Therapeutic Delivery of Placental Stem Cells to Modulate Vasculature and Promote Fracture Repair

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Introduction: Blood supply to a bone fracture is a critical determinant of the rate and extent of healing. The incidence of patients with delayed or non-unions when fractures occur in conjunction with vascular injury is 46%, as compared to a 10% occurrence amongst the normal fracture population. These observations indicate that enhancing vascularity may stimulate fracture repair. Vascular remodeling occurs naturally in the placenta during pregnancy in a process orchestrated by a placental progenitor population called the trophoblast stem cells (TSCs). TSCs differentiate into two cell types - Trophoblast Giant Cells (TGCs) and Syncytiotrophoblasts (SynT) which have distinct biological functions. TGCs invade the maternal vasculature and remodel local arteries via endovascular invasion, while SynTs provide a source of trophic factors that help promote nutrient exchange at the placenta. Together these cells generate a low pressure, high volume, blood flow that greatly increases the exchange potential at the feto-maternal interface. We hypothesized that the therapeutic application of TSCs to a fracture site could enable vascular remodeling and/or provide paracrine factors to improve the rate or extent of bone repair.

Methods: All in vivo work was approved by our IACUC. Fractures were made in the mid-diaphysis of SCID Beige mice tibia using a customized 3-point bending fixture and allowed to heal without stabilization. Immediately after injury mice were injected with β-gal labeled TSCs (10⁶ cells in 10μL), or a saline control, at the fracture site and animals survived for 7-21 days. Tibia were harvested, fixed in 4% PFA, decalcified for 14 days, then embedded into paraffin or OCT for serial sectioning. Paraffin embedded tibia were stained with safranin-O/fast green and Milligan’s Trichrome to quantify the volume of cartilage and bone in the fracture callus by histomorphometery. PECAM (1:500 BD Parmigen #553370) immunohistochemistry was performed on adjacent sections to visualize blood vessels at the fracture site. Frozen samples were stained with x-gal solution to localize the TSCs. Gene array data was completed on the TGC and SynT populations to identify highly active genes related to bone repair.

Results: To test the ability of trophoblasts to modulate fracture repair we injected undifferentiated β-gal labeled TSCs (1x10⁶ cells/10μl) locally at a murine tibia fracture and allowed the bones to repair through endochondral ossification. After 48 hours we demonstrate persistence of the β-gal labeled cells and show engraftment into the vasculature (Fig. 1). Initial data suggest that TSCs promote considerable vascular remodeling in the early phase of fracture repair with blood islands, similar to those of the placenta, seen by histology near the fracture site at day 10 (not shown), and noticeably dilated vessels in TSC injected fractures at day 14 (Fig. 2A-B) compared to control (Fig. 2C). A gene array of the trophoblast’s show the transcriptome of these cells is highly angiogenic and also associated with moderately high levels of chondro- and osteo- inductive genes (Fig. 3). Quantification of the fracture callus suggests the TSC treatment has a trend toward faster and more extensive healing, with more cartilage at day 7 and more bone at day 14 relative to control animals (Fig. 4).

Discussion: Previously we have demonstrated that ischemia significantly alters the fracture repair process by inducing periosteal apoptosis, delaying and reducing bone and cartilage formation, and ultimately producing a delayed union that is characterized by a large amount of fibrous, adipose, and cartilage tissue, but a small amount of bone. Further, we have determined that hyperoxia can stimulate angiogenesis and promote healing in the ischemic fracture environment. These data suggest that enhancing delivery of oxygen to the fracture site is one mechanism by which the blood supply contributes to repair. In addition, we and others1 have determined that various bone morphogenetic proteins (BMPs) are expressed by the vasculature in the fracture callus, and demonstrated that exogenous BMP7 stimulates endochondral and intramembranous ossification, but did not increase vascular density, in our ischemic model. In vitro we have found that secreted molecules from endothelial cells are sufficient promote mineralization of cartilage callus explants. Together these results indicate that soluble signals from the vasculature may also participate in stimulating fracture healing. Collectively, these outcomes reveal the important role that the vasculature plays in the normal course of bone repair, and suggests that manipulating the vasculature may provide therapeutic approaches to treat patients. Our data presented here shows, for the first time, that placental derived stem cells (TSCs) can be applied therapeutically to promote localized vascular remodeling. Furthermore, TSCs are transcriptionally regulated by hypoxia in a fashion that will promote vascular invasion under ischemic conditions5, such as those observed fetal development and traumatic injuries.

Significance: To our knowledge this is the first study evaluating the therapeutic potential of trophoblasts, specifically their ability to modulate the post-natal vasculature. These results suggest that TSCs may offer a distinct therapeutic role relative to other...
stem cells such as MSCs, which demonstrate a very poor engraftment. Our results have the potential to not only enhance clinical outcomes in skeletal trauma where there is often poor vascular perfusion, but may also have a significant impact on the broad field of regenerative medicine, since tissue necrosis and loss of organ function is often intimately tied to compromised vascularity (e.g. stroke, myocardial infarction).

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References: In the spirit of anonymous reviews our work is not cited. ¹Matsubara, H. et al. Vascular tissues are a primary source of BMP2 expression during bone formation induced by distraction osteogenesis. Bone 51 (2012). ²Maltepe, E. et al. Hypoxia-inducible factor-dependent histone deacetylase activity determines stem cell fate in the placenta. Development 132.
FIGURE 3: Gene expression array of TGCs and SynTs. Microarray gene expression of angiogenic and trophic genes in TGCs (green) and SynTs (blue) relative to the highly expressed keratin7 gene.

FIGURE 4: TSC treatment accelerates fracture repair. (A) Cartilage and (B) bone volume in fracture callus. Safranin-O staining of day 14 fracture (C) control or (D) TSC treated fractures show more cartilage (white arrows) in control fractures.

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