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How CRISPR Is Revolutionizing the Generation of New Models for Cancer Research

Francisco J. Sánchez Rivera[1,2] and Lukas E. Dow[3,4,5]

[1]David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02142, USA

[2]Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02142, USA

[3]Sandra and Edward Meyer Cancer Center, Weill Cornell Medicine, New York, New York 10065, USA

[4]Department of Biochemistry, Weill Cornell Medicine, New York, New York 10065, USA

[5]Department of Medicine, Weill Cornell Medicine, New York, New York 10065, USA

Correspondence: fsr@mit.edu; lud2005@med.cornell.edu

Cancers arise through acquisition of mutations in genes that regulate core biological processes like cell proliferation and cell death. Decades of cancer research have led to the identification of genes and mutations causally involved in disease development and evolution, yet defining their precise function across different cancer types and how they influence therapy responses has been challenging. Mouse models have helped define the in vivo function of cancer-associated alterations, and genome-editing approaches using CRISPR have dramatically accelerated the pace at which these models are developed and studied. Here, we highlight how CRISPR technologies have impacted the development and use of mouse models for cancer research and discuss the many ways in which these rapidly evolving platforms will continue to transform our understanding of this disease.

Cancers arise through the accumulation of genetic and nongenetic alterations that regulate fundamental biological processes such as cell proliferation, death, and differentiation, among others (Hanahan and Weinberg 2000). While we have cataloged many of the genes and mutations that are causally involved in malignant progression, defining the functional role that specific cancer-associated changes play across different cancer types has remained a major challenge. Revealing the key alterations and biological processes that drive cancer progression is central to understanding cancer and "precision oncology" paradigms that aim to tailor specific cancer therapies to individual patient genotypes (Hyman et al. 2017).

Genetically engineered mouse models (GEMMs) have played a vital role in the functional characterization of genomic alterations involved in tumorigenesis (Frese and Tuveson 2007). GEMMs harboring gene-specific knockouts have led to the validation of tumor suppressors like *TP53* (Donehower et al. 1992; Jacks et al. 1994) and *RB1* (Jacks et al. 1992), while knockin or transgene alleles have been used to probe the oncogenic potential of putative cancer drivers like

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KRAS (Johnson et al. 2001; Tuveson et al. 2004) and *EGFR* (Politi et al. 2006). Over the past decade, the development of genome-editing approaches using clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) enzymes has revolutionized biomedical research (Jinek et al. 2012; Hsu et al. 2014). CRISPR technologies have had a rapid and tangible impact on the way we design and implement experiments in the mouse. It has both accelerated the pace of model development using established experimental approaches and given rise to entirely new ways to study cancer biology in vivo. Here, we highlight how CRISPR technologies have impacted the development and use of mice in cancer research and how these rapidly evolving platforms are key to a fundamental understanding of human malignancies.

FROM MICROBES TO MICE

Although CRISPR is relatively new to our field, it has been an evolving component of bacterial immunity for many, many years (Garneau et al. 2010). We now appreciate that there is an enormous diversity of natural CRISPR systems in prokaryotes, and even phages (Al-Shayeb et al. 2022), and systematic metagenomic and molecular biology studies continue to expand the repertoire of both natural and engineered CRISPR systems at a breakneck speed (Koonin et al. 2017; Makarova et al. 2020; Al-Shayeb et al. 2022).

CRISPR tools used for genome engineering are composed of at least two modular components: an RNA-guided DNA-binding Cas protein (e.g., Cas9) and a sequence-specific guide RNA (gRNA) (Fig. 1; Hsu et al. 2014). In its

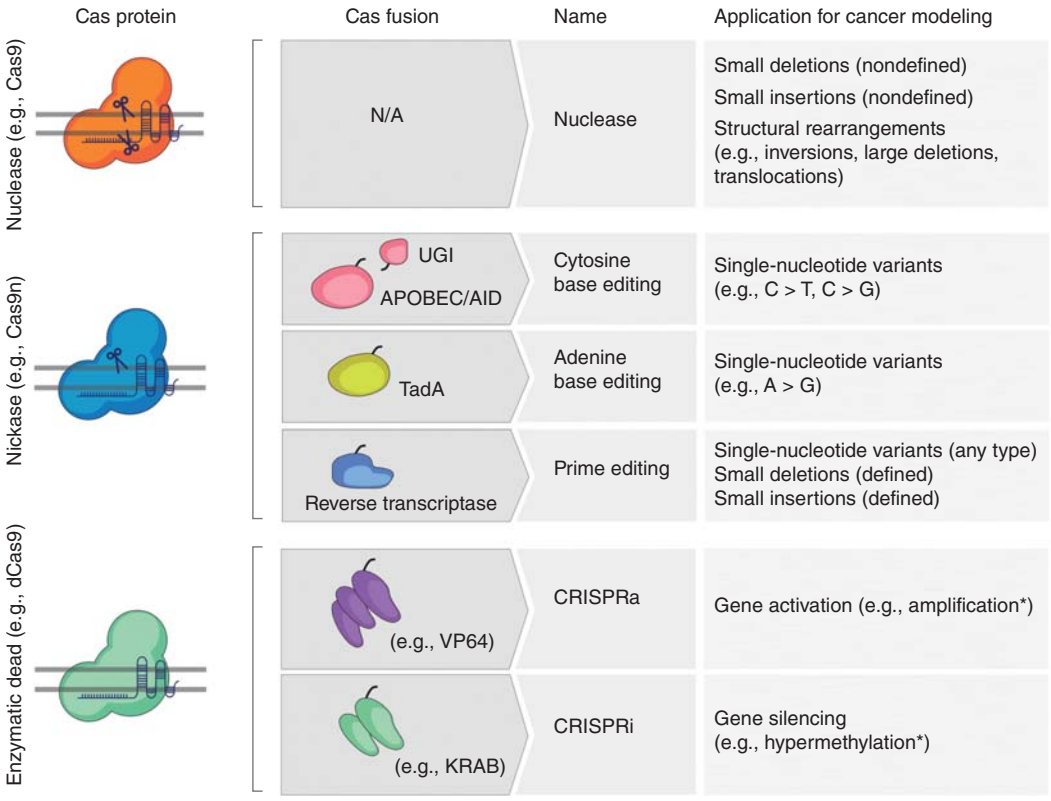

Figure 1. Predominant CRISPR-based tools used in mice and their application for cancer modeling. *CRISPRa and CRISPRi tools may approximate the effects of amplification and gene hypermethylation, respectively, but the mechanism of regulation is different than seen in cancers.

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native context, Cas-gRNA complexes recognize genomic sites through RNA-DNA base pairing, where target recognition activates Cas nuclease activity, leading to a targeted double-strand break (DSB). Sequence engagement is dependent on near-perfect base pairing over a 17–21-bp "protospacer" sequence and the presence of a protospacer-adjacent motif (PAM) at the 3′ end of the target. The canonical PAM for *Streptococcus pyogenes*–derived Cas9 (SpCas9) is NGG, although Cas proteins from other bacterial species have their own distinct PAM preferences (Esvelt et al. 2013; Hou et al. 2013; Ran et al. 2015; Hirano et al. 2016; Müller et al. 2016; Harrington et al. 2017; Kim et al. 2017a; Koonin et al. 2017; Chatterjee et al. 2018; Edraki et al. 2019; Makarova et al. 2020; Al-Shayeb et al. 2022).

In addition to naturally occurring Cas variants, experimental and computational efforts integrating metagenomics, structure-based rational engineering, and directed experimental evolution have led to an explosion of both natural and synthetic CRISPR systems that increase the flexibility of genomic targeting (Kleinstiver et al. 2015a; Hu et al. 2018b; Nishimasu et al. 2018; Miller et al. 2020; Walton et al. 2020). The breadth of orthologous CRISPR systems has been extensively reviewed elsewhere (Anzalone et al. 2020; Chen and Liu 2022; Katti et al. 2022a); thus, we will focus our discussion on how these technologies have directly impacted cancer modeling in the mouse.

The specificity, modularity, and programmability of CRISPR systems have triggered the development of a large and growing list of sophisticated molecular tools (Fig. 1). Unlike native Cas9, these systems often engage DNA targets using nuclease-defective or "dead" Cas9 (dCas9) and include variants for gene repression (e.g., CRISPRi [Gilbert et al. 2013; Qi et al. 2013]), gene activation (e.g., CRISPRa [Maeder et al. 2013; Mali et al. 2013; Perez-Pinera et al. 2013; Gilbert et al. 2014]), chromatin modification (Hilton et al. 2015; Kearns et al. 2015; Amabile et al. 2016; Cano-Rodriguez et al. 2016; Choudhury et al. 2016; Liu et al. 2016; Vojta et al. 2016; Xu et al. 2016; Bennett-Baker and Mueller 2017; Lei et al. 2017; Nuñez et al. 2021), RNA targeting/editing (Bartolomé et al. 2015; Abudayyeh et al. 2017; Cox et al. 2017; Konermann et al. 2018; Xu et al. 2020; Li et al. 2021a) (e.g., Cas12/13), protein labeling (Gao et al. 2018; Myers et al. 2018), single-nucleotide transitions and transversions (Komor et al. 2016; Gaudelli et al. 2017; Koblan et al. 2021; Kurt et al. 2021; Chen et al. 2022; Neugebauer et al. 2022) (for reviews, see Anzalone et al. 2020; Chen and Liu 2022), as well as complex insertions or deletions (Anzalone et al. 2019; Chen et al. 2021; Chen and Liu 2022; Nelson et al. 2022).

CRISPR, BETTER, FASTER, STRONGER

While virtually all CRISPR technologies have been developed in simpler cell systems (i.e., bacteria or mammalian cell lines), most have been successfully adapted for implementation in more complex in vivo settings. While each present their own challenges, the advances described below demonstrate that most (if not all) CRISPR technologies are readily adaptable to in vivo systems and can be used to model most types of alterations observed in human cancer (Fig. 1; Box 1).

Germline Engineering

Now commonplace in most basic research laboratories worldwide, before 2012, CRISPR was relatively unheard of in cancer research (Jinek et al. 2012). In the pre-CRISPR era, the generation of new targeted genetic alleles in mice relied on in vitro modification of mESCs (Smithies et al. 1985; Thomas et al. 1986; Mansour et al. 1988) and production of mice by blastocyst injection or tetraploid aggregation (Fig. 2). This process requires substantial experience with mESC culture and can be laborious and expensive. While such traditional approaches remain effective today, CRISPR has fundamentally changed the way mouse germline engineering is performed. For those who do opt for mESC targeting, CRISPR streamlines the creation of knockin and knockout alleles (Wang et al. 2013). Gene disruptions can be easily created through introduction of small insertions or deletions (indels) with a single Cas9-gRNA combination, or by larger genomic excisions using

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BOX 1. GLOSSARY

Protospacer-adjacent motif (PAM)
A nucleotide sequence immediately adjacent to the CRISPR target sequence that is required for Cas proteins to recognize, bind, and perform a specific molecular operation (e.g., cleavage). The canonical PAM for *S. pyogenes*–derived Cas9 (SpCas9) is NGG, although Cas proteins from other bacterial species have their own distinct PAM preferences.

Insertions or deletions (indels)
Mutations that arise due to small insertions or deletions of DNA sequences often as a result of a nonhomologous end joining pathway (NHEJ; see below). Indels are the most common type of mutation introduced by CRISPR-Cas nucleases (e.g., SpCas9).

Nonhomologous end joining pathway (NHEJ)
An error-prone cellular DNA repair pathway that is engaged upon introduction of a DSB at a genomic site. NHEJ involves modification, addition/removal of nucleotides, and subsequent error-prone ligation of two broken DNA ends, often leading to indels.

Microhomology-mediated end joining (MMEJ)
MMEJ is another error-prone cellular DNA repair pathway that relies on exposed microhomologous sequences flanking a DSB that get aligned, modified, and subsequently ligated, leading to indels and, in some instances, chromosomal translocations.

Homology-directed repair (HDR)
A precise cellular DNA repair pathway that physiologically relies on homologous chromosomes or sister chromatids to repair DSBs but that can be experimentally engaged upon introduction of exogenous donor DNA templates.

Cre recombinase
Originally derived from the P1 bacteriophage, *Cre* is a site-specific tyrosine recombinase that catalyzes recombination between *lox* sites. Other types of commonly used site-specific recombinases are *Flp* (which recognizes *FRT* sites) and *Dre* (which recognizes *rox* sites).

Mouse embryonic stem cells (mESCs)
Cells derived from the inner cell mass of a developing blastocyst that are able to self-renew indefinitely in vitro while retaining the potential to reconstitute all embryonic lineages. These cells are used for generating GEMMs.

Genetically engineered mouse models (GEMMs)
Mice produced using mESCs modified to express either exogenous (e.g., Cas9) or endogenous mutated genes.

Hydrodynamic tail vein injection (HTVI)
Also called "hydrodynamic gene transfer," HTVI is a high-pressure injection that allows for efficient delivery of DNA to liver cells through hydrodynamic pressure-mediated enhancement of endothelial and parenchymal cell permeability.

Virus-like particles (VLPs)
Nanoscale particles generated through self-assembly of proteins encoding viral capsids, cores, and envelopes, as well as defined protein cargoes of interest (e.g., Cas9 protein).

Organ/tissue electroporation
Conceptually similar to in vitro electroporation, tissue electroporation involves the use of electrodes to deliver short (microsecond to millisecond) high electric field pulses that increase the membrane permeability of cells within specific organs, allowing the delivery of single or multiplexed defined cargoes (e.g., plasmid DNA).

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Copy number alterations (CNAs)
Large (often chromosome-level) genomic alterations that often perturb the physiological ploidy of cells through loss (deletion) or gain (amplification) of specific genomic regions, or even entire chromosomes.

Linked (genetic linkage)
In genetic terms, linkage refers to genes or otherwise DNA sequences that are physically located in the same chromosome.

Positive–negative selection
A strategy commonly used in mESC engineering to increase the efficiency of correct gene targeting by using vectors simultaneously encoding positive selection cassettes (e.g., antibiotic resistance) to enrich for cells with an integrated construct and negative selection cassettes (e.g., diphteria toxin receptor [DTR], herpes simplex virus 1 thymidine kinase/HSV-TK) to eliminate cells with off-target or inappropriate genomic integration.

DNA integrases
Enzymes that catalyze the linkage of double-stranded DNA molecules into another DNA molecule (e.g., genome).

Single-nucleotide variants (SNVs)
Genetic variants that differ between each other (e.g., wild-type vs. mutant) due to a single base pair difference.

Missense mutations
Mutations that result in altered amino acid sequences. Most missense mutations are SNVs.

Transversions
Single-nucleotide changes involving a base pair change from purine to a pyrimidine, or vice versa.

Transitions
Single-nucleotide changes involving a base pair change from purine to purine or pyrimidine to pyrimidine.

Nonsense mutations
Single-nucleotide changes that generate a nonsense (stop) codon (TAA, TAG, and TGA), leading to premature translation termination and truncating mutations.

Hypomorphic mutations
Mutations that result in partial loss of function or reduction in gene function. Hypomorphic mutations can be experimentally modeled in some contexts using RNA interference (RNAi) and CRISPR interference (CRISPRi), although the precise nature of the hypomorphic mutation may have effects that go beyond just lower gene dosage.

Gain-of-function (GOF) mutations
Mutations that result in a new molecular function for the altered gene product. Some GOF mutations can be experimentally modeled using gene overexpression methods like cDNA overexpression constructs and CRISPR activation (CRISPRa), although the precise nature of many GOF mutations can have effects that go beyond just increased gene dosage (e.g., altered enzyme activity, new protein–protein interactions, etc.). Most GOF mutations are dominant (e.g., $Kras^{G12D}$).

Separation-of-function mutations
Mutations that confer loss of a particular biological property (e.g., a biochemical activity) without necessarily altering other functions of the altered gene product.

Neoepitopes
A nonself antigen presented by the major histocompatibility complex often derived from SNVs, or insertions or deletions (indels) in expressed DNA that create new peptide fragments.

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Figure 2. Advantages and disadvantages of different types of germline and in utero engineering approaches with CRISPR.

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pairs of gRNAs flanking relevant regions. Similarly, the creation of more complicated conditional or knockin alleles by HDR is significantly enhanced using CRISPR due to its ability to create targeted DSBs that can be repaired through incorporation of exogenous DNA templates. In addition to individual gene disruptions, CRISPR also makes it possible to build complex genetic systems. Early studies by Jaenisch and colleagues demonstrated that CRISPR could be used to simultaneously knockout multiple genes or knockin various types of genetic elements, including protein tags, Cre recombinase recognition sites, and fluorescent reporters (Wang et al. 2013; Yang et al. 2013).

The efficiency of genome engineering with CRISPR has also driven the development of strategies for direct manipulation of mouse zygotes. These approaches most commonly employ direct injection of RNA or RNA:protein (ribonucleoprotein [RNP]) complexes to fertilized zygotes, and enable targeting of a variety of alternate murine strains that have been recalcitrant to ESC culture (Fig. 2; Clark et al. 2020). Easi-CRISPR (Quadros et al. 2017) and CRISPR-EZ (Modzelewski et al. 2018) combine preassembled RNP Cas9-gRNA complexes with or without single-stranded DNA (ssDNA) donor templates to engineer insertions, deletions, point mutations, and knockin alleles in fertilized zygotes collected from superovulated females. Other methods like CRISPR-READI (Chen et al. 2015) use adeno-associated viruses (AAVs) as DNA donor templates to engineer diverse alleles via HDR. GONAD bypasses the collection and ex vivo engineering of zygotes altogether (Takahashi et al. 2015; Ohtsuka et al. 2018; Sato et al. 2020), allowing direct one-step or sequential genome editing of preimplantation mouse embryos within intact murine oviducts by electroporation (Fig. 2).

The combined practical outcome of CRISPR germline engineering technologies has been the democratization of mouse modeling. What was once the domain of specialized laboratories and dedicated commercial entities has become standard practice at most institutional gene-targeting core facilities and used for producing hundreds of constitutive and conditional knockout and knockin models (Gurumurthy et al. 2019). The significant increase in the speed and efficiency of model generation is also catalyzing the adoption of new inducible–reversible gene-disruption strategies based on regulation of protein stability using small molecules. For instance, auxin-inducible degrons (Nishimura et al. 2009) and degradation tags (dTAGs) (Winter et al. 2015; Nabet et al. 2018; Abuhashem et al. 2022) can be fused to Cas effectors (e.g., Cas9 nuclease) to induce protein degradation upon addition of indole-3-acetic acid (IAA) or specific dTAG molecules, respectively (Kleinjan et al. 2017; Sreekanth et al. 2020), and CRISPR itself is now commonly used to generate endogenous gene–degron fusion alleles (Macdonald et al. 2022). The versatility of CRISPR approaches has also led to the creation of large collections of genetically modified mouse strains (Han et al. 2021; Wei et al. 2021; Abuhashem et al. 2022) that would have been considered science fiction only a decade earlier.

Somatic Engineering

The capacity of CRISPR systems for efficient and precise genome engineering in cells to introduce different types of mutations (e.g., insertions, deletions, point mutations) or cancer-associated chromosomal rearrangements (e.g., segmental deletions, translocations, inversions) (Blasco et al. 2014; Choi and Meyerson 2014; Ghezraoui et al. 2014; Han et al. 2017; Vanoli et al. 2017) motivated several groups to build systems for somatic editing directly in tissues of living animals (Fig. 3; for reviews, see Sánchez-Rivera and Jacks 2015; Ventura and Dow 2018). Early studies showed that transient delivery of Cas9 and gRNA-containing DNA plasmids to the livers of adult mice via hydrodynamic tail vein injection (HTVI) could be used to generate liver cancers with diverse oncogenic drivers (Xue et al. 2014). Similarly, stable delivery of "all-in-one" (Cas9 and gRNA) CRISPR lentiviruses to the lung (Blasco et al. 2014; Platt et al. 2014; Sánchez-Rivera et al. 2014), pancreas (Chiou et al. 2015; Mazur et al. 2015), colon (Roper et al. 2017), and breast (Annunziato et al. 2016) of adult mice leads to de novo development of ge-

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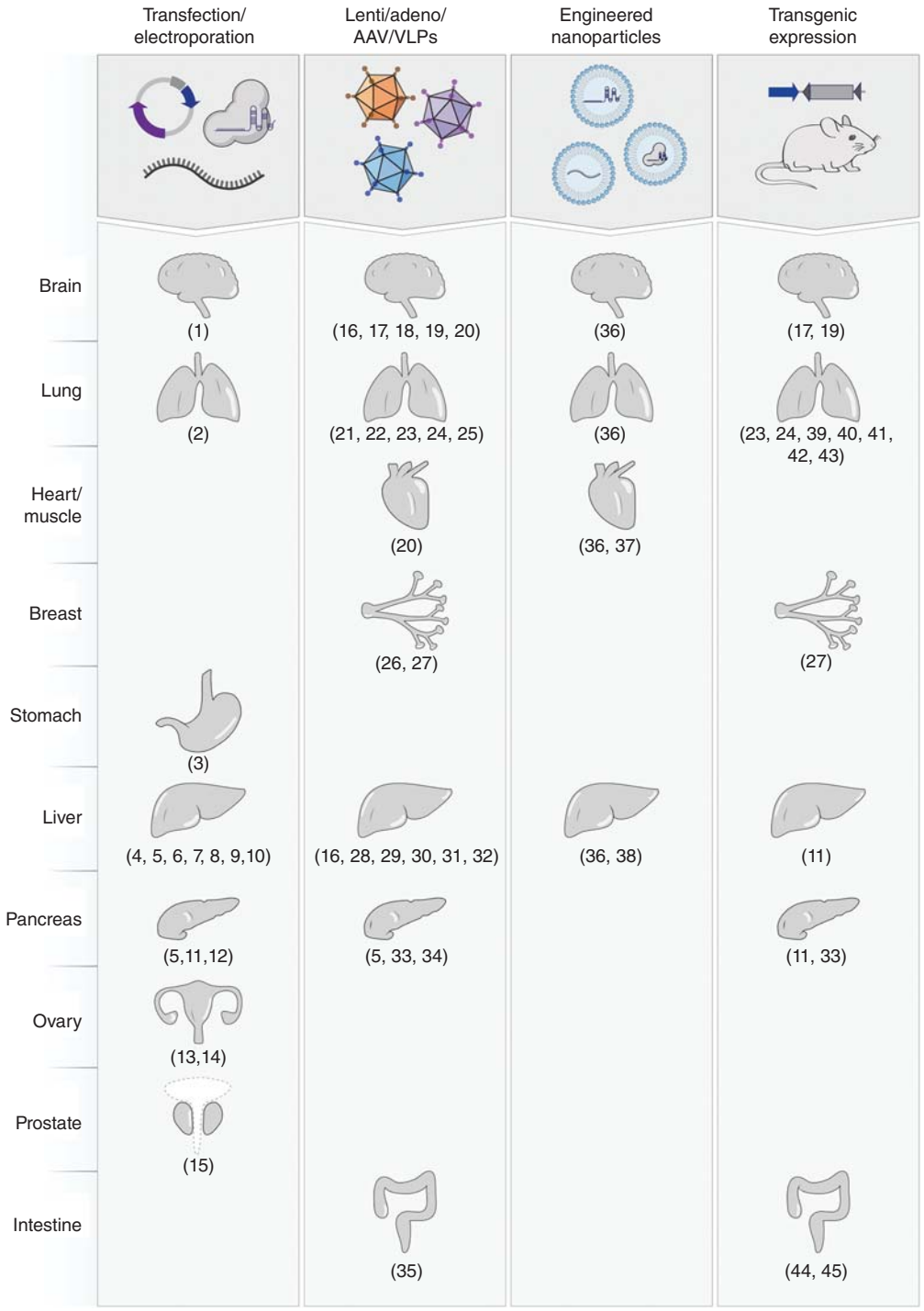

Figure 3. Modalities for somatic editing in different tissues in mice. Numbered references are provided as example cases but do not reflect an exhaustive list of all mouse modeling with indicated modalities. Numbers correspond to the references as follows: 1. Zuckermann et al. 2015; 2. Alapati et al. 2019; 3. Leibold et al. 2022; 4. Edraki et al. 2019; 5. Kaltenbacher et al. 2022; 6. Kastenhuber et al. 2017; 7. Seehawer et al. 2018; 8. Xue et al. 2014; 9. Zafra et al. 2018; 10. Zheng et al. 2022; 11. Katti et al. 2022b; 12. Maresch et al. 2016; 13. Paffenholz et al. 2022; 14. Teng et al. 2021; 15. Leibold et al. 2020; 16. Banskota et al. 2022; 17. Chow et al. 2017; 18. Chow et al. 2017; 19. Hu et al. 2021; 20. Levy et al. 2020; 21. Blasco et al. 2014; 22. Maddalo et al. 2014; 23. Ng et al. 2020; 24. Platt et al. 2014; 25. Sanchez-Rivera et al. 2014; 26. Annunziato et al. 2016; 27. Annunziato et al. 2020; 28. Jia et al. 2022; 29. Keys and Knouse 2022; 30. Rossidis et al. 2018; 31. Wang et al. 2015; 32. Wang et al. 2018; 33. Chiou et al. 2015; 34. Mazur et al. 2015; 35. Roper et al. 2017; 36. Wei et al. 2020; 37. Lee et al. 2017; 38. Mangeot et al. 2019; 39. Ely et al. 2023; 40. Rogers et al. 2017; 41. Rogers et al. 2018; 42. Winters et al. 2017; 43. Yang et al. 2022; 44. Dow et al. 2015; 45. Han et al. 2017.

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netically defined primary tumors in otherwise wild-type host tissues. Alternatively, CRISPR components can be inducibly expressed from genomically integrated alleles (Platt et al. 2014; Dow et al. 2015), avoiding potential immunogenic effects of Cas proteins (Annunziato et al. 2016; Simhadri et al. 2018; Charlesworth et al. 2019; Ferdosi et al. 2019; Wagner et al. 2019; Dubrot et al. 2021), or be transiently delivered to mouse tissues in utero (Fig. 2) or adult organs (Fig. 3) using nonintegrating viral vectors like adenoviruses (Maddalo et al. 2014; Wang et al. 2015; Rossidis et al. 2018) and AAVs (Platt et al. 2014; Chow et al. 2017; Wang et al. 2018; Hu et al. 2021; Kaltenbacher et al. 2022), VLPs (Mangeot et al. 2019; Banskota et al. 2022), nanoparticles (Lee et al. 2017; Mangeot et al. 2019; Wei et al. 2020), and even in naked plasmid format via direct organ electroporation (Zuckermann et al. 2015; Maresch et al. 2016; Seehawer et al. 2018; Alapati et al. 2019; Leibold et al. 2020, 2022; Teng et al. 2021; Kaltenbacher et al. 2022; Katti et al. 2022b; Paffenholz et al. 2022). There are now multiple examples of such electroporation-based GEMMs (EPO-GEMMs) (Kaltenbacher et al. 2022), including pancreatic (Maresch et al. 2016; Katti et al. 2022b), liver (Seehawer et al. 2018), prostate (Leibold et al. 2020), ovarian (Teng et al. 2021; Paffenholz et al. 2022), and gastric (Leibold et al. 2022) cancer models. These and other strategies can also be combined. For instance, plasmid-based somatic CRISPR engineering can be combined with sleeping beauty transposons to build immunocompetent liver cancer GEMMs expressing defined model antigens (Ruiz de Galarreta et al. 2019).

Chromosome Engineering

One specific area of somatic genome engineering where CRISPR has had an outsized impact is the creation and interrogation of large CNAs. For instance, specific chromosomal regions, some of which contain thousands of genetically linked genes and regulatory elements, are recurrently deleted, amplified, translocated, or otherwise rearranged across many human cancers (Mitelman 2000; Beroukhim et al. 2010; Zack et al. 2013). Whether some (or all) genes and genomic regions located in recurrently deleted or amplified chromosome regions play causal roles in cancer phenotypes has been very difficult to assess. In part, this is due to technical limitations of chromosome engineering methods, but also reflects the lack of available cell-based models for CNAs that are seen in a small fraction of cancers (Alonso and Dow 2021). In this context, CRISPR is a critical tool for building model systems to engineer and study rare but potentially targetable genomic variants.

Before CRISPR, modeling chromosome rearrangements in vivo required complex, time-consuming rounds of sequential gene targeting in mESCs to introduce recognition motifs for site-specific recombinases (e.g., *loxP* sites for Cre) or homing endonucleases (e.g., I-Sce I) (Kmita et al. 2000; Langer et al. 2002; Smith et al. 2002; Zhang and Lutz 2002; Forster et al. 2003, 2005; Oberdoerffer et al. 2003; Adams et al. 2004; Bagchi et al. 2007; Wallace et al. 2007; Mangeot et al. 2019). Post-CRISPR, there was an explosion of chromosome engineering studies in mouse and human cells, as well as in vivo, showing that combining Cas9 with a pair of gRNAs could be used to engineer chromosomal deletions, inversions, and translocations (Xiao et al. 2013; Essletzbichler et al. 2014; Ghezraoui et al. 2014; Han et al. 2014; Maddalo et al. 2014; Torres et al. 2014; Lagutina et al. 2015; Li et al. 2015; Lekomtsev et al. 2016; Zhu et al. 2016; Han et al. 2017; Kastenhuber et al. 2017; Spraggon et al. 2017; Cullot et al. 2019; Xu et al. 2022). Further, recent methods like MACHETE (Barriga et al. 2022) can significantly increase chromosome deletion efficiency and precision through positive–negative selection strategies. MACHETE uses CRISPR-based targeting in two sequential steps. The first step establishes the cell model, using CRISPR-driven HDR to knock in a synthetic cassette carrying positive (e.g., puromycin resistance) and negative (e.g., DTR) selection markers into a defined genome locus. The second step allows CRISPR-mediated deletion of a genomic region harboring the cassette, and enrichment of those modified cells by removing nondeleted cells through negative selection. Deletions of different sizes (including megabase-scale deletions) can be engineered by simply

varying the pair of sgRNAs used for the negative selection step. On the other hand, engineering chromosomal amplifications (including extrachromosomal circular DNA [eccDNA]) remains notoriously difficult, and most studies have employed artificial transgene-based approaches (Noer et al. 2022). While CRISPRa strategies are an option for driving endogenous overexpression, such approaches do not mimic the cellular (e.g., micronuclei formation) and genomic structural changes, including coamplified chromosomal segments that accompany most CNAs of this type, some of which have been shown to uniquely influence phenotypes associated with metastasis and immune surveillance (Harding et al. 2017; Mackenzie et al. 2017; Bakhoum et al. 2018; Wang et al. 2021). Beyond subchromosomal CNAs, new CRISPR-based methods to manipulate entire chromosomes could be used to engineer and study specific cancer aneuploidies. For instance, a method called KaryoCreate (karyotype CRISPR engineered aneuploidy technology) combines sgRNAs targeting chromosome-specific centromeric α-satellite repeats with dCas9 fused to the KNL1 kinetochore protein to engineer chromosome-specific aneuploidies (Bosco et al. 2023).

The recent discovery and engineering of new DNA integrases that exhibit modular compatibility with other CRISPR-based platforms like prime editing (PE) may offer a strategy to more efficiently manipulate large genomic segments (Durrant et al. 2022; Yarnall et al. 2022). For instance, the PASTE platform (Yarnall et al. 2022) combines DNA integrases with PE to insert specific integrase-recognition sites followed by integrase-mediated introduction of DNA fragments of interest. In principle, this approach could be coupled with positive–negative selection strategies to significantly boost integration efficiency and drive robust engineering of both chromosomal deletions and amplifications. Human tumors typically harbor dozens of CNAs that can impact up to a third of the genome (Mitelman 2000; Beroukhim et al. 2010; Zack et al. 2013). Thus, understanding which CNAs are critical drivers in human cancer and the molecular mechanisms by which they do so is of both fundamental and clinical importance.

Mo' CRISPR, Mo' Models

The above studies have collectively signaled a new direction for the creation of genetically defined mouse models of cancer. Generating complex—but specific—cancer genotypes without the need for creation or extensive breeding of pre-engineered murine alleles have enabled a wave of in vivo somatic functional genomic screens that aim to define true genetic driver mutations among the hundreds that have been identified from tumor genome sequencing efforts (for review, see Winters et al. 2018). Effective multiplexed in vivo engineering of loss-of-function (LOF) genetic alterations or modulation of gene expression has been demonstrated using lentiviral (Chow et al. 2017; Rogers et al. 2017, 2018; Cai et al. 2021; Foggetti et al. 2021; Blair et al. 2022; Keys and Knouse 2022; Yousefi et al. 2022; Lee et al. 2023), AAV (Chow et al. 2017; Wang et al. 2018), and plasmid-based (Weber et al. 2015; Maresch et al. 2016; Xu et al. 2017; Jia et al. 2022) pools of CRISPR constructs or multifunctional Cas12a systems (Breinig et al. 2019), while smaller, focused AAV libraries have also been used to screen specific oncogenic SNVs via multiplexed HDR (Winters et al. 2017). In combination with genetic barcoding and computational tools, orthogonal CRISPR-based effectors capable of multiplexed gene inhibition (Gilbert et al. 2013; Qi et al. 2013), activation (Maeder et al. 2013; Mali et al. 2013; Perez-Pinera et al. 2013; Gilbert et al. 2014), chromatin modification (Hilton et al. 2015; Kearns et al. 2015; Amabile et al. 2016; Cano-Rodriguez et al. 2016; Choudhury et al. 2016; Liu et al. 2016; Vojta et al. 2016; Xu et al. 2016; Bennett-Baker and Mueller 2017; Lei et al. 2017; Nuñez et al. 2021), or other types of genome/transcriptome engineering (Bartolomé et al. 2015; Komor et al. 2016; Abudayyeh et al. 2017; Cox et al. 2017; Gaudelli et al. 2017; Konermann et al. 2018; Anzalone et al. 2019, 2020; Xu et al. 2020; Chen et al. 2021; Koblan et al. 2021; Kurt et al. 2021; Li et al. 2021a; Chen and Liu 2022; Chen et al. 2022; Nelson et al. 2022; Neugebauer et al. 2022) will allow systematic and quantitative assessment of the oncogenicity of recurrently altered single genes or gene combinations (Rogers et al. 2017;

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Winters et al. 2018; Blair et al. 2022; Lee et al. 2023). A comprehensive overview of multiplexed and quantitative cancer modeling in the mouse is covered in the review by Tang et al. (2023).

TEACHING A NEW DOG NEW TRICKS

While tumors exhibit substantial genetic and genomic complexity, including the large structural rearrangements, insertions, and deletions (discussed above), most recurrent mutations across cancer genomes are SNVs (Alexandrov et al. 2013, 2020). Contrary to mouse knockout alleles, SNVs rarely induce complete gene loss and, in many cases, produce hypomorphic, GOF, or even neomorphic changes to protein function (Lek et al. 2016; Hopf et al. 2017; Hess et al. 2019; Muiños et al. 2021). For instance, mounting evidence suggests that different cancer-associated SNVs in genes like *KRAS* and *TP53* can produce significantly different cellular and molecular phenotypes depending on the precise alteration and the context in which they arise (Winters et al. 2017; Kotler et al. 2018; Boettcher et al. 2019; Poulin et al. 2019; Gorelick et al. 2020; Zafra et al. 2020; Hassin et al. 2022; Sánchez-Rivera et al. 2022; Ursu et al. 2022). Understanding the impact of these diverse alterations requires empirical testing, and the mouse is an ideal platform to do this.

As outlined above, CRISPR nuclease activity-based strategies can be used to engineer specific mutations like SNVs, but they have important limitations. Although certain Cas9 nuclease: gRNA combinations can lead to reproducible installations of specific mutations via NHEJ or MMEJ (Shen et al. 2018; Chen et al. 2019), most editing outcomes produce small indels and SNVs are exceedingly rare. Although HDR offers a path to engineer precise SNVs, it remains difficult to multiplex across different genomic targets and, as efficiencies are typically low, most edits are heterozygous. Both of these approaches also rely on the introduction of DSBs, which can be toxic in many cell types (Wang et al. 2014; Aguirre et al. 2016) and in vivo (Chan et al. 2019). Fortunately, the development of CRISPR-based "precision genome editing" technologies like base editing (BE) (Komor et al. 2016; Gaudelli et al. 2017) and PE (Komor et al. 2016; Gaudelli et al. 2017; Anzalone et al. 2019; for reviews, see Anzalone et al. 2020; Chen and Liu 2022) offer an effective alternative to nuclease-based systems and may change the landscape of cancer model generation in mice.

Base Editing Enters the Chat

CRISPR BE enzymes are a direct fusion between DSB-deficient Cas proteins (e.g., Cas9n or "nickase") and DNA-modifying enzymes such as APOBEC (Komor et al. 2016), AID (Hess et al. 2016; Ma et al. 2016; Nishida et al. 2016; for review, see Anzalone et al. 2020), or engineered variants such as TadA (Richter et al. 2020; Chen et al. 2022; Neugebauer et al. 2022). When targeted to a genomic site, BEs install C·G to T·A (cytosine base editors [CBEs]), A·T to G·C (adenine base editors [ABEs]), or C·G to G·C (CGBEs) (Koblan et al. 2021; Kurt et al. 2021) mutations within a small but variable window of the protospacer. Given the modular nature of these systems, recent studies have reported new BE variants with narrower (Kim et al. 2017c; Liu et al. 2020) or wider (Hess et al. 2016; Jiang et al. 2018; Thuronyi et al. 2019) targeting windows, broader PAM compatibility (Kleinstiver et al. 2015a,b; Gao et al. 2017; Hu et al. 2018b; Nishimasu et al. 2018; Kim et al. 2020; Miller et al. 2020; Walton et al. 2020), and dual-activity variants capable of performing both cytosine and adenine BE (Grünewald et al. 2020; Li et al. 2020; Zhang et al. 2020; Chen et al. 2022; Neugebauer et al. 2022). BE platforms continue to evolve quite rapidly, giving rise to a diverse menu of flexible base editors (Anzalone et al. 2020).

Many studies have demonstrated the power of BE to introduce precise SNVs across a variety of mammalian systems, including stem cells, cancer cell lines, and organoids (Anzalone et al. 2020). In mice, BE has been used effectively in fertilized zygotes and in utero to create germline mutations in a variety of genes and generate non-cancer models (Kim et al. 2017b; Liang et al. 2017; Bester et al. 2018; Li et al. 2018; Sasaguri et al. 2018; Zhang et al. 2018; Lee et al. 2019; Bose

et al. 2021; Caso and Davies 2022). We used BE mRNA in zygotes to engineer a recurrent nonsense mutation in the *Apc* tumor-suppressor gene, revealing how distinct Apc truncations dictate responses to WNT pathway inhibitors in vivo (Schatoff et al. 2019). A similar approach was used in pancreatic organoids to demonstrate that mutant *Trp53* expression and *Trp53* loss of heterozygosity cooperate to regulate the mevalonate pathway in pancreatic cancer (Oni et al. 2020).

In vivo somatic BE has been adapted to model liver (Zafra et al. 2018; Katti et al. 2022b), breast (Annunziato et al. 2020), and pancreatic cancer (Katti et al. 2022b), among others. Nevertheless, the large size of base editors has somewhat restricted their in vivo application and prompted the development of split enzymes that can be more easily packaged in viral vector systems (Guo et al. 2015; Nishimasu et al. 2018; Villiger et al. 2018; Bajaj et al. 2020; Levy et al. 2020; Berríos et al. 2021). Like their original counterparts, split BE AAVs can induce SNVs in hepatocytes, muscles, eyes, and the brain of mice (Fig. 3; Levy et al. 2020). Although no studies to date have used AAV-BEs to model cancer development in vivo, they are only theoretically limited by possible routes of delivery and potential immunogenicity of Cas proteins (Annunziato et al. 2016; Simhadri et al. 2018; Charlesworth et al. 2019; Ferdosi et al. 2019; Wagner et al. 2019; Dubrot et al. 2021). An alternate approach to viral editor expression is conditional BE transgene expression. For instance, Jonkers and colleagues created a Cre-inducible BE3 allele and showed that it could be used to engineer both oncogene and tumor-suppressor mutations in the mammary gland (Annunziato et al. 2020).

In both AAV and constitutive transgene models, sustained expression of the BE enzyme has potential drawbacks, increasing the risk of off-target mutations (Grünewald et al. 2019a,b; Rees et al. 2019; Katti et al. 2020; Yu et al. 2020; Yan et al. 2023) and unwanted "on-target" indels (Annunziato et al. 2020). To circumvent these issues, we recently reported a doxycycline (dox)-inducible "iBE" mouse allele (Katti et al. 2022b) that enables widespread and transient induction of target gene editing across multiple murine tissues and organoids. We showed that reversible dox-mediated induction of an expression-optimized BE enzyme can be used to rapidly generate somatic, genetically defined primary tumors in the livers and pancreata of adult mice using a combination of plasmid DNA constructs and synthetic sgRNAs (Katti et al. 2022b). In contrast to mice with constitutive expression of the BE3 enzyme (Yan et al. 2023), iBE mice showed no increase in unwanted C > T DNA editing and low-level, transient RNA editing. The ability to control BE activity in systems like this offers the opportunity for sequential or multistep genome editing, thereby mimicking the stepwise acquisition of mutations as cancers evolve. Non-integrating VLPs encoding base editors will complement these approaches by allowing transient in vitro and in vivo genome engineering (Joung et al. 2020; Syed et al. 2021; Banskota et al. 2022). With continued evolution of these and other technologies, we expect BE will become a go-to technology to build cancer models via multiplexed and/or multistep gene editing.

Is It Prime Time for Mouse Models?

PE (Anzalone et al. 2019; Chen et al. 2021; Chen and Liu 2022; Nelson et al. 2022) is a particularly powerful precision genome-editing technology that can, in theory, be used to engineer any type of focal genetic alteration. Compared to the gRNA architecture of standard CRISPR systems, which mainly consist of a variable 17–21-nucleotide-long sequence in a fixed scaffold, pegRNAs harbor an additional 3′ extension region (Anzalone et al. 2019; Chen et al. 2021; Chen and Liu 2022; Nelson et al. 2022) that serves as a primer for reverse transcription and extension of a template that encodes the mutation of interest (Anzalone et al. 2019). There are many features that go into pegRNA design, and the rules that dictate maximum pegRNA efficiency and precision are the subject of active investigation (Bhagwat et al. 2020; Anderson et al. 2021; Chow et al. 2021b; Hsu et al. 2021; Hwang et al. 2021; Li et al. 2021b; Morris et al. 2021; Standage-Beier et al. 2021; Gould and Sánchez-Rivera 2022).

Despite having more "moving parts" than standard CRISPR approaches, PE has been quick-

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ly deployed across various in vitro and in vivo contexts to engineer a range of SNVs and indels (for review, see Chen and Liu 2022). In fact, while current PE efficiencies remain lower, almost every BE application described above has already been demonstrated using PE, including precision genome engineering of cell lines, organoids, mESCs, zygotes, and even somatic tissues in adult mice (Abbosh et al. 2017; Anzalone et al. 2019; Aird et al. 2021; Liu et al. 2021, 2022; Gao et al. 2022; Jang et al. 2022; Jiang et al. 2022a,b; Zheng et al. 2022; Ely et al. 2023). Illustrating the versatility of this technology, several groups have developed variants of PE that use two or more pegRNAs to engineer defined indels and structural genomic rearrangements of significant size (Lin et al. 2021; Anzalone et al. 2022; Choi et al. 2022b; Jiang et al. 2022a; Kweon et al. 2022; Tao et al. 2022a,b; Wang et al. 2022; Zhuang et al. 2022). In addition, there are hybrid platforms that combine prime editors and site-specific serine integrases to perform programmable insertion and deletion of specific DNA payloads (Anzalone et al. 2022; Yarnall et al. 2022), paving the way for precision chromosome-level engineering.

Like BEs, several groups have also developed split PEs that can be efficiently coexpressed in vitro or in vivo using plasmids, RNA, and/or AAVs (Abbosh et al. 2017; Aird et al. 2021; Gao et al. 2022; Jiang et al. 2022b). Most in vivo studies to date have focused on somatic engineering or correction of disease-associated mutations (Anzalone et al. 2019; Liu et al. 2021; Jang et al. 2022; Jiang et al. 2022a; Liu et al. 2022; Zheng et al. 2022), although one study has already demonstrated that PE delivery by HTVI can be used to model liver cancer in mice (Liu et al. 2021). Ely et al. (2023) constructed a GEMM carrying a Cre-inducible prime editor and used it to generate mice harboring autochthonous tumors driven by diverse *Kras* and *Trp53* mutant SNVs in vivo.

Engineered Transplant Systems: Taking the Model out of the Mouse

Germline and somatic GEMMs remain a gold standard in mouse modeling, but even with CRISPR, they still trail ex vivo systems in speed and flexibility. In many cases, syngeneic cell and organoid transplant models provide a happy medium of in vitro flexibility with in vivo physiology. Ex vivo genome engineering of cells or organoids followed by subcutaneous or orthotopic engraftment can be used to rapidly generate new genetically defined cancer mouse models (Sánchez-Rivera and Jacks 2015). Indeed, CRISPR-mediated engineering of mutations or chromosomal rearrangements has been used to build transplantable models of lymphoma and leukemia (Malina et al. 2013; Chen et al. 2014; Heckl et al. 2014), as well as colorectal (Drost et al. 2015; Matano et al. 2015; O'Rourke et al. 2017; Roper et al. 2017; Han et al. 2020a), breast (Dekkers et al. 2020), pancreas (Zafra et al. 2020), prostate (Feng et al. 2021), lung (Ng et al. 2020; Ciampricotti et al. 2021; Naranjo et al. 2022), and brain cancer (Cook et al. 2017; Ogawa et al. 2018). The key advantages of these models are that they are typically fast, scalable, homogeneous, and synchronizable. They are also flexible in that cells can be transplanted at various anatomical sites, including subcutaneous, orthotopically into the organ of interest, or seeded into the circulation or metastatic site. Further, in a transplant setting, recipient mice can be varied (e.g., immunocompetent vs. immunodeficient recipients) to study the contribution of nontumor cells to malignant progression or therapy response. In addition to transplantation of engineered culture models, Poirier and colleagues demonstrated the use of CRISPR to directly engineer the genome of patient-derived xenografts (PDXs) without extended ex vivo culture, providing much needed flexibility for interrogating the genetic mechanisms of cancer progression and therapy responses in human cancer models in vivo (Hulton et al. 2020).

Genome engineering in transplant models can be achieved using transient or stable methods. In vitro transcribed (IVT) mRNA preparations or purified recombinant proteins encoding genome editors like Cas9 can be coupled with chemically modified synthetic sgRNAs to perform transient genome engineering in cells and organoids followed by transplantation into recipient mice to generate genotype-specific cancer models. Nonintegrating adenoviruses (Mad-

dalo et al. 2014; Wang et al. 2015; Rossidis et al. 2018), AAVs (Platt et al. 2014; Chow et al. 2017; Wang et al. 2018; Hu et al. 2021; Kaltenbacher et al. 2022), integration-defective lentivirus (Ortinski et al. 2017; Hu et al. 2018a; Apolonia 2020), and VLPs encoding single or multiple genome-editing components can also be used to perform transient genome engineering. Alternatively, integrating viral vectors like retroviruses and lentiviruses encoding genome-editing components can be used to perform stable genome engineering in cells and organoids. While both approaches work, it is important to consider that transient editing avoids two critical issues that are relevant to in vivo modeling: (1) stable expression of genome editing machinery (e.g., Cas9s, BEs, and PEs) can cause subtle but meaningful changes in cellular fitness (Enache et al. 2020), and (2) Cas proteins can drive adaptive immune responses in immunocompetent mice (Annunziato et al. 2016; Simhadri et al. 2018; Charlesworth et al. 2019; Ferdosi et al. 2019; Wagner et al. 2019; Dubrot et al. 2021). In principle, transient editing also offers the advantage of virtually unlimited iterative editing of endogenous genes, which has been exploited by some groups for modeling multistep colorectal tumorigenesis (Drost et al. 2015; Matano et al. 2015) and, more recently, to develop genetically complex organoid cultures to produce new mouse models of epithelial cancers (Han et al. 2020a; Naranjo et al. 2022).

THE NEXT FRONTIER OF CRISPR-BASED MOUSE MODELING

Welcome to the post-CRISPR era. An enormous body of work over the past decade has built a powerful and growing collection of diverse CRISPR-based molecular effectors that can be used for cancer research. Now, in cells, organoids, zygotes, tissues, and even entire organisms, it is possible to disrupt, delete, inhibit, activate, modify, or otherwise mutate any coding or noncoding region of the mammalian genome with incredible precision. Better still, these technologies can be mixed and matched in increasingly sophisticated ways. So, what advances should we expect during the next wave of CRISPR innovation and how will they impact cancer research?

High-Throughput Quantitative Interrogation of Specific Cancer Mutations

Single-nucleotide editing technologies (e.g., BE and PE) have enormous potential for creating models that more faithfully recapitulate the vast majority of mutations seen in human cancers. To date, these tools have not been adapted for combinatorial genome editing in vivo, but the success of multiplexed nuclease-based methods like Tuba-seq (Rogers et al. 2017; Winters et al. 2018; Blair et al. 2022; Lee et al. 2023) and the recent implementation of both BE and PE for high-throughput genetic screens in mammalian cells (Cuella-Martin et al. 2021; Hanna et al. 2021; Erwood et al. 2022; Kim et al. 2022; Sánchez-Rivera et al. 2022) suggests a straight path moving forward. For instance, plasmid or viral pools of BE sgRNAs or pegRNAs designed to engineer specific cancer-associated SNVs could be delivered somatically to different tissues of adult mice followed by quantitative assessment of fitness properties produced by different variants in resulting tumors.

While stable integration and sequencing of sgRNAs, pegRNAs, or guide-specific barcodes in principle allow easier identification and quantification of specific mutations, nonintegrating strategies using plasmid and AAV pools could also be coupled with multiplexed, targeted capture sequencing of target loci (Chow et al. 2017). Thus, instead of only assessing the effects of LOF of hundreds, or even thousands of genes using the Cas9 nuclease, precision genome-editing technologies provide an opportunity to interrogate patient-specific mutations in vivo, including potential hypomorphic, GOF, neomorphic, and separation-of-function alleles observed in oncogenes, tumor-suppressor genes, and other types of coding and noncoding cancer-associated loci. These methods could also be used to engineer and study potential neo-epitopes produced as a byproduct of both driver and passenger mutations. Collectively, these applications will be critical to assess variant-specific biology across diverse contexts, including different genetic

backgrounds, immunocompetent versus immunodeficient hosts, and in the context of chemotherapies, targeted therapies, and immunotherapies.

Charting Tumor Evolution through Space and Time Using CRISPR Molecular Recording

Tumors are dynamic and heterogeneous ecosystems that exhibit significant intratumoral heterogeneity and can be composed of a diversity of phenotypically plastic cell types and cell "states" that arise over time (Lawson et al. 2018; Gupta et al. 2019). Achieving a complete and dynamic understanding of how cancer cells transition between different states (or fates) in vivo and how this can vary depending on the context (e.g., tissue type, therapy) requires integrative experimental and computational approaches (Sankaran et al. 2022). While bulk genome-profiling studies can be used to map the genetic and molecular diversity present within tumors, they provide little functional information on how specific perturbations (e.g., mutations) dynamically influence the evolving structure of these ecosystems. Cell and tissue atlases constructed using single-cell RNA sequencing (scRNA-seq) have proven invaluable for understanding cellular complexity in vivo, although these approaches only provide a static snapshot of a given biological state and provide little information on genetic relationships (Wagner et al. 2016; Regev et al. 2017; Tanay and Regev 2017; Schiller et al. 2019; Taylor et al. 2019; Han et al. 2020b; LaFave et al. 2020; Marjanovic et al. 2020). Further, although mitochondrial DNA variation has recently emerged as a powerful, naturally evolving barcode for retrospective lineage tracing in humans (Ludwig et al. 2019; Xu et al. 2019), the inability to engineer additional molecular recording diversity over time limits its utility in models with low mitochondrial genetic variation.

Exploiting the ability of genome-editing technologies to "write" information into the genome, CRISPR systems (often in combination with scRNA-seq) have been coopted to perform quantitative recording, measurement, and reconstruction of phylogenetic and transcriptional relationships within tissues, tumors, and even entire organisms at single-cell resolution (Fig. 4; McKenna et al. 2016; Frieda et al. 2017; Alemany et al. 2018; Kalhor et al. 2018; Raj et al. 2018; Spanjaard et al. 2018; Tang and Liu 2018; Chan et al. 2019; Bowling et al. 2020; Zafar et al. 2020; Chow et al. 2021a; Loveless et al. 2021b; Quinn et al. 2021; Simeonov et al. 2021; Choi et al. 2022a; Yang et al. 2022; for review, see Sankaran et al. 2022). These emerging technologies have already produced key insights into the mechanisms by which cancers may initiate, progress, and metastasize (Quinn et al. 2021; Simeonov et al. 2021; Yang et al. 2022). For instance, a recent study integrated an evolving, scRNA-seq-compatible CRISPR-based lineage-tracing system with a panel of Cre-inducible oncogenic *Kras*-driven GEMMs of lung cancer to chart tumor evolution from single cells, and demonstrated that transcriptional plasticity of transformed alveolar type 2 cells produces phenotypically distinct clones with very high metastatic potential (Yang et al. 2022).

Given the versatility of this technology with other types of GEMMs, future studies could explore whether germline and/or somatic variation can modulate the molecular trajectories taken by cancer cells at different stages of tumor evolution and upon distinct types of perturbation or therapeutic interventions. It is possible that specific genetic events differentially influence the number and type of cells that can follow a given trajectory, the number of available trajectories, and the plasticity of cell states along these trajectories. Work from Yang et al. (2022) suggests that different cancer driver combinations (e.g., oncogenic *Kras* mutations coupled with *Trp53*, *Apc*, or *Lkb1* mutations) can shape clonal evolution and disease progression in vivo. Lineage-tracing approaches that exploit BE and PE for molecular recording have already been developed (Tang and Liu 2018; Farzadfard et al. 2019; Askary et al. 2020; Loveless et al. 2021a; Choi et al. 2022a), setting the stage for increasingly sophisticated, temporally dynamic multi-platform cancer modeling and lineage-tracing studies.

The growing diversity of orthogonal CRISPR platforms has also opened the door for increasingly complex combinatorial genome-engineer-

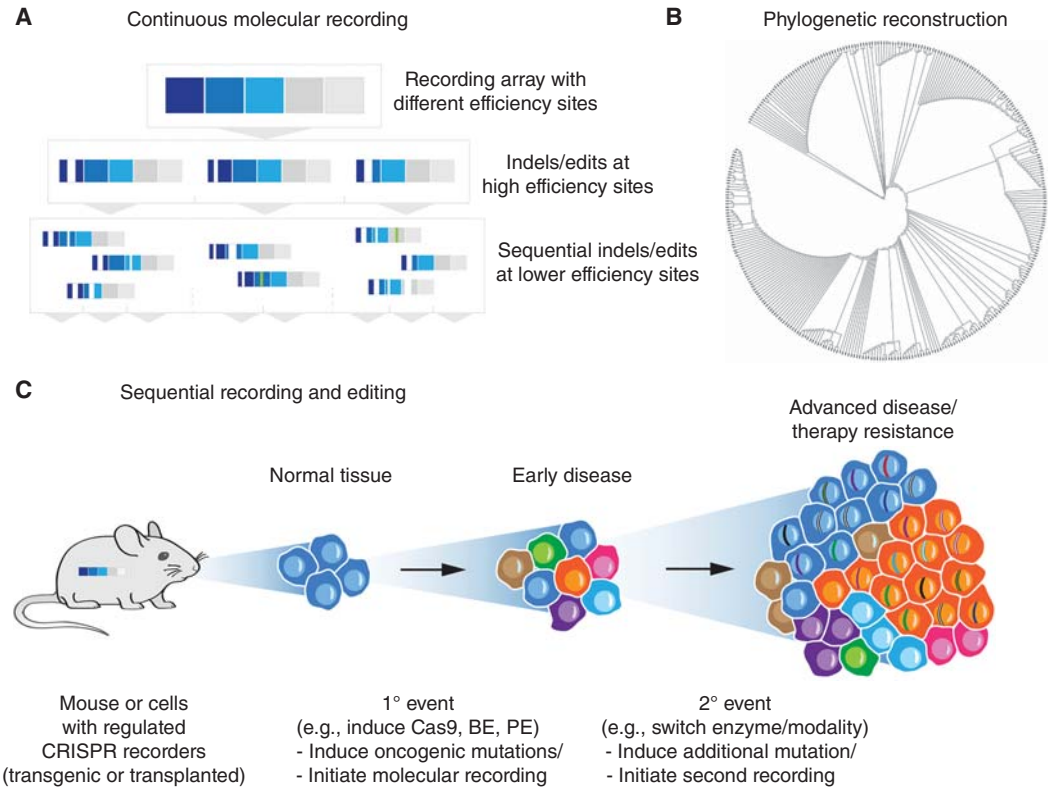

Figure 4. Molecular recording in mouse cancer models using CRISPR. (*A*) Schematic of a CRISPR molecular recording module where different colors represent different efficiency of Cas9 targeting. White and green changes represent deletions and insertions that accumulate over time. (*B*) Example of phylogenetic reconstruction from evolving molecular barcodes in a tumor engraftment model. CRISPR-based recording enables reconstruction of cell lineages with high resolution. (*C*) Hypothetical example of using inducible or orthogonal CRISPR systems in mice to enable temporal mutation and/or molecular recording. In this example, cell lineage changes can be linked to specific genetic events induced by the same CRISPR tools used for molecular recording.

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ing approaches that could be used to perform different types of molecular operations in vivo in a spatiotemporal manner. As an example, combining different types of CRISPR effectors that recognize different PAM sequences could be used to perform multiple independent molecular operations in cells that coexpress these molecules (Fig. 4). These operations can be temporally uncoupled if the expression or activity of one or more CRISPR effectors is inducible.

For instance, one could initiate and trace the early development of tumors harboring a defined genetic event followed by inducible engineering of a second genetic event that is coupled to activation of a second molecular recorder to trace subclonal expansion (Fig. 4). Double-traced tumor-bearing mice could then be perturbed genetically or pharmacologically while concomitantly activating a third molecular recorder to accurately identify and distinguish cells and phylogenetic lineages that respond or not to a given perturbation. Taking it a step further, we speculate that these approaches will be combined with emerging spatial genomics technologies like MERFISH (Canver et al. 2015), epigenomic MERFISH (Abbosh et al. 2017), DNA seqFISH^{+} (Takei et al. 2021), in situ genome sequencing (Payne et al. 2021), and many others (Zhuang 2021), including functional screening methods (Feldman et al. 2019, 2022; Dhainaut et al. 2022;

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Funk et al. 2022). Such studies could provide important quantitative insights into cancer cell plasticity and the degree of resilience present within complex tumor ecosystems composed of genetically, phenotypically, and geographically heterogeneous cells that fuel tumor evolution, maintenance, and therapeutic resistance.

OUTLOOK

We are amid a golden age of functional genomics in the mouse. Through CRISPR technologies, we have the potential to engineer and interrogate any and every type of mutation observed or hypothesized to play a role in cancer. Cancer-associated genes are no longer studied exclusively through the lens of knockout phenotypes but are interrogated as they exist in cancer cells. Moreover, cancer phenotypes can now be studied in physiologically relevant settings using dynamic quantitative approaches like molecular recording and single-cell CRISPR screening. These could be combined with orthogonal CRISPR-based technologies to dynamically chart in vivo tumor evolution with single-cell resolution and elucidate the different ways through which specific mutations regulate tumor development, progression, and therapy responses. We expect the approaches described here will not only continue to expand the power and utility of mice in cancer research, but also allow the rapid development of nontraditional animal models for cancer research. Together, CRISPR-based animal models will have a major role to play in understanding cancer and developing the next generation of effective treatment options.

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