cohorts of patients with CLL refractory to, or relapsing after, CD19 CAR therapy. Downregulation or loss of target antigen after CAR therapy has been observed in patients with ALL and multiple myeloma after infusion of CAR T cells targeting CD19, CD22, or BCMA. The full spectrum of mechanisms leading to antigen escape remains to be defined.

Fraietta and colleagues confirmed the common occurrence of memory T cell depletion in peripheral blood T cells from patients with CLL and revealed how these affect CAR T cell manufacturing in vitro and antitumor efficacy in vivo. These findings explain, at least in part, the lesser performance of CD19 CAR T cells in CLL relative to ALL (Table 1). Pending confirmation in larger patient cohorts, these insights may inform better management of patients with CLL undergoing CD19 CAR therapy and guide the design of improved therapeutic strategies.

Spotlight

CRISPR: Stressed about p53?

Miguel Foronda1 and Lukas E. Dow1,2,*

Two recent reports show that, in some contexts, CRISPR-mediated genome editing can lead to a p53-mediated stress response and cell-cycle arrest. These findings may help to explain why CRISPR-mediated genetic manipulation in different cell types leads to dissimilar outcomes, and highlights the need for a better understanding of the factors that influence effective genome editing in vitro and in vivo.

Tumor protein 53 (TP53) is arguably the most studied gene in biology. It is a potent tumor suppressor that is mutated in more than half of all human cancers, and regulates a myriad of cellular functions such as metabolism, apoptosis, senescence, and DNA damage repair [1]. Thus, it should come as no surprise that p53 is a key player in CRISPR/Cas9-based genome editing. What is perhaps startling is that it took so long to recognize. This month, two studies in *Nature Medicine* report that p53 induction in select pluripotent and immortalized human cells drives a DNA damage response to CRISPR-mediated cleavage that can limit genome-editing efficiency (Figure 1).

To say that CRISPR has had an impact on biomedical science is certainly an understatement. It is now the gold-standard tool for genetic manipulation in cultured cells, in vivo animal models, and has become the go-to approach for genome-wide forward genetic screens. In fact, Haapaniemi et al. [2] reached their findings by following a curious result from a genome-wide CRISPR dropout screen in immortalized human retinal pigment epithelial (RPE1) cells. Unlike previous screens, they noted a limited depletion of guide RNAs (gRNAs) targeting essential genes (such as ribosomal genes). Instead, they saw enrichment of gRNAs targeting TP53, CDKN1A, and RB1, and speculated that the cells were capable of CRISPR-editing, but that p53- or RB-dependent cell-cycle arrest could be preventing the proliferation of other gRNA-targeted cells in the screen. When they repeated the screen in p53−/− cells, ribosomal genes were efficiently depleted. The authors conclude that p53 activity, in part through mediating DNA damage-induced cell-cycle arrest, limits genome editing in RPE1 cells.

Ihry and colleagues [3] reached a similar conclusion, albeit by a different path. Using multiple different inducible or transient Cas9 delivery methods, they noted profound suppression of proliferation in human pluripotent stem cells (hPSCs) following genome targeting with several different gRNAs. This was not due to downstream consequences of target editing because the effect was seen even for genes that are not expressed and/or are dispensable for hPSC growth. The authors find modest, but consistent, increases in DNA damage (γ-H2AX) foci and subsequent p21 induction in hPSCs, which they show to be at least partially dependent on p53; using p53−/− hESCs or human inducible PSCs (hiPSCs) significantly improved the efficiency of genome targeting.

References

Before the two studies mentioned above, others had described severe deleterious consequences of Cas9-induced DNA damage, although in most cases this was dependent on focal clusters of DNA double-strand breaks (DSBs) [4,5]. What is perhaps most surprising about the new work is the claim that a single targeted DNA break is sufficient to arrest or kill a cell. It is important to note that these findings have yet to be replicated in a variety of cell types. Indeed, it is unlikely to be a universal phenomenon because there are many examples of successful editing in p53 wild-type cells, both in vitro and in vivo [6]. However, even if only restricted to hPSCs, these observations are an important piece of the puzzle for the efficient manipulation of pluripotent cells for regenerative medicine. Indeed, Ihry et al. provide evidence that suppression of p53 activity can dramatically improve gene targeting in these cells. It would thus be of great interest if short-term use of p53 inhibitors (such as pifithrin-μ, or a TP53-siRNA) could provide a window in which genome editing is temporarily permissive, and then allow p53 restoration as the cells expand.

One important question that has not been addressed is whether Cas9 variants such as recently reported base editors (BEs) [7] also induce p53 activity and subsequent cell cycle arrest because these enzymes do not rely on DSB resolution and non-homologous end-joining (NHEJ)/homology-directed repair (HDR). Most BEs also contain a UGI motif from Bacillus subtilis that inhibits cellular uracil-N-glycosylase (UNG), and thus are predicted to prevent activation of the UNG-dependent DNA damage response (DDR) machinery [8]. BEs enable precise editing at a single base-pair resolution, and through recent optimization efforts represent a good alternative to Cas9-based approaches for disease allele correction. If p53 proves to be a significant roadblock to direct DNA editing in more cell types, non-DSB-inducing methods such as CRISPR interference (CRISPRi) and short hairpin RNAs (shRNAs) are obvious alternatives for loss-of-function genetics.

This is not the first time p53 has played the villain in stem cell biology. In 2009, p53 was identified by five independent groups as a primary barrier to iPSC reprogramming [9]. While these discoveries carried possible repercussions for the development of iPSC-based regenerative medicine efforts, they were not scrutinized for their clinical implications in the same way the CRISPR studies have been. Initial press releases and online media reports have exaggerated the conclusions of the current studies and focused on the potential for adverse events from clinical CRISPR applications.
What then is the reality, and what is the fear?

In both research and clinical settings, the primary concern is that any editing of p53 wild-type cells will be challenging and may be biased toward those cells in the population that have compromised p53 activity. If the modified cultures are to be used for cell replacement therapies, they could be predisposed to tumor development as a result of selection for p53 dysfunction. While such clonal selection could be identified and eliminated through careful screening of ex vivo modified cells, this could not be avoided if the same phenomenon occurred during in vivo somatic gene correction (Figure 1). It is important to note that neither study in Nature Medicine demonstrates that CRISPR modification selects for pre-existing TP53 mutant cells during in vitro cell expansion, nor impacts on the outcome of in vivo modification. The bottom line is that we simply do not know how many p53 mutant cells may be present in otherwise normal tissues in any given individual, or whether this is a real concern for ex vivo or in vivo clinical CRISPR applications. In some contexts, disruption of a tumor suppressor may actually enhance therapeutic efficacy. There is at least one recent example reporting outgrowth of an effective chimeric antigen receptor T cell (CAR-T) clone carrying a viral insertion in the TET2 tumor-suppressor gene, in this case actually leading to complete remission of disease in this patient [10]. We do not know how p53 loss in a similar circumstance would affect cell function or tumor predisposition, but it is likely not a risk worth taking. At a minimum it will be important to examine the tumorigenic potential of ex vivo CRISPR-edited cells in animal models, and directly examine the consequences of concurrent p53 loss.

Unintended consequences of CRISPR-based genome-editing strategies are an important consideration for experimental and clinical application of this technology, and moving forward it would be prudent to be mindful of this. At the same time, no causal relationship has been demonstrated between CRISPR editing and tumorigenesis, and we must therefore be careful not to extrapolate so far from these cautionary tales that it stifles clinical progress of otherwise promising therapies.

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Spotlight
pSTAT3+ Reactive Astrocytes Promote Brain Metastasis

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The tumor microenvironment contains heterogeneous populations of stromal cells with important roles in cancer progression and metastasis. In a recent study published in Nature Medicine, pSTAT3+ reactive astrocytes were found to promote brain metastasis by altering the tumor microenvironment, and represent a promising target for the treatment of brain metastasis.

The progression of cancer involves a complex interplay between tumor cells and various stromal cells. This is particularly relevant in the development of metastasis in distant organs [1], as metastatic cancer cells need to survive and thrive in a stromal environment that is often drastically from that of the primary tumor [2]. Decades of tumor microenvironment research has identified many stromal cell types that can promote or inhibit metastasis [3]. Notably, many stromal cell types, such as macrophages, neutrophils, and fibroblasts, can play context-dependent and sometimes even opposite roles in metastasis. Therefore, development of new metastasis-targeting therapeutics critically depends on identifying subpopulations of stromal cells with defined molecular mechanisms in promoting organ-specific metastasis. In a recent study in Nature Medicine, Priego et al. combined elegant mouse models and clinical studies in human patients to elucidate an important role of STAT3-active astrocytes in brain metastasis [4].