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MEETING REPORTS

Pre-clinical Fracture Repair Studies: Meeting Report from the 31st Annual Meeting of the American Society for Bone and Mineral Research

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Fracture repair is a complex process that involves numerous cellular players whose roles remain poorly understood. A number of pre-clinical studies at the recent ASBMR Annual Meeting have shed light on the importance of different cell lineages in the bone healing process. These advances allow for more directed fracture healing therapies to target the relevant cell types with new biological agents.

An inflammatory response, concomitant with most musculoskeletal injuries, involves the recruitment of immune cells including macrophages, lymphocytes, and granulocytes. While a key function of these cells during bone repair is to prevent infection, these cells may act to support or diminish bone formation.

Osteal macrophages or 'osteomacs' have been identified lining bone surfaces and may be important in bone homeostasis (1;2). These cells are ablated in the Macrophage-Fas-Induced Apoptosis (MAFIA) mouse system, and this can have profound effects on bone remodeling. Ablation of macrophages using this mouse model profoundly disrupted bone repair in a tibial defect model. Moreover, local treatment with the pro-inflammatory cytokine colony stimulating factor 1 (CSF-1) could augment bone repair (3). While the aforementioned experiments support the concept of a population of osteal macrophages that can promote bone healing, more explicit and directed experiments are required to establish this paradigm and harness these cells therapeutically.

Lymphocytes are also present with inflammatory injury, and their role was examined in *Rag1*(-/-) mice that lack mature B and T cells (4). In a thorough fracture healing study, closed femoral fractures were generated in wild type and *Rag1*(-/-) mice and samples were collected at multiple time points for μCT analysis, strength testing, and decalcified histology. Unexpectedly, fracture repair was accelerated in *Rag1*(-/-) mice, indicating that lymphocytes can antagonize bone healing. The mechanism of this effect is unclear, but may involve the secretion of specific cytokines by B and T cells that are detrimental to osteoprogenitor recruitment or differentiation.

One important question in orthopedics is the contribution of circulating cells to the repair of fractures and other bone defects. While circulating osteoprogenitors have been described in humans (5), mouse models of parabiosis have previously indicated only a minor role for these cells in fracture healing (6). A similar parabiotic mouse model was utilized to investigate whether the participation of smooth-muscle alpha actin (SMAA) osteoprogenitors (7) in bone healing primarily involved the circulation (8). No contribution by these SMAA+ osteoprogenitor was observed in tibial fractures, indicating that any involvement of these cells arose directly from local tissues rather than via the blood supply. This study again reinforces the concept that local osteoprogenitors are likely to be the major contributors to bone repair.

One emerging possibility is that some of these local osteoprogenitors may derive from adjacent muscle tissues (9). In a mouse open fracture model, it was demonstrated that muscle-derived osteoprogenitors are able to make a significant contribution to bone healing. Myogenic progenitors were permanently labelled using a MyoD-Cre;Z/AP reporter mouse system. In open fractures where the periosteum was stripped, 30-50% of the cells that contributed to early fracture repair were from the muscle lineage (10). Muscle cell contribution was greatly reduced in closed fractures with an intact periosteal layer. These data show a new and potentially important role for muscle cells in bone repair, although more work will be required to find ways to mobilize these cells therapeutically.

Muscle-derived osteoprogenitors may also be a target of GDF-8 (myostatin), a negative regulator of bone and muscle. A GDF-8 inhibitor (decoy receptor) was shown to prevent microgravity-induced bone and muscle loss in mice, and the study professed to show the first video footage of experimental mice in free orbit (11). In another study, an inhibitory GDF-8 propeptide was found to dramatically improve bone healing in a fibular osteotomy model (12). The frequency of union at day 15 was increased by more than 50% and the callus volume increased by 30%. While it is unclear whether the propeptide acts on collective or distinct muscle and bone progenitor populations, its robust effects on fracture healing make this a new and exciting therapeutic strategy for orthopedic intervention.

An increasingly widespread strategy is to apply anti-osteoporosis drugs to models of fracture healing situations. Nevertheless, fundamental differences exist in the cell types involved with homeostasis versus healing that can affect the eventual outcome. One example of this is TGF-β, a molecule now purported to couple resorption with marrow stromal cell recruitment for bone maintenance (13;14). In cultured cells, TGF-β is a strong inhibitor of osteogenic differentiation, suggesting it would be unfavorable for bone tissue engineering (15). However, in a BMP-2-induced ectopic bone formation model, TGF-β was able to potentiate the effects of BMP-2 *in vivo* (16). TGF-β may augment osteoprogenitor recruitment in a bone healing context or may act upon differentiated osteoblasts and/or osteoclasts, although more studies will be required to validate the exact mechanism.

Despite the distinction between skeletal maintenance and repair, the emerging osteoporotic therapy of anti-sclerostin antibody (Scl-Ab) may have orthopedic relevance. Researchers from Amgen presented data suggesting beneficial effects for Scl-Ab in a rat closed fracture model (17). Furthermore, in a bilateral tibial defect model in primates (cynomolgus monkeys), treatment with Scl-Ab generated a global increase in bone formation rate and specific benefits to callus volume and mechanical strength (18). Together these studies allude to a negative regulatory role of osteocytes on bone healing via sclerostin that has yet to be fully characterized.

In conclusion, while osteoblasts and osteoclasts are the traditional “key players” in fracture repair, other cell types may have important roles in modulating bone healing. Pre-clinical studies at the recent ASBMR meeting have started to address the roles of inflammatory cells, muscle cells, circulating cells, and osteocytes in bone repair, illustrating a complex array of cellular interactions that influence the healing process. It is likely that a greater understanding of the roles and contributions of these cell lineages will lead to new and innovative treatments for fracture repair.

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