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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.

STEM CELLS TRANSLATIONAL MEDICINE

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Mutations in the dystrophin-encoding gene prompt the development of Duchenne muscular dystrophy (DMD), a severe inherited form of muscular dystrophy primarily affecting young males. Wild-type dystrophin, a structural myofiber sarcolemma protein,[1] links the extracellular matrix to cytoskeletal proteins; however, the mutation-induced loss of function or loss of dystrophin disrupts this interaction and induces problems in the skeletal, cardiac, and respiratory muscles that can progress to multiorgan failure and premature death.[2,3] While we currently lack effective treatment options for DMD,[4] multiple therapeutic strategies remain under development. Gene therapies aiming to restore dystrophin levels (eg, exon skipping, microdystrophin gene delivery via adeno-associated viruses, and CRISPR/Cas9-mediated gene-editing) suffer from limitations that include limited patient applicability, the induction of adverse immune responses, and an inadequate impact on affected muscles. While stem cell-based therapies for DMD treatment have provided encouraging results, significant drawbacks include limited or short-term cell engraftment, adverse immune responses, and side effects associated with supportive immunosuppressive therapy. What are the next steps we can take in the development of safe and effective treatment strategies for this devastating disease? In the first of our Featured Articles published this month in *STEM CELLS Translational Medicine*, Siemionow et al describe human dystrophin-expressing chimeric (DEC) cells derived from myoblasts of normal and DMD-affected donors as a novel long-term therapeutic approach for DMD that protects against the deteriorating function of crucial muscle types.[5] In a Related Article published in *STEM CELLS*, Matre et al employed CRISPR/Cas9-mediated genome editing of muscle progenitors to uncover a new role for dystrophin and raise the possibility of a novel therapeutic approach for DMD patients.[6]

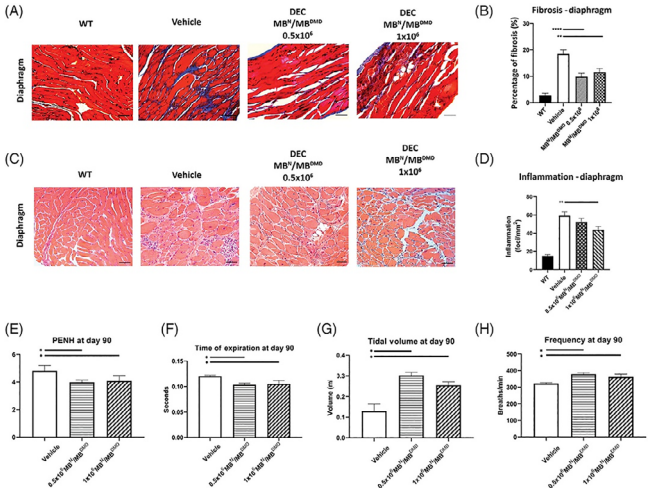
Reactive oxygen species (ROS), which include peroxides, superoxide, hydroxyl radical, and singlet oxygen, are highly reactive chemicals derived from O_2 that are formed as byproducts of normal intracellular metabolism. ROS generally exist at low levels in normally functioning cells where they play critical roles in cell signaling and cellular homeostasis; however, the significant elevation of ROS levels in response to oxidative stress induced by aging or disease, for example, can prompt irreversible intracellular damage that limits cell function and induces apoptosis. ROS play dual roles in the realms of stem cells and regenerative medicine, as they can regulate both stem cell potency and lineage commitment/differentiation.[7] As an example, low levels of ROS promote hematopoietic stem progenitor cell (HSPC) quiescence and support their differentiation potential, while elevated ROS levels induce hematopoietic differentiation and HSPC exhaustion.[8,9] Overall, these findings suggest the vital importance of redox signaling to normal hematopoietic function,[10] and ongoing research aims in this area include evaluating the impact of ROS in the induced mobilization of HSPCs and exploring effective means to reduce ROS levels in stressed HSPCs to support hematopoietic function. In the second of our Featured Articles published this month in *STEM CELLS Translational Medicine*, So et al describe how glucose oxidase (GO) administration enhances the peripheral mobilization of HSPCs via ROS signaling and maintains HSPC functions in conditioned recipient animals.[11] In a Related Article published in *STEM CELLS*, Lin et al reported how the accelerated recovery of stressed HSPCs following exposure to the mTOR inhibitor rapamycin associates with reduced ROS and DNA damage levels, thereby supporting rapamycin treatment as a clinically viable means to treat/prevent hematopoietic injury.[12]

FEATURED ARTICLES

Dystrophin-Expressing Chimeric Cell Therapy: A New Hope for DMD Patients

Researchers from the group of Maria Siemionow (University of Illinois at Chicago, Chicago, Illinois) previously demonstrated that the local transplantation of DEC cells into a mouse model of DMD enhanced dystrophin expression and prompted significant improvements in muscle strength and function[13,14]; furthermore, they established that intraosseous DEC transplantation improved cardiac function.[15] Their new *STEM CELLS Translational Medicine* article now describes their most recent research, which evaluated how the intraosseous transplantation of human DEC cells impacts the long-term functional decline of DMD-affected muscles.[5] The authors generated DEC cells by fusing myoblasts isolated from normal and DMD donors and subsequently evaluated engraftment and therapeutic output at 90 days post-intraosseous DEC transplantation in a mouse model of DMD via

the assessment of functional outcomes, histology, and muscle pathology in the cardiac, respiratory, and skeletal muscles. The authors linked an increase in dystrophin expression in severely affected DMD muscles following DEC therapy to improvements in muscle function, which included the preservation of ejection fraction and fractional shortening via echocardiography, an improvement in respiratory function via plethysmography, and an increase in limb skeletal muscles strength and function. Encouragingly, improved muscle histopathology (including reduced DMD pathology, fibrosis, and inflammation) and preserved muscle morphology and architecture all correlated with improvements to DMD-affected muscles. Overall, these hugely encouraging findings suggest that DEC therapy may provide a protective effect in DMD-affected muscles and could represent an effective treatment approach for human DMD patients.



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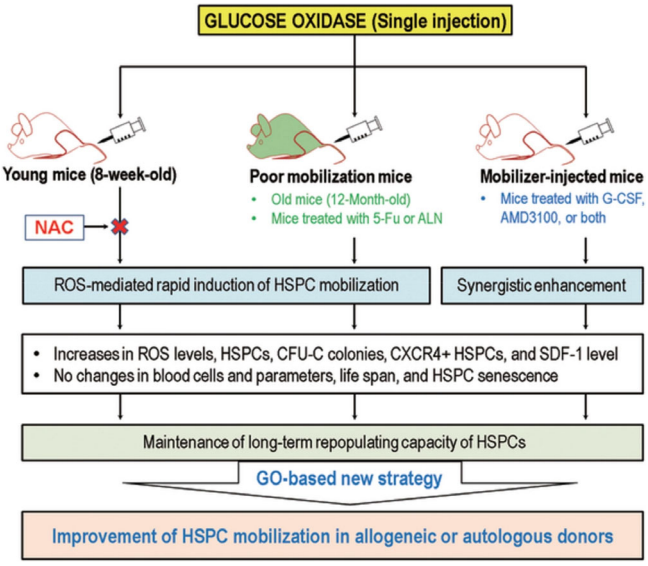
GO! GO! GO! How Glucose Oxidase and ROS Get HSPCs Mobilized

While the mobilization of HSPCs in response to agents such as granulocyte colony-stimulating factor and AMD3100[16,17] had been linked to ROS production, whether ROS directly mobilize HSPCs remains unknown. To explore this hypothesis, researchers led by Jeong-Chae Lee and Sung-Ho Kook (Jeonbuk National University, Jeonju, South Korea) evaluated HSPC mobilization following exposure to GO, an oxidoreductase that converts glucose into glucuronic acid and hydrogen peroxide and produces the mild and prolonged generation of ROS.[18] Their findings, published in a recent *STEM CELLS Translational Medicine* article, now establish the power of GO treatment alone or in synergy with stem cell mobilizing agents and highlight the importance of ROS to stem cell mobilization.[11] So et al revealed that a single administration of GO induced the mobilization of HPSCs that displayed long-term reconstituting and differentiating activity in conditioned mice. Transplanted mice lived a normal life without any signs of stem cell senescence, hematopoietic disorders, or alterations to blood parameters. Encouragingly, the authors observed a similar positive therapeutic effect of GO exposure in animals that generally display poor HSPC mobilization, such as older or chemotherapy-treated mice, and also discovered that treatments combining GO with stem cell mobilizers induced a synergistic effect on HSPCs. Importantly, the inhibition of GO-induced improvements following antioxidant administration (N-acetyl cysteine or NAC) underscored the general importance of ROS to HSPC mobilization. Overall, the authors provide robust evidence for the general importance of ROS signaling in HSPC mobilization and highlight complementary GO treatment as an exciting means of boosting HSPC mobilization in poorly mobilizing allogeneic or autologous donors.



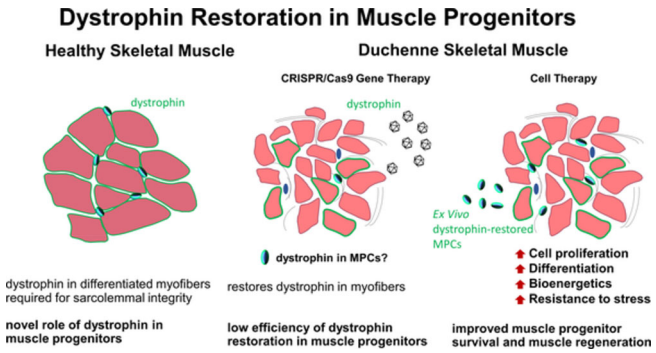
https://doi.org/10.1002/sctm.20-0514

RELATED ARTICLES

New Dystrophin Role Uncovered Through the Power of Gene Editing

While research had suggested novel roles for dystrophin in muscle stem cells, thereby providing evidence for DMD as a stem cell disease,[19,20] any impact of dystrophin on mitochondrial/metabolic activity and stress tolerance in stem cells remained unclear. To explore this critical area, researchers from the laboratory of Johnny Huard (Steadman Philippon Research Institute, Vail, Colorado) studied how muscle progenitors (or MPCs) from DMD model mice responded to the correction of the dystrophin mutation. In their recent *STEM CELLS* article,[6] Matre et al first corrected the dystrophin mutation by excising the mutated exon 23 of the *Dmd* mouse gene in muscle progenitors via CRISPR/Cas9-mediated genome editing. In vitro analysis

suggested that the correction of this mutation fostered significant improvements in muscle progenitor proliferation, multilineage differentiation, mitochondrial bioenergetics, and resistance to both oxidative and endoplasmic reticulum stress in vitro, suggesting that corrected muscle progenitors may exhibit an improved ability to survive, self-renew, and regenerate myofibers within diseased muscles. In agreement with this hypothesis, the authors revealed significantly improved engraftment and dystrophic muscle regeneration following the transplantation of gene-corrected muscle progenitors into the muscles of DMD model mice, suggesting the possible direct or indirect contribution of transplanted gene-corrected muscle progenitors to the muscle stem/progenitor compartment. The authors of this exciting study hoped that their findings would impact both the development of new DMD therapies and our understanding of dystrophin's role in muscle stem cells/progenitors and stem cell biology.



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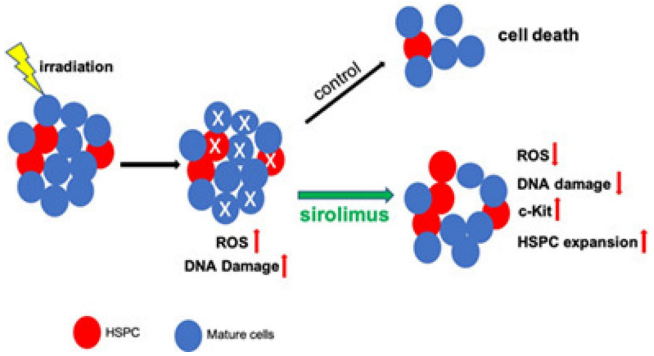
Rapamycin Reduces ROS in HSPCs to Boost Recovery

Previous research led by Xingmin Feng (National Institutes of Health, Bethesda, Maryland) established that rapamycin (also known as sirolimus) could prevent immune-mediated bone marrow failure in mice and protect hematopoiesis thanks to the depletion of clonogenic T cells and the preservation of immunosuppressive regulatory T cells.[21] In their recent *STEM CELLS* article,[12] the authors evaluated the possibility of employing rapamycin to protect HSPCs from external stresses (such as irradiation or chemotherapy) and maintain their function. Lin et al discovered that HSPCs derived from rapamycin-treated mice that had undergone total body irradiation (and hence bone marrow injury) expressed higher levels of hematopoiesis-associated genes and could engraft well into lethally irradiated recipient mice. Interestingly, decreased levels of the DNA damage marker γ-H2AX, increased expression of DNA repair genes, reduced cell death, and the enhanced clearance of ROS all accompanied the rapamycin-induced improvements observed in HSPCs. Excitingly, xenotransplantation studies established that rapamycin treatment improved the engraftment of irradiated human HSPCs following transplantation into mice to boost the production of mature blood cells. Finally, the authors also highlighted the ability of rapamycin to improve HSPC recovery in mice exposed to common hemotoxic chemotherapeutic agents (busulfan and 5-fluorouracil). Overall, the findings of this exciting study provide evidence for rapamycin as an effective means to treat/prevent hematopoietic injury, in part by reducing ROS levels.



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