Apyrase as a novel therapeutic inhibitor of heterotopic ossification

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Heterotopic ossification (HO) is a disabling disorder where endochondral bone forms in soft tissue (1). Genetic diseases, traumatic injuries, or severe burns can induce this pathological condition and can lead to severe immobility (2). While the mechanisms by which the bony lesions arise are not completely understood, intense inflammation associated with musculoskeletal injury and/or highly invasive orthopaedic surgery is thought to induce HO. The incidence of HO has been reported between 3\% and 90\% following total hip arthroplasty or severe fracture of the long bones (3). While the vast majority of these cases are asymptomatic, some patients will present decreased range of motion and painful swelling around the affected joints leading to severe immobility. In severe cases, ectopic bone formation may be involved in implant failure, leading to costly and painful revision surgery (4). Currently, no treatment modalities exist for HO and attempts to prevent the ectopic formation of bone have had only limited success. Early diagnosis of the formation of ectopic bone is complicated by the absence of calcium in the matrix during the first few weeks following surgery, making X-ray detection of ectopic bone very difficult. Prophylactic radiation therapy, anti-inflammatory, and bisphosphonates agents have shown some promise in preventing HO, but their effects are mild to moderate at best and complicated with adverse effects (5).

Bone morphogenetic proteins (BMP) such as BMP2, BMP4, and BMP9 have been shown to promote HO when injected into the muscle tissue of mice (6-8) and are highly expressed in human lesions of heterotopic bone (9,10). In individuals suffering from fibrodysplasia ossificans progressiva (FOP), a rare genetic disorder where patients exhibit massive HO and form an ectopic skeleton (2), a single point mutation in the \textit{ACVR1} gene causing a change in amino acid 206 (R206H) of the BMP type I receptor activin-like kinase 2 (ALK2) disrupts an \(\alpha\)-helix in the glycine-serine rich domain, rendering ALK2 constitutively active (11,12). ALK2 activity causes phosphorylation of Smad proteins (13) and has been shown to regulate endothelial-mesenchymal transition (EndMT) and stem cell differentiation to form new skeletal tissue (7,14).

The discovery of the ALK2 mutation in FOP has provided the most direct therapeutic strategy by inhibiting this BMP receptor. Studies using chemical inhibitors of ALK2 called dorsomorphin and LDN-193189 have been successful in preventing HO in mice (15). A further study showed that the BMP type II receptor is required for HO in mutant ALK2 transgenic mice (16), providing another therapeutic target.

Other studies have shown that mice with defective sensory neurons show impaired BMP-induced HO (17). A neuropeptide released by sensory neurons called Substance P, which causes recruitment and degranulation of mast cells, plays a significant role in HO. RP-67580, a Substance P antagonist, significantly reduces BMP4-induced HO in mice (18). An anti-histamine called Cromolyn, which blocks mast cell degranulation, also significantly reduces HO in mice (17). Interestingly, retinoic acid receptor-\(\gamma\) agonists have been identified to be tremendously effective pharmacological inhibitors of HO by impairing
chondrogenesis and endochondral ossification and represent the most promising drugs to date for potential clinical application to prevent HO (19). However, novel therapeutic strategies are needed to avoid potential side effects of such inhibitors.

In a recent issue of *Science Translational Medicine*, Peterson *et al.* show that burn injury-induced HO can be treated through a novel therapeutic called apyrase, which causes ATP hydrolysis and modulation of Smad1/5/8 phosphorylation (20). The authors analyzed adipose tissue and adipose-derived mesenchymal stem cells (MSCs) from burn patients and found elevated BMP-dependent Smad signaling and osteogenic differentiation compared to control groups. In a mouse burn model (dorsal scald burn) the injury showed similar increased BMP signaling and osteogenic potential. Addition of the ALK2 inhibitor LDN-193189 decreased the osteogenic potential of MSCs from the burn site. Elevated levels of ATP and decreased levels of cAMP were observed after burn injury (20). cAMP has been described to inhibit Smad1/5/8 phosphorylation (21). The authors used topical apyrase, an ATP hydrolyzing agent, at the burn site and showed that it inhibited BMP signaling and osteogenic differentiation of MSCs, thereby preventing HO at the site of injury. Interestingly, application of apyrase to the burn site also inhibited HO caused by Achilles tenotomy (20). Small molecule therapeutics such as LDN-193189 may result in severe off-target effects thereby impairing their clinical utility in preventing HO (22).

It would be beneficial to test whether apyrase would alleviate HO in an animal model of FOP or in patients afflicted with this debilitating disorder. Clinical studies should also be done to assess the effects of apyrase in patients with various forms of trauma-induced HO. Targeting ATP hydrolysis using apyrase has great potential for clinical application in HO treatment and will likely improve current therapeutic strategies.

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