Skeletal Muscle Regeneration by Fibromodulin Reprogrammed Cells without Tumorigenic Risks

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PURPOSE: Skeletal muscle, one of the most commonly injured tissue, is easily lost by severe injuries from car accidents, natural disasters, or salvage surgeries for tumors. Unfortunately, the skeletal muscle mass has limited repair capacity. Direct transplantation of committed myoblasts is hindered by inadequate cell availability, limited cell spreading, and poor survivability of implanted cells. In addition, using mesenchymal stem cells for tissue regeneration is always accompanied by the painful, invasive procedures (i.e., tissue biopsy, bone marrow aspiration, and liposuction) that potentially cause severe complications or fatal outcomes. The tumorigenic risk of pluripotent cells also remains as the major concern for clinical application, and intramuscular injection is one of the most common routes for teratoma formation that validates the pluripotency in vivo in skeletal muscle regeneration. Previously, we have established a novel platform technology using a single molecule, fibromodulin (FMOD), to reprogram human dermal fibroblast into a multipotent state while circumventing oncogene usage and genome integrating. The yielded FMOD ReProgrammed (FReP) cells hold significant potential for myogenic differentiation both in vitro and in vivo. Our current study focused on its tumorigenic risk assessment.

METHODS: RNA-seq was performed to compare global gene expression of FReP cells and induced pluripotent stem cells (iPSCs) which holds the high tumorigenic risks. Differential mRNA expressions were identified by TopHat-Cufflinks package, functionally annotated via DAVID Bioinformatics Resource, and aligned with human proto-oncogenes and tumor suppressor genes listed in the UniProt database. Soft agar colony formation assay, the standard tumorigenicity test, was used to examine the cellular survival ability in an anchorage-independent manner under low nutritional and oxygen concentration microenvironment in vitro. On top of intramuscular injection, intratesticular injection was also carried out to further evaluate the tumorigenic potential of FReP cells, as intratesticular stromal cells produce more supportive environment that fosters implanted cells in comparison with subcutaneous and intramuscular microenvironment.

RESULTS: Functional analysis of more than 2300 differential genes between FReP cells and iPSCs by KEGG pathways revealed enrichment of genes involved in the ‘Pathways in cancer’ with significant similarity of term overlap (Kappa value = 1.0). Notably, FReP cells showed lower expression of more proto-oncogenes but higher expression of more tumor suppressor genes when compared to iPSCs. Unlike iPSCs, FReP cell neither proliferated nor formed colonies in soft agar after 14-day cultivation. Furthermore, in intramuscular injection, 2 of 8 iPSC-implanted animals (25%) ended up with tumor formation instead of skeletal muscle generation, while none of FReP cell-implanted animals presented tumor formation. Intratesticular injection of iPSCs resulted in 100% (10/10) teratoma formation, but FReP cells showed 0% (0/10) tumor formation in 4 months.

CONCLUSIONS: Our in vitro and in vivo studies collectively showed that FReP cells are less likely to generate tumors in vivo, which suggested that FReP cells is a safe cell source for skeletal muscle regeneration.

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PURPOSE: Severe musculoskeletal trauma as observed in crush injuries and prolonged ischemia as seen in flap reconstruction and composite tissue allotransplantation is associated with ischemia-reperfusion (IR) injury leading to an aberrant inflammatory response. While smaller muscle injuries heal by complete muscle regeneration, complex injuries result in aberrant healing with muscle fibrosis. However, the inflammatory cascade that is governing this process remains incompletely understood. Recently, neutrophil extracellular traps (NETs), extracellular DNA structures released by neutrophils, have been implicated in the initial phases of ischemic inflammation. In this study, we develop a mouse extremity polytrauma model of combined muscle trauma and IR injury to assess the initial inflammatory response.

METHODS: Male C57BL/6 mice were randomized into three treatment groups: 1. Fibrosis model: Cardiotoxin (CTX) injection into the tibialis anterior (known fibrosis model); 2. IR model: hindlimb ischemia by occluding the femoral artery using a microvascular clamp for 3.5 hours followed by reperfusion for up to 5 days 3. Polytrauma model: IR plus CTX injection (n=4 mice/group). Bioluminescent imaging for myeloperoxidase as a readout of inflammation was analyzed as well as neutrophil elastase. Additionally, lower extremity muscle was harvested for flow cytometry to assess inflammatory cell subpopulations and histology.

RESULTS: Macroscopic evaluation of muscle specimens revealed an evident area of necrosis and apparent muscle changes while the CTX alone and IR alone muscles appeared normal. In-vivo imaging revealed significantly increased inflammation as measured by myeloperoxidase and neutrophil elastase activity in mice undergoing IR+CTX than either of the other groups. This was further corroborated by flow cytometry where we found differences in inflammatory cell subpopulations with more inflammatory monocytes/macrophages and neutrophils recruited to the muscle injury site in the IR+CTX group (p(Monocytes: IR+CTX vs. CTX only)=.000002 and p(Neutrophils: IR+CTX vs. CTX only)=.037).

CONCLUSIONS: Our findings demonstrate that combined IR+muscle injury (CTX) results in significantly increased muscle injury than either ischemia or muscle injury alone. This was characterized by an elevated inflammatory response with amplified recruitment of pro-inflammatory macrophages and neutrophils. This model can be utilized to examine the effects of musculoskeletal polytrauma on muscle regeneration and fibrosis. A better understanding and description of the inflammatory cascade after extremity IR injury will enable early intervention and prevention of post-traumatic muscle fibrosis.

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Violence Against Women: Facial Fractures Secondary to Assault in the Urban Female Population

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PURPOSE: Assault has been frequently indicated as a major cause of facial trauma in the United States and around the world. Facial fractures secondary to assault have been shown to occur at a much higher rate in the male population as opposed to females. While these injuries may occur less frequently in females, they remain a significant medical problem within this demographic as facial trauma is one of the most frequently reported injuries resulting from domestic violence against women. Numerous studies have examined facial fractures in the general population, but few have assessed these injuries in females specifically. The objective of this study is to assess facial fractures secondary to assault in the female population. We intend to examine the prevalence and specific mechanism of action of these injuries in order to develop effective management strategies and decrease the likelihood of future injury.

METHODS: All facial fractures between the years 2001 and 2011 were retrospectively reviewed based on International Classification of Disease (ICD-9) codes. The facial fractures included in this study were the result of assault in the female population in an urban, level 1 trauma center (University Hospital, Newark, NJ). Results were categorized by patient demographics, location of fractures, concomitant injuries, length of hospital stay, critical complications, and surgical management strategies.