Reduction in Angiogenic Support is the Primary Defect of Mesenchymal Stromal Cells Resident at the Site of Long Bone Nonunion

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Purpose: The underlying biological mechanisms that contribute to atrophic long bone nonunion are poorly understood. Mesenchymal stromal cells (MSCs) are widely accepted as key contributors to new bone formation and are also recognized as important mediators of blood vessel formation. This study examines the role of MSCs in tissue formation at the site of long bone nonunion. By comparing this tissue to induced periosteum, a highly osteogenic tissue rich in MSCs, and examining MSCs isolated from both nonunion and induced periosteum, we hope to gain new insights into disease pathogenesis.

Methods: Tissue and MSCs from the site of nonunion (n = 20) compared to induced periosteum (n = 15) and MSCs harvested from iliac crest bone marrow aspirates (n = 8). MSC colony and differentiation assays were used to assess MSC content and differentiation capacity. Flow cytometry and histological analysis was used to assess differences in overall cell content and vasculature between nonunion and induced periosteum. Real-time polymerase chain reaction was used to detect differences between expanded MSCs isolated from nonunion, induced periosteum, and bone marrow.

Results: Nonunion tissue was a rich source of MSCs that had osteogenic and chondrogenic potential comparable to bone marrow-derived MSCs. Compared to induced periosteum, nonunion tissue contained a 2.8-fold greater proportion of pericytes ($P = 0.036$), 3.3-fold more endothelial cells ($P = 0.016$), and 3.3-fold fewer lymphocytes ($P = 0.007$). Histological examination showed that blood vessels were 2.4-fold more numerous ($P = 0.001$) but had 2.9-fold smaller median luminal area ($P = 0.046$) in nonunion compared to induced membrane tissue. Transcript analysis revealed altered expression of several transcripts with primarily angiogenic roles, including FLT1, PTN, and ANGPTL4.

Conclusion: Nonunion tissue contains MSCs that do not display any impairment of in vitro differentiation capacity. The primary difference between nonunion and induced periosteum seems to be related to the vascular network, possibly due to altered expression of several angiogenic regulatory genes in nonunion-derived MSCs. We suggest that deficiency in angiogenic support rather than differentiation capacity is the primary defect in nonunion-derived MSCs.