

NEWS AND VIEWS

Revival of gene therapy

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In front of the inherited diseases, it seems we are at our wit's end, despite that we enjoy ourselves for the great achievements in fighting against the nature's devil. For once we placed our hope on gene therapy. However, on Friday, September 17th, 1998, at 2:30 PM, the death of Jesse Gelsinger depressed the whole community of the gene therapy. In order to cure the ornithine transcarbamylase deficiency, an X-linked genetic disease of the liver, the 18-year-old boy Gelsinger was involved in a clinical trial, in which an adenovirus carrying a corrected gene was applied to reverse the genetic defects. Four days after the injection, the adenoviral vector triggered severe immune responses, causing multiple organ failures and brain death. Despite of the following discussion, investigation, rebuttal and compensation, this first publicly identified death in clinical trial of gene therapy not only raised questions about the clinical outcome of this approach, but also challenged the related safety procedures and ethical guidance, especially on the applicability of the adenovirus vector for gene therapy or other human-related purpose.

It is now well-characterized that many diseases, particularly inherited disorders, are caused by gene mutations. With the advancement of molecular biology and genetics, scientists have proposed to correct the genetic malfunctions by introducing a beneficial gene, inactivating a deleterious gene, and/or replacing an abnormal gene. Although significant methodology progresses have been made and intensive animal studies have been conducted, gene therapy remains a risky and controversial approach for clinical use. Even the representative success of gene therapy trial—treating X-linked severe combined immunodeficiency (X-SCID)—was accompanied with the development of leukemia due to the activation of cancer-triggering gene(s) by retrovirus-mediated gene insertion. The unexpected outcome led to the 'on hold' of related clinical trials in the USA (Kaiser, 2003; Marshall, 2003). Gene therapy has been for a while at its low tide.

Recently, beacons of the gene therapy was lightened up again by Jay Neitz's group in the USA, who successfully cured the red-green color blindness in adult primates using an adeno-associated virus (AAV)-based approach (Mancuso et al., 2009), and by Patrick Aubourg's group in France, who reported promising clinical outcome on treating X-linked adrenoleukodystrophy (ALD) by lentiviral vector-mediated

hematopoietic stem cell (HSC) gene therapy (Cartier et al., 2009).

Humans distinguish colors through three kinds of photopigments that are located in the corresponding cone cells in retina. The individual photopigment has high sensitivity to short, medium (M) or long (L) wavelengths of lights and the three photopigments cooperate to provide full visible spectra; malfunction of a single photopigment will cause defects in recognizing blue, green or yellow (commonly mis-mentioned as red) color, respectively. In particular, absence of M and/or L wavelength photopigment will lead to red-green blindness. Color blindness is one of the most common genetic disorders and is primarily due to X chromosome mutation(s). For this reason, color blindness affects much more men than women. Despite of being cute and lovely, all male and some female squirrel monkeys (*Saimiri sciureus*) are born to be red-green blindness due to the missing of *L-opsin* gene. In this study, to rescue the phenotype, two adult red-green blinded squirrel monkeys, named Sam and Dalton (in memory of the English chemist. John Dalton, for his contribution on understanding color blindness over 200 years ago), were subretinally injected with an adeno-associated virus (AAV) that contains human *L-opsin* gene, whose expression is controlled by L/M-opsin the enhancer and promoter and is optimized in M cones. Twenty weeks after the injection, L-opsin showed appropriate expression in Sam and Dalton and they were no longer color-blinded as tested by a computer-based behavior examination.

Using subretinal injection of AAV carrying *RPE65* gene, the Jean Bennett group has demonstrated promising results for treating *RPE65*-associated Leber's congenital amaurosis, an inherited disorder with severe vision loss, while the outcome is age-dependent (Maguire et al., 2008, 2009). It was generally believed that congenital vision dysfunctions have to be treated at young age, when the neuronal connection is still under development.

Monkeys are close vertebrates to humans on the earth. Although it took months to take effects and the long-term safety parameters still remain to be investigated in the Neitz group's report, the regaining of full color spectra for Sam and Dalton reveals the hope for AAV-based gene therapy for treating vision disorders in human.

Owing to the capability of transducing non-dividing cells, HIV-derived lentiviral vectors represent another major gene

deliver approach for gene therapy. Recently, Cartier et al. (2009) reported their breakthrough of using lentivirus-based gene transfer into the hematopoietic stem cell to treat X-linked adrenoleukodystrophy (ALD, also known as Addison-Schilder disease, Siemerling-Creutzfeldt disease and Schilder's disease), which is a rare inherited fatal disorder with severe brain demyelination and only affects boys. ALD is caused by the malfunction of ALD protein (an ATP-binding cassette transporter encoded by *ABCD1* gene) and ALD patients barely survive to adolescence. Currently, the only established procedure to treat ALD is allogeneic hematopoietic cell transplantation (HCT) that potentially provides 'good' brain microglial cells from the donor bone marrow myelo-monocytic cells. Benefited from previous studies *in vitro* and *in vivo*, Cartier et al. applied gene transfer approach on two ALD boys (7 and 7.5 years old) to whom matched HCT donors are unavailable. Autologous CD34⁺ cells were obtained from the peripheral blood mononuclear cells (PBMCs) of the patients, transduced by lentiviral vector carrying wild type *ABCD1* gene, and transplanted back to the patients. The transplanted HSCs were able to self-renew and repopulate into multiple lineages, and expressions of ALD protein were detected in hematopoietic cells over the 24 and 30 months after infusion. Importantly, the HSC gene therapy showed significant neurological benefits that are comparable to HCT approach—the progression of brain demyelination and other malignant ALP symptoms were successfully prevented. These outcomes not only bring an alternate therapeutic rationale for ALD, but also promise the potential of lentivirus-based gene therapy in clinical application.

In spite of the obstacles, failures and frustrations, gene therapy is still considered to have optimistic prospects to conquer the most difficult diseases, such as inherited disorders and cancers. The recent breakthroughs will inevitably inspire researchers to keep investigating and optimizing gene therapy approach, and hopefully the gene therapy will bring routine clinical benefits in near future.

REFERENCES

- Cartier, N., Hacein-Bey-Abina, S., Bartholomae, C.C., Veres, G., Schmidt, M., Kutschera, I., Vidaud, M., Abel, U., Dal-Cortivo, L., Caccavelli, L., *et al.* (2009). Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy. *Science* 326, 818–823.
- Kaiser, J. (2003). Seeking the cause of induced leukemias in X-SCID trial. *Science* 299, 495.
- Maguire, A.M., High, K.A., Auricchio, A., Wright, J.F., Pierce, E.A., Testa, F., Mingozzi, F., Bennicelli, J.L., Ying, G.S., Rossi, S., *et al.* (2009). Age-dependent effects of RPE65 gene therapy for Leber's congenital amaurosis: a phase 1 dose-escalation trial. *Lancet* 374, 1597–1605.
- Maguire, A.M., Simonelli, F., Pierce, E.A., Pugh, E.N., Jr, Mingozzi, F., Bennicelli, J., Banfi, S., Marshall, K.A., Testa, F., Surace, E.M., *et al.* (2008). Safety and efficacy of gene transfer for Leber's congenital amaurosis. *N Engl J Med* 358, 2240–2248.
- Mancuso, K., Hauswirth, W.W., Li, Q., Connor, T.B., Kuchenbecker, J. A., Mauck, M.C., Neitz, J., and Neitz, M. (2009). Gene therapy for red-green colour blindness in adult primates. *Nature* 461, 784–787.
- Marshall, E. (2003). Second child in French trial is found to have leukemia. *Science* 299, 320.