improvements, however, there are still many short term risks and potential long term toxicities. As a consequence, the rapid emergence of alternative therapies (including new drugs, enzyme and gene therapies), necessitate constant re-evaluation of the risk/benefit ratio for each disease and hence the appropriateness of SCT. This review describes the major aspects of the transplant process, indications for transplantation, outcome statistics, and areas where alternative therapies are becoming available.

Sukoyan, M. A., et al. (2002). "Establishment of new murine embryonic stem cell lines for the generation of mouse models of human genetic diseases." Braz J Med Biol Res **35**(5): 535-542.

Embryonic stem cells are totipotent cells derived from the inner cell mass of blastocysts. Recently, the development of appropriate culture conditions for the differentiation of these cells into specific cell types has permitted their use as potential therapeutic agents for several diseases. In addition, manipulation of their genome in vitro allows the creation of animal models of human genetic diseases and for the study of gene function in vivo. We report

the establishment of new lines of murine embryonic stem cells from preimplantation stage embryos of 129/Sv mice. Most of these cells had a normal karyotype and an XY sex chromosome composition. The pluripotent properties of the cell lines obtained were analyzed on the basis of their alkaline phosphatase activity and their capacity to form complex embryoid bodies with rhythmically contracting cardiomyocytes. Two lines, USP-1 and USP-3, with the best in vitro characteristics of pluripotency were used in chimera-generating experiments. The capacity to contribute to the germ line was demonstrated by the USP-1 cell line. This cell line is currently being used to generate mouse models of human diseases.

Swaminathan, V. V., et al. (2022). "Treosulfan-Based Conditioning in Matched Family, Unrelated and Haploidentical Hematopoietic Stem Cell Transplantation for Genetic Hemophagocytic Lymphohistiocytosis: Experience and Outcomes over 10 Years from India." Indian J Hematol Blood Transfus **38**(1): 84-91.

We aimed to analyze data in children with primary hemophagocytic lymphohistiocytosis (HLH) who underwent hematopoietic stem cell transplantation (HSCT). We performed a retrospective study where children up to 18 years, with primary HLH and who underwent HSCT from January 2011 to December 2019, were included. Twenty-five children with genetic HLH underwent HSCT, including variants (Griscelli syndrome (GS2) 7, Chediak-Higashi syndrome (CHS) 2, XIAP mutation 2). Donors were matched family 8 (32%), umbilical cord blood unit 3 (12%), matched unrelated 2 (8%), haploidentical HSCT 12 (48%), (TCR alpha/beta depletion 2 and post-transplant cyclophosphamide 10). With treosulfan-based conditioning, engraftment was achieved in 23/25 (92%) transplants (100% in haplo-HSCT), with sustained complete chimerism in 87%. Disease-free survival was noted in 2/3 children with stable mixed chimerism. Graft-versus-host disease (GVHD) of grade I/II was noted in 6 (24%), grade III in 3 (13%); chronic limited skin GVHD in 2 (12%) children. Overall survival was 72% (87.5% in matched donor, 66.7% in the haplo-HSCT), 71% in GS2, 50% in CHS, 100% in XIAP. HSCT is curative in primary HLH with acceptable disease-free survival with mixed chimerism. Haplo-HSCT is a viable option for those without matched family or unrelated donors.

Tadokoro, M., et al. (2010). "[Genetic basis for skeletal disease. Stem cell therapy for genetic bone disorders]." Clin Calcium **20**(8): 1228-1235.

Mesenchymal stem cells (MSCs) can show osteogenic differentiation capability when implanted in vivo , as well as cultured in vitro; therefore we attempted to use allogeneic MSCs for a patient with hypophosphatasia, which is caused by mutations in tissue non-specific alkaline phosphatase (TNSALP) gene. Donor MSCs were obtained by culture expansion of fresh marrow from the patient's father. Some of the MSCs were further cultured under osteogenic conditions on a culture dish or porous hydroxyapatite ceramics, resulting in cultured osteoblasts and osteogenic constructs, respectively. After traditional bone marrow transplantation, The donor MSCs and osteoblasts were injected into the patient and the constructs were implanted subcutaneously or intraosseous lesions. The patient's respiratory condition improved and donor cells were detected in newly formed bone tissue. These findings showed the importance of allogeneic MSC transplantation for the hypophosphatasia patient.

Taei, A., et al. (2010). "Derivation of new human embryonic stem cell lines from preimplantation genetic screening and diagnosis-analyzed embryos." In Vitro Cell Dev Biol Anim **46**(3-4): 395-402.

In this study, we focused on the derivation of human embryonic stem cell (hESC) from preimplantation genetic screening (PGS)-analyzed and preimplantation genetic diagnosis (PGD)-analyzed embryos. Out of 62 fresh PGD/PGS-analyzed embryos, 22 embryos reached the blastocyst stage. From 12 outgrowth blastocysts, we derived four hESC lines onto a feeder layer. Surprisingly, karyotype analysis showed that hESC lines derived from aneuploid embryos had diploid female karyotype. One hESC line was found to carry a balanced Robertsonian translocation. All the cell lines showed hESC markers and had the pluripotent ability to differentiate into derivatives of the three embryonic germ layers. The established lines had clonal propagation with 22-31% efficiency in the presence of ROCK inhibitor. These results further indicate that hESC lines can be derived from PGD/PGS-analyzed embryos that are destined to be discarded and can serve as an alternative source for normal euploid lines.

Tagami, M., et al. (2003). "Genetic and ultrastructural demonstration of strong reversibility in human mesenchymal stem cell." Cell Tissue Res **312**(1): 31-40.

We examined human bone marrow mesenchymal stem cells by applying real-time quantitative polymerase chain reaction (PCR) (RT-PCR) technology and electron-microscopic techniques. Our RT-PCR demonstrated that the values of peroxisome proliferation-activated receptor gamma2 (PPARgamma2) and lipoprotein lipase (LPL) mRNA dramatically increased according to adipogenic stimulation. The expressions of both PPARgamma2

and LPL mRNA were significantly reduced (P<0.01) and almost disappeared after stimulation had ceased. The expressions of both genes, however, increased again by stimulation even though the cells were in a dedifferentiated state for a month. In the ultrastructural study, over 80% of the cells proceeded into morphologically well-developed adipocytes at the 12th day of induction/maintenance which were packed with lipid droplets and clusters. In the next process these lipid products were excreted from the cell bodies and the peripheral small parts containing numerous droplets were torn from the greater parts, which stuck tightly to each other and adhered to culture dishes. Adipocytes were not detected in the culture media during the final stage. The total cell number was equal to and over 90% of the cells dedifferentiated into fibroblast-like stem cells during the final maintenance period of 1 month. Furthermore the dedifferentiated cells quickly differentiated again into adipocytes by stimulation even if they were quiescent for 1 month. Thus we conclude that mesenchymal stem cells have strong reversibility from both the genetic and morphological points of view.

Takashima, S. and V. Hartenstein (2012). "Genetic control of intestinal stem cell specification and development: a comparative view." Stem Cell Rev Rep **8**(2): 597-608.

Stem cells of the adult vertebrate intestine (ISCs) are responsible for the continuous replacement of intestinal cells, but also serve as site of origin of intestinal neoplasms. The interaction between multiple signaling pathways, including Wnt/Wg, Shh/Hh, BMP, and Notch, orchestrate mitosis, motility, and differentiation of ISCs. Many fundamental questions of how these pathways carry out their function remain unanswered. One approach to gain more insight is to look at the development of stem cells, to analyze the "programming" process which these cells undergo as they emerge from the large populations of embryonic progenitors. This review intends to summarize pertinent data on vertebrate intestinal stem cell biology, to then take a closer look at recent studies of intestinal stem cell development in Drosophila. Here, stem cell pools and their niche environment consist of relatively small numbers of cells, and questions concerning the pattern of cell division, niche-stem cell contacts, or differentiation can be addressed at the single cell level. Likewise, it is possible to analyze the emergence of stem cells during development more easily than in vertebrate systems: where in the embryo do stem cells arise, what structures in their environment do they interact with, and what signaling pathways are active sequentially as a result of these interactions. Given the high degree of conservation among genetic mechanisms controlling stem cell behavior in all animals, findings in Drosophila will provide answers that inform research in the vertebrate stem cell field.

Takayanagi, S., et al. (2006). "Genetic marking of hematopoietic stem and endothelial cells: identification of the Tmtsp gene encoding a novel cell surface protein with the thrombospondin-1 domain." Blood **107**(11): 4317-4325.

Using an in silico database search, we identified a novel gene encoding a cell surface molecule with a thrombospondin domain, and designated the gene as transmembrane molecule with thrombospondin module (Tmtsp). Expression profiling of Tmtsp using specific monoclonal antibodies and Venus, a variant of yellow fluorescent protein knock-in mice in the Tmtsp locus, demonstrated its specific expression in hematopoietic and endothelial cells. In lymphohematopoietic cells, Tmtsp was predominantly expressed in hematopoietic stem and progenitor cells, and the level of expression gradually declined as the cells differentiated. Venus expression faithfully traced the expression of Tmtsp, and the level of Venus expression correlated well to the in vitro hematopoietic activity as well as the in vivo bone marrow repopulating capacity. Notably, Venus expression marked the development of definitive hematopoiesis in both the extraembryonic yolk sac and the intraembryonic aorta-gonad-mesonephros (AGM) region and, in combination with CD41, strikingly promoted the enrichment of developing progenitors in the CD41(+)Venus(high) fraction at embryonic day 10.5 (E10.5). In this context, Tmtsp is a novel marker gene for primitive hematopoietic cells and endothelial cells, and Tmtsp(Venus/)(+) mice would serve as a valuable mouse model for the analysis of both embryonic and adult hematopoiesis, as well as for vascular biology.

Tang, Z. H., et al. (2016). "Genetic Correction of Induced Pluripotent Stem Cells From a Deaf Patient With MYO7A Mutation Results in Morphologic and Functional Recovery of the Derived Hair Cell-Like Cells." Stem Cells Transl Med **5**(5): 561-571.

The genetic correction of induced pluripotent stem cells (iPSCs) induced from somatic cells of patients with sensorineural hearing loss (caused by hereditary factors) is a promising method for its treatment. The correction of gene mutations in iPSCs could restore the normal function of cells and provide a rich source of cells for transplantation. In the present study, iPSCs were generated from a deaf patient with compound heterozygous MYO7A mutations (c.1184G>A and c.4118C>T; P-iPSCs), the asymptomatic father of the patient (MYO7A c.1184G>A mutation; CF-iPSCs), and a normal donor (MYO7A(WT/WT); C-iPSCs). One of MYO7A

mutation sites (c.4118C>T) in the P-iPSCs was corrected using CRISPR/Cas9. The corrected iPSCs (CP-iPSCs) retained cell pluripotency and normal karyotypes. Hair cell-like cells induced from CP-iPSCs showed restored organization of stereocilia-like protrusions; moreover, the electrophysiological function of these cells was similar to that of cells induced from C-iPSCs and CF-iPSCs. These results might facilitate the development of iPSC-based gene therapy for genetic disorders. SIGNIFICANCE: Induced pluripotent stem cells (iPSCs) were generated from a deaf patient with compound heterozygous MYO7A mutations (c.1184G>A and c.4118C>T). One of the MYO7A mutation sites (c.4118C>T) in the iPSCs was corrected using CRISPR/Cas9. The genetic correction of MYO7A mutation resulted in morphologic and functional recovery of hair cell-like cells derived from iPSCs. These findings confirm the hypothesis that MYO7A plays an important role in the assembly of stereocilia into stereociliary bundles. Thus, the present study might provide further insight into the pathogenesis of sensorineural hearing loss and facilitate the development of therapeutic strategies against monogenic disease through the genetic repair of patient-specific iPSCs.

ten Brink, M. H., et al. (2013). "Effect of genetic variants GSTA1 and CYP39A1 and age on busulfan clearance in pediatric patients undergoing hematopoietic stem cell transplantation." Pharmacogenomics **14**(14): 1683-1690.

BACKGROUND: Busulfan is used in preparative regimens prior to stem cell transplantation in pediatric patients. There is significant interpatient variability in busulfan pharmacokinetics (PK) and exposure is related to outcome. To date, only polymorphisms in genes encoding for glutathione-S-transferases were studied, but could only explain a small portion of the variability in PK. AIM: To investigate the effect of seven genetic markers on busulfan clearance and the effect of ontogenesis on these genetic variants in a pediatric population. MATERIALS & METHODS: In an earlier study of our group seven genetic markers in GSTA1, CYP2C19, CYP39A1, ABCB4, SLC22A4 and SLC7A8 were associated with busulfan clearance in adult patients. Eighty four pediatric patients were genotyped for these markers and genotype was associated with busulfan clearance. RESULTS & CONCLUSION: GSTA1 and CYP39A1 were found to be associated with busulfan clearance. When combined, the two haplotypes explained 17% of the variability in busulfan clearance. Furthermore, the effect of GSTA1 haplotype on clearance was dependent on age.

Tiewsiri, K., et al. (2020). "The First Asian, Single-Center Experience of Blastocyst Preimplantation Genetic Diagnosis with HLA Matching in Thailand for the Prevention of Thalassemia and Subsequent Curative Hematopoietic Stem Cell Transplantation of Twelve Affected Siblings." Biomed Res Int **2020**: 5292090.

RESULTS: In 221 cycles from 138 patients (104 cycles requiring HLA matching), 90.5% had embryo(s) biopsied for genetic testing. There were 119 embryo transfers for thalassemia (76) and thalassemia-HLA cases (43), respectively, resulting in overall clinical pregnancy rates of 54.6%, implantation rates of 45.7%, and live birth rates of 44.1%. Our dataset included fifteen PGD-HLA live births with successful HSCT in twelve affected siblings, 67% using umbilical cord blood stem cells (UCBSC) as the only SC source. CONCLUSIONS: We report favorable thalassemia PGD and PGD-HLA laboratory and clinical outcomes from a single center. The ultimate success in PGD-HLA is of course the cure of a thalassemia-affected sibling by HSCT. Our PGD-HLA HSCT series is the first and largest performed entirely in Asia with twelve successful and two pending cures and predominant UCBSC use.

Tonti, G. A. and F. Mannello (2008). "From bone marrow to therapeutic applications: different behaviour and genetic/epigenetic stability during mesenchymal stem cell expansion in autologous and foetal bovine sera?" Int J Dev Biol **52**(8): 1023-1032.

Bone marrow-derived mesenchymal stem cells are a multipotent adult cellular population endowed with broad differentiation potential. Their regeneration capability, ease to undergo gene modifications, and immuno-suppressive capacity makes them optimal tools for tissue engineering, gene- and immuno-therapy. Due to the ever-increasing number of studies on the clinical applications of mesenchymal stem cells in regenerative medicine, these cells have become attractive targets in clinical transplantation. However, the identification and definition of mesenchymal stem cell culture media for their clinical application in cell therapy is currently a matter of strong discussion. Up to now, clinical studies have been conducted with mesenchymal stem cells cultured in foetal calf serum, and the chance of contamination or immunological reaction towards xenogeneic compounds must be taken into consideration. On the other hand, a serum-free medium without the addition of growth factors is not able to expand these cells in vitro; so the evaluation of which is best, among foetal calf serum, human serum (whether autologous or allogeneic) and platelet-rich plasma, is a hot topic urgently needing further research efforts. The need for the establishment of standardized

protocols for mesenchymal stem cell preparations, in order not to interfere with their self-renewal and differentiation processes, assuring durable engraftment and long-term therapeutic effects, is evidently crucial. Therefore, the search for optimal culture conditions for the effective clinical-scale production of vast numbers of mesenchymal stem cells for cellular therapy is of paramount importance and the need for a robust passage from basic to translational research is fundamental.

Tropel, P., et al. (2010). "High-efficiency derivation of human embryonic stem cell lines following pre-implantation genetic diagnosis." In Vitro Cell Dev Biol Anim **46**(3-4): 376-385.

Pre-implantation genetic diagnosis allows the characterisation of embryos that carry a gene responsible for a severe monogenic disease and to transfer to the mother's uterus only the unaffected one(s). The genetically affected embryos can be used to establish human embryonic stem cell (hESC) lines. We are currently establishing a cell bank of ESC lines carrying specific disease-causing mutant genes. These cell lines are available to the scientific community. For this purpose, we have designed a technique that requires only minimal manipulation of the embryos. At the blastocyst stage, we just removed the zona pellucida before seeding the embryo as a whole on a layer of feeder cells. This approach gave a good success rate (>20%), whatever the quality of the embryos, and allowed us to derive 11 new hESC lines, representing seven different pathologies. Full phenotypic validation of the cell lines according to ISCI guidelines confirmed their pluripotent nature, as they were positive for hESC markers and able to differentiate in vitro in all three germ layers derivatives. Nine out of 11 stem cell lines had normal karyotypes. Our results indicate that inner cell mass isolation is not mandatory for hESC derivation and that minimal manipulation of embryos can lead to high success rate.

Tsai, M., et al. (2002). "Mast cells derived from embryonic stem cells: a model system for studying the effects of genetic manipulations on mast cell development, phenotype, and function in vitro and in vivo." Int J Hematol **75**(4): 345-349.

Large quantities of highly enriched populations of mast cells can be generated from mouse embryonic stem (ES) cells using an in vitro differentiation system. These embryonic stem cell-derived mast cells (ESMCs) exhibit many similarities to mouse bone marrow-derived cultured mast cells (BMCMCs), including the abilities to survive and to orchestrate immunologically specific immunoglobulin E (IgE)-dependent reactions in vivo after transplantation into genetically mast cell-deficient KitW/KitW-v mice. Coupled with the current spectrum of techniques for genetically manipulating ES cells, ESMCs represent a unique model system to analyze the effects of specific alterations in gene structure, expression, or function, including embryonic lethal mutations, on mast cell development, phenotype, and function in vitro and in vivo.

Tsamadou, C., et al. (2022). "Donor genetic determinant of thymopoiesis rs2204985 impacts clinical outcome after single HLA mismatched hematopoietic stem cell transplantation." Bone Marrow Transplant **57**(10): 1539-1547.

A common genetic variant within the T cell receptor alpha (TCRA)-T cell receptor delta (TCRD) locus (rs2204985) has been recently found to associate with thymic function. Aim of this study was to investigate the potential impact of donor rs2204985 genotype on patient's outcome after unrelated hematopoietic stem cell transplantation (uHSCT). 2016 adult patients were retrospectively analyzed. rs2204985 genotyping was performed by next generation sequencing, $p < 0.05$ was considered significant and donor rs2204985 GG/AG genotypes were set as reference vs. the AA genotype. Multivariate analysis of the combined cohort regarding the impact of donor's rs2204985 genotype indicated different risk estimates in 10/10 and 9/10 HLA matched transplantations. A subanalysis on account of HLA incompatibility revealed that donor AA genotype in single HLA mismatched cases ($n = 624$) associated with significantly inferior overall- (HR: 1.48, $p = 0.003$) and disease-free survival (HR: 1.50, $p = 0.001$). This effect was driven by a combined higher risk of relapse incidence (HR: 1.40, $p = 0.026$) and non-relapse mortality (HR: 1.38, $p = 0.042$). This is the first study to explore the role of rs2204985 in a clinical uHSCT setting. Our data suggest that donor rs2204985 AA genotype in combination with single HLA mismatches may adversely impact post-HSCT outcome and should thus be avoided.

Tsao, J. L., et al. (1997). "Intestinal stem cell division and genetic diversity. A computer and experimental analysis." Am J Pathol **151**(2): 573-579.

Somatic mutations are expected to arise with age. This process is accelerated in mice lacking the DNA mismatch repair gene Pms2. The distributions of microsatellite alleles present in small patches of normal Pms2 -/- intestines revealed a general increase in genetic diversity or the number of mutations with age. However, the patterns were complex with different distributions and variances present within a single mouse. Computer simulations indicate that the experimental data are consistent with mutation rates between 0.0020 and 0.0025 mutations per division,

nonrandom cell death, and an effective population size of 20 or fewer cells. Small numbers of cells exacerbate the random accumulation of mutations expected of a stochastic mutation process. The computer simulations and experimental data are consistent with known patterns of intestinal development and renewal by small numbers of stem cells and demonstrate relatively high mutation rates in histologically normal epithelium. These findings provide background for the analysis of microsatellite mutations in normal and tumor tissue lacking mismatch repair and further support the hypothesis that microsatellite loci can function as molecular tumor clocks.

Turetsky, T., et al. (2008). "Laser-assisted derivation of human embryonic stem cell lines from IVF embryos after preimplantation genetic diagnosis." Hum Reprod **23**(1): 46-53.

BACKGROUND: Human embryonic stem cells (hESCs) suitable for future transplantation therapy should preferably be developed in an animal-free system. Our objective was to develop a laser-based system for the isolation of the inner cell mass (ICM) that can develop into hESC lines, thereby circumventing immunosurgery that utilizes animal products. METHODS: Hatching was assisted by micromanipulation techniques through a laser-drilled orifice in the zona pellucida of 13 abnormal preimplantation genetic diagnosed blastocysts. ICMs were dissected from the trophectoderm by a laser beam and plated on feeders to derive hESC lines. RESULTS: eight ICMs were isolated from nine hatched blastocysts and gave rise to three hESC lines affected by myotonic dystrophy type 1, hemophilia A and a carrier of cystic fibrosis 405 + 1G > A mutation. Five blastocysts that collapsed during assisted hatching or ICM dissection were plated whole, giving rise to an additional line affected by fragile X. All cell lines expressed markers of pluripotent stem cells and differentiated in vitro and in vivo into the three germ layers. CONCLUSIONS: These hESC lines can serve as an important model of the genetic disorders that they carry. Laser-assisted isolation of the ICMs may be applied for the derivation of new hESC lines in a xeno-free system for future clinical applications.

Turpeinen, H., et al. (2009). "Genetic similarity of chromosome 6 between patients receiving hematopoietic stem cell transplantation and HLA matched sibling donors." Haematologica **94**(4): 528-535.

BACKGROUND: Matching for HLA genes located on chromosome 6 is required in hematopoietic stem cell transplantation to reduce the incidence of graft-versus-host disease. However, a considerable proportion of patients still suffer from it, obviously due to genetic differences outside the HLA gene region. DESIGN AND METHODS: We studied the similarity of almost 4,000 single nucleotide polymorphisms on chromosome 6 between patients receiving hematopoietic stem cell transplantation and their HLA-matched sibling donors. RESULTS: We observed that as a result of routine HLA matching the siblings in fact shared surprisingly long chromosomal fragments with similar single nucleotide polymorphism genotypes--from 11.65 Mb to 134.66 Mb. The number of genes mapped on these shared fragments varied from 402 to 1,302. Considering the whole chromosome 6, the HLA-matched siblings were apparently identical for 65.2-97.8% of the single nucleotide polymorphisms. CONCLUSIONS: Potentially, genes similar in some transplantation pairs while different in others might have a significant role in determining the outcome after hematopoietic stem cell transplantation.

Tzatsos, A. and N. Bardeesy (2008). "Ink4a/Arf regulation by let-7b and Hmga2: a genetic pathway governing stem cell aging." Cell Stem Cell **3**(5): 469-470.

Stem cell self-renewal capacity declines with age. In a recent issue of Cell, Nishino and colleagues (2008) show that Hmga2 maintains neural stem cell (NSC) function in young mice through repression of the Ink4a/Arf locus; in contrast, during aging, elevated let-7b blocks Hmga2 and contributes to declining NSC function.

Ugai, T., et al. (2017). "Role of Genetic Polymorphism of ALDH2 in Hematopoietic Stem Cell Transplantation." Biol Blood Marrow Transplant **23**(8): 1374-1380.

Aldehyde dehydrogenase 2 (ALDH2) is involved in critically important biological processes, such as the metabolism of aldehydes and aldehyde-induced genotoxicity in hematopoietic stem cells. Given its role in these biological processes, we hypothesized that a functional ALDH2 polymorphism could affect transplantation outcomes after hematopoietic stem cell transplantation. Here, we analyzed the Japanese national registry data for 409 patients who underwent allogeneic bone marrow transplantation (BMT) from HLA-matched unrelated donors. To evaluate the impact of the recipient and donor ALDH2 polymorphism on transplantation outcomes, we estimated hazard ratios (HRs) and 95% confidence intervals (CIs) adjusted for potential confounders. The recipient ALDH2 Lys/Lys genotype was significantly associated with higher transplantation-related mortality (TRM), with an HR relative to Glu/Glu genotype of 2.45 (95% CI, 1.22 to 4.90). The recipient Lys/Lys genotype also tended to be associated with delayed platelet engraftment (HR, .66;

95% CI, .43 to 1.03). In conclusion, we observed increased TRM among recipients with the ALDH2 Lys/Lys genotype in HLA fully matched BMT. We also observed a suggestive association with delayed platelet engraftment, which warrants further examination. These results may suggest that the recipient ALDH2 genotype affects the metabolism of endogenous aldehydes, leading to a significant impact on transplantation outcomes.

Ule, G. and M. Bauer (1983). "Dystrophy of Purkinje cell dendrites and vacuolar myelinopathy of the upper brain stem in young Turkish twin girls: secondary encephalopathy or genetic disorder?" Clin Neuropathol **2**(1): 23-30.

Two Turkish twin sisters exhibited alteration of muscle tonus and coordination together with a disturbance of eye motility and a tendency toward central tonoclonic seizures at the age of 2 months. The two children died at the ages of 8 1/2 and 12 months. Microscopic findings in the cerebellum of one of the cases showed cactuslike thickenings of the Purkinje cell dendrites and grumose degeneration in the nucleus dentatus. In addition, vacuolar myelinopathy of the myelinated fiber systems of the upper brain stem was observed. A hereditary disorder of the central nervous system is discussed.

Upadhaya, S., et al. (2018). "New genetic tools for the in vivo study of hematopoietic stem cell function." Exp Hematol **61**: 26-35.

The production of blood cells is dependent on the activity of a rare stem cell population that normally resides in the bone marrow (BM) of the organism. These hematopoietic stem cells (HSCs) have the ability to both self-renew and differentiate, ensuring this lifelong hematopoiesis. Determining the regulation of HSC functions should thus provide critical insight to advancing regenerative medicine. Until quite recently, HSCs were primarily studied using in vitro studies and transplantations into immunodeficient hosts. Indeed, the definition of a bona fide HSC is its ability to reconstitute lymphopenic hosts. In this review, we discuss the development of novel, HSC-specific genetic reporter systems that enable the prospective identification of HSCs and the study of their functions in the absence of transplantation. Coupled with additional technological advances, these studies are now defining the fundamental properties of HSCs in vivo. Furthermore, complex cellular and molecular mechanisms that regulate HSC dormancy, self-renewal, and differentiation are being identified and further dissected. These novel reporter systems represent a major technological advance for the stem cell field and allow new questions to be addressed.

Uppugunduri, C. R., et al. (2014). "The association of cytochrome P450 genetic polymorphisms with sulfolane formation and the efficacy of a busulfan-based conditioning regimen in pediatric patients undergoing hematopoietic stem cell transplantation." Pharmacogenomics J **14**(3): 263-271.

Cytochrome P450 enzymes (CYPs) and flavin-containing monooxygenases (FMOs) likely have a role in the oxidation of intermediate metabolites of busulfan (Bu). In vitro studies to investigate the involvement of these enzymes are cumbersome because of the volatile nature of the intermediate metabolite tetrahydrothiophene (THT) and the lack of sensitive quantitation methods. This study explored the association between the CYP2C9, CYP2C19, CYP2B6 and FMO3 genotypes and sulfolane (Su, a water soluble metabolite of Bu) plasma levels in children undergoing hematopoietic stem cell transplantation (HSCT). The relationship between these genotypes and the effectiveness of myeloablative conditioning was also analyzed. Sixty-six children receiving an intravenous Bu-based myeloablative conditioning regimen were genotyped for common functional variant alleles in CYP2C9 (*2 and *3), CYP2C19 (*2 and *17), FMO3 (rs2266780, rs2266782 and rs1736557) and CYP2B6 (*5 and *9). The plasma levels of Bu and its metabolite Su were measured after the ninth Bu dose in a subset of 44 patients for whom plasma samples were available. The ratio of Bu to Su was considered the metabolic ratio (MR) and was compared across the genotype groups. Higher MRs were observed in CYP2C9*2 and *3 allele carriers (mean+/-s.d.: 7.8+/-3.6 in carriers vs 4.4+/-2.2 in non-carriers; P=0.003). An increased incidence of graft failure was observed among patients with an MR>5 compared with those with MR values <5 (20% vs 0%; P=0.02). In contrast, a significantly higher incidence of relapse and graft failure (evaluated as event-free survival) was observed in patients with malignant disease who carried CYP2B6 alleles with reduced function on both chromosomes compared with carriers of at least one normal allele (100% vs 40%; P=0.0001). These results suggest that CYP2C9 has a role in the oxidation reactions of THT and indicate that it may be possible to predict the efficacy of Bu-based myeloablative conditioning before HSCT on the basis of CYP genotypes and Bu MRs.

van den Boom, V., et al. (2012). "Genetic and epigenetic alterations that drive leukemic stem cell self-renewal." J Stem Cells **7**(3): 155-179.

Acute myeloid leukemia has emerged as a paradigm for the concept of the cancer stem cell. This hypothesis presumes that the disease is maintained by a rare population of leukemia-initiating stem cells which have acquired genetic or epigenetic changes. It is most

likely that a single (epi)genetic event will not be sufficient to cause leukemia, but that a number of sequential events are required. Similar to normal hematopoietic stem cells, both intrinsic as well as extrinsic factors that arise from the bone marrow niche, provide essential cues that regulate cell fate decisions such as leukemic stem cell self-renewal and differentiation. In this chapter, we will review the current understanding of genetic and epigenetic abnormalities that underlie the process of leukemic transformation, and will discuss which events potentially co-operate to induce leukemia.

Van Zant, G., et al. (1983). "Genetic control of hematopoietic kinetics revealed by analyses of allophenic mice and stem cell suicide." Cell **35**(3 Pt 2): 639-645.

The pluripotential hematopoietic stem cell is influenced by at least one gene that differs between DBA/2 and C3H/He, and C57BL/6 inbred mouse strains. This gene(s) manifests itself by its effect on susceptibility to killing of spleen colony forming cells (CFU-S) caused by hydroxyurea (HU). In strains DBA/2 and C3H/He 20% of the CFU-S population is normally in S phase whereas practically none from strain C57BL/6 are synthesizing DNA. On the other hand, in C57BL/6 in equilibrium DBA/2 allophenic mice we observed that the proportion of DBA/2 erythrocytes was higher than the proportion of DBA/2 lymphocytes; the fraction of platelets and neutrophils with the DBA/2 genotype fell between the values for erythrocytes and lymphocytes. Control experiments using mice congenic at the Fv-2 locus confirm that in both situations we are examining effects of a gene(s) other than Fv-2. For the effect on the S phase fraction of CFU-S, we refer to the gene(s) as Stk (stem cell kinetics). We suggest that the observed skewing in composition among the various mature blood cell types in C57BL/6 in equilibrium DBA/2 allophenic mice is caused by allelic variants of the Stk gene. Such variation would favor the formation of DBA/2 erythrocytes, platelets, and neutrophils over those of the C57BL/6 genotype.

Wang, J., et al. (2010). "The potential landscape of genetic circuits imposes the arrow of time in stem cell differentiation." Biophys J **99**(1): 29-39.

Differentiation from a multipotent stem or progenitor state to a mature cell is an essentially irreversible process. The associated changes in gene expression patterns exhibit time-directionality. This "arrow of time" in the collective change of gene expression across multiple stable gene expression patterns (attractors) is not explained by the regulated activation, the suppression of individual genes which are bidirectional molecular processes, or by the standard dynamical models of the underlying gene circuit which only account for local stability of attractors. To capture the global dynamics of this nonequilibrium system and gain insight in the time-asymmetry of state transitions, we computed the quasipotential landscape of the stochastic dynamics of a canonical gene circuit that governs branching cell fate commitment. The potential landscape reveals the global dynamics and permits the calculation of potential barriers between cell phenotypes imposed by the circuit architecture. The generic asymmetry of barrier heights indicates that the transition from the uncommitted multipotent state to differentiated states is inherently unidirectional. The model agrees with observations and predicts the extreme conditions for reprogramming cells back to the undifferentiated state.

Wang, L., et al. (2006). "Genetic deletion of Cdc42GAP reveals a role of Cdc42 in erythropoiesis and hematopoietic stem/progenitor cell survival, adhesion, and engraftment." Blood **107**(1): 98-105.

Rho family GTPases are key signal transducers in cell regulation. Although a body of literature has implicated the Rho family members Rac1 and Rac2 in multiple hematopoietic-cell functions, the role of Cdc42 in hematopoiesis remains unclear. Here we have examined the hematopoietic properties and the hematopoietic stem/progenitor cell (HSP) functions of gene-targeted mice carrying null alleles of cdc42gap, a negative regulator of Cdc42. The Cdc42GAP-/- fetal liver and bone marrow cells showed a 3-fold increase in Cdc42 activity but normal Rac and RhoA activities, indicating that Cdc42GAP knockout resulted in a gain of Cdc42 activity in the hematopoietic tissues. Cdc42GAP-/- mice were anemic. The cellularity of fetal liver and bone marrow, the number and composition percentage of HSPs, and the erythroid blast-forming unit and colony-forming unit (BFU-E/CFU-E) activities were significantly reduced in the homozygous mice. The decrease in HSP number was associated with increased apoptosis of the Cdc42GAP-/- HSPs and the activation of JNK-mediated apoptotic machinery. Moreover, homozygous HSPs showed impaired cortical F-actin assembly, deficiency in adhesion and migration, and defective engraftment. These results provide evidence that Cdc42 activity is important for erythropoiesis and for multiple HSP functions, including survival, adhesion, and engraftment.

Wang, Y., et al. (2018). "Establishment of TUSMi003-A, an induced pluripotent stem cell (iPSC) line from a 62-year old Chinese Han patient with Alzheimer's disease with ApoE3/4 genetic background." Stem Cell Res **27**: 57-60.

A 62-year old Chinese Han Alzheimer's disease (AD) female patient with ApoE3/4 genetic background donated her Peripheral blood mononuclear cells (PBMC). The non-integrating episomal vector system was used to reprogrammed PBMCs with the human OKSM transcription factors. The pluripotency of transgene-free iPSCs was confirmed by immunocytochemistry for pluripotency markers and by the ability of the iPSCs to differentiate spontaneously into 3 germ layers in vitro. In addition, the iPSC line displayed a normal karyotype. Our model might offer a good platform to further study the pathological mechanisms, to identify early biomarkers, and also for drug testing studies in AD.

Wang, Y., et al. (2017). "Generation of a human induced pluripotent stem cell line from a 65-year old healthy female donor with Chinese Han genetic background." Stem Cell Res **24**: 33-35.

Peripheral blood mononuclear cells (PBMC) were collected from a 65-year old healthy woman with Chinese Han genetic background. The PBMCs were reprogrammed with the human OKSM transcription factors using the non-integrating episomal vector system. The transgene-free iPSC showed pluripotency verified by immunocytochemistry for pluripotency markers and differentiated spontaneously toward the 3 germ layers in vitro. Furthermore, the iPSC line showed normal karyotype. The iPSC line can be used as control in disease mechanism studies. Resource table.

Wanner, M., et al. (2009). "Losing the genetic twin: donor grief after unsuccessful unrelated stem cell transplantation." BMC Health Serv Res **9**: 2.

BACKGROUND: Stem cell transplantations from related or unrelated donors are used to cure leukaemia and other blood diseases. When a patient dies after an unsuccessful transplantation, interested unrelated donors are informed about the failure by their donor centre. Studies focussing on failed related donations show that donors undergo an intense grieving process. As there are only two investigations about reactions from unrelated donors, knowledge about their reactions is less comprehensive. METHODS: We conducted a prospective study of reactions of unrelated donors to the information of failed transplantations, subject to various communication methods (letter, phone). Questionnaires were sent to 395 unrelated donors who received the news of their recipients' deaths between November 2005 and August 2006. In addition, twelve in-depth interviews with selected donors were carried out. RESULTS: Unrelated donors were emotionally affected by the recipients' deaths, and it is appropriate to speak about a "Donor Grief" phenomenon, as the results of 325 returned questionnaires (return rate 82.3%) and in-depth interviews show. Donors demonstrated a range of feelings such as sadness, disappointment, grief, and helplessness. These feelings were often unexpectedly intense given the fact that the recipient was a stranger. Although the news caused grief, donors underlined that they nevertheless wanted to be informed. They preferred knowledge of the failure to uncertainty. The method of providing the information is only of secondary importance. Most donors favoured the way of communication they had experienced. CONCLUSION: This result indicates that both phone and letter communication can be justified. However, phone communication seems to be superior with respect to aspects of sensitivity. In spite of transplantation failure and the associated negative feelings, most donors were happy to have donated and would be willing to do so again. Our results underline the special responsibility of donor centres for informing and supporting unrelated volunteer donors in case their recipients have died.

Weber, T., et al. (2013). "Genetic fate mapping of type-1 stem cell-dependent increase in newborn hippocampal neurons after electroconvulsive seizures." Hippocampus **23**(12): 1321-1330.

Electroconvulsive therapy (ECT) is a uniquely effective treatment for major depressive disorder. An increase in hippocampal neurogenesis is implicated in the recovery from depression. We used an inducible genetic mouse model in which only GFAP-expressing stem-like cells (type-1 cells) and their progeny are selectively labeled with the reporter protein beta-galactosidase to track the process of neurogenesis in the dentate gyrus over 3 months following electroconvulsive seizures (ECS), the mouse equivalent of ECT. All ECS protocols tested induced a transient increase in type-1 cell divisions. While this led to an expansion of the type-1 cell pool after high-frequency ECS sessions for 5 consecutive days (5-ECS), asymmetric divisions drove neurogenesis by giving rise to Doublecortin (DCX)-expressing neuroblasts that matured into NeuN+ neurons. Significantly, the increase in newly generated DCX+ and NeuN+ cells after 5-ECS could be traced back to proliferating type-1 cells. Low-frequency continuation ECS (c-ECS) consisting of five single ECS sessions administered every 2 weeks resulted in a similar increase in newborn neurons as the high-frequency 5-ECS protocol. Moreover, the combination of 5-ECS and c-ECS led to a further significant increase in newborn neurons, suggesting a cellular mechanism responsible for the propitious effects of high-frequency ECT followed by continuation ECT in severely depressed patients. The ability of high- and low-frequency ECS to induce normally quiescent type-1 cells to proliferate and generate new neurons sets it apart from other

antidepressant treatments and may underlie the superior clinical efficacy of ECT.

Wedge, E., et al. (2021). "Allogeneic Hematopoietic Stem Cell Transplantation for Chronic Myelomonocytic Leukemia: Clinical and Molecular Genetic Prognostic Factors in a Nordic Population." Transplant Cell Ther **27**(12): 991 e991-991 e999.

Chronic myelomonocytic leukemia (CMML) is an aggressive disease in which survival after allogeneic hematopoietic stem cell transplantation (HCT) remains relatively poor. An assessment of prognostic factors is an important part of treatment decision making and has the potential to be greatly improved by the inclusion of molecular genetics. However, there is a significant knowledge gap in the interpretation of mutational patterns. This study aimed to describe outcomes of allogeneic HCT in patients with CMML in relation to clinical and molecular genetic risk factors. This retrospective study included 64 patients with CMML who underwent allogeneic HCT between 2008 and 2018, with a median follow-up of 5.4 years. Next-generation sequencing using targeted myeloid panels was carried out on saved material from 51 patients from the time of transplantation. Kaplan-Meier and Cox regression were used for analysis of overall survival (OS), and cumulative incidence with competing risks and Fine and Gray models were used for analysis of relapse and nonrelapse mortality (NRM). Mutations were detected in 48 patients (94%), indicating high levels of minimal residual disease (MRD) positivity at transplantation, even among those in complete remission (CR) (n = 14), 86% of whom had detectable mutations. The most frequently mutated genes were ASXL1 (37%), TET2 (37%), RUNX1 (33%), SRSF2 (26%), and NRAS (20%). Risk stratification using the CMML-specific Prognostic Scoring System molecular score (CPSS-Mol) resulted in 45% of patients moving to a higher risk-group compared with risk stratification using the CPSS. High leucocyte count (>/=13 x 10(9)/L), transfusion requirement, and previous intensive chemotherapy were associated with higher incidence of relapse. Being in CR was not linked to better outcomes. Neither ASXL1 nor RUNX1 mutation was associated with a difference in OS, relapse, or NRM, despite being high risk in the nontransplantation setting. TET2 mutations were associated with a significantly higher 3-year OS (73% versus 40%; P = .039). Achieving MRD-negative CR was rare in this CMML cohort, which may explain why we did not observe better outcomes for those in CR. This merits further investigation. Our analyses suggest that the negative impact of ASXL1 and RUNX1 mutations can be overcome by allogeneic HCT; however, risk stratification is complex in CMML and requires larger cohorts and multivariate models, presenting an ongoing challenge in this rare disease.

Wehman, A. M., et al. (2005). "Genetic dissection of the zebrafish retinal stem-cell compartment." Dev Biol **281**(1): 53-65.

In a large-scale forward-genetic screen, we discovered that a limited number of genes are required for the regulation of retinal stem cells after embryogenesis in zebrafish. In 18 mutants out of almost 2000 F2 families screened, the eye undergoes normal embryonic development, but fails to continue growth from the ciliary marginal zone (CMZ), the post-embryonic stem-cell niche. Class I-A mutants (5 loci) display lower amounts of proliferation in the CMZ, while nearly all cells in the retina appear differentiated. Class I-B mutants (2 loci) have a reduced CMZ with a concomitant expansion in the retinal pigmented epithelium (RPE), suggesting a common post-embryonic stem cell is the source for these neighboring cell types. Class II encompasses three distinct types of mutants (11 loci) with expanded CMZ, in which the progenitor population is arrested in the cell cycle. We also show that in at least one combination, the reduced CMZ phenotype is genetically epistatic to the expanded CMZ phenotype, suggesting that Class I genes are more likely to affect the stem cells and Class II the progenitor cells. Finally, a comparative mapping analysis demonstrates that the new genes isolated do not correspond to genes previously implicated in stem-cell regulation. Our study suggests that embryonic and post-embryonic stem cells utilize separable genetic programs in the zebrafish retina.

Wilke, G., et al. (2019). "A Stem-Cell-Derived Platform Enables Complete Cryptosporidium Development In Vitro and Genetic Tractability." Cell Host Microbe **26**(1): 123-134 e128.

Despite being a frequent cause of severe diarrheal disease in infants and an opportunistic infection in immunocompromised patients, Cryptosporidium research has lagged due to a lack of facile experimental methods. Here, we describe a platform for complete life cycle development and long-term growth of C. parvum in vitro using "air-liquid interface" (ALI) cultures derived from intestinal epithelial stem cells. Transcriptomic profiling revealed that differentiating epithelial cells grown under ALI conditions undergo profound changes in metabolism and development that enable completion of the parasite life cycle in vitro. ALI cultures support parasite expansion > 100-fold and generate viable oocysts that are transmissible in vitro and to mice, causing infection and animal death. Transgenic parasite lines created using CRISPR/Cas9 were used to complete a genetic cross in vitro, demonstrating Mendelian segregation of

chromosomes during meiosis. ALI culture provides an accessible model that will enable innovative studies into Cryptosporidium biology and host interactions.

Wu, Q., et al. (2014). "[Neural stem cell-specific peroxisome proliferator-activated receptor gamma knockout mice: breeding and genetic identification]." Nan Fang Yi Ke Da Xue Xue Bao **34**(12): 1768-1771.

OBJECTIVE: To breed neual stem cell-specific peroxisome proliferator-activated receptor gamma (PPARgamma) knockout mice. METHODS: Two transgenic mouse models, namely B6.PPARgammaloxp/loxp and B6.Nestin-Cre were interbred, and the first- generation offsprings were backcrossed with B6.PPARgammaloxp/loxp to obtain the second-generation mice. Genomic DNA was extracted from the second-generation mice for PCR to amplify the loxp and Cre gene fragments followed by agarose gel electrophoresis to verify their sizes. The mice with the PPARgammaloxp/loxp.Nestin-Cre (KO) genotype were selected as the neural stem cell-specific knockout PPARgamma mice, with B6.PPARgammaloxp/loxp (loxp) mice as the control. Tissue samples were collected from specific regions of the mouse brain and peripheral tissue for detecting the expression of PPARgamma mRNA using RT-PCR and real-time quantitative PCR. RESULTS AND CONCLUSION: Genotyping results showed PPARgammaloxp and Cre bands in the knockout mice, which showed obviously decreased mRNA expression of PPARgamma, suggesting successful establishment of neural stem cell-specific PPARgamma knockout mice. The two transgenic mice we used were fertile, and their breeding pattern followed the laws of Mendelian inheritance.

Wu, X., et al. (2009). "Genetic variation in the prostate stem cell antigen gene PSCA confers susceptibility to urinary bladder cancer." Nat Genet **41**(9): 991-995.

We conducted a genome-wide association study on 969 bladder cancer cases and 957 controls from Texas. For fast-track validation, we evaluated 60 SNPs in three additional US populations and validated the top SNP in nine European populations. A missense variant (rs2294008) in the PSCA gene showed consistent association with bladder cancer in US and European populations. Combining all subjects (6,667 cases, 39,590 controls), the overall P-value was 2.14 x 10(-10) and the allelic odds ratio was 1.15 (95% confidence interval 1.10-1.20). rs2294008 alters the start codon and is predicted to cause truncation of nine amino acids from the N-terminal signal sequence of the primary PSCA translation product. In vitro reporter gene assay showed that the variant allele significantly reduced promoter activity. Resequencing of the PSCA genomic region showed that rs2294008 is the only common missense SNP in PSCA. Our data identify rs2294008 as a new bladder cancer susceptibility locus.

Xiao, H., et al. (2012). "Genetic variations in T-cell activation and effector pathways modulate alloimmune responses after allogeneic hematopoietic stem cell transplantation in patients with hematologic malignancies." Haematologica **97**(12): 1804-1812.

BACKGROUND: Recently, several important polymorphisms have been identified in T-cell activation and effector pathway genes and have been reported to be associated with inter-patient variability in alloimmune responses. The present study was designed to assess the impact of these genetic variations on the outcomes of allogeneic hematopoietic stem cell transplantation. DESIGN AND METHODS: We first investigated ten single nucleotide polymorphisms in six genes, CD28, inducible co-stimulator, cytotoxic T-lymphocyte antigen 4, granzyme B, Fas and Fas ligand, in 138 pairs of patients and their unrelated donors and a second cohort of 102 pairs of patients and their HLA-identical sibling donors. RESULTS: We observed that patients receiving stem cells from a donor with the cytotoxic T-lymphocyte antigen 4 gene CT60 variant allele (AA genotype) had a reduced incidence of grades II-IV acute graft-versus-host disease; however, they experienced early cytomegalovirus infection and relapsed more frequently, which suggested an interaction between the donor cytotoxic T-lymphocyte antigen 4 gene CT60 AA genotype and reduced T-cell alloreactivity. Furthermore, an unrelated donor with the granzyme B +55 variant genotype (AA) was an independent risk factor for development of grades II-IV acute graft-versus-host disease (P=0.024, RR=1.811). Among patients with acute myelogenous leukemia, those with the Fas -670 TT genotype were at higher risk of relapse (P=0.003, RR=3.823). The presence of these susceptible alleles in the donor and/or patient resulted in worse overall survival (54.9% versus 69.5%, P=0.029). CONCLUSIONS: Our data suggest that genotype analysis of T-cell activation and effector pathway genes can be used for risk assessment for patients with hematologic malignancies before hematopoietic stem cell transplantation.

Xiao, L. L., et al. (2004). "[A probability analysis for HLA matching in adult stem cell transplantation treating nervous genetic diseases]." Zhongguo Shi Yan Xue Ye Xue Za Zhi **12**(6): 845-848.

The aim of this study was to investigate the clinical feasibility of adult stem cell transplantation for lethal mono-gene inherited disease, Duchenne muscular dystrophy (DMD). A total of 30 blood samples from DMD patients were genotyped with HLA-A,-B and -DR alleles by means of polymerase

chain reaction-reverse sequence specific oligonucleotide (PCR-RSSO). The HLA gene types in 30 DMD patients were compared with those of 668 unrelated donors from Umbilical Cord Blood Center of Guangdong Province and 34 910 unrelated donors from Chinese Bone Marrow Bank. The results showed that HLA gene of the DMD group was inherited in normal distribution. There was no striking difference of HLA-A, -B and -DR alleles expression between the DMD patients group and control healthy group. 25% of the DMD patients got suitable donors for stem cell transplantation, in which 15 patients found donors with >or= 5/6 HLA match at the Umbilical Cord Blood Center of Guangdong Province, i.e. occupying 50% of the total. Eight patients got 6/6 HLA matching donors at the Chinese Bone Marrow Bank, i.e. occupying 26% of the total. It is concluded that stem cells transplantation therapy for DMD patients is feasible, which will benefit these patients suffered from the lethal neuromuscular disease, and create a new way to treat this tough nervous system disease.

Xie, X., et al. (2007). "Genetic modification of embryonic stem cells with VEGF enhances cell survival and improves cardiac function." Cloning Stem Cells **9**(4): 549-563.

Cardiac stem cell therapy remains hampered by acute donor cell death posttransplantation and the lack of reliable methods for tracking cell survival in vivo. We hypothesize that cells transfected with inducible vascular endothelial growth factor 165 (VEGF(165)) can improve their survival as monitored by novel molecular imaging techniques. Mouse embryonic stem (ES) cells were transfected with an inducible, bidirectional tetracycline (Bi-Tet) promoter driving VEGF(165) and renilla luciferase (Rluc). Addition of doxycycline induced Bi-Tet expression of VEGF(165) and Rluc significantly compared to baseline ($p<0.05$). Expression of VEGF(165) enhanced ES cell proliferation and inhibited apoptosis as determined by Annexin-V staining. For noninvasive imaging, ES cells were transduced with a double fusion (DF) reporter gene consisting of firefly luciferase and enhanced green fluorescence protein (Fluc-eGFP). There was a robust correlation between cell number and Fluc activity ($R(2)=0.99$). Analysis by immunostaining, histology, and RT-PCR confirmed that expression of Bi-Tet and DF systems did not affect ES cell self-renewal or pluripotency. ES cells were differentiated into beating embryoid bodies expressing cardiac markers such as troponin, Nkx2.5, and beta-MHC. Afterward, 5 x 10(5) cells obtained from these beating embryoid bodies or saline were injected into the myocardium of SV129 mice (n=36) following ligation of the left anterior descending (LAD) artery. Bioluminescence imaging (BLI) and echocardiography showed that VEGF(165) induction led to significant improvements in both transplanted cell survival and cardiac function ($p<0.05$). This is the first study to demonstrate imaging of embryonic stem cell-mediated gene therapy targeting cardiovascular disease. With further validation, this platform may have broad applications for current basic research and further clinical studies.

Yadav, A., et al. (2016). "Association of cancer stem cell markers genetic variants with gallbladder cancer susceptibility, prognosis, and survival." Tumour Biol **37**(2): 1835-1844.

Genes important to stem cell progression have been involved in the genetics and clinical outcome of cancers. We investigated germ line variants in cancer stem cell (CSC) genes to predict susceptibility and efficacy of chemoradiotherapy treatment in gallbladder cancer (GBC) patients. In this study, we assessed the effect of SNPs in CSC genes (surface markers CD44, ALCAM, EpCAM, CD133) and (molecular markers NANOG, SOX-2, LIN-28A, ALDH1A1, OCT-4) with GBC susceptibility and prognosis. Total 610 GBC patients and 250 controls were genotyped by using PCR-RFLP, ARMS-PCR, and TaqMan allelic discrimination assays. Chemotoxicity graded 2-4 in 200 patients and tumor response was recorded in 140 patients undergoing neoadjuvant chemotherapy (NACT). Differences in genotype and haplotype frequency distributions were calculated by binary logistic regression. Gene-gene interaction model was analyzed by generalized multifactor dimensionality reduction (GMDR). Overall survival was assessed by Kaplan-Meier survival curve and multivariate Cox-proportional methods. ALCAM Ars1157Crs10511244 ($P = 0.0035$) haplotype was significantly associated with GBC susceptibility. In GMDR analysis, ALCAM rs1157G>A, EpCAM rs1126497T>C emerged as best significant interaction model with GBC susceptibility and ALDH1A1 rs13959T>G with increased risk of grade 3-4 hematological toxicity. SOX-2 rs11915160A>C, OCT-4 rs3130932T>G, and NANOG rs11055786T>C were found best gene-gene interaction model for predicting response to NACT. In both Cox-proportional and recursive partitioning ALCAM rs1157GA+AA genotype showed higher mortality and hazard ratio. ALCAM gene polymorphisms associated with GBC susceptibility and survival while OCT-4, SOX-2, and NANOG variants showed an interactive role with treatment response.

Yamada, S., et al. (2008). "Embryonic stem cell therapy of heart failure in genetic cardiomyopathy." Stem Cells **26**(10): 2644-2653.

Pathogenic causes underlying nonischemic cardiomyopathies are increasingly being resolved, yet

repair therapies for these commonly heritable forms of heart failure are lacking. A case in point is human dilated cardiomyopathy 10 (CMD10; Online Mendelian Inheritance in Man #608569), a progressive organ dysfunction syndrome refractory to conventional therapies and linked to mutations in cardiac ATP-sensitive K(+) (K(ATP)) channel subunits. Embryonic stem cell therapy demonstrates benefit in ischemic heart disease, but the reparative capacity of this allogeneic regenerative cell source has not been tested in inherited cardiomyopathy. Here, in a Kir6.2-knockout model lacking functional K(ATP) channels, we recapitulated under the imposed stress of pressure overload the gene-environment substrate of CMD10. Salient features of the human malignant heart failure phenotype were reproduced, including compromised contractility, ventricular dilatation, and poor survival. Embryonic stem cells were delivered through the epicardial route into the left ventricular wall of cardiomyopathic stressed Kir6.2-null mutants. At 1 month of therapy, transplantation of 200,000 cells per heart achieved teratoma-free reversal of systolic dysfunction and electrical synchronization and halted maladaptive remodeling, thereby preventing end-stage organ failure. Tracked using the lacZ reporter transgene, stem cells engrafted into host heart. Beyond formation of cardiac tissue positive for Kir6.2, transplantation induced cell cycle activation and halved fibrotic zones, normalizing sarcomeric and gap junction organization within remuscularized hearts. Improved systemic function induced by stem cell therapy translated into increased stamina, absence of anasarca, and benefit to overall survivorship. Embryonic stem cells thus achieve functional repair in nonischemic genetic cardiomyopathy, expanding indications to the therapy of heritable heart failure. Disclosure of potential conflicts of interest is found at the end of this article.

Yamamoto, T., et al. (2022). "Correlation Between Genetic Abnormalities in Induced Pluripotent Stem Cell-Derivatives and Abnormal Tissue Formation in Tumorigenicity Tests." Stem Cells Transl Med **11**(5): 527-538.

Cell therapy using induced pluripotent stem cell (iPSC) derivatives may result in abnormal tissue generation because the cells undergo numerous cycles of mitosis before clinical application, potentially increasing the accumulation of genetic abnormalities. Therefore, genetic tests may predict abnormal tissue formation after transplantation. Here, we administered iPSC derivatives with or without single-nucleotide variants (SNVs) and deletions in cancer-related genes with various genomic copy number variant (CNV) profiles into immunodeficient mice and examined the relationships between mutations and abnormal tissue formation after transplantation. No positive correlations were found between the presence of SNVs/deletions and the formation of abnormal tissues; the overall predictivity was 29%. However, a copy number higher than 3 was correlated, with an overall predictivity of 86%. Furthermore, we found CNV hotspots at 14q32.33 and 17q12 loci. Thus, CNV analysis may predict abnormal tissue formation after transplantation of iPSC derivatives and reduce the number of tumorigenicity tests.

Yang, H., et al. (2012). "Genetic variations in stem cell-related genes and colorectal cancer prognosis." J Gastrointest Cancer **43**(4): 584-593.

BACKGROUND: Many properties of cancer cells are reminiscent of those in normal stem cells. Genes important to stem cell development have been significantly implicated in the etiology and clinical outcome of colorectal cancer (CRC). However, the associations of genetic variations in these genes with CRC prognosis have not yet been elucidated. METHODS: We analyzed the effects of eight potentially functional single nucleotide polymorphisms (SNPs) in six stem cell-related genes on the prognosis of a well-characterized population of 380 Chinese CRC patients diagnosed from February 2006 to January 2010. RESULTS: The most significant finding was related to rs879882, a variant in the 5' region of POU5F1 gene which encodes a protein essential for embryonic stem cell self-renewal and pluripotency, and induced pluripotent stem cell reprogramming. The variant-containing genotypes of rs879882 were associated with an increased risk of recurrence (hazard ratio [HR] = 2.10, 95% confidence interval [CI] 1.17-3.76, P = 0.01). In chemotherapy-stratified analysis, the association remained borderline significant in patients receiving chemotherapy (HR = 1.97, 95% CI 0.89-4.34, P = 0.09). In addition, a nonsynonymous SNP of APC gene was also significantly associated with recurrence risk in chemotherapy-treated patients (HR = 2.63, 95% CI 1.14-6.06 P = 0.02). Further analyses showed a combined effect of the two SNPs in predicting CRC recurrence in patients receiving chemotherapy (P = 0.04) but not in those without chemotherapy (P = 0.43). Moreover, an exploratory multivariate assessment model indicated that these two variants enhanced the power to predict recurrence after chemotherapy. CONCLUSION: We presented one of the first epidemiologic studies showing that stem cell-related genetic variants may impact CRC clinical outcomes, especially in chemotherapy-treated patients.

Yao, L., et al. (2017). "Molecular Profiling of Human Induced Pluripotent Stem Cell-Derived Hypothalamic Neurones Provides Developmental Insights into Genetic Loci for Body Weight Regulation." J Neuroendocrinol **29**(2).

Recent data suggest that common genetic risks for metabolic disorders such as obesity may be human-specific and exert effects via the central nervous system. To overcome the limitation of human tissue access for study, we have generated induced human pluripotent stem cell (hiPSC)-derived neuronal cultures that recapture many features of hypothalamic neurones within the arcuate nucleus. In the present study, we have comprehensively characterised this model across development, benchmarked these neurones to in vivo events, and demonstrate a link between obesity risk variants and hypothalamic development. The dynamic transcriptome across neuronal maturation was examined using microarray and RNA sequencing methods at nine time points. K-means clustering of the longitudinal data was conducted to identify co-regulation and microRNA control of biological processes. The transcriptomes were compared with those of 103 samples from 13 brain regions reported in the Genotype-Tissue Expression database (GTEx) using principal components analysis. Genes with proximity to body mass index (BMI)-associated genetic variants were mapped to the developmentally expressed genesets, and enrichment significance was assessed with Fisher's exact test. The human neuronal cultures have a transcriptional and physiological profile of neuropeptide Y/agouti-related peptide arcuate nucleus neurones. The neuronal transcriptomes were highly correlated with adult hypothalamus compared to any other brain region from the GTEx. Also, approximately 25% of the transcripts showed substantial changes in expression across neuronal development and potential co-regulation of biological processes that mirror neuronal development in vivo. These developmentally expressed genes were significantly enriched for genes in proximity to BMI-associated variants. We confirmed the utility of this in vitro human model for studying the development of key hypothalamic neurones involved in energy balance and show that genes at loci associated with body weight regulation may share a pattern of developmental regulation. These data support the need to investigate early development to elucidate the human-specific central nervous system pathophysiology underlying obesity susceptibility.

Ye, L., et al. (2009). "Induced pluripotent stem cells offer new approach to therapy in thalassemia and sickle cell anemia and option in prenatal diagnosis in genetic diseases." Proc Natl Acad Sci U S A **106**(24): 9826-9830.

The innovation of reprogramming somatic cells to induced pluripotent stem cells provides a possible new approach to treat beta-thalassemia and other genetic diseases such as sickle cell anemia. Induced pluripotent stem (iPS) cells can be made from these patients' somatic cells and the mutation in the beta-globin gene corrected by gene targeting, and the cells differentiated into hematopoietic cells to be returned to the patient. In this study, we reprogrammed the skin fibroblasts of a patient with homozygous beta(0) thalassemia into iPS cells, and showed that the iPS cells could be differentiated into hematopoietic cells that synthesized hemoglobin. Prenatal diagnosis and selective abortion have been effective in decreasing the number of beta-thalassemia births in some countries that have instituted carrier screening and genetic counseling. To make use of the cells from the amniotic fluid or chorionic villus sampling that are used for prenatal diagnosis, we also showed that these cells could be reprogrammed into iPS cells. This raises the possibility of providing a new option following prenatal diagnosis of a fetus affected by a severe illness. Currently, the parents would choose either to terminate the pregnancy or continue it and take care of the sick child after birth. The cells for prenatal diagnosis can be converted into iPS cells for treatment in the perinatal periods. Early treatment has the advantage of requiring much fewer cells than adult treatment, and can also prevent organ damage in those diseases in which damage can begin in utero or at an early age.

Yoon, J. K., et al. (2018). "Direct Control of Stem Cell Behavior Using Biomaterials and Genetic Factors." Stem Cells Int **2018**: 8642989.

Stem cells have recently emerged as an important candidate for cell therapy. However, some major limitations still exist such as a small quantity of cell supply, senescence, and insufficient differentiation efficiency. Therefore, there is an unmet need to control stem cell behavior for better clinical performance. Since native microenvironment factors including stem cell niche, genetic factors, and growth factors direct stem cell fate cooperatively, user-specified in vitro settings are required to understand the regulatory roles and effects of each factor, thereby applying the factors for improved cell therapy. Among others, various types of biomaterials and transfection method have been employed as key tools for development of the in vitro settings. This review focuses on the current strategies to improve stemness maintenance, direct differentiation, and reprogramming using biomaterials and genetic factors without any aids from additional biochemicals and growth factors.

Yoon, K. J., et al. (2014). "Modeling a genetic risk for schizophrenia in iPSCs and mice reveals neural stem cell deficits associated with adherens junctions and polarity." Cell Stem Cell **15**(1): 79-91.

Defects in brain development are believed to contribute toward the onset of neuropsychiatric disorders, but identifying specific underlying

mechanisms has proven difficult. Here, we took a multifaceted approach to investigate why 15q11.2 copy number variants are prominent risk factors for schizophrenia and autism. First, we show that human iPSC-derived neural progenitors carrying 15q11.2 microdeletion exhibit deficits in adherens junctions and apical polarity. This results from haploinsufficiency of CYFIP1, a gene within 15q11.2 that encodes a subunit of the WAVE complex, which regulates cytoskeletal dynamics. In developing mouse cortex, deficiency in CYFIP1 and WAVE signaling similarly affects radial glial cells, leading to their ectopic localization outside of the ventricular zone. Finally, targeted human genetic association analyses revealed an epistatic interaction between CYFIP1 and WAVE signaling mediator ACTR2 and risk for schizophrenia. Our findings provide insight into how CYFIP1 regulates neural stem cell function and may contribute to the susceptibility of neuropsychiatric disorders.

Yoshizato, T. (2019). "[Impact of genetic alterations on prognosis in myelodysplastic syndrome patients undergoing stem cell transplantation]." Rinsho Ketsueki **60**(8): 960-967.

Myelodysplastic syndromes (MDS) constitute a group of heterogeneous disorders of hematopoietic stem cells, characterized by defective hematopoiesis and multilineage dysplasia. While low-risk subtypes normally exhibit a relatively chronic clinical course, high-risk subtypes harbor unfavorable prognosis in which hematopoietic stem cell transplantation (HCT) is the only curative therapy. Nevertheless, transplantation-related mortality is relatively high and should be weighed against the potential benefits of HCT. Hence, it is vital to precisely stratify the prognostic risks before HCT for predicting and enhancing their prognosis. Recently, our understanding of the genetic basis of MDS has substantially advanced, through which a full spectrum of major mutational targets was delineated. Moreover, its effects in the setting of HCT have also been assessed besides the conventional predictive factors. While clinical factors account for as much as 70% of the total hazard of MDS cases treated with HCT, the remaining 30% is explicated by genetic factors. The integration of genetic test and conventional clinical factors could be useful for precise stratification of the prognostic risks and, therefore, treatment decision in MDS.

Yoshizato, T., et al. (2017). "Genetic abnormalities in myelodysplasia and secondary acute myeloid leukemia: impact on outcome of stem cell transplantation." Blood **129**(17): 2347-2358.

Genetic alterations, including mutations and copy-number alterations, are central to the pathogenesis of myelodysplastic syndromes and related diseases (myelodysplasia), but their roles in allogeneic stem cell transplantation have not fully been studied in a large cohort of patients. We enrolled 797 patients who had been diagnosed with myelodysplasia at initial presentation and received transplantation via the Japan Marrow Donor Program. Targeted-capture sequencing was performed to identify mutations in 69 genes, together with copy-number alterations, whose effects on transplantation outcomes were investigated. We identified 1776 mutations and 927 abnormal copy segments among 617 patients (77.4%). In multivariate modeling using Cox proportional-hazards regression, genetic factors explained 30% of the total hazards for overall survival; clinical characteristics accounted for 70% of risk. TP53 and RAS-pathway mutations, together with complex karyotype (CK) as detected by conventional cytogenetics and/or sequencing-based analysis, negatively affected posttransplant survival independently of clinical factors. Regardless of disease subtype, TP53-mutated patients with CK were characterized by unique genetic features and associated with an extremely poor survival with frequent early relapse, whereas outcomes were substantially better in TP53-mutated patients without CK. By contrast, the effects of RAS-pathway mutations depended on disease subtype and were confined to myelodysplastic/myeloproliferative neoplasms (MDS/MPNs). Our results suggest that TP53 and RAS-pathway mutations predicted a dismal prognosis, when associated with CK and MDS/MPNs, respectively. However, for patients with mutated TP53 or CK alone, long-term survival could be obtained with transplantation. Clinical sequencing provides vital information for accurate prognostication in transplantation.

Yuan, R., et al. (2005). "Genetic regulation of hematopoietic stem cell exhaustion during development and growth." Exp Hematol **33**(2): 243-250.

OBJECTIVE: During aging, hematopoietic stem cell (HSC) exhaustion is more severe in BALB/cByJ (BALB) mice than in C57BL/6J (B6) mice. Our objective is to determine whether HSC exhaustion during development from fetus to adult also is more severe for BALB than for B6 mice. MATERIALS AND METHODS: Hematopoietic stem cells from fetal liver cells (FLCs) and from young adult bone marrow cells (BMCs) were compared using the competitive repopulation assay to measure long-term repopulating ability (LTRA) and HSC expansion after serial transplantation. LTRAs were measured in repopulating units (RU), as the ability to produce donor-type erythrocytes and lymphocytes in lethally irradiated recipients relative to the congenic fresh marrow competitor. To test expansion, FLCs or BMCs

were serially transplanted into lethally irradiated carriers whose marrow cells were compared using fluorescence-activated cell staining (FACS), and subsequently tested for LTRA. RESULTS: BALB and B6 FLCs, respectively, repopulated 2.6 and 13.5 times as well as BMCs. LTRAs correlated with HSC expansion for BALB, but not B6. Per million donor cells, CD34(-) HSC-enriched fractions (HEFs) and total RU values were 6.8 and 4.6 times higher for FLCs than for BMCs in BALB carriers, while these ratios were only 1.2 and 0.97 higher in B6 carriers. CONCLUSION: In B6 HSC development, LTRA is dissociated from expansion. Although 1 x 10(6) BMCs have much lower LTRA, they expand HSCs as well as 1 x 10(6) FLCs. HSC expansion is partly exhausted in BALB, but not B6, during development.

Zanetta, C., et al. (2014). "Molecular, genetic and stem cell-mediated therapeutic strategies for spinal muscular atrophy (SMA)." J Cell Mol Med **18**(2): 187-196.

Spinal muscular atrophy (SMA) is an autosomal recessive motor neuron disease. It is the first genetic cause of infant mortality. It is caused by mutations in the survival motor neuron 1 (SMN1) gene, leading to the reduction of SMN protein. The most striking component is the loss of alpha motor neurons in the ventral horn of the spinal cord, resulting in progressive paralysis and eventually premature death. There is no current treatment other than supportive care, although the past decade has seen a striking advancement in understanding of both SMA genetics and molecular mechanisms. A variety of disease modifying interventions are rapidly bridging the translational gap from the laboratory to clinical trials. In this review, we would like to outline the most interesting therapeutic strategies that are currently developing, which are represented by molecular, gene and stem cell-mediated approaches for the treatment of SMA.

Zaret, K. S. (2008). "Genetic programming of liver and pancreas progenitors: lessons for stem-cell differentiation." Nat Rev Genet **9**(5): 329-340.

The liver and pancreas arise from a common multipotent population of endoderm cells and share many aspects of their early development. Yet each tissue originates from multiple spatial domains of the endoderm, under the influence of different genes and inductive cues, and obtains different regenerative capacities. Emerging genetic evidence is illuminating the ability of newly specified hepatic and pancreatic progenitors to reverse their course and develop into gut progenitors. Understanding how tissue programming can be reversed and how intrinsic regenerative capacities are determined should facilitate the discovery of the basis of cellular plasticity and aid in the targeted programming and growth of stem cells.

Zhang, Z. P., et al. (2020). "Double sperm cloning (DSC) is a promising strategy in mammalian genetic engineering and stem cell research." Stem Cell Res Ther **11**(1): 388.

Embryonic stem cells (ESCs) derived from somatic cell nuclear transfer (SCNT) and induced pluripotent stem cells (iPSCs) are promising tools for meeting the personalized requirements of regenerative medicine. However, some obstacles need to be overcome before clinical trials can be undertaken. First, donor cells vary, and the reprogramming procedures are diverse, so standardization is a great obstacle regarding SCNT and iPSCs. Second, somatic cells derived from a patient may carry mitochondrial DNA mutations and exhibit telomere instability with aging or disease, and SCNT-ESCs and iPSCs retain the epigenetic memory or epigenetic modification errors. Third, reprogramming efficiency has remained low. Therefore, in addition to improving their success rate, other alternatives for producing ESCs should be explored. Producing androgenetic diploid embryos could be an outstanding strategy; androgenic diploid embryos are produced through double sperm cloning (DSC), in which two capacitated sperms (XY or XX, sorted by flow cytometer) are injected into a denucleated oocyte by intracytoplasmic sperm injection (ICSI) to reconstruct embryo and derive DSC-ESCs. This process could avoid some potential issues, such as mitochondrial interference, telomere shortening, and somatic epigenetic memory, all of which accompany somatic donor cells. Oocytes are naturally activated by sperm, which is unlike the artificial activation that occurs in SCNT. The procedure is simple and practical and can be easily standardized. In addition, DSC-ESCs can overcome ethical concerns and resolve immunological response matching with sperm providers. Certainly, some challenges must be faced regarding imprinted genes, epigenetics, X chromosome inactivation, and dosage compensation. In mice, DSC-ESCs have been produced and have shown excellent differentiation ability. Therefore, the many advantages of DSC make the study of this process worthwhile for regenerative medicine and animal breeding.

Zhao, L., et al. (2019). "Genetic communication by extracellular vesicles is an important mechanism underlying stem cell-based therapy-mediated protection against acute kidney injury." Stem Cell Res Ther **10**(1): 119.

Stem cell-based therapy appears to be a promising new candidate for acute kidney injury (AKI) management. Traditionally, it has been accepted that the mechanism underlying the regenerative effect of

stem cells is based on their paracrine/endocrine activity, including release of bioactive factors that act on injured renal cells and presentation of proangiogenic, antiapoptotic, antioxidative, and immunomodulatory effects. Recently, multiple studies have confirmed that extracellular vesicles (EVs) are a kind of vesicle rich in a broad variety of biologically active molecules, including lipids, proteins, and, in particular, nucleic acids. EVs are able to transfer genetic information to target cells, alter target gene regulatory networks, and exert biological effects. Stem cell-derived EVs (SC-EVs) are emerging as potent genetic information sources that deliver mRNAs and miRNAs to injured renal cells and exert renoprotective effects during AKI. On the other hand, EVs originating from injured renal cells also contain genetic information that is believed to be able to influence phenotypic and functional changes in stem cells, favoring renal recovery. In this review, we summarize studies providing evidence of genetic communication during the application of stem cells in preclinical AKI models, aiming to clarify the mechanism and describe the therapeutic effects of stem cell-based therapy in AKI patients.

Zhou, X., et al. (2015). "The Genetic Landscape of Hematopoietic Stem Cell Frequency in Mice." Stem Cell Reports **5**(1): 125-138.

Prior efforts to identify regulators of hematopoietic stem cell physiology have relied mainly on candidate gene approaches with genetically modified mice. Here we used a genome-wide association study (GWAS) strategy with the hybrid mouse diversity panel to identify the genetic determinants of hematopoietic stem/progenitor cell (HSPC) frequency. Among 108 strains, we observed approximately 120- to 300-fold variation in three HSPC populations. A GWAS analysis identified several loci that were significantly associated with HSPC frequency, including a locus on chromosome 5 harboring the homeodomain-only protein gene (Hopx). Hopx previously had been implicated in cardiac development but was not known to influence HSPC biology. Analysis of the HSPC pool in Hopx-/- mice demonstrated significantly reduced cell frequencies and impaired engraftment in competitive repopulation assays, thus providing functional validation of this positional candidate gene. These results demonstrate the power of GWAS in mice to identify genetic determinants of the hematopoietic system.

Zhu, Y. and X. Feng (2018). "Genetic contribution to mesenchymal stem cell dysfunction in systemic lupus erythematosus." Stem Cell Res Ther **9**(1): 149.

Allogeneic mesenchymal stem cell (MSC) transplantation has recently become a promising therapy for patients with systemic lupus erythematosus (SLE). MSCs are a kind of multipotent stem cell than can efficiently modulate both innate and adaptive immune responses, yet those from SLE patients themselves fail to maintain the balance of immune cells, which is partly due to the abnormal genetic background. Clarifying genetic factors associated with MSC dysfunction may be helpful to delineate SLE pathogenesis and provide new therapeutic targets. In this review, the scientific evidence on the genetic contribution to MSC dysfunction in SLE is summarized.

Zou, C., et al. (2012). "Efficient derivation and genetic modifications of human pluripotent stem cells on engineered human feeder cell lines." Stem Cells Dev **21**(12): 2298-2311.

Derivation of pluripotent stem cells (iPSCs) induced from somatic cell types and the subsequent genetic modifications of disease-specific or patient-specific iPSCs are crucial steps in their applications for disease modeling as well as future cell and gene therapies. Conventional procedures of these processes require co-culture with primary mouse embryonic fibroblasts (MEFs) to support self-renewal and clonal growth of human iPSCs as well as embryonic stem cells (ESCs). However, the variability of MEF quality affects the efficiencies of all these steps. Furthermore, animal sourced feeders may hinder the clinical applications of human stem cells. In order to overcome these hurdles, we established immortalized human feeder cell lines by stably expressing human telomerase reverse transcriptase, Wnt3a, and drug resistance genes in adult mesenchymal stem cells. Here, we show that these immortalized human feeders support efficient derivation of virus-free, integration-free human iPSCs and long-term expansion of human iPSCs and ESCs. Moreover, the drug-resistance feature of these feeders also supports nonviral gene transfer and expression at a high efficiency, mediated by piggyBac DNA transposition. Importantly, these human feeders exhibit superior ability over MEFs in supporting homologous recombination-mediated gene targeting in human iPSCs, allowing us to efficiently target a transgene into the AAVS1 safe harbor locus in recently derived integration-free iPSCs. Our results have great implications in disease modeling and translational applications of human iPSCs, as these engineered human cell lines provide a more efficient tool for genetic modifications and a safer alternative for supporting self-renewal of human iPSCs and ESCs.

Zuo, L., et al. (2014). "Association of a common genetic variant in prostate stem cell antigen with cancer risk." Arch Med Sci **10**(3): 425-433.

INTRODUCTION: Polymorphisms in the prostate stem cell antigen (PSCA) gene have been

hypothesized to increase the genetic susceptibility to cancers. The common sequence variation in PSCA rs2294008 (C>T) has been implicated in cancer risk. However, results of the relevant published studies were somewhat underpowered and controversial in general. MATERIAL AND METHODS: To evaluate the role of PSCA rs2294008 (C>T) genotype in global cancer, we performed a pooled analysis of all the available published studies involving 22,817 cancer patients and 27,753 control subjects. RESULTS: The results showed evidence that PSCA rs2294008 (C>T) was associated with increased total cancer risk in the overall comparisons. Stratified analysis by cancer type indicated that PSCA rs2294008 T is associated with increased risk of gastric cancer (OR = 1.24, 95% CI = 1.09-1.42, p heterogeneity < 0.001, I (2) = 88.0%) and bladder cancer (OR = 1.07, 95% CI = 1.04-1.11, p heterogeneity = 0.108, I (2) = 55.0%) by allelic contrast. Furthermore, in stratified analysis by histological types of gastric cancer, this PSCA variant showed significant associations with diffuse type (OR = 1.81, 95% CI = 1.16-2.81, p heterogeneity < 0.001, I (2) = 88.9%) but not intestinal type (OR = 1.29, 95% CI = 0.95-1.74, p heterogeneity < 0.001, I (2) = 85.2%) in a dominant genetic model. Similar results were found in Asian and European descendents and population-based studies. CONCLUSIONS: In all, our meta-analysis suggests that PSCA rs2294008 (C>T) may play allele-specific roles in cancer development. Further prospective studies with larger numbers of participants worldwide should be performed in different kinds of cancer and other descendents in more detail.

The above contents are the collected information from Internet and public resources to offer to the people for the convenient reading and information disseminating and sharing.

References

[1]. Baidu. http://www.baidu.com. 2022.
[2]. Cancer Biology. http://www.cancerbio.net. 2022.
[3]. Google. http://www.google.com. 2022.
[4]. Journal of American Science. http://www.jofamericanscience.org. 2022.
[5]. Life Science Journal. http://www.lifesciencesite.com. 2022.
[6]. Ma H, Chen G. Stem cell. The Journal of American Science 2005;1(2):90-92. doi:10.7537/marsjas010205.14. http://www.jofamericanscience.org/journals/am-sci/0102/14-mahongbao.pdf.
[7]. Ma H, Cherng S. Eternal Life and Stem Cell. Nature and Science. 2007;5(1):81-96. doi:10.7537/marsnsj050107.10. http://www.sciencepub.net/nature/0501/10-0247-mahongbao-eternal-ns.pdf.
[8]. Ma H, Cherng S. Nature of Life. Life Science Journal 2005;2(1):7-15. doi:10.7537/marslsj020105.03. http://www.lifesciencesite.com/lsj/life0201/life-0201-03.pdf.
[9]. Ma H, Yang Y. Turritopsis nutricula. Nature and Science 2010;8(2):15-20. doi:10.7537/marsnsj080210.03. http://www.sciencepub.net/nature/ns0802/03_1279_hongbao_turritopsis_ns0802_15_20.pdf.
[10]. Ma H. The Nature of Time and Space. Nature and science 2003;1(1):1-11. doi:10.7537/marsnsj010103.01. http://www.sciencepub.net/nature/0101/01-ma.pdf.
[11]. Marsland Press. http://www.sciencepub.net. 2022.
[12]. Marsland Press. http://www.sciencepub.org. 2022.
[13]. National Center for Biotechnology Information, U.S. National Library of Medicine. http://www.ncbi.nlm.nih.gov/pubmed. 2022.
[14]. Nature and Science. http://www.sciencepub.net/nature. 2022.
[15]. Stem Cell. http://www.sciencepub.net/stem. 2022.
[16]. Wikipedia. The free encyclopedia. http://en.wikipedia.org. 2022.

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