Correcting the aberrant Fanconi anemia transcriptional program by gene therapy

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Fanconi anemia (FA) is a multisystem disorder, but hematopoietic derangements are the most common causes of morbidity and mortality, which include bone marrow failure and leukemic transformation. FA is a genetically diverse disorder with 22 associated genes.[1] *FANCA* is the most commonly mutated gene, and about 60% of the patients have mutations in this gene.

All FA genes are involved in DNA repair.[2] *FANCA* is part of nine FA genes that are recruited to fork-like DNA structures, and form a single large nuclear protein called "core complex". The core complex functions as a ubiquitin ligase that monoubiquinates a downstream heterodimer composed of two FA proteins, FANCI and FANCD2, which bind to an arrested replication fork at an interstrand DNA crosslink. The monoubiquitinated FANCI and FANCD2 form a binding interface for single- and double-stranded DNA and downstream effector complexes with additional FA and other proteins to cleave DNA interstrand crosslinks, resulting in DNA adducts and double-stranded DNA breaks. The latter aberrant DNA fragments are resolved by exonucleases and by homologous recombination, both involving additional FA proteins.

Currently, the only curative treatment for the hematopoietic failure in FA is hematopoietic stem cell transplantation. However, short-term and long-term complications (e.g., serious infections, organ failure and graft-*versus*-host disease) are not uncommon.[3] Furthermore, hematopoietic stem cell transplantation significantly increases the risk of solid tumors in this population of patients.[4]

Due to the limited treatment options and their associated risks, gene therapy provides an attractive alternative possibility. However, there are several challenges with gene therapy which are specific to inherited bone marrow failure syndromes such as FA. For example, although mobilization, collection and cryopreservation of sufficient peripheral blood CD34$^+$ cells from FA patients are feasible with the administration of granulocyte colony-stimulating factor or plerixafor,[5] this process is challenging for most such patients. The reason is that most FA patients are diagnosed when they have substantial cytopenia and their hematopoietic stem cells are depleted.[6]

Several gene therapy trials have been conducted in FA and various others are currently being conducted in Europe and the USA. Preliminary data showed that *FANCA* gene therapy without conditioning therapy is safe in FA and corrected cells are engrafted and manifest a proliferative advantage.[5,7,8] Furthermore, reasonable blood counts were sustained for several years.[5] However, long-term follow-up studies are lacking. Therefore, we do not know whether gene therapy can abrogate the FA phenotype of a gradual depletion of hematopoietic stem cells and decline in blood cell counts. Furthermore, we do not know whether gene therapy can provide hematopoietic stem cells with the long-term ability to maintain normal or sufficient molecular and functional properties. We also do not know how long corrected hematopoietic stem cells can generate hematopoietic stem and progenitor cells with normal or sufficient molecular and functional properties.

A study by Lasaga and colleagues, published in the current issue of *Haematologica*,[9] aimed to address some of the above issues by investigating four previously described FA patients with *FANCA* mutations who had successfully undergone gene therapy using a PGK-FANCA-WPRE lentiviral vector.[5] The vector contains a phosphoglycerate kinase (PGK) promoter, woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) sequence that enhances expression, and *FANCA*. Bone marrow CD34$^+$ cells were harvested from the patients, transduced with the vector, and then infused back into the patients without conditioning.

The analysis in the work by Lasaga *et al.* was performed 2-5 years after the gene therapy. The corrected cells were estimated to constitute 26-77% of bone marrow CD34$^+$ cells. Using single-cell RNA sequencing the authors demonstrated that the lentiviral-mediated gene therapy resulted in substantial correction of the transcriptional program in hematopoietic stem and progenitor cells of patients with FA, bringing it closer to the transcriptional program of normal hematopoietic stem and progenitor cells.[9] Importantly,

the expression of several genes that had been shown to be upregulated and mediate bone marrow failure in FA, such as *p21*[10] and *TGFβ*,[11] were normalized after gene therapy. The authors also showed that gene therapy improved cellular properties, including reduced chromosomal breakage of peripheral blood T cells in response to diepoxybutane, and a slower rate of telomere length reduction in peripheral blood cells, as determined by quantitative polymerase chain reaction, compared to the cellular properties of a control group of patients who were not engrafted after cell transduction. Furthermore, gene therapy improved cellular function, specifically survival of hematopoietic progenitors plated in cultures with mitomycin C and their ability to form colonies, which likely facilitates a progressive increase in the number of bone marrow and peripheral blood gene-corrected cells.

The findings described in the paper by Lasaga *et al.* are important and document positive effects of gene therapy. Although the results are very promising, there are still questions that need to be addressed. For example, the ability of gene therapy to provide sufficient hematopoietic stem and progenitor cell numbers and blood cell counts for the full lifespan of a patient needs to be documented. The sustainability of the transcriptional program, DNA integrity and telomere length also still need to be shown.

Disclosures

No conflicts of interest to disclose.

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