ΔModulating the Vasculature at a Fracture Through the Therapeutic Application of Placental Stem Cells

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Purpose: Blood supply to a fracture is a critical determinant of the rate and extent of healing. Therapies designed promote bone healing by stimulating angiogenesis have been proposed for a long time, yet to date no effective treatments are available. In this work, we capitalize on a transient process that modifies the vasculature during pregnancy to support the developing fetus. Placental progenitors, trophoblast stem cells (TSCs), promote vasculogenesis in response to fetal hypoxia by physically remodeling the maternal arterioles and secreting angiogenic factors to generate a high-volume, low-pressure fluid exchange. We hypothesize that the therapeutic application of TSCs will promote vascular remodeling and enhance fracture healing.

Methods: All murine studies were approved by the Institutional Animal Care and Use Committee. TSCs were isolated from the day E3.5 mouse blastocyst, transfected with eGFP (enhanced green fluorescent protein) and b-gal (beta-galactosidase) reporter constructs, expanded, and maintained in an undifferentiated state in vitro using published methodology. Nonstable fractures were created in the mid-diaphysis of immunocompromised mice (10-14 weeks, male, SCID Beige mice). Fractures were given and injection of 1 x 10^6 TSCs in 10 mL of PBS (phosphate-buffered saline), or PBS alone as a control. Fracture healing was evaluated by histology and quantitative stereology 5-28 days post injury. Immunohistochemistry was used to localize the cells following injections, and gene expression arrays were used to determine highly expressed genes from TSC that could benefit fracture repair.

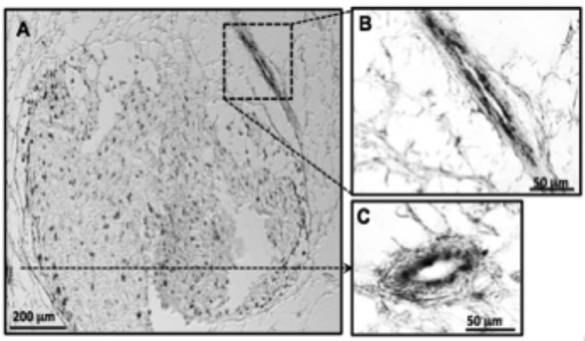

Figure 1. TSC Engraftment and endovascular invasion. (A) Bolus of β-gal labeled TSC located adjacent to fracture. (B-C) Insets of β-gal TSCs intercalated within vascular endothelium.

Results: Our data show that TSCs injected to nonstabilized murine fractures engraft into the vasculature (Fig. 1) and enhance the local blood supply (Fig. 2). Furthermore, injection of TSCs increased the volume of the cartilage callus 7 days post fracture, leading to more bone after 14 days of healing (Fig. 3)

Δ OTA Grant

- The FDA has not cleared this drug and/or medical device for the use described in this presentation (i.e., the drug or medical device is being discussed for an "off label" use). For full information, refer to page 600.

Conclusion: To our knowledge, this is the first study evaluating the therapeutic potential of TSCs. Our results have the potential to enhance clinical outcomes in skeletal trauma, where there is often poor vascular perfusion. Importantly, this work may also have a significant impact on the broader function that is often intimately tied to compromised vascularity.

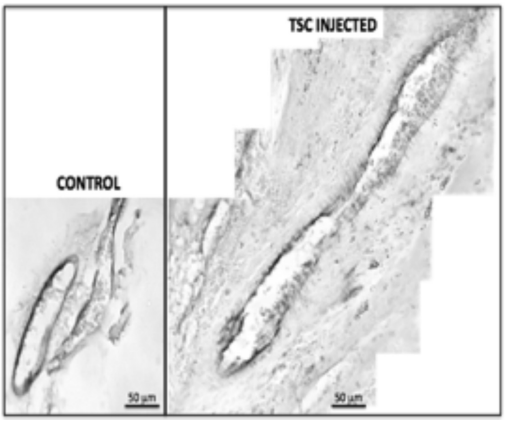

Figure 2. Comparison of average blood vessel diameter near fracture in control versus TSC injected animals shows vasodilation following TSC injection.

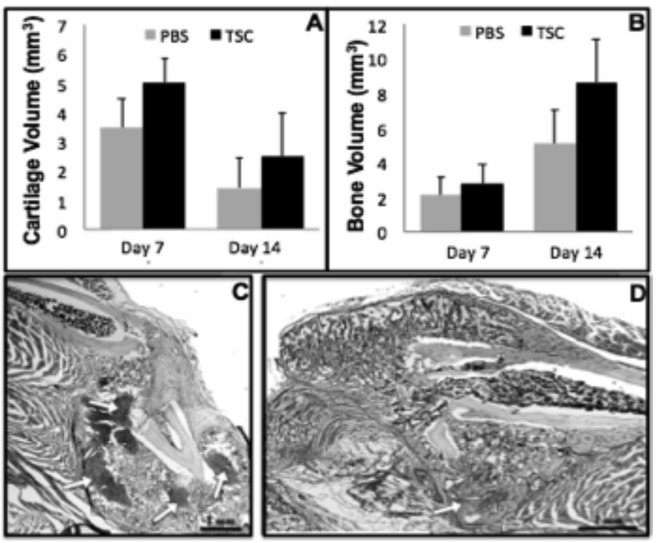

Figure 3. TSC treatment accelerates fracture repair. (A) Cartilage and (B) volume in fracture callus. Safranin-O staining of day 14 fracture (C) control, or (D) TSC treated fractures

See pages 99 - 147 for financial disclosure information.