BIOTECHNOLOGY

A metabolic safety switch for cell therapy

The utility of cell therapies could be increased by safeguards that control donor cell proliferation. However, existing safeguards use transgenes that can induce immunogenicity or be disrupted by inactivating mutations. In a study in *Nature Biotechnology*, Wiebking et al. describe a novel metabolic 'safety switch' based on the disruption of uridine monophosphate synthetase (UMPS) — an enzyme that catalyses the biosynthesis of the nucleotide UMP — and engineer therapeutically relevant cell types to depend on exogenous uridine to proliferate.

The authors first designed a CRISPR–Cas9-based system to disrupt UMPS activity, using a single guide RNA (sgRNA) specific to exon 1 of *UMPS*. Introduction of ribonucleoproteins (RNPs) consisting of the sgRNA and Cas9 into activated T cells inhibited their proliferation in media lacking UMP or uridine.

In order to produce a homogenous cell population with biallelic knockout of *UMPS*, the authors introduced a Cas9/sgRNA RNP targeted to the *UMPS* locus into K562 cells, this time in conjunction with two recombinant adeno-associated virus (rAAV) vectors each carrying a distinct expression cassette containing either genes encoding GFP and firefly luciferase (FLuc) or a truncated form of neural growth factor receptor (tNGFR). Cells positive for both tNGFR and GFP — suggesting integration of both vectors into the *UMPS* gene and therefore biallelic knockout — were purified using fluorescence-activated cell sorting (FACS). These $UMPS^{-/-}$ cells were sensitive to uridine deprivation, confirming the loss of UMPS activity.

As well as synthesizing UMP, UMPS also converts the prodrug 5-fluoroorotic acid (5-FOA) to its toxic metabolite, fluorouracil (5-FU). The authors hypothesized that 5-FOA could allow the enrichment of $UMPS^{-/-}$ cells in vitro by killing cells with UMPS activity. Indeed, following treatment of a mixed population of wild-type and $UMPS^{-/-}$ cells, cells with functional UMPS were lost on 5-FOA treatment as determined by FACS, with increasing concentrations of 5-FOA leading to enhanced enrichment of the $UMPS^{-/-}$ cells.

> “Disruption of UMPS can create a ‘safety switch’ that could be used to control the proliferation of cell-based therapies in response to uridine levels”

The authors then focused on using their double *UMPS* knockout strategy in cells with therapeutic applications. They first engineered human T cells using Cas9 RNPs and two rAAV vectors, each carrying a cassette corresponding to either tNGFR or a truncated epidermal growth factor receptor (tEGFR), and purified $EGFR^{+}NGFR^{+}$ T cells using magnetic-activated cell sorting. The purified cells only proliferated when supplemented with uridine, and were resistant to 5-FOA — confirming successful induction of uridine auxotrophy and 5-FOA insensitivity. Disruption of *UMPS* with Cas9 RNPs in human embryonic stem cells (hESCs) similarly showed cells with biallelic frameshift indels in the *UMPS* gene were unable to grow in the absence of uridine, with proliferation increasing in a uridine dose-dependent manner. $UMPS^{-/-}$ hESCs were resistant to 5-FOA treatment, which enriched $UMPS^{KO/KO}$ cells in a mixed population of GFP-labelled $UMPS^{-/-}$ hESCs and unlabelled wild-type cells. These data collectively show that these techniques can be used to engineer both uridine dependency and 5-FOA insensitivity in therapeutically important cell types.

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The authors went on to test the sensitivity of $UMPS^{-/-}$ cells to uridine in vivo, first by implanting $UMPS^{-/-}$ K562 cells engineered to express FLuc into immunodeficient mice subcutaneously. Mice were given either regular food or food enriched with uridine triacetate (UTA) — a prodrug for uridine known to markedly increase uridine levels in the serum at safe doses. Only mice fed UTA developed $UMPS^{-/-}$ K562 cell-derived tumours, as visualized by the presence of luciferase bioluminescence. Similarly, teratomas formed following the injection of $UMPS^{-/-}$ hESCs into the hind legs of mice were markedly larger in mice supplemented with UTA.

Finally, implantation of immunodeficient mice with $UMPS^{-/-}$ T cells — enriched in vitro before implantation using 5-FOA — allowed for successful engraftment in mice regardless of UTA treatment, with expansion of the human T cell population dependent on administration of UTA. All mice implanted with $UMPS^{-/-}$ T cells in the absence of UTA survived beyond 40 days, whereas mice implanted with *CCR5*-knockout T cells succumbed to graft-versus-host disease in this timeframe.

Together, these data show that disruption of *UMPS* can create a 'safety switch' that could be used to control the proliferation of cell-based therapies in response to uridine levels. Furthermore, *UMPS* disruption allows enrichment of engineered cells before implantation through treatment with 5-FOA. The authors plan to investigate how this system might aid therapies based on chimeric antigen receptor T cells by allowing metabolic control of their proliferation and persistence.

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ORIGINAL ARTICLE Wiebking, V. et al. Metabolic engineering generates a transgene-free safety switch for cell therapy. *Nat. Biotechnol.* https://doi.org/10.1038/s41587-020-0580-6 (2020)
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