GENE THERAPY

Fine tuning

High efficiency of gene delivery and long-term expression are key requirements for a good gene therapy vector. Adenoviral vectors come close to meeting this specification — they are among the most efficient delivery vectors, they have a broad range of targets and removing most of the virus's own genes has rendered them non-toxic and mostly non-immunogenic. However, gene expression from the adenoviral genome, which is linear and remains extrachromosomal, is unstable and is almost completely lost within a year. Yant *et al.* now report a substantial improvement to this gene delivery method — by combining adenovirus-mediated delivery with transposon-mediated integration, they achieve high levels of long-term transgene expression in mice.

In a previous study, the authors showed that the *Sleeping Beauty* (*SB*) transposase could cause a plasmid-borne *SB* transposon to integrate randomly into a mouse chromosome *in vivo*. Although the integrated transgene was expressed over long periods of time, the applicability of this approach for gene therapy was limited because plasmids, unlike adenoviruses, cannot be delivered efficiently to cells *in vivo*. Yant *et al.* therefore engineered a transposon-containing transgene in an adenoviral vector; however, they soon discovered that transposition is efficient only from circular templates — adenoviral DNA is linear. To overcome this problem, the authors introduced an Flp/FRT recombination step into their procedure — Flp recombinase, expressed from a separate vector, was used to mediate recombination between FRT sites that flanked the transposon-containing transgene to circularize it. This circular construct was then a target for *SB* transposase, which integrates it into the genome.

Once Yant *et al.* were satisfied that the system worked reliably, they tested it for the persistence of transgene expression by including the *lacZ* ORF in the *SB* transposon. The constructs were delivered into the tail vein of immunocompromised mice and their livers were examined five weeks later for β-galactosidase expression. Up to 45% of hepatocytes showed expression. Also, expression was maintained after several rounds of cell division, confirming the stability of the integrated transgene. Because the activity of *SB* transposase depends on zinc, transgene expression can be turned on or off by regulating zinc levels in the water that the mice drink.

The system was finally tested for expression efficiency by placing a gene for human coagulation factor IX — the protein that is deficient in haemophilia B — in the transposon vector. Even after six months, the level of human coagulation factor IX in transgenic mice was ~135-fold higher than in controls. Importantly, similar levels had previously been shown to be sufficiently therapeutic in a mouse model of haemophilia B.

The authors are quick to point out the advantages of their system. A major limitation of adenoviral vectors — their instability — has been overcome, but it also turns out that transposon-mediated integration has an important advantage over other types of integration as it doesn't cause chromosomal rearrangements. Far from being ready to rest on their laurels, Yant *et al.* have already begun working on improving the system by developing a single vector to integrate the properties of the two that were used in this study.

Magdalena Skipper

References and links

ORIGINAL RESEARCH PAPER Yant, S. R. *et al.* Transposition from a gutless adeno-transposon vector stabilizes transgene expression *in vivo*. *Nature Biotechnol.* **10**, 999–1005 (2002)

ETHICS WATCH

A shifting concept of family?

During the past decade, much has been written about the potential ramifications, both good and bad, of the "genetic revolution" as a broad social phenomenon. The tremendous advances that have occurred in the realm of genetics will undoubtedly have an impact on a broad spectrum of society. The products and language of genetics are everywhere. It has been suggested that this omnipresence might, however, cause us to overemphasize inappropriately the role of genetics and to de-emphasize the social, economic and other environmental factors that are relevant to human development[1].

It has also been suggested that this "geneticization" phenomenon might have an impact on the legal and social definition of family[2]. Throughout this century, most jurisdictions have embraced an increasingly inclusive notion of family, one that stresses the importance of social and emotional bonds over that of biological relatedness. Might the *ad hoc* application of genetics in the realm of family law cause the concept of "biological family" to become pre-eminent again, thus narrowing the conception of family to those with whom we share our biological heritage? Given the socially complex nature of the concept of family, such an emphasis on biology could hardly be considered a constructive trend, but is there any evidence for this shift in focus?

In Canada, a recent survey of family-law cases found that the courts are increasingly turning to the language of genetics as a justification for ordering paternity tests[3]. Specifically, the judiciary is compelling individuals to be tested because of the perceived health benefits, among other things, of knowing one's "genetic" parents. In these cases, the courts never referred to a specific medical condition or even to a specific application of the genetic information. Rather, the courts seem to be merely guessing that significant medical or genetic information might become available.

In addition, the courts are also influenced by the idea that there might be negative emotional consequences that would result from not knowing one's biological parents and that individuals have a right to know their genetic heritage. We clearly need to be sensitive to the understandable desire of individuals to know their biological relations. However, we also need to be aware of the potential cultural influence that the application of genetics in such settings can have, particularly when it has the potential to alter legal obligations and rights. As we move deeper into the "genetic era", law-makers need to become increasingly careful about how they interpret and use genetic information. Indeed, as suggested by Alta Charo: "While the law may find biology one useful factor in its classification of persons and their rights, it cannot afford to ignore the purpose for which those rights and rules are created."[4]

Tim Caulfield

Timothy Caulfield is at the Health Law Institute at the University of Alberta, Canada.

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