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Autologous Stem Cell Transplant with Gene Therapy for Friedreich Ataxia

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Abstract

We advance the overarching hypothesis that stem cell therapy is a potent treatment for Friedreich's ataxia (FRDA). Here, we discuss the feasibility of autologous transplantation in FRDA, highlighting the need for the successful isolation of the FRDA patient's bone marrow-derived mesenchymal stem cells, followed by characterization that these cells maintain the GAA repeat expansion and the reduced FXN mRNA expression, both hallmark features of FRDA. Next, we discuss the need for assessment of the proliferative capability and pluripotency of FRDA patient's bone marrow-derived mesenchymal stem cells. In particular, we view the need for characterizing the *in vitro* differentiation of bone marrow-derived mesenchymal stem cells into the two cell types primarily affected in FRDA, peripheral neurons and cardiomyocytes. Finally, we discuss the need to test the application of bone marrow-derived mesenchymal stem cells as potent autologous donor cells for FRDA. The demonstration of the functional correction of the mutated gene in these cells will be a critical endpoint of determining the potential of stem cell therapy in FRDA. We envision a gene-based cell transplant strategy as a likely therapeutic approach for FRDA, involving stable insertion of functional human bacterial artificial chromosomes or BACs containing the intact FXN gene into stem cells, thereafter leading to the expression of frataxin protein in differentiated neurons/cardiomyocytes.

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Conflicts of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Introduction

Friedreich ataxia (FRDA) is an autosomal recessive disorder caused by a trinucleotide GAA triplet expansions or point mutations in the FXN gene on chromosome 9q13 [1, 2]. Such anomalies results in reduced synthesis of FXN mRNA and the corresponding protein product, frataxin [3, 4]. While the exact function of frataxin is unknown, it is believed to be important to mitochondrial function and mitochondrial iron homeostasis [5, 6, 7, 8]. Individuals with FRDA typically present with frataxin levels ranging from 4% to 29% of normal levels [9]. FRDA is the most common form of hereditary ataxia, affecting about 1 in every 50,000 individuals in the United States. FRDA is characterized by neurodegeneration and cardiomyopathy [3]. Degeneration occurs in the spinal cord, peripheral nerves, and the cerebellum, thus an early clinical symptom of FRDA is gait ataxia. As the disease progresses, patients exhibit loss of sensation in the extremities which slowly spreads to the arms, trunk, and other parts of the body. In general, individuals are confined to a wheelchair within 10 to 20 years of the initial onset of symptoms, and may experience complete incapacitation in the later stages of the disease. In parallel, FRDA patients often exhibit chest pain, shortness of breath, and heart palpitations resulting from various concomitant forms of heart disease including hypertrophic cardiomyopathy (enlargement of the heart), myocardial fibrosis (formation of fiber-like material in the muscles of the heart), and cardiac failure. Such associated forms of heart disease are the most common cause of death of FRDA patients.

Currently, there is no cure or effective treatment for FRDA. Transplantation of mesenchymal stem cells has been proposed as an experimental treatment for various neurodegenerative disorders including FRDA [10, 11]. An autologous transplantation, using the patients' own bone marrow, would circumvent the adverse effects of graft rejection inherent in transplantations using donor-recipient mismatch. However, in FRDA the patient-derived bone marrow-derived mesenchymal stem cells may display the characteristic disease hallmarks, such as GAA repeat expansion and reduced FXN mRNA expression. Accordingly, in order to realize autologous transplantation in FRDA, the patient's bone marrow-derived mesenchymal stem cells must undergo genetic correction that will allow replacement of the mutated gene with the intact FXN gene in the harvested stem cells.

Hypothesis

We hypothesize transplantation of autologous bone marrow-derived mesenchymal stem cells that have undergone correction of the mutated FXN gene will be viable, and effectively attenuate the severity of FRDA via increased frataxin expression.

Discussion of the hypothesis

FRDA is a neurodegenerative disorder caused by GAA triplet expansions or point mutations in the FXN gene. These mutations lead to underexpression of the protein frataxin, which is believed to be responsible for iron homeostasis in the mitochondria [9, 12]. In yeast, the involvement of frataxin in iron homeostasis has been demonstrated [12]. Frataxin knockout yeast has been shown to accumulate 10 times more iron than wild type yeast, and is

incapable of oxidative phosphorylation [13]. When yeast frataxin was introduced to the cells, accumulated mitochondrial iron was rapidly exported into the cytosol as free iron [13]. It is thought that frataxin serves a similar function in human mitochondria, and that increased expression of frataxin may attenuate or even reverse the symptoms of FRDA. In a study conducted by Tan and colleagues, lymphoblasts of FRDA compound heterozygotes (FRDA-CH) were transfected with the frataxin gene to produce FRDA-CH-t cells. When compared to the FRDA-CH cells, FRDA-CH-t cells showed near normal mRNA and protein levels, decreased mitochondrial iron accumulation, and decreased sensitivity to oxidative stress [14]. Additionally, Pook and colleagues demonstrated in a rodent model that frataxin deficient cells can be rescued through increased frataxin expression [15]. While FRDA symptoms will certainly be attenuated through increased frataxin expression, it is still unclear whether an autologous bone marrow transplant is feasible in FRDA patients. For autologous transplantation to be feasible for gene therapy for FRDA patients, the bone marrow-derived mesenchymal stem cells should maintain the hallmark features of FRDA, namely GAA repeat expansion and reduced FXN mRNA expression. Such retention will form the basis for genetically correcting the mutated FXN gene and advancing autologous transplantation for FRDA. Although FRDA cell lines from patients have previously been generated, the cell lines were harvested from lymphoblast and fibroblast cells, which are not primarily affected despite reduced levels of frataxin expression [16]. Recently, however, induced pluripotent stem cells retaining the characteristic GAA repeat expansion and decreased FXN mRNA expression have been generated from skin fibroblasts of FRDA patients [3]. This suggests that FRDA bone marrow-derived stem cells may also retain the characteristic defects of FRDA. The proliferative capacity and pluripotency of FRDA patient bone marrow-derived mesenchymal stem cells should be evaluated to determine their capacity to differentiate into the two cell types primarily affected in FRDA, peripheral neurons and cardiomyocytes. Demonstration of differentiation into neurons and cardiomyocytes, arguably the two major cell populations necessary for gene- and cell-based therapy, will establish the relevance of an autologous transplantation regimen for the treatment of FRDA. As noted above, FRDA is accompanied by neurodegeneration and cardiomyopathy [3], with progressive cell death in the spinal cord, peripheral nerves, and the cerebellum associated with the early clinical symptom of gait ataxia [17,18], and heart diseases, such as hypertrophic cardiomyopathy, myocardial fibrosis, and cardiac failure, as the major underlying cause of death [19,20]. With this in mind, treatment of FRDA should be approached by abrogating both neurodegeneration and cardiomyopathy. To this end, systemic delivery of stem cells may allow targeted deposition of the grafts to brain and heart of FRDA patients. Lastly, functional correction of the mutated gene in FRDA bone marrow-derived mesenchymal stem cells leading to increased frataxin expression must be demonstrated in differentiated neurons and cardiomyocytes. To be considered an appropriate source for autologous transplantation, the bone marrow-derived mesenchymal stem cells from FRDA patients, subjected to genetic correction, should display phenotypes of healthy neurons and cardiomyocytes and be free of FRDA-associated disease hallmarks. Successful transfection and integration of a human bacterial artificial chromosome containing the FRDA locus has been demonstrated in human umbilical cord blood-derived multipotent stem cells [21]. Furthermore, the transfected cells retained the ability to differentiate into cells from all three germ line layers. Accordingly, such multipotent stem cells harvested

from FRDA patients may serve as efficacious stem cell donors for autologous transplantation in FRDA.

Conclusion

The transfected bone marrow-derived mesenchymal stem cells are expected to retain the ability to differentiate into neurons and cardiomyocytes, as demonstrated by phenotypic markers noted above. More importantly, determination of genetic correction of GAA repeats, FXN mRNA levels, and frataxin protein expression will be key outcome measures of a robust stem cell donor for FRDA. The basic science, translational, and clinical significance of this envisioned gene-based stem cell therapy is the demonstration that bone marrow-derived mesenchymal stem cells from FRDA patients subjected to genetic correction will display phenotypes of healthy neurons and cardiomyocytes and free of FRDA-associated disease hallmarks, thereby representing a novel source of transplantable autologous cells for FRDA patients.

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References

1. Epplen C, Epplen JT, Frank G, Miterski B, Santos EJ, Schöls L. Differential stability of the (GAA) n tract in the Friedreich ataxia (STM7) gene. *Human genetics*. 1997; 99(6):834–836. [PubMed: 9187683]
2. Cossée M, Schmitt M, Campuzano V, et al. Evolution of the Friedreich's ataxia trinucleotide repeat expansion: founder effect and premutations. *Proceedings of the National Academy of Sciences*. 1997; 94(14):7452–7457.
3. Liu J, Verma PJ, Evans-Galea, et al. Generation of induced pluripotent stem cell lines from Friedreich ataxia patients. *Stem Cell Reviews and Reports*. 2011; 7(3):703–713. [PubMed: 21181307]
4. Selak MA, Lyver E, Micklow E, et al. Blood cells from Friedreich ataxia patients harbor frataxin deficiency without a loss of mitochondrial function. *Mitochondrion*. 2011; 11(2):342–350. [PubMed: 21147271]
5. Ristow M, Pfister MF, Yee AJ, et al. Frataxin activates mitochondrial energy conversion and oxidative phosphorylation. *Proceedings of the National Academy of Sciences*. 2000; 97(22):12239–12243.
6. Sanchez-Casis G, Cote M, Barbeau A. Pathology of the heart in Friedreich's ataxia: review of the literature and report of one case. *The Canadian journal of neurological sciences. Le journal canadien des sciences neurologiques*. 1976; 3(4):349.
7. Lamarche JB, Shapcott D, Cote M, Lemieux B. Cardiac iron deposits in Friedreich's ataxia. *Neurological disease and therapy*. 1993; 16:453–453.
8. Radisky DC, Babcock MC, Kaplan J. The Yeast Frataxin Homologue Mediates Mitochondrial Iron Efflux evidence for a mitochondrial iron cycle. *Journal of Biological Chemistry*. 1999; 274(8): 4497–4499. [PubMed: 9988680]
9. Campuzano V, Montermini L, Lutz Y, et al. Frataxin is reduced in Friedreich ataxia patients and is associated with mitochondrial membranes. *Human molecular genetics*. 1997; 6(11):1771–1780. [PubMed: 9302253]

10. Jones J, Jaramillo-Merchán J, Bueno C, Pastor D, Viso-León M, Martínez S. Mesenchymal stem cells rescue Purkinje cells and improve motor functions in a mouse model of cerebellar ataxia. *Neurobiology of disease*. 2010; 40(2):415–423. [PubMed: 20638477]
11. Park HJ, Bang G, Lee BR, Kim HO, Lee PH. Neuroprotective effect of human mesenchymal stem cells in an animal model of double toxin-induced multiple system atrophy parkinsonism. *Cell transplantation*. 2011; 20(6):827–835. [PubMed: 21054946]
12. Bulteau AL, O'Neill HA, Kennedy MC, Ikeda-Saito M, Isaya G, Szewda LI. Frataxin acts as an iron chaperone protein to modulate mitochondrial aconitase activity. *Science*. 2004; 305(5681): 242–245. [PubMed: 15247478]
13. Babcock M, de Silva D, Oaks R, et al. Regulation of mitochondrial iron accumulation by Yfh1p, a putative homolog of frataxin. *Science*. 1997; 276(5319):1709–1712. [PubMed: 9180083]
14. Tan G, Chen LS, Lonnerdal B, Gellera C, Taroni FA, Cortopassi GA. Frataxin expression rescues mitochondrial dysfunctions in FRDA cells. *Human molecular genetics*. 2001; 10(19):2099–2107. [PubMed: 11590127]
15. Pook MA, Al-Mahdawi S, Carroll CJ, et al. Rescue of the Friedreich's ataxia knockout mouse by human YAC transgenesis. *Neurogenetics*. 2001; 3(4):185–193. [PubMed: 11714098]
16. Sturm B, Bistrich U, Schranzhofer M, et al. Friedreich's ataxia, no changes in mitochondrial labile iron in human lymphoblasts and fibroblasts: a decrease in antioxidative capacity? *Journal of Biological Chemistry*. 2005; 280(8):6701–6708. [PubMed: 15615730]
17. Koeppen AH, Mazurkiewicz JE. Friedreich ataxia: neuropathology revised. *J Neuropathol Exp Neurol*. 2013; 72(2):78–90. [PubMed: 23334592]
18. Koeppen AH, Morral JA, McComb RD, Feustel PJ. The neuropathology of late-onset Friedreich's ataxia. *Cerebellum*. 2011; 10(1):96–103. [PubMed: 21128039]
19. Evans-Galea MV, Pebay A, Dottori M, Corben LA, Ong SH, Lockhart PJ, Delatycki MB. Cell and gene therapy for Friedreich ataxia - progress to date. *Hum Gene Ther*. 2014 [Epub ahead of print] PubMed PMID: 24749505.
20. Perdomini M, Belbellaa B, Monassier L, et al. Prevention and reversal of severe mitochondrial cardiomyopathy by gene therapy in a mouse model of Friedreich's ataxia. *Nat Med*. 2014; 20(5): 542–547. [PubMed: 24705334]
21. Zaibak F, Kozlovski J, Vadolas J, Sarsero JP, Williamson R, Howden SE. Integration of functional bacterial artificial chromosomes into human cord blood-derived multipotent stem cells. *Gene therapy*. 2009; 16(3):404–414. [PubMed: 19177134]