Genome Editing using CRISPR/Cas9 and Cancer Research Literatures

Ma Hongbao, PhD

Brookdale University Hospital and Medical Center, Brooklyn, New York 11212, USA, ma8080@gmail.com

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. CRISPR is a family of DNA sequences in bacteria. The sequences contain snippets of DNA from viruses that have attacked the bacterium. These snippets are used by the bacterium to detect and destroy DNA from similar viruses during subsequent attacks. These sequences play a key role in a bacterial defense system, and form the basis of a technology known as CRISPR/Cas9 that effectively and specifically changes genes within organisms.

Key words: cancer; life; research; literature; cell; CRISPR/Cas9; genome editing

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.

CRISPR is a family of DNA sequences in bacteria. The sequences contain snippets of DNA from viruses that have attacked the bacterium. These snippets are used by the bacterium to detect and destroy DNA from similar viruses during subsequent attacks. These sequences play a key role in a bacterial defense system, and form the basis of a technology known as CRISPR/Cas9 that effectively and specifically changes genes within organisms.

The CRISPR/Cas system is a prokaryotic immune system that confers resistance to foreign genetic elements such as those present within plasmids and phages that provides a form of acquired immunity. RNA harboring the spacer sequence helps Cas (CRISPR-associated) proteins recognize and cut exogenous DNA. Other RNA-guided Cas proteins cut foreign RNA. CRISPRs are found in approximately 40% of sequenced bacterial genomes and 90% of sequenced archaea.

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

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.

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.

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.

Chen, S., et al. (2016). "CRISPR-Cas9: from Genome Editing to Cancer Research." Int J Biol Sci **12**(12): 1427-1436.

Cancer development is a multistep process triggered by innate and acquired mutations, which cause the functional abnormality and determine the initiation and progression of tumorigenesis. Gene editing is a widely used engineering tool for generating mutations that enhance tumorigenesis. The recent developed clustered regularly interspaced short palindromic repeats-CRISPR-associated 9 (CRISPR-Cas9) system renews the genome editing approach into a more convenient and efficient way. By rapidly introducing genetic modifications in cell lines, organs and animals, CRISPR-Cas9 system extends the gene editing into whole genome screening, both in loss-of-function and gain-of-function manners. Meanwhile, the system accelerates the establishment of animal cancer models, promoting in vivo studies for cancer research. Furthermore, CRISPR-Cas9 system is modified into diverse innovative tools for observing the dynamic bioprocesses in cancer studies, such as image tracing for targeted DNA, regulation of transcription activation or repression. Here, we view recent technical advances in the application of CRISPR-Cas9 system in cancer genetics, large-scale cancer driver gene hunting, animal cancer modeling and functional studies.

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.

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.

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.

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.

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.

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, 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.

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.

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.

Lin, A., et al. (2017). "CRISPR/Cas9 mutagenesis invalidates a putative cancer dependency targeted in on-going clinical trials." Elife **6**.

The Maternal Embryonic Leucine Zipper Kinase (MELK) has been reported to be a genetic dependency in several cancer types. MELK RNAi and small-molecule inhibitors of MELK block the proliferation of various cancer cell lines, and MELK knockdown has been described as particularly effective against the highly-aggressive basal/triple-negative subtype of breast cancer. Based on these preclinical results, the MELK inhibitor OTS167 is currently being tested as a novel chemotherapy agent in several clinical trials. Here, we report that mutagenizing MELK with

CRISPR/Cas9 has no effect on the fitness of basal breast cancer cell lines or cell lines from six other cancer types. Cells that harbor null mutations in MELK exhibit wild-type doubling times, cytokinesis, and anchorage-independent growth. Furthermore, MELK-knockout lines remain sensitive to OTS167, suggesting that this drug blocks cell division through an off-target mechanism. In total, our results undermine the rationale for a series of current clinical trials and provide an experimental approach for the use of CRISPR/Cas9 in preclinical target validation that can be broadly applied.

Liu, Y., et al. (2014). "Synthesizing AND gate genetic circuits based on CRISPR-Cas9 for identification of bladder cancer cells." Nat Commun **5**: 5393.

The conventional strategy for cancer gene therapy offers limited control of specificity and efficacy. A possible way to overcome these limitations is to construct logic circuits. Here we present modular AND gate circuits based on CRISPR-Cas9 system. The circuits integrate cellular information from two promoters as inputs and activate the output gene only when both inputs are active in the tested cell lines. Using the luciferase reporter as the output gene, we show that the circuit specifically detects bladder cancer cells and significantly enhances luciferase expression in comparison to the human telomerase reverse transcriptase-renilla luciferase construct. We also test the modularity of the design by replacing the output with other cellular functional genes including hBAX, p21 and E-cadherin. The circuits effectively inhibit bladder cancer cell growth, induce apoptosis and decrease cell motility by regulating the corresponding gene. This approach provides a synthetic biology platform for targeting and controlling bladder cancer cells in vitro.

Lu, X. J., et al. (2015). "Modeling cancer processes with CRISPR-Cas9." Trends Biotechnol **33**(6): 317-319.

CRISPR-Cas9 is an RNA-guided site-specific DNA editing tool which, together with its reprogrammed versions such as nickase Cas9 and dead Cas9, enables quick modeling of desired combinations of cancer-associated genomic and/or epigenetic aberrations simultaneously or sequentially, thus facilitating massive functional interrogations and therapy testing.

Luo, J. (2016). "CRISPR/Cas9: From Genome Engineering to Cancer Drug Discovery." Trends Cancer **2**(6): 313-324.

Advances in translational research are often driven by new technologies. The advent of microarrays, next-generation sequencing, proteomics and RNA interference (RNAi) have led to breakthroughs in our understanding of the mechanisms of cancer and the discovery of new cancer drug targets. The discovery of the bacterial clustered regularly interspaced palindromic repeat (CRISPR) system and its subsequent adaptation as a tool for mammalian genome engineering has opened up new avenues for functional genomics studies. This review will focus on the utility of CRISPR in the context of cancer drug target discovery.

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.

Matano, M., et al. (2015). "Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids." Nat Med **21**(3): 256-262.

Human colorectal tumors bear recurrent mutations in genes encoding proteins operative in the WNT, MAPK, TGF-beta, TP53 and PI3K pathways. Although these pathways influence intestinal stem cell niche signaling, the extent to which mutations in these pathways contribute to human colorectal carcinogenesis remains unclear. Here we use the CRISPR-Cas9 genome-editing system to introduce multiple such mutations into organoids derived from normal human intestinal epithelium. By modulating the culture conditions to mimic that of the intestinal niche, we selected isogenic organoids harboring mutations in the tumor suppressor genes APC, SMAD4 and TP53, and in the oncogenes KRAS and/or PIK3CA. Organoids engineered to express all five mutations grew independently of niche factors in vitro, and they formed tumors after implantation under the kidney subcapsule in mice. Although they formed micrometastases containing dormant tumor-initiating cells after injection into the spleen of mice, they failed to colonize in the liver. In contrast, engineered organoids derived from chromosome-instable human adenomas formed macrometastatic colonies. These results suggest that 'driver' pathway mutations enable stem cell maintenance in the hostile tumor microenvironment, but that additional molecular lesions are required for invasive behavior.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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., 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.

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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