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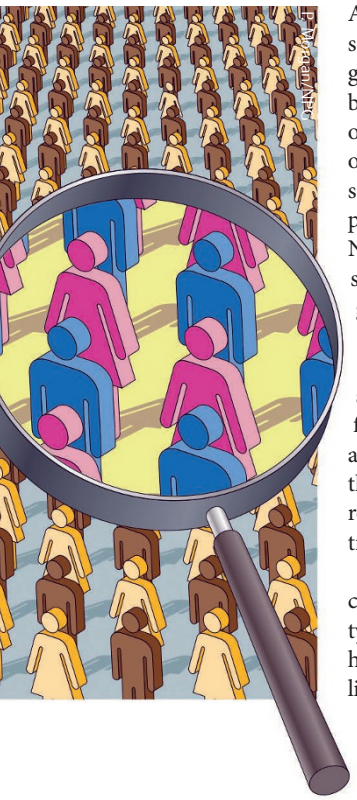
GENE THERAPY

In vivo selection of gene-corrected cells



A new system that enables *in vivo* selection and amplification of genetically modified hepatocytes has been reported in mice. Gene therapy offers promise for treating a number of genetic disorders, but low efficacy, safety concerns and transgene persistence are common problems. Nygaard *et al.* hypothesized that the selection of cells with the desired genetic modification could overcome issues associated with current gene therapy methods. In this study, hepatocytes were modified for resistance to a toxic drug (as well as expression of the transgene) and then selectively amplified in mice, resulting in significantly increased transgene expression.

Hereditary tyrosinaemia type 1 is caused by an inability to metabolize tyrosine owing to fumarylacetoacetate hydrolase (FAH) deficiency in the liver. As a result, fumarylacetoacetate (FAA) accumulates in hepatocytes and causes DNA damage and apoptosis. In the livers of some individuals with FAH deficiency, however, some cells act as 'healthy' cells and still express FAH. This effect has been attributed to gene reversion, whereby a spontaneous back-mutation results in the correction of the pathogenic variant. Notably, cells in which gene reversion has occurred can replace diseased tissue owing to a selective advantage. "This potent selection does not occur in most liver disorders, and therefore we wanted to artificially create a selective environment," explains Markus Grompe, lead author of the study.

"To get selection, we used a promoterless vector that can express a protective small hairpin RNA (shRNA) only if it integrates into the precise, intended chromosomal location," explains Grompe. Cells producing the shRNA cannot accumulate FAA and have a selective advantage when the toxic drug is administered, allowing for *in vivo* selection of gene-edited hepatocytes. The initial targeting frequency with this approach was <1%, although notably, after selection, approximately half of the hepatocytes expressed the transgene without the need to use any nuclease for gene editing.

This work demonstrates proof-of-principle of this selective approach, whereby a transgene co-expressed with a protective shRNA can be selected for in an expanding population of gene-corrected cells, although further research is needed. The team plan on conducting further *in vivo* experiments, as well as optimizing their approach for human hepatocytes using chimeric mice, to test for safety and efficacy of other US Food and Drug Administration (FDA)-approved compounds.

Bryony Jones

ORIGINAL ARTICLE Nygaard, S. *et al.* A universal system to select gene-modified hepatocytes *in vivo*. *Sci. Transl. Med.* http://dx.doi.org/10.1126/scitranslmed.aad8166 (2016)