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CRISPR–Cas-related technologies in basic and translational liver research

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Abstract

CRISPR–Cas9 has revolutionized biomedical research. Studies in the past few years have achieved notable successes in hepatology, such as correction of genetic disease genes and generation of liver cancer animal models. Where does this technology stand at the frontier of basic and translational liver research?

CRISPR genome editing tools are paving the way for new innovation in liver research[1,2]. The highly programmable nature of CRISPR is based on the Cas9 nuclease and guide RNA (sgRNA) complex that recognizes genomic sequences with a protospacer-adjacent motif (PAM, the DNA sequence immediately following the genomic sequence targeted by Cas9). The commonly used *Streptococcus pyogenes* Cas9 (SpCas9) recognizes an 'NGG' PAM. Other Cas9 proteins such as SaCas9, NmeCas9 and Cpf1 that recognize different PAMs and/or are more compact in size have expanded the utility of CRISPR[1]. Double-stranded DNA breaks generated by Cas9 are often repaired by non-homologous end-joining (NHEJ), which induces small insertions or deletions that inactivate the target gene, or by precise repair through homology directed repair (HDR) using template DNA that is homologous to the genomic region[1]. Furthermore, dCas9 fusion proteins, such as those incorporating transcriptional domains or deaminase[3] activity, can confer on CRISPR novel functions such as base editing ability, which introduces single-base DNA changes without double-stranded DNA breaks (FIG. 1a).

Herein, we briefly explore the latest applications of CRISPR–Cas in liver research with an emphasis on *in vivo* somatic genome editing in animal models. In addition to somatic genome editing, CRISPR–Cas9 has been applied in liver cell lines and in germline liver disease models through zygote injection, although these studies are not discussed here.

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Competing interests

The authors declare no competing interests.

SUPPLEMENTARY INFORMATION

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ALL LINKS ARE ACTIVE IN THE ONLINE PDF

Although CRISPR has tremendous potential in basic liver research, translational applications remain a challenge.

Disease gene correction in mice

The most exciting translational use of CRISPR–Cas9 is the correction of mutations in genetic diseases (reviewed elsewhere[2]). This approach has the potential to provide long-term therapy after a single treatment. Various delivery vehicles and methods, such as hydrodynamic injection, adeno-associated virus (AAV) and lipid nanoparticles (LNPs), can deliver CRISPR–Cas9 and the HDR template to the liver. First, CRISPR–Cas9 can be used to correct loss-of-function mutations. HDR, microhomology-mediated end-joining (MMEJ) and metabolic reprogramming have been tested in a *Fah* mutant mouse model of tyrosinaemia (Supplementary Table 1). CRISPR is also being explored for other liver diseases. For example, X-linked deficiency of ornithine transcarbamylase has been corrected by HDR in newborn and adult mice using a dual AAV delivery system (Supplementary Table 1).

Second, CRISPR–Cas9 can be used to deplete disease-inducing genes or HBV viral DNA. For example, adenovirus, AAV and FNP delivery of Cas9, as well as base editing, have demonstrated successful *Pcsk9* depletion in mice (Supplementary Table 1). Of note, structure-guided chemical modification of single guide RNA (e-sgRNA) enables potent genome editing in the mouse liver without the use of viral vectors[4]. A single dose of FNP-encapsulated Cas9 mRNA and e-sgRNA led to >80% gene editing in the liver and substantially reduced serum cholesterol levels[4]. These studies demonstrate that CRISPR–Cas9 has the potential to treat both loss-of-function and gain-of-function liver diseases.

CRISPR for liver cancer modelling

Liver cancer is the second most lethal cancer type worldwide. A major challenge in liver cancer is to identify crucial driver genes among the vast number of mutated genes discovered by cancer genome sequencing. However, this effort has been limited by the cost of traditional mouse models and the time required to generate them. CRISPR provides a rapid and simple genetic platform to identify cancer genes and model cancer mutations in vivo[5]. CRISPR delivery via hydrodynamic injection has been used to model the two major liver cancer types in mice, hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) (Supplementary Table 1). Importantly, CRISPR can be combined with transposon or Cre–*loxP* mouse strains to establish compound models to better understand the cooperative effects of cancer genes. Notably, despite low editing efficiency, hydrodynamic injection is sufficient for generating cancer models because of the proliferation advantage of cancer cells.

Besides HCC and CCA, CRISPR has also been used to model fibrolamellar hepatocellular carcinoma (FLHCC), a rare liver cancer subtype that predominantly affects young adults (Supplementary Table 1). Hydrodynamic injections of two sgRNAs in mouse liver induced a 400 kb genomic deletion that leads to *Dnajb1–Prkaca* fusion and tumours resembling FLHCC.

In addition to single gene validation, unbiased CRISPR screens have been performed to study liver cancer. For example, an *ex vivo* CRISPR screen using a genome-wide sgRNA library newly identified *Nf1, Plxnb1, Flrt2* and *B9d1* as suppressors of liver tumour formation in mouse models[6]. Tumour *Nf1* expression is correlated with survival of patients with liver cancer. Together, these studies demonstrate the power of CRISPR in modelling various types of liver cancer and in discovering new cancer genes.

Future challenges

Despite the tremendous progress in engineering the CRISPR–Cas9 system, challenges remain.

A major issue is the delivery of CRISPR *in vivo*. Although the liver is amenable to AAV and LNP delivery, future work is required to develop safe and efficient delivery of CRISPR components and the HDR template in patients. This step will be crucial for exploring the use of CRISPR to correct liver disease genes in patients. In addition, concerns remain about the safety of CRISPR, for example, regarding the immune response to the Cas9 protein[7] and potential AAV integration at DNA breaks.

Precision genome editing requires minimal off-target effects, and therefore monitoring and reducing off-target effects is an important effort in the CRISPR field. Genome-wide analysis of Cas9 off-target sites such as GUIDE-seq has been reported to be an effective tool for this analysis[8]. Many groups have developed technologies to reduce off-target events, such as paired Cas9 nickase (single-strand cuts at off-target sites will usually be repaired seamlessly), high-fidelity Cas9 and truncated sgRNAs (for example, a 17 nt sgRNA reduces off-target compared with a regular 20 nt sgRNA). In addition to off-target editing, some sgRNAs can induce partial exon skipping or exon deletion. This risk further enforces the caution that some sgRNAs designed for loss of function can partially induce unexpected phenotypes such as new mRNA isoforms or even gain of function.

Many studies have demonstrated that the HDR rate is low compared with the NHEJ rate in the liver. For liver diseases that require a high percentage of gene correction, it remains unknown whether CRISPR can restore protein levels to a therapeutic threshold. Therefore, increasing HDR efficiency is an essential task to make CRISPR broadly applicable for liver diseases. Base editing and homology-independent targeted integration (HITI, a NHEJ-mediated gene integration at Cas9 cleavage site) are promising alternative strategies[9] (FIG. 1a,b).

With the development of new versions of CRISPR such as Casl3, which enables RNA editing[10] (FIG. 1c), the CRISPR toolbox is still expanding. This technology will continue to accelerate our understanding of liver diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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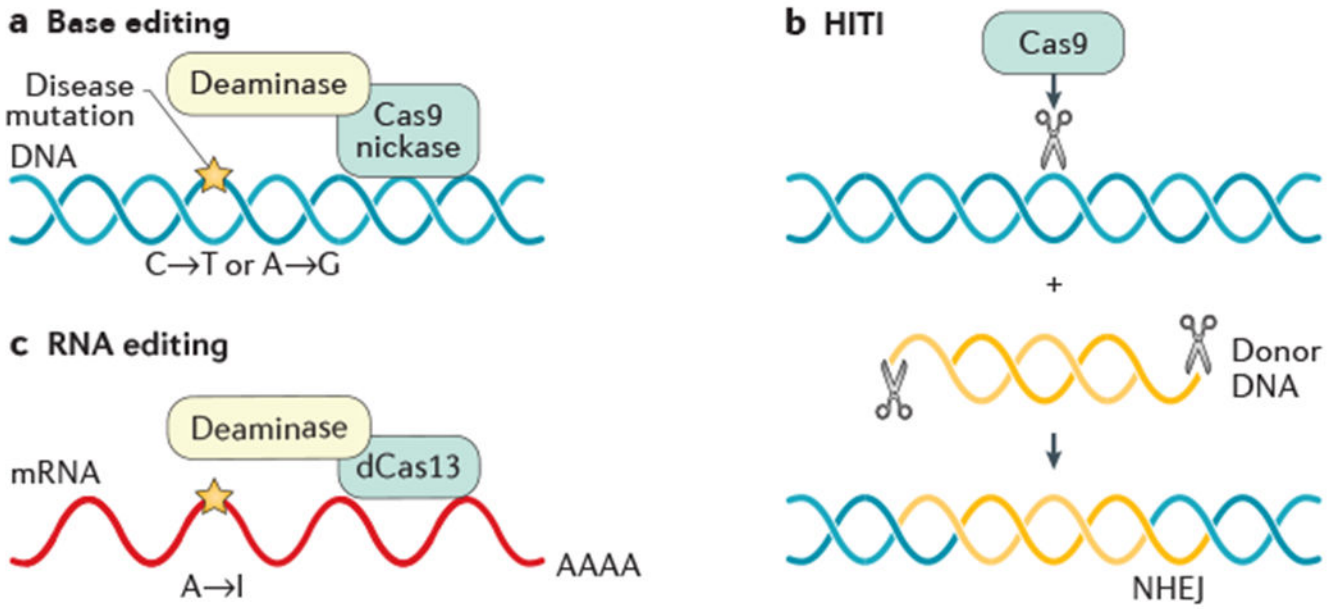

Fig. 1 |. New CRISPR–Cas9-based technologies.

a | Base editing using a Cas9–deaminase fusion. **b** | Homology-independent targeted integration. **c** | CRISPR–Cas13-mediated RNA editing. NHEJ, non-homologous end-joining.