Fabry Disease
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Vector Divergence to Convergence?

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Gene therapy. Both the term itself and the associated therapeutic concept have a broad scope. Many different gene delivery systems are employed and a variety of cells and tissues are targeted to hopefully overcome manifestations and underlying pathophysiological deficiencies of both inherited and acquired disorders. More delivery systems along with additional target genes and disorders are being appended to the roster all the time. Some manipulations are fairly complex and involve *ex vivo* transduction followed by transplantation, for example, or even formation of artificially engineered 'depot organs' to provide sustained levels of corrective factors. Yet, a recent paper by Park *et al*,[1] from the NINDS at the NIH in Bethesda suggests that perhaps a more minimalist approach might be most beneficial, at least in the context of one inherited disorder where many different delivery vehicles have been used and distinct curative tactics have been undertaken by a number of research groups. Perhaps this recent report is the reduction of all divergent approaches to a single point of convergence.

Fabry disease is a pan-ethnic, X-linked, inherited disorder of lipid catabolism. It is the second-most prevalent lysosomal storage disorder (LSD). Unlike many LSDs, limited primary central nervous system involvement is seen in Fabry patients. The enzyme deficient in Fabry disease is a homodimeric lysosomal hydrolase (α-galactosidase A; α-gal A), and accumulation of galactosyl-terminal lipid substrates (mainly globotriaosylceramide; Gb3) leads to the disease manifestations. Until recently, treatment for Fabry disease was only palliative and patients succumbed in mid-life to cardiovascular, cerebrovascular, or renal disease. The key recent development was the actualization of ideas that enzyme replacement therapy (ERT) would be a practical intervention. This is based on metabolic cooperativity or 'cross-correction'. This is a phenomenon, seen with some other LSDs as well, wherein the corrective lysosomal enzyme in the circulation can be taken up, through mannose-6-phosphate-mediated endocytosis, and functionally utilized by cells and tissues relevant to the disorder. Metabolic cooperativity also forms the platform for the development of gene therapy for Fabry disease.

So, for the application of gene therapy towards Fabry disease, what is the best delivery vector system and what is the optimal target cell population or tissue? Herein is the divergence. Interestingly, Fabry disease (and associated mouse model) is somewhat unique in gene therapy in that many of the major viral and nonviral delivery systems have been directed at correction of the disorder.[2] Hematopoietic cells have been targeted as circulating delivery vehicles for α-gal A after transduction by recombinant oncoretroviral vectors,[3] for example, as have other localized tissues such as plasmid-injected[4] or AAV-transduced muscle[5] or Ad-transduced lung[6] as 'depot organs' to produce α-gal A. Ad vectors encoding α-gal A have also been directly injected i.v.[7] Yet some of these schemas require fairly complex manipulations or have demonstrated short-term or somewhat localized enzymatic correction or lipid reduction.

Enter the results of the new study by Park *et al*.[1] In that work, the authors constructed an AAV vector that engineers the expression of α-gal A driven by the chicken α-actin promoter. They administered a single i.v. injection of the recombinant vector into recipient Fabry mice. The relative number of recombinant AAV genomes injected was fairly low (10^{10} particles; as measured by PCR). Recipient animals were then followed for up to 24 weeks. Significant increases in α-gal A activity and Gb3 reduction were observed in a number of organs. Indeed, supra-normal levels of α-gal A activity were observed in liver, heart, and spleen (up to a level of >5-fold higher than the activity in normal mice in the liver).

So is this the point of vector convergence on this model and disorder? The levels of correction in the Park *et al*[1] study, both enzymatic and in the reduction of Gb3, are higher than others have observed using this model, albeit with different delivery schemas. Furthermore, some other parameters need to be further investigated: AAV vector localization after administration, vector persistence, and plasma α-gal A activity. Examination of these dimensions will address what is the relative contribution of transduced localized cells *versus* circulating α-gal A in correction of key tissues. Along these lines, when the same vector in the Park *et al*,[1] study was tested on Fabry fibroblasts in culture, an incomplete reduction of Gb3 was observed at an m.o.i. of 50; yet full reduction of Gb3 levels is observed in some organs where the effective m.o.i. (on a per cell basis) would likely be much lower.[1] This study also serves to highlight some other enigmatic issues that need to be resolved. For example, how does one standardize results when treatment methods such as ERT and gene therapy are applied to a disorder like this? Especially since the mouse model of Fabry disease is relatively long-lived and healthy even without corrective intervention. This problem has also been highlighted in the development of ERT for Fabry disease and different centers have not agreed on what exactly constitutes clinical correction of the disorder. In addition, even in the same animal disease model using similar vectors, completely different results can be obtained. Takahashi *et al* [5] found that intramuscular injection of a similar (but not identical) recombinant AAV construct leads to sustained circulating levels of α-gal A, but Park *et al*[1] suggested that no systemic α-gal A activity could be obtained by this method of delivery. Clearly, additional experiments are required to resolve these topics.

Fabry disease and the associated preclinical model are a good experimental platform to test a variety of delivery systems and outcomes. Strategies targeting secreted effector platforms and their associated developmental requirements, such as how to optimize circulation of corrective factors, or how to optimize stability or organ-specific targeting, can all be modeled here. In addition, since the α-gal A enzyme assay itself is relatively easy and the reduction of Gb3 can also be readily tracked, this model should be beneficial for many years as new delivery systems are developed and implemented. ■

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1 Park J *et al. Proc Natl Acad Sci USA* 2003; **100**: 3450–3454.
2 Siatskas C, Medin JA. *J Inherit Metab Dis* 2001; **24**: 25–41.
3 Takenaka T *et al. Proc Natl Acad Sci USA* 2000; **97**: 7515–7520.
4 Novo FJ, Gorecki DC, Goldspink G, MacDermot KD. *Gene Therapy* 1997; **4**: 488–492.
5 Takahashi H *et al. Proc Natl Acad Sci USA* 2002; **99**: 13777–13782.
6 Li C *et al. Mol Ther* 2002; **5**: 745–754.
7 Ziegler RJ *et al. Hum Gene Ther* 1999; **10**: 1667–1682.