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CRISPRing or RISKing? Dangers Arising from Gene Editing with CRISPR-Cas9

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Editorial

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Editorial

Since its first experimental demonstration in 2007 [1], CRISPR-Cas9 (short for Clustered Regularly Interspaced Short Palindromic Repeats – CRISPR-Associated protein 9) brought a revolution in biology, especially in the fields of gene editing [2] and precision medicine [3]. CRISPR offers several advantages, namely accuracy [4], flexibility [5], versatility [6] and cost reduction [7] that collectively designate it as the most popular approach for gene editing [8], supplanting in parallel other technologies such as TALENs [9] and ZFNs [10].

Similar to TALEN/ZNFs, CRISPR-Cas9 relies on the precise introduction of DSBs (Double Strand Breaks) in the target genome [11], facilitated by the interaction of complementary small RNAs, known as sgRNAs (Single Guide RNAs) with the Cas9 effector protein [12]. The sgRNA/Cas9 ribonucleoprotein complex interacts with genomic DNA via sgRNA/genomic DNA R-loop formation [13, 14], guiding wild type Cas9 to introduce DSBs via its nuclease activity in a precise manner [15]. Host genome DNA repair mechanisms, such as Non-Homologous-End Joining (NHEJ) and/or Homology-Directed Repair (HDR) recognise and repair DBS formation, resulting either in the introduction of mutating Insertions/Deletions (InDels) in the case of NHEJ [16], or precision gene editing and Single Nucleotide Polymorphism (SNP) repair in the case of HDR [17].

Targeted mutations can restrict or completely abolish cas9 nuclease activity, converting it into a nickase (nCas9, [18,19]) or a catalytically inactive protein (dCas9, [20]) that retains its DNA-interacting properties. These engineered flavours of Cas9 contribute to the reduction of its greater side-effect, generation of off-target effects [21], while expanding the repertoire of CRISPR applications from a mere gene editing tool to a technology that allows in vivo biotagging [22] and/or transcriptional transactivation/repression through direct endogenous promoter interactions [23].

Applications of CRISPR-Cas9 greatly expanded our knowledge in various organisms such as bacteria [24], yeast [25], worms [26], insects [27], plants [28] and animals [29-32], fuelling an active debate as to whether this technology is safe enough for human gene editing clinical trials [33]. The benefits are obvious: precision medicines, treatment of hereditary disease, stalling/curing of cancer progression [34], are just a few of the exciting opportunities arising from the technology [35].

However two recent papers published in Nature Medicine highlight the important biases and dangers that accompany CRISPR-Cas9 mediated editing in human cells. The first paper refers to a CRISPR-Cas9 dropout screen in retinal pigment epithelium cells (RPE1) [36]. Such screens rely on a lentiviral pool of sgRNAs, designed to target hundreds to thousands of genes, for transducing Cas9-expressing cells. The successfully transduced cells are left to propagate for a limited period before being subjected to Next Generation Sequencing (NGS)-based screening. The concept is obvious: sgRNAs targeting genes that are pivotal for the survival of cells under the screening conditions will be eliminated from the pool resulting into

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a detectable distortion in the composition of sgRNA expressing cells in the NGS results, leading towards the identification of the corresponding target genes [37].

The authors report that Cas9-induced DSS activate p53, a well known tumor-suppressor that acts as a master regulator of DNA damage response [38], leading to cell cycle arrest and introduction of a growth disadvantage that eventually shifts the balance from HDR (precision editing) to NHEJ (imperfect repair/introduction of InDels) [36]. The growth defect was chemically dampened with the use of nutlin-3a, an inhibitor of p53 [36]. However general inhibition of p53 via nutlin-3a leaves the recipient cell vulnerable to major genetic abrogations, such as chromosome rearrangement/loss, accumulation of mutations and malignant transformation, renting this approach unsuited for safe therapeutic use of CRISPR-Cas9 mediated gene editing.

In a parallel study, scientists from Novartis conducted genetics screens in human pluripotent stem cells (hPSCs). Despite of their great therapeutic potential, screens in hPSCs have been impeded on the generally low efficiency of these cells to genetic engineering [39]. CRISPR-based editing offers great precision and together with the pluripotency of hPSCs provides an attractive therapeutic tool. Strikingly, the increased mutagenic efficiency of CRISPR in the hPSCs in the case of the Novartis study was accompanied with severe toxicity leading to a dramatic decrease in the percentage of surviving cells [39]. This toxicity was tightly linked to the generation of DSBs that were induced by targeting sgRNAs, persisted even under short induction of Cas9 expression and was not observed in hPSCs transduced with scrambled sgRNAs [39]. Additional experiments narrowed down the cause of this DSB-induced toxicity again to a P53-depended action [39].

These two studies collectively highlight the risk of deploying CRISPR-Cas9 as a therapeutic gene editing tool, nailing down the cause of the observed toxicity into the role of the p53 tumor-suppressor gene. CRISPR-Cas9 holds a great therapeutic potential, calling for a need to develop novel chemical and/or genetic approaches so as to eliminate the observed p53 complications. Our designing efforts should not focus on the general inhibition of p53 since this would increase the tumorigenicity of the engineered cell. Instead we should develop compounds that transiently block p53 function and/or block Cas9 mediated genotoxic stress ensuring both efficient gene editing and subsequent p53 anti-tumorigenic protection for the engineered human stem cell.

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