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PREVIEWS

Previews highlight research articles published in the current issue of STEM CELLS TRANSLATIONAL MEDICINE, putting the results in context for readers.

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Patients carrying point mutations in the β-globin gene suffer from sickle cell disease, a condition in which the mutant globin protein produced promotes red blood cell deformation into a sickle or crescent shape under stress-inducing conditions. This deformation can induce premature death of red blood cells or the blockage of small blood vessels, leading to symptoms that can range from hemolytic anemia to multiorgan damage and early mortality.[1] The synergism of patient-derived induced pluripotent stem cell (iPSC) generation, gene editing via zinc finger nucleases, transcription activator-like effector nucleases, and CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9, and directed hematopoietic differentiation aims to provide an alternative source of both corrected red blood cells/platelets for transfusion and hematopoietic stem/progenitor cells for transplantation.[2] How far have we advanced toward reaching this lofty aim in the realm of sickle cell disease? And can we apply what we currently understand to other related diseases? In the first of our Featured Articles published this month in *STEM CELLS Translational Medicine*, Haro-Mora et al describe both the gene correction of a sickle cell disease mutation in patient-derived iPSCs and their differentiation into corrected erythrocytes employing a feeder- and serum-free methodology and the generation of a hemangioblast-like structure containing hematopoietic-like spherical cells.[3] In a Related Article published recently in *STEM CELLS*, Huang et al. employed CRISPR/Cas9-mediated gene editing to correct a sickle cell disease mutation in patient-derived iPSCs and permit the production of erythrocytes expressing the appropriate globin protein from the corrected allele.[4]

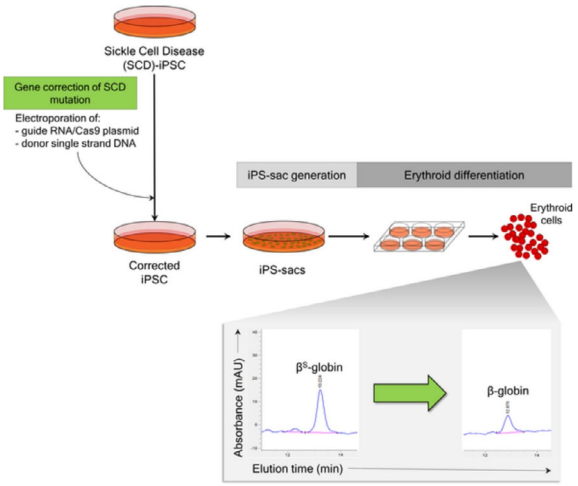
Autophagy, a catabolic protein degradation and recycling pathway that ensures cellular homeostasis and proper function, removes unnecessary or dysfunctional cellular components through the generation and activity of double-membrane vesicles called autophagosomes that engulf cellular proteins and organelles for delivery to the lysosome.[5] Interestingly, studies into the role of autophagy in disease, including cancer, inflammatory bowel disease, and neurodegeneration,[6] have established that autophagy can promote both cell survival and cell death in a context-specific manner. In contrast, a general reduction in autophagy accompanies the normal aging process. Overall, an extensive research base has provided evidence that the targeted modulation of autophagic activity may represent an exciting means to improve therapeutic outcomes in multiple conditions. In the realm of stem cells, autophagic function appears to balance quiescence, self-renewal, and differentiation under normal physiological and stress-inducing conditions[7]; therefore, can the modulation of autophagy in stem cells also foster enhanced regenerative outcomes? In the second of our Featured Articles published this month in *STEM CELLS Translational Medicine*, Zhu et al demonstrate that the administration of engineered neural stem cells (NSCs) can decrease autophagy, reverse the hypoxic microenvironment, and reduce central nervous system glial scar formation to permit improvements to locomotor function following spinal cord injury.[8] In a Related Article published recently in *STEM CELLS*, Matsumoto et al described how a signaling cascade involving autophagy, short telomeres, and p53 regulates the age-associated detrimental changes in the cell fate of cardiac stem/progenitor cells (CPCs) and identified a means to rejuvenate aged CPCs and improve cardiac therapy.[9]

FEATURED ARTICLES

Novel Culture Approach Enhances the Production of Corrected Erythroid Cells from Sickle Cell iPSCs

Previous research from the laboratory of Naoya Uchida (National Institutes of Health, Bethesda, Maryland) established that hematopoietic-like spherical cells derived from human embryonic stem cell-derived hemangioblast-like structures (ES-sacs)[10] could be differentiated into definitive β-globin-expressing erythroid cells for potential exploitation in transfusions.[11,12] The team's new *STEM CELLS Translational Medicine* article sought to advance this research by exploring the ability of gene-corrected iPSCs derived from sickle cell patients to form definitive erythroid cells in an analogous manner.[3] Haro-Mora et al began their approach with the biallelic β-globin gene correction in sickle cell disease iPSCs through the implementation of an electroporation-based viral vector-free method following culture under feeder-free conditions. Next, the

authors differentiated gene-corrected iPSCs under extensively optimized serum-free "iPS-sac" culture conditions, which, encouragingly, provided for the generation of normal β-globin-expressing erythroid cells. Interestingly, the team also highlighted that their optimized feeder- and serum-free iPS-sac protocol elicited higher yields of sac-structures, hematopoietic-like spherical cells, and erythroid cells when compared with the standard ES-sac protocol, which employs both mouse embryonic fibroblast feeders and fetal bovine serum containing medium. Overall, these findings highlight the combination of gene correction and differentiation via an optimized iPS-sac protocol as an exciting means to develop clinically relevant regenerative transfusion therapies for sickle cell disease patients.



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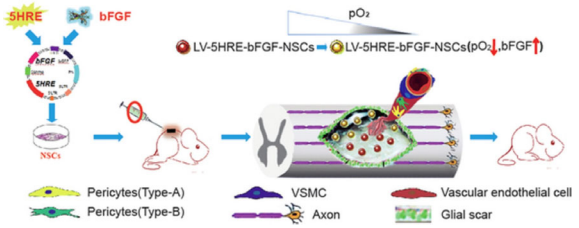
Autophagy as a Key Target for Spinal Cord Repair by Engineered Neural Stem Cells

A progressive decrease in spinal cord blood flow and the subsequent severe hypoxia and ischemic insult represent some of the characteristics of spinal cord injury, a condition that currently suffers from a lack of effective treatment approaches. Given this situation, researchers led by Jian Xiao (Wenzhou Medical University, Wenzhou, Zhejiang, China) aimed to fully characterize the spinal cord injury microenvironment in a rat model to explore possible novel therapeutic strategies. In their recent *STEM CELLS Translational Medicine* article,[8] Zhu et al describe how they encountered a pattern of hypoxia with increased cell autophagy throughout the injured spinal cord.[13] With this in mind, the team engineered NSCs to express basic fibroblast growth factor, a growth factor that possesses anti-ischemic and neuropromoting properties,[14,15] under the regulation of hypoxia-responsive elements to specifically target hypoxic loci. Encouragingly, the treatment of spinal cord injury rats with engineered NSCs prompted improvements in neuronal survival and the inhibition of autophagy in spinal cord lesions due to the reversal of hypoxic conditions. Furthermore, the authors also observed neuron regeneration, glial scar inhibition, and evidence of axonal regeneration across the scar boundary, which all prompted enhanced functional restoration. Overall, this fascinating new study suggests that targeting autophagy and hypoxia may represent an efficient means to improve spinal cord regeneration by stem cell therapy in human patients.

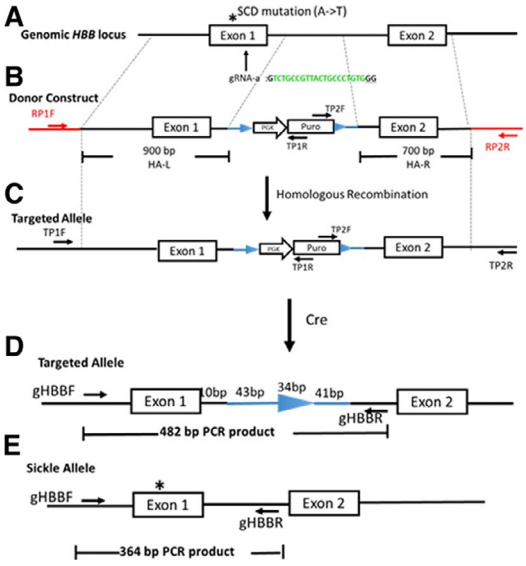


https://doi.org/10.1002/sctm.19-0282

RELATED ARTICLES

Genome Editing of Sickle Cell iPSCs Allows for Gene-Corrected Erythrocyte Production

While several research groups had employed a variety of gene-editing technologies to target and correct mutations in the β-globin gene in cancer cell lines and human iPSCs,[16-18] the specificity and efficiencies of these approaches remained to be fully analyzed. With this knowledge in hand, researchers led by Linzhao Cheng (Johns Hopkins, Baltimore, Maryland) sought to demonstrate that CRISPR/Cas9-mediated gene editing of the β-globin locus in human iPSCs represents a more efficient strategy when compared to zinc finger nucleases and transcription activator-like effector nucleases. In their recent *STEM CELLS* article,[4] Huang et al employed a specific guide RNA and Cas9 to correct one mutant allele of the β-globin gene in human iPSCs via homologous recombination with a donor DNA template containing wild-type β-globin DNA and a selection cassette, which the team subsequently removed to avoid any possible interference. Next, employing fully optimized xeno-free and feeder-free culture conditions, the team differentiated iPSC clones with one corrected β-globin allele into erythrocytes; encouragingly, they discovered that resultant cells expressed the corrected β-globin protein from the corrected allele and that approximately 6% to 10% of erythrocytes displayed signs of maturation into reticulocytes. Overall, the authors hoped that their new advance might accelerate the clinical application of genome-edited patient-derived iPSCs and their derivatives to sickle cell disease and other relevant human disorders.



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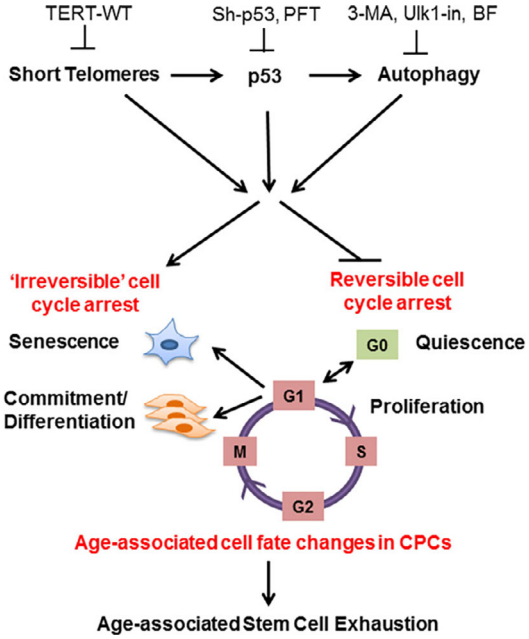

https://doi.org/10.1002/stem.2793

Rejuvenating Cardiac Progenitor Cells by Targeting Autophagy

While CPCs display promise in the treatment of cardiac disease via myocardial repair and regeneration, the normal aging process can induce senescence, reduce stem-like characteristics, and impair regenerative potential.[19,20] In the hope of delineating the mechanisms controlling the loss of regenerative potential during aging, researchers led by Nirmala Hariharan (University of California at Davis, California) recently evaluated the influence of age-associated factors, such as telomere length, p53 expression, and autophagy in modulating CPC fate through comparative analyses of model mice displaying greatly differing telomere lengths. Reporting in *STEM CELLS*,[9] Matsumoto et al established that mice with short telomeres displayed early cardiac aging (cardiac dysfunction, hypertrophy, fibrosis, and senescence), altered cell fate (characterized by cell cycle arrest, senescence, basal commitment, and a loss of quiescence), and induced autophagy, as evidenced by reduced p62 levels and an increase in the accumulation of autophagic puncta. However, the authors noted that inhibition of autophagosome formation prompted the appearance of a more youthful CPC phenotype, while p53 inhibition also reversed some of the signs of aging in CPCs, coincident with the attenuation of autophagy. Overall, these findings suggested a mechanism by which short telomeres activate p53 and autophagy, thereby inducing CPC differentiation, senescence, and eventual exhaustion. While this study provides a mechanism for the lack of regenerative potential of aging CPCs, it also highlights potential means to prevent senescence and enhance CPC therapy in the failing heart.

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