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Cancer Biology

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Cancer Biology Research Literatures (3)

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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

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. This article introduces recent research reports as references in the related studies.

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

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.

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.

Chen, Y. and Y. Zhang (2018). "Application of the CRISPR/Cas9 System to Drug Resistance in Breast Cancer." Adv Sci (Weinh) **5**(6): 1700964.

Clinical evidence indicates that drug resistance is a great obstacle in breast cancer therapy. It renders the disease uncontrollable and causes high mortality. Multiple mechanisms contribute to the development of drug resistance, but the underlying cause is usually a shift in the genetic composition of tumor cells. It is increasingly feasible to engineer the genome with the clustered regularly interspaced short palindromic repeats (CRISPR)/associated (Cas)9 technology recently developed, which might be advantageous in overcoming drug resistance. This article discusses how the CRISPR/Cas9 system might revert resistance gene mutations and identify potential resistance targets in drug-resistant breast cancer. In addition, the challenges that impede the clinical applicability of this technology and highlight the CRISPR/Cas9 systems are presented. The CRISPR/Cas9 system is poised to play an important role in preventing drug resistance in breast cancer therapy and will become an essential tool for personalized medicine.

Chen, Z., et al. (2017). "Targeted Delivery of CRISPR/Cas9-Mediated Cancer Gene Therapy via Liposome-Templated Hydrogel Nanoparticles." Adv Funct Mater **27**(46).

Due to its simplicity, versatility, and high efficiency, the clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 technology has emerged as one of the most promising approaches for treatment of a variety of genetic diseases, including human cancers. However, further translation of CRISPR/Cas9 for cancer gene therapy requires development of safe approaches for efficient, highly specific delivery of both Cas9 and single guide RNA to tumors. Here, novel core-shell nanostructure, liposome-templated hydrogel nanoparticles (LHNPs) that are optimized for efficient codelivery of Cas9 protein and nucleic acids is reported. It is demonstrated that, when coupled with the minicircle DNA technology, LHNPs deliver CRISPR/Cas9 with efficiency greater than commercial agent Lipofectamine 2000 in cell culture and can be engineered for targeted inhibition of genes in tumors, including tumors the brain. When CRISPR/Cas9 targeting a model therapeutic gene, polo-like kinase 1 (PLK1), is delivered, LHNPs effectively inhibit tumor growth and improve tumor-bearing mouse survival. The results suggest LHNPs as versatile CRISPR/Cas9-delivery tool that can be adapted for experimentally studying the biology of cancer as well as for clinically translating cancer gene therapy.

Cheung, A. H., et al. (2018). "Specific targeting of point mutations in EGFR L858R-positive lung cancer by CRISPR/Cas9." Lab Invest **98**(7): 968-976.

Cancer cells are defined genetically by the mutations they harbor, commonly single nucleotide substitutions. Therapeutic approaches which specifically target cancer cells by recognizing these defining genetic aberrations are expected to exhibit minimal side-effects. However, current protein-based targeted therapy is greatly limited by the range of genes that can be targeted, as well as by acquired resistance. We hypothesized that a therapeutic oligonucleotide-based strategy may address this need of specific cancer targeting. We used CRISPR/Cas9 system to target a commonly occurring EGFR point mutation, L858R, with an oligonucleotide guide that recognizes L858R as the suitable protospacer-adjacent motif (PAM) sequence for DNA cleavage. We found that this strategy, which utilized PAM to differentiate cancer mutation from normal, afforded high specificity to the extent of a single nucleotide substitution. The anti-L858R vehicle resulted in selective genome cleavage only in L858R mutant cells, as detected by Sanger sequencing and T7 Endonuclease I assay. Wild-type cells were unaffected by the same treatment. Digital PCR revealed 37.9 +/- 8.57% of L858R gene copies were targeted in mutant. Only treated mutant cells, but not wild-type cells, showed reduction in EGFR expression and decreased cell proliferation. Treated mutant cells also formed smaller tumor load in vivo. This targeting approach is expected to be able to target a significant subset of the 15-35% cancer mutations with C > G, A > G, and T > G point mutations. Thus, this strategy may serve as a useful approach to target cancer-defining mutations with specificity, to the extent of differentiating the change of a single nucleotide.

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.

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.

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.

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.

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.

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.

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.

Lee, W., et al. (2018). "Selective targeting of KRAS oncogenic alleles by CRISPR/Cas9 inhibits proliferation of cancer cells." Sci Rep **8**(1): 11879.

Mutations within the KRAS oncogene are associated with the proliferation of various cancers. Therapeutic approaches for treating cancers with such mutations have focused on targeting the downstream protein effectors of KRAS. However, to date, no approved treatment has targeted the mutated KRAS oncogene directly. Presently, we used the selectivity of the CRISPR/Cas9 system to directly target mutated KRAS alleles. We designed single-guide RNAs (sgRNAs) to target two specific single-nucleotide missense mutations on KRAS codon-12 located in the

seed region adjacent to a protospacer adjacent motif (PAM). Lentiviral transduction of Cas9 and the sgRNAs into cancer cells with respective KRAS mutations resulted in high frequency of indels in the seed region. Indel-associated disruption of the mutant KRAS alleles correlated with reduced viability of the cancer cells. The results indicate that CRISPR-Cas9-mediated genome editing can potentially be used for the treatment of cancer patients, specifically those with oncogenic KRAS mutations.

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.

Ling, K., et al. (2018). "Nanog interaction with the androgen receptor signaling axis induce ovarian cancer stem cell regulation: studies based on the CRISPR/Cas9 system." J Ovarian Res **11**(1): 36.

BACKGROUND: Ovarian cancer stem cells (OCSCs) contribute to the poor prognosis of ovarian cancer. Involvement of the androgen receptor (AR) in the malignant behaviors of other tumors has been reported. However, whether AR associates with Nanog (a stem cell marker) and participates in OCSC functions remain unclear. In this study, we investigated the interaction of Nanog with AR and examined whether this interaction induced stem-like properties in ovarian cancer cells. METHODS: AR and Nanog expression in ovarian tumors was evaluated. Using the CRISPR/Cas9 system, we constructed a Nanog green fluorescent protein (GFP) marker cell model to investigate the expression and co-localization of Nanog and AR. Then, we examined the effect of androgen on the Nanog promoter in ovarian cancer cell lines (A2780 and SKOV3). After androgen or anti-androgen treatment, cell proliferation, migration, sphere formation, colony formation and tumorigenesis were assessed in vitro and in vivo. RESULTS: Both AR and Nanog expression were obviously high in ovarian tumors. Our results showed that Nanog expression was correlated with AR expression. The androgen 5alpha-dihydrotestosterone (DHT) activated Nanog promoter transcription. Meanwhile, Nanog GFP-positive cells treated with DHT exhibited higher levels of proliferation, migration, sphere formation and colony formation. We also observed that the tumorigenesis of Nanog GFP-positive cells was significantly higher than that of the GFP-negative cells. Xenografts of Nanog GFP-positive cells showed significant differences when treated with androgen or anti-androgen drugs in vivo. CONCLUSIONS: The interaction of Nanog with the AR signaling axis might induce or contribute to OCSC regulation. In addition, androgen might promote stemness characteristics in ovarian cancer cells by activating the Nanog promoter. This finding merits further study because it may provide a new understanding of OCSC regulation from a hormone perspective and lead to the reevaluation of stem cell therapy for ovarian cancer.

Liu, X. and Y. Zhao (2018). "CRISPR/Cas9 genome editing: Fueling the revolution in cancer immunotherapy." Curr Res Transl Med **66**(2): 39-42.

The development of genomic editing technologies expands the landscape of T cell engineering for adoptive cell therapy. Among the multiple tools that can be used, CRISPR/Cas9 has been shown to be relatively easy to use, simple to design and cost effective with highly efficient multiplex genome engineering capabilities. Allogeneic universal chimeric antigen receptor (CAR) T cells can be produced by disrupting T cell receptor (TCR) and beta-2-microglobulin (B2M) in CAR T cells or by directly knocking in a CAR at the disrupted TRAC locus. The anti-tumor function can be further boosted by simultaneous ablation of PD-1 and CTLA-4. The anti-tumor activities and safety of TCR-transferred T cells can be improved by knocking out endogenous TCR, which avoids the use of affinity-enhanced TCRs that may lose specificity and cause severe adverse effects. Therefore, CRISPR/Cas9 technology holds enormous promise to advance the field of adoptive cell therapy.

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.

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.

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.

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.

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.

Norouzi-Barough, L., et al. (2018). "CRISPR/Cas9, a new approach to successful knockdown of ABCB1/P-glycoprotein and reversal of chemosensitivity in human epithelial ovarian cancer cell line." Iran J Basic Med Sci **21**(2): 181-187.

Objectives: Multidrug resistance (MDR) is a major obstacle in the successful chemotherapy of ovarian cancer. Inhibition of P-glycoprotein (P-gp), a member of ATP-binding cassette (ABC) transporters, is a well-known strategy to overcome MDR in cancer.

The aim of this study was to investigate the efficiency and ability of CRISPR/Cas9 genome editing technology to knockdown ABCB1 gene expression in adriamycin resistant (A2780/ADR) ovarian cancer cell line and evaluate the sensitivity changes to doxorubicin. Materials and Methods: Three single-guide RNAs (sgRNAs) targeting the fourth and fifth exons of human ABCB1 gene were designed in this study. Expression level of ABCB1 was detected using quantitative real time PCR (qRT-PCR) after co-transfection of all three sgRNAs into A2780/ADR cell line and subsequent antibiotic selection. Drug sensitivity to doxorubicin was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Results: The results showed that CRISPR/Cas9 system could significantly reduce the expression of P-gp. The dramatic decline in ABCB1 gene expression was associated with increased sensitivity of cells transfected with sgRNAs to doxorubicin. Conclusion: Based on the results of this study, it is concluded that the CRISPR-based systems, used in the present study, effectively down-regulated the target gene and acted as an ideal and cost-effective tool for gene editing of A2780/ADR cell line resulting in restoration of nonmalignant phenotype.

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.

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.

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.

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.

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.

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.

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.

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, 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.

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.

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.

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.

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 non-coding RNA genes in Cancer Research." Hum Gene Ther.

Long non-coding RNAs (LncRNA), a class of transcripts with lengths>200nt, 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 evidences show that LncRNAs can be targeted by the CRISPR/Cas9 system used for evaluation the function. Thus, the CRISPR/Cas9 systems provide a novel gene-editing strategy for the modification of LncRNA expression. In this review, we summarize current knowledge of the functions and underlying mechanisms of LncRNA by CRISPR/Cas9. We also discuss emerging strategies for non-viral/viral delivery of CRISPR/Cas9 in a clinical context.

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

Targeted therapy results in objective responses in cervical cancer. However, the responses are short. In contrast, treatment with immune checkpoint inhibitors results in a lower responses rate, but the responses tend to be more durable. Based on these findings, we hypothesized that HPV16 E6/E7-targeted therapy may synergize with the PD-1 pathway blockade to enhance antitumor activity. To test the hypothesis, we described the effects of CRISPR/Cas9 which targeted to the HPV and PD1 in vitro and in vivo. Our data showed that gRNA/cas9 targeted HPV16 E6/E7 induced cervical cancer cell SiHa apoptosis, and suggested that overexpression of PD-L1, induced by HPV16 E6/E7, may be responsible for lymphocyte dysfunction. In established SiHa cell-xenografted humanized SCID mice, Administration of gRNA-PD-1 together with gRNA-HPV16 E6/E7 treatment improved the survival and suppressed the tumor growth obviously. Additionally, combination treatment increased the population of dendritic cells, CD8(+) and CD4(+) T lymphocyte cells. Accordingly, it enhanced the expression of Th1-associated immune-stimulating genes while reducing the transcription of regulatory/suppressive immune genes, reshaping tumor microenvironment from an immunosuppressive to a stimulatory state. These results demonstrate potent synergistic effects of combination therapy using HPV16 E6/E7-targeted therapy and immune checkpoint blockade PD1, supporting a direct translation of this combination strategy in clinic for the treatment of cervical cancer.

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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