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Opinion Article

Osteopontin-Mediated Macrophage Polarization in Fracture Healing and Bone Regeneration

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DESCRIPTION

Fracture healing represents a complex biological process involving multiple cell types and signaling pathways that must be precisely coordinated to restore bone integrity. Macrophages play essential roles in fracture healing through their diverse functions including debris clearance, inflammatory modulation, and tissue remodeling. Osteopontin (OPN), a phosphorylated glycoprotein abundant in bone matrix, has emerged as a key regulator of macrophage function. This investigation examined the role of osteopontin in modulating macrophage polarization during fracture healing and its impact on bone regeneration outcomes.

Closed femoral fractures were created in 8-week-old C57BL/6J mice using a standardized three-point bending technique with intramedullary fixation. Wild-type and Osteopontin-deficient (OPN-/-) mice were compared to determine the specific contributions of osteopontin to fracture healing. Fracture healing was monitored using radiography, micro-computed tomography, and histological analysis at 7, 14, 21, and 28 days post-fracture.

Flow cytometric analysis of fracture callus tissue revealed distinct macrophage populations with different polarization states. $CD68^+$ $CD86^+$ M1 macrophages predominated during the early inflammatory phase (days 1-7), while $CD68^+$ $CD206^+$ M2 macrophages increased during the repair phase (days 14-21). In wild-type mice, M2 macrophages constituted 65% of total macrophages by day 14, compared to only 32% in OPN-/- mice, indicating impaired M2 polarization in the absence of osteopontin.

Immunofluorescence staining confirmed the spatial distribution of macrophage populations within fracture calluses. M1 macrophages were concentrated at the fracture site periphery, while M2 macrophages localized to areas of active bone and cartilage formation. OPN-/- mice showed persistent M1 macrophage infiltration and reduced M2 macrophage presence in reparative tissues, correlating with delayed healing progression. *In vitro* studies using Bone Marrow-Derived Macrophages (BMDMs) demonstrated that osteopontin directly promoted M2 polarization through integrin-mediated signaling. Osteopontin treatment (10 μg/ml) increased expression of M2 markers including Arg1 (3.2-fold), Il10 (2.8-fold), and Fizz1 (4.1-fold), while suppressing M1 markers including Nos2 (68% reduction) and Il6 (74% reduction). These effects were blocked by integrin αvβ3 antagonists, confirming integrin-dependent mechanisms.

Mechanical testing of healed fractures revealed impaired biomechanical properties in OPN-/- mice. Ultimate load was reduced by 47%, while energy to failure decreased by 56% compared to wild-type controls. Stiffness measurements showed 39% reduction in OPN-/- mice, indicating compromised mechanical restoration. These biomechanical deficits correlated with histological evidence of impaired bone formation and delayed callus remodeling.

Transcriptomic analysis of fracture callus tissue identified distinct gene expression profiles associated with osteopontin-mediated macrophage polarization. Wild-type mice showed enhanced expression of genes involved in tissue repair, angiogenesis, and bone formation, including *Vegfa*, *Bmp2*, and *Runx2*. In contrast, OPN-/- mice exhibited prolonged expression of inflammatory genes including *Il1b*, *Tnfa*, and *Mmp9*, suggesting persistent inflammatory signaling.

Cytokine analysis of fracture callus homogenates revealed that osteopontin deficiency altered the local cytokine environment. Pro-inflammatory cytokines IL-1β and TNF-α remained elevated in OPN-/- mice through day 21, while anti-inflammatory cytokines IL-10 and TGF-β were significantly reduced. This imbalanced cytokine profile correlated with impaired bone formation and delayed soft callus mineralization.

Therapeutic studies using exogenous osteopontin administration demonstrated potential for improving fracture healing outcomes. Local injection of recombinant osteopontin (50 μg) at the fracture site enhanced M2 macrophage polarization and accelerated healing in both wild-type and OPN-/- mice. Treated fractures showed 34% improvement in ultimate load and 28% increase in bone volume compared to vehicle controls.

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Mechanistic investigations revealed that osteopontin promotes M2 polarization through activation of the PI3K/Akt signaling pathway. Osteopontin treatment increased Akt phosphorylation by 267% within 30 minutes, leading to downstream activation of mTOR and enhanced oxidative metabolism characteristic of M2 macrophages.

CONCLUSION

Osteopontin plays a crucial role in fracture healing through promotion of M2 macrophage polarization and resolution of inflammation. The impaired healing observed in osteopontin-deficient mice demonstrates the essential nature of this protein in coordinating immune responses during bone regeneration. These findings suggest that osteopontin-based therapies could enhance fracture healing outcomes, particularly in cases of delayed or impaired bone repair. These metabolic changes were accompanied by increased mitochondrial biogenesis and enhanced anti-inflammatory cytokine production.