CRISPR and Cancer Biology Research Literatures

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Abstract: Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries. This article introduces recent research reports as references in the related studies.
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Key words: cancer; life; research; literature; cell; CRISPR; Cas9; Genome editing

1. Introduction

Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries.

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

Adelmann, C. H., et al. (2019). "Genome-Wide CRISPR/Cas9 Screening for Identification of Cancer Genes in Cell Lines." Methods Mol Biol **1907**: 125-136.

In this protocol, pooled sgRNA libraries targeting thousands of genes are computationally designed, generated using microarray-based synthesis techniques, and packaged into lentiviral particles. Target cells of interest are transduced with the lentiviral sgRNA pools to generate a collection of knockout mutants-via Cas9-mediated genomic cleavage-and screened for a phenotype of interest. The relative abundance of each mutant in the population can be monitored over time through high-throughput sequencing of the integrated sgRNA expression cassettes. Using this technique, we outline strategies for the identification of cancer driver genes and genes mediating drug response.

Ahn, J., et al. (2016). "Target sequencing and CRISPR/Cas editing reveal simultaneous loss of UTX and UTY in urothelial bladder cancer." Oncotarget **7**(39): 63252-63260.

UTX is a histone demethylase gene located on the X chromosome and is a frequently mutated gene in urothelial bladder cancer (UBC). UTY is a paralog of UTX located on the Y chromosome. We performed target capture sequencing on 128 genes in 40 non-metastatic UBC patients. UTX was the most frequently mutated gene (30%, 12/40). Of the genetic alterations identified, 75% were truncating mutations. UTY copy number loss was detected in 8 male patients (22.8%, 8/35). Of the 9 male patients with UTX mutations, 6 also had copy number loss (66.7%). To evaluate the functional roles of UTX and UTY in tumor progression, we designed UTX and UTY single knockout and UTX-UTY double knockout experiments using a CRISPR/Cas9 lentiviral system, and compared the proliferative capacities of two UBC cell lines in vitro. Single UTX or UTY knockout increased cell proliferation as compared to UTX-UTY wild-type cells. UTX-UTY double knockout cells exhibited greater proliferation than single knockout cells. These findings suggest both UTX and UTY function as dose-dependent suppressors of UBC development. While UTX escapes X chromosome inactivation in females, UTY may function as a male homologue of UTX, which could compensate for dosage imbalances.

Albitar, A., et al. (2018). "The Application of CRISPR/Cas Technology to Efficiently Model Complex Cancer Genomes in Stem Cells." J Cell Biochem **119**(1): 134-140.

CRISPR/Cas gene editing technologies have emerged as powerful tools in the study of oncogenic transformation. The system's specificity, versatility, and ease of implementation allow researchers to identify important molecular markers and pathways

which grant cancers stem cell like properties. This technology has already been applied to researching specific cancers, but has seen restricted therapeutic applications due to inherent ethical and technical limitations. Active development and adaptation of the CRISPR/Cas system has produced new methods to take advantage of both non-homologous end joining and homologous recombination repair mechanisms in attempts to remedy these limitations and improve the versatility of gene edits that can be created. Nonetheless, until issues with specificity and in vivo efficiency are resolved, utilization of CRISPR/Cas systems would be best employed in the modeling and study of various cancer genes. While it may have potential therapeutic applications to targeted cancer therapies in the future, presently CRISPR/Cas is a remarkable technique that can be utilized for easy and efficient gene editing when it comes to cancer research. J. Cell. Biochem. 119: 134-140, 2018. (c) 2017 Wiley Periodicals, Inc.

Aquino-Jarquin, G. (2017). "Emerging Role of CRISPR/Cas9 Technology for MicroRNAs Editing in Cancer Research." Cancer Res **77**(24): 6812-6817.

MicroRNAs (miRNA) are small, noncoding RNA molecules with a master role in the regulation of important tasks in different critical processes of cancer pathogenesis. Because there are different miRNAs implicated in all the stages of cancer, for example, functioning as oncogenes, this makes these small molecules suitable targets for cancer diagnosis and therapy. RNA-mediated interference has been one major approach for sequence-specific regulation of gene expression in eukaryotic organisms. Recently, the CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 system, first identified in bacteria and archaea as an adaptive immune response to invading genetic material, has been explored as a sequence-specific molecular tool for editing genomic sequences for basic research in life sciences and for therapeutic purposes. There is growing evidence that small noncoding RNAs, including miRNAs, can be targeted by the CRISPR/Cas9 system despite their lacking an open reading frame to evaluate functional loss. Thus, CRISPR/Cas9 technology represents a novel gene-editing strategy with compelling robustness, specificity, and stability for the modification of miRNA expression. Here, I summarize key features of current knowledge of genomic editing by CRISPR/Cas9 technology as a feasible strategy for globally interrogating miRNA gene function and miRNA-based therapeutic intervention. Alternative emerging strategies for nonviral delivery of CRISPR/Cas9 core components into human cells in a clinical context are also analyzed critically. Cancer Res; 77(24); 6812-7. (c)2017 AACR.

Baliou, S., et al. (2018). "CRISPR therapeutic tools for complex genetic disorders and cancer (Review)." Int J Oncol **53**(2): 443-468.

One of the fundamental discoveries in the field of biology is the ability to modulate the genome and to monitor the functional outputs derived from genomic alterations. In order to unravel new therapeutic options, scientists had initially focused on inducing genetic alterations in primary cells, in established cancer cell lines and mouse models using either RNA interference or cDNA overexpression or various programmable nucleases [zinc finger nucleases (ZNF), transcription activator-like effector nucleases (TALEN)]. Even though a huge volume of data was produced, its use was neither cheap nor accurate. Therefore, the clustered regularly interspaced short palindromic repeats (CRISPR) system was evidenced to be the next step in genome engineering tools. CRISPR-associated protein 9 (Cas9)-mediated genetic perturbation is simple, precise and highly efficient, empowering researchers to apply this method to immortalized cancerous cell lines, primary cells derived from mouse and human origins, xenografts, induced pluripotent stem cells, organoid cultures, as well as the generation of genetically engineered animal models. In this review, we assess the development of the CRISPR system and its therapeutic applications to a wide range of complex diseases (particularly distinct tumors), aiming at personalized therapy. Special emphasis is given to organoids and CRISPR screens in the design of innovative therapeutic approaches. Overall, the CRISPR system is regarded as an eminent genome engineering tool in therapeutics. We envision a new era in cancer biology during which the CRISPR-based genome engineering toolbox will serve as the fundamental conduit between the bench and the bedside; nonetheless, certain obstacles need to be addressed, such as the eradication of side-effects, maximization of efficiency, the assurance of delivery and the elimination of immunogenicity.

Baylis, F. and M. McLeod (2017). "First-in-human Phase 1 CRISPR Gene Editing Cancer Trials: Are We Ready?" Curr Gene Ther **17**(4): 309-319.

A prospective first-in-human Phase 1 CRISPR gene editing trial in the United States for patients with melanoma, synovial sarcoma, and multiple myeloma offers hope that gene editing tools may usefully treat human disease. An overarching ethical challenge with first-in-human Phase 1 clinical trials, however, is knowing when it is ethically acceptable to initiate such trials on the basis of safety and efficacy data obtained from pre-clinical studies. If the pre-clinical studies that inform trial design are themselves poorly designed - as a result of which the quality of pre-clinical evidence is

deficient - then the ethical requirement of scientific validity for clinical research may not be satisfied. In turn, this could mean that the Phase 1 clinical trial will be unsafe and that trial participants will be exposed to risk for no potential benefit. To assist sponsors, researchers, clinical investigators and reviewers in deciding when it is ethically acceptable to initiate first-in-human Phase 1 CRISPR gene editing clinical trials, structured processes have been developed to assess and minimize translational distance between pre-clinical and clinical research. These processes draw attention to various features of internal validity, construct validity, and external validity. As well, the credibility of supporting evidence is to be critically assessed with particular attention to optimism bias, financial conflicts of interest and publication bias. We critically examine the pre-clinical evidence used to justify the first-inhuman Phase 1 CRISPR gene editing cancer trial in the United States using these tools. We conclude that the proposed trial cannot satisfy the ethical requirement of scientific validity because the supporting pre-clinical evidence used to inform trial design is deficient.

Belvedere, R., et al. (2018). "miR-196a Is Able to Restore the Aggressive Phenotype of Annexin A1 Knock-Out in Pancreatic Cancer Cells by CRISPR/Cas9 Genome Editing." Int J Mol Sci **19**(7).

Annexin A1 (ANXA1) is a Ca (2+)-binding protein that is involved in pancreatic cancer (PC) progression. It is able to mediate cytoskeletal organization maintaining a malignant phenotype. Our previous studies showed that ANXA1 Knock-Out (KO) MIA PaCa-2 cells partially lost their migratory and invasive capabilities and also the metastatization process appeared affected in vivo. Here, we investigated the microRNA (miRNA) profile in ANXA1 KO cells finding that the modification in miRNA expression suggests the significant involvement of ANXA1 in PC development. In this study, we focused on miR-196a which appeared down modulated in absence of ANXA1. This miRNA is a well known oncogenic factor in several tumour models and it is able to trigger the agents of the epithelial to mesenchymal transition (EMT), like ANXA1. Our results show that the reintroduction in ANXA1 KO cells of miR-196a through the mimic sequence restored the early aggressive phenotype of MIA PaCa-2. Then, ANXA1 seems to support the expression of miR-196a and its role. On the other hand, this miRNA is able to mediate cytoskeletal dynamics and other protein functions promoting PC cell migration and invasion. This work describes the correlation between ANXA1 and specific miRNA sequences, particularly miR-196a. These results could lead to further information on ANXA1 intracellular role in PC, explaining other aspects that are apart from its tumorigenic behaviour.

Biagioni, A., et al. (2018). "Delivery systems of CRISPR/Cas9-based cancer gene therapy." J Biol Eng **12**: 33.

CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats) is today one of the most reliable method for gene-editing, supporting previous gene therapies technologies such as TALEN, Meganucleases and ZFNs. There is a growing up number of manuscripts reporting several successful gene-edited cancer cell lines, but the real challenge is to translate this technique to the clinical practice. While treatments for diseases based on a single gene mutation is closer, being possible to target and repair the mutant allele in a selective way generating specific guide RNAs (gRNAs), many steps need to be done to apply CRISPR to face cancer. In this review, we want to give a general overview to the recent advancements in the delivery systems of the CRISPR/Cas9 machinery in cancer therapy.

Blake, D. J., et al. (2018). "Ablation of the CD9 receptor in human lung cancer cells using CRISPR/Cas alters migration to chemoattractants including IL-16." Cytokine **111**: 567-570.

CD9, a member of the tetraspanin superfamily, has been implicated in regulating various physiological processes, including cell motility, adhesion, apoptosis and metastasis. Recently, interleukin-16 (IL-16), a pro-inflammatory cytokine released by normal airway and alveolar epithelial cells, has been implicated as a possible ligand for CD9 as an alternative receptor. In this study, using A549 cells as a model of human alveolar epithelium, CD9 expression was ablated using CRISPR/Cas technology. Decreased expression of CD9 mRNA and protein levels was confirmed through RT-qPCR and flow cytometry, respectively. Individual clones were generated that expressed high levels of CD9 (wild-type, WT) or significantly less CD9 (knockdown, KD). Both wild-type and knockdown A549 cell migration was quantified using a FluoroBloc transwell chemotaxis assay. Our results indicate that wild-type A549 cells migrated towards chemoattractants. Moreover, CD9 expression was required in this process since the CD9 knockdown cells had a significantly reduced migration towards growth serum and IL-16. These results support the migratory properties for CD9 in human lung cells and support the hypothesis that CD9 serves as an alternative receptor for IL-16.

Blanas, A., et al. (2018). "Transcriptional activation of fucosyltransferase (FUT) genes using the

CRISPR-dCas9-VPR technology reveals potent N-glycome alterations in colorectal cancer cells." Glycobiology.

Aberrant fucosylation in cancer cells is considered as a signature of malignant cell transformation and it is associated with tumor progression, metastasis and resistance to chemotherapy. Specifically, in colorectal cancer cells, increased levels of the fucosylated Lewisx antigen are attributed to the deregulated expression of pertinent fucosyltransferases, like fucosyltransferase 4 (FUT4) and fucosyltransferase 9 (FUT9). However, the lack of experimental models closely mimicking cancer-specific regulation of fucosyltransferase gene expression has, so far, limited our knowledge regarding the substrate specificity of these enzymes and the impact of Lewisx synthesis on the glycome of colorectal cancer cells. Therefore, we sought to transcriptionally activate the Fut4 and Fut9 genes in the well-known murine colorectal cancer cell line, MC38, which lacks expression of the FUT4 and FUT9 enzymes. For this purpose, we utilized a physiologically relevant, guide RNA-based model of de novo gene expression, namely the CRISPR-dCas9-VPR system. Induction of the Fut4 and Fut9 genes in MC38 cells using CRISPR-dCas9-VPR resulted in specific neo-expression of functional Lewisx antigen on the cell surface. Interestingly, Lewisx was mainly carried by N-linked glycans in both MC38-FUT4 and MC38-FUT9 cells, despite pronounced differences in the biosynthetic properties and the expression stability of the induced enzymes. Moreover, Lewisx expression was found to influence core-fucosylation, sialylation, antennarity and the subtypes of N-glycans in the MC38-glycovariants. In conclusion, exploiting the CRISPR-dCas9-VPR system to augment glycosyltransferase expression is a promising method of transcriptional gene activation with broad application possibilities in glycobiology and oncology research.

Chiou, S. H., et al. (2015). "Pancreatic cancer modeling using retrograde viral vector delivery and in vivo CRISPR/Cas9-mediated somatic genome editing." Genes Dev **29**(14): 1576-1585.

Pancreatic ductal adenocarcinoma (PDAC) is a genomically diverse, prevalent, and almost invariably fatal malignancy. Although conventional genetically engineered mouse models of human PDAC have been instrumental in understanding pancreatic cancer development, these models are much too labor-intensive, expensive, and slow to perform the extensive molecular analyses needed to adequately understand this disease. Here we demonstrate that retrograde pancreatic ductal injection of either adenoviral-Cre or lentiviral-Cre vectors allows titratable initiation of pancreatic neoplasias that progress into invasive and metastatic PDAC. To enable in vivo CRISPR/Cas9-mediated gene inactivation in the pancreas, we generated a Cre-regulated Cas9 allele and lentiviral vectors that express Cre and a single-guide RNA. CRISPR-mediated targeting of Lkb1 in combination with oncogenic Kras expression led to selection for inactivating genomic alterations, absence of Lkb1 protein, and rapid tumor growth that phenocopied Cre-mediated genetic deletion of Lkb1. This method will transform our ability to rapidly interrogate gene function during the development of this recalcitrant cancer.

Chow, R. D. and S. Chen (2018). "Cancer CRISPR Screens In Vivo." Trends Cancer **4**(5): 349-358.

Clustered regularly interspaced short palindromic repeats (CRISPR) screening is a powerful toolset for investigating diverse biological processes. Most CRISPR screens to date have been performed with in vitro cultures or cellular transplant models. To interrogate cancer in animal models that more closely recapitulate the human disease, autochthonous direct in vivo CRISPR screens have recently been developed that can identify causative drivers in the native tissue microenvironment. By empowering multiplexed mutagenesis in fully immunocompetent animals, direct in vivo CRISPR screens enable the rapid generation of patient-specific avatars that can guide precision medicine. This Opinion article discusses the current status of in vivo CRISPR screens in cancer and offers perspectives on future applications.

Cook, P. J. and A. Ventura (2018). "Cancer diagnosis and immunotherapy in the age of CRISPR." Genes Chromosomes Cancer.

The explosion in genome editing technologies that has occurred in the past decade has revolutionized cancer research and promises to improve cancer diagnosis and therapy. Ongoing efforts include engineering of chimeric antigen receptor-T cells using clustered regularly interspaced short palindromic repeats (CRISPR) to generate a safer, more effective therapy with improved performance in immunologically "cold" tumors, as well as clever adaptations of CRISPR enzymes to allow fast, simple, and sensitive detection of specific nucleotide sequences. While still in their infancy, CRISPR-based cancer therapeutics and diagnostics are developing at an impressive speed and it is likely they will soon impact clinical practice. Here, we summarize their history and the most recent developments.

Cui, Y., et al. (2017). "Knockdown of EPHA1 Using CRISPR/CAS9 Suppresses Aggressive

Properties of Ovarian Cancer Cells." Anticancer Res **37**(8): 4415-4424.

BACKGROUND/AIM: Overexpression of erythropoietin-producing hepatocellular A1 (EPHA1), a member of the EPH super family, is frequently observed in various cancer types. The dysregulated interaction of EPHA1 with its ligand Ephrin A1 has been linked to the progression of ovarian cancer (OC). However, the contribution of EPHA1 in the regulation of the aggressive properties of OC cells remains unknown. MATERIALS AND METHODS: In this study we investigated the differential expression of EPHA1 in human OC cells. The EPHA1 gene was knocked-down using the CRISPR/Cas9 technique to evaluate its effect on the progressive properties of OC cells. RESULTS: After EPHA1 was knocked-down using a CRISPR/CAS9 genomic editing system in OC cells (SKOV3 and COV504), we observed cell-cycle arrest at the G0/G1 phases in both OC cell lines. Knockdown of EPHA1 in the two OC cells inhibited their aggressive traits, including proliferation, invasion and migration, as well as improving their attachment to extracellular matrix. EPHA1 may play a role in OC through its regulation of multiple signaling pathways, such as matrix metalloproteinase-2 (MMP2), extracellular signal-regulated kinase 2 (ERK2) and proto-oncogene c-MYC. CONCLUSION: EPHA1 may promote the aggression of some OC cells and, thus, be considered a potential therapeutic target for the treatment of malignant OC.

Drost, J. and H. Clevers (2016). "Who Is in the Driver's Seat: Tracing Cancer Genes Using CRISPR-Barcoding." Mol Cell **63**(3): 352-354.

Intratumor heterogeneity is thought to be the driving force of tumor evolution and therapy resistance. Yet tools to study these processes are limited. In this issue, Guernet et al. (2016) devised clustered regularly interspaced short palindromic repeats (CRISPR)-barcoding to functionally annotate specific mutations and study clonal evolution in heterogeneous cell populations.

Drost, J., et al. (2017). "Use of CRISPR-modified human stem cell organoids to study the origin of mutational signatures in cancer." Science **358**(6360): 234-238.

Mutational processes underlie cancer initiation and progression. Signatures of these processes in cancer genomes may explain cancer etiology and could hold diagnostic and prognostic value. We developed a strategy that can be used to explore the origin of cancer-associated mutational signatures. We used CRISPR-Cas9 technology to delete key DNA repair genes in human colon organoids, followed by delayed subcloning and whole-genome sequencing. We found that mutation accumulation in organoids deficient in the mismatch repair gene MLH1 is driven by replication errors and accurately models the mutation profiles observed in mismatch repair-deficient colorectal cancers. Application of this strategy to the cancer predisposition gene NTHL1, which encodes a base excision repair protein, revealed a mutational footprint (signature 30) previously observed in a breast cancer cohort. We show that signature 30 can arise from germline NTHL1 mutations.

Estevao, D., et al. (2018). "CRISPR-Cas9 therapies in experimental mouse models of cancer." Future Oncol **14**(20): 2083-2095.

The CRISPR-Cas9, a part of the defence mechanism from bacteria, has rapidly become the simplest, fastest and the most precise genome-editing tool available. The therapeutic applications of CRISPR are boundless: correction of mutations in several disorders, inactivation of oncogenes and viral oncoproteins, and activation of tumor suppressor genes. In this review, we expose recent advances concerning animal models of cancer that use CRISPR-Cas9, addressing also the current efforts to develop CRISPR-Cas9-based therapies, focusing on proof-of-concept studies. Finally, the review exposes some of the main challenges that this genome-editing tool faces. The key issue remains: does CRISPR-Cas9 have real potential for therapeutic application or will it just remain a wonderful research tool?

Fei, T., et al. (2017). "Genome-wide CRISPR screen identifies HNRNPL as a prostate cancer dependency regulating RNA splicing." Proc Natl Acad Sci U S A **114**(26): E5207-E5215.

Alternative RNA splicing plays an important role in cancer. To determine which factors involved in RNA processing are essential in prostate cancer, we performed a genome-wide CRISPR/Cas9 knockout screen to identify the genes that are required for prostate cancer growth. Functional annotation defined a set of essential spliceosome and RNA binding protein (RBP) genes, including most notably heterogeneous nuclear ribonucleoprotein L (HNRNPL). We defined the HNRNPL-bound RNA landscape by RNA immunoprecipitation coupled with next-generation sequencing and linked these RBP-RNA interactions to changes in RNA processing. HNRNPL directly regulates the alternative splicing of a set of RNAs, including those encoding the androgen receptor, the key lineage-specific prostate cancer oncogene. HNRNPL also regulates circular RNA formation via back splicing. Importantly, both HNRNPL and its RNA targets are aberrantly expressed in human prostate tumors, supporting their

clinical relevance. Collectively, our data reveal HNRNPL and its RNA clients as players in prostate cancer growth and potential therapeutic targets.

Gebler, C., et al. (2017). "Inactivation of Cancer Mutations Utilizing CRISPR/Cas9." J Natl Cancer Inst **109**(1).

Although whole-genome sequencing has uncovered a large number of mutations that drive tumorigenesis, functional ratification for most mutations remains sparse. Here, we present an approach to test functional relevance of tumor mutations employing CRISPR/Cas9. Combining comprehensive sgRNA design and an efficient reporter assay to nominate efficient and selective sgRNAs, we establish a pipeline to dissect roles of cancer mutations with potential applicability to personalized medicine and future therapeutic use.

Gnanamony, M. and C. S. Gondi (2018). "Targeting the Expression of Cathepsin B Using CRISPR/Cas9 System in Mammalian Cancer Cells." Methods Mol Biol **1731**: 123-131.

Cathepsin B belongs to a family of cathepsins and plays an important role in normal physiological functions in the cell. However, overexpression of cathepsin B has been associated with different malignancies, and this has made it an attractive pharmacological target. The advent of CRISPR-Cas9 technology has allowed researchers to efficiently knock down genes with very less nonspecific activity compared to earlier methods. The protocol described below will enable investigators to develop cathepsin B knockdown stable cells and explains ways to study the knockdown.

Goodspeed, A., et al. (2018). "A Whole-genome CRISPR Screen Identifies a Role of MSH2 in Cisplatin-mediated Cell Death in Muscle-invasive Bladder Cancer." Eur Urol.

BACKGROUND: The response to first-line, platinum-based treatment of muscle-invasive bladder cancer has not improved in 3 decades. OBJECTIVE: To identify genes that influence cisplatin resistance in bladder cancer. DESIGN, SETTING, AND PARTICIPANTS: We performed a whole-genome CRISPR screen in a bladder cancer cell line to identify genes that mediate resistance to cisplatin. OUTCOME MEASUREMENTS AND STATISTICAL ANALYSIS: Targeted validation was performed in two bladder cancer cell lines. The top gene candidate was validated in a publicly available bladder cancer dataset. RESULTS AND LIMITATIONS: From the CRISPR screen, we identified MSH2 as the most significantly enriched gene and mismatch repair as the most significantly enriched pathway that promoted resistance to cisplatin. Bladder cancer cells with knockdown of MSH2 showed a reduction in cisplatin-mediated apoptosis. MSH2 loss did not impact the sensitivity to other chemotherapies, including the cisplatin analog oxaliplatin. Bladder tumors with low MSH2 protein levels, quantified using reverse-phase protein array, showed poorer survival when treated with cisplatin- or carboplatin-based therapy; these results require future validation using immunohistochemistry. Additionally, results are retrospective from patients with primarily high-grade tumors; thus, validation in a controlled clinical trial is needed. CONCLUSIONS: We generated in vitro evidence that bladder cancer cell lines depleted of MSH2 are more resistant to cisplatin. We additionally found an association between low MSH2 in bladder tumors and poorer patient survival when treated with platinum-based chemotherapy. If successfully validated prospectively, MSH2 protein level could assist in the selection of patients for chemotherapy. PATIENT SUMMARY: We report the first evidence that MSH2 protein level may contribute to chemotherapy resistance observed in muscle-invasive bladder cancer. MSH2 has potential as a biomarker predictive of response to platinum-based therapy.

Granados-Riveron, J. T. and G. Aquino-Jarquin (2018). "CRISPR-Cas13 Precision Transcriptome Engineering in Cancer." Cancer Res **78**(15): 4107-4113.

The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated genes (Cas) system has been rapidly harnessed to perform various genomic engineering tasks. Recently, it has been demonstrated that a novel RNA-targeting CRISPR effector protein, called Cas13, binds and cleaves RNA rather than DNA substrates analogously to the eukaryotic RNA interference system. The known Cas13a-Cas13d effectors are able to efficiently cleave complementary target single-stranded RNAs, which represent a potentially safer alternative to deoxyribonuclease Cas9, because it induces loss-of-function phenotypes without genomic loss of the targeted gene. Furthermore, through the improvement in Cas13 effector functionalities, a system called REPAIR has been developed to edit full-length transcripts containing pathogenic mutations, thus providing a promising opportunity for precise base editing. Moreover, advanced engineering of this CRISPR effector also permits nucleic acid detection, allowing the identification of mutations in cell-free tumor DNA through a platform termed Specific High Sensitivity Enzymatic Reporter Unlocking. All of these properties give us a glimpse about the potential of the CRISPR toolkit for precise transcriptome engineering, possibly leading to an expansion of

CRISPR technologies for cancer therapeutics and diagnostics. Here, we examine previously unaddressed aspects of the CRISPR-based RNA-targeting approach as a feasible strategy for globally interrogating gene function in cancer in a programmable manner. Cancer Res; 78(15); 4107-13. (c)2018 AACR.

Guernet, A. and L. Grumolato (2017). "CRISPR/Cas9 editing of the genome for cancer modeling." Methods **121-122**: 130-137.

The CRISPR/Cas9 revolution has democratized access to genome editing in many biological fields, including cancer research. Cancer results from the multistep accumulation of mutations that confer to the transformed cells certain biological hallmarks typical of the malignant phenotype. One of the major goals in cancer research is to characterize such mutations and assess their implication in the oncogenic process. Through CRISPR/Cas9 technology, genetic aberrations identified in a patient's tumor can now be easily recreated in experimental models, which can then be used for basic research or for more translational applications. Here we review the different CRISPR/Cas9 strategies that have been implemented to recapitulate oncogenic mutations in both in vitro and in vivo systems, including novel strategies to model tumor evolution and genetic heterogeneity.

Guo, Y., et al. (2018). "CRISPR-mediated deletion of prostate cancer risk-associated CTCF loop anchors identifies repressive chromatin loops." Genome Biol **19**(1): 160.

BACKGROUND: Recent genome-wide association studies (GWAS) have identified more than 100 loci associated with increased risk of prostate cancer, most of which are in non-coding regions of the genome. Understanding the function of these non-coding risk loci is critical to elucidate the genetic susceptibility to prostate cancer. RESULTS: We generate genome-wide regulatory element maps and performed genome-wide chromosome confirmation capture assays (in situ Hi-C) in normal and tumorigenic prostate cells. Using this information, we annotate the regulatory potential of 2,181 fine-mapped prostate cancer risk-associated SNPs and predict a set of target genes that are regulated by prostate cancer risk-related H3K27Ac-mediated loops. We next identify prostate cancer risk-associated CTCF sites involved in long-range chromatin loops. We use CRISPR-mediated deletion to remove prostate cancer risk-associated CTCF anchor regions and the CTCF anchor regions looped to the prostate cancer risk-associated CTCF sites, and we observe up to 100-fold increases in expression of genes within the loops when the prostate cancer risk-associated CTCF anchor regions are deleted. CONCLUSIONS: We identify GWAS risk loci involved in long-range loops that function to repress gene expression within chromatin loops. Our studies provide new insights into the genetic susceptibility to prostate cancer.

Han, K., et al. (2017). "Synergistic drug combinations for cancer identified in a CRISPR screen for pairwise genetic interactions." Nat Biotechnol **35**(5): 463-474.

Identification of effective combination therapies is critical to address the emergence of drug-resistant cancers, but direct screening of all possible drug combinations is infeasible. Here we introduce a CRISPR-based double knockout (CDKO) system that improves the efficiency of combinatorial genetic screening using an effective strategy for cloning and sequencing paired single guide RNA (sgRNA) libraries and a robust statistical scoring method for calculating genetic interactions (GIs) from CRISPR-deleted gene pairs. We applied CDKO to generate a large-scale human GI map, comprising 490,000 double-sgRNAs directed against 21,321 pairs of drug targets in K562 leukemia cells and identified synthetic lethal drug target pairs for which corresponding drugs exhibit synergistic killing. These included the BCL2L1 and MCL1 combination, which was also effective in imatinib-resistant cells. We further validated this system by identifying known and previously unidentified GIs between modifiers of ricin toxicity. This work provides an effective strategy to screen synergistic drug combinations in high-throughput and a CRISPR-based tool to dissect functional GI networks.

Haraguchi, M., et al. (2015). "CRISPR/Cas9n-Mediated Deletion of the Snail 1Gene (SNAI1) Reveals Its Role in Regulating Cell Morphology, Cell-Cell Interactions, and Gene Expression in Ovarian Cancer (RMG-1) Cells." PLoS One **10**(7): e0132260.

Snail1 is a transcription factor that induces the epithelial to mesenchymal transition (EMT). During EMT, epithelial cells lose their junctions, reorganize their cytoskeletons, and reprogram gene expression. Although Snail1 is a prominent repressor of E-cadherin transcription, its precise roles in each of the phenomena of EMT are not completely understood, particularly in cytoskeletal changes. Previous studies have employed gene knockdown systems to determine the functions of Snail1. However, incomplete protein knockdown is often associated with these systems, which may cause incorrect interpretation of the data. To more precisely evaluate the functions of Snail1, we generated a stable cell line with a targeted ablation of Snail1 (Snail1 KO) by using the CRISPR/Cas9n system. Snail1 KO cells show increased cell-cell adhesion, decreased cell-substrate adhesion and cell

migration, changes to their cytoskeletal organization that include few stress fibers and abundant cortical actin, and upregulation of epithelial marker genes such as E-cadherin, occludin, and claudin-1. However, morphological changes were induced by treatment of Snail1 KO cells with TGF-beta. Other transcription factors that induce EMT were also induced by treatment with TGF-beta. The precise deletion of Snail1 by the CRISPR/Cas9n system provides clear evidence that loss of Snail1 causes changes in the actin cytoskeleton, decreases cell-substrate adhesion, and increases cell-cell adhesion. Treatment of RMG1 cells with TGF-beta suggests redundancy among the transcription factors that induce EMT.

Hart, T., et al. (2015). "High-Resolution CRISPR Screens Reveal Fitness Genes and Genotype-Specific Cancer Liabilities." Cell **163**(6): 1515-1526.

The ability to perturb genes in human cells is crucial for elucidating gene function and holds great potential for finding therapeutic targets for diseases such as cancer. To extend the catalog of human core and context-dependent fitness genes, we have developed a high-complexity second-generation genome-scale CRISPR-Cas9 gRNA library and applied it to fitness screens in five human cell lines. Using an improved Bayesian analytical approach, we consistently discover 5-fold more fitness genes than were previously observed. We present a list of 1,580 human core fitness genes and describe their general properties. Moreover, we demonstrate that context-dependent fitness genes accurately recapitulate pathway-specific genetic vulnerabilities induced by known oncogenes and reveal cell-type-specific dependencies for specific receptor tyrosine kinases, even in oncogenic KRAS backgrounds. Thus, rigorous identification of human cell line fitness genes using a high-complexity CRISPR-Cas9 library affords a high-resolution view of the genetic vulnerabilities of a cell.

He, Z. Y., et al. (2018). "In Vivo Ovarian Cancer Gene Therapy Using CRISPR-Cas9." Hum Gene Ther **29**(2): 223-233.

Clustered regularly interspaced short palindromic repeats (CRISPR)-caspase 9 (Cas9) genome editing technology holds great promise for the field of human gene therapy. However, a lack of safe and effective delivery systems restricts its biomedical application. Here, a folate receptor-targeted liposome (F-LP) was used to deliver CRISPR plasmid DNA co-expressing Cas9 and single-guide RNA targeting the ovarian cancer-related DNA methyltransferase 1 (DNMT1) gene (gDNMT1). F-LP efficiently bound the gDNMT1 plasmid and formed a stable complex (F-LP/gDNMT1) that was safe for injection. F-LP/gDNMT1 effectively mutated endogenous DNMT1 in vitro, and then expressed the Cas9 endonuclease and downregulated DNMT1 in vivo. The tumor growth of both paclitaxel-sensitive and -resistant ovarian cancers were inhibited by F-LP/gDNMT1, which shows fewer adverse effects than paclitaxel injection. Therefore, CRISPR-Cas9-targeted DNMT1 manipulation may be a potential therapeutic regimen for ovarian cancer, and lipid-mediated delivery systems represent promising delivery vectors of CRISPR-Cas9 technology for precise genome editing therapeutics.

Hou, C., et al. (2018). "Knockdown of Trio by CRISPR/Cas9 suppresses migration and invasion of cervical cancer cells." Oncol Rep **39**(2): 795-801.

Triple functional domain protein (Trio) is an evolutionarily conserved protein with guanine nucleotide exchange factors that regulate different physiological processes in some types of cancer. However, the expression and function of Trio in cervical cancer are still unknown. The purpose of this study was to detect the expression of Trio in cervical cancer tissues and to evaluate its clinical value. Furthermore, the effects of the Trio on the migration and invasion of cervical cancer cells and its mechanism were investigated in vitro. The results of the present study revealed that Trio expression levels were significantly higher in most of the clinical cervical cancer samples than in adjacent tissues. The clinicopathological significance of Trio expression was also analyzed, and the results revealed that high expression levels in cervical cancer were correlated with lymph node metastasis (P=0.005). The CRISPR/Cas9 system was used to knockdown the endogenous Trio. The inhibition of Trio significantly decreased the migration and invasion abilities of cervical cancer cells. Meanwhile, levels of RhoA/ROCK signaling factors (RhoA, Rock, and p-LIMK), which contributed to cell migration and invasion, were decreased along with the inhibition of Trio. Therefore, Trio may regulate the migration and invasion of cervical cancer through the RhoA/ROCK signaling pathway.

Hsu, D. S., et al. (2018). "Targeting HPV16 DNA using CRISPR/Cas inhibits anal cancer growth in vivo." Future Virol **13**(7): 475-482.

Aim: The goal of this study was to determine if a single AAV vector, encoding Cas9 and guide RNAs specific for the HPV16 E6 and E7 genes, could inhibit the growth of an HPV16-induced tumor in vivo. Materials & methods: We grew HPV16(+), patient-derived anal cancer explants in immunodeficient mice and then challenged these by injection of AAV-based vectors encoding Cas9 and control or HPV16-specific guide RNAs. Results & conclusion: We observed a

significant and selective reduction in tumor growth when the HPV16 E6 and E7 genes were targeted using Cas9. These studies provide proof of principle for the hypothesis that CRISPR/Cas has the potential to be used to selectively treat HPV-induced tumors in humans.

Hu, Z., et al. (2018). "Ligase IV inhibitor SCR7 enhances gene editing directed by CRISPR-Cas9 and ssODN in human cancer cells." Cell Biosci **8**: 12.

Background: Precise genome editing is essential for both basic and translational research. The recently developed CRISPR/Cas9 system can specifically cleave a designated site of target gene to create a DNA double-strand break, which triggers cellular DNA repair mechanism of either inaccurate non-homologous end joining, or site-specific homologous recombination. Unfortunately, homology-directed repair (HDR) is challenging due to its very low efficiency. Herein, we focused on improving the efficiency of HDR using a combination of CRISPR/Cas9, eGFP, DNA ligase IV inhibitor SCR7, and single-stranded oligodeoxynucleotides (ssODN) in human cancer cells. Results: When Cas9, gRNA and eGFP were assembled into a co-expression vector, the disruption rate more than doubled following GFP-positive cell sorting in transfected cells compared to those unsorted cells. Using ssODNs as templates, SCR7 treatment increased targeted insertion efficiency threefold in transfected cells compared to those without SCR7 treatment. Moreover, this combinatorial approach greatly improved the efficiency of HDR and targeted gene mutation correction at both the GFP-silent mutation and the beta-catenin Ser45 deletion mutation cells. Conclusion: The data of this study suggests that a combination of co-expression vector, ssODN, and ligase IV inhibitor can markedly improve the CRISPR/Cas9-directed gene editing, which should have significant application in targeted gene editing and genetic disease therapy.

Hu, Z., et al. (2014). "Disruption of HPV16-E7 by CRISPR/Cas system induces apoptosis and growth inhibition in HPV16 positive human cervical cancer cells." Biomed Res Int **2014**: 612823.

High-risk human papillomavirus (HR-HPV) has been recognized as a major causative agent for cervical cancer. Upon HPV infection, early genes E6 and E7 play important roles in maintaining malignant phenotype of cervical cancer cells. By using clustered regularly interspaced short palindromic repeats-(CRISPR-) associated protein system (CRISPR/Cas system), a widely used genome editing tool in many organisms, to target HPV16-E7 DNA in HPV positive cell lines, we showed for the first time that the HPV16-E7 single-guide RNA (sgRNA) guided CRISPR/Cas system could disrupt HPV16-E7 DNA at specific sites, inducing apoptosis and growth inhibition in HPV positive SiHa and Caski cells, but not in HPV negative C33A and HEK293 cells. Moreover, disruption of E7 DNA directly leads to downregulation of E7 protein and upregulation of tumor suppressor protein pRb. Therefore, our results suggest that HPV16-E7 gRNA guided CRISPR/Cas system might be used as a therapeutic strategy for the treatment of cervical cancer.

Huang, C. H., et al. (2018). "Applications of CRISPR-Cas Enzymes in Cancer Therapeutics and Detection." Trends Cancer **4**(7): 499-512.

Cancer is a complex disease caused by combinations of cellular genetic alterations and heterogeneous microenvironments. The use of the robust and programmable CRISPR-Cas systems has greatly improved genome editing for precision cancer modeling and enabled multiplexed genetic manipulation for cancer treatment and mutation detection. In this review, we outline the current CRISPR-Cas toolkit, and discuss the promises and hurdles in translating this revolutionary technology into effective and safe clinical applications for cancer treatment and diagnosis.

Huang, L. C., et al. (2018). "CRISPR/Cas9 Genome Editing of Epidermal Growth Factor Receptor Sufficiently Abolished Oncogenicity in Anaplastic Thyroid Cancer." Dis Markers **2018**: 3835783.

Anaplastic carcinoma of the thyroid (ATC), also called undifferentiated thyroid cancer, is the least common but most aggressive and deadly thyroid gland malignancy of all thyroid cancers. The aim of this study is to explore essential biomarker and use CRISPR/Cas9 with lentivirus delivery to establish a gene-target therapeutic platform in ATC cells. At the beginning, the gene expression datasets from 1036 cancers from CCLE and 8215 tumors from TCGA were collected and analyzed, showing EGFR is predominantly overexpressed in thyroid cancers than other type of cancers ($P = 0.017$ in CCLE and $P = 0.001$ in TCGA). Using CRISPR/Cas9 genomic edit system, ATC cells with EGFR sgRNA lentivirus transfection obtained great disruptions on gene and protein expression, resulting in cell cycle arrest, cell growth inhibition, and most importantly metastasis turn-off ability. In addition, the FDA-approved TKI of afatinib for EGFR targeting also illustrates great anticancer activity on cancer cell death occurrence, cell growth inhibition, and cell cycle arrest in SW579 cells, an EGFR expressing human ATC cell line. Furthermore, off-target effect of using EGFR sgRNAs was measured and found no genomic editing can be detected in off-target candidate gene. To conclude, this

study provides potential ATC therapeutic strategies for current and future clinical needs, which may be possible in increasing the survival rate of ATC patients by translational medicine.

Huang, X., et al. (2017). "An enhanced hTERT promoter-driven CRISPR/Cas9 system selectively inhibits the progression of bladder cancer cells." Mol Biosyst **13**(9): 1713-1721.

The current therapies for treating tumors are lacking in efficacy and specificity. Synthetic biology principles may bring some new possible methods for curing cancer. Here we present a synthetic logic circuit based on the CRISPR/Cas9 system. The CRISPR/Cas9 technology has been applied in many biological fields, including cancer research. In this study, the expression of Cas9 nuclease was controlled indirectly by an enhanced hTERT promoter using the GAL4/upstream activating sequence (UAS) binding system. Cas9 was driven by 5XUAS, single guide RNA (sgRNA) was used to target mutant or wild-type HRAS, and the fusion gene GAL4-P65 was driven by the enhanced hTERT promoter. The system was tested in bladder cancer cells (T24 and 5637) and the results showed that the enhanced hTERT promoter could drive the expression of GAL4-P65 in these bladder cancer cell lines. Then all these devices were packed into lentivirus and the results of quantitative real-time PCR showed that the mRNA expression level of HRAS was selectively inhibited in the T24 and 5637 cells. The results of functional experiments suggested that the proliferation, cell migration and invasion were selectively suppressed, and that the apoptosis rate was increased in bladder cancer cells but not in human foreskin fibroblasts (HFF). In conclusion, we successfully constructed an enhanced hTERT promoter-driven CRISPR/Cas9 system and data showed that it could selectively suppress the progression of bladder cancer cells.

Hudacsek, V. and B. Gyorffy (2018). "[Genome engineering using the CRISPR-Cas9 system and applications in cancer research]." Magy Onkol **62**(2): 119-127.

Today, we have to investigate the effects of selected mutations and molecular alterations - this is made possible by the new genome editing technologies. In these, either a section of the DNA is deleted or moved, or a targeted mutation is introduced into the cell. Here, we describe in detail the CRISPR-Cas9 genome editing method. The technology is based on a feature used by bacteria to combat recurrent viral infections. At present, commencing a set of modifications, the method is functional and can be applied in eukaryotic cells as well. The method has three major steps: first, the system has to be carefully designed and constructed, then the construct has to be introduced into the target cells by transfection, and finally, the achieved effect has to be functionally validated. The reliability of the method enables multiple applications in oncology, including the detailed and efficient investigation of oncogenes, tumor suppressor genes, chromosomal translocations and other molecular changes. At the moment, available CRISPR-Cas9 protocols enable both in vitro and in vivo application. All these already made CRISPR-Cas9 one of the basic methods required for future-proof oncology research.

Huo, W., et al. (2017). "Lentiviral CRISPR/Cas9 vector mediated miR-21 gene editing inhibits the epithelial to mesenchymal transition in ovarian cancer cells." J Cancer **8**(1): 57-64.

CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats) mediated genome editing is a powerful approach for loss of function studies. Here we report that lentiviral CRISPR/Cas9 vectors are highly efficient in introducing mutations in the precursor miRNA sequence, thus leading to the loss of miRNA expression and function. We constructed four different lentiviral CRISPR/Cas9 vectors that target different regions of the precursor miR-21 sequence and found that these lentiviral CRISPR/Cas9 miR-21 gRNA vectors induced mutations in the precursor sequences as shown by DNA surveyor mutation assay and Sanger sequencing. Two miR-21 lentiviral CRISPR/Cas9 gRNA vectors were selected to probe miR-21 function in ovarian cancer SKOV3 and OVCAR3 cell lines. Our data demonstrate that disruption of pre-miR-21 sequences leads to reduced cell proliferation, migration and invasion. Moreover, CRISPR/Cas9-mediated miR-21 gene editing sensitizes both SKOV3 and OVCAR3 cells to chemotherapeutic drug treatment. Disruption of miR-21 leads to the inhibition of epithelial to mesenchymal transition (EMT) in both SKOV3 and OVCAR3 cells as evidenced by the upregulation of epithelial cell marker E-cadherin and downregulation of mesenchymal marker genes, vimentin and Snai2. The miR-21 target genes PDCD4 and SPRY2 were upregulated in cells transduced with miR-21gRNAs compared to controls. Our study indicates that lentiviral CRISPR/Cas9-mediated miRNA gene editing is an effective approach to address miRNA function, and disruption of miR-21 inhibits EMT in ovarian cancer cells.

Jubair, L. and N. A. J. McMillan (2017). "The Therapeutic Potential of CRISPR/Cas9 Systems in Oncogene-Addicted Cancer Types: Virally Driven Cancers as a Model System." Mol Ther Nucleic Acids **8**: 56-63.

The field of gene editing is undergoing unprecedented growth. The first ex vivo human clinical trial in China started in 2016, more than 1000 US patents have been filed, and there is exponential growth in publications. The ability to edit genes with high fidelity is promising for the development of new treatments for a range of diseases, particularly inherited conditions, infectious diseases, and cancers. For cancer, a major issue is the identification of driver mutations and oncogenes to target for therapeutic effect, and this requires the development of robust models with which to prove their efficacy. The challenge is that there is rarely a single critical gene. However, virally driven cancers, in which cells are addicted to the expression of a single viral oncogene in some cases, may serve as model systems for CRISPR/Cas therapies, as they did for RNAi. These models and systems offer an excellent opportunity to test both preclinical models and clinical conditions to examine the effectiveness of gene editing, and here we review the options and offer a way forward.

Kanda, T., et al. (2016). "Highly Efficient CRISPR/Cas9-Mediated Cloning and Functional Characterization of Gastric Cancer-Derived Epstein-Barr Virus Strains." J Virol **90**(9): 4383-4393.

UNLABELLED: The Epstein-Barr virus (EBV) is etiologically linked to approximately 10% of gastric cancers, in which viral genomes are maintained as multicopy episomes. EBV-positive gastric cancer cells are incompetent for progeny virus production, making viral DNA cloning extremely difficult. Here we describe a highly efficient strategy for obtaining bacterial artificial chromosome (BAC) clones of EBV episomes by utilizing a CRISPR/Cas9-mediated strand break of the viral genome and subsequent homology-directed repair. EBV strains maintained in two gastric cancer cell lines (SNU719 and YCCEL1) were cloned, and their complete viral genome sequences were determined. Infectious viruses of gastric cancer cell-derived EBVs were reconstituted, and the viruses established stable latent infections in immortalized keratinocytes. While Ras oncoprotein overexpression caused massive vacuolar degeneration and cell death in control keratinocytes, EBV-infected keratinocytes survived in the presence of Ras expression. These results implicate EBV infection in predisposing epithelial cells to malignant transformation by inducing resistance to oncogene-induced cell death. IMPORTANCE: Recent progress in DNA-sequencing technology has accelerated EBV whole-genome sequencing, and the repertoire of sequenced EBV genomes is increasing progressively. Accordingly, the presence of EBV variant strains that may be relevant to EBV-associated diseases has begun to attract interest. Clearly, the determination of additional disease-associated viral genome sequences will facilitate the identification of any disease-specific EBV variants. We found that CRISPR/Cas9-mediated cleavage of EBV episomal DNA enabled the cloning of disease-associated viral strains with unprecedented efficiency. As a proof of concept, two gastric cancer cell-derived EBV strains were cloned, and the infection of epithelial cells with reconstituted viruses provided important clues about the mechanism of EBV-mediated epithelial carcinogenesis. This experimental system should contribute to establishing the relationship between viral genome variation and EBV-associated diseases.

Kannan, R. and A. Ventura (2015). "The CRISPR revolution and its impact on cancer research." Swiss Med Wkly **145**: w14230.

A revolution in cancer research is underway. Spurred by the advent of the CRISPR-Cas9 technology, new methods to probe the mammalian genomes are being developed. By providing simple, flexible, and cost-effective ways to edit and manipulate the genome of somatic cells of adult animals, these new methods present the opportunity to model cancer progression in vivo with an unprecedented degree of sophistication. Here we provide a brief overview of this exciting and fast-moving field.

Kardooni, H., et al. (2018). "CRISPR-Mediated Reactivation of DKK3 Expression Attenuates TGF-beta Signaling in Prostate Cancer." Cancers (Basel) **10**(6).

The DKK3 gene encodes a secreted protein, Dkk-3, that inhibits prostate tumor growth and metastasis. DKK3 is downregulated by promoter methylation in many types of cancer, including prostate cancer. Gene silencing studies have shown that Dkk-3 maintains normal prostate epithelial cell homeostasis by limiting TGF-beta/Smad signaling. While ectopic expression of Dkk-3 leads to prostate cancer cell apoptosis, it is unclear if Dkk-3 has a physiological role in cancer cells. Here, we show that treatment of PC3 prostate cancer cells with the DNA methyltransferase (DNMT) inhibitor decitabine demethylates the DKK3 promoter, induces DKK3 expression, and inhibits TGF-beta/Smad-dependent transcriptional activity. Direct induction of DKK3 expression using CRISPR-dCas9-VPR also inhibited TGF-beta/Smad-dependent transcription and attenuated PC3 cell migration and proliferation. These effects were not observed in C4-2B cells, which do not respond to TGF-beta. TGF-beta signals can regulate gene expression directly via SMAD proteins and indirectly by increasing DNMT expression, leading to promoter methylation. Analysis of genes downregulated by promoter methylation and

predicted to be regulated by TGF-beta found that DKK3 induction increased expression of PTGS2, which encodes cyclooxygenase-2. Together, these observations provide support for using CRISPR-mediated induction of DKK3 as a potential therapeutic approach for prostate cancer and highlight complexities in Dkk-3 regulation of TGF-beta signaling.

Katz, G. and P. J. Pitts (2017). "Implications of CRISPR-Based Germline Engineering for Cancer Survivors." Ther Innov Regul Sci **51**(6): 672-682.

Cancer survivors can carry germline mutations that will be transmitted to their progeny. Today, many of these mutations have been identified and can be tracked. With the recent development of genome-editing technologies and CRISPR (clustered regularly interspaced short palindromic repeats), the possibility of genetically modifying the human germline-gametes and embryos-has never been closer. This perspective has sparked a controversy within the scientific community with reactions ranging from calls for a ban on germline modification to cautious approval of further research. This Editorial analyzes the possible adoption of CRISPR-based germline engineering to prevent the spread of cancer predispositions in the human population. We discuss whether the genomic edition of human sperm and eggs would contribute to rectifying or altering the heritable genome. We anticipate the emergence of a new form of liberal eugenics fueled by a logic of offer and demand from stakeholders such as cancer survivors and their relatives and offspring, but also from fertility clinics, biotech firms, insurers, and clinicians. From a regulatory perspective, validating the clinical safety and utility of CRISPR-based germline engineering is an essential step. However, with time, gradually perfecting the technology and assessing the economic benefits for stakeholders could soften society's resistance and align opinions in support of genomic decontamination of human germlines. This progressive shift would be justified in the name of cancer prevention as well as a moral obligation to facilitate the conception of cancer-free children at a cost that is acceptable to individuals and health systems.

Kawamura, N., et al. (2015). "CRISPR/Cas9-mediated gene knockout of NANOG and NANOGP8 decreases the malignant potential of prostate cancer cells." Oncotarget **6**(26): 22361-22374.

NANOG expression in prostate cancer is highly correlated with cancer stem cell characteristics and resistance to androgen deprivation. However, it is not clear whether NANOG or its pseudogenes contribute to the malignant potential of cancer. We established NANOG- and NANOGP8-knockout DU145 prostate cancer cell lines using the CRISPR/Cas9 system. Knockouts of NANOG and NANOGP8 significantly attenuated malignant potential, including sphere formation, anchorage-independent growth, migration capability, and drug resistance, compared to parental DU145 cells. NANOG and NANOGP8 knockout did not inhibit in vitro cell proliferation, but in vivo tumorigenic potential decreased significantly. These phenotypes were recovered in NANOG- and NANOGP8-rescued cell lines. These results indicate that NANOG and NANOGP8 proteins are expressed in prostate cancer cell lines, and NANOG and NANOGP8 equally contribute to the high malignant potential of prostate cancer.

Khan, F. A., et al. (2016). "CRISPR/Cas9 therapeutics: a cure for cancer and other genetic diseases." Oncotarget **7**(32): 52541-52552.

Cancer is caused by a series of alterations in genome and epigenome mostly resulting in activation of oncogenes or inactivation of cancer suppressor genes. Genetic engineering has become pivotal in the treatment of cancer and other genetic diseases, especially the formerly-niche use of clustered regularly interspaced short palindromic repeats (CRISPR) associated with Cas9. In defining its superior use, we have followed the recent advances that have been made in producing CRISPR/Cas9 as a therapy of choice. We also provide important genetic mutations where CRISPRs can be repurposed to create adaptive immunity to fight carcinomas and edit genetic mutations causing it. Meanwhile, challenges to CRISPR technology are also discussed with emphasis on ability of pathogens to evolve against CRISPRs. We follow the recent developments on the function of CRISPRs with different carriers which can efficiently deliver it to target cells; furthermore, analogous technologies are also discussed along CRISPRs, including zinc-finger nuclease (ZFN) and transcription activator-like effector nucleases (TALENs). Moreover, progress in clinical applications of CRISPR therapeutics is reviewed; in effect, patients can have lower morbidity and/or mortality from the therapeutic method with least possible side-effects.

Kim, S. M., et al. (2017). "Cancer-derived exosomes as a delivery platform of CRISPR/Cas9 confer cancer cell tropism-dependent targeting." J Control Release **266**: 8-16.

An intracellular delivery system for CRISPR/Cas9 is crucial for its application as a therapeutic genome editing technology in a broad range of diseases. Current vehicles carrying CRISPR/Cas9 limit in vivo delivery because of low tolerance and immunogenicity; thus, the in vivo delivery of genome editing remains challenging. Here,

we report that cancer-derived exosomes function as natural carriers that can efficiently deliver CRISPR/Cas9 plasmids to cancer. Compared to epithelial cell-derived exosomes, cancer-derived exosomes provide potential vehicles for effective in vivo delivery via selective accumulation in ovarian cancer tumors of SKOV3 xenograft mice, most likely because of their cell tropism. CRISPR/Cas9-loaded exosomes can suppress expression of poly (ADP-ribose) polymerase-1 (PARP-1), resulting in the induction of apoptosis in ovarian cancer. Furthermore, the inhibition of PARP-1 by CRISPR/Cas9-mediated genome editing enhances the chemosensitivity to cisplatin, showing synergistic cytotoxicity. Based on these results, tumor-derived exosomes may be very promising for cancer therapeutics in the future.

Krachulec, J. M., et al. (2016). "Footprintless disruption of prosurvival genes in aneuploid cancer cells using CRISPR/Cas9 technology." Biochem Cell Biol **94**(3): 289-296.

CRISPR/Cas9 has emerged as a powerful methodology for the targeted editing of genomic DNA sequences. Nevertheless, the intrinsic inefficiency of transfection methods required to use this technique with cultured cells requires the selection and isolation of successfully modified cells, which invariably subjects the cells to stress. Here we report a workflow that allows the isolation of genomically modified cells, even where loss of functional alleles constitutes a selective disadvantage owing to impaired ability to survive stress. Using targeted disruption of the Id1 and Id3 genes in murine B16-F10 and Ret melanoma cell lines as an example, we show that the method allows for the footprintless isolation of CRISPR/Cas9-modified aneuploid cancer cells. We also provide evidence that serial CRISPR/Cas9 modifications can occur, for example when initial homologous recombination events introduce cryptic PAM sequences, and demonstrate that multiple alleles can be successfully targeted in aneuploid cancer cells. By sequencing individual alleles we also found evidence for CRISPR/Cas9-induced transposable element insertion, albeit at a low frequency. This workflow should have broad application in the functional analysis of prosurvival gene function in cultured cells.

Manguso, R. T., et al. (2017). "In vivo CRISPR screening identifies Ptpn2 as a cancer immunotherapy target." Nature **547**(7664): 413-418.

Immunotherapy with PD-1 checkpoint blockade is effective in only a minority of patients with cancer, suggesting that additional treatment strategies are needed. Here we use a pooled in vivo genetic screening approach using CRISPR-Cas9 genome editing in transplantable tumours in mice treated with immunotherapy to discover previously undescribed immunotherapy targets. We tested 2,368 genes expressed by melanoma cells to identify those that synergize with or cause resistance to checkpoint blockade. We recovered the known immune evasion molecules PD-L1 and CD47, and confirmed that defects in interferon-gamma signalling caused resistance to immunotherapy. Tumours were sensitized to immunotherapy by deletion of genes involved in several diverse pathways, including NF-kappaB signalling, antigen presentation and the unfolded protein response. In addition, deletion of the protein tyrosine phosphatase PTPN2 in tumour cells increased the efficacy of immunotherapy by enhancing interferon-gamma-mediated effects on antigen presentation and growth suppression. In vivo genetic screens in tumour models can identify new immunotherapy targets in unanticipated pathways.

Maresch, R., et al. (2016). "Multiplexed pancreatic genome engineering and cancer induction by transfection-based CRISPR/Cas9 delivery in mice." Nat Commun **7**: 10770.

Mouse transgenesis has provided fundamental insights into pancreatic cancer, but is limited by the long duration of allele/model generation. Here we show transfection-based multiplexed delivery of CRISPR/Cas9 to the pancreas of adult mice, allowing simultaneous editing of multiple gene sets in individual cells. We use the method to induce pancreatic cancer and exploit CRISPR/Cas9 mutational signatures for phylogenetic tracking of metastatic disease. Our results demonstrate that CRISPR/Cas9-multiplexing enables key applications, such as combinatorial gene-network analysis, in vivo synthetic lethality screening and chromosome engineering. Negative-selection screening in the pancreas using multiplexed-CRISPR/Cas9 confirms the vulnerability of pancreatic cells to Brca2-inactivation in a Kras-mutant context. We also demonstrate modelling of chromosomal deletions and targeted somatic engineering of inter-chromosomal translocations, offering multifaceted opportunities to study complex structural variation, a hallmark of pancreatic cancer. The low-frequency mosaic pattern of transfection-based CRISPR/Cas9 delivery faithfully recapitulates the stochastic nature of human tumorigenesis, supporting wide applicability for biological/preclinical research.

Martinez-Lage, M., et al. (2018). "CRISPR/Cas9 for Cancer Therapy: Hopes and Challenges." Biomedicines **6**(4).

Cancer is the second leading cause of death globally and remains a major economic and social burden. Although our understanding of cancer at the

molecular level continues to improve, more effort is needed to develop new therapeutic tools and approaches exploiting these advances. Because of its high efficiency and accuracy, the CRISPR-Cas9 genome editing technique has recently emerged as a potentially powerful tool in the arsenal of cancer therapy. Among its many applications, CRISPR-Cas9 has shown an unprecedented clinical potential to discover novel targets for cancer therapy and to dissect chemical-genetic interactions, providing insight into how tumours respond to drug treatment. Moreover, CRISPR-Cas9 can be employed to rapidly engineer immune cells and oncolytic viruses for cancer immunotherapeutic applications. Perhaps more importantly, the ability of CRISPR-Cas9 to accurately edit genes, not only in cell culture models and model organisms but also in humans, allows its use in therapeutic explorations. In this review, we discuss important considerations for the use of CRISPR/Cas9 in therapeutic settings and major challenges that will need to be addressed prior to its clinical translation for a complex and polygenic disease such as cancer.

Meyers, R. M., et al. (2017). "Computational correction of copy number effect improves specificity of CRISPR-Cas9 essentiality screens in cancer cells." Nat Genet **49**(12): 1779-1784.

The CRISPR-Cas9 system has revolutionized gene editing both at single genes and in multiplexed loss-of-function screens, thus enabling precise genome-scale identification of genes essential for proliferation and survival of cancer cells. However, previous studies have reported that a gene-independent antiproliferative effect of Cas9-mediated DNA cleavage confounds such measurement of genetic dependency, thereby leading to false-positive results in copy number-amplified regions. We developed CERES, a computational method to estimate gene-dependency levels from CRISPR-Cas9 essentiality screens while accounting for the copy number-specific effect. In our efforts to define a cancer dependency map, we performed genome-scale CRISPR-Cas9 essentiality screens across 342 cancer cell lines and applied CERES to this data set. We found that CERES decreased false-positive results and estimated sgRNA activity for both this data set and previously published screens performed with different sgRNA libraries. We further demonstrate the utility of this collection of screens, after CERES correction, for identifying cancer-type-specific vulnerabilities.

Mollanoori, H., et al. (2018). "CRISPR/Cas9 and CAR-T cell, collaboration of two revolutionary technologies in cancer immunotherapy, an instruction for successful cancer treatment." Hum Immunol **79**(12): 876-882.

Clustered regularly interspaced short palindromic repeats/CRISPR associated nuclease9 (CRISPR/Cas9) technology, an acquired immune system in bacteria and archaea, has provided a new tool for accurately genome editing. Using only a single nuclease protein in complex with 2 short RNA as a site-specific endonuclease made it a simple and flexible genome editing tool to target nearly any genomic locus. Due to recent developments in therapeutic engineered T cell and effective responses of CD19-directed chimeric antigen receptor T cells (CART19) in patients with B-cell leukemia and lymphoma, adoptive T cell immunotherapy, particularly CAR-T cell therapy became a rapidly growing field in cancer therapy and recently Kymriah and Yescarta (CD19-directed CAR-T cells) were approved by FDA. Therefore, the combination of CRISPR/Cas9 technology as a genome engineering tool and CAR-T cell therapy (engineered T cells that express chimeric antigen receptors) may lead to further improvement in efficiency and safety of CAR-T cells. This article reviews mechanism and therapeutic application of CRISPR/Cas9 technology, accuracy of this technology, cancer immunotherapy by CAR T cells, the application of CRISPR technology for the production of universal CAR T cells, improving their antitumor efficacy, and biotech companies that invested in CRISPR technology for CAR-T cell therapy.

Moses, C., et al. (2018). "Hallmarks of cancer: The CRISPR generation." Eur J Cancer **93**: 10-18.

The hallmarks of cancer were proposed as a logical framework to guide research efforts that aim to understand the molecular mechanisms and derive treatments for this highly complex disease. Recent technological advances, including comprehensive sequencing of different cancer subtypes, have illuminated how genetic and epigenetic alterations are associated with specific hallmarks of cancer. However, as these associations are purely descriptive, one particularly exciting development is the emergence of genome editing technologies, which enable rapid generation of precise genetic and epigenetic modifications to assess the consequences of these perturbations on the cancer phenotype. The most recently developed of these tools, the system of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), consists of an RNA-guided endonuclease that can be repurposed to edit both genome and epigenome with high specificity, and facilitates the functional interrogation of multiple loci in parallel. This system has the potential to dramatically accelerate progress in cancer research, whether by modelling the genesis and progression of cancer in vitro and in vivo, screening for novel therapeutic targets, conducting functional

genomics/epigenomics, or generating targeted cancer therapies. Here, we discuss CRISPR research on each of the ten hallmarks of cancer, outline potential barriers for its clinical implementation and speculate on the advances it may allow in cancer research in the near future.

Mou, H., et al. (2015). "Precision cancer mouse models through genome editing with CRISPR-Cas9." Genome Med **7**(1): 53.

The cancer genome is highly complex, with hundreds of point mutations, translocations, and chromosome gains and losses per tumor. To understand the effects of these alterations, precise models are needed. Traditional approaches to the construction of mouse models are time-consuming and laborious, requiring manipulation of embryonic stem cells and multiple steps. The recent development of the clustered regularly interspersed short palindromic repeats (CRISPR)-Cas9 system, a powerful genome-editing tool for efficient and precise genome engineering in cultured mammalian cells and animals, is transforming mouse-model generation. Here, we review how CRISPR-Cas9 has been used to create germline and somatic mouse models with point mutations, deletions and complex chromosomal rearrangements. We highlight the progress and challenges of such approaches, and how these models can be used to understand the evolution and progression of individual tumors and identify new strategies for cancer treatment. The generation of precision cancer mouse models through genome editing will provide a rapid avenue for functional cancer genomics and pave the way for precision cancer medicine.

Munoz, D. M., et al. (2016). "CRISPR Screens Provide a Comprehensive Assessment of Cancer Vulnerabilities but Generate False-Positive Hits for Highly Amplified Genomic Regions." Cancer Discov **6**(8): 900-913.

UNLABELLED: CRISPR/Cas9 has emerged as a powerful new tool to systematically probe gene function. We compared the performance of CRISPR to RNAi-based loss-of-function screens for the identification of cancer dependencies across multiple cancer cell lines. CRISPR dropout screens consistently identified more lethal genes than RNAi, implying that the identification of many cellular dependencies may require full gene inactivation. However, in two aneuploid cancer models, we found that all genes within highly amplified regions, including nonexpressed genes, scored as lethal by CRISPR, revealing an unanticipated class of false-positive hits. In addition, using a CRISPR tiling screen, we found that sgRNAs targeting essential domains generate the strongest lethality phenotypes and thus provide a strategy to rapidly define the protein domains required for cancer dependence. Collectively, these findings not only demonstrate the utility of CRISPR screens in the identification of cancer-essential genes, but also reveal the need to carefully control for false-positive results in chromosomally unstable cancer lines. SIGNIFICANCE: We show in this study that CRISPR-based screens have a significantly lower false-negative rate compared with RNAi-based screens, but have specific liabilities particularly in the interrogation of regions of genome amplification. Therefore, this study provides critical insights for applying CRISPR-based screens toward the systematic identification of new cancer targets. Cancer Discov; 6(8); 900-13. (c)2016 AACR.See related commentary by Sheel and Xue, p. 824See related article by Aguirre et al., p. 914This article is highlighted in the In This Issue feature, p. 803.

Naert, T. and K. Vleminckx (2018). "Cancer Models in Xenopus tropicalis by CRISPR/Cas9 Mediated Knockout of Tumor Suppressors." Methods Mol Biol **1865**: 147-161.

The recent advent of CRISPR/Cas9 as a straightforward genome editing tool has allowed the establishment of the first bona fide genetic cancer models within the diploid aquatic model organism Xenopus tropicalis (X. tropicalis). Within this chapter, we demonstrate the methods for targeting tumor suppressors with the CRISPR/Cas9 system in the developing X. tropicalis embryo. We further illustrate genotyping and phenotyping of the resulting tumor-bearing F0 mosaic mutant animals (crispants). We focus in detail on the histopathological analysis of cancer neoplasms, the methodology to illustrate high proliferative index by proliferation marker immunofluorescence and how to isolate specific (tumor) cell populations by laser capture microdissection. As such, the described pipeline allows for rapid establishment of novel cancer models by CRISPR/Cas9 targeting of established tumor suppressor genes, or novel candidates obtained from clinical data. In conclusion, we thus provide the methodology for modeling human cancer with the highly efficient CRISPR/Cas9 system in F0 X. tropicalis.

O'Donnell, K. A. (2018). "Advances in functional genetic screening with transposons and CRISPR/Cas9 to illuminate cancer biology." Curr Opin Genet Dev **49**: 85-94.

Large-scale genome sequencing studies have identified a wealth of mutations in human tumors and have dramatically advanced the field of cancer genetics. However, the functional consequences of an

altered gene in tumor progression cannot always be inferred from mutation status alone. This underscores the critical need for complementary methods to assign functional significance to mutated genes in cancer. Transposons are mobile genetic elements that serve as powerful tools for insertional mutagenesis. Over the last decade, investigators have employed mouse models with on-demand transposon-mediated mutagenesis to perform unbiased genetic screens to identify clinically relevant genes that participate in the pathogenesis of human cancer. Two distinct DNA transposon mutagenesis systems, Sleeping Beauty (SB) and PiggyBac (PB), have been applied extensively in vivo and more recently, in ex vivo settings. These studies have informed our understanding of the genes and pathways that drive cancer initiation, progression, and metastasis. This review highlights the latest progress on cancer gene identification for specific cancer subtypes, as well as new technological advances and incorporation of the CRISPR/Cas9 toolbox into transposon-mediated functional genetic studies.

Olive, J. F., et al. (2018). "Accounting for tumor heterogeneity when using CRISPR-Cas9 for cancer progression and drug sensitivity studies." PLoS One **13**(6): e0198790.

Gene editing protocols often require the use of a subcloning step to isolate successfully edited cells, the behavior of which is then compared to the aggregate parental population and/or other non-edited subclones. Here we demonstrate that the inherent functional heterogeneity present in many cell lines can render these populations inappropriate controls, resulting in erroneous interpretations of experimental findings. We describe a novel CRISPR/Cas9 protocol that incorporates a single-cell cloning step prior to gene editing, allowing for the generation of appropriately matched, functionally equivalent control and edited cell lines. As a proof of concept, we generated matched control and osteopontin-knockout Her2+ and Estrogen receptor-negative murine mammary carcinoma cell lines and demonstrated that the osteopontin-knockout cell lines exhibit the expected biological phenotypes, including unaffected primary tumor growth kinetics and reduced metastatic outgrowth in female FVB mice. Using these matched cell lines, we discovered that osteopontin-knockout mammary tumors were more sensitive than control tumors to chemotherapy in vivo. Our results demonstrate that heterogeneity must be considered during experimental design when utilizing gene editing protocols and provide a solution to account for it.

Park, M. Y., et al. (2017). "Generation of lung cancer cell lines harboring EGFR T790M mutation by CRISPR/Cas9-mediated genome editing." Oncotarget **8**(22): 36331-36338.

Tyrosine kinase inhibitors (TKIs) such as gefitinib and erlotinib are effective against lung adenocarcinomas harboring epidermal growth factor receptor (EGFR) mutations. However, cancer cells can develop resistance to these agents with prolonged exposure; in over 50% of cases, this is attributable to the EGFR T790M mutation. Moreover, additional resistance mutations can arise with the use of new drugs. Cancer cell lines with specific mutations can enable the study of resistance mechanisms. In this study, we introduced the EGFR T790M mutation into the PC9 human lung cancer cell line-which has a deletion in exon 19 of the EGFR gene-by clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas)9-mediated genome editing. EGFR pyrosequencing and peptide nucleic acid clamping revealed that PC9 cells with EGFR T790M generated by CRISPR/Cas 9 had a higher T790M mutation rate than those with the same mutation generated by long-term exposure to gefitinib (PC9-G); moreover, resistance to gefitinib in these clones was higher than that in PC9-G cells. The clones were also highly sensitive to the 3rd-generation EGFR TKI AZD9291, which is cytotoxic to lung cancer cells with EGFR T790M. The CRISPR/Cas9 programmable nuclease system can be used to generate various cancer cell lines with specific mutations that can facilitate studies on resistance mechanisms and drug efficacy.

Peng, L., et al. (2018). "A tetracycline-inducible CRISPR/Cas9 system, targeting two long non-coding RNAs, suppresses the malignant behavior of bladder cancer cells." Oncol Lett **16**(4): 4309-4316.

Clustered regularly interspaced short palindromic repeats (CRISPR) associated protein 9 (Cas9) technology has been applied in varied biological studies, including cancer studies. However, stable mRNA expression of Cas9 has potential risks in future gene therapy. Therefore, in the present study, a tetracycline-inducible switch was used to control the mRNA expression of Cas9. Long non-coding RNAs (lncRNAs) may be important functional regulators in tumor development, including in bladder cancer. RNA was designed to simultaneously target two lncRNAs, PVT1 and ANRIL, which are considered to be bladder cancer oncogenes. The mRNA expression of Cas9 was controlled by doxycycline. Reverse transcription-quantitative polymerase chain reaction revealed that the expression of PVT1 and ANRIL was significantly inhibited by the tetracycline-inducible CRISPR/Cas9 system. Functional assays demonstrated that this

system could inhibit proliferation, induce apoptosis and suppress cell migration. Therefore, the tetracycline-inducible CRISPR/Cas9 system was demonstrated to repress the malignant behavior of bladder cancer cells by controlling the expression of Cas9 and simultaneously targeting two oncogenic lncRNAs.

Platt, R. J., et al. (2014). "CRISPR-Cas9 knockin mice for genome editing and cancer modeling." Cell **159**(2): 440-455.

CRISPR-Cas9 is a versatile genome editing technology for studying the functions of genetic elements. To broadly enable the application of Cas9 in vivo, we established a Cre-dependent Cas9 knockin mouse. We demonstrated in vivo as well as ex vivo genome editing using adeno-associated virus (AAV)-, lentivirus-, or particle-mediated delivery of guide RNA in neurons, immune cells, and endothelial cells. Using these mice, we simultaneously modeled the dynamics of KRAS, p53, and LKB1, the top three significantly mutated genes in lung adenocarcinoma. Delivery of a single AAV vector in the lung generated loss-of-function mutations in p53 and Lkb1, as well as homology-directed repair-mediated Kras (G12D) mutations, leading to macroscopic tumors of adenocarcinoma pathology. Together, these results suggest that Cas9 mice empower a wide range of biological and disease modeling applications.

Ratan, Z. A., et al. (2018). "CRISPR-Cas9: a promising genetic engineering approach in cancer research." Ther Adv Med Oncol **10**: 1758834018755089.

Bacteria and archaea possess adaptive immunity against foreign genetic materials through clustered regularly interspaced short palindromic repeat (CRISPR) systems. The discovery of this intriguing bacterial system heralded a revolutionary change in the field of medical science. The CRISPR and CRISPR-associated protein 9 (Cas9) based molecular mechanism has been applied to genome editing. This CRISPR-Cas9 technique is now able to mediate precise genetic corrections or disruptions in in vitro and in vivo environments. The accuracy and versatility of CRISPR-Cas have been capitalized upon in biological and medical research and bring new hope to cancer research. Cancer involves complex alterations and multiple mutations, translocations and chromosomal losses and gains. The ability to identify and correct such mutations is an important goal in cancer treatment. In the context of this complex cancer genomic landscape, there is a need for a simple and flexible genetic tool that can easily identify functional cancer driver genes within a comparatively short time. The CRISPR-Cas system shows promising potential for modeling, repairing and correcting genetic events in different types of cancer. This article reviews the concept of CRISPR-Cas, its application and related advantages in oncology.

Rattanapornsompong, K., et al. (2019). "Generation of Human Pyruvate Carboxylase Knockout Cell Lines Using Retrovirus Expressing Short Hairpin RNA and CRISPR-Cas9 as Models to Study Its Metabolic Role in Cancer Research." Methods Mol Biol **1916**: 273-288.

We report two protocols to generate human pyruvate carboxylase knockdown and knockout cell lines using short hairpin RNA (shRNA) and CRISPR-Cas9 technologies. The first protocol involved cloning of a shRNA cassette targeted to human pyruvate carboxylase (PC) under the control of a U6 promoter in a retrovirus-based vector. The stable knockdown cells were achieved following infection of retroviruses expressing shRNA in target cells followed by selecting these in medium containing puromycin. The second protocol describes a CRISPR Cas9-knockout cell constructed by cloning of single guide RNA (gRNA) targeted to the human pyruvate carboxylase gene placed adjacent to Cas 9 in the pSpCas9(BB)-2A-GFP vector. The knockout cells can be selected by sorting the cells expressing GFP. We also describe protocols for detecting the level of PC mRNA and protein in the knockdown or knockout cells using qPCR and Western blot analyses, respectively. The above protocols allow investigators to create PC deficient cell lines as a tool to study role of this enzyme in cancer research.

Reinshagen, C., et al. (2018). "CRISPR-enhanced engineering of therapy-sensitive cancer cells for self-targeting of primary and metastatic tumors." Sci Transl Med **10**(449).

Tumor cells engineered to express therapeutic agents have shown promise to treat cancer. However, their potential to target cell surface receptors specific to the tumor site and their posttreatment fate have not been explored. We created therapeutic tumor cells expressing ligands specific to primary and recurrent tumor sites (receptor self-targeted tumor cells) and extensively characterized two different approaches using (i) therapy-resistant cancer cells, engineered with secretable death receptor-targeting ligands for "off-the-shelf" therapy in primary tumor settings, and (ii) therapy-sensitive cancer cells, which were CRISPR-engineered to knock out therapy-specific cell surface receptors before engineering with receptor self-targeted ligands and reapplied in autologous models of recurrent or metastatic disease. We show that both approaches allow high expression of targeted ligands that induce tumor cell killing and translate into

marked survival benefits in mouse models of multiple cancer types. Safe elimination of therapeutic cancer cells after treatment was achieved by co-engineering with a prodrug-converting suicide system, which also allowed for real-time in vivo positron emission tomography imaging of therapeutic tumor cell fate. This study demonstrates self-tumor tropism of engineered cancer cells and their therapeutic potential when engineered with receptor self-targeted molecules, and it establishes a roadmap toward a safe clinical translation for different cancer types in primary, recurrent, and metastatic settings.

Roper, J., et al. (2018). "Colonoscopy-based colorectal cancer modeling in mice with CRISPR-Cas9 genome editing and organoid transplantation." Nat Protoc **13**(2): 217-234.

Most genetically engineered mouse models (GEMMs) of colorectal cancer are limited by tumor formation in the small intestine, a high tumor burden that limits metastasis, and the need to generate and cross mutant mice. Cell line or organoid transplantation models generally produce tumors in ectopic locations-such as the subcutaneous space, kidney capsule, or cecal wall-that do not reflect the native stromal environment of the colon mucosa. Here, we describe detailed protocols to rapidly and efficiently induce site-directed tumors in the distal colon of mice that are based on colonoscopy-guided mucosal injection. These techniques can be adapted to deliver viral vectors carrying Cre recombinase, CRISPR-Cas9 components, CRISPR-engineered mouse tumor organoids, or human cancer organoids to mice to model the adenoma-carcinoma-metastasis sequence of tumor progression. The colonoscopy injection procedure takes approximately 15 min, including preparation. In our experience, anyone with reasonable hand-eye coordination can become proficient with mouse colonoscopy and mucosal injection with a few hours of practice. These approaches are ideal for a wide range of applications, including assessment of gene function in tumorigenesis, examination of tumor-stroma interactions, studies of cancer metastasis, and translational research with patient-derived cancers.

Sachdeva, M., et al. (2015). "CRISPR/Cas9: molecular tool for gene therapy to target genome and epigenome in the treatment of lung cancer." Cancer Gene Ther **22**(11): 509-517.

Although varied drugs and therapies have been developed for lung cancer treatment, in the past 5 years overall survival rates have not improved much. It has also been reported that lung cancer is diagnosed in most of the patients when it is already in the advanced stages with heterogeneous tumors where single therapy is mostly ineffective. A combination of therapies are being administered and specific genes in specific tissues are targeted while protecting normal cell, but most of the therapies face drawbacks for the development of resistance against them and tumor progression. Therefore, therapeutic implications for various therapies need to be complemented by divergent strategies. This review frames utilization of CRISPR/Cas9 for molecular targeted gene therapy leading to long-term repression and activation or inhibition of molecular targets linked to lung cancer, avoiding the cycles of therapy.

Sanchez-Rivera, F. J. and T. Jacks (2015). "Applications of the CRISPR-Cas9 system in cancer biology." Nat Rev Cancer **15**(7): 387-395.

The prokaryotic type II CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats-CRISPR-associated 9) system is rapidly revolutionizing the field of genetic engineering, allowing researchers to alter the genomes of a large range of organisms with relative ease. Experimental approaches based on this versatile technology have the potential to transform the field of cancer genetics. Here, we review current approaches for functional studies of cancer genes that are based on CRISPR-Cas, with emphasis on their applicability for the development of next-generation models of human cancer.

Sayin, V. I. and T. Papagiannakopoulos (2017). "Application of CRISPR-mediated genome engineering in cancer research." Cancer Lett **387**: 10-17.

Cancer is a multistep process that arises from a series of genetic and epigenetic events. With recent technological advances there has been a burst in genome sequencing and epigenetic studies revealing a plethora of alterations that may contribute to cancer. However, the great challenge for the cancer research community is the systematic functional characterization of these genetic and epigenetic events to assess their role in cancer initiation and progression. Recent advances in genome engineering using CRISPR/Cas9, an ancient bacterial immune-like system, have revolutionized cancer genetics. Here we highlight the breakthroughs in the effective use of these novel genome-editing techniques, and we discuss the challenges and potential applications of these tools for cancer biology.

Shi, J., et al. (2015). "Discovery of cancer drug targets by CRISPR-Cas9 screening of protein domains." Nat Biotechnol **33**(6): 661-667.

CRISPR-Cas9 genome editing technology holds great promise for discovering therapeutic targets in

cancer and other diseases. Current screening strategies target CRISPR-Cas9-induced mutations to the 5' exons of candidate genes, but this approach often produces in-frame variants that retain functionality, which can obscure even strong genetic dependencies. Here we overcome this limitation by targeting CRISPR-Cas9 mutagenesis to exons encoding functional protein domains. This generates a higher proportion of null mutations and substantially increases the potency of negative selection. We also show that the magnitude of negative selection can be used to infer the functional importance of individual protein domains of interest. A screen of 192 chromatin regulatory domains in murine acute myeloid leukemia cells identifies six known drug targets and 19 additional dependencies. A broader application of this approach may allow comprehensive identification of protein domains that sustain cancer cells and are suitable for drug targeting.

Slipek, N. J., et al. (2019). "CRISPR/Cas9-Based Positive Screens for Cancer-Related Traits." Methods Mol Biol **1907**: 137-144.

Since the advent of large-scale, detailed descriptive cancer genomics studies at the beginning of the century, such as The Cancer Genome Atlas (TCGA), labs around the world have been working to make this data useful. Data like these can be made more useful by comparison with complementary functional genomic data. One new example is the application of CRISPR/Cas9-based library screening for cancer-related traits in cell lines. Such screens can reveal genome-wide suppressors of tumorigenesis and metastasis. Here we describe the use of widely available lentiviral libraries for such screens in cultured cell lines.

Su, S., et al. (2016). "CRISPR-Cas9 mediated efficient PD-1 disruption on human primary T cells from cancer patients." Sci Rep **6**: 20070.

Strategies that enhance the function of T cells are critical for immunotherapy. One negative regulator of T-cell activity is ligand PD-L1, which is expressed on dentritic cells (DCs) or some tumor cells, and functions through binding of programmed death-1 (PD-1) receptor on activated T cells. Here we described for the first time a non-viral mediated approach to reprogram primary human T cells by disruption of PD-1. We showed that the gene knockout of PD-1 by electroporation of plasmids encoding sgRNA and Cas9 was technically feasible. The disruption of inhibitory checkpoint gene PD-1 resulted in significant reduction of PD-1 expression but didn't affect the viability of primary human T cells during the prolonged in vitro culture. Cellular immune response of the gene modified T cells was characterized by up-regulated IFN-gamma production and enhanced cytotoxicity. These results suggest that we have demonstrated an approach for efficient checkpoint inhibitor disruption in T cells, providing a new strategy for targeting checkpoint inhibitors, which could potentialy be useful to improve the efficacy of T-cell based adoptive therapies.

Su, S., et al. (2017). "CRISPR-Cas9-mediated disruption of PD-1 on human T cells for adoptive cellular therapies of EBV positive gastric cancer." Oncoimmunology **6**(1): e1249558.

The successful use of immune cell checkpoint inhibitors PD-1 and PD-L1, over the past 5 y has raised the concern of using immunotherapy to treat various cancers. Epstein-Barr virus-associated gastric cancer (EBVaGC) exhibits high infiltration of lymphocytes and high amplification of immune-related genes including PD-L1 as distinguished from Epstein-Barr virus-non-associated gastric cancer (EBVnGC). Here, we presume that this PD-1/PD-L1 pathway may hinder the efficacy of adoptive T cell therapy toward EBVaGC. These studies reveal possibility of generating PD-1-disrupted CTL by CRISPR-Cas9 system and demonstrate enhanced immune response of these PD-1-disrupted CTLs to the EBV-LMP2A antigen and superior cytotoxicity to the EBV-positive gastric cancer cell. In addition, when combined with low-dose radiotherapy, these PD-1-disrupted CTLs mediated an impressive antitumor effect in a xenograft mouse model of EBVaGC. Taken together, these studies illustrate PD-1/PD-L1-mediated immune tolerance of EBVaGC and provide a new strategy for targeting immune checkpoints to break the tolerance for the T cell-based adoptive therapy.

Szlachta, K., et al. (2018). "CRISPR knockout screening identifies combinatorial drug targets in pancreatic cancer and models cellular drug response." Nat Commun **9**(1): 4275.

Predicting the response and identifying additional targets that will improve the efficacy of chemotherapy is a major goal in cancer research. Through large-scale in vivo and in vitro CRISPR knockout screens in pancreatic ductal adenocarcinoma cells, we identified genes whose genetic deletion or pharmacologic inhibition synergistically increase the cytotoxicity of MEK signaling inhibitors. Furthermore, we show that CRISPR viability scores combined with basal gene expression levels could model global cellular responses to the drug treatment. We develop drug response evaluation by in vivo CRISPR screening (DREBIC) method and validated its efficacy using large-scale experimental data from independent experiments. Comparative analyses demonstrate that DREBIC predicts drug response in cancer cells from a wide range of tissues with high accuracy and identifies

therapeutic vulnerabilities of cancer-causing mutations to MEK inhibitors in various cancer types.

Takao, A., et al. (2018). "Generation of PTENknockout (/) murine prostate cancer cells using the CRISPR/Cas9 system and comprehensive gene expression profiling." Oncol Rep **40**(5): 2455-2466.

Phosphatase and tensin homolog (PTEN) deficiency is associated with development, progression, and metastasis of various cancers. However, changes in gene expression associated with PTEN deficiency have not been fully characterized. To explore genes with altered expression in PTENdeficient cells, the present study generated a PTENknockout cell line (DeltaPTEN) from a mouse prostate cancerderived cell line using the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPRassociated protein 9 (CRISPR/Cas9) gene editing system. Following transfection of the CRISPR/Cas9 construct, DNA sequencing was performed to identify deletion of the Pten locus and PTEN inactivation was verified by western blotting. The DeltaPTEN cell line exhibited enhanced RACalpha serine/threonineprotein kinase phosphorylation and cyclin D1 expression. In addition, an increase in cell proliferation and colony formation was observed in the DeltaPTEN cell line. Gene expression profiling experiments were analyzed with microarray and microRNA (miRNA) arrays. In the microarray analysis, 111 genes exhibited >/=10fold increased expression compared with the parent strain and mock cell line and 23 genes were downregulated. The only miRNA with increased expression of 10fold or more was mmumiR2103p. Genes with enhanced expression included genes involved in the development, progression, and metastasis of cancer such as Tet methylcytosine dioxygenase 1, twist family BHLH transcription factor 2, Cfosinduced growth factor and WinglessType MMTV Integration Site Family, Member 3, and genes involved in immunosuppression such as Arginase 1. The results of the present study suggest that PTEN deficiency mobilizes a variety of genes critical for cancer cell survival and host immune evasion.

Tanihara, F., et al. (2018). "Generation of a TP53-modified porcine cancer model by CRISPR/Cas9-mediated gene modification in porcine zygotes via electroporation." PLoS One **13**(10): e0206360.

TP53 (which encodes p53) is one of the most frequently mutated genes in cancers. In this study, we generated TP53-mutant pigs by gene editing via electroporation of the Cas9 protein (GEEP), a process that involves introducing the Cas9 protein and single-guide RNA (sgRNA) targeting exon 3 and intron 4 of TP53 into in vitro-fertilized zygotes. Zygotes modified by the sgRNAs were transferred to recipients, two of which gave birth to a total of 11 piglets. Of those 11 piglets, 9 survived. Molecular genetic analysis confirmed that 6 of 9 live piglets carried mutations in TP53, including 2 piglets with no wild-type (WT) sequences and 4 genetically mosaic piglets with WT sequences. One mosaic piglet had 142 and 151 bp deletions caused by a combination of the two sgRNAs. These piglets were continually monitored for 16 months and three of the genome-edited pigs (50%) exhibited various tumor phenotypes that we presumed were caused by TP53 mutations. Two mutant pigs with no WT sequences developed mandibular osteosarcoma and nephroblastoma. The mosaic pig with a deletion between targeting sites of two sgRNAs exhibited malignant fibrous histiocytoma. Tumor phenotypes of TP53 mosaic mutant pigs have not been previously reported. Our results indicated that the mutations caused by gene editing successfully induced tumor phenotypes in both TP53 mosaic- and bi-allelic mutant pigs.

Thomenius, M. J., et al. (2018). "Small molecule inhibitors and CRISPR/Cas9 mutagenesis demonstrate that SMYD2 and SMYD3 activity are dispensable for autonomous cancer cell proliferation." PLoS One **13**(6): e0197372.

A key challenge in the development of precision medicine is defining the phenotypic consequences of pharmacological modulation of specific target macromolecules. To address this issue, a variety of genetic, molecular and chemical tools can be used. All of these approaches can produce misleading results if the specificity of the tools is not well understood and the proper controls are not performed. In this paper we illustrate these general themes by providing detailed studies of small molecule inhibitors of the enzymatic activity of two members of the SMYD branch of the protein lysine methyltransferases, SMYD2 and SMYD3. We show that tool compounds as well as CRISPR/Cas9 fail to reproduce many of the cell proliferation findings associated with SMYD2 and SMYD3 inhibition previously obtained with RNAi based approaches and with early stage chemical probes.

Torres-Ruiz, R. and S. Rodriguez-Perales (2015). "CRISPR-Cas9: A Revolutionary Tool for Cancer Modelling." Int J Mol Sci **16**(9): 22151-22168.

The cancer-modelling field is now experiencing a conversion with the recent emergence of the RNA-programmable CRISPR-Cas9 system, a flexible methodology to produce essentially any desired modification in the genome. Cancer is a multistep process that involves many genetic mutations and

other genome rearrangements. Despite their importance, it is difficult to recapitulate the degree of genetic complexity found in patient tumors. The CRISPR-Cas9 system for genome editing has been proven as a robust technology that makes it possible to generate cellular and animal models that recapitulate those cooperative alterations rapidly and at low cost. In this review, we will discuss the innovative applications of the CRISPR-Cas9 system to generate new models, providing a new way to interrogate the development and progression of cancers.

Van Treuren, T. and J. K. Vishwanatha (2018). "CRISPR deletion of MIEN1 in breast cancer cells." PLoS One **13**(10): e0204976.

Migration and Invasion Enhancer (MIEN1) is an oncogene which is involved in facilitating motility of cancer cells through actin dynamics and gene expression. Increased MIEN1 expression in many types of tumors leads to disease progression and metastatic propensity. It is unclear precisely how MIEN1 is involved in this process and more studies are required to tease out the mechanisms. Here we show that Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) genome editing effectively produced specific genomic deletions in the MIEN1 gene which led to the abrogation of its expression in breast cancer cells. The single guide RNAs (sgRNAs) mediated targeting of MIEN1 was specific and none of the clones screened for off-target cleavage revealed any insertions or deletions (indels). Additionally, disruption of the MIEN1 gene did not alter the cell morphology, growth, proliferation or survival. Knocking out MIEN1 in these breast cancer cells will allow future studies to determine the exact role MIEN1 plays in breast tumor metastasis, which might lead to production of novel therapeutics to treat this and other cancers.

Vorvis, C., et al. (2016). "Transcriptomic and CRISPR/Cas9 technologies reveal FOXA2 as a tumor suppressor gene in pancreatic cancer." Am J Physiol Gastrointest Liver Physiol **310**(11): G1124-1137.

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive cancer with low survival rates and limited therapeutic options. Thus elucidation of signaling pathways involved in PDAC pathogenesis is essential for identifying novel potential therapeutic gene targets. Here, we used a systems approach to elucidate those pathways by integrating gene and microRNA profiling analyses together with CRISPR/Cas9 technology to identify novel transcription factors involved in PDAC pathogenesis. FOXA2 transcription factor was found to be significantly downregulated in PDAC relative to control pancreatic tissues. Functional experiments revealed that FOXA2 has a tumor suppressor function through inhibition of pancreatic cancer cell growth, migration, invasion, and colony formation. In situ hybridization analysis revealed miR-199a to be significantly upregulated in pancreatic cancer. Bioinformatics and luciferase analyses showed that miR-199a negatively but directly regulates FOXA2 expression through binding in its 3'-untranslated region (UTR). Evaluation of the functional importance of miR-199a on pancreatic cancer revealed that miR-199a acts as an inhibitor of FOXA2 expression, inducing an increase in pancreatic cancer cell proliferation, migration, and invasion. Additionally, gene ontology and network analyses in PANC-1 cells treated with a small interfering RNA (siRNA) against FOXA2 revealed an enrichment for cell invasion mechanisms through PLAUR and ERK activation. FOXA2 deletion (FOXA2Delta) by using two CRISPR/Cas9 vectors in PANC-1 cells induced tumor growth in vivo resulting in upregulation of PLAUR and ERK pathways in FOXA2Delta xenograft tumors. We have identified FOXA2 as a novel tumor suppressor in pancreatic cancer and it is regulated directly by miR-199a, thereby enhancing our understanding of how microRNAs interplay with the transcription factors to affect pancreatic oncogenesis.

Wang, D. Y., et al. (2016). "[The application of CRISPR/Cas9 genome editing technology in cancer research]." Yi Chuan **38**(1): 1-8.

The CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein-9 nuclease) genome editing technology has become more and more popular in gene editing because of its simple design and easy operation. Using the CRISPR/Cas9 system, researchers can perform site-directed genome modification at the base level. Moreover, it has been widely used in genome editing in multiple species and related cancer research. In this review, we summarize the application of the CRISPR/Cas9 system in cancer research based on the latest research progresses as well as our understanding of cancer research and genome editing techniques.

Wang, G., et al. (2018). "Mapping a functional cancer genome atlas of tumor suppressors in mouse liver using AAV-CRISPR-mediated direct in vivo screening." Sci Adv **4**(2): eaao5508.

Cancer genomics consortia have charted the landscapes of numerous human cancers. Whereas some mutations were found in classical oncogenes and tumor suppressors, others have not yet been functionally studied in vivo. To date, a comprehensive assessment of how these genes influence oncogenesis is lacking. We performed direct high-throughput in vivo mapping of functional variants in an

autochthonous mouse model of cancer. Using adeno-associated viruses (AAVs) carrying a single-guide RNA (sgRNA) library targeting putative tumor suppressor genes significantly mutated in human cancers, we directly pool-mutagenized the livers of Cre-inducible CRISPR (clustered regularly interspaced short palindromic repeats)-associated protein 9 (Cas9) mice. All mice that received the AAV-mTSG library developed liver cancer and died within 4 months. We used molecular inversion probe sequencing of the sgRNA target sites to chart the mutational landscape of these tumors, revealing the functional consequence of multiple variants in driving liver tumorigenesis in immunocompetent mice. AAV-mediated autochthonous CRISPR screens provide a powerful means for mapping a provisional functional cancer genome atlas of tumor suppressors in vivo.

Weber, J., et al. (2015). "CRISPR/Cas9 somatic multiplex-mutagenesis for high-throughput functional cancer genomics in mice." Proc Natl Acad Sci U S A **112**(45): 13982-13987.

Here, we show CRISPR/Cas9-based targeted somatic multiplex-mutagenesis and its application for high-throughput analysis of gene function in mice. Using hepatic single guide RNA (sgRNA) delivery, we targeted large gene sets to induce hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC). We observed Darwinian selection of target genes, which suppress tumorigenesis in the respective cellular/tissue context, such as Pten or Cdkn2a, and conversely found low frequency of Brca1/2 alterations, explaining mutational spectra in human ICC/HCC. Our studies show that multiplexed CRISPR/Cas9 can be used for recessive genetic screening or high-throughput cancer gene validation in mice. The analysis of CRISPR/Cas9-induced tumors provided support for a major role of chromatin modifiers in hepatobiliary tumorigenesis, including that of ARID family proteins, which have recently been reported to be mutated in ICC/HCC. We have also comprehensively characterized the frequency and size of chromosomal alterations induced by combinatorial sgRNA delivery and describe related limitations of CRISPR/Cas9 multiplexing, as well as opportunities for chromosome engineering in the context of hepatobiliary tumorigenesis. Our study describes novel approaches to model and study cancer in a high-throughput multiplexed format that will facilitate the functional annotation of cancer genomes.

Wei, C., et al. (2018). "CRISPR/Cas9 targeting of the androgen receptor suppresses the growth of LNCaP human prostate cancer cells." Mol Med Rep **17**(2): 2901-2906.

Androgens have been recognized to be primary causative agents of prostate cancer. Following binding to the androgen receptor (AR), androgens serve important roles in the carcinogenesis of prostate cancers. ARs serve an important role during all stages of prostate cancer, and inhibiting their function may help to slow prostate cancer growth. In the present study, the AR gene was targeted in androgenpositive prostate cancer cells using the clustered regularly interspaced short palindromic repeatsassociated protein (CRISPR/Cas) system. A total of three different singleguide RNAs (sgRNAs) were designed according to the three different target sites in the AR gene. The optimal sgRNA with a specific target effect was effectively screened to cleave the AR gene in androgenpositive prostate cancer cell lines, and to suppress the growth of androgensensitive prostate cancer in vitro. The ARsgRNAguided CRISPR/Cas system was able to disrupt the AR at specific sites and inhibit the growth of androgensensitive prostate cancer cells; further studies demonstrated that the decreased cell proliferation was due to cellular apoptosis. The results of the present study suggested that the CRISPR/Cas system may be a useful therapeutic strategy for the treatment of prostate cancer.

Wen, W. S., et al. (2016). "CRISPR-Cas9 systems: versatile cancer modelling platforms and promising therapeutic strategies." Int J Cancer **138**(6): 1328-1336.

The RNA-guided nuclease CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats-CRISPR associated nuclease 9) and its variants such as nickase Cas9, dead Cas9, guide RNA scaffolds and RNA-targeting Cas9 are convenient and versatile platforms for site-specific genome editing and epigenome modulation. They are easy-to-use, simple-to-design and capable of targeting multiple loci simultaneously. Given that cancer develops from cumulative genetic and epigenetic alterations, CRISPR-Cas9 and its variants (hereafter referred to as CRISPR-Cas9 systems) hold extensive application potentials in cancer modeling and therapy. To date, they have already been applied to model oncogenic mutations in cell lines (e.g., Choi and Meyerson, Nat Commun 2014;5:3728) and in adult animals (e.g., Xue et al., Nature 2014;514:380-4), as well as to combat cancer by disabling oncogenic viruses (e.g., Hu et al., Biomed Res Int 2014;2014:612823) or by manipulating cancer genome (e.g., Liu et al., Nat Commun 2014;5:5393). Given the importance of epigenome and transcriptome in tumourigenesis, manipulation of cancer epigenome and transcriptome for cancer modeling and therapy is a promising area in the future. Whereas (epi)genetic modifications of cancer microenvironment with CRISPR-Cas9 systems

for therapeutic purposes represent another promising area in cancer research. Herein, we introduce the functions and mechanisms of CRISPR-Cas9 systems in genome editing and epigenome modulation, retrospect their applications in cancer modelling and therapy, discuss limitations and possible solutions and propose future directions, in hope of providing concise and enlightening information for readers interested in this area.

White, M. K. and K. Khalili (2016). "CRISPR/Cas9 and cancer targets: future possibilities and present challenges." Oncotarget **7**(11): 12305-12317.

All cancers have multiple mutations that can largely be grouped into certain classes depending on the function of the gene in which they lie and these include oncogenic changes that enhance cellular proliferation, loss of function of tumor suppressors that regulate cell growth potential and induction of metabolic enzymes that confer resistance to chemotherapeutic agents. Thus the ability to correct such mutations is an important goal in cancer treatment. Recent research has led to the developments of reagents which specifically target nucleotide sequences within the cellular genome and these have a huge potential for expanding our anticancer armamentarium. One such a reagent is the clustered regulatory interspaced short palindromic repeat (CRISPR)-associated 9 (Cas9) system, a powerful, highly specific and adaptable tool that provides unparalleled control for editing the cellular genome. In this short review, we discuss the potential of CRISPR/Cas9 against human cancers and the current difficulties in translating this for novel therapeutic approaches.

Wu, H. Y. and C. Y. Cao (2018). "The application of CRISPR-Cas9 genome editing tool in cancer immunotherapy." Brief Funct Genomics.

Clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (CRISPR-Cas9) system was originally discovered in prokaryotes functioned as a part of the adaptive immune system. Because of its high efficiency and easy operability, CRISPR-Cas9 system has been developed to be a powerful and versatile gene editing tool shortly after its discovery. Given that multiple genetic alterations are the main factors that drive genesis and development of tumor, CRISPR-Cas9 system has been applied to correct cancer-causing gene mutations and deletions and to engineer immune cells, such as chimeric antigen receptor T (CAR T) cells, for cancer immunotherapeutic applications. Recently, CRISPR-Cas9-based CAR T-cell preparation has been an important breakthrough in antitumor therapy. Here, we summarize the mechanism, delivery and the application of CRISPR-Cas9 in gene editing, and discuss the challenges and future directions of CRISPR-Cas9 in cancer immunotherapy.

Xia, A. L., et al. (2019). "Applications and advances of CRISPR-Cas9 in cancer immunotherapy." J Med Genet **56**(1): 4-9.

Immunotherapy has emerged as one of the most promising therapeutic strategies in cancer. The clustered regularly interspaced short palindromic repeat (CRISPR)-associated protein 9 (CRISPR-Cas9) system, as an RNA-guided genome editing technology, is triggering a revolutionary change in cancer immunotherapy. With its versatility and ease of use, CRISPR-Cas9 can be implemented to fuel the production of therapeutic immune cells, such as construction of chimeric antigen receptor T (CAR-T) cells and programmed cell death protein 1 knockout. Therefore, CRISPR-Cas9 technology holds great promise in cancer immunotherapy. In this review, we will introduce the origin, development and mechanism of CRISPR-Cas9. Also, we will focus on its various applications in cancer immunotherapy, especially CAR-T cell-based immunotherapy, and discuss the potential challenges it faces.

Xu, X., et al. (2017). "CRISPR-ON-Mediated KLF4 overexpression inhibits the proliferation, migration and invasion of urothelial bladder cancer in vitro and in vivo." Oncotarget **8**(60): 102078-102087.

Kruppel like factor 4 (KLF4), a transcription factor associated with carcinogenesis and tumor progression, plays an important role in various malignancies. In the present study, we utilized the CRISPR-ON system to upregulate KLF4 expression level and subsequently investigated the effect and mechanism of KLF4 in the carcinogenesis and progression of urothelial bladder cancer (UBC). Immunohistochemistry (IHC) and quantitative RT-PCR (qRT-PCR) were used to evaluate the expression of KLF4. The CpG methylation status of the promoter region was analyzed using bisulfite-sequencing PCR (BSP). CRISPR-ON system comprised sgRNA and dCas9 protein combined with a transcriptional activation domain. The cell proliferation and cell cycle were assessed by CCK-8 assay, flow cytometry and colony formation assay. The cell motility ability was evaluated using trans-well assay. In vivo tumorigenesis assay and lung metastasis model were also performed. The KLF4 expression was significantly downregulated in UBC tissues. The high CpG methylation status in the promoter of KLF4 was confirmed using BSP. KLF4 overexpression was successfully achieved via CRISPR-ON system, which inhibited the proliferation and induced G1-phase arrest

in T24 cells through the regulation of AKT/p21 signal. Furthermore, enforced expression of KLF4 also abrogated the migration and invasion of T24 cells by suppressing EMT progression. Finally, in vivo models indicated that the upregulation of KLF4 could inhibit tumorigenesis and lung metastasis in nude mice. In conclusion, KLF4 overexpression mediated by CRISPR-ON inhibits tumorigenesis and EMT progression in UBC cells, representing a potential therapeutic target, and CRISPR-ON system could be a therapeutic strategy for UBC in the future.

Xu, Y., et al. (2018). "[Knocking-out of HIF1alpha gene by CRISPR/cas9 inhibits proliferation and invasiveness of prostate cancer DU145 cells]." Zhonghua Yi Xue Yi Chuan Xue Za Zhi **35**(2): 160-164.

OBJECTIVE: To explore the role of HIF1alpha gene in prostate cancer cell line DU145 by knocking it out with a novel gene-editing tool CRISPR/cas9 system. METHODS: A CRISPR/cas9 system with two sgRNAs targeting exon 1 of the HIF1alpha gene was constructed for the knock out experiment. CCK8 assay and transwell experiment were carried out to assess the effect of the knock out on the proliferation, migration and invasiveness of DU145 cells. RESULTS: The efficiency of gene-targeting was measured through a T7E1 assaying and sequence analysis, which confirmed that the partial knock out was successful and has led to a significant decrease in the expression of HIF1alpha and inhibition of cell proliferation, migration and invasiveness. CONCLUSION: A CRISPR/cas9 system for the knock out of HIF1alpha has been successfully constructed, which could inhibit the proliferation and migration of DU145 cells. The system can facilitate further studies of the HIF1alpha gene and its roles in tumorigenesis.

Xue, W., et al. (2014). "CRISPR-mediated direct mutation of cancer genes in the mouse liver." Nature **514**(7522): 380-384.

The study of cancer genes in mouse models has traditionally relied on genetically-engineered strains made via transgenesis or gene targeting in embryonic stem cells. Here we describe a new method of cancer model generation using the CRISPR/Cas (clustered regularly interspaced short palindromic repeats/CRISPR-associated proteins) system in vivo in wild-type mice. We used hydrodynamic injection to deliver a CRISPR plasmid DNA expressing Cas9 and single guide RNAs (sgRNAs) to the liver that directly target the tumour suppressor genes Pten (ref. 5) and p53 (also known as TP53 and Trp53) (ref. 6), alone and in combination. CRISPR-mediated Pten mutation led to elevated Akt phosphorylation and lipid accumulation in hepatocytes, phenocopying the effects of deletion of the gene using Cre-LoxP technology. Simultaneous targeting of Pten and p53 induced liver tumours that mimicked those caused by Cre-loxP-mediated deletion of Pten and p53. DNA sequencing of liver and tumour tissue revealed insertion or deletion mutations of the tumour suppressor genes, including bi-allelic mutations of both Pten and p53 in tumours. Furthermore, co-injection of Cas9 plasmids harbouring sgRNAs targeting the beta-catenin gene and a single-stranded DNA oligonucleotide donor carrying activating point mutations led to the generation of hepatocytes with nuclear localization of beta-catenin. This study demonstrates the feasibility of direct mutation of tumour suppressor genes and oncogenes in the liver using the CRISPR/Cas system, which presents a new avenue for rapid development of liver cancer models and functional genomics.

Yang, H., et al. (2018). "Break Breast Cancer Addiction by CRISPR/Cas9 Genome Editing." J Cancer **9**(2): 219-231.

Breast cancer is the leading diagnosed cancer for women globally. Evolution of breast cancer in tumorigenesis, metastasis and treatment resistance appears to be driven by the aberrant gene expression and protein degradation encoded by the cancer genomes. The uncontrolled cancer growth relies on these cellular events, thus constituting the cancerous programs and rendering the addiction towards them. These programs are likely the potential anticancer biomarkers for Personalized Medicine of breast cancer. This review intends to delineate the impact of the CRSPR/Cas-mediated genome editing in identification and validation of these anticancer biomarkers. It reviews the progress in three aspects of CRISPR/Cas9-mediated editing of the breast cancer genomes: Somatic genome editing, transcription and protein degradation addictions.

Yao, S., et al. (2015). "CRISPR/Cas9-Mediated Genome Editing of Epigenetic Factors for Cancer Therapy." Hum Gene Ther **26**(7): 463-471.

Advances in engineered recombinant nuclease have provided facile and reliable methods for genome editing. Especially with the development of the CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated protein-9 nuclease) system, the discovery of various versions of Cas9 proteins and delivery carriers, it is now practicable to introduce desired mutations into the genome, to correct disease-related mutations, and to activate or suppress genes of interest. Epigenetic regulators are often disturbed in cancer cells and are essential for the transformation of normal to cancerous cells. Tumor-related epigenetic alterations or epigenetic factor mutations play a major part during

the various steps of carcinogenesis and affect a variety of cancer-related genes and a wide range of cancerous phenotypes. Therefore, epigenetic regulatory enzymes might be candidate targets for cancer therapy. In this review, we discuss prospects of CRISPR/Cas9-based genome editing in targeting epigenetics for cancer gene therapy.

Ye, R., et al. (2017). "CRISPR/Cas9 targeting of GPRC6A suppresses prostate cancer tumorigenesis in a human xenograft model." J Exp Clin Cancer Res **36**(1): 90.

BACKGROUND: GPRC6A is implicated in the pathogenesis of prostate cancer, but its role remains uncertain because of a purported tolerant gene variant created by substitution of a K..Y polymorphism in the 3rd intracellular loop (IL) that evolved in the majority of humans and replaces the ancestral RKLP present in 40% of humans of African descent and all other species. METHODS: We determined whether the K..Y polymorphism is present in human-derived prostate cancer cell lines by sequencing the region of the 3rd IL and assessed the cellular localization of a "humanized" mouse GPRC6A containing the K..Y sequence by immunofluorescence. We assessed functions of GPRC6A in PC-3 cells expressing endogenous GPRC6A and in GPRC6A-deficient PC-3 cells created using CRISPR/Cas9 technology. The effect of GPRC6A on basal and ligand stimulated cell proliferation and migration was evaluated in vitro in wild-type and PC-3-deficient cell lines. The effect of editing GPRC6A on prostate cancer growth and progression in vivo was assessed in a Xenograft mouse model implanted with wild-type and PC-3 deficient cells and treated with the GPRC6A ligand osteocalcin. RESULTS: We found that all of the human prostate cancer cell lines tested endogenously express the "K..Y" polymorphism in the 3rd IL. Comparison of mouse wild-type GPRC6A with a "humanized" mouse GPRC6A construct created by replacing the "RKLP" with the "K..Y" sequence, found that both receptors were predominantly expressed on the cell surface. The transfected "humanized" GPRC6A receptor, however, preferentially activated mTOR compared to ERK signaling in HEK-293 cells. In contrast, in PC-3 cells expressing the endogenous GPRC6A with the "K..Y" polymorphism, the ligand osteocalcin stimulated ERK, AKT and mTOR phosphorylation, promoted cell proliferation and migration, and upregulated genes regulating testosterone biosynthesis. Targeting GPRC6A in PC-3 cells by CRISPR/Cas9 significantly blocked these responses in vitro. In addition, GPRC6A deficient PC-3 xenografts exhibited significantly less growth and were resistant to osteocalcin-induced prostate cancer progression compared to control PC-3 cells expressing GPRC6A. CONCLUSIONS: Human GPRC6A is a functional osteocalcin and testosterone sensing receptor that promotes prostate cancer progression. GPRC6A may contribute to racial disparities in prostate cancer, and is a potential therapeutic target to develop antagonists to treat prostate cancer.

Yi, L. and J. Li (2016). "CRISPR-Cas9 therapeutics in cancer: promising strategies and present challenges." Biochim Biophys Acta **1866**(2): 197-207.

Cancer is characterized by multiple genetic and epigenetic alterations that drive malignant cell proliferation and confer chemoresistance. The ability to correct or ablate such mutations holds immense promise for combating cancer. Recently, because of its high efficiency and accuracy, the CRISPR-Cas9 genome editing technique has been widely used in cancer therapeutic explorations. Several studies used CRISPR-Cas9 to directly target cancer cell genomic DNA in cellular and animal cancer models which have shown therapeutic potential in expanding our anticancer protocols. Moreover, CRISPR-Cas9 can also be employed to fight oncogenic infections, explore anticancer drugs, and engineer immune cells and oncolytic viruses for cancer immunotherapeutic applications. Here, we summarize these preclinical CRISPR-Cas9-based therapeutic strategies against cancer, and discuss the challenges and improvements in translating therapeutic CRISPR-Cas9 into clinical use, which will facilitate better application of this technique in cancer research. Further, we propose potential directions of the CRISPR-Cas9 system in cancer therapy.

Zare, K., et al. (2018). "CRISPR/Cas9 Knockout Strategies to Ablate CCAT1 lncRNA Gene in Cancer Cells." Biol Proced Online **20**: 21.

Background: With the increasing discovery of long noncoding RNAs (lncRNAs), the application of functional techniques that could have very specific, efficient, and robust effects and readouts is necessary. Here, we have applied and analyzed three gene knockout (KO) strategies to ablate the CCAT1 gene in different colorectal adenocarcinoma cell lines. We refer to these strategies as "CRISPR excision", "CRISPR HDR", and "CRISPR du-HITI". Results: In order to obstruct the transcription of lncRNA or to alter its structure, in these strategies either a significant segment of the gene is removed, or a transcription termination signal is inserted in the target gene. We use RT-qPCR, RNA-seq, MTT, and colony formation assay to confirm the functional effects of CCAT1 gene ablation in knockout colorectal adenocarcinoma cell lines. We applied three different CRISPR/Cas9 mediated knockout strategies to abolish the

transcription of CCAT1 lncRNA. CCAT1 knockout cells displayed dysregulation of genes involved in several biological processes, and a significant reduction for anchorage-independent growth. The du-HITI strategy introduced in this study removes a gene segment and inserts a reporter and a transcription termination signal in each of the two target alleles. The preparation of donor vector for this strategy is much easier than that in "CRISPR HDR", and the selection of cells in this strategy is also much more practical than that in "CRISPR excision". In addition, use of this technique in the first attempt of transfection, generates single cell knockouts for both alleles. Conclusions: The strategies applied and introduced in this study can be used for the generation of CCAT1 knockout cell lines and in principle can be applied to the deletion of other lncRNAs for the study of their function.

Zhan, H., et al. (2018). "Synthesizing a Genetic Sensor Based on CRISPR-Cas9 for Specifically Killing p53-Deficient Cancer Cells." ACS Synth Biol **7**(7): 1798-1807.

Cancer is still one of the greatest medical challenges in the world. The p53 protein plays an important role in the process of cancer formation. In addition, p53 is found as the most common mutant gene in cancers. Because of the central role of p53 in oncology, it is necessary to construct effective sensors to detect this protein. However, there are few methods to detect wild type p53 protein (WTP53) or to distinguish the wild type and mutant p53 proteins. In our study, we designed and constructed a p53 genetic sensor that detected the expression of WTP53 in cells. Moreover, we combined the p53 sensor with diphtheria toxin using the CRISPR-Cas9 system to construct a p53 genetic sensor that specifically killed p53-deficient cells such as tumor cells. Our study therefore developed a new way to treat cancers by using an available genetic sensor based on p53 protein.

Zhan, T., et al. (2018). "CRISPR/Cas9 for cancer research and therapy." Semin Cancer Biol.

CRISPR/Cas9 has become a powerful method for making changes to the genome of many organisms. First discovered in bacteria as part of an adaptive immune system, CRISPR/Cas9 and modified versions have found a widespread use to engineer genomes and to activate or to repress the expression of genes. As such, CRISPR/Cas9 promises to accelerate cancer research by providing an efficient technology to dissect mechanisms of tumorigenesis, identify targets for drug development, and possibly arm cells for cell-based therapies. Here, we review current applications of the CRISPR/Cas9 technology for cancer research and therapy. We describe novel Cas9 variants and how they are used in functional genomics to discover novel cancer-specific vulnerabilities. Furthermore, we highlight the impact of CRISPR/Cas9 in generating organoid and mouse models of cancer. Finally, we provide an overview of the first clinical trials that apply CRISPR/Cas9 as a therapeutic approach against cancer.

Zhang, Y. Q., et al. (2018). "CRISPR/Cas9-mediated knockout of the PDEF gene inhibits migration and invasion of human gastric cancer AGS cells." Biomed Pharmacother **111**: 76-85.

Gastric cancer is one of the most common malignant tumors worldwide and has the second highest incidence and mortality rate among malignant tumors in China. Prostate-derived Ets factor (PDEF) is a member of the Ets family of transcription factors. Although PDEF plays an important role in tumorigenesis, its biological function in gastric cancer is still unclear. Here, we evaluated PDEF expression in 30 cases of human gastric carcinoma and the corresponding peritumoral tissues, using immunohistochemistry and immunofluorescence. Significantly higher levels of PDEF were detected in tumors compared to peritumoral tissues. We then investigated PDEF expression in the gastric cancer cell lines SGC and AGS and the normal gastric epithelial cell line GES; The CRISPR/Cas9 genome-editing system was used to knockout PDEF in AGS cells as a model for gastric cancer. Cell proliferation, apoptosis, migration, and invasion of PDEF-knockout AGS cells were evaluated using CCK-8, flow cytometry, scratch wound, and transwell assays, respectively. The results illustrated that PDEF-knockout inhibited AGS cell proliferation, migration, and invasion. Taken together, the results imply that PDEF plays important roles in the proliferation, migration, and invasion of AGS cells and may serve as a new treatment target in gastric cancer.

Zhao, G., et al. (2017). "Lentiviral CRISPR/Cas9 nickase vector mediated BIRC5 editing inhibits epithelial to mesenchymal transition in ovarian cancer cells." Oncotarget **8**(55): 94666-94680.

BIRC5 encodes the protein survivin, a member of the inhibitor of apoptosis family. Survivin is highly expressed in a variety of cancers but has very low expression in the corresponding normal tissues, and its expression is often associated with tumor metastasis and chemoresistance. We report that survivin was highly expressed in ovarian cancer and strongly correlated with patient overall poor survival. For the first time, we provide experimental evidence that survivin is involved in epithelial to mesenchymal transition (EMT) in ovarian cancer cells. Lentiviral CRISPR/Cas9 nickase vector mediated BIRC5 gene editing led to the inhibition of EMT by upregulating

epithelial cell marker, cytokeratin 7 and downregulating mesenchymal markers: snail2, beta-catenin, and vimentin in both ovarian cancer SKOV3 and OVCAR3 cells. Consistent with this molecular approach, pharmacological treatment of ovarian cancer cells using a small molecule survivin inhibitor, YM155 also inhibited EMT in these ovarian cancer cell lines. Overexpression of BIRC5 promoted EMT in SKOV3 cells. Using molecular or pharmacological approaches, we found that cell proliferation, migration, and invasion were significantly inhibited following BIRC5 disruption in both cell lines. Inhibition of BIRC5 expression also sensitized cell responses to paclitaxel treatment. Moreover, loss of BIRC5 expression attenuated TGFbeta signaling in both SKOV3 and OVCAR3 cells. Collectively, our studies demonstrated that disruption of BIRC5 expression inhibited EMT by attenuating the TGFbeta pathway in ovarian cancer cells.

Zhao, Q., et al. (2018). "Role of BMI1 in epithelial ovarian cancer: investigated via the CRISPR/Cas9 system and RNA sequencing." J Ovarian Res **11**(1): 31.

BACKGROUND: B-cell-specific Moloney murine leukemia virus integration site 1 (BMI1) might be an appropriate biomarker in the management of epithelial ovarian cancer (EOC). However, the biological role of BMI1 and its relevant molecular mechanism needs further elaboration. Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system is an excellent genome-editing tool and is scarcely used in EOC studies. METHODS: We first applied CRISPR/Cas9 technique to silence BMI1 in EOC cells; thereafter we accomplished various in vivo and in vitro experiments to detect biological behaviors of ovarian cancer cells, including MTT, flow cytometry, Transwell, real-time polymerase chain reaction and western blotting assays, etc.; eventually, we used RNA sequencing to reveal the underlying molecular traits driven by BMI1 in EOC. RESULTS: We successfully shut off the expression of BMI1 in EOC cells using CRISPR/Cas9 system, providing an ideal cellular model for investigations of target gene. Silencing BMI1 could reduce cell growth and metastasis, promote cell apoptosis, and enhance the platinum sensitivity of EOC cells. BMI1 might alter extracellular matrix structure and angiogenesis of tumor cells through regulating Focal adhesion and PI3K/AKT pathways. CONCLUSION: BMI1 is a potential biomarker in EOC management, especially for tumor progression and chemo-resistance. Molecular traits, including BMI1 and core genes in Focal adhesion and PI3K/AKT pathways, might be alternatives as therapeutic targets for EOC.

Zhao, X., et al. (2018). "A CRISPR-Cas13a system for efficient and specific therapeutic targeting of mutant KRAS for pancreatic cancer treatment." Cancer Lett **431**: 171-181.

Mutant KRAS is a known driver oncogene in pancreatic cancer. However, this protein remains an "undruggable" therapeutic target. Inhibiting mutated KRAS expression at the mRNA level is a potentially effective strategy. Recently, a novel CRISPR-Cas effector, Cas13a has been reported to specifically knock down mRNA expression under the guidance of a single CRISPR-RNA in mammalian cells. Here we demonstrate that the CRISPR-Cas13a system can be engineered for targeted therapy of mutant KRAS in pancreatic cancer. In initial screening, we show that the bacterial Cas13a protein and crRNA significantly knock down mutant KRAS mRNA expression, identifying a CRISPR-Cas13a system that can induce up to a 94% knockdown efficiency. Introducing a single mismatch into the crRNA-target duplex enabled the CRISPR-Cas13a system to specifically recognize KRAS-G12D mRNA with no detectable effects on wild-type KRAS mRNA. More importantly, CRISPR-Cas13a-mediated KRAS-G12D mRNA knockdown potently induced apoptosis in vitro and elicited marked tumor shrinkage in mice. Our work describes an optimization strategy for the development of a CRISPR-Cas13a system to affect efficient and specific knockdown of the oncogenic mRNA, establishing the CRISPR-Cas13a system as a flexible, targeted therapeutic tool.

Zhen, S., et al. (2017). "Inhibition of long non-coding RNA UCA1 by CRISPR/Cas9 attenuated malignant phenotypes of bladder cancer." Oncotarget **8**(6): 9634-9646.

CRISPR/Cas9 is a novel and effective genome editing technique, but its application is not widely expanded to manipulate long non-coding RNA (lncRNA) expression. The lncRNA urothelial carcinoma-associated 1 (UCA1) is upregulated in bladder cancer and promotes the progression of bladder cancer. Here, we design gRNAs specific to UCA1 and construct CRISPR/Cas9 systems targeting UCA1. Single CRISPR/Cas9-UCA1 can effectively inhibit UCA1 expression when transfected into 5637 and T24 bladder cancer cells, while the combined transfection of the two most effective CRISPR/Cas9-UCA1s can generate more satisfied inhibitory effect. CRISPR/Cas9-UCA1s attenuate UCA1 expression via targeted genome-specific DNA cleavage, resulting in the significant inhibition of cell proliferation, migration and invasion in vitro and in vivo. The mechanisms associated with the inhibitory effect of CRISPR/Cas9-UCA1 on malignant phenotypes of

bladder cancer are attributed to the induction of cell cycle arrest at G1 phase, a substantial increase of apoptosis, and an enhanced activity of MMPs. Additionally, urinary UCA1 can be used as a non-invasive diagnostic marker for bladder cancer as revealed by a meta-analysis. Collectively, our data suggest that CRISPR/Cas9 technique can be used to down-modulate lncRNA expression, and urinary UCA1 may be used as a non-invasive marker for diagnosis of bladder cancer.

Zhen, S., et al. (2014). "In vitro and in vivo growth suppression of human papillomavirus 16-positive cervical cancer cells by CRISPR/Cas9." Biochem Biophys Res Commun **450**(4): 1422-1426.

Deregulated expression of high-risk human papillomavirus oncogenes (E6 and E7) is a pivotal event for pathogenesis and progression in cervical cancer. Both viral oncogenes are therefore regarded as ideal therapeutic targets. In the hope of developing a gene-specific therapy for HPV-related cancer, we established CRISPR/Cas9 targeting promoter of HPV 16 E6/E7 and targeting E6, E7 transcript, transduced the CRISPR/Cas9 into cervical HPV-16-positive cell line SiHa. The results showed that CRISPR/Cas9 targeting promoter, as well as targeting E6 and E7 resulted in accumulation of p53 and p21 protein, and consequently remarkably reduced the abilities of proliferation of cervical cancer cells in vitro. Then we inoculated subcutaneously cells into nude mice to establish the transplanted tumor animal models, and found dramatically inhibited tumorigenesis and growth of mice incubated by cells with CRISPR/Cas9 targeting (promoter+E6+E7)-transcript. Our results may provide evidence for application of CRISPR/Cas9 targeting HR-HPV key oncogenes, as a new treatment strategy, in cervical and other HPV-associated cancer therapy.

Zhen, S. and X. Li (2017). "Oncogenic Human Papillomavirus: Application of CRISPR/Cas9 Therapeutic Strategies for Cervical Cancer." Cell Physiol Biochem **44**(6): 2455-2466.

Oncogenic human papillomaviruses (HPVs) cause different types of cancer especially cervical cancer. HPV-associated carcinogenesis provides a classical model system for clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) based cancer therapies since the viral oncogenes E6 and E7 are exclusively expressed in cancerous cells. Sequence-specific gene knockdown/knockout using CRISPR/Cas9 shows promise as a novel therapeutic approach for the treatment of a variety of diseases that currently lack effective treatments. However, CRISPR/Cas9-based targeting therapy requires further validation of its efficacy in vitro and in vivo to eliminate the potential off-target effects, necessitates verification of the delivery vehicles and the combinatory use of conventional therapies with CRISPR/Cas9 to ensure the feasibility and safety. In this review we discuss the potential of combining CRISPR/Cas9 with other treatment options as therapies for oncogenic HPVs-associated carcinogenesis. and present our assessment of the promising path to the development of CRISPR/Cas9 therapeutic strategies for clinical settings.

Zhen, S. and X. Li (2018). "Application of CRISPR-Cas9 for Long Noncoding RNA Genes in Cancer Research." Hum Gene Ther.

Long noncoding RNAs (LncRNA), a class of transcripts with lengths >200 nt, play a master role in the regulation of cancer pathogenesis. Recently, the CRISPR-Cas9 system has been explored as a revolutionary genome editing tool for molecular biology. Growing evidence shows that LncRNAs can be targeted by the CRISPR-Cas9 system used for evaluating its function. Thus, the CRISPR-Cas9 systems provide a novel gene-editing strategy for the modification of LncRNA expression. This review summarizes current knowledge of the functions and underlying mechanisms of LncRNA by CRISPR-Cas9. Emerging strategies for non-viral/viral delivery of CRISPR-Cas9 in a clinical context are also discussed.

Zhen, S., et al. (2018). "Synergistic Antitumor Effect on Bladder Cancer by Rational Combination of Programmed Cell Death 1 Blockade and CRISPR-Cas9-Mediated Long Non-Coding RNA Urothelial Carcinoma Associated 1 Knockout." Hum Gene Ther **29**(12): 1352-1363.

Targeted therapy produces objective responses in bladder cancer patients, although the responses can be short. Meanwhile, response rates to immune therapy are lower, but the effects are more durable. Based on these findings, it was hypothesized that urothelial carcinoma associated 1 (UCA1)-targeted therapy could synergize with programmed cell death 1 (PD-1) blockade to enhance antitumor activity. To test this hypothesis, the effects of CRISPR-Cas9 targeting of UCA1 and PD-1 were assessed in vitro and in vivo. It was found that gRNA/cas9-targeted UCA1 induced apoptosis of 5637 bladder cancer cells, whereas PD-1 gene knockout could be achieved by electroporation of gRNA/cas9 targeting PD-1, as detected by polymerase chain reaction. In 5637 cell-xenografted humanized SCID mice, stimulation with CRISPR-Cas9 systems, immune phenotypes, and cytokine expression of human dendritic cells (DCs) was detected by flow cytometry, and polymerase chain reaction, respectively. The results of these assays suggested that the gRNA/cas9 treatment upregulated expression of

CD80, CD83, and CD86 and significantly increased interleukin (IL)-6, IL-12, and IL-23 and tumor necrosis factor alpha mRNA levels. Co-administration of anti-PD-1 and anti-UCA1 treatment suppressed tumor growth and markedly improved survival of 5637 xenografted mice. Additionally, the combination treatment increased interferon gamma production by T cells that subsequently enhanced the expression of Th1-associated immune-stimulating genes to reduce transcription of regulatory/suppressive immune genes and reshape the tumor microenvironment from an immunosuppressive to a stimulatory state. Finally, anti-UCA1 treatment was shown to induce interferon gamma-dependent programmed cell death ligand 1 expression within 5637 xenograft tumors in vivo. Together, these results demonstrate potent synergistic effects of a combination therapy using LncRNA UCA1-targeted therapy and immune checkpoint blockade of PD-1, thus supporting the translational potential of this combination strategy for clinical treatment of bladder cancer.

Zhen, S., et al. (2018). "WITHDRAWN: CRISPR/Cas9 mediated HPV and PD1 inhibition produces a synergistic anti-tumor effect on cervical cancer." Arch Biochem Biophys.

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Zhen, S., et al. (2016). "In Vitro and In Vivo Synergistic Therapeutic Effect of Cisplatin with Human Papillomavirus16 E6/E7 CRISPR/Cas9 on Cervical Cancer Cell Line." Transl Oncol **9**(6): 498-504.

PURPOSE: Human papillomavirus (HPV) type 16 is one of the major etiologic factors of cervical cancer. Our study aims to investigate the potentiality of the antiviral clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated Cas9 system (CRISPR/Cas9) targeting the E6 and E7 oncogenes of HPV16 as a potential chemosensitizer of cisplatin (cis-diaminedichloroplatinum II; CDDP) for cervical cancer. METHODS: Specifically, the therapeutic efficacy of combination of CDDP and HPV16 E6 + E7-CRISPR/Cas9 was assessed in cervical cancer cells and cervical cancer xenograft models. RESULTS: In vitro experiments showed that long-term exposure of SiHa cells to the HPV16 E6 + E7-CRISPR/Cas9 induced apoptosis, and its pro-apoptosis effect became more obvious when combined with CDDP. In vivo study found the efficacy of the combination of HPV16 E6 + E7-CRISPR/Cas9 and CDDP were superior to either of the treatments in term of apoptosis induction and metastasis inhibition. CONCLUSION: Collectively, our results suggested that HPV16 E6 + E7-CRISPR/Cas9 could be an effective sensitizer of CDDP chemotherapy in cervical cancer.

Zhen, S., et al. (2017). "Targeted delivery of CRISPR/Cas9 to prostate cancer by modified gRNA using a flexible aptamer-cationic liposome." Oncotarget **8**(6): 9375-9387.

The potent ability of CRISPR/Cas9 system to inhibit the expression of targeted gene is being exploited as a new class of therapeutics for a variety of diseases. However, the efficient and safe delivery of CRISPR/Cas9 into specific cell populations is still the principal challenge in the clinical development of CRISPR/Cas9 therapeutics. In this study, a flexible aptamer-liposome-CRISPR/Cas9 chimera was designed to combine efficient delivery and increased flexibility. Our chimera incorporated an RNA aptamer that specifically binds prostate cancer cells expressing the prostate-specific membrane antigen as a ligand. Cationic liposomes were linked to aptamers by the post-insertion method and were used to deliver therapeutic CRISPR/Cas9 that target the survival gene, polo-like kinase 1, in tumor cells. We demonstrate that the aptamer-liposome-CRISPR/Cas9 chimeras had a significant cell-type binding specificity and a remarkable gene silencing effect in vitro. Furthermore, silencing promoted a conspicuous regression of prostate cancer in vivo. Importantly, the approach described here provides a universal means of cell type-specific CRISPR/Cas9 delivery, which is a critical goal for the widespread therapeutic applicability of CRISPR/Cas9 or other nucleic acid drugs.

Zhigalova, N. A., et al. (2017). "[CRISPR/Cas9-editing-based modeling of hypoxia in renal cancer cells]." Mol Biol (Mosk) **51**(5): 836-840.

Uncontrolled growth in the cell mass of malignant tumors induces intensive angiogenesis. However, the demands of the cancer cells for nutrients and oxygen remain only partially met. Hypoxia is a process that accompanies malignant transformation and evokes changes in the DNA methylation profile in solid tumors. To a certain extent, these changes, including the hypermethylation of tumor suppressor gene promoters, are related to the decrease in the activity of Tet proteins under the conditions of oxygen and free radical deficit. Stabilization, accumulation, and nuclear translocation of the transcription factor HIF1alpha are the key molecular events in hypoxia. We modified the clear-cell renal cancer cell line Caki1 to stabilize the HIF1alpha protein and characterized a model cell line that will enable the studies of the

mechanisms of changes of the DNA methylation level at a constant activity of Tet proteins and a gene transcription profile characteristic of hypoxia. The CRISPR/Cas9 DNA editing system was used to edit the VHL gene. The mutant VHL protein contained a disrupted alpha-helix at the C-terminus and could not participate in the molecular pathway of proteasomal degradation of the HIF1alpha factor; therefore, the latter accumulated in the nucleus and activated the specific target genes. An analysis of gene transcription revealed the induction of hypoxia-associated genes in the modified cell line. The developed capital ES, Cyrillicaki-1/VHLmut model can be used to discriminate between the effects evoked by oxygen-suppressed hydroxylases of the Tet family and other hypoxia-associated mechanisms of DNA methylation/demethylation.

Zuckermann, M., et al. (2017). "Applications of the CRISPR/Cas9 system in murine cancer modeling." Brief Funct Genomics **16**(1): 25-33.

Advanced biological technologies allowing for genetic manipulation of the genome are increasingly being used to unravel the molecular pathogenesis of human diseases. The clustered regulatory interspaced short palindromic repeat/CRISPR-associated protein (CRISPR/Cas) technology started a revolution of this field owing to its flexibility and relative ease of use. Recently, application of the CRISPR/Cas9 system has been extended to in vivo approaches, leveraging its potential for human disease modeling. Particularly in oncological research, where genetic defects in somatic cells are tightly linked to etiology and pathological phenotypes, the CRISPR/Cas technology is being used to recapitulate various types of genetic aberrations. Here we review murine cancer models that have been developed via combining the CRISPR/Cas9 technology with in vivo somatic gene transfer approaches. Exploiting these methodological advances will further accelerate detailed investigations of tumor etiology and treatment.

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