Somatic Stem Cell 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; 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.

Adams, J. G., et al. (1991). "Disappearance of the protein of a somatic mutation: a possible example of stem cell inactivation." Am J Physiol **261**(3 Pt 1): C448-454.

The low concentration of the hemoglobin variant, Hb Vicksburg (leucine-beta-75 deleted), and a profound deficit of its mRNA led us to postulate that a beta (+)-thalassemia mutation existed in cis to the coding region mutation, suppressing its synthesis. We examined blood from this patient 6, 8, and 10 yr after our initial studies, using methods of analysis unavailable initially. We found 1) mutations causing beta (+)-(-88 C----T) and beta 0-(849 A----G) thalassemia; 2) that the proportion of Hb Vicksburg in erythrocytes fell over time, from 8 to 4%, and ultimately disappeared; and 3) that the mutation causing Hb Vicksburg was not detectable in genomic DNA isolated from blood leukocytes when this variant was present in hemolysate. We postulate that Hb Vicksburg arose from a somatic mutation of a beta (+)-thalassemia gene in an erythroid-committed stem cell. Its gradual disappearance suggests the cycling of stem cells, with inactivation of different clones over time.

Adorno, M., et al. (2013). "Usp16 contributes to somatic stem-cell defects in Down's syndrome." Nature **501**(7467): 380-384.

Down's syndrome results from full or partial trisomy of chromosome 21. However, the consequences of the underlying gene-dosage imbalance on adult tissues remain poorly understood. Here we show that in Ts65Dn mice, which are trisomic for 132 genes homologous to genes on human chromosome 21, triplication of Usp16 reduces the self-renewal of haematopoietic stem cells and the expansion of mammary epithelial cells, neural progenitors and fibroblasts. In addition, Usp16 is associated with decreased ubiquitination of Cdkn2a and accelerated senescence in Ts65Dn fibroblasts. Usp16 can remove ubiquitin from histone H2A on lysine 119, a critical mark for the maintenance of multiple somatic tissues. Downregulation of Usp16, either by mutation of a single normal Usp16 allele or by short interfering RNAs, largely rescues all of these defects. Furthermore, in human tissues overexpression of USP16 reduces the expansion of normal fibroblasts and postnatal neural progenitors, whereas downregulation of USP16 partially rescues the proliferation defects of Down's syndrome fibroblasts. Taken together, these results suggest that USP16 has an important role in antagonizing the self-renewal and/or senescence pathways in Down's syndrome and could serve as an attractive target to ameliorate some of the associated pathologies.

Aktas, M., et al. (2010). "Good manufacturing practice-grade production of unrestricted somatic stem cell from fresh cord blood." Cytotherapy **12**(3): 338-348.

BACKGROUND AIMS: The discovery of unrestricted somatic stem cells (USSC), a non-hematopoietic stem cell population, brought cord blood (CB) to the attention of regenerative medicine for defining more protocols for non-hematopoietic indications. We demonstrate that a reliable and reproducible method for good manufacturing practice (GMP)-conforming generation of USSC is possible that fulfils safety requirements as well as criteria for clinical applications, such as adherence of strict regulations on cell isolation and expansion. METHODS: In order to maintain GMP conformity, the automated cell processing system Sepax (Biosafe) was implemented for mononucleated cell (MNC) separation from fresh CB. After USSC generation, clinical-scale expansion was achieved by multi-layered CellSTACKs (Costar/Corning). Infectious disease markers, pyrogen and endotoxin levels, immunophenotype, potency, genetic stability and sterility of the cell product were evaluated. RESULTS: The MNC isolation and cell cultivation methods used led to safe and reproducible GMP-conforming USSC production while maintaining somatic stem cell character. CONCLUSIONS: Together with implemented in-process controls guaranteeing contamination-free products with adult stem cell character, USSC produced as suggested here may serve as a universal allogeneic stem cell source for future cell treatment and clinical settings.

Albert, E. A., et al. (2018). "Direct control of somatic stem cell proliferation factors by the Drosophila testis stem cell niche." Development **145**(17).

Niches have traditionally been characterised as signalling microenvironments that allow stem cells to maintain their fate. This definition implicitly assumes that the various niche signals are integrated towards a binary fate decision between stemness and differentiation. However, observations in multiple systems have demonstrated that stem cell properties, such as proliferation and self-renewal, can be uncoupled at the level of niche signalling input, which is incompatible with this simplified view. We have studied the role of the transcriptional regulator Zfh1, a shared target of the Hedgehog and Jak/Stat niche signalling pathways, in the somatic stem cells of the Drosophila testis. We found that Zfh1 binds and downregulates salvador and kibra, two tumour suppressor genes of the Hippo/Wts/Yki pathway, thereby restricting Yki activation and proliferation to the Zfh1(+) stem cells. These observations provide an unbroken link from niche signal input to an individual aspect of stem cell behaviour that does not, at any step, involve a fate decision. We discuss the relevance of these findings for an overall concept of stemness and niche function.

Amoyel, M., et al. (2016). "Somatic stem cell differentiation is regulated by PI3K/Tor signaling in response to local cues." Development **143**(21): 3914-3925.

Stem cells reside in niches that provide signals to maintain self-renewal, and differentiation is viewed as a passive process that depends on loss of access to these signals. Here, we demonstrate that the differentiation of somatic cyst stem cells (CySCs) in the Drosophila testis is actively promoted by PI3K/Tor signaling, as CySCs lacking PI3K/Tor activity cannot differentiate properly. We find that an insulin peptide produced by somatic cells immediately outside of the stem cell niche acts locally to promote somatic differentiation through Insulin-like receptor (InR) activation. These results indicate that there is a local 'differentiation' niche that upregulates PI3K/Tor signaling in the early daughters of CySCs. Finally, we demonstrate that CySCs secrete the Dilp-binding protein ImpL2, the Drosophila homolog of IGFBP7, into the stem cell niche, which blocks InR activation in CySCs. Thus, we show that somatic cell differentiation is controlled by PI3K/Tor signaling downstream of InR and that the local production of positive and negative InR signals regulates the differentiation niche. These results support a model in which leaving the stem cell niche and initiating differentiation are actively induced by signaling.

Anisimov, S. V. (2009). "[Cell therapy for Parkinson's disease: II. Somatic stem cell-based applications]." Adv Gerontol **22**(1): 150-166.

Motor dysfunctions in Parkinson's disease are believed to be primarily due to the degeneration of dopaminergic neurons located in the substantia nigra pars compacta. Numerous cell replacement therapy approaches have been developed and tested, including these based on donor cell transplantation (embryonic and adult tissue-derived), adult mesenchymal stem cells (hMSCs)-, neural stem cells (hNSCs)- and finally human embryonic stem cells (hESCs)-based. Despite the progress achieved, numerous difficulties prevent wider practical application of stem cell-based therapy approaches for the treatment of Parkinson's disease. Among the latter, ethical, safety and technical issues stand out. Current series of reviews (Cell therapy for Parkinson's disease: I. Embryonic and adult donor tissue-based applications; II. Adult stem cell-based applications; III. Neonatal, fetal and embryonic stem cell-based applications; IV. Risks and future trends)

aims providing a balanced and updated view on various issues associated with cell types (including stem cells) in regards to their potential in the treatment of Parkinson's disease. Essential features of the individual cell subtypes, principles of available cell handling protocols, transplantation, and safety issues are discussed extensively.

Anne Cook, H., et al. (2000). "Crypt-restricted metallothionein immunopositivity in murine colon: validation of a model for studies of somatic stem cell mutation." J Pathol **191**(3): 306-312.

The ability to visualize the cellular effects of a somatic mutation is relevant to studies of cell kinetics and carcinogenesis. In the colon, mutagen administration leads to scattered crypt-restricted loss of activity of the X-linked enzyme glucose-6-phosphate dehydrogenase (G6PD); it has been shown that this is due to somatic mutation in the G6PD gene. Mutagen-induced crypt-restricted immunopositivity for metallothionein (MT) has been reported in one study in the mouse colon; if this is also due to somatic mutation, it provides a simple method for studying the phenomenon which could be carried out on paraffin sections. This study shows that, as in the G6PD model, the frequency of crypt-restricted immunopositivity for MT is very low in untreated animals, but increases proportionately with the dose of mutagen administered. There is a good overall correlation of a range of MT-positive crypt frequencies with those derived from studies using G6PD. As with the G6PD model, the MT-positive crypt phenotype evolves over time after mutagen administration; initially individual crypts include both positive and negative phenotype cells, but later almost all involved crypts are composed entirely of MT-positive cells. The frequency of MT-positive crypts stabilizes after a few weeks and remains at the same level 6 months later. All these observations are qualitatively identical to those found using the G6PD model and provide strong evidence that stable, crypt-restricted immunopositivity for MT results from a mutation affecting expression of the metallothionein gene in a colonic stem cell. This model will provide a useful tool to study factors influencing stem cell mutation frequency and cell kinetics in the colon.

Araki, R., et al. (2017). "The Number of Point Mutations in Induced Pluripotent Stem Cells and Nuclear Transfer Embryonic Stem Cells Depends on the Method and Somatic Cell Type Used for Their Generation." Stem Cells **35**(5): 1189-1196.

Induced pluripotent stem cells hold great promise for regenerative medicine but point mutations have been identified in these cells and have raised serious concerns about their safe use. We generated nuclear transfer embryonic stem cells (ntESCs) from both mouse embryonic fibroblasts (MEFs) and tail-tip fibroblasts (TTFs) and by whole genome sequencing found fewer mutations compared with iPSCs generated by retroviral gene transduction. Furthermore, TTF-derived ntESCs showed only a very small number of point mutations, approximately 80% less than the number observed in iPSCs generated using retrovirus. Base substitution profile analysis confirmed this greatly reduced number of point mutations. The point mutations in iPSCs are therefore not a Yamanaka factor-specific phenomenon but are intrinsic to genome reprogramming. Moreover, the dramatic reduction in point mutations in ntESCs suggests that most are not essential for genome reprogramming. Our results suggest that it is feasible to reduce the point mutation frequency in iPSCs by optimizing various genome reprogramming conditions. We conducted whole genome sequencing of ntES cells derived from MEFs or TTFs. We thereby succeeded in establishing TTF-derived ntES cell lines with far fewer point mutations. Base substitution profile analysis of these clones also indicated a reduced point mutation frequency, moving from a transversion-predominance to a transition-predominance. Stem Cells 2017;35:1189-1196.

Arnaud, D., et al. (1993). "A panel of deleted mouse X chromosome somatic cell hybrids derived from the embryonic stem cell line HD3 shows preferential breakage in the Hprt-DXHX254E region." Genomics **18**(3): 520-526.

A panel of 91 somatic cell hybrids containing deleted mouse X chromosomes and falling into seven nested intervals has been isolated and characterized from fusions involving the murine embryonic stem cell HD3. Many of the X chromosome breakpoints present in these hybrids fall within regions in which few or no other hybrids were previously available. The apparent enrichment for breakpoints lying within the Hprt-DXHX254E region is discussed in relation to both the nature of the embryonic stem cell fusions and the presence of the Fmr1 gene associated with FRAXA in man within this span.

Bejar, R., et al. (2014). "Somatic mutations predict poor outcome in patients with myelodysplastic syndrome after hematopoietic stem-cell transplantation." J Clin Oncol **32**(25): 2691-2698.

PURPOSE: Recurrently mutated genes in myelodysplastic syndrome (MDS) are pathogenic drivers and powerfully associated with clinical phenotype and prognosis. Whether these types of mutations predict outcome after allogeneic hematopoietic stem-cell transplantation (HSCT) in patients with MDS is not known. PATIENTS AND METHODS: We used massively parallel sequencing

to examine tumor samples collected from 87 patients with MDS before HSCT for coding mutations in 40 recurrently mutated MDS genes. RESULTS: Mutations were identified in 92% of patients, most frequently in the ASXL1 (29%), TP53 (21%), DNMT3A (18%), and RUNX1 (16%) genes. In univariable analyses, only TP53 mutations were associated with shorter overall (OS; hazard ratio [HR], 3.74; P <.001) and progression-free survival (HR, 3.97; P <.001). After adjustment for clinical variables associated with these end points, mutations in TP53 (HR, 2.30; P =.027), TET2 (HR, 2.40; P =.033), and DNMT3A (HR, 2.08; P =.049) were associated with decreased OS. In multivariable analysis including clinical variables, complex karyotype status, and candidate genes, mutations in TP53 (HR, 4.22; P </=.001) and TET2 (HR, 1.68; P =.037) were each independently associated with shorter OS. Nearly one half of patients (46%) carried a mutation in TP53, DNMT3A, or TET2 and accounted for 64% of deaths. Three-year OS in patients without these mutations was 59% (95% CI, 43% to 72%), versus 19% (95% CI, 9% to 33%) in patients with these mutations. CONCLUSION: Mutations in TP53, TET2, or DNMT3A identify patients with MDS with shorter OS after HSCT.

Bhartiya, D., et al. (2012). "Stem cell interaction with somatic niche may hold the key to fertility restoration in cancer patients." Obstet Gynecol Int **2012**: 921082.

The spontaneous return of fertility after bone marrow transplantation or heterotopic grafting of cryopreserved ovarian cortical tissue has surprised many, and a possible link with stem cells has been proposed. We have reviewed the available literature on ovarian stem cells in adult mammalian ovaries and presented a model that proposes that the ovary harbors two distinct populations of stem cells, namely, pluripotent, quiescent, very small embryonic-like stem cells (VSELs), and slightly larger "progenitor" ovarian germ stem cells (OGSCs). Besides compromising the somatic niche, oncotherapy destroys OGSCs since, like tumor cells, they are actively dividing; however VSELs persist since they are relatively quiescent. BMT or transplanted ovarian cortical tissue may help rejuvenate the ovarian niche, which possibly supports differentiation of persisting VSELs resulting in neo-oogenesis and follicular development responsible for successful pregnancies. Postnatal oogenesis in mammalian ovary from VSELs may be exploited for fertility restoration in cancer survivors including those who were earlier deprived of gametes and/or gonadal tissue cryopreservation options.

Biteau, B., et al. (2011). "Maintaining tissue homeostasis: dynamic control of somatic stem cell activity." Cell Stem Cell **9**(5): 402-411.

Long-term maintenance of tissue homeostasis relies on the accurate regulation of somatic stem cell activity. Somatic stem cells have to respond to tissue damage and proliferate according to tissue requirements while avoiding overproliferation. The regulatory mechanisms involved in these responses are now being unraveled in the intestinal epithelium of Drosophila, providing new insight into strategies and mechanisms of stem cell regulation in barrier epithelia. Here, we review these studies and highlight recent findings in vertebrate epithelia that indicate significant conservation of regenerative strategies between vertebrate and fly epithelia.

Bonde, S., et al. (2010). "Cell fusion of bone marrow cells and somatic cell reprogramming by embryonic stem cells." FASEB J **24**(2): 364-373.

Bone marrow transplantation is a curative treatment for many diseases, including leukemia, autoimmune diseases, and a number of immunodeficiencies. Recently, it was claimed that bone marrow cells transdifferentiate, a much desired property as bone marrow cells are abundant and therefore could be used in regenerative medicine to treat incurable chronic diseases. Using a Cre/loxP system, we studied cell fusion after bone marrow transplantation. Fused cells were chiefly Gr-1(+), a myeloid cell marker, and found predominantly in the bone marrow; in parenchymal tissues. Surprisingly, fused cells were most abundant in the kidney, Peyer's patches, and cardiac tissue. In contrast, after cell fusion with embryonic stem cells, bone marrow cells were reprogrammed into new tetraploid pluripotent stem cells that successfully differentiated into beating cardiomyocytes. Together, these data suggest that cell fusion is ubiquitous after cellular transplants and that the subsequent sharing of genetic material between the fusion partners affects cellular survival and function. Fusion between tumor cells and bone marrow cells could have consequences for tumor malignancy.

Boonkaew, B., et al. (2018). "Induced pluripotent stem cell line MUSIi006-A derived from hair follicle keratinocytes as a non-invasive somatic cell source." Stem Cell Res **31**: 79-82.

In this study, we used hair follicle keratinocytes for reprogramming. Collection of plucked hairs offers advantages over other somatic cells because no medical professional or operation room is required. Keratinocytes were isolated from plucked hairs of a 21-year-old healthy woman and characterized for the expression of cytokeratin 14 (CK14). Reprogramming of keratinocytes was performed using Sendai virus.

Further characterization of the keratinocyte-derived iPSC line (designated as MUSIi006-A) confirmed that the cell line was pluripotent, free from Sendai viral genome and transgenes, and retained normal karyotype. Our method represents an easy, non-invasive and efficient approach for iPSC generation from hair samples.

Bosse, R., et al. (1997). "Good manufacturing practice production of human stem cells for somatic cell and gene therapy." Stem Cells **15 Suppl 1**: 275-280.

Peripheral blood stem cells (PBSC) are used for transplantation to reconstitute the hematopoietic system after high-dose chemotherapy. PBSC are harvested from peripheral blood upon successful mobilization by cytokines and/or chemotherapy. Further in vitro manipulation steps like enrichment of CD34+ PBSC or gene transfer can be performed. To ensure the quality and safety of the final cell preparations intended for transplantation, national and international guidelines and regulations have been issued. Herein the implementation of a quality assurance program including the principles of good manufacturing practice (GMP) and a quality control (QC) system is one major concern. GMP regulations apply to all phases of cell collection, processing and storage as well as documentation, training of personnel, and the laboratory facility. QC measures have to be taken to ensure consistent quality and safety with an emphasis on preventing any deficiencies.

Byrne, J. A., et al. (2007). "Producing primate embryonic stem cells by somatic cell nuclear transfer." Nature **450**(7169): 497-502.

Derivation of embryonic stem (ES) cells genetically identical to a patient by somatic cell nuclear transfer (SCNT) holds the potential to cure or alleviate the symptoms of many degenerative diseases while circumventing concerns regarding rejection by the host immune system. However, the concept has only been achieved in the mouse, whereas inefficient reprogramming and poor embryonic development characterizes the results obtained in primates. Here, we used a modified SCNT approach to produce rhesus macaque blastocysts from adult skin fibroblasts, and successfully isolated two ES cell lines from these embryos. DNA analysis confirmed that nuclear DNA was identical to donor somatic cells and that mitochondrial DNA originated from oocytes. Both cell lines exhibited normal ES cell morphology, expressed key stem-cell markers, were transcriptionally similar to control ES cells and differentiated into multiple cell types in vitro and in vivo. Our results represent successful nuclear reprogramming of adult somatic cells into pluripotent ES cells and demonstrate proof-of-concept for therapeutic cloning in primates.

Byrne, J. A., et al. (2003). "Nuclei of adult mammalian somatic cells are directly reprogrammed to oct-4 stem cell gene expression by amphibian oocytes." Curr Biol **13**(14): 1206-1213.

Nuclear reprogramming by the transplantation of somatic cell nuclei to eggs (in second meiotic metaphase) is always followed by a phase of chromosome replication and cell division before new gene expression is seen. To help understand the mechanism of nuclear reprogramming, we have asked whether the nuclei of normal, nontransformed, nondividing, and terminally differentiated mammalian cells can be directly reprogrammed, without DNA replication, by Xenopus oocytes. We find that nuclei of adult mouse thymocytes and of adult human blood lymphocytes, injected into Xenopus oocytes, are induced to extinguish a differentiation marker and to strongly express oct-4, the most diagnostic mammalian stem cell/pluripotency marker. In the course of 2 days at 18 degrees C, the mammalian oct-4 transcripts are spliced to mature mRNA. We conclude that normal mammalian nuclei can be directly reprogrammed by the nucleus (germinal vesicle) of amphibian oocytes to express oct-4 at a rate comparable to that of oct-4 in mouse ES cells. To our knowledge, this is the first demonstration of a stem cell marker being induced in a differentiated adult human cell nucleus. This is an early step toward the long-term aim of developing a procedure for reprogramming readily accessible human adult cells for cell replacement therapy.

Campbell, F., et al. (1998). "No difference in stem cell somatic mutation between the background mucosa of right- and left-sided sporadic colorectal carcinomas." J Pathol **186**(1): 31-35.

Epidemiological, morphological, and molecular differences exist between carcinomas of the right and left sides of the large bowel. To investigate whether this is reflected in differences in somatic mutation frequency in the background mucosa, mutation of the neutral O-acetyltransferase gene (oat) was quantified in histologically normal resection margins from 20 informative (heterozygous) patients with caecal or ascending colon cancer (11 males, median age 75 years) and 20 with sigmoid colon or rectal cancer (10 males, median age 70 years). Mutant discordant crypts lacking O-acetyltransferase activity were visualized by mPAS staining and classified as wholly or partially involved by the mutant phenotype; median frequencies (x10(-4) were compared (Mann-Whitney U-test) after assessing a sample of more than 10,000 crypts per case. No significant difference was found between the frequencies of wholly involved mPAS-positive crypts

in background mucosa of left- and right-sided cancers ($p = 0.4569$), indicating that tumours on both sides of the colon are associated with similar levels of lifetime-accumulated stem cell mutational load. However, partially involved mPAS-positive crypts were significantly more frequent in mucosa from left-sided cancers ($p < 0.04$), indicating increased mutational activity during the previous 12 months. Analysis of mucosa proximal and distal to left-sided cancers showed that this increase was due to a statistically higher frequency of partially involved crypts in proximal mucosa, which probably resulted from the obstructive effects of the tumour causing increased exposure of the proximal mucosa to luminal carcinogens and/or epithelial regeneration in response to low-grade inflammation or ischaemia. The findings indicate that although left-sided colonic cancer is commoner than right-sided cancer in the British population, carcinomas on both sides of the large bowel arise in a background of similar levels of stem cell mutational activity.

Campbell, F., et al. (1998). "Increased stem cell somatic mutation in the non-neoplastic colorectal mucosa of patients with familial adenomatous polyposis." Hum Pathol **29**(12): 1531-1535.

Colorectal tumorigenesis in familial adenomatous polyposis (FAP) results from somatic mutation of either the normal APC allele or another growth control gene in epithelial cells bearing a germline APC defect. The rate at which tumors develop is therefore dependent on the somatic mutation frequency; it is not known whether this is normal or elevated in FAP. We aimed to quantify stem cell somatic mutation in FAP, comparing it with hereditary nonpolyposis colorectal cancer (HNPCC) and Crohn's disease (CD). Stem cell somatic mutation frequency was studied in 47 FAP patients, 5 HNPCC patients, and 13 CD patients, all younger than 49 years, by quantifying crypt-restricted loss of O-acetyltransferase activity in sections of morphologically normal colonic mucosa from individuals heterozygous for this monogenically inherited polymorphism. Median stem cell somatic mutation frequency was significantly higher in FAP than HNPCC (4.2 x 10(-4) v 1.4 x 10(-4), Mann-Whitney U, P <.02). The level in CD (4.0 x 10(-4)) was similar to FAP. Mutated crypts occurred in groups more frequently in FAP (22%) than HNPCC (12%) or CD (10%), suggesting an increase in stem cell division associated with crypt fission in FAP. We conclude that stem cell somatic mutation frequency is raised in non-neoplastic colorectal mucosa in FAP. This is probably related to increased stem cell proliferation and contributes to the high rate of tumor formation in this condition.

Corti, S., et al. (2004). "Somatic stem cell research for neural repair: current evidence and emerging perspectives." J Cell Mol Med **8**(3): 329-337.

Recent evidence supports the existence of adult mammalian stem cell subpopulations, particularly within the bone marrow, that may be able to "transdifferentiate" across tissue lineage boundaries, thus offering an accessible source for therapeutic applications even for neural tissue repair. However, the difficulties in reproducing some experimental data, the rarity of the transdifferentiation events and observations that cell fusion may be an alternative explanation argue against the idea of stem cell plasticity. Investigations going beyond descriptive experiments and more mechanicistic approaches may provide a more solid foundation to adult stem cell therapeutic potential.

Dabelsteen, S., et al. (2009). "Epithelial cells derived from human embryonic stem cells display p16INK4A senescence, hypermotility, and differentiation properties shared by many P63+ somatic cell types." Stem Cells **27**(6): 1388-1399.

Human embryonic stem (hES) cells can generate cells expressing p63, K14, and involucrin, which have been proposed to be keratinocytes. Although these hES-derived, keratinocyte-like (hESderK) cells form epithelioid colonies when cultured in a fibroblast feeder system optimal for normal tissue-derived keratinocytes, they have a very short replicative lifespan unless engineered to express HPV16 E6E7. We report here that hESderK cells undergo senescence associated with p16(INK4A) expression, unrelated to telomere status. Transduction to express bmi1, a repressor of the p16(INK4A)/p14(ARF) locus, conferred upon hESderK cells and keratinocytes a substantially extended lifespan. When exposed to transforming growth factor beta or to an incompletely processed form of Laminin-332, three lifespan-extended or immortalized hESderK lines that we studied became directionally hypermotile, a wound healing and invasion response previously characterized in keratinocytes. In organotypic culture, hESderK cells stratified and expressed involucrin and K10, as do epidermal keratinocytes in vivo. However, their growth requirements were less stringent than keratinocytes. We then extended the comparison to endoderm-derived, p63(+)/K14(+) urothelial and tracheobronchial epithelial cells. Primary and immortalized lines of these cell types had growth requirements and hypermotility responses similar to keratinocytes and bmi1 expression facilitated their immortalization by engineering to express the catalytic subunit of telomerase (TERT). In organotypic culture, they stratified and exhibited squamous metaplasia, expressing involucrin and K10. Thus, hESderK cells

proved to be distinct from all three normal p63(+) cell types tested. These results indicate that hESderK cells cannot be identified conclusively as keratinocytes or even as ectodermal cells, but may represent an incomplete form of, or deviation from, normal p63(+) lineage development.

Davis, E. A. and M. J. Dailey (2018). "A direct effect of the autonomic nervous system on somatic stem cell proliferation?" Am J Physiol Regul Integr Comp Physiol.

Regulation of somatic stem cell proliferation is critical for the maintenance of tissue and organ function throughout the body. Modulators of this process include nutrients and peptides, but the role of a neural influence on stem cell proliferation has been neglected. This article describes the literature in support of autonomic nervous system (ANS) influence on somatic stem cells, with emphasis on intestinal epithelial stem cells (IESCs) as a representative somatic stem cell. Based on the current available data, models for the direct influence of both branches of the ANS, the sympathetic and parasympathetic nervous systems, on intestinal epithelial stem cells (IESCs) are outlined. Finally, the prospect of treatments derived from ANS influence on somatic stem cells is explored.

Deleyrolle, L. P., et al. (2011). "Determination of somatic and cancer stem cell self-renewing symmetric division rate using sphere assays." PLoS One **6**(1): e15844.

Representing a renewable source for cell replacement, neural stem cells have received substantial attention in recent years. The neurosphere assay represents a method to detect the presence of neural stem cells, however owing to a deficiency of specific and definitive markers to identify them, their quantification and the rate they expand is still indefinite. Here we propose a mathematical interpretation of the neurosphere assay allowing actual measurement of neural stem cell symmetric division frequency. The algorithm of the modeling demonstrates a direct correlation between the overall cell fold expansion over time measured in the sphere assay and the rate stem cells expand via symmetric division. The model offers a methodology to evaluate specifically the effect of diseases and treatments on neural stem cell activity and function. Not only providing new insights in the evaluation of the kinetic features of neural stem cells, our modeling further contemplates cancer biology as cancer stem-like cells have been suggested to maintain tumor growth as somatic stem cells maintain tissue homeostasis. Indeed, tumor stem cell's resistance to therapy makes these cells a necessary target for effective treatment. The neurosphere assay mathematical model presented here allows the assessment of the rate malignant stem-like cells expand via symmetric division and the evaluation of the effects of therapeutics on the self-renewal and proliferative activity of this clinically relevant population that drive tumor growth and recurrence.

Della Porta, M. G., et al. (2016). "Clinical Effects of Driver Somatic Mutations on the Outcomes of Patients With Myelodysplastic Syndromes Treated With Allogeneic Hematopoietic Stem-Cell Transplantation." J Clin Oncol **34**(30): 3627-3637.

PURPOSE: The genetic basis of myelodysplastic syndromes (MDS) is heterogeneous, and various combinations of somatic mutations are associated with different clinical phenotypes and outcomes. Whether the genetic basis of MDS influences the outcome of allogeneic hematopoietic stem-cell transplantation (HSCT) is unclear. PATIENTS AND METHODS: We studied 401 patients with MDS or acute myeloid leukemia (AML) evolving from MDS (MDS/AML). We used massively parallel sequencing to examine tumor samples collected before HSCT for somatic mutations in 34 recurrently mutated genes in myeloid neoplasms. We then analyzed the impact of mutations on the outcome of HSCT. RESULTS: Overall, 87% of patients carried one or more oncogenic mutations. Somatic mutations of ASXL1, RUNX1, and TP53 were independent predictors of relapse and overall survival after HSCT in both patients with MDS and patients with MDS/AML (P values ranging from.003 to.035). In patients with MDS/AML, gene ontology (ie, secondary-type AML carrying mutations in genes of RNA splicing machinery, TP53-mutated AML, or de novo AML) was an independent predictor of posttransplantation outcome (P =.013). The impact of ASXL1, RUNX1, and TP53 mutations on posttransplantation survival was independent of the revised International Prognostic Scoring System (IPSS-R). Combining somatic mutations and IPSS-R risk improved the ability to stratify patients by capturing more prognostic information at an individual level. Accounting for various combinations of IPSS-R risk and somatic mutations, the 5-year probability of survival after HSCT ranged from 0% to 73%. CONCLUSION: Somatic mutation in ASXL1, RUNX1, or TP53 is independently associated with unfavorable outcomes and shorter survival after allogeneic HSCT for patients with MDS and MDS/AML. Accounting for these genetic lesions may improve the prognostication precision in clinical practice and in designing clinical trials.

Do, E. K., et al. (2014). "Reptin regulates pluripotency of embryonic stem cells and somatic cell reprogramming through Oct4-dependent mechanism." Stem Cells **32**(12): 3126-3136.

Oct4 has been implicated in regulation of pluripotency in embryonic stem cells (ESCs) and reprogramming of somatic cells into induced pluripotent stem cells. However, the molecular mechanisms involved in Oct4-dependent regulation of pluripotency and reprogramming have not been clear. To gain insight into the mechanism of regulation of Oct4-mediated self-renewal of ESCs and reprogramming of somatic cells, we attempted to identify Oct4-binding proteins using affinity purification and mass spectrometry. We identified Reptin, a key component of ATP-dependent chromatin remodeling complexes, as an Oct4-binding protein. Depletion of endogenous Reptin using lentiviral short hairpin RNA (shRNA) led to a decrease in the number and size of alkaline phosphatase-positive colonies of mouse ESCs. In addition, shRNA-mediated silencing of Reptin resulted in decreased expression of pluripotency-specific marker genes, including Oct4, Sox2, Nanog, and SSEA-1. Results of the Oct4 reporter assay showed synergism between Oct4 and Reptin, and depletion of endogenous Reptin abolished Oct4 transcriptional activity. Results of a chromatin immunoprecipitation assay showed the overlapping interaction of Reptin and Oct4 to CR4 in the Oct4 enhancer in ESCs. Knockdown of Reptin using shRNA suppressed the reprogramming of mouse embryonic fibroblasts to induced pluripotent stem cells, whereas overexpression of Reptin resulted in enhanced efficiency of induced pluripotent stem cell generation. These results strongly suggest that Reptin plays a key role in maintaining the pluripotency of ESCs and in establishing the pluripotency during reprogramming of somatic cells by regulation of Oct4-mediated gene regulation.

Dominado, N., et al. (2016). "Rbf Regulates Drosophila Spermatogenesis via Control of Somatic Stem and Progenitor Cell Fate in the Larval Testis." Stem Cell Reports **7**(6): 1152-1163.

The Drosophila testis has been fundamental to understanding how stem cells interact with their endogenous microenvironment, or niche, to control organ growth in vivo. Here, we report the identification of two independent alleles for the highly conserved tumor suppressor gene, Retinoblastoma-family protein (Rbf), in a screen for testis phenotypes in X chromosome third-instar lethal alleles. Rbf mutant alleles exhibit overproliferation of spermatogonial cells, which is phenocopied by the molecularly characterized Rbf (11) null allele. We demonstrate that Rbf promotes cell-cycle exit and differentiation of the somatic and germline stem cells of the testes. Intriguingly, depletion of Rbf specifically in the germline does not disrupt stem cell differentiation, rather Rbf loss of function in the somatic lineage drives overproliferation and differentiation defects in both lineages. Together our observations suggest that Rbf in the somatic lineage controls germline stem cell renewal and differentiation non-autonomously via essential roles in the microenvironment of the germline lineage.

Easley, C. A., et al. (2014). "Gamete derivation from embryonic stem cells, induced pluripotent stem cells or somatic cell nuclear transfer-derived embryonic stem cells: state of the art." Reprod Fertil Dev **27**(1): 89-92.

Generating gametes from pluripotent stem cells (PSCs) has many scientific justifications and several biomedical rationales. Here, we consider several strategies for deriving gametes from PSCs from mice and primates (human and non-human) and their anticipated strengths, challenges and limitations. Although the 'Weismann barrier', which separates the mortal somatic cell lineages from the potentially immortal germline, has long existed, breakthroughs first in mice and now in humans are artificially creating germ cells from somatic cells. Spermatozoa with full reproductive viability establishing multiple generations of seemingly normal offspring have been reported in mice and, in humans, haploid spermatids with correct parent-of-origin imprints have been obtained. Similar progress with making oocytes has been published using mouse PSCs differentiated in vitro into primordial germ cells, which are then cultured after xenografting reconstructed artificial ovaries. Progress in making human oocytes artificially is proving challenging. The usefulness of these artificial gametes, from assessing environmental exposure toxicity to optimising medical treatments to prevent negative off-target effects on fertility, may prove invaluable, as may basic discoveries on the fundamental mechanisms of gametogenesis.

Eve, D. J., et al. (2018). "Human Somatic Stem Cell Neural Differentiation Potential." Results Probl Cell Differ **66**: 21-87.

Human somatic stem cells can be identified and isolated from different types of tissues and are grouped here based on their developmental maturation and ability to undergo neural differentiation. The first group will represent afterbirth somatic tissues, which are perinatal stem cells including placental blood and tissue, amniotic fluid and tissue, and umbilical cord blood- and umbilical cord tissue-derived cells. The second group of cells discussed in this chapter is the adult stem cells, generally those in a transient period of development, thus placing them in the special position of transitioning from the perinatal to young somatic tissue, and they include the menstrual blood-,

the peripheral blood-, and the bone marrow-derived stem cells.

Faast, R., et al. (2006). "Use of adult mesenchymal stem cells isolated from bone marrow and blood for somatic cell nuclear transfer in pigs." Cloning Stem Cells **8**(3): 166-173.

Mesenchymal stem cells (MSCs) isolated from bone marrow were used to examine the hypothesis that a less differentiated cell type could increase adult somatic cell nuclear transfer (SCNT) efficiencies in the pig. SCNT embryos were produced using a fusion before activation protocol described previously and the rate at which these developed to the blastocyst stage compared with that using fibroblasts obtained from ear tissue from the same animal. The use of bone marrow MSCs did not increase cleavage rates compared with adult fibroblasts. However, the percentage of embryos that developed to the blastocyst stage was almost doubled, providing support for the hypothesis that a less differentiated cell can increase cloning efficiencies. As MSCs are relatively difficult to isolate from the bone marrow of live animals, a second experiment was undertaken to determine whether MSCs could be isolated from the peripheral circulation and used for SCNT. Blood MSCs were successfully isolated from four of the five pigs sampled. These cells had a similar differentiation capacity and marker profile to those isolated from bone marrow but did not result in increased rates of development. This is the first study to our knowledge, to report that MSCs can be derived from peripheral blood and used for SCNT for any species. These cells can be readily obtained under relatively sterile conditions compared with adult fibroblasts and as such, may provide an alternative cell type for cloning live animals.

Foshay, K. M., et al. (2012). "Embryonic stem cells induce pluripotency in somatic cell fusion through biphasic reprogramming." Mol Cell **46**(2): 159-170.

It is a long-held paradigm that cell fusion reprograms gene expression but the extent of reprogramming and whether it is affected by the cell types employed remain unknown. We recently showed that the silencing of somatic genes is attributable to either trans-acting cellular environment or cis-acting chromatin context. Here, we examine how trans- versus cis-silenced genes in a somatic cell type behave in fusions to another somatic cell type or to embryonic stem cells (ESCs). We demonstrate that while reprogramming of trans-silenced somatic genes occurs in both cases, reprogramming of cis-silenced somatic genes occurs only in somatic-ESC fusions. Importantly, ESCs reprogram the somatic genome in two distinct phases: trans-reprogramming occurs rapidly, independent of DNA replication, whereas cis-reprogramming occurs with slow kinetics requiring DNA replication. We also show that pluripotency genes Oct4 and Nanog are cis-silenced in somatic cells. We conclude that cis-reprogramming capacity is a fundamental feature distinguishing ESCs from somatic cells.

Frydman, H. M., et al. (2006). "Somatic stem cell niche tropism in Wolbachia." Nature **441**(7092): 509-512.

Wolbachia are intracellular bacteria found in the reproductive tissue of all major groups of arthropods. They are transmitted vertically from the female hosts to their offspring, in a pattern analogous to mitochondria inheritance. But Wolbachia phylogeny does not parallel that of the host, indicating that horizontal infectious transmission must also occur. Insect parasitoids are considered the most likely vectors, but the mechanism for horizontal transfer is largely unknown. Here we show that newly introduced Wolbachia cross several tissues and infect the germline of the adult Drosophila melanogaster female. Through investigation of bacterial migration patterns during the course of infection, we found that Wolbachia reach the germline through the somatic stem cell niche in the D. melanogaster germarium. In addition, our data suggest that Wolbachia are highly abundant in the somatic stem cell niche of long-term infected hosts, implying that this location may also contribute to efficient vertical transmission. This is, to our knowledge, the first report of an intracellular parasite displaying tropism for a stem cell niche.

Funayama, N. (2010). "The stem cell system in demosponges: insights into the origin of somatic stem cells." Dev Growth Differ **52**(1): 1-14.

The stem cell system is one of the unique systems that have evolved only in multicellular organisms. Major questions about this system include what type (s) of stem cells are involved (pluri-, multi- or uni-potent stem cells), and how the self-renewal and differentiation of stem cells are regulated. To understand the origin of the stem cell system in metazoans and to get insights into the ancestral stem cell itself, it is important to discover the molecular and cellular mechanisms of the stem cell system in sponges (Porifera), the evolutionarily oldest extant metazoans. Histological studies here provided a body of evidence that archeocytes are the stem cells in sponges, and recent molecular studies of sponges, especially the finding of the expression of Piwi homologues in archeocytes and choanocytes in a freshwater sponge, Ephydatia fluviatilis, have provided critical insights into the stem cell system in demosponges. Here I introduce archeocytes and

discuss (i) modes of archeocyte differentiation, (ii) our current model of the stem cell system in sponges composed of both archeocytes and choanocytes based on our molecular analysis and previous microscopic studies suggesting the maintenance of pluripotency in choanocytes, (iii) the inference that the Piwi and piRNA function in maintaining stem cells (which also give rise to gametes) may have already been achieved in the ancestral metazoan, and (iv) possible hypotheses about how the migrating stem cells arose in the urmetazoan (protometazoan) and about the evolutionary origin of germline cells in the urbilaterian (protobilaterian).

Gheisari, Y., et al. (2013). "Human unrestricted somatic stem cell administration fails to protect nude mice from cisplatin-induced acute kidney injury." Nephron Exp Nephrol **123**(3-4): 11-21.

BACKGROUND: Kidney failure is a debilitating disorder with limited treatment options. The kidney-protective effects of stem cells have been vastly investigated and promising results have been achieved with various sources of stem cells. However, in spite of beneficial effects on other disease models, the renoprotective potential of human cord blood-derived unrestricted somatic stem cells (USSC) has not been examined so far. METHODS: In the present study, acute kidney failure was induced in female nude mice and the effect of USSC transplantation on kidney function and structure was assessed. Furthermore, the expression of some cytokine genes was examined by real-time PCR. Homing of the transplanted cells into kidneys was assessed by flow cytometry, immunohistochemistry, and real-time PCR. RESULTS: USSC-conditioned medium did not attenuate the in vitro nephrotoxic effects of cisplatin. Transplantation of USSC to nude mice did not protect kidney function and was associated with worsened kidney structural damage. USSC transplantation was also associated with a decline in the renal expression of VEGF-A gene. In spite of these effects, the transplanted cells could not be detected in the kidneys by any of the exploited methods and they were mainly entrapped in the lungs. CONCLUSION: These data indicate that USSC are not suitable for cell therapy in the setting of acute kidney injury. Also, this study shows that these stem cells are able to affect damaged kidneys even if they are not homed there.

Ghule, P. N., et al. (2009). "The subnuclear organization of histone gene regulatory proteins and 3' end processing factors of normal somatic and embryonic stem cells is compromised in selected human cancer cell types." J Cell Physiol **220**(1): 129-135.

Human histone gene expression is controlled at the level of transcription initiation and subsequent 3'end processing to generate non-polyadenylated stem-loop containing histone mRNAs. Transcription is controlled at the G1/S phase transition by the Cyclin E/CDK2 mediated induction of p220(NPAT)/HiNF-P complexes at subnuclear domains designated Histone Locus Bodies (HLBs) that associate with histone gene clusters. Histone mRNA maturation is mediated by Lsm10 containing U7snRNP complexes. In normal human somatic and embryonic stem cells, the 6p histone locus, the transcription marker p220(NPAT) and the 3'end processing marker Lsm10 (but not the Cajal Body marker coilin) co-localize, reflecting the assembly of an integrated factory for histone gene expression. Using in situ immuno-fluorescence microscopy and fluorescence in situ hybridization (FISH), we show that this subnuclear organization is compromised in some cancer cell lines. In aneuploid cells, the presence of HLBs correlates with the number of histone gene loci. More importantly, the in situ co-localization of p220(NPAT) and Lsm10 is disrupted in HeLa S3 cervical carcinoma cells and MCF7 breast adenocarcinoma cells, with most Lsm10 residing in Cajal Bodies. The finding that the subnuclear integration of transcriptional initiation and 3'end processing of histone gene transcripts is deregulated may be causally linked to tumor-related modifications in molecular pathways controlling histone gene expression during the cell cycle.

Glanzner, W. G., et al. (2016). "Exposure of Somatic Cells to Cytoplasm Extracts of Porcine Oocytes Induces Stem Cell-Like Colony Formation and Alters Expression of Pluripotency and Chromatin-Modifying Genes." Cell Reprogram **18**(3): 137-146.

Cell permeabilization followed by exposure to cytoplasmic extracts of oocytes has been proposed as an alternative to transduction of transcription factors for inducing pluripotency in cultured somatic cells. The main goal in this study was to investigate the effect of treating porcine fibroblast cells with cytoplasmic extracts of GV-stage oocyte (OEx) followed by inhibition of histone deacetylases with Scriptaid (Scrip) on the formation of stem cell-like colonies and expression of genes encoding pluripotency and chromatin-modifying enzymes. Stem cell-like colonies start developing approximately 2 weeks after treatment in cells exposed to OEx or OEx + Scrip. The number of cell colonies at the first day of appearance and 48 hours later was also similar between OEx and OEx + Scrip treatments. Transcripts for Nanog, Rex1, and c-Myc genes were detected in most cell samples that were analyzed on different days after OEx treatment. However, Sox2 transcripts were not detected and only a small proportion of samples

had detectable levels of Oct4 mRNA after OEx treatment. A similar pattern of transcripts for pluripotency genes was observed in cells treated with OEx alone or OEx + Scrip. Transcript levels for Dnmt1 and Ezh2 were reduced at Day 3 after treatment in cells exposed to OEx. These findings revealed that: (a) exposure to OEx can induce a partial reprogramming of fibroblast cells toward pluripotency, characterized by colony formation and activation of pluripotency genes; and (b) inhibition of histone deacetylases does not improve the reprogramming effect of OEx treatment.

Greber, B. and H. Scholer (2008). "[A breakthrough in stem cell research? Reprogramming somatic cells into pluripotent stem cells]." Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz **51**(9): 1005-1013.

Embryonic stem (ES) cells are capable of generating all cell types and tissues of the body. As such they represent an attractive source for therapeutic approaches. However, transplanted cells may be rejected by the immune system. One way to address this problem is to generate patient-specific ES cells. This, however, requires the transformation of the genetic program of somatic cells back to that of an early embryonic state. The field of stem cell research and reprogramming is rapidly evolving. This article aims at providing background information to understand some of the most exciting recent developments. Subsequently, the different existing strategies of converting somatic cells into ES-like cells are reviewed and evaluated.

Hadjimichael, C., et al. (2017). "Promyelocytic Leukemia Protein Is an Essential Regulator of Stem Cell Pluripotency and Somatic Cell Reprogramming." Stem Cell Reports **8**(5): 1366-1378.

Promyelocytic leukemia protein (PML), the main constituent of PML nuclear bodies, regulates various physiological processes in different cell types. However, little is known about its functions in embryonic stem cells (ESC). Here, we report that PML contributes to ESC self-renewal maintenance by controlling cell-cycle progression and sustaining the expression of crucial pluripotency factors. Transcriptomic analysis and gain- or loss-of-function approaches showed that PML-deficient ESC exhibit morphological, metabolic, and growth properties distinct to naive and closer to the primed pluripotent state. During differentiation of embryoid bodies, PML influences cell-fate decisions between mesoderm and endoderm by controlling the expression of Tbx3. PML loss compromises the reprogramming ability of embryonic fibroblasts to induced pluripotent stem cells by inhibiting the transforming growth factor beta pathway at the very early stages. Collectively, these results designate PML as a member of the regulatory network for ESC naive pluripotency and somatic cell reprogramming.

Haller, S., et al. (2017). "mTORC1 Activation during Repeated Regeneration Impairs Somatic Stem Cell Maintenance." Cell Stem Cell **21**(6): 806-818 e805.

The balance between self-renewal and differentiation ensures long-term maintenance of stem cell (SC) pools in regenerating epithelial tissues. This balance is challenged during periods of high regenerative pressure and is often compromised in aged animals. Here, we show that target of rapamycin (TOR) signaling is a key regulator of SC loss during repeated regenerative episodes. In response to regenerative stimuli, SCs in the intestinal epithelium of the fly and in the tracheal epithelium of mice exhibit transient activation of TOR signaling. Although this activation is required for SCs to rapidly proliferate in response to damage, repeated rounds of damage lead to SC loss. Consistently, age-related SC loss in the mouse trachea and in muscle can be prevented by pharmacologic or genetic inhibition, respectively, of mammalian target of rapamycin complex 1 (mTORC1) signaling. These findings highlight an evolutionarily conserved role of TOR signaling in SC function and identify repeated rounds of mTORC1 activation as a driver of age-related SC decline.

Hirose, M., et al. (2018). "Aberrant imprinting in mouse trophoblast stem cells established from somatic cell nuclear transfer-derived embryos." Epigenetics **13**(7): 693-703.

Although phenotypic abnormalities frequently appear in the placenta following somatic cell nuclear transfer (SCNT), mouse trophoblast stem cells (TSCs) established from SCNT embryos reportedly show no distinct abnormalities compared with those derived from normal fertilization. In this study, we reexamined SCNT-TSCs to identify their imprinting statuses. Placenta-specific maternally imprinted genes (Gab1, Slc38a4, and Sfmbt2) consistently showed biallelic expression in SCNT-TSCs, suggesting their loss of imprinting (LOI). The LOI of Gab1 was associated with decreased DNA methylation, and that of Sfmbt2 was associated with decreased DNA methylation and histone H3K27 trimethylation. The maternal allele of the intergenic differentially methylated region (IG-DMR) was aberrantly hypermethylated following SCNT, even though this region was prone to demethylation in TSCs when established in a serum-free chemically defined medium. These findings indicate that the development of cloned embryos is

associated with imprinting abnormalities specifically in the trophoblast lineage from its initial stage, which may affect subsequent placental development.

Ichikawa, C., et al. (2013). "Rat hair follicle-constituting cells labeled by a newly-developed somatic stem cell-recognizing antibody: a possible marker of hair follicle development." Histol Histopathol **28**(2): 257-268.

A3 was generated as an antibody recognizing somatic stem cells in rat tissues. We investigated the distribution of A3-positive cells in developing rat hair follicles by immunolabeling. A3-positive cells began to be seen in the hair germ and peg in fetuses and neonates; the positive cells were epithelial cells above basal cells. Furthermore, A3-positive cells were seen in the outer root sheath adjacent to the bulge in mature hair follicles. Double immunofluorescence revealed that these A3-positive epithelial cells reacted to E-cadherin (for all epithelial elements) but not to CK15 (for basal cells/epithelial stem cells) or to nestin (for stem cells), indicating that A3-positive epithelial cells are suprabasal cells in the developing epidermic hair follicle. Additionally, spindle-shaped mesenchymal cells surrounding the hair peg and mature hair follicle reacted to A3; in double immunofluorescence, the A3-positive cells were located outside collagen type IV-positive glassy membrane, and reacted to vimentin (for mesenchmal cells), Thy-1 (for immature mesenchymal cells), CD34 (for stem cells) and nestin, but not to alpha-smooth muscle actin (for myofibroblasts); the positive cells were regarded as immature mesenchymal cells with stem cell nature in the connective tissue sheath of developing hair follicles. A3-positive epithelial and mesenchymal cells did not show proliferating activity. Collectively, it is considered that A3-positive cells seen in developing rat hair follicles may be quiescent post-progenitor cells with the potential to differentiate into either highly-differentiated epithelial or mesenchymal cells. A3 would become a useful antibody to know the kinetics of rat hair follicle-constituting cells.

Ikeda, M. and M. Ohme-Takagi (2014). "TCPs, WUSs, and WINDs: families of transcription factors that regulate shoot meristem formation, stem cell maintenance, and somatic cell differentiation." Front Plant Sci **5**: 427.

In contrast to somatic mammalian cells, which cannot alter their fate, plant cells can dedifferentiate to form totipotent callus cells and regenerate a whole plant, following treatment with specific phytohormones. However, the regulatory mechanisms and key factors that control differentiation-dedifferentiation and cell totipotency have not been completely clarified in plants. Recently, several plant transcription factors that regulate meristem formation and dedifferentiation have been identified and include members of the TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR (TCP), WUSCHEL (WUS), and WOUND INDUCED DEDIFFERENTIATION (WIND1) families. WUS and WIND positively control plant cell totipotency, while TCP negatively controls it. Interestingly, TCP is a transcriptional activator that acts as a negative regulator of shoot meristem formation, and WUS is a transcriptional repressor that positively maintains totipotency of the stem cells of the shoot meristem. We describe here the functions of TCP, WUS, and WIND transcription factors in the regulation of differentiation-dedifferentiation by positive and negative transcriptional regulators.

Ikemoto, Y., et al. (2017). "Somatic mosaicism containing double mutations in PTCH1 revealed by generation of induced pluripotent stem cells from nevoid basal cell carcinoma syndrome." J Med Genet **54**(8): 579-584.

BACKGROUND: Nevoid basal cell carcinoma syndrome (NBCCS) is an autosomal dominant disorder characterised by developmental defects and tumorigenesis, such as medulloblastomas and basal cell carcinomas, caused by mutations of the patched-1 (PTCH1) gene. In this article, we seek to demonstrate a mosaicism containing double mutations in PTCH1 in an individual with NBCCS. METHODS AND RESULTS: A de novo germline mutation of PTCH1 (c.272delG) was detected in a 31-year-old woman with NBCCS. Gene analysis of two out of four induced pluripotent stem cell (iPSC) clones established from the patient unexpectedly revealed an additional mutation, c.274delT. Deep sequencing confirmed a low-prevalence somatic mutation (5.5%-15.6% depending on the tissue) identical to the one found in iPSC clones. CONCLUSIONS: This is the first case of mosaicism unequivocally demonstrated in NBCCS. Furthermore, the mosaicism is unique in that the patient carries one normal and two mutant alleles. Because these mutations are located in close proximity, reversion error is likely to be involved in this event rather than a spontaneous mutation. In addition, this study indicates that gene analysis of iPSC clones can contribute to the detection of mosaicism containing a minor population carrying a second mutation.

Issigonis, M. and E. Matunis (2012). "The Drosophila BCL6 homolog Ken and Barbie promotes somatic stem cell self-renewal in the testis niche." Dev Biol **368**(2): 181-192.

Stem cells sustain tissue regeneration by their remarkable ability to replenish the stem cell pool and to generate differentiating progeny. Signals from local

microenvironments, or niches, control stem cell behavior. In the Drosophila testis, a group of somatic support cells called the hub creates a stem cell niche by locally activating the Janus Kinase-Signal Transducer and Activator of Transcription (JAK-STAT) pathway in two adjacent types of stem cells: germline stem cells (GSCs) and somatic cyst stem cells (CySCs). Here, we find that ken and barbie (ken) is autonomously required for the self-renewal of CySCs but not GSCs. Furthermore, Ken misexpression in the CySC lineage induces the cell-autonomous self-renewal of somatic cells as well as the nonautonomous self-renewal of germ cells outside the niche. Thus, Ken, like Stat92E and its targets ZFH1 (Leatherman and Dinardo, 2008) and Chinmo (Flaherty et al., 2010), is necessary and sufficient for CySC renewal. However, ken is not a JAK-STAT target in the testis, but instead acts in parallel to Stat92E to ensure CySC self-renewal. Ken represses a subset of Stat92E targets in the embryo (Arbouzova et al., 2006) suggesting that Ken maintains CySCs by repressing differentiation factors. In support of this hypothesis, we find that the global JAK-STAT inhibitor Protein tyrosine phosphatase 61F (Ptp61F) is a JAK-STAT target in the testis that is repressed by Ken. Together, our work demonstrates that Ken has an important role in the inhibition of CySC differentiation. Studies of ken may inform our understanding of its vertebrate orthologue B-Cell Lymphoma 6 (BCL6) and how misregulation of this oncogene leads to human lymphomas.

Jacobs, K., et al. (2014). "Low-grade chromosomal mosaicism in human somatic and embryonic stem cell populations." Nat Commun **5**: 4227.

Current knowledge on chromosomal mosaicism in human cell cultures is mostly based on cytogenetic banding methods. The recent development of high-resolution full-genome analysis methods applicable to single cells is providing new insights into genetic and cellular diversity. Here we study the genetic content of 92 individual human cells, including fibroblasts, amniocytes and embryonic stem cells (hESCs), using single-cell array-based comparative genomic hybridization (aCGH). We find that human somatic and embryonic stem cell cultures show significant fractions of cells carrying unique megabase-scale chromosomal abnormalities, forming genetic mosaics that could not have been detected by conventional cytogenetic methods. These findings are confirmed by studying seven clonal hESC sub-lines by aCGH. Furthermore, fluorescent in situ hybridisation reveals an increased instability of the subtelomeric regions in hESC as compared to somatic cells. This genetic heterogeneity may have an impact on experimental results and, in the case of hESC, on their potential clinical use.

Kania, G., et al. (2005). "Somatic stem cell marker prominin-1/CD133 is expressed in embryonic stem cell-derived progenitors." Stem Cells **23**(6): 791-804.

Prominin-1/CD133 is a plasma membrane marker found in several types of somatic stem cells, including hematopoietic and neural stem cells. To study its role during development and with differentiation, we analyzed its temporal and spatial expression (mRNA and protein) in preimplantation embryos, undifferentiated mouse embryonic stem (ES) cells, and differentiated ES cell progeny. In early embryos, prominin-1 was expressed in trophoblast but not in cells of the inner cell mass; however, prominin-1 transcripts were detected in undifferentiated ES cells. Both ES-derived cells committed to differentiation and early progenitor cells coexpressed prominin-1 with early lineage markers, including the cytoskeletal markers (nestin, cytokeratin 18, desmin), fibulin-1, and valosin-containing protein. After spontaneous differentiation at terminal stages, prominin-1 expression was downregulated and no coexpression with markers characteristic for neuroectodermal, mesodermal, and endodermal cells was found. Upon induction of neuronal differentiation, some prominin-1-positive cells, which coexpressed nestin and showed the typical morphology of neural progenitor cells, persisted until terminal stages of differentiation. However, no coexpression of prominin-1 with markers of differentiated neural cells was detected. In conclusion, we present the somatic stem cell marker prominin-1 as a new parameter to define ES-derived committed and early progenitor cells.

Katiyar, S., et al. (2007). "Somatic excision demonstrates that c-Jun induces cellular migration and invasion through induction of stem cell factor." Mol Cell Biol **27**(4): 1356-1369.

Cancer cells arise through sequential acquisition of mutations in tumor suppressors and oncogenes. c-Jun, a critical component of the AP-1 complex, is frequently overexpressed in diverse tumor types and has been implicated in promoting cellular proliferation, migration, and angiogenesis. Functional analysis of candidate genetic targets using germ line deletion in murine models can be compromised through compensatory mechanisms. As germ line deletion of c-jun induces embryonic lethality, somatic deletion of the c-jun gene was conducted using floxed c-jun (c-jun (f/f)) conditional knockout mice. c-jun-deleted cells showed increased cellular adhesion, stress fiber formation, and reduced cellular migration. The reduced migratory velocity and migratory

directionality was rescued by either c-Jun reintroduction or addition of secreted factors from wild-type cells. An unbiased analysis of cytokines and growth factors, differentially expressed and showing loss of secretion upon c-jun deletion, identified stem cell factor (SCF) as a c-Jun target gene. Immunoneutralizing antibody to SCF reduced migration of wild-type cells. SCF addition rescued the defect in cellular adhesion, cellular velocity, directional migration, transwell migration, and cellular invasion of c-jun (-/-) cells. c-Jun induced SCF protein, mRNA, and promoter activity. Induction of the SCF promoter required the c-Jun DNA-binding domain. c-Jun bound to the SCF promoter in chromatin immunoprecipitation assays. Mutation of the c-Jun binding site abolished c-Jun-mediated induction of the SCF promoter. These studies demonstrate an essential role of c-Jun in cellular migration through induction of SCF.

Kiger, A. A., et al. (2000). "Somatic support cells restrict germline stem cell self-renewal and promote differentiation." Nature **407**(6805): 750-754.

Stem cells maintain populations of highly differentiated, short-lived cell-types, including blood, skin and sperm, throughout adult life. Understanding the mechanisms that regulate stem cell behaviour is crucial for realizing their potential in regenerative medicine. A fundamental characteristic of stem cells is their capacity for asymmetric division: daughter cells either retain stem cell identity or initiate differentiation. However, stem cells are also capable of symmetric division where both daughters remain stem cells, indicating that mechanisms must exist to balance self-renewal capacity with differentiation. Here we present evidence that support cells surrounding the stem cells restrict self-renewal and control stem cell number by ensuring asymmetric division. Loss of function of the Drosophila Epidermal growth factor receptor in somatic cells disrupted the balance of self-renewal versus differentiation in the male germline, increasing the number of germline stem cells. We propose that activation of this receptor specifies normal behaviour of somatic support cells; in turn, the somatic cells play a guardian role, providing information that prevents self-renewal of stem cell identity by the germ cell they enclose.

Kim, B. O., et al. (2005). "Cell transplantation improves ventricular function after a myocardial infarction: a preclinical study of human unrestricted somatic stem cells in a porcine model." Circulation **112**(9 Suppl): I96-104.

BACKGROUND: Cell transplantation offers the promise in the restoration of ventricular function after an extensive myocardial infarction, but the optimal cell type remains controversial. Human unrestricted somatic stem cells (USSCs) isolated from umbilical cord blood have great potential to differentiate into myogenic cells and induce angiogenesis. The present study evaluated the effect of USSCs on myocardial regeneration and improvement of heart function after myocardial infarction in a porcine model. METHOD AND RESULTS: The distal left anterior descending artery of Yorkshire pigs (30 to 35 kg) was occluded by endovascular implantation of a coil. Four weeks after infarction, single-photon emission computed tomography technetium 99m sestamibi scans (MIBI) and echocardiography were performed. USSCs (100 x 10(6)) or culture media were then directly injected into the infarcted region (n=8 per group). Pigs were immunosuppressed by daily administration of cyclosporin A. At 4 weeks after transplantation, MIBI and echocardiography were repeated and heart function was also assessed with a pressure-volume catheter. The infarcted myocardium and implanted cells were studied histologically. MIBI showed improved regional perfusion ($P<0.05$) and wall motion ($P<0.05$) of the infarct region in the transplant group compared with the control. Ejection fraction evaluated by both MIBI and echocardiography decreased in the control group but increased in the transplant group ($P<0.01$). Scar thickness of the transplant group was higher than the control. The grafted cells were detected 4 weeks after transplantation by both immunohistochemistry and in situ hybridization. CONCLUSIONS: Engrafted USSCs were detected in the infarct region 4 weeks after cell transplantation, and the implanted cells improved regional and global function of the porcine heart after a myocardial infarction. This study suggests that the USSC implantation will be efficacious for cellular cardiomyoplasty.

Kim, D., et al. (2015). "In vitro culture of stem-like cells derived from somatic cell nuclear transfer bovine embryos of the Korean beef cattle species, HanWoo." Reprod Fertil Dev.

We established and maintained somatic cell nuclear transfer embryo-derived stem-like cells (SCNT-eSLCs) from the traditional Korean beef cattle species, HanWoo (Bos taurus coreanae). Each SCNT blastocyst was placed individually on a feeder layer with culture medium containing three inhibitors of differentiation (3i). Primary colonies formed after 2-3 days of culture and the intact colonies were passaged every 5-6 days. The cells in each colony showed embryonic stem cell-like morphologies with a distinct boundary and were positive to alkaline phosphatase staining. Immunofluorescence and reverse transcription-polymerase chain reaction analyses also confirmed that these colonies expressed pluripotent

markers. The colonies were maintained over 50 passages for more than 270 days. The cells showed normal karyotypes consisting of 60 chromosomes at Passage 50. Embryoid bodies were formed by suspension culture to analyse in vitro differentiation capability. Marker genes representing the differentiation into three germ layers were expressed. Typical embryonal carcinoma was generated after injecting cells under the testis capsule of nude mice, suggesting that the cultured cells may also have the potential of in vivo differentiation. In conclusion, we generated eSLCs from SCNT bovine embryos, using a 3i system that sustained stemness, normal karyotype and pluripotency, which was confirmed by in vitro and in vivo differentiation.

Kim, J. Y., et al. (2005). "Counting human somatic cell replications: methylation mirrors endometrial stem cell divisions." Proc Natl Acad Sci U S A **102**(49): 17739-17744.

Cell proliferation may be altered in many diseases, but it is uncertain exactly how to measure total numbers of divisions. Although it is impossible to count every division directly, potentially total numbers of stem cell divisions since birth may be inferred from numbers of somatic errors. The idea is that divisions are surreptitiously recorded by random errors that occur during replication. To test this "molecular clock" hypothesis, epigenetic errors encoded in certain methylation patterns were counted in glands from 30 uteri. Endometrial divisions can differ among women because of differences in estrogen exposures or numbers of menstrual cycles. Consistent with an association between mitotic age and methylation, there was an age-related increase in methylation with stable levels after menopause, and significantly less methylation was observed in lean or older multiparous women. Methylation patterns were diverse and more consistent with niche rather than immortal stem cell lineages. There was no evidence for decreased stem cell survival with aging. An ability to count lifetime numbers of stem cell divisions covertly recorded by random replication errors provides new opportunities to link cell proliferation with aging and cancer.

Kim, K., et al. (2007). "Recombination signatures distinguish embryonic stem cells derived by parthenogenesis and somatic cell nuclear transfer." Cell Stem Cell **1**(3): 346-352.

Parthenogenesis and somatic cell nuclear transfer (SCNT) are two methods for deriving embryonic stem (ES) cells that are genetically matched to the oocyte donor or somatic cell donor, respectively. Using genome-wide single nucleotide polymorphism (SNP) analysis, we demonstrate distinct signatures of genetic recombination that distinguish parthenogenetic ES cells from those generated by SCNT. We applied SNP analysis to the human ES cell line SCNT-hES-1, previously claimed to have been derived by SCNT, and present evidence that it represents a human parthenogenetic ES cell line. Genome-wide SNP analysis represents a means to validate the genetic provenance of an ES cell line.

Kim, M. K. (2009). "Oversight framework over oocyte procurement for somatic cell nuclear transfer: comparative analysis of the Hwang Woo Suk case under South Korean bioethics law and U.S. guidelines for human embryonic stem cell research." Theor Med Bioeth **30**(5): 367-384.

We examine whether the current regulatory regime instituted in South Korea and the United States would have prevented Hwang's potential transgressions in oocyte procurement for somatic cell nuclear transfer, we compare the general aspects and oversight framework of the Bioethics and Biosafety Act in South Korea and the US National Academies' Guidelines for Human Embryonic Stem Cell Research, and apply the relevant provisions and recommendations to each transgression. We conclude that the Act would institute centralized oversight under governmental auspices while the Guidelines recommend politically-independent, decentralized oversight bodies including a special review body for human embryonic stem cell research at an institutional level and that the Guidelines would have provided more vigorous protection for the women who had undergone oocyte procurement for Hwang's research than the Act. We also suggest additional regulations to protect those who provide oocytes for research in South Korea.

Kim, N., et al. (2017). "Immobilized pH in culture reveals an optimal condition for somatic cell reprogramming and differentiation of pluripotent stem cells." Reprod Med Biol **16**(1): 58-66.

Aim: One of the parameters that greatly affects homeostasis in the body is the pH. Regarding reproductive biology, germ cells, such as oocytes or sperm, are exposed to severe changes in pH, resulting in dramatic changes in their characteristics. To date, the effect of the pH has not been investigated regarding the reprogramming of somatic cells and the maintenance and differentiation of pluripotent stem cells. Methods: In order to investigate the effects of the pH on cell culture, the methods to produce induced pluripotent stem cells (iPSCs) and to differentiate embryonic stem cells (ESCs) into mesendoderm and neuroectoderm were performed at each medium pH from 6.6 to 7.8. Using the cells of the Oct4-GFP (green fluorescent protein) carrying mouse, the effects of pH changes were examined on the timing and

colony formation at cell reprogramming and on the cell morphology and direction of the differentiation of the ESCs. Results: The colony formation rate and timing of the reprogramming of the somatic cells varied depending on the pH of the culture medium. In addition, mesendodermal differentiation of the mouse ESCs was enhanced at the high pH level of 7.8. Conclusion: These results suggest that the pH in the culture medium is one of the key factors in the induction of the reprogramming of somatic cells and in the differentiation of pluripotent stem cells.

Kim, S., et al. (2010). "Establishment and characterization of embryonic stem-like cells from porcine somatic cell nuclear transfer blastocysts." Zygote **18**(2): 93-101.

This study was aimed to establish embryonic stem (ES)-like cells from blastocysts derived from somatic cell nuclear transfer (SCNT) in pig. Somatic cells isolated from both day-30 fetus and neonatal cloned piglet were used for donor cells. A total of 60 blastocysts (46 and 14 derived from fetal and neonatal fibroblast donor cells, respectively) were seeded onto a mitotically inactive mouse embryonic fibroblast (MEF) monolayer and two ES-like cell lines, one from each donor cell type, were established. They remained undifferentiated over more than 52 (fetal fibroblast-derived) and 48 (neonatal fibroblast-derived) passages, while retaining alkaline phosphatase activity and reactivity with ES specific markers Oct-4, stage-specific embryonic antigen-1 (SSEA-1), SSEA-4, TRA-1-60 and TRA-1-81. These ES-like cells maintained normal diploid karyotype throughout subculture and successfully differentiated into embryoid bodies that expressed three germ layer-specific genes (ectoderm: beta-III tubulin; endoderm: amylase; and mesoderm: enolase) after culture in leukemia inhibitory factor-free medium. Microsatellite analysis confirmed that they were genetically identical to its donor cells. Combined with gene targeting, our results may contribute to developing an efficient method for producing transgenic pigs for various purposes.

King, F. J. and H. Lin (1999). "Somatic signaling mediated by fs (1)Yb is essential for germline stem cell maintenance during Drosophila oogenesis." Development **126**(9): 1833-1844.

Drosophila oogenesis starts when a germline stem cell divides asymmetrically to generate a daughter germline stem cell and a cystoblast that will develop into a mature egg. We show that the fs (1)Yb gene is essential for the maintenance of germline stem cells during oogenesis. We delineate fs (1)Yb within a 6.4 kb genomic region by transgenic rescue experiments. fs (1)Yb encodes a 4.1 kb RNA that is present in the third instar larval, pupal and adult stages, consistent with its role in regulating germline stem cells during oogenesis. Germline clonal analysis shows that all fs (1)Yb mutations are soma-dependent. In the adult ovary, fs (1)Yb is specifically expressed in the terminal filament cells, suggesting that fs (1)Yb acts in these signaling cells to maintain germline stem cells. fs (1)Yb encodes a novel hydrophilic protein with no potential signal peptide or transmembrane domains, suggesting that this protein is not itself a signal but a key component of the signaling machinery for germline stem cell maintenance.

Kirilly, D., et al. (2005). "BMP signaling is required for controlling somatic stem cell self-renewal in the Drosophila ovary." Dev Cell **9**(5): 651-662.

BMP signaling is essential for promoting self-renewal of mouse embryonic stem cells and Drosophila germline stem cells and for repressing stem cell proliferation in the mouse intestine and skin. However, it remains unknown whether BMP signaling can promote self-renewal of adult somatic stem cells. In this study, we show that BMP signaling is necessary and sufficient for promoting self-renewal and proliferation of somatic stem cells (SSCs) in the Drosophila ovary. BMP signaling is required in SSCs to directly control their maintenance and division, but is dispensable for proliferation of their differentiated progeny. Furthermore, BMP signaling is required to control SSC self-renewal, but not survival. Moreover, constitutive BMP signaling prolongs the SSC lifespan. Therefore, our study clearly demonstrates that BMP signaling directly promotes SSC self-renewal and proliferation in the Drosophila ovary. Our work further suggests that BMP signaling could promote self-renewal of adult stem cells in other systems.

Kishigami, S., et al. (2006). "Cloned mice and embryonic stem cell establishment from adult somatic cells." Hum Cell **19**(1): 2-10.

Cloning methods are now well described and becoming routine. Yet the frequency at which cloned offspring are produced remains below 2% irrespective of nucleus donor species or cell type. Especially in the mouse, few laboratories can make clones from adult somatic cells, and most mouse strains never succeed to produce cloned mice. On the other hand, nuclear transfer can be used to generate embryonic stem (ntES) cell lines from a patient's own somatic cells. We have shown that ntES cells can be generated relatively easily from a variety of mouse genotypes and cell types of both sexes, even though it may be more difficult to generate clones directly. Several reports have already demonstrated that ntES cells can be used in regenerative medicine in order to rescue immune deficient or infertile phenotypes. However, it is

unclear whether ntES cells are identical to fertilized embryonic stem (ES) cells. In general, ntES cell techniques are expected to be applicable to regenerative medicine, however, these techniques can also be used for the preservation of the genetic resources of mouse strains instead of preserving such resources in embryos, oocytes or spermatozoa. This review seeks to describe the phenotype, application, and possible abnormalities of cloned mice and ntES cell lines.

Kogler, G., et al. (2004). "A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential." J Exp Med **200**(2): 123-135.

Here a new, intrinsically pluripotent, CD45-negative population from human cord blood, termed unrestricted somatic stem cells (USSCs) is described. This rare population grows adherently and can be expanded to 10(15) cells without losing pluripotency. In vitro USSCs showed homogeneous differentiation into osteoblasts, chondroblasts, adipocytes, and hematopoietic and neural cells including astrocytes and neurons that express neurofilament, sodium channel protein, and various neurotransmitter phenotypes. Stereotactic implantation of USSCs into intact adult rat brain revealed that human Tau-positive cells persisted for up to 3 mo and showed migratory activity and a typical neuron-like morphology. In vivo differentiation of USSCs along mesodermal and endodermal pathways was demonstrated in animal models. Bony reconstitution was observed after transplantation of USSC-loaded calcium phosphate cylinders in nude rat femurs. Chondrogenesis occurred after transplanting cell-loaded gelfoam sponges into nude mice. Transplantation of USSCs in a noninjury model, the preimmune fetal sheep, resulted in up to 5% human hematopoietic engraftment. More than 20% albumin-producing human parenchymal hepatic cells with absence of cell fusion and substantial numbers of human cardiomyocytes in both atria and ventricles of the sheep heart were detected many months after USSC transplantation. No tumor formation was observed in any of these animals.

Kossowska-Tomaszczuk, K. and C. De Geyter (2013). "Cells with stem cell characteristics in somatic compartments of the ovary." Biomed Res Int **2013**: 310859.

Antral follicular growth in the ovary is characterized by rapid expansion of granulosa cells accompanied by a rising complexity of their functionality. Within two weeks the number of human granulosa cells increases from less than 500,000 to more than 50 millions cells per follicle and differentiates into groups of cells with a variety of specialized functions involved in steroidogenesis, nursing the oocyte, and forming a functional syncitium. Both the rapid proliferation and different specialized functions of the granulosa cells can only be explained through the involvement of stem cells. However, luteinizing granulosa cells were believed to be terminally differentiated cells. Only recently, stem and progenitor cells with FSH-receptor activity were identified in populations of luteinizing granulosa cells obtained during oocyte collected for assisted reproduction. In the presence of the leukaemia-inhibiting factor (LIF), it was possible to culture a subpopulation of the luteinizing granulosa cells over prolonged time periods. Furthermore, when embedded in a matrix consisting of collagen type I, these cells continued to express the FSH receptor over prolonged time periods, developed globular formations that surrogated as follicle-like structures, providing a promising tool for reproductive biology.

Kruglova, A. A., et al. (2010). "Dominance of parental genomes in embryonic stem cell/fibroblast hybrid cells depends on the ploidy of the somatic partner." Cell Tissue Res **340**(3): 437-450.

Two dozen hybrid clones were produced by fusion of diploid embryonic stem (ES) cells positive for green fluorescent protein (GFP) with tetraploid fibroblasts derived from DD/c and C57BL-I (I)1RK mice. Cytogenetic analysis demonstrated that most cells from these hybrid clones contained near-hexaploid chromosome sets. Additionally, the presence of chromosomes derived from both parental cells was confirmed by polymerase chain reaction (PCR) analysis of polymorphic microsatellites. All hybrid cells were positive for GFP and demonstrated growth characteristics and fibroblast-like morphology. In addition, most hybrid cells were positive for collagen type I, fibronectin, and lamin A/C but were negative for Oct4 and Nanog proteins. Methylation status of the Oct4 and Nanog gene promoters was evaluated by bisulfite genomic sequencing analysis. The methylation sites (CpG-sites) of the Oct4 and Nanog gene promoters were highly methylated in hybrid cells, whereas the CpG-sites were unmethylated in the parental ES cells. Thus, the fibroblast genome dominated the ES genome in the diploid ES cell/tetraploid fibroblast hybrid cells. Immunofluorescent analysis of the pluripotent and fibroblast markers demonstrated that establishment of the fibroblast phenotype occurred shortly after fusion and that the fibroblast phenotype was further maintained in the hybrid cells. Fusion of karyoplasts and cytoplast derived from tetraploid fibroblasts with whole ES cells demonstrated that karyoplasts were able to establish the fibroblast phenotype of the reconstructed cells but not fibroblast cytoplasts. Thus,

these data suggest that the dominance of parental genomes in hybrid cells of ES cell/somatic cell type depends on the ploidy of the somatic partner.

Kues, W. A., et al. (2005). "From fibroblasts and stem cells: implications for cell therapies and somatic cloning." Reprod Fertil Dev **17**(1-2): 125-134.

Pluripotent embryonic stem cells (ESCs) from the inner cell mass of early murine and human embryos exhibit extensive self-renewal in culture and maintain their ability to differentiate into all cell lineages. These features make ESCs a suitable candidate for cell-replacement therapy. However, the use of early embryos has provoked considerable public debate based on ethical considerations. From this standpoint, stem cells derived from adult tissues are a more easily accepted alternative. Recent results suggest that adult stem cells have a broader range of potency than imagined initially. Although some claims have been called into question by the discovery that fusion between the stem cells and differentiated cells can occur spontaneously, in other cases somatic stem cells have been induced to commit to various lineages by the extra- or intracellular environment. Recent data from our laboratory suggest that changes in culture conditions can expand a subpopulation of cells with a pluripotent phenotype from primary fibroblast cultures. The present paper critically reviews recent data on the potency of somatic stem cells, methods to modify the potency of somatic cells and implications for cell-based therapies.

Kuniakova, M., et al. (2015). "Somatic stem cell aging and malignant transformation--impact on therapeutic application." Cell Mol Biol Lett **20**(5): 743-756.

Somatic stem cells possess unique properties of self-renewal and plasticity which make them promising candidates for use in tissue engineering and regenerative medicine, in addition to serving as efficient delivery vehicles in site-specific therapy. In the case of therapeutic application, it is essential to isolate and culture stem cells in vitro, to obtain them in sufficient quantities. Although long-term cultivation provides an adequate number of cells, it has been shown that this approach is associated with increased risk of transformation of cultured cells, which presents a significant biological hazard. This article reviews information about biological features and cellular events which occur during long-term cultivation of somatic stem cells, with respect to their safe utilization in potential clinical practice.

Kuraguchi, M., et al. (2001). "Differences in susceptibility to colonic stem cell somatic mutation in three strains of mice." J Pathol **193**(4): 517-521.

Different species and different strains of animals commonly show very different sensitivities to carcinogenic regimes, which are often unexplained. A major possible contributory factor is variation in susceptibility to mutation, but this has not been directly demonstrated. This study therefore quantified the colonic stem cell mutation frequency in three strains of mice using two carcinogens. Stem cell mutations were identified using loss of function of glucose 6-phosphate dehydrogenase (G6PD) in individual crypts, a technique validated by several previous studies. The carcinogens dimethylhydrazine (DMH) and ethyl nitrosurea (ENU) were given to Balb/C, C57BL/6J, and C3H mice. In response to DMH, Balb/C mice were most susceptible, with approximately double the stem cell mutation frequency found in C3H and more than ten-fold that found in C57BL/6J (3.3+/-0.71 vs. 1.5+/-0.52 vs. 0.28+/-0.8x10(-4)). In response to ENU, Balb/C mice and C3H mice were equally susceptible, showing a stem cell mutation frequency approximately twice that of C57BL/6J (3.1+/-0.4 vs. 3.1+/-0.65 vs. 1.63+/-0.28x10(-4)). The observed differences among the strains with respect to somatic mutation following DMH treatment are likely to be due to the previously documented differences in metabolic conversion to the active metabolite. However, as ENU is a directly acting, rapidly inactivated mutagen, strain differences in response to ENU are unlikely to be due to strain-dependent metabolism of the mutagen and are likely to reflect differences in DNA repair efficiency, or possibly in stem cell kinetics among the strains studied. Susceptibility to the induction of colonic stem cell mutation is an important factor in susceptibility to carcinogens, whether due to differences in DNA repair or to other factors. Direct quantification of stem cell mutation frequency allows the separate identification of this component of the carcinogenic cascade and shows that it can make a major contribution to the differing susceptibility of different mouse strains.

Langerova, A., et al. (2013). "Somatic cell nuclear transfer-derived embryonic stem cell lines in humans: pros and cons." Cell Reprogram **15**(6): 481-483.

The recent paper, published by Mitalipov's group in Cell (Tachibana et al., 2013), reporting the production of human somatic cell nuclear transfer (SCNT) embryonic stem cells (ESCs), opens again the debate if, in the era of induced pluripotent stem cells (iPSCs), the production of these cells is indeed necessary and, if so, whether they are different from ESCs produced from spare embryos and iPSCs. It is our opinion that these questions are very difficult to answer because it is still unclear whether and how normal ESCs differ from iPSCs.

Langroudi, L., et al. (2015). "MiR-371-373 cluster acts as a tumor-suppressor-miR and promotes cell cycle arrest in unrestricted somatic stem cells." Tumour Biol **36**(10): 7765-7774.

Recent advances in small RNA research have implicated microRNAs (miRNAs) as important regulators of proliferation and development. The miR-371-373 cluster is prominently expressed in human embryonic stem cells (ESCs) and rapidly decreases after cell differentiation. MiR-371-373 cluster was investigated as one of the key factors of stem cell maintenance and pluripotency in unrestricted somatic stem cells (USSCs) using a lentivirus system. Gene expression showed a dual effect on proliferation, which revealed a transient cell cycle progression and consequent repression in pluripotency factors and cell cycle genes. Cell proliferation analysis with CFU, MTT, and DNA content assays further confirmed the dual effect of cluster after prolonged exposure. Analyzing the course of action, it seems that miR-371-373 cluster acts as an onco/tumor suppressor-miR. MiR371-373 cluster acts by modulating the function of these factors and limiting the excessive cell cycle propagation upon oncogenic stimuli to protect cells from replicative stress, but also activate CDK inhibitors and transcriptional repressors of the retinoblastoma family to cause cell cycle arrest. In contrast to the previous studies, we believe that miR-371-373 cluster functions as a self-renewal miRNA to induce and maintain the pluripotent state but also to potentially inhibit dysregulated proliferation through cell cycle arrest. It seems that miR-371-373 cluster presents with a dual effect in this cellular context which may possess different actions in various cells. This not only expands the basic knowledge of the cluster but may offer a great chance for therapeutic interventions.

Leatherman, J. L. and S. Dinardo (2008). "Zfh-1 controls somatic stem cell self-renewal in the Drosophila testis and nonautonomously influences germline stem cell self-renewal." Cell Stem Cell **3**(1): 44-54.

The ability of adult stem cells to maintain their undifferentiated state depends upon residence in their niche. While simple models of a single self-renewal signal are attractive, niche-stem cell interactions are likely to be more complex. Many niches have multiple cell types, and the Drosophila testis is one such complex niche with two stem cell types, germline stem cells (GSCs) and somatic cyst progenitor cells (CPCs). These stem cells require chemokine activation of Jak/STAT signaling for self-renewal. We identified the transcriptional repressor Zfh-1 as a presumptive somatic target of Jak/STAT signaling, demonstrating that it is necessary and sufficient to maintain CPCs. Surprisingly, sustained zfh-1 expression or intrinsic STAT activation in somatic cells caused neighboring germ cells to self-renew outside their niche. In contrast, germline-intrinsic STAT activation was insufficient for GSC renewal. These data reveal unexpected complexity in cell interactions in the niche, implicating CPCs in GSC self-renewal.

Lee, J. H., et al. (2014). "Somatic transcriptome priming gates lineage-specific differentiation potential of human-induced pluripotent stem cell states." Nat Commun **5**: 5605.

Human-induced pluripotent stem cells (hiPSCs) provide an invaluable source for regenerative medicine, but are limited by proficient lineage-specific differentiation. Here we reveal that hiPSCs derived from human fibroblasts (Fibs) versus human cord blood (CB) exhibit indistinguishable pluripotency, but harbour biased propensities for differentiation. Genes associated with germ layer specification were identical in Fib- or CB-derived iPSCs, whereas lineage-specific marks emerge upon differentiation induction of hiPSCs that were correlated to the cell of origin. Differentiation propensities come at the expense of other lineages and cannot be overcome with stimuli for alternative cell fates. Although incomplete DNA methylation and distinct histone modifications of lineage-specific loci correlate to lineage-specific transcriptome priming, transitioning hiPSCs into naive state of pluripotency removes iPSC-memorized transcriptome. Upon re-entry to the primed state, transcriptome memory is restored, indicating a human-specific phenomenon whereby lineage gated developmental potential is not permanently erased, but can be modulated by the pluripotent state.

Lenhart, K. F. and S. DiNardo (2015). "Somatic cell encystment promotes abscission in germline stem cells following a regulated block in cytokinesis." Dev Cell **34**(2): 192-205.

In many tissues, the stem cell niche must coordinate behavior across multiple stem cell lineages. How this is achieved is largely unknown. We have identified delayed completion of cytokinesis in germline stem cells (GSCs) as a mechanism that regulates the production of stem cell daughters in the Drosophila testis. Through live imaging, we show that a secondary F-actin ring is formed through regulation of Cofilin activity to block cytokinesis progress after contractile ring disassembly. The duration of this block is controlled by Aurora B kinase. Additionally, we have identified a requirement for somatic cell encystment of the germline in promoting GSC abscission. We suggest that this non-autonomous role promotes coordination between stem cell lineages.

These findings reveal the mechanisms by which cytokinesis is inhibited and reinitiated in GSCs and why such complex regulation exists within the stem cell niche.

Li, C., et al. (2015). "Ci antagonizes Hippo signaling in the somatic cells of the ovary to drive germline stem cell differentiation." Cell Res **25**(10): 1152-1170.

Many stem cell populations are tightly regulated by their local microenvironment (niche), which comprises distinct types of stromal cells. However, little is known about mechanisms by which niche subgroups coordinately determine the stem cell fate. Here we identify that Yki, the key Hippo pathway component, is essential for escort cell (EC) function in promoting germline differentiation in Drosophila ovary. We found that Hedgehog (Hh) signals emanating primarily from cap cells support the function of ECs, where Cubitus interruptus (Ci), the Hh signaling effector, acts to inhibit Hippo kinase cascade activity. Mechanistically, we found that Ci competitively interacts with Hpo and impairs the Hpo-Wts signaling complex formation, thereby promoting Yki nuclear localization. The actions of Ci ensure effective Yki signaling to antagonize Sd/Tgi/Vg-mediated default repression in ECs. This study uncovers a mechanism explaining how subgroups of niche cells coordinate to determine the stem cell fate via Hh-Hippo signaling crosstalk, and enhances our understanding of mechanistic regulations of the oncogenic Yki/YAP signaling.

Lim, J. M. and S. P. Gong (2013). "Somatic cell transformation into stem cell-like cells induced by different microenvironments." Organogenesis **9**(4): 245-248.

Development of induced pluripotent stem cell (iPSC) technology introduced a novel way to derive pluripotent stem cells, but the genetic manipulation required to generate iPSCs may lead to uncontrolled tumorigenesis of the established cells and thus limit clinical feasibility of the technology. Numerous attempts have been made to date, and alternative reprogramming of somatic cells to reactivate cellular plasticity after differentiation has been suggested. As a result, it had become clear that cell-to-cell interactions and specific acellular environments can be utilized for somatic cell reprogramming. In our previous studies, embryonic stem cell (ESC)-like cells could be derived from transforming ovarian cells and fetal fibroblasts by cell-to-cell interaction or specific cell-mediated microenvironmental factor (s). This cellular event was induced without undertaking genetic manipulation of progenitor cells. Several differences were found between the cellular properties of niche-induced, ESC-like cells and those of genetically manipulated iPSCs and the referenced ESCs. Thus, we provided evidence that terminally differentiated somatic cells either acquire pluripotency-like activity or possess cellular and genetic plasticity under a specific microenvironment and/or cell-to-cell interaction. In this minireview, we discuss derivation of stem cell-like cells under specific microenvironmental conditions in terms of technical perspectives and limitations.

Loeffler, M., et al. (1993). "Somatic mutation, monoclonality and stochastic models of stem cell organization in the intestinal crypt." J Theor Biol **160**(4): 471-491.

Among highly proliferating tissues the intestinal tissue is of particular interest. Techniques are available that permit an insight into how intestinal crypts as the basic macroscopic tissue unit are regenerated from a small population of self-maintaining stem cells. However, neither the precise number of these stem cells nor their properties are known. We have recently suggested a model of stem cell organization which explains the life cycle of murine intestinal crypts, their birth (by crypt fission) and extinction rates, as well as their size distribution on a quantitative basis (Loeffler & Grossman, 1991). The model assumptions involve two stochastic branching processes, one for the growth of several independent indistinguishable stem cells and a second for a threshold dependent crypt fission process. New data have now become available challenging the above concept. They relate to the conversion of crypts to monoclonal phenotypic expression after mutagenic events, presumably taking place in single stem cells. A detailed analysis of these data is shown here utilizing a more elaborate version of the above model. The new data are consistent with this model within the range of parameters predicted previously. We conclude that the cellular regeneration of intestinal crypts can be explained on the basis of several indistinguishable stem cells which can replace each other.

Markoulaki, S., et al. (2008). "Somatic cell nuclear transfer and derivation of embryonic stem cells in the mouse." Methods **45**(2): 101-114.

Addressing the fundamental questions of nuclear equivalence in somatic cells has fascinated scientists for decades and has resulted in the development of somatic cell nuclear transfer (SCNT) or animal cloning. SCNT involves the transfer of the nucleus of a somatic cell into the cytoplasm of an egg whose own chromosomes have been removed. In the mouse, SCNT has not only been successfully used to address the issue of nuclear equivalence, but has been used as a model system to test the hypothesis that embryonic

stem cells (ESCs) derived from NT blastocysts have the potential to correct--through genetic manipulations--degenerative diseases. This paper aims to provide a comprehensive description of SCNT in the mouse and the derivation of ESCs from blastocysts generated by this technique. SCNT is a very challenging and inefficient procedure because it is technically complex, it bypasses the normal events of gamete interactions and egg activation, and it depends on adequate reprogramming of the somatic cell nucleus in vivo. Improvements in any or all those aspects may enhance the efficiency and applicability of SCNT. ESC derivation from SCNT blastocysts, on the other hand, requires the survival of only a few successfully reprogrammed cells, which have the capacity to proliferate indefinitely in vitro, maintain correct genetic and epigenetic status, and differentiate into any cell type in the body--characteristics that are essential for transplantation therapy or any other in vivo application.

Marthaler, A. G., et al. (2013). "Reprogramming to pluripotency through a somatic stem cell intermediate." PLoS One **8**(12): e85138.

Transcription factor-based reprogramming can lead to the successful switching of cell fates. We have recently reported that mouse embryonic fibroblasts (MEFs) can be directly reprogrammed into induced neural stem cells (iNSCs) after the forced expression of Brn4, Sox2, Klf4, and Myc. Here, we tested whether iNSCs could be further reprogrammed into induced pluripotent stem cells (iPSCs). The two factors Oct4 and Klf4 were sufficient to induce pluripotency in iNSCs. Immunocytochemistry and gene expression analysis showed that iNSC-derived iPSCs (iNdiPSCs) are similar to embryonic stem cells at the molecular level. In addition, iNdiPSCs could differentiate into cells of all three germ layers, both in vitro and in vivo, proving that iNdiPSCs are bona fide pluripotent cells. Furthermore, analysis of the global gene expression profile showed that iNdiPSCs, in contrast to iNSCs, do not retain any MEF transcriptional memory even at early passages after reprogramming. Overall, our results demonstrate that iNSCs can be reprogrammed to pluripotency and suggest that cell fate can be redirected numerous times. Importantly, our findings indicate that the induced pluripotent cell state may erase the donor-cell type epigenetic memory more efficiently than other induced somatic cell fates.

Michel, M., et al. (2012). "Hh signalling is essential for somatic stem cell maintenance in the Drosophila testis niche." Development **139**(15): 2663-2669.

In the Drosophila testis, germline stem cells (GSCs) and somatic cyst stem cells (CySCs) are arranged around a group of postmitotic somatic cells, termed the hub, which produce a variety of growth factors contributing to the niche microenvironment that regulates both stem cell pools. Here we show that CySC but not GSC maintenance requires Hedgehog (Hh) signalling in addition to Jak/Stat pathway activation. CySC clones unable to transduce the Hh signal are lost by differentiation, whereas pathway overactivation leads to an increase in proliferation. However, unlike cells ectopically overexpressing Jak/Stat targets, the additional cells generated by excessive Hh signalling remain confined to the testis tip and retain the ability to differentiate. Interestingly, Hh signalling also controls somatic cell populations in the fly ovary and the mammalian testis. Our observations might therefore point towards a higher degree of organisational homology between the somatic components of gonads across the sexes and phyla than previously appreciated.

Miki, T., et al. (2011). "Wnt/beta-catenin signaling in embryonic stem cell self-renewal and somatic cell reprogramming." Stem Cell Rev **7**(4): 836-846.

Embryonic stem cells and induced pluripotent stem (iPS) cells are characterized by their ability to self-renew and to generate differentiated cells of all three germ layers. This potential makes them an attractive source to address question of developmental and also for use in clinical regenerative medicine. Although the culture conditions to maintain pluripotency and reprogramming technologies have been established, the underlying molecular mechanisms are incompletely understood. Accumulating evidence indicates that the Wnt/beta-catenin signaling pathway plays a pivotal role in the maintenance of pluripotency as well as in the process of somatic cell reprogramming. Reciprocally, Wnt/beta-catenin signaling also plays a critical role in the lineage decision/commitment process. These dramatically different outcomes upon activation of the Wnt signaling cascade has fueled enormous controversy concerning the role of Wnt signaling in the maintenance of potency and induction of differentiation in stem cells. Here, we discuss and explore the divergent roles of the Wnt signaling pathways based on findings from our lab. Accumulated results from our lab indicate the usage of a critical switching mechanism that regulates the divergent Wnt/catenin transcriptional programs associated with either maintenance of potency or initiation of differentiation.

Monahan, A. J. and M. Starz-Gaiano (2016). "Apontic regulates somatic stem cell numbers in Drosophila testes." BMC Dev Biol **16**: 5.

BACKGROUND: Microenvironments called niches maintain resident stem cell populations by balancing self-renewal with differentiation, but the genetic regulation of this process is unclear. The niche of the Drosophila testis is well-characterized and genetically tractable, making it ideal for investigating the molecular regulation of stem cell biology. The JAK/STAT pathway, activated by signals from a niche component called the hub, maintains both germline and somatic stem cells. RESULTS: This study investigated the molecular regulation of the JAK/STAT pathway in the stem cells of the Drosophila testis. We determined that the transcriptional regulator Apontic (Apt) acts in the somatic (cyst) stem cells (CySCs) to balance differentiation and maintenance. We found Apt functions as a negative feedback inhibitor of STAT activity, which enables cyst cell maturation. Simultaneous loss of the STAT regulators apt and Socs36E, or the Stat92E-targeting microRNA miR-279, expanded the somatic stem cell-like population. CONCLUSIONS: Genetic analysis revealed that a conserved genetic regulatory network limits JAK/STAT activity in the somatic stem cells of Drosophila testis. In these cells, we determined JAK/STAT signaling promotes apt expression. Then, Apt functions through Socs36E and miR-279 to attenuate pathway activation, which is required for timely CySC differentiation. We propose that Apt acts as a core component of a STAT-regulatory circuit to prevent stem cell overpopulation and allow stem cell maturation.

Morillo Prado, J. R., et al. (2012). "Polycomb group genes Psc and Su (z)2 maintain somatic stem cell identity and activity in Drosophila." PLoS One **7**(12): e52892.

Adult stem cells are essential for the proper function of many tissues, yet the mechanisms that maintain the proper identity and regulate proliferative capacity in stem cell lineages are not well understood. Polycomb group (PcG) proteins are transcriptional repressors that have recently emerged as important regulators of stem cell maintenance and differentiation. Here we describe the role of Polycomb Repressive Complex 1 (PRC1) genes Posterior sex combs (Psc) and Suppressor of zeste two (Su (z)2) in restricting the proliferation and maintaining the identity of the Cyst Stem Cell (CySC) lineage in the Drosophila testis. In contrast, Psc and Su (z)2 seem to be dispensable for both germline stem cell (GSC) maintenance and germ cell development. We show that loss of Psc and Su (z)2 function in the CySC lineage results in the formation of aggregates of mutant cells that proliferate abnormally, and display abnormal somatic identity correlated with derepression of the Hox gene Abdominal-B. Furthermore, we show that tumorigenesis in the CySC lineage interferes non-cell autonomously with maintenance of GSCs most likely by displacing them from their niche.

Mullen, A. C. and J. L. Wrana (2017). "TGF-beta Family Signaling in Embryonic and Somatic Stem-Cell Renewal and Differentiation." Cold Spring Harb Perspect Biol **9**(7).

Soon after the discovery of transforming growth factor-beta (TGF-beta), seminal work in vertebrate and invertebrate models revealed the TGF-beta family to be central regulators of tissue morphogenesis. Members of the TGF-beta family direct some of the earliest cell-fate decisions in animal development, coordinate complex organogenesis, and contribute to tissue homeostasis in the adult. Here, we focus on the role of the TGF-beta family in mammalian stem-cell biology and discuss its wide and varied activities both in the regulation of pluripotency and in cell-fate commitment.

Munsie, M. J., et al. (2000). "Isolation of pluripotent embryonic stem cells from reprogrammed adult mouse somatic cell nuclei." Curr Biol **10**(16): 989-992.

Pluripotent human stem cells isolated from early embryos represent a potentially unlimited source of many different cell types for cell-based gene and tissue therapies [1-3]. Nevertheless, if the full potential of cell lines derived from donor embryos is to be realised, the problem of donor-recipient tissue matching needs to be overcome. One approach, which avoids the problem of transplant rejection, would be to establish stem cell lines from the patient's own cells through therapeutic cloning [3,4]. Recent studies have shown that it is possible to transfer the nucleus from an adult somatic cell to an unfertilised oocyte that is devoid of maternal chromosomes, and achieve embryonic development under the control of the transferred nucleus [5-7]. Stem cells isolated from such a cloned embryo would be genetically identical to the patient and pose no risk of immune rejection. Here, we report the isolation of pluripotent murine stem cells from reprogrammed adult somatic cell nuclei. Embryos were generated by direct injection of mechanically isolated cumulus cell nuclei into mature oocytes. Embryonic stem (ES) cells isolated from cumulus-cell-derived blastocysts displayed the characteristic morphology and marker expression of conventional ES cells and underwent extensive differentiation into all three embryonic germ layers (endoderm, mesoderm and ectoderm) in tumours and in chimaeric foetuses

and pups. The ES cells were also shown to differentiate readily into neurons and muscle in culture. This study shows that pluripotent stem cells can be derived from nuclei of terminally differentiated adult somatic cells and offers a model system for the development of therapies that rely on autologous, human pluripotent stem cells.

Nagata, N. and S. Yamanaka (2014). "Perspectives for induced pluripotent stem cell technology: new insights into human physiology involved in somatic mosaicism." Circ Res **114**(3): 505-510.

Induced pluripotent stem cell technology makes in vitro reprogramming of somatic cells from individuals with various genetic backgrounds possible. By applying this technology, it is possible to produce pluripotent stem cells from biopsy samples of arbitrarily selected individuals with various genetic backgrounds and to subsequently maintain, expand, and stock these cells. From these induced pluripotent stem cells, target cells and tissues can be generated after certain differentiation processes. These target cells/tissues are expected to be useful in regenerative medicine, disease modeling, drug screening, toxicology testing, and proof-of-concept studies in drug development. Therefore, the number of publications concerning induced pluripotent stem cells has recently been increasing rapidly, demonstrating that this technology has begun to infiltrate many aspects of stem cell biology and medical applications. In this review, we discuss the perspectives of induced pluripotent stem cell technology for modeling human diseases. In particular, we focus on the cloning event occurring through the reprogramming process and its ability to let us analyze the development of complex disease-harboring somatic mosaicism.

Nogales, F. F., et al. (2018). "Germ cell tumour growth patterns originating from clear cell carcinomas of the ovary and endometrium: a comparative immunohistochemical study favouring their origin from somatic stem cells." Histopathology **72**(4): 634-647.

AIMS: To report a series of 11 ovarian and one endometrial neoplasm in elderly patients with mixed clear cell tumour and germ cell tumour (GCT) components, to compare their immunohistochemical profiles and demonstrate a putative stem cell population. METHODS AND RESULTS: The clear cell tumours included 11 clear cell carcinomas (CCC) and one borderline clear cell tumour, while the GCT always included glandular yolk sac tumour (YST). In four cases, there were also foci of teratoma with immature neuroepithelial and endodermal tissues and undifferentiated areas showing true embryoids. To distinguish between the clear cell and YST components, the following antibodies were used: HNF1-beta, napsin-A, cytokeratin 7 (CK7), PAX8, EMA, AFP, SALL4, villin, glypican-3 (GPC-3), GATA3, HepPar-1, OCT4, CDX2, CD30 and SOX2. HNF1-beta, CK7, EMA and GPC-3 were often expressed in both components. Other markers had higher specificity for each cellular lineage; napsin-A and PAX8 were expressed only in CCC, while SALL4, villin, AFP and HepPar-1 were positive in the glandular YST component but negative in the clear cell component. OCT4 expression occurred in six of 10 cases and consistently in teratoma (four of four). CONCLUSIONS: There is considerable immunophenotypical overlap between the two components in these mixed neoplasms, and a panel of markers should be used to facilitate the distinction. We propose that OCT4-expressing somatic cancer cells differentiate into GCT and represent spontaneously induced pluripotent stem cells, possibly conditioned by age-related epigenetic factors. These neoplasms have features of prepubertal type GCT showing lack of 12p gain, preponderance of YST and coexistence with immature neuroectoderm. However, there may also be undifferentiated stem cell areas with embryoid bodies, of the type seen in postpubertal testicular GCT, but lacking a complete embryonal carcinoma immunophenotype.

Oda, M., et al. (2009). "Establishment of trophoblast stem cell lines from somatic cell nuclear-transferred embryos." Proc Natl Acad Sci U S A **106**(38): 16293-16297.

Placental abnormalities occur frequently in cloned animals. Here, we attempted to isolate trophoblast stem (TS) cells from mouse blastocysts produced by somatic cell nuclear transfer (NT) at the blastocyst stage (NT blastocysts). Despite the predicted deficiency of the trophoblast cell lineage, we succeeded in isolating cell colonies with typical morphology of TS cells and cell lines from the NT blastocysts (ntTS cell lines) with efficiency as high as that from native blastocysts. The established 10 ntTS cell lines could be maintained in the undifferentiated state and induced to differentiate into several trophoblast subtypes in vitro. A comprehensive analysis of the transcriptional and epigenetic traits demonstrated that ntTS cells were indistinguishable from control TS cells. In addition, ntTS cells contributed exclusively to the placenta and survived until term in chimeras, indicating that ntTS cells have developmental potential as stem cells. Taken together, our data show that NT blastocysts contain cells that can produce TS cells in culture, suggesting that proper commitment to the trophoblast cell lineage in NT embryos occurs by the blastocyst stage.

Oka, M., et al. (2013). "Differential role for transcription factor Oct4 nucleocytoplasmic dynamics in somatic cell reprogramming and self-renewal of embryonic stem cells." J Biol Chem **288**(21): 15085-15097.

Oct4 is a member of the POU family of transcription factors and plays a critical role in both maintenance of the undifferentiated state of embryonic stem (ES) cells and in the reprogramming of somatic cells to induced pluripotent stem cells. Oct4 is imported into the nucleus where it functions as a transcription factor; however, the spatiotemporal dynamic behavior of Oct4 remains largely unknown. In the present study we show that Oct4 is a nucleocytoplasmic shuttling protein. Furthermore, although Oct4 mutants with altered nuclear import/export activity were able to maintain the self-renewal of ES cells, they displayed limited potential for cellular reprogramming. These results indicate that the intracellular localization of Oct4, which is dependent on nucleocytoplasmic shuttling, must be more strictly regulated for cellular reprogramming, suggesting that Oct4 plays differential roles in the self-renewal of ES cells and in somatic cell reprogramming.

Oosterhuis, J. W., et al. (2013). "Patient with two secondary somatic-type malignancies in a late recurrence of a testicular non-seminoma: illustration of potential and flaw of the cancer stem cell therapy concept." Int J Dev Biol **57**(2-4): 153-157.

Here, we report the case of a patient with a non-seminoma of the left testicle, with an intestinal-type adenocarcinoma and a low grade leiomyosarcoma in a late recurrence 19 years after initial diagnosis. The history of the patient, alive with disease 21 years after initial treatment, illustrates the potential and flaw of the cancer stem cell therapy concept. In addition, it is proposed that residual mature teratoma can be regarded as normalization of cancer due to embryonic patterning, and the development of a secondary somatic-type malignancy as failure of normalization.

Othman, E. R., et al. (2018). "Stem Cell Markers Describe a Transition From Somatic to Pluripotent Cell States in a Rat Model of Endometriosis." Reprod Sci **25**(6): 873-881.

OBJECTIVE: To study Thy1 as a fibroblast marker, SSEA1 as a marker of intermediate pluripotency, and Oct4 as a marker of established pluripotency in rat model of endometriosis. DESIGN: In vivo animal study. MATERIALS AND METHODS: Endometriosis was induced in 20 albino female rats through autologous transplantation of one uterine horn to mesentery of intestine. Other 20 rats had their horn removed without transplantation (controls). Rats were sacrificed 4 weeks after induction surgery. Ectopic, eutopic, and control endometria were harvested from endometriosis and control animals respectively. Quantitative syber green based RT-PCR was used to detect expression of Thy-1 (CD90), FUT4 (SSEA1), and POU5F1 (Oct4) genes in tissues. Relative expression was normalized to that of beta actin. Thy1, SSEA1, and Oct4 protein expression were detected by immunohistochemistry. RESULTS: Ectopic endometrium expressed significantly higher mRNA of Oct4 and SSEA1 as compared to control endometrium. Expression levels of Oct4 and SSEA1 were comparable between ectopic and eutopic endometria and between eutopic and control endometria. Thy1 (CD90) gene expression level was comparable among ectopic, eutopic, and control endometria. Oct4 immunoscore were significantly higher in ectopic (6.6+/-0.91) than eutopic (2.5+/-0.78) or control endometrium (3.7+/-0.1) (P value 0.02). Thy1 and SSEA1 immunoscores were comparable among all three types of endometria. CONCLUSIONS: Using rat model of endometriosis, ectopic endometrium showed significantly higher Oct4, and SSEA1, but similar Thy1 gene expression to that of control endometrium. This indicates increased transition from somatic to pluripotent cell states in ectopic endometrium which may play a role in endometriosis pathogenesis.

Panopoulos, A. D., et al. (2012). "The metabolome of induced pluripotent stem cells reveals metabolic changes occurring in somatic cell reprogramming." Cell Res **22**(1): 168-177.

Metabolism is vital to every aspect of cell function, yet the metabolome of induced pluripotent stem cells (iPSCs) remains largely unexplored. Here we report, using an untargeted metabolomics approach, that human iPSCs share a pluripotent metabolomic signature with embryonic stem cells (ESCs) that is distinct from their parental cells, and that is characterized by changes in metabolites involved in cellular respiration. Examination of cellular bioenergetics corroborated with our metabolomic analysis, and demonstrated that somatic cells convert from an oxidative state to a glycolytic state in pluripotency. Interestingly, the bioenergetics of various somatic cells correlated with their reprogramming efficiencies. We further identified metabolites that differ between iPSCs and ESCs, which revealed novel metabolic pathways that play a critical role in regulating somatic cell reprogramming. Our findings are the first to globally analyze the metabolome of iPSCs, and provide mechanistic insight into a new layer of regulation involved in inducing pluripotency, and in evaluating iPSC and ESC equivalence.

Pelosi, E., et al. (2011). "Germ cell formation from embryonic stem cells and the use of somatic cell nuclei in oocytes." Ann N Y Acad Sci **1221**: 18-26.

Embryonic stem cells (ESCs) have remarkable properties of pluripotency and self-renewal, along with the retention of chromosomal integrity. Germ cells function as a kind of "transgenerational stem cells," transmitting genetic information from one generation to the next. The formation of putative primordial germ cells (PGCs) and germ cells from mouse and human ESCs (hESCs) has, in fact, been shown, and the apparent derivation of functional mouse male gametes has also been described. Additionally, investigators have successfully reprogrammed somatic nuclei into a pluripotent state by inserting them into ESCs or oocytes. This would enable the generation of ESCs genetically identical to the somatic cell donor and their use in cell therapy. However, these methodologies are still inefficient and their mechanisms poorly understood. Until full comprehension of these processes is obtained, clinical applications remain remote. Nevertheless, they represent promising tools in the future, enhancing methods of therapeutic cloning and infertility treatment.

Plickert, G., et al. (2012). "Hydractinia, a pioneering model for stem cell biology and reprogramming somatic cells to pluripotency." Int J Dev Biol **56**(6-8): 519-534.

Hydractinia, a representative marine colonial hydroid, was the first organism in the history of biology in which migratory precursors of germ cells were described and termed "stem cells" (Weismann, 1883). These stem cells, now known as interstitial cells (i-cells), are thought to remain pluripotent throughout their life. Using animals depleted of their own stem cells and repopulated with allogeneic mutant donor stem cells, it was shown that Hydractinia i-cells differentiate into any cell type including epithelial cells and germ cells that express germ line markers such as Vasa, Piwi and Nanos. In Hydra, i-cells also provide germ cells and somatic cells with the exception of epithelial cells. The latter derive from two subpopulations of differentiated epithelial cells with self-renewal capacity. In Hydractinia, forced expression of the Oct4-like transcription factor, Polynem (Pln), in epithelial cells transforms them into stem cells that develop neoplasms. I-cells express the Wnt-receptor Frizzled and are Wnt responsive. Activation of Wnt signaling induces the production of numerous nematocytes (stinging cells) and nerve cells. In parallel, supernumerary tentacles develop. I-cells also express Myc and Nanos. Their misexpression causes severe developmental defects. Hydractinia polyp buds arise from aggregating stem cells, in contrast to Hydra buds, which derive from evaginating epithelial cells. Wnt activation increases budding frequency and the emergence of ectopic head structures. The potential of stem cells to invade neighbors may have provided selection pressure for the evolution of allorecognition and histo-incompatibility. Hence, Hydractinia have now attained the position of a powerful model in stem cell research, axis formation and allorecognition.

Pralong, D., et al. (2005). "A novel method for somatic cell nuclear transfer to mouse embryonic stem cells." Cloning Stem Cells **7**(4): 265-271.

Nuclear reprogramming by somatic cell nuclear transfer (SCNT) provides a practical approach for generating autologous pluripotent cells from adult somatic cells. It has been shown that murine somatic cells can also be reprogrammed to a pluripotent-like state by fusion with embryonic stem (ES) cells. Typically, the first step in SCNT involves enucleation of the recipient cell. However, recent evidence suggests that enucleated diploid ES cells may lack reprogramming capabilities. Here we have developed methods whereby larger tetraploid ES cells are first generated by fusion of two mouse ES cell lines transfected with plasmids carrying different antibiotic-resistance cassettes, followed by double antibiotic selection. Tetraploid ES cells grown on tissue culture disks or wells can be efficiently enucleated (up to 99%) using a combination of cytochalasin B treatment and centrifugation, with cytoplasts generated from these cells larger than those obtained from normal diploid ES cells. Also, we show that the enucleation rate is dependent on centrifugation time and cell ploidy. Further, we demonstrate that normal diploid ES cells can be fused to tetraploid ES cells to form heterokaryons, and that selective differential centrifugation conditions can be applied where the tetraploid nucleus is removed while the diploid donor nucleus is retained. This technology opens new avenues for generating autologous, diploid pluripotent cells, and provides a dynamic model for studying nuclear reprogramming in ES cells.

Ravindran, S. and A. George (2015). "Biomimetic extracellular matrix mediated somatic stem cell differentiation: applications in dental pulp tissue regeneration." Front Physiol **6**: 118.

Dental caries is one of the most widely prevalent infectious diseases in the world. It affects more than half of the world's population. The current treatment for necrotic dental pulp tissue arising from dental caries is root canal therapy. This treatment results in loss of tooth sensitivity and vitality making it prone for secondary infections. Over the past decade, several tissue-engineering approaches have attempted regeneration of the dental pulp tissue. Although

several studies have highlighted the potential of dental stem cells, none have transitioned into a clinical setting owing to limited availability of dental stem cells and the need for growth factor delivery systems. Our strategy is to utilize the intact ECM of pulp cells to drive lineage specific differentiation of bone marrow derived mesenchymal stem cells. From a clinical perspective, pulp ECM scaffolds can be generated using cell lines and patient specific somatic stem cells can be used for regeneration. Our published results have shown the feasibility of using pulp ECM scaffolds for odontogenic differentiation of non-dental mesenchymal cells. This focused review discusses the issues surrounding dental pulp tissue regeneration and the potential of our strategy to overcome these issues.

Rebscher, N., et al. (2012). "Hidden in the crowd: primordial germ cells and somatic stem cells in the mesodermal posterior growth zone of the polychaete Platynereis dumerillii are two distinct cell populations." Evodevo **3**: 9.

BACKGROUND: In the polychaete Platynereis, the primordial germ cells (PGCs) emerge from the vasa, piwi, and PL10 expressing mesodermal posterior growth zone (MPGZ) at the end of larval development, suggesting a post-embryonic formation from stem cells. METHODS: In order to verify this hypothesis, embryos and larvae were pulse labeled with the proliferation marker 5-ethynyl-2'-deoxyuridine (EdU) at different stages of development. Subsequently, the PGCs were visualized in 7-day-old young worms using antibodies against the Vasa protein. RESULTS: Surprisingly, the primordial germ cells of Platynereis incorporate EdU only shortly before gastrulation (6-8 hours post fertilization (hpf)), which coincides with the emergence of four small blastomeres from the mesoblast lineage. We conclude that these so-called 'secondary mesoblast cells' constitute the definitive PGCs in Platynereis. In contrast, the cells of the MPGZ incorporate EdU only from the pre-trochophore stage onward (14 hpf). CONCLUSION: While PGCs and the cells of the MPGZ in Platynereis are indistinguishable in morphology and both express the germline markers vasa, nanos, and piwi, a distinct cluster of PGCs is detectable anterior of the MPGZ following EdU pulse-labeling. Indeed the PGCs form independently from the stem cells of the MPGZ prior to gastrulation. Our data suggest an early PGC formation in the polychaete by preformation rather than by epigenesis.

Redmer, T., et al. (2011). "E-cadherin is crucial for embryonic stem cell pluripotency and can replace OCT4 during somatic cell reprogramming." EMBO Rep **12**(7): 720-726.

We report new functions of the cell-adhesion molecule E-cadherin in murine pluripotent cells. E-cadherin is highly expressed in mouse embryonic stem cells, and interference with E-cadherin causes differentiation. During cellular reprogramming of mouse fibroblasts by OCT4, SOX2, KLF4 and c-MYC, fully reprogrammed cells were exclusively observed in the E-cadherin-positive cell population and could not be obtained in the absence of E-cadherin. Moreover, reprogrammed cells could be established by viral E-cadherin in the absence of exogenous OCT4. Thus, reprogramming requires spatial cues that cross-talk with essential transcription factors. The cell-adhesion molecule E-cadherin has important functions in pluripotency and reprogramming.

Rodriguez-Alvarez, L., et al. (2013). "Constitutive expression of the embryonic stem cell marker OCT4 in bovine somatic donor cells influences blastocysts rate and quality after nucleus transfer." In Vitro Cell Dev Biol Anim **49**(9): 657-667.

Nuclear transfer (NT) is associated with epigenetic reprogramming of donor cells. Expression of certain genes in these cells might facilitate their expression in the NT embryo. This research was aimed to investigate the effect of constitutive expression of OCT4 in bovine somatic cells used for NT on the developmental potential of derived cloned embryos as well as in the expression of pluripotency markers in the Day-7 resulting embryos. Cloned blastocysts were generated from five cell lines that expressed OCT4. Pools of blastocysts were screened to detect OCT4, SOX2, and NANOG by qPCR. In vitro-fertilized time-matched blastocysts were used as controls. The development potential was assessed on the basis of blastocysts rate; grading and total cell counts at Day 7. OCT4 expression in the cell lines positively correlates with blastocysts rate ($r = 0.92$; $p = 0.02$), number of grade I blastocysts ($r = 0.96$; $p = 0.01$), and total cell number ($r = 0.98$; $p = 0.002$). The high expression of OCT4 in the cell line did not improve the final outcome of cloning. Somatic expression of OCT4 lead to increased expression of OCT4 and SOX2 in cloned grade I blastocysts; however, there was a bigger variability in OCT4 and SOX2 ($p = 0.03$; $p = 0.02$) expression in the embryos generated from cells expressing highest levels of OCT4. Probably the higher variability in OCT4 expression in cloned embryos is due to incorrect reprogramming and incapability of the oocyte to correct for higher OCT4 levels. For that reason, we concluded that OCT4 expression in somatic cells is not a good prognosis marker for selecting cell lines.

Roese-Koerner, B., et al. (2013). "Pluripotent stem cell-derived somatic stem cells as tool to study

the role of microRNAs in early human neural development." Curr Mol Med **13**(5): 707-722.

The in vitro differentiation of human pluripotent stem cells represents a convenient approach to generate large numbers of neural cells for basic and translational research. We recently described the derivation of homogeneous populations of long-term self-renewing neuroepithelial-like stem cells from human pluripotent stem cells (lt-NES (R) cells). These cells constitute a suitable source of neural stem cells for in vitro modelling of early human neural development. Recent evidence demonstrates that microRNAs are important regulators of stem cells and nervous system development. Studies in several model organisms suggest that microRNAs contribute to different stages of neurogenesis - from progenitor self-renewal to survival and function of differentiated neurons. However, the understanding of the impact of microRNA-based regulation in human neural development is still at its dawn. Here, we give an overview on the current state of microRNA biology in stem cells and neural development and examine the role of the neural-associated miR-124, miR- 125b and miR-9/9* in human lt-NES (R) cells. We show that overexpression of miR-124, as well as overexpression of miR-125b, impair lt-NES (R) cell self-renewal and induce differentiation into neurons. Overexpression of the miR-9/9* locus also impairs self-renewal of lt-NES (R) cells and supports their commitment to neuronal differentiation. A detailed examination revealed that overexpression of miR-9 promotes differentiation, while overexpression of miR-9* affects both proliferation and differentiation of lt-NES (R) cells. This work provides insights into the regulation of early human neuroepithelial cells by microRNAs and highlights the potential of controlling differentiation of human stem cells by modulating the expression of selected microRNAs.

Rouhani, F. J., et al. (2016). "Mutational History of a Human Cell Lineage from Somatic to Induced Pluripotent Stem Cells." PLoS Genet **12**(4): e1005932.

The accuracy of replicating the genetic code is fundamental. DNA repair mechanisms protect the fidelity of the genome ensuring a low error rate between generations. This sustains the similarity of individuals whilst providing a repertoire of variants for evolution. The mutation rate in the human genome has recently been measured to be 50-70 de novo single nucleotide variants (SNVs) between generations. During development mutations accumulate in somatic cells so that an organism is a mosaic. However, variation within a tissue and between tissues has not been analysed. By reprogramming somatic cells into induced pluripotent stem cells (iPSCs), their genomes and the associated mutational history are captured. By sequencing the genomes of polyclonal and monoclonal somatic cells and derived iPSCs we have determined the mutation rates and show how the patterns change from a somatic lineage in vivo through to iPSCs. Somatic cells have a mutation rate of 14 SNVs per cell per generation while iPSCs exhibited a ten-fold lower rate. Analyses of mutational signatures suggested that deamination of methylated cytosine may be the major mutagenic source in vivo, whilst oxidative DNA damage becomes dominant in vitro. Our results provide insights for better understanding of mutational processes and lineage relationships between human somatic cells. Furthermore it provides a foundation for interpretation of elevated mutation rates and patterns in cancer.

Ruiz, S., et al. (2015). "Limiting replication stress during somatic cell reprogramming reduces genomic instability in induced pluripotent stem cells." Nat Commun **6**: 8036.

The generation of induced pluripotent stem cells (iPSC) from adult somatic cells is one of the most remarkable discoveries in recent decades. However, several works have reported evidence of genomic instability in iPSC, raising concerns on their biomedical use. The reasons behind the genomic instability observed in iPSC remain mostly unknown. Here we show that, similar to the phenomenon of oncogene-induced replication stress, the expression of reprogramming factors induces replication stress. Increasing the levels of the checkpoint kinase 1 (CHK1) reduces reprogramming-induced replication stress and increases the efficiency of iPSC generation. Similarly, nucleoside supplementation during reprogramming reduces the load of DNA damage and genomic rearrangements on iPSC. Our data reveal that lowering replication stress during reprogramming, genetically or chemically, provides a simple strategy to reduce genomic instability on mouse and human iPSC.

Santourlidis, S., et al. (2011). "Unrestricted somatic stem cells (USSC) from human umbilical cord blood display uncommitted epigenetic signatures of the major stem cell pluripotency genes." Stem Cell Res **6**(1): 60-69.

Unrestricted somatic stem cells (USSC) from human cord blood display a broad differentiation potential for ectodermal, mesodermal, and endodermal cell types. The molecular basis for these stem cell properties is unclear and unlike embryonic stem cells (ESC) none of the major stem cell factors OCT4, SOX2, and NANOG exhibits significant expression in USSC. Here, we report that these key stem cell genes hold an epigenetic state in between that of an ESC and a terminally differentiated cell type. DNA methylation

analysis exhibits partial demethylation of the regulatory region of OCT4 and a demethylated state of the NANOG and SOX2 promoter/enhancer regions. Further genome-wide DNA methylation profiling identified a partially demethylated state of the telomerase gene hTERT. Moreover, none of the pluripotency factors exhibited a repressive histone signature. Notably, SOX2 exhibits a bivalent histone signature consisting of the opposing histone marks dimeH3K4 and trimeH3K27, which is typically found on genes that are "poised" for transcription. Consequently, ectopic expression of OCT4 in USSC led to rapid induction of expression of its known target gene SOX2. Our data suggest that incomplete epigenetic repression and a "poised" epigenetic status of pluripotency genes preserves the USSC potential to be able to react adequately to distinct differentiation and reprogramming cues.

Schira, J., et al. (2012). "Significant clinical, neuropathological and behavioural recovery from acute spinal cord trauma by transplantation of a well-defined somatic stem cell from human umbilical cord blood." Brain **135**(Pt 2): 431-446.

Stem cell therapy is a potential treatment for spinal cord injury and different stem cell types have been grafted into animal models and humans suffering from spinal trauma. Due to inconsistent results, it is still an important and clinically relevant question which stem cell type will prove to be therapeutically effective. Thus far, stem cells of human sources grafted into spinal cord mostly included barely defined heterogeneous mesenchymal stem cell populations derived from bone marrow or umbilical cord blood. Here, we have transplanted a well-defined unrestricted somatic stem cell isolated from human umbilical cord blood into an acute traumatic spinal cord injury of adult immune suppressed rat. Grafting of unrestricted somatic stem cells into the vicinity of a dorsal hemisection injury at thoracic level eight resulted in hepatocyte growth factor-directed migration and accumulation within the lesion area, reduction in lesion size and augmented tissue sparing, enhanced axon regrowth and significant functional locomotor improvement as revealed by three behavioural tasks (open field Basso-Beattie-Bresnahan locomotor score, horizontal ladder walking test and CatWalk gait analysis). To accomplish the beneficial effects, neither neural differentiation nor long-lasting persistence of the grafted human stem cells appears to be required. The secretion of neurite outgrowth-promoting factors in vitro further suggests a paracrine function of unrestricted somatic stem cells in spinal cord injury. Given the highly supportive functional characteristics in spinal cord injury, production in virtually unlimited quantities at GMP grade and lack of ethical concerns, unrestricted somatic stem cells appear to be a highly suitable human stem cell source for clinical application in central nervous system injuries.

Schmidt, R. and K. Plath (2012). "The roles of the reprogramming factors Oct4, Sox2 and Klf4 in resetting the somatic cell epigenome during induced pluripotent stem cell generation." Genome Biol **13**(10): 251.

Somatic cell reprogramming to induced pluripotent stem (iPS) cells by defined factors is a form of engineered reverse development carried out in vitro. Recent investigation has begun to elucidate the molecular mechanisms whereby these factors function to reset the epigenome.

Schwalie, P. C., et al. (2017). "Cross-Tissue Identification of Somatic Stem and Progenitor Cells Using a Single-Cell RNA-Sequencing Derived Gene Signature." Stem Cells **35**(12): 2390-2402.

A long-standing question in biology is whether multipotent somatic stem and progenitor cells (SSPCs) feature molecular properties that could guide their system-independent identification. Population-based transcriptomic studies have so far not been able to provide a definite answer, given the rarity and heterogeneous nature of these cells. Here, we exploited the resolving power of single-cell RNA-sequencing to develop a computational model that is able to accurately distinguish SSPCs from differentiated cells across tissues. The resulting classifier is based on the combined expression of 23 genes including known players in multipotency, proliferation, and tumorigenesis, as well as novel ones, such as Lcp1 and Vgll4 that we functionally validate in intestinal organoids. We show how this approach enables the identification of stem-like cells in still ambiguous systems such as the pancreas and the epidermis as well as the exploration of lineage commitment hierarchies, thus facilitating the study of biological processes such as cellular differentiation, tissue regeneration, and cancer. Stem Cells 2017;35:2390-2402.

Secher, J. O., et al. (2017). "Evaluation of porcine stem cell competence for somatic cell nuclear transfer and production of cloned animals." Anim Reprod Sci **178**: 40-49.

Porcine somatic cell nuclear transfer (SCNT) has been used extensively to create genetically modified pigs, but the efficiency of the methodology is still low. It has been hypothesized that pluripotent or multipotent stem cells might result in increased SCNT efficacy as these cells are closer than somatic cells to the epigenetic state found in the blastomeres and therefore need less reprogramming. Our group has worked with porcine SCNT during the last 20 years

and here we describe our experience with SCNT of 3 different stem cell lines. The porcine stem cells used were: Induced pluripotent stem cells (iPSCs) created by lentiviral doxycycline-dependent reprogramming and cultered with a GSK3beta- and MEK-inhibitor (2i) and leukemia inhibitor factor (LIF) (2i LIF DOX-iPSCs), iPSCs created by a plasmid-based reprogramming and cultured with 2i and fibroblast growth factor (FGF) (2i FGF Pl-iPSCs) and embryonic germ cells (EGCs), which have earlier been characterized as being multipotent. The SCNT efficiencies of these stem cell lines were compared with that of the two fibroblast cell lines from which the iPSC lines were derived. The blastocyst rates for the 2i LIF DOX-iPSCs were 14.7%, for the 2i FGF Pl-iPSC 10.1%, and for the EGCs 34.5% compared with the fibroblast lines yielding 36.7% and 25.2%. The fibroblast- and EGC-derived embryos were used for embryo transfer and produced live offspring at similar low rates of efficiency (3.2 and 4.0%, respectively) and with several instances of malformations. In conclusion, potentially pluripotent porcine stem cells resulted in lower rates of embryonic development upon SCNT than multipotent stem cells and differentiated somatic cells.

Seipel, K., et al. (2004). "The germ line and somatic stem cell gene Cniwi in the jellyfish Podocoryne carnea." Int J Dev Biol **48**(1): 1-7.

In most animal phyla from insects to mammals, there is a clear division of somatic and germ line cells. This is however not the case in plants and some animal phyla including tunicates, flatworms and the basal phylum Cnidaria, where germ stem cells arise de novo from somatic cells. Piwi-like genes represent essential stem cell genes in diverse multicellular organisms. The cnidarian Piwihomolog Cniwiwas cloned from Podocoryne carnea, a hydrozoan with a full life cycle. CniwiRNA is present in all developmental stages with highest levels in the egg and the medusa. In the adult medusa, Cniwi expression is prominent in the gonads where it likely functions as a germ stem cell gene. The gene is also expressed, albeit at low levels, in differentiated somatic cells like the striated muscle of the medusa. Isolated striated muscle cells can be induced to transdifferentiate into smooth muscle cells which proliferate and differentiate into nerve cells. Cniwi expression is upregulated transiently after induction of transdifferentiation and again when the emerging smooth muscle cells proliferate and differentiate. The continuous low-level expression of an inducible stem cell gene in differentiated somatic cells may underlie the ability to form medusa buds from polyp cells and explain the extraordinary transdifferentation and regeneration potential of Podocoryne carnea.

Shah, S. M., et al. (2015). "Development of buffalo (Bubalus bubalis) embryonic stem cell lines from somatic cell nuclear transferred blastocysts." Stem Cell Res **15**(3): 633-639.

We developed buffalo embryonic stem cell lines from somatic cell nuclear transfer derived blastocysts, produced by hand-guided cloning technique. The inner cell mass of the blastocyst was cut mechanically using a Microblade and cultured onto feeder cells in buffalo embryonic stem (ES) cell culture medium at 38 degrees C in a 5% CO2 incubator. The stem cell colonies were characterized for alkaline phosphatase activity, karyotype, pluripotency and self-renewal markers like OCT4, NANOG, SOX2, c-Myc, FOXD3, SSEA-1, SSEA-4, TRA-1-60, TRA-1-81 and CD90. The cell lines also possessed the capability to differentiate across all the three germ layers under spontaneous differentiation conditions.

Shah, S. N., et al. (2012). "HMGA1 reprograms somatic cells into pluripotent stem cells by inducing stem cell transcriptional networks." PLoS One **7**(11): e48533.

BACKGROUND: Although recent studies have identified genes expressed in human embryonic stem cells (hESCs) that induce pluripotency, the molecular underpinnings of normal stem cell function remain poorly understood. The high mobility group A1 (HMGA1) gene is highly expressed in hESCs and poorly differentiated, stem-like cancers; however, its role in these settings has been unclear. METHODS/PRINCIPAL FINDINGS: We show that HMGA1 is highly expressed in fully reprogrammed iPSCs and hESCs, with intermediate levels in ECCs and low levels in fibroblasts. When hESCs are induced to differentiate, HMGA1 decreases and parallels that of other pluripotency factors. Conversely, forced expression of HMGA1 blocks differentiation of hESCs. We also discovered that HMGA1 enhances cellular reprogramming of somatic cells to iPSCs together with the Yamanaka factors (OCT4, SOX2, KLF4, cMYC - OSKM). HMGA1 increases the number and size of iPSC colonies compared to OSKM controls. Surprisingly, there was normal differentiation in vitro and benign teratoma formation in vivo of the HMGA1-derived iPSCs. During the reprogramming process, HMGA1 induces the expression of pluripotency genes, including SOX2, LIN28, and cMYC, while knockdown of HMGA1 in hESCs results in the repression of these genes. Chromatin immunoprecipitation shows that HMGA1 binds to the promoters of these pluripotency genes in vivo. In addition, interfering with HMGA1 function using a short hairpin RNA or a dominant-negative construct blocks cellular reprogramming to a pluripotent state.

CONCLUSIONS: Our findings demonstrate for the first time that HMGA1 enhances cellular reprogramming from a somatic cell to a fully pluripotent stem cell. These findings identify a novel role for HMGA1 as a key regulator of the stem cell state by inducing transcriptional networks that drive pluripotency. Although further studies are needed, these HMGA1 pathways could be exploited in regenerative medicine or as novel therapeutic targets for poorly differentiated, stem-like cancers.

Shemesh, N., et al. (2013). "Germline stem cell arrest inhibits the collapse of somatic proteostasis early in Caenorhabditis elegans adulthood." Aging Cell **12**(5): 814-822.

All cells rely on highly conserved protein folding and clearance pathways to detect and resolve protein damage and to maintain protein homeostasis (proteostasis). Because age is associated with an imbalance in proteostasis, there is a need to understand how protein folding is regulated in a multicellular organism that undergoes aging. We have observed that the ability of Caenorhabditis elegans to maintain proteostasis declines sharply following the onset of oocyte biomass production, suggesting that a restricted protein folding capacity may be linked to the onset of reproduction. To test this hypothesis, we monitored the effects of different sterile mutations on the maintenance of proteostasis in the soma of C. elegans. We found that germline stem cell (GSC) arrest rescued protein quality control, resulting in maintenance of robust proteostasis in different somatic tissues of adult animals. We further demonstrated that GSC-dependent modulation of proteostasis requires several different signaling pathways, including hsf-1 and daf-16/kri-1/tcer-1, daf-12, daf-9, daf-36, nhr-80, and pha-4 that differentially modulate somatic quality control functions, such that each signaling pathway affects different aspects of proteostasis and cannot functionally complement the other pathways. We propose that the effect of GSCs on the collapse of proteostasis at the transition to adulthood is due to a switch mechanism that links GSC status with maintenance of somatic proteostasis via regulation of the expression and function of different quality control machineries and cellular stress responses that progressively lead to a decline in the maintenance of proteostasis in adulthood, thereby linking reproduction to the maintenance of the soma.

Shenoy, A. and R. H. Blelloch (2014). "Regulation of microRNA function in somatic stem cell proliferation and differentiation." Nat Rev Mol Cell Biol **15**(9): 565-576.

microRNAs (miRNAs) are important modulators of development. Owing to their ability to simultaneously silence hundreds of target genes, they have key roles in large-scale transcriptomic changes that occur during cell fate transitions. In somatic stem and progenitor cells--such as those involved in myogenesis, haematopoiesis, skin and neural development--miRNA function is carefully regulated to promote and stabilize cell fate choice. miRNAs are integrated within networks that form both positive and negative feedback loops. Their function is regulated at multiple levels, including transcription, biogenesis, stability, availability and/or number of target sites, as well as their cooperation with other miRNAs and RNA-binding proteins. Together, these regulatory mechanisms result in a refined molecular response that enables proper cellular differentiation and function.

Shields, A. R., et al. (2014). "The actin-binding protein profilin is required for germline stem cell maintenance and germ cell enclosure by somatic cyst cells." Development **141**(1): 73-82.

Specialized microenvironments, or niches, provide signaling cues that regulate stem cell behavior. In the Drosophila testis, the JAK-STAT signaling pathway regulates germline stem cell (GSC) attachment to the apical hub and somatic cyst stem cell (CySC) identity. Here, we demonstrate that chickadee, the Drosophila gene that encodes profilin, is required cell autonomously to maintain GSCs, possibly facilitating localization or maintenance of E-cadherin to the GSC-hub cell interface. Germline specific overexpression of Adenomatous Polyposis Coli 2 (APC2) rescued GSC loss in chic hypomorphs, suggesting an additive role of APC2 and F-actin in maintaining the adherens junctions that anchor GSCs to the niche. In addition, loss of chic function in the soma resulted in failure of somatic cyst cells to maintain germ cell enclosure and overproliferation of transit-amplifying spermatogonia.

Shin, D. H., et al. (2017). "Characterization of Tetraploid Somatic Cell Nuclear Transfer-Derived Human Embryonic Stem Cells." Dev Reprod **21**(4): 425-434.

Polyploidy is occurred by the process of endomitosis or cell fusion and usually represent terminally differentiated stage. Their effects on the developmental process were mainly investigated in the amphibian and fishes, and only observed in some rodents as mammalian model. Recently, we have established tetraploidy somatic cell nuclear transfer-derived human embryonic stem cells (SCNT-hESCs) and examined whether it could be available as a research model for the polyploidy cells existed in the human tissues. Two tetraploid hESC lines were artificially acquired by reintroduction of remained 1st polar body during the establishment of SCNT-hESC

using MII oocytes obtained from female donors and dermal fibroblasts (DFB) from a 35-year-old adult male. These tetraploid SCNT-hESC lines (CHA-NT1 and CHA-NT3) were identified by the cytogenetic genotyping (91, XXXY,-6, t [2:6] / 92,XXXY,-12,+20) and have shown of indefinite proliferation, but slow speed when compared to euploid SCNT-hESCs. Using the eight Short Tendem Repeat (STR) markers, it was confirmed that both CHA-NT1 and CHA-NT3 lines contain both nuclear and oocyte donor genotypes. These hESCs expressed pluripotency markers and their embryoid bodies (EB) also expressed markers of the three embryonic germ layers and formed teratoma after transplantation into immune deficient mice. This study showed that tetraploidy does not affect the activities of proliferation and differentiation in SCNT-hESC. Therefore, tetraploid hESC lines established after SCNT procedure could be differentiated into various types of cells and could be an useful model for the study of the polyploidy cells in the tissues.

Sidorov, I., et al. (2009). "Leukocyte telomere dynamics and human hematopoietic stem cell kinetics during somatic growth." Exp Hematol **37**(4): 514-524.

OBJECTIVE: A central question in stem cell research is knowing the frequency of human hematopoietic stem cells (HSC) replication in vivo. MATERIALS AND METHODS: We have constructed a model that characterizes HSC kinetics and the relative sizes of the hematopoietic progenitor cell (HPC) and HSC pools from birth onward. The model capitalizes on leukocyte telomere length (LTL) data and body weight-gain charts from birth to the age of 20 years. The core premise of the model is that during human growth, LTL dynamics (birth LTL and age-dependent LTL shortening afterward) chronicle the expansions of the HSC and HPC pools. RESULTS: The model estimates that by the end of the first year of life, HSC have replicated approximately 17 times and they replicate approximately 2.5 times/year between the ages of 3 and 13 years. Subsequently, HSC replication slows considerably. In adults HSC replicate at a rate of approximately 0.6 times/year. In addition, the model predicts that newborns with small birth weight would have shorter LTL as adults and that women would have longer LTL than men. CONCLUSION: Our findings will be useful in bone marrow transplantations and might explain a body of clinical observations related to LTL distribution in the general population.

Somoza, R. A. and F. J. Rubio (2012). "Cell therapy using induced pluripotent stem cells or somatic stem cells: this is the question." Curr Stem Cell Res Ther **7**(3): 191-196.

A lot of effort has been developed to bypass the use of embryonic stem cells (ES) in human therapies, because of several concerns and ethical issues. Some unsolved problems of using stem cells for human therapies, excluding the human embryonic origin, are: how to regulate cell plasticity and proliferation, immunological compatibility, potential adverse side-effects when stem cells are systemically administrated, and the in vivo signals to rule out a specific cell fate after transplantation. Currently, it is known that almost all tissues of an adult organism have somatic stem cells (SSC). Whereas ES are primary involved in the genesis of new tissues and organs, SSC are involved in regeneration processes, immuno-regulatory and homeostasis mechanisms. Although the differentiating potential of ES is higher than SSC, several studies suggest that some types of SSC, such as mesenchymal stem cells (MSC), can be induced epigenetically to differentiate into tissue-specific cells of different lineages. This unexpected pluripotency and the variety of sources that they come from, can make MSC-like cells suitable for the treatment of diverse pathologies and injuries. New hopes for cell therapy came from somatic/mature cells and the discovery that could be reprogrammed to a pluripotent stage similar to ES, thus generating induced pluripotent stem cells (iPS). For this, it is necessary to overexpress four main reprogramming factors, Sox2, Oct4, Klf4 and c-Myc. The aim of this review is to analyze the potential and requirements of cellular based tools in human therapy strategies, focusing on the advantage of using MSC over iPS.

Strelchenko, N., et al. (2006). "Reprogramming of human somatic cells by embryonic stem cell cytoplast." Reprod Biomed Online **12**(1): 107-111.

Somatic cell nuclear transfer (SCNT) provides the basis for the development of patient-specific stem cell lines. Recent progress in SCNT suggested the presence of reprogramming factors in human embryonic stem (hES) cells, although no method is currently available for replacement of nuclei of hES cells by somatic cell nuclei. An original technique has been developed, involving the fusion of different types of somatic cells with hES cells, which allowed a complete replacement of the nuclei of hES cells by nuclei of somatic cells. The resulting 'cybrids' were demonstrated to have the genotype of the donor somatic cells and 'stemness' of the recipient hES cells. However, the colonies isolated from the resulting fusion contained a mixture of these cybrid cells with the cells with the recipient nuclei, as well as hybrid cells containing both donor and recipient nuclei, so future purification will be necessary before the technique can be considered for future practical application.

Su, Y. H., et al. (2009). "Auxin-induced WUS expression is essential for embryonic stem cell renewal during somatic embryogenesis in Arabidopsis." Plant J **59**(3): 448-460.

Somatic embryogenesis requires auxin and establishment of the shoot apical meristem (SAM). WUSCHEL (WUS) is critical for stem cell fate determination in the SAM of higher plants. However, regulation of WUS expression by auxin during somatic embryogenesis is poorly understood. Here, we show that expression of several regulatory genes important in zygotic embryogenesis were up-regulated during somatic embryogenesis of Arabidopsis. Interestingly, WUS expression was induced within the embryonic callus at a time when somatic embryos could not be identified morphologically or molecularly. Correct WUS expression, regulated by a defined critical level of exogenous auxin, is essential for somatic embryo induction. Furthermore, it was found that auxin gradients were established in specific regions that could then give rise to somatic embryos. The establishment of auxin gradients was correlated with the induced WUS expression. Moreover, the auxin gradients appear to activate PIN1 polar localization within the embryonic callus. Polarized PIN1 is probably responsible for the observed polar auxin transport and auxin accumulation in the SAM and somatic embryo. Suppression of WUS and PIN1 indicated that both genes are necessary for embryo induction through their regulation of downstream gene expression. Our results reveal that establishment of auxin gradients and PIN1-mediated polar auxin transport are essential for WUS induction and somatic embryogenesis. This study sheds new light on how auxin regulates stem cell formation during somatic embryogenesis.

Subramanian, V. (1989). "A malignant, stem cell-like somatic hybrid between a mouse teratocarcinoma and a rat ascitic hepatoma is differentiation competent." Cell Differ Dev **27**(3): 197-214.

A hybrid clone was developed by the fusion of a pluripotent mouse teratocarcinoma cell line PCC-4 AzaR to the Zajdela ascitic hepatoma (ZAH) of rat origin. This hybrid cell line, F2231A, possessed a predominantly teratocarcinoma morphology with a large nucleus and prominent nucleoli, and grew in nests. F2231A cells formed undifferentiated tumours in irradiated Sv/129 mice. It formed aggregates when subcultured at high densities in bacteriological Petri dishes. The hybrid cell line differentiated in response to retinoic acid and also underwent spontaneous differentiation upon overgrowth. Karyological analysis showed the presence of several rat chromosomes in the hybrid and upon isozyme analysis it was found that only the rat variant of the X-linked enzyme HGPRT was expressed. Analysis of the genomic DNA with a cloned probe, specific for rat repetitive sequences, gave strong positive signals in the hepatoma parent and F2231A cells while the parental embryonal carcinoma (EC) cells were negative. The hybrid cell line, like the PCC-4 cells, expressed the SSEA-1 surface marker but not SSEA-3, intercellular fibronectin and EGF receptors. Upon differentiation of F2231A cells there was a loss of expression of SSEA-1. The mRNA for alpha-fetoprotein was expressed by the hybrid cell line and in this respect it resembled the hepatoma parent. Albumin mRNA was not detectable in the hybrid cell line. The mRNA for the transformation-related protein, p53, was expressed at a high level in F2231A cells. The hybrid cell line F2231A retained several of the biochemical and immunological properties of the teratocarcinoma cells.

Sullivan, S., et al. (2006). "Nuclear reprogramming of somatic cells by embryonic stem cells is affected by cell cycle stage." Cloning Stem Cells **8**(3): 174-188.

Hybrid embryonic stem (ES)-like clones were generated by fusion of murine ES cells with somatic cells that carried a neo resistance gene under the transcriptional control of the Oct-4 promoter. The Oct-4 promoter was reactivated in hybrid ES cells formed by fusion with fetal fibroblasts, and all hybrid colonies were of ES rather than fibroblast phenotype, suggesting efficient reprogramming of fibroblast chromosomes. Like normal diploid murine ES cells, hybrid lines expressed alkaline phosphatase activity and formed differentiated cells derived from the three embryonic germ layers both in vitro and in vivo. Treatments thought to affect nuclear transfer efficiency (ES cell confluence and serum starvation of primary embryonic fibroblasts) were investigated to determine whether they had an effect on reprogramming in cell hybrids. Serum starvation of primary embryonic fibroblasts increased hybrid colony number 50-fold. ES cells were most effective at reprogramming when they contained a high proportion of cells in the S and G2/M phases of the cell cycle. These data suggest that nuclear reprogramming requires an initial round of somatic DNA replication of quiescent chromatin in the presence of ES-derived factors produced during S and G2/M phases.

Sumer, H. and P. J. Verma (2015). "Embryonic stem cell-somatic cell fusion and postfusion enucleation." Methods Mol Biol **1222**: 61-69.

Embryonic stem (ES) cells are able to reprogram somatic cells following cell fusion. The resulting cell hybrids have been shown to have similar properties to pluripotent cells. It has also been shown that

transcriptional changes can occur in a heterokaryon, without nuclear hybridization. However it is unclear whether these changes can be sustained following removal of the dominant ES nucleus. In this chapter, methods are described for the cell fusion of mouse tetraploid ES cells with somatic cells and enrichment of the resulting heterokaryons. We next describe the conditions for the differential removal of the ES cell nucleus, allowing for the recovery of somatic cells.

Sung, L. Y., et al. (2014). "Telomere elongation and naive pluripotent stem cells achieved from telomerase haplo-insufficient cells by somatic cell nuclear transfer." Cell Rep **9**(5): 1603-1609.

Haplo-insufficiency of telomerase genes in humans leads to telomere syndromes such as dyskeratosis congenital and idiopathic pulmonary fibrosis. Generation of pluripotent stem cells from telomerase haplo-insufficient donor cells would provide unique opportunities toward the realization of patient-specific stem cell therapies. Recently, pluripotent human embryonic stem cells (ntESCs) have been efficiently achieved by somatic cell nuclear transfer (SCNT). We tested the hypothesis that SCNT could effectively elongate shortening telomeres of telomerase haplo-insufficient cells in the ntESCs with relevant mouse models. Indeed, telomeres of telomerase haplo-insufficient (Terc (+/-)) mouse cells are elongated in ntESCs. Moreover, ntESCs derived from Terc (+/-) cells exhibit naive pluripotency as evidenced by generation of Terc (+/-) ntESC clone pups by tetraploid embryo complementation, the most stringent test of naive pluripotency. These data suggest that SCNT could offer a powerful tool to reprogram telomeres and to discover the factors for robust restoration of telomeres and pluripotency of telomerase haplo-insufficient somatic cells.

Sung, L. Y., et al. (2006). "Differentiated cells are more efficient than adult stem cells for cloning by somatic cell nuclear transfer." Nat Genet **38**(11): 1323-1328.

Since the creation of Dolly via somatic cell nuclear transfer (SCNT), more than a dozen species of mammals have been cloned using this technology. One hypothesis for the limited success of cloning via SCNT (1%-5%) is that the clones are likely to be derived from adult stem cells. Support for this hypothesis comes from the findings that the reproductive cloning efficiency for embryonic stem cells is five to ten times higher than that for somatic cells as donors and that cloned pups cannot be produced directly from cloned embryos derived from differentiated B and T cells or neuronal cells. The question remains as to whether SCNT-derived animal clones can be derived from truly differentiated somatic cells. We tested this hypothesis with mouse hematopoietic cells at different differentiation stages: hematopoietic stem cells, progenitor cells and granulocytes. We found that cloning efficiency increases over the differentiation hierarchy, and terminally differentiated postmitotic granulocytes yield cloned pups with the greatest cloning efficiency.

Svahn, J., et al. (2016). "Somatic, hematologic phenotype, long-term outcome, and effect of hematopoietic stem cell transplantation. An analysis of 97 Fanconi anemia patients from the Italian national database on behalf of the Marrow Failure Study Group of the AIEOP (Italian Association of Pediatric Hematology-Oncology)." Am J Hematol **91**(7): 666-671.

We analyzed 97 Fanconi anemia patients from a clinic/biological database for genotype, somatic, and hematologic phenotype, adverse hematological events, solid tumors, and treatment. Seventy-two patients belonged to complementation group A. Eighty percent of patients presented with mild/moderate somatic phenotype and most with cytopenia. No correlation was seen between somatic/hematologic phenotype and number of missense mutations of FANCA alleles. Over follow-up, 33% of patients improved or maintained mild/moderate cytopenia or normal blood count, whereas remaining worsened cytopenia. Eleven patients developed a hematological adverse event (MDS, AML, pathological cytogenetics) and three developed solid tumors. 10 years cumulative risk of death of the whole cohort was 25.6% with median follow-up 5.8 years. In patients eligible to hematopoietic stem cell transplantation because of moderate cytopenia, mortality was significantly higher in subjects transplanted from matched unrelated donor over nontransplanted subjects, whereas there was no significant difference between matched sibling donor transplants and nontransplanted patients. In patients eligible to transplant because of severe cytopenia and clonal disease, mortality risk was not significantly different in transplanted from matched unrelated versus matched sibling donor versus nontransplanted subjects. The decision to transplant should rely on various elements including, type of donor, HLA matching, patient comorbidities, impairment, and clonal evolution of hematopoiesis. Am. J. Hematol. 91:666-671, 2016. (c) 2016 Wiley Periodicals, Inc.

Tsao, J. L., et al. (1998). "Tracing cell fates in human colorectal tumors from somatic microsatellite mutations: evidence of adenomas with stem cell architecture." Am J Pathol **153**(4): 1189-1200.

Occult aspects of tumor proliferation are likely recorded genetically as their microsatellite (MS) loci become polymorphic. However, MS mutations

generated by division may also be eliminated with death as noncoding MS loci lack selective value. Therefore, highly polymorphic MS loci cannot exist unless mutation rates are high, or unless mutation losses are inherently minimized. Mutations accumulate differently when cell fates are determined intrinsically before or extrinsically after division. Stem cell (asymmetrical division as in intestinal crypts) and random (asymmetrical and symmetrical division) proliferation, respectively, represent simulated cell fates determined before or after division. Whereas mutations regardless of selection systematically persist once inherited with stem cell proliferation, mutations are eliminated by the symmetrical losses of both daughter cells with random proliferation. Therefore, greater genetic diversity or MS variance accumulate with stem cell compared with random proliferation. MS loci in normal murine intestinal mucosa and xenografts of cancer cell lines accumulated mutations, respectively, consistent with stem cell and random proliferation. Tumors from patients with hereditary nonpolyposis colorectal cancer (HNPCC) demonstrated polymorphic MS loci. Overall, three of five adenomas and one of six cancers exhibited high MS variances. Assuming mutation rates are not significantly greater in adenomas than in cancers, these studies suggest the stem cell proliferation and hierarchy of normal intestines persists in many HNPCC adenomas and some cancers. An adenoma stem cell architecture can explain the complex polymorphic MS loci observed in HNPCC adenomas and account for many adenoma features. In contrast, cancers may lose intrinsic control of cell fate. These studies illustrate a feasible phylogenetic approach to unravel and describe occult aspects of human tumor proliferation. The switch from predominantly stem cell to random proliferation may be a critical and defining characteristic of malignancy.

Tseng, C. Y., et al. (2018). "Smad-Independent BMP Signaling in Somatic Cells Limits the Size of the Germline Stem Cell Pool." Stem Cell Reports **11**(3): 811-827.

In developing organisms, proper tuning of the number of stem cells within a niche is critical for the maintenance of adult tissues; however, the involved mechanisms remain largely unclear. Here, we demonstrate that Thickveins (Tkv), a type I bone morphogenetic protein (BMP) receptor, acts in the Drosophila developing ovarian soma through a Smad-independent pathway to shape the distribution of BMP signal within the niche, impacting germline stem cell (GSC) recruitment and maintenance. Somatic Tkv promotes Egfr signaling to silence transcription of Dally, which localizes BMP signals on the cell surface. In parallel, Tkv promotes Hh signaling, which promotes escort cell cellular protrusions and upregulates expression of the Drosophila BMP homolog, Dpp, forming a positive feedback loop that enhances Tkv signaling and strengthens the niche boundary. Our results reveal a role for non-canonical BMP signaling in the soma during GSC establishment and generally illustrate how complex, cell-specific BMP signaling mediates niche-stem cell interactions.

Vasilkova, A. A., et al. (2007). "Dominant manifestation of pluripotency in embryonic stem cell hybrids with various numbers of somatic chromosomes." Mol Reprod Dev **74**(8): 941-951.

Developmental potential was assessed in 8 intra-specific and 20 inter-specific hybrid clones obtained by fusion of embryonic stem (ES) cells with either splenocytes or fetal fibroblasts. Number of chromosomes derived from ES cells in these hybrid clones was stable while contribution of somatic partner varied from single chromosomes to complete complement. This allowed us to compare pluripotency of the hybrid cells with various numbers of somatic chromosomes. Three criteria were used for the assessment: (i) expression of Oct-4 and Nanog genes; (ii) analyses of teratomas generated by subcutaneous injections of the tested cells into immunodeficient mice; (iii) contribution of the hybrid cells in chimeras generated by injection of the tested cells into C57BL blastocysts. All tested hybrid clones showed expression of Oct-4 and Nanog at level comparable to ES cells. Histological and immunofluorescent analyses demonstrated that most teratomas formed from the hybrid cells with different number of somatic chromosomes contained derivatives of three embryonic layers. Tested hybrid clones make similar contribution in various tissues of chimeras in spite of significant differences in the number of somatic chromosomes they contained. The data indicate that pluripotency is manifested as a dominant trait in the ES hybrid cells and does not depend substantially on the number of somatic chromosomes. The latter suggests that the developmental potential derived from ES cells is maintained in ES-somatic cell hybrids by cis-manner and is rather resistant to trans-acting factors emitted from the somatic one.

Vinoth, K. J., et al. (2015). "Differential resistance of human embryonic stem cells and somatic cell types to hydrogen peroxide-induced genotoxicity may be dependent on innate basal intracellular ROS levels." Folia Histochem Cytobiol **53**(2): 169-174.

Previously, we demonstrated that undifferentiated human embryonic stem cells (hESC) displayed higher resistance to oxidative and genotoxic stress compared to somatic cells, but did not further probe the underlying mechanisms. Using H (2)O (2)-

induced genotoxicity as a model, this study investigated whether higher resistance of hESC to oxidative and genotoxic stress could be due to lower innate basal intracellular levels of reactive oxygen species (ROS), as compared to their differentiated fibroblastic progenies (H1F) and two other somatic cell types - human embryonic palatal mesenchymal (HEPM) cells and peripheral blood lymphocytes (PBL). Comet assay demonstrated that undifferentiated hESC consistently sustained lower levels of DNA damage upon acute exposure to H (2)O (2) for 30 min, compared to somatic cells. DCFDA and HE staining with flow cytometry showed that undifferentiated hESC had lower innate basal intracellular levels of reactive oxygen species compared to somatic cells, which could lead to their higher resistance to genotoxic stress upon acute exposure to H (2)O (2).

Virts, E. L., et al. (2015). "AluY-mediated germline deletion, duplication and somatic stem cell reversion in UBE2T defines a new subtype of Fanconi anemia." Hum Mol Genet **24**(18): 5093-5108.

Fanconi anemia (FA) is a rare inherited disorder clinically characterized by congenital malformations, progressive bone marrow failure and cancer susceptibility. At the cellular level, FA is associated with hypersensitivity to DNA-crosslinking genotoxins. Eight of 17 known FA genes assemble the FA E3 ligase complex, which catalyzes monoubiquitination of FANCD2 and is essential for replicative DNA crosslink repair. Here, we identify the first FA patient with biallelic germline mutations in the ubiquitin E2 conjugase UBE2T. Both mutations were aluY-mediated: a paternal deletion and maternal duplication of exons 2-6. These loss-of-function mutations in UBE2T induced a cellular phenotype similar to biallelic defects in early FA genes with the absence of FANCD2 monoubiquitination. The maternal duplication produced a mutant mRNA that could encode a functional protein but was degraded by nonsense-mediated mRNA decay. In the patient's hematopoietic stem cells, the maternal allele with the duplication of exons 2-6 spontaneously reverted to a wild-type allele by monoallelic recombination at the duplicated aluY repeat, thereby preventing bone marrow failure. Analysis of germline DNA of 814 normal individuals and 850 breast cancer patients for deletion or duplication of UBE2T exons 2-6 identified the deletion in only two controls, suggesting aluY-mediated recombinations within the UBE2T locus are rare and not associated with an increased breast cancer risk. Finally, a loss-of-function germline mutation in UBE2T was detected in a high-risk breast cancer patient with wild-type BRCA1/2. Cumulatively, we identified UBE2T as a bona fide FA gene (FANCT) that also may be a rare cancer susceptibility gene.

Vitaloni, M., et al. (2014). "MicroRNAs contribute to induced pluripotent stem cell somatic donor memory." J Biol Chem **289**(4): 2084-2098.

Induced pluripotent stem cells (iPSCs) maintain during the first few culture passages a set of epigenetic marks and metabolites characteristic of their somatic cell of origin, a concept defined as epigenetic donor memory. These residual somatic features are lost over time after extensive culture passaging. Therefore, epigenetic donor memory may be responsible for the higher differentiation efficiency toward the tissue of origin observed in low passage iPSCs versus high passage iPSC or iPSCs derived from a different tissue source. Remarkably, there are no studies on the relevance of microRNA (miRNA) memory following reprogramming, despite the established role of these molecules in the context of pluripotency and differentiation. Using hematopoietic progenitors cells as a model, we demonstrated that miRNAs play a central role in somatic memory retention in iPSCs. Moreover, the comparison of the miRNA expression profiles among iPSCs from different sources allowed for the detection of a set of candidate miRNAs responsible for the higher differentiation efficiency rates toward blood progenitors observed in low passage iPSCs. Combining bioinformatic predictive algorithms with biological target validation, we identified miR-155 as a key player for the in vitro differentiation of iPSC toward hematopoietic progenitors. In summary, this study reveals that during the initial passages following reprogramming, iPSCs maintained the expression of a miRNA set exclusive to the original somatic population. Hence the use of these miRNAs might hold a direct application toward our understanding of the differentiation process of iPSCs toward hematopoietic progenitor cells.

Vo, L. T. and G. Q. Daley (2015). "De novo generation of HSCs from somatic and pluripotent stem cell sources." Blood **125**(17): 2641-2648.

Generating human hematopoietic stem cells (HSCs) from autologous tissues, when coupled with genome editing technologies, is a promising approach for cellular transplantation therapy and for in vitro disease modeling, drug discovery, and toxicology studies. Human pluripotent stem cells (hPSCs) represent a potentially inexhaustible supply of autologous tissue; however, to date, directed differentiation from hPSCs has yielded hematopoietic cells that lack robust and sustained multilineage potential. Cellular reprogramming technologies represent an alternative platform for the de novo generation of HSCs via direct conversion from

heterologous cell types. In this review, we discuss the latest advancements in HSC generation by directed differentiation from hPSCs or direct conversion from somatic cells, and highlight their applications in research and prospects for therapy.

Voog, J., et al. (2008). "Multipotent somatic stem cells contribute to the stem cell niche in the Drosophila testis." Nature **454**(7208): 1132-1136.

Adult stem cells reside in specialized microenvironments, or niches, that have an important role in regulating stem cell behaviour. Therefore, tight control of niche number, size and function is necessary to ensure the proper balance between stem cells and progenitor cells available for tissue homeostasis and wound repair. The stem cell niche in the Drosophila male gonad is located at the tip of the testis where germline and somatic stem cells surround the apical hub, a cluster of approximately 10-15 somatic cells that is required for stem cell self-renewal and maintenance. Here we show that somatic stem cells in the Drosophila testis contribute to both the apical hub and the somatic cyst cell lineage. The Drosophila orthologue of epithelial cadherin (DE-cadherin) is required for somatic stem cell maintenance and, consequently, the apical hub. Furthermore, our data indicate that the transcriptional repressor escargot regulates the ability of somatic cells to assume and/or maintain hub cell identity. These data highlight the dynamic relationship between stem cells and the niche and provide insight into genetic programmes that regulate niche size and function to support normal tissue homeostasis and organ regeneration throughout life.

Wakao, H., et al. (2008). "In vitro induction of natural killer T cells from embryonic stem cells prepared using somatic cell nuclear transfer." FASEB J **22**(7): 2223-2231.

The ectopic expression of the Notch receptor ligand delta-like 1 on stromal cells allows the induction of T cells from embryonic stem cells (ESCs). However, these in vitro-generated T cells are not transplantable because they are too immature to mount an immune response in an immunocompromised animal. We efficiently generated a subset of T cells called invariant natural killer T (iNKT) cells from ESCs derived from peripheral iNKT cells using somatic cell nuclear transfer (ntESCs). These iNKT cells matured autonomously in vivo and exhibited an adjuvant effect accompanying the production of interferon-gamma in an antigen-specific manner. This adjuvant effect culminated in the inhibition of inoculated tumor cell growth. Our results indicate that ntESC-derived iNKT cells are transplantable lymphocytes that will be beneficial for the induction of immune tolerance and the treatment of autoimmune diseases, tumors, and infections.

Wakayama, T., et al. (2001). "Differentiation of embryonic stem cell lines generated from adult somatic cells by nuclear transfer." Science **292**(5517): 740-743.

Embryonic stem (ES) cells are fully pluripotent in that they can differentiate into all cell types, including gametes. We have derived 35 ES cell lines via nuclear transfer (ntES cell lines) from adult mouse somatic cells of inbred, hybrid, and mutant strains. ntES cells contributed to an extensive variety of cell types, including dopaminergic and serotonergic neurons in vitro and germ cells in vivo. Cloning by transfer of ntES cell nuclei could result in normal development of fertile adults. These studies demonstrate the full pluripotency of ntES cells.

Wang, L., et al. (2013). "The THO complex regulates pluripotency gene mRNA export and controls embryonic stem cell self-renewal and somatic cell reprogramming." Cell Stem Cell **13**(6): 676-690.

Embryonic stem cell (ESC) self-renewal and differentiation are governed by a broad-ranging regulatory network. Although the transcriptional regulatory mechanisms involved have been investigated extensively, posttranscriptional regulation is still poorly understood. Here we describe a critical role of the THO complex in ESC self-renewal and differentiation. We show that THO preferentially interacts with pluripotency gene transcripts through Thoc5 and is required for self-renewal at least in part by regulating their export and expression. During differentiation, THO loses its interaction with those transcripts due to reduced Thoc5 expression, leading to decreased expression of pluripotency proteins that facilitates exit from self-renewal. THO is also important for the establishment of pluripotency, because its depletion inhibits somatic cell reprogramming and blastocyst development. Together, our data indicate that THO regulates pluripotency gene mRNA export to control ESC self-renewal and differentiation, and therefore uncover a role for this aspect of posttranscriptional regulation in stem cell fate specification.

Wang, Z. (2011). "Derivation of mouse embryonic stem cell lines from blastocysts produced by fertilization and somatic cell nuclear transfer." Methods Mol Biol **770**: 529-549.

This chapter describes a detailed protocol for deriving mouse embryonic stem (ES) cell lines from blastocysts that are produced either by fertilization or by somatic cell nuclear transfer (NT or cloning). Rather than function as a scientific communication

with the experts in this field, this protocol is written with researchers who are new to mouse ES cell line derivation in mind. Therefore, researchers who want to establish such methods in laboratories where mouse ES cell line derivation has never been done before should find this chapter helpful.

Wedrychowicz, A. and J. Starzyk (2013). "[Thyroid gland dysfunction, disorders of somatic and sexual development, disturbances of fertility after hematopoietic stem cell transplantation]." Pediatr Endocrinol Diabetes Metab **19**(1): 29-34.

Since the 1980s, hematopoietic stem cell transplantation (HSCT) has been performed for malignant and non-malignant disorders leading to increasing numbers of long-term survivors. Some of them develop long-term posttransplantation complications, among them endocrine complications that arise many years after HSCT and demand to be treated till the end of patients life. In the paper "classical", observed several years after HSCT had been used as a treatment procedure, endocrine complications are discussed and the review of literature regarding this problem is presented. Thyroid dysfunction, disorders of somatic and sexual development are presented in details. Gonad dysfunction with the problem of fertility disturbances is reported. The paper presents the etiopathogenesis, methods of prevention, as well as treatment and the results of the treatment of these endocrine complications after HSCT. Moreover actual recommendations for screening and prevention of endocrine complications in long-term HCT survivors are presented.

Wernet, P., et al. (2010). "Lentiviral labeling reveals three germ layer differentiation potential of a single unrestricted somatic stem cell from human cord blood." Exp Hematol **38**(11): 1099-1104.

OBJECTIVE: Generation and expression of unrestricted somatic stem cells (USSC) from human cord blood as well as their in vitro functional characterization at the clonal level. MATERIALS AND METHODS: USSC generation was initiated from fresh cord blood followed by lentiviral transfection and clone generation via limiting dilution. Individual clones were analyzed for lentiviral genomic integration patterns by ligation-mediated polymerase chain reaction. In vitro differentiation of clonal USSC was performed into mesodermal, endodermal, and ectodermal lineages according to our published protocols. Respective osteogenic, hepatic, and neuronal lineage-specification was documented by immunohistochemistry and tissue-specific protein expression was analyzed by Western blotting. MicroRNA expression analysis was achieved using the TaqMan microRNA Megaplex array. RESULTS: Lentivirally labeled USSC cultures were successfully subjected to limiting dilution cloning. One clone containing a single lentiviral integration site was identified (clone 4) and used for further differentiation experiments. Ligation-mediated polymerase chain reaction results from mesodermally, endodermally, and ectodermally differentiated USSC clone 4 consistently showed only the primary single lentiviral integration site. Lineage-specific differentiation experiments were confirmed by morphology and cell-fate-specific monoclonal antibodies in immunocytochemistry. MicroRNA expression profiles did not reveal dramatic differences between clonal and nonclonal USSC. CONCLUSIONS: The proof of the clonal existence of USSC is important for the assessment of biological properties unique for these unrestricted human stem cell candidates. As clones they can be subjected to advanced methods that enable defining of the multilayer nature of regulatory mechanisms through single-cell analysis.

Wijaya, L., et al. (2011). "Reversing breast cancer stem cell into breast somatic stem cell." Curr Pharm Biotechnol **12**(2): 189-195.

Stem cells have an important role in cell biology, allowing tissues to be renewed by freshly created cells throughout their lifetime. The specific micro-environment of stem cells is called stem cell niche; this environment influences the development of stem cells from quiescence through stages of differentiation. Recent advance researches have improved the understanding of the cellular and molecular components of the micro-environment--or niche--that regulates stem cells. We point out an important trend to the study of niche activity in breast cancers. Breast cancer has long been known to conserve a heterogeneous population of cells. While the majority of cells that make up tumors are destined to differentiate and eventually stop dividing, only minority populations of cells, termed cancer stem cell, possess extensive self renewal capability. These cancer stem cells possess characteristics of both stem cells and cancer cells. Breast cancer stem cells reversal to breast somatic stem cells offer a new therapy, that not only can stop the spread of breast cancer cells, but also can differentiate breast cancer stem cells into normal breast somatic stem cells. These can replace damaged breast tissue. Nevertheless, the complexity of realizing this therapy approach needs further research.

Williams, E. D., et al. (1992). "A stem cell niche theory of intestinal crypt maintenance based on a study of somatic mutation in colonic mucosa." Am J Pathol **141**(4): 773-776.

In both large and small intestine, mutagen administration leads to the occurrence of isolated crypts that are completely populated by a mutated phenotype; therefore, it has been proposed that crypts are maintained by a single stem cell. We show in mice that a single dose of mutagen leads to an early transient increase in frequency of colonic crypts that show a partial mutated phenotype and a later increase in frequency of crypts that show a complete mutated phenotype. This increase reaches a plateau at about the same time as the disappearance of partially mutated crypts. The same is true in the small intestine, but the time course is much slower. We propose an explanation based on multiple crypt stem cells that occupy a "stem cell niche," with random cell loss after stem cell division. A small difference in the number of crypt stem cells that occupy the niche provides a simple explanation for the surprisingly large difference in the time course of phenotypic changes in the large and small intestines after administration of a single dose of mutagen.

Wolf, D. P., et al. (2017). "Concise Review: Embryonic Stem Cells Derived by Somatic Cell Nuclear Transfer: A Horse in the Race?" Stem Cells **35**(1): 26-34.

Embryonic stem cells (ESC) hold promise for the treatment of human medical conditions but are allogeneic. Here, we consider the differences between autologous pluripotent stem cells produced by nuclear transfer (NT-ESCs) and transcription factor-mediated, induced pluripotent stem cells (iPSCs) that impact the desirability of each of these cell types for clinical use. The derivation of NT-ESCs is more cumbersome and requires donor oocytes; however, the use of oocyte cytoplasm as the source of reprogramming factors is linked to a key advantage of NT-ESCs-the ability to replace mutant mitochondrial DNA in a patient cell (due to either age or inherited disease) with healthy donor mitochondria from an oocyte. Moreover, in epigenomic and transcriptomic comparisons between isogenic iPSCs and NT-ESCs, the latter produced cells that more closely resemble bona fide ESCs derived from fertilized embryos. Thus, although NT-ESCs are more difficult to generate than iPSCs, the ability of somatic cell nuclear transfer to replace aged or diseased mitochondria and the closer epigenomic and transcriptomic similarity between NT-ESCs and bona fide ESCs may make NT-ESCs superior for future applications in regenerative medicine. Stem Cells 2017;35:26-34.

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