Precision Engineering in Oncology: Advances and Applications of CRISPR Technology in Cancer Modeling Using Murine Model

Abstract

The innovative CRISPR-Cas9 system, which was awarded the Nobel Prize in Chemistry in 2020, has altered genetic manipulation, allowing researchers to better understand human illnesses. The CRISPR/Cas system, or "genetic scissors," developed by Nobel laureates Emmanuelle Charpentier and Jennifer Doudna, enables flexible and straightforward genome editing. CRISPR/Cas9 has been widely used in cancer research, but it has now been extended to in vivo techniques, improving human disease modeling. Despite advances in cancer therapy, current medications have substantial toxicity and low success rates.Cancer researchers get insight into intricate tumor biology within dynamic physiological systems by using transgenic mice models. Because of the complexities of the cancer genome, which includes multiple mutations, translocations, and chromosomal changes, exact models are required for thorough knowledge. CRISPR-Cas9 and its variations are RNA-guided nucleases that provide diverse and user-friendly platforms for site-specific genome editing, revolutionising gene editing by imitating genetic processes in human cancer cells.CRISPR high throughput genetic screening and barcoding uncover genes associated with treatment resistance, metastasis, and carcinogenesis, allowing the monitoring and research of cancer cell adaptations. This review focuses on how CRISPR-Cas9 has been used to create precise germline and somatic mice models, allowing researchers to better understand the evolution and course of individual tumours. The successes and pitfalls of these techniques are discussed, emphasising their promise for improving functional cancer genomics and altering the landscape of precision cancer therapy. Future CRISPR breakthroughs promise more precise genome editing and complex cancer models, which will help understand tumor progression and design successful therapies

Keywords: CRISPR-Cas9 , In vivo Modeling, Transgenic Mice Models,Precision Cancer Therapy ,High Throughput Genetic Screening,Ethical considerations , Tumor Evolution,Carcinogenesis Pathways,CRISPR Barcoding,Cancer research,

A Discovery Timeline of CRISPR Cas9 System

CRISPR, an acronym for clustered regularly interspaced short palindromic repeats, was discovered in the DNA sequences of Escherichia coli bacterium in 1987 [Ishino et al.,1987] at Osaka University in Japan. Despite the difficulty of sequencing these DNA snippets at the time, the origin and importance within the bacterial cell were not immediately appreciated by the researchers. Although the biological role of the CRISPR system was unknown in the early stages of study, scientists proposed using the information encoded in CRISPR loci to genotype various bacterial strains. This application was first investigated in Mycobacterium TB [Groenen et al.,1993] and then in Streptococcus pyogenes [Hoe et al.,1999].In 1995, Francisco Mojica of the University of Alicante, Spain, identified comparable structures in the archaeal genome of Haloferax mediterranei, which was important in understanding the biological role of CRISPR loci [Mojica et al.,1993]. The occurrence of these components across evolutionarily distant areas of life hinted to their significant functional significance, prompting additional investigation. Mojica noticed similarities between the

new archaeal components and previously identified DNA repeats in bacterial genomes. [144, 145] He was among the first to postulate that these unique loci are made up of foreign DNA pieces and are part of bacteria and archaea's immune systems [Mojica et al.,2005]. In the same year, two additional independent laboratories found identical conclusions, kicking off active investigation into this extraordinary natural phenomenon [Bolotin et al.,2005; Pourcel et al.,2005].In accordance with the notion of the prokaryotic immune system, clusters of viral DNA fragments known as "spacers," ranging from 17 to 84 bases in length and separated by short palindromic repeats (23-50 bases) [Popkov et al.,2016], are located in intergenic areas. This arrangement creates a library of potentially dangerous genetic information, forming a microbial antiviral arsenal [Isaey et al.,2021:Isaey et al.,2021]. Initially, it was assumed that the system worked by RNA interference. However, Marraffini and Sontheimer empirically established for the first time that the prokaryotic immune system's actual target is foreign DNA rather than mRNA. This finding showed that such a technology may be used in the laboratory for genome editing [Marraffini et al.,2008]. Subsequent research indicated that certain CRISPR systems do interact directly with RNA molecules [Shmakov et al.,2015: ,Shmakovet al .,2017], allowing selective deactivation of particular transcripts in cell [Abudayyeh et al.,2016: Abudayyeh et al.,2017].The collaborative research of two French food scientists, Rodolphe Barrangou and Philippe Horvath, yielded the first insights into the operational mechanism of the CRISPR system in 2007. Their research using Streptococcus thermophilus bacteria in yoghurt for the Danish corporation Danisco was a watershed moment [Barrangou et al.,2007]. Using the company's enormous bacterial strain collection amassed since the 1980s, the researchers followed the historical history of bacterial spacer acquisition at the CRISPR locus in response to viral attacks by bacteriophages. The inclusion of new spacers during their research provided acquired immunity to novel types of bacteriophages in S. thermophilus. This observation resulted in the authors obtaining one of the first CRISPR patents [Horvath et al.,2017]. Danisco began vaccination of bacterial strains in 2005 using CRISPR [Isaacson et al.,2021].CRISPR repetitions are found in approximately half of the analysed bacterial genomes but not in eukaryotic or viral DNA sequences. The existence of CRISPR repeats in mitochondria was postulated in early studies that also introduced CRISPR in cyanobacteria [Masepohl et al.,1996]. This idea was based on the sequencing of mitochondrial plasmids from Vicia faba L. beans [Flamand et al.,1992], with Mojica et al. citing these findings [Mojica et al.,2000]. However, further research has failed to validate these findings [Popkov et al.,2016].

During the original discoveries, different scientific groups used multiple acronyms for CRISPR, hindering the retrieval of early articles. Jansen et al. officially coined the word "CRISPR" in 2002, and Mojica recommended it in correspondence amongst the collaborating scientific groups [Mojica et al .,2000].The discovery of this distinctive adaptive immune defence system altered the modelling of many biological processes and earned the Nobel Prize in 2020.

Need for Precision Mouse Cancer Models

Cancer is the second leading cause of death worldwide behind cardiovascular disease (GBD 2015).Despite significant advances in cancer surgery, physical interventions have been demonstrated to enhance the likelihood of metastatic recurrences by encouraging cancer cell dissemination [Yamaguchi et al .,2000]. These therapies also increase pro-inflammatory mediators, angiogenic factors [Van Der Bij et al., 2009:,Oh et al.,2014], and cancer-dependent adhesion molecules. As a result, while many treatments strive to improve tumour control specificity, genome editing technology has lately emerged as a feasible option to other, more invasive procedures such as surgeries, chemotherapies, and immunotherapies. Prior to genetic mouse models, cancer research

depended on human tumour cell lines cultured in culture systems. Despite providing useful information, these systems limit the study of physiological tumour relationships. Many research use immunocompromised mice or subcutaneous implantation, which impedes immune responses and site-

specific interactions. Ideal mice systems develop tumours that are genetically and morphologically similar to human equivalents. The use of mice models is critical for investigating tumour start, development, progression, and treatment response.Cancer biologists now have several genetic manipulation strategies at their disposal. Choosing the proper technique for developing mouse cancer models is a critical first step, as is specifying the intended aims of individual models [Jansen et al ., 2002: ,Knudson et al.,2001].Cell and animal models are critical for improving our understanding of tumour biology [Hanahan et al .,2011] and serve as powerful preclinical platforms for evaluating novel drugs [Tuveson et al.,2002: Sharpless et al.,2006].

Historical Development of Mouse Cancer Models

Cancer modelling has evolved through several stages, including the use of human tumour cell lines, xenografts, and genetically modified models [Bergers et al.,2008]. Transgene expression or homologous recombination have historically been used in genetic manipulation techniques [Frese et al.,2007: Capecchi et al.,1989]. Over the last decade, programmable nucleases such as zinc-finger nucleases (ZFNs) and transcription-activator-like effector nucleases (TALENs) have improved precision [Gaj et al.,2013]. By employing single-stranded guide RNA for specific genome targeting, the CRISPR-Cas9 system has exceeded ZFNs and TALENs. Cas9 reprogramming is made easier using RNA, allowing for specific genomic alterations. This ground-breaking approach has been used in a variety of experimental models, including human, mouse, rat, zebrafish, fruit fly, and rhesus monkey [Hsu et al.,2014].

Introducing CRISPR/Cas 9 System

The CRISPR/Cas9 system consists of the Cas9 operon, which includes repetitions and spacers, and tracrRNA, a unique RNA that is situated close to the Cas9 operon and resembles several sequences in the system [Chylinski et al.,2013]. When a virus infects bacteria, a portion of the virus's genetic code known as the spacer sequence is introduced to the bacterial genome [Modell et al.,2018].Following that, the Cas9 operon assists in the formation of a segment known as pre crRNA, which combines the newly inserted spacer with the host's DNA [Burmistrz et al.,2020]. This pre-crRNA contains host and viral components [Dyda et al.,2015]. Pre-crRNA converts into mature guide RNA (gRNA) by a series of steps involving tracrRNA, RNase III, Cas1, and Cas2 nucleases [Lone et al .,2018].To make the CRISPR/Cas9 system work, the Cas9 endonuclease attaches to the gRNA, forming a Cas9/gRNA complex. This complex, led by the gRNA, accurately cuts the target DNA at certain sites [Vakulskas et al.,2019]. Cas9 proteins from different bacteria recognise diverse PAM sequences. Cas9 from Streptococcus pyogenes, for example, recognises both the 'NGG' and the less robust 'NAG' PAM sequences [Hsu et al.,2013]. The cut in the DNA occurs a few bases distant from the PAM sequence due to the action of two nuclease domains, the HNH domain and the RuvC-like nuclease [Murovec et al .,2017]. Cas9 stays inactive unless guided by the gRNA, assuring precision [Jiang et al.,2017]. After non-homologous end joining, the genomic sequence changes by adding or deleting base pairs, resulting in insertions or deletions (indels). This mechanism can cause changes in the genomic sequence, potentially resulting in loss of-function mutations in the targeted protein [Jinek et al.,2013; Cho et al .,2013: Hsu et al2013:Platt et al.,2014].Alternatively, homologous end joining repair can be used in conjunction with a "repair

template." This allows a desired genomic sequence to be added to the target of interest [Kim H et al.,2015].These are the basic principles behind CRISPR’s genome editing

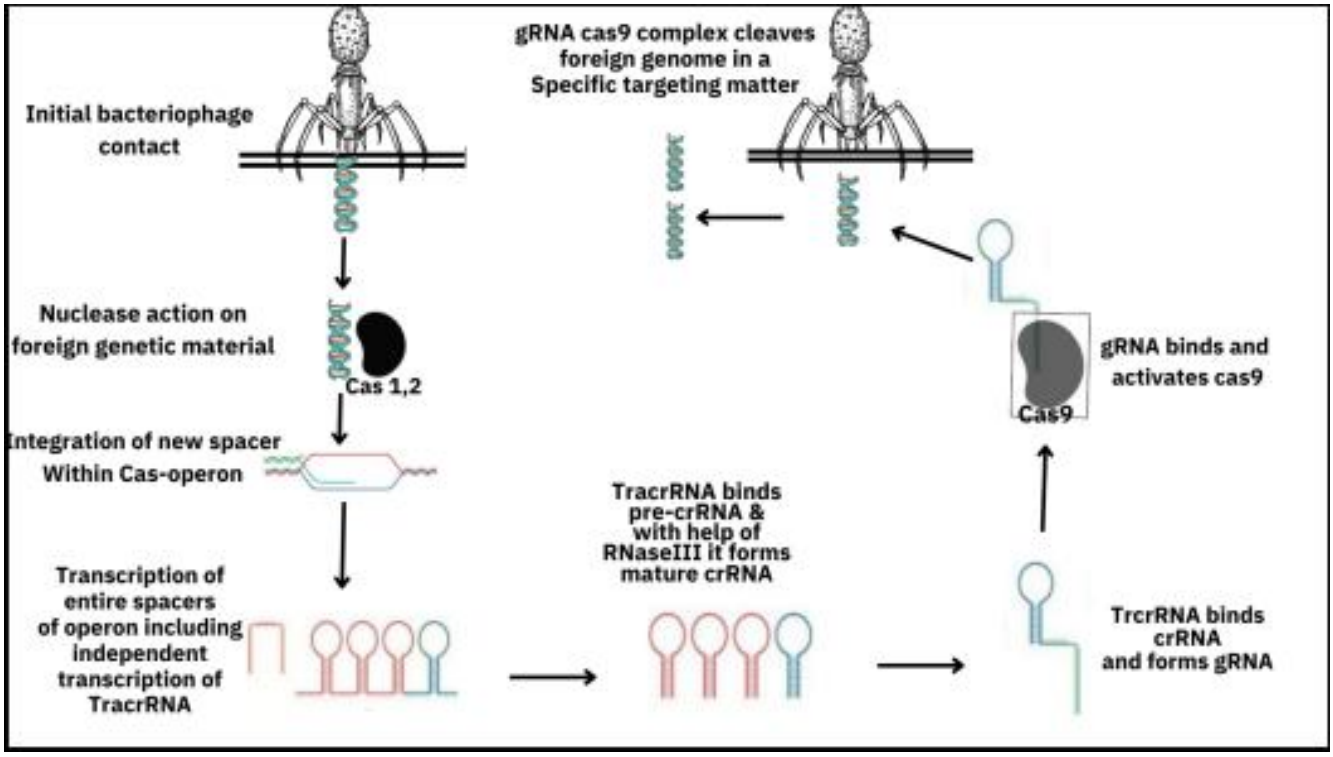


fig. 1 CRISPR/Cas9 Mechanism of Action

Versions of CRISPR/Cas 9 System

The **wt-CRISPR-Cas9 (wild type)** system is a microbial adaptive immune defence system product [Kim H et al.,2015]. The Cas9 nuclease and a single guide RNA (sgRNA), a hybrid of trans-Nat Commun et al.,2014:Sanchez-Rivera FJ wt al .,2014: Xue W et al.,2014]. The sgRNA connects with the target strand by base-pairing when the opposing DNA strand carries the protospacer-adjacent motif (PAM) sequence. This allows Cas9 to cut the DNA site-specifically, resulting in DNA double strand breaks (DSBs) [Yang H et al.,2014] .The two catalytic domains of the wt-Cas9 nuclease, HNH and RuvC, cleave the target DNA strand while RuvC cleaves the opposing strand [Shao Y et al.,2014,Sanchez-Rivera FJ wt al .,2014].

wt-Cas9 becomes **dCas9 (dead)** [Gilbert LA et al.,2013] due to inactive HNH and RuvC domains When combined with effectors such as fluorescent proteins or epigenetic modifiers [Gilbert LA et al.,2013: Maeder ML et al .,2013: Konermann S et al.,2015:Gilbert LA et al.,2014], dCas9, as a DNA binding tool. Referred to as epigenome editing [Rusk N et al .,2014], this dCas9-effector system facilitates future therapeutic epigenetic modifications or the study of gene expressions linked to cancer.By combining dCas9 with different activator or repressor domains, this variation was created to be able to target any region of the genome without cleavage and to either up- or down-regulate the transcription of target genes. Chen and Huang identified an additional use for the dCas9 system (Chen B et al.,2014).There are drawbacks to the dCas9-effector approach. In the first

place, all target loci are activated or suppressed by its one-directional control. Furthermore, there is a need to enhance its regulatory efficacy.

Cas9D10A (nickase) is a Cas9 variation intended for increased HDR efficiency [Cong L et al.,2013]. The activation of NHEJ is reduced by this mutant because it cleaves only one DNA strand. Combining it with sgRNA and a homologous repair DNA template enables high-fidelity HDR, which makes it possible to precisely modify and replace genes in the mouse genome, something that was difficult to achieve with conventional transgenesis techniques.

Type II CRISPR, which is the source of CRISPR-Cas9, mainly targets DNA, although type III-B CRISPR can also cut RNA. The PAM sequence on the opposing DNA strand is necessary for sgRNA recognition, making targeting RNA seem implausible. The programmable single-strand RNA (ssRNA) cleavage tool **RCas9** [O'Connell MR et al.,2014]can be created from CRISPR-Cas9 by inserting an exogenous PAM-containing oligonucleotide (PAMmer) as an artificial "opposite DNA strand". By inactivating its catalytic domains, RCas9 may be further reprogrammed into **dRCas9**, which functions as a site-specific ssRNA binding domain when fused to effectors

Table 1: **Versions of CRISPR**

Version	Description	Application
wt-CRISPR-Cas9	The wild-type CRISPR-Cas9 system is a microbial adaptive immune defence mechanism. Cas9 nuclease and single guide RNA (sgRNA) are used to cause DNA double strand breaks (DSBs).	Site-specific DNA cleavage, genetic alterations, gene knockouts.
dCas9	Cas9 with deactivated HNH and RuvC domains. When coupled with effectors, it can be used to attach to DNA	Epigenome editing, gene expression research, and possible therapeutic epigenetic changes
Cas9D10A	Cas9D10A is a nickase variant of Cas9 that cleaves just one DNA strand, reducing NHEJ activation and increasing homologous directed repair effectiveness	High-fidelity HDR, precise gene modification and substitution in the mouse genome.
RCas9	RCas9 is a programmable single-strand RNA (ssRNA) cleavage tool generated by introducing an exogenous PAM-containing oligonucleotide (PAMmer) into CRISPR-Cas9	Targeting and cleaving small RNAs
dRCas9	When RCas9 is fused to effectors, its inactivated catalytic domains function as a site-specific ssRNA binding domain	Site-specific ssRNA binding and RNA studies

Techniques of CRISPR/Cas 9 delivery

The liver was the focus of a pioneering CRISPR/Cas9 in vivo investigation. Hepatocytes were able to express Cas9 protein and sgRNA through **intravenous injection of plasmids** [Weber et al.,2015:Xue et al.,2014]. CRISPR caused PTEN and Trp53 mutations, which led to liver cancer.Plasmid delivery has the benefit of carrying a substantial payload because the coding sequence of the Cas9 protein is just 4.1 kbp long.Intravenous infusion of Cas9-edited hematopoietic stem progenitor cells has been used successfully to mimic myeloid malignancies in mice (Heckl D et al.,2014) and a Burkitt lymphoma model (Aubrey BJ et al.,2015).

Several viruses are used, **lentivirus** being the first, because of their capacity to transport heavy loads [Counsell et al.,2017]. The target cell can get sgRNA and the Cas9 genomic code thanks to lentivirus, which then integrates into the host genome to allow for ongoing expression. This guarantees high CRISPR effectiveness, but because guides are persistently expressed alongside Cas9, there are worries about off-target alterations [,Pattanayak et al.,2013]. Brain, pancreatic, lung, and breast malignancies have all been induced by lentivirus [Roper et al.,2015:Annunziato et al.,2016: Chiou et al .,2015: Sánchez-Rivera et al.,2014]. Although viral integration functions as a "fingerprint" and verifies sgRNA sequences, it may also encourage the onset of cancer, which presents a problem for the distribution of lentiviruses [Dong et al.,2021].

To prevent viral genome integration into target cells, **adenoviruses** and adeno-associated viruses deliver CRISPR/Cas9. Their ability to multiply during cell division, combined with their temporary presence and cell tropism, makes them advantageous for targeting many organ [Crystal et al.,2014]. s. Adenoviruses are extremely harmful and can cause tissue damage and cell death[Crystal et al.,2014]. They are appropriate for the delivery of sgRNA and Cas9 because they have a broad tropism and can transport substantial payload.Adenoviruses are frequently used to transmit sgRNA and Cas9, causing in vivo cell transformation, especially in mice for brain and lung cancer [Cheng et al.,2014].

Adeno-associated virus (AAV) differs from AV in that it is only capable of reproducing in cells that are also infected with AV [Day et al.,2008]. AAV is not harmful, seldom integrates into the host

genome, and can live in infected cells for more than a year. Integration normally happens at a specific place without interfering with gene expression [Day et al.,2008]. Different AAV serotypes show great tropism for particular cell types, which enhances transduction and induces cancer at specific places [Wu et al.,2006]. AAV's cargo size limitation is that each virus particle may only contain about 5 kilobases. Because the Cas9 coding sequence is 4.1 kilobases long, the possibilities for delivering numerous sgRNAs with Cas9 are limited.

Fertilised eggs or blastocysts (for transformed ES cells) are classically **microinjected** to create transgenic mouse models harbouring CRISPR/Cas9-induced alterations in every cell in the body (Dow LE).For NSCLC, basic epithelial cell **transfection** has also been employed to target genomic rearrangements (Choi PS et al .,2015).

.Electroporation is an effective method of delivering modified sgRNAs in contact with the Cas9 protein

(Cas9/sgRNA ribonucleoprotein complexes, or RNPs) in vitro [Hendel et al.,2015]. RNPs, however, are ineffective for in vivo applications because their fast degradation and negative charge impair uptake [Wei et al.,2015].Consequently, RNPs are packed into **lipid nanoparticles** for distribution to various organs.It has been possible to successfully modify genes to cause cancer in a variety of organs, including the brain, liver, and lung. There have also been some reported successful attempts recently to **electroporate** pronuclear zygotes (Qin W et al.,2015,:Hashimoto M et al et a;.,2015).

The hepatic delivery of CRISPR components targeting Pten and Trp53 was achieved in wild type mice treated with carbon tetrachloride as an accelerator of cancer using **hydrodynamic tail vein injection**. The animals developed cholangiocarcinomas that had histopathological characteristics that were very similar to those seen in conventional GEMM [Yang H et al.,2015].

Cell-Penetrating Peptides (CPP), in conjunction with other transfection agents, were used in a recent study [Ramakrishna S et al.,2014] to successfully transport sgRNA and recombinant Cas9 protein into human cells. Previous studies have demonstrated that CPPs are efficient mediators for in vivo transport of RNA [Li H et al .,2014] and protein [Boisguerin P et al .,2011], indicating a possible novel pathway for the administration of CRISPR-Cas9 components in vivo.

The development of a **Cre-dependent Cas9** mouse by Platt et al. [Maddalo et al .,2014] provided a method for multigene pairings. The Rosa26 locus was altered to accept a floxed-stopped Cas9 expression cassette. They created successive genetic lesions in this animal by expressing three sgRNAs using a single AAV serotype9, an HDR donor template with the KrasG12D mutation, and Cre recombinase, proving that constitutive or tissue-specific Cas9 expression had no negative effects. Two months after infection, lung tumours developed in 100% of treated rats due to multiplex sgRNA delivery, which specifically targeted Kras, p53, and Lkb1..The avoidance of immunological reactions against somatic Cas9 expression, which may result in inflammation and the elimination of Cas9-expressing cells, is an advantage of Cas9 knockin mice [Annunziato S etal .,2008: Wang D et al.,2015].Analogously, reported on the application of Cas9 and Cas9D10A animal models that are doxycycline-inducible for inducible genome editing in mice.[Dow LE et al.,2015]

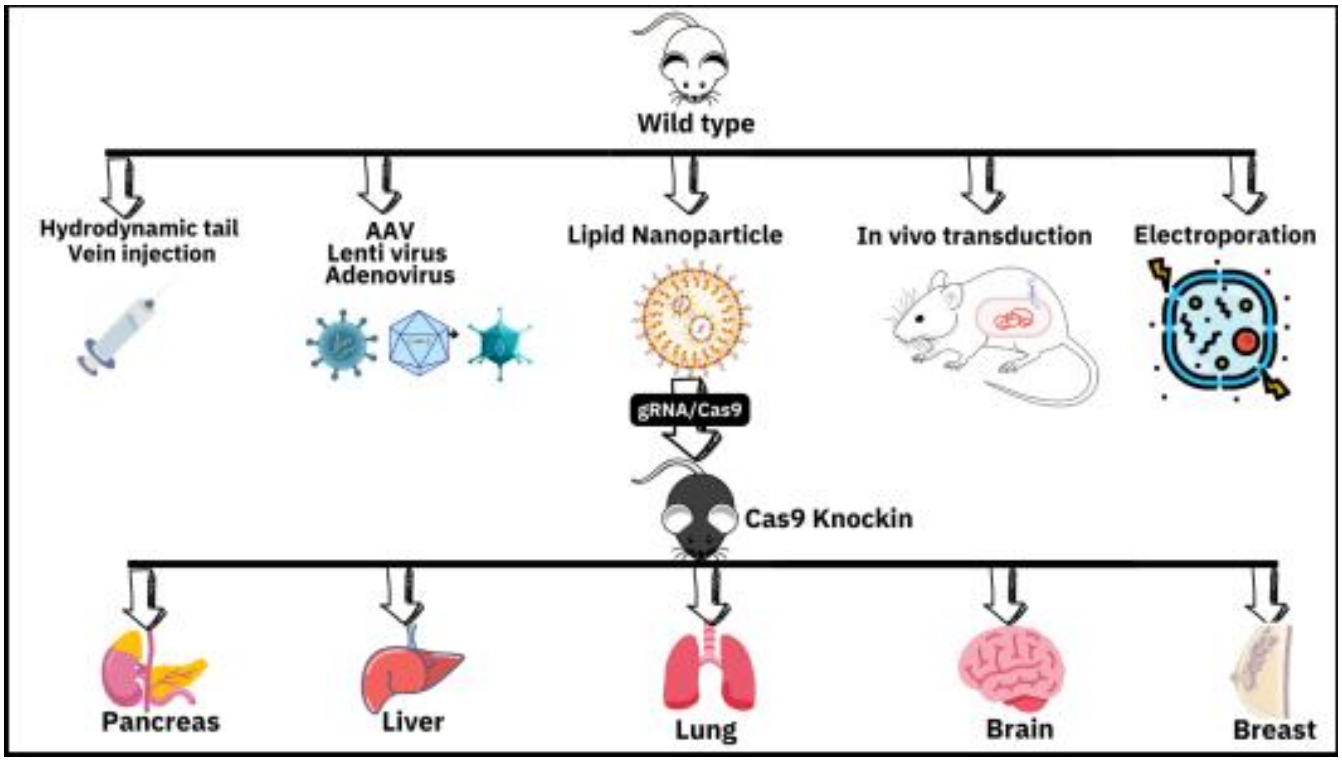


fig.2 Creating murine tumour models using various CRISPR/Cas delivery techniques.

CRISPR Cancer Models

Hepatocellular carcinomas that resembled those seen in conventionally generated mice with liver specific Pten and Trp53 loss were formed as a result of the hydrodynamic tail vein injection method of administering CRISPR/Cas9 components targeting Pten and Trp53 to the liver via a plasmid expressing Cas9 and sgRNAs [Yang H et al.,2015].The animals developed cholangiocarcinoma that exhibited histopathological characteristics that were similar to those seen in conventional GEMM [Yang H et al.,2015].

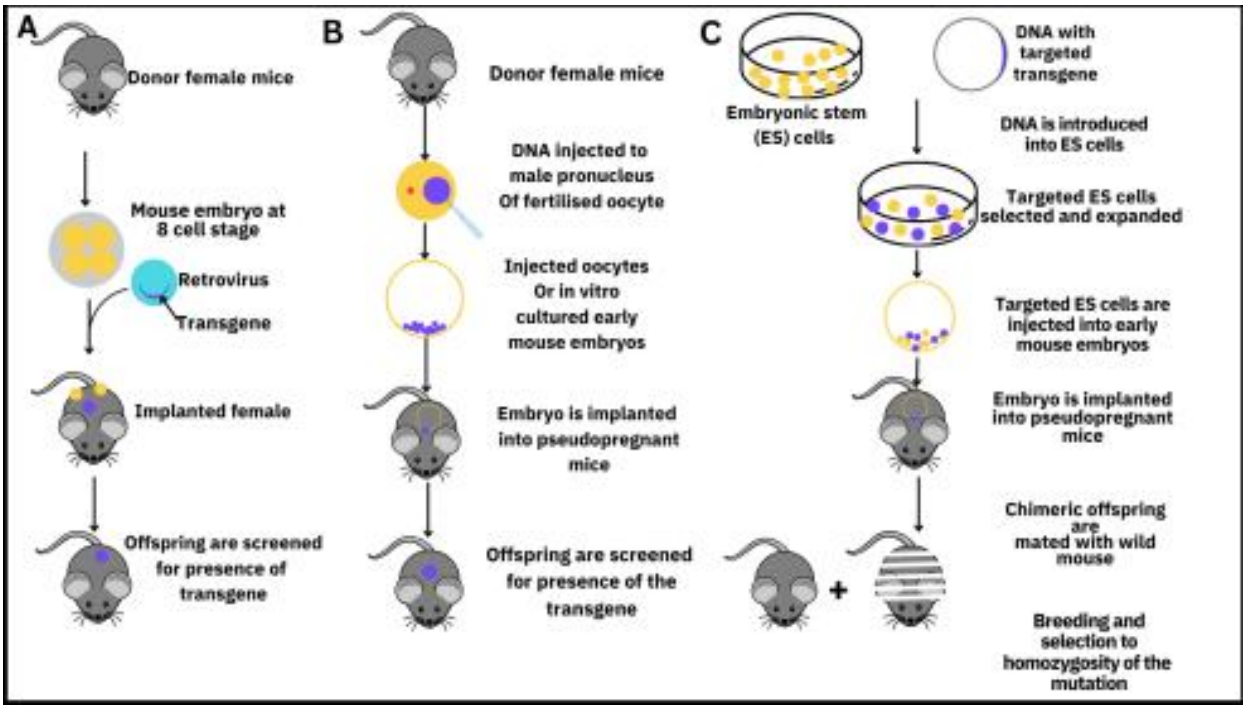
Alb-Cre;KrasLSL-G12D/+ mice were multiplexed and hydrodynamic tail vein injection was performed using ten different CRISPR-SB vectors. Each vector, including Cas9 and one of 10

distinct sgRNAs flanked by Sleeping Beauty (SB) transposon repeats, was accompanied by an SB transposase vector to facilitate genomic integration. **Hepatocellular carcinoma** and **intrahepatic cholangiocarcinoma** were the results of this strategy [Weber J et al.,2015].

Using PEI-mediated transfection of the neonatal cerebellum or in utero electroporation of the developing prosencephalon, CRISPR **cancer modelling in the brain** was first accomplished [Zuckermann M et al.,2015]. While glioblastoma was produced by targeting Nf1, Pten, and Trp53, **medulloblastoma** was formed when the sonic hedgehog receptor Ptch1 was inactivated [Zuckermann M et al.,2015]. Subsequent research used cerebral injection of viral vectors containing CRISPR to imitate high-grade glioma [O'Rourke KP et al.,2017: Chow RD et al.,2017:Annunziato S et al.,2016].

Pancreatic cancer modelling strategies included retrograde pancreatic ductal [Chiou SH et al.,2015], direct injection of CRISPR-carrying viruses into the pancreas [Mazur PK et al.,2015: Ideno N et al.,2019], and CRISPR plasmid injection followed by in vivo electroporation [Maresch R et al.,2016: Mueller S et al.,2018]. Even if it is ineffective, in vivo electroporation allows transfected cells to absorb several plasmids, which facilitates in vivo multiplex mutagenesis. This method confirmed negative selection for Brca2 inactivation in KrasG12D-driven pancreatic cancer, enabling the first direct in vivo synthetic lethality screening [Maresch R et al., 2016].

fig.3 Various ways for making transgenic mice: (A) retroviral method, hardly used; (B) typical transgene strategy, introducing DNA into the genome non-specifically; and (C) gene-targeted



Blasco et al. [Yang et al.,2015] showed that CRISPR-Cas9 is a viable tool for producing gene mutations as well as for designing malignant chromosomal rearrangements in mice and maybe other species in vivo. The Eml4-Alk gene rearrangement recurrent in non-small-cell **lung tumours** was successfully produced. In order to introduce CRISPR components into adult mouse lung tissue, lentiviral particles were used either intrapulmonaryly or intratracheally. Two single-strand breaks (DSBs) caused by targeting the Eml4 and Alk genes on mouse chromosome 17 can occasionally result in 10-Mb inversions (1.5 rearrangements/106 cells).Maddalo et al. reported producing the Eml4-Alk inversion in adult mouse lung somatic cells in a different work [Schwank et al.,2013].In order to produce loss-of-function mutations in p53, Lkb1, and HDR-mediated KrasG12D alterations, which mimicked the pathophysiology of l**ung adenocarcinoma**, a single AAV vector was delivered into the lung [Platt RJ et al.,2014].

Hematopoietic stem cells are used in ex vivo engineering, wherein they are transduced in vitro using CRISPR-Cas9 viral vectors and then transplanted into recipient mice. This permits the quick generation of novel mice models for haematological malignancies [Heckl D et al.,2014: Malina A et al.,2013:Chen C et al.,2014:Aubrey BJ et al.,2015]. In a recent work, delivering combinations of sgRNAs and Cas9 via a lentiviral vector resulted in effective alteration of up to five genes in HSCs [Heckl D et al., 2014]. Utilising this method produced models of **acute myeloid leukaemia** through clonal expansion and the emergence of myeloid malignancy.

Using the CRISPR-Cas9 method, Kraft et al. [Kraft K et al.,2015] recently rearranged selected genomic intervals at six loci (H2afy, Bmp2, Ihh, Pitx1, Laf4, and Epha4) that are associated to human illnesses. The genomic intervals ranged from 1 kb to 1.6 Mb. Deletions and inversions were identified at all sites, with duplications occurring in Pitx1 (0.7%) and Laf4 (28.1%). Notably, **human malformation syndromes and neurological abnormalities** were replicated by the deletion of 353 kb and 1.6 Mb genomic regions at the Laf4 and Epha4 loci, respectively [Kraft K et al.,2015].

Annunziato et al. utilised a mouse model with Wap promoter-driven Cre recombinase to achieve targeted Cas9 expression in the mammary epithelium. Four months after these mice received a mammary duct

injection of a lentivirus expressing a sgRNA targeting Pten along with conditional Cdh1 gene deletion (E-cadherin), these mice developed tumours that resembled **human lobular breast carcinomas** [Annunziato S et al.,2016].

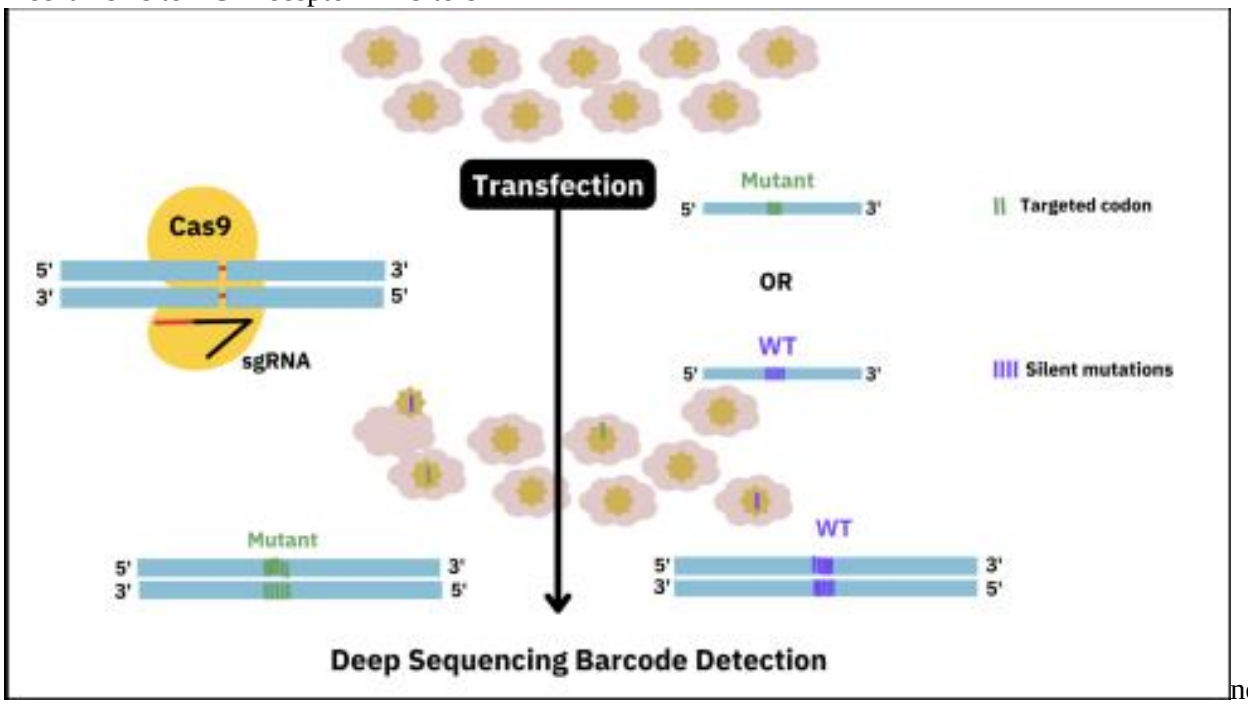
CRISPR-Cas9 High-Throughput Genetic Screening

When screening a large number of genes, CRISPR/Cas9 gives a special advantage.In 2014, Zhang et al. carried out two important in vitro investigations [Konermann et al.,2015: Lagutina et al.,2015]. First, genes linked to vemurafenib resistance in melanoma were identified by using the CRISPR-Cas9 knockout (GeCKO) library, which targets approximately 18,000 human genes for targeting. Both proven and fresh candidates were identified by this investigation. The second study used a sgRNA library to screen for genes that activate vemurafenib resistance. Using a genome wide loss-of-function sgRNA library, Zhang and Sharp [Xiao et al.,2013] expanded on this strategy by methodically screening genes linked to metastasis. They mutated a non-metastatic lung cancer cell line, transplanted the mutant cells into mice, and used deep sequencing to identify a collection of genes consistently related with tumour growth and metastasis. In vitro screens have proved effective in laboratories due to the capacity to use a large number of cells, ensuring complete coverage of gene libraries holding 50,000 to 100,000 distinct sgRNAs. Applying these libraries to in vivo research is difficult, though. In response, particular gene groupings are the focus of in vivo screening. In an AAV vector, for example, Chow and Wang (2017 & 2018) generated a library including over 250 tumour suppressor genes, each represented by 5 distinct sgRNAs. Through the use of Tpr53-deficient mice and targeted genes such as Pen, they were able to effectively explore the formation of gliomas and HCCs [Wang et al .,2018: Chow et al.,2017].

CRISPR Barcoding

The limited efficiency of HDR genome editing is exploited by CRISPR/Cas9 [Y. Lin et al.,2014] to introduce oncogenic mutations selectively, monitoring modified cells through the use of a genetic barcode.We inserted a unique code, readable by sophisticated instruments, to track these transformed cells. This gave us the opportunity to investigate how cancer cells adapt and test various cancer treatments. This method uses a certain subgroup as an internal control. Since both control and mutant cells express the same sgRNA, off-target effects are prevented and specificity is increased. Different barcodes in the same cell's

stranded deoxyribonucleotides) separately with plasmids containing Cas9 and sgRNA. We demonstrated the usefulness of the approach in evaluating cancer medication combinations by applying it to tumour cell lines, where we generated TP53 mutations and amplified cells by Nutlin 3 treatment.The approach also studies effects on proliferation, invasion, and tumorigenicity in immunodeficient mice, modelling resistance mechanisms to EGF receptor inhibitors in



non-small cell lung cancer [A. Guernet et al.,2016].

Fig 4: CRISPR barcoding for genetic tracking

CRISPR Challenges in Genome Editing

Non-Homologous End Joining (NHEJ) methods produce different indels in current CRISPR/Cas9- based gene editing for minor deletions and insertions, resulting in significant sequence changes in the resulting allelic series. Although indels usually result in loss-of-function alleles, they can also cause in-frame or out-of-frame indels due to their unpredictability and lack of control during base
editing via NHEJ. This unpredictability poses complications, as most indels cause frame-shift mutations, but 3-base indels selectively modify amino acids without disturbing the reading frame

The CRISPR/Cas9 system's off-target editing activity, which has the potential to change the genome in unexpected ways, is a major cause for concern. Although research on human cells [Yang H et al.,2013: Zetsche B et al.,2015] has shown that off-target events occur somewhat often, preliminary findings in mouse embryos suggest that CRISPR/Cas9 off-target events are uncommon . Reduce and carefully monitor these off-target effects, especially in therapeutic applications, since they can cause epigenetic modifications, disturbances in gene activity, and instability of the genome. The transcription of the targeted gene may be inhibited by off-target effects, which might appear as binding to partly complementary target sequences but not cleaving them [Y. Lin et al.,2014].

One issue in utilising CRISPR/Cas9 to simulate cancer in living creatures is successfully distributing the

restriction, with the colon's crypt structure providing unique challenges. Current approaches entail sophisticated procedures such as introducing Adeno-Associated Virus (AAV) to the mouse's prostatic lobes via surgery and complex injections. It is possible for this process to fail and for unwanted tumours to grow in other organs or to fail entirely, leaving the targeted organ without tumour start. Enhancing the efficiency of in vivo genome editing can be achieved by using split Cas9 [Zetsche B et al.,2015:Wright AV et al.,2015] and better CRISPR delivery strategies, such as CRISPR proteins or mRNA. Furthermore, investigating smaller-sized Cas9 proteins may simplify the packaging of CRISPR viral vectors [Esvelt KM et al.,2013: Hou Z et al.,2013:Ran FA et al.,2015].

Furthermore, despite the fact that CRISPR-Cas9 has shown to be an adaptable research tool, the scientific community is still concerned about Cas9's safety [Baltimore BD et al.,2015]. More research is needed to thoroughly assess CRISPR-Cas9 safety.Furthermore, ethical concerns about manipulating the human germline will undoubtedly come up in the future [Tsai SQ et al.,2015].

Potential Solutions

The use of CRISPR/Cas9 raises the possibility of unforeseen consequences, or "off-target effects." Advanced techniques like ChIP-seq[Fu Y et al.,2013], GUIDE-seq[Tsai SQ et al.,2015], and Digenome-seq[Kim D et al.,2015] are needed to identify these problems because the conventional methods may overlook some of them. Wang and colleagues[Wang X wt al.,2015] presented a lentiviral vector-based approach to identify Cas9's off-target effects.Researchers are attempting several strategies to reduce these unintentional consequences. Purified Cas9 proteins can be used to mitigate difficulties instead of introducing Cas9 genes into cells. This is because the proteins degrade rapidly, leading to fewer long-lasting issues with the instructions inside the cell. Utilising a "inducible" Cas9 is a further option; it reduces the possibility of unforeseen consequences by functioning momentarily. Reducing these effects has also been demonstrated to be possible with a modified version of Cas9. The guide RNA (sgRNA) may be made more responsive to alterations by trimming a tiny portion of it, which will lessen any unwanted consequences.To avoid tumour growth in secondary tissues, Cas9 expression might be tissue specific. Tissue-specific Cre expression can be implemented through the use of transgenic mice under Cre induction for Cas9. Conditional Cas9-expressing mice can be intercrossed with strains of tissue-specific animals for Cre expression. An approach is to clone a tissue-specific promoter, transport it within a viral particle, and use it to express Cre.
For in vivo CRISPR-Cas9 delivery, methods such as nanoparticles[Roy I et al.,2003], cell penetrating peptides [Ramakrishna S et al.,2014], and ultrasound-mediated gene transfer[Lu Q et al.,2003: Bekeredjian R et al .,2003]which increases cell membrane permeability by using ultrasound—show promise. Without the need for additional transfection agents, a recent work effectively delivered sgRNA and recombinant Cas9 protein into human cells via CPP [Ramakrishna S et al.,2014]].

Future Horizons in Crispr Technology

Epigenetic enzymes that regulate epigenetic alterations such as methylation are being combined with dCas9 by researchers. For instance, dCas9 combined with the demethylase enzyme TET1 was utilised to induce the activation of a reporter gene by removing methylation from certain DNA locations in mice. A different research methylated a particular section of the mice's genome by fusing dCas9 with the DNA methyltransferase MQ1. These dCas9-enzyme fusions are anticipated

to be important in the future for cancer modelling, as aberrant DNA methylation has been connected to cance rthus conducting “epi-modelling” of cancer or exerting therapeutic functions. [Liu XS et al :2016:Lei Y et al.,2017].Through a technique called CRISPRa, dCas9 fusions may be used to increase gene expression. In order to do this, dCas9 is attached to activators such as VP64 and p65. These activators, when directed by sgRNAs, target certain DNA areas and enhance gene activity. In a CRISPRa study, for example, resistance mechanisms in a melanoma cell line with the BRAFV600E mutation were examined. Novel and well-known resistance mechanisms were detected by the screen. It is anticipated that CRISPRi/a systems will soon be used in vivo with mice models of cancer because reversible regulation of gene expression offers a closer match to human tumour genetics [Gilbert LA et al.,2014].CRISPR interference, or CRISPRi, is an additional technique for studying loss of function. It functions by preventing the production of the targeted genes and directing repressors (such as the Kruppel associated box, or KRAB) to the transcriptional start site. Key regulators of early mesoderm development, such as the transcription factor FOXA2, were identified, for example, in a CRISPRi research conducted in human ES cells [Liu SJ et al.,2013].sgRNAs that target 5,689 lncRNA loci's beginnings were employed in a human GBM cell line that expressed dCas9-KRAB in a recent in vitro CRISPRi investigation. As a result, several lncRNAs that increase glioma cells' sensitivity to radiation were discovered [Nihongaki Y et al.,2015].
Advances in the CRISPR-Cas9 system will eventually allow for more accurate genome editing, which will increase our capacity to develop sophisticated cancer models. There may be other advantages to creating novel Cas9 fusion proteins, such as light-inducible dCas9[Polstein LR et al.,2015:Zender L et al.,2008]. Like with RNA-interference techniques[Chen S et al.,2014], in vivo CRISPR screens [MacLeod RS et al.,2019]could reveal novel cancer driver genes.Furthermore, the development of accurate cancer mouse models using genome editing techniques is something we anticipate will help us better understand how individual tumours advance and devise efficient treatment plans.Precision cancer mouse models will play a crucial role in advancing precision cancer medicine.

Conclusion

To sum up, the revolutionary CRISPR-Cas9 system has evolved to be an adaptable and strong instrument in cancer modelling, providing hitherto unseen potential for genome editing and investigating many uses.It has greatly increased our understanding of oncogenic pathways, from inducing germline editing and somatic editing for modelling gene knockouts, knock-ins, and chromosomal rearrangements to systematic genome-wide study. Despite the immense promise of constructing sophisticated cancer models, obstacles like as off-target effects, delivery modalities, and the requirement for precise control continue. CRISPR high-throughput genetic screening and CRISPR Barcoding are effective methods for identifying genes related with drug resistance, metastasis, and carcinogenesis, allowing for the monitoring and research of cancer cell adaptations, as well as the testing of therapies. In any case, the dynamics of the CRISPR/Cas field, which was sparked by pioneering, groundbreaking work that transformed a bacterial immune system into a game-changing bio- technological tool, indicate that it is likely to undergo further unanticipated developments that will add new milestones to the tumour modelling toolbox.

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Details of the AI usage are given below:

1.

2.

3.

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