Picking a bone with heterotopic ossification: translational progress current and future

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Heterotopic ossification (HO) represents a serious clinical and basic science conundrum. It is a frequent complication of some of the most common orthopaedic procedures including joint replacement, fracture repair, and amputations. Furthermore it causes costly debilitation following trauma, burns, brain and spinal injuries, and is one of the defining injuries of modern military conflicts (1). However, despite the commonality and burden of HO, relatively little is known about its mechanism. This leads to a dearth of effective clinical prophylactic and therapeutic strategies.

A recent editorial discussing new additions to the bank of knowledge on HO highlighted some of the questions that remain and were indeed posed by our recent article on targeting ATP hydrolysis and BMP signaling blockade as potential therapeutic pathways to prevent ectopic bone formation (2,3). The purpose of this article is to expound upon some of those questions and lay a framework for some of the future translational research that is critically needed in HO.

Broadly speaking, we know that HO is a reactive process in response to inflammatory insult. Furthermore, because ectopic bone is a highly differentiated tissue that develops in an endochondral fashion and is complete with its own marrow cavity, it likely develops from multipotent stem cells capable of forming bone. We have shown that histologically and spectroscopically HO resembles mature orthotopic bone but, because it is a dysregulated process, there are differences in the mineral content, function, and strength (4). To date there is no clear consensus on the specific identity of the multipotent cells responsible for forming HO, despite many elegant studies suggesting a range of local or circulating progenitors (5).

We have used adipose stromal cells (ASC) as a proxy for the still unidentified HO progenitor cell because it is well-described and easily manipulated mesenchymal stem cell. Hence, we believe that until the HO progenitor is definitively identified, ASCs represent an adequate vehicle for *in vitro* study of biologic mechanism and osteogenic modifiers. We do not suggest that ASCs are ultimately the primary cells responsible for HO.

One of the interesting results in our study was the effect of a remote burn injury on ASCs distant from the burn site. We saw increased osteogenecity of the ASCs from mice that sustained a burn injury. This highlights the importance of a systemic inflammatory insult to the permissive niche necessary for ectopic bone formation.

These results are consistent with the more clinically relevant data we presented on gene expression in adipose tissue from burn patients in which osteogenic genes, including *SMAD*s and RUNX2, were upregulated within the first 96 hours following burn injury. While we agree with Kan *et al.* (2) that these samples were not pure MSC populations, the significantly elevated expression of osteogenic genes in a heterogenous population of cells highlights how influential the post-injury inflammatory environment is to the signaling pathways that lead to bone formation. Similarly, in our mouse model of HO formation, which consists of a focused musculoskeletal injury (achilles tenotomy) and a distant burn injury, we noted significant, but not complete, reductions in HO development by treating the burn injury site with a targeted anti-inflammatory drug—apyrase. One important implication of these results is that the initial mitigation of inflammation

may be of paramount importance to the prevention of HO.

While the general anti-inflammatory effects of apyrase are certainly contributing to the overall reduction in reactive osteogenicity, we also believe that its specific action—inactivation of ATP through cleavage of phosphate groups—is central to its efficacy in preventing HO while not inhibiting wound healing. We were somewhat surprised at the intracellular signaling effects of apyrase which itself primarily functions extracellularly. Cyclic adenosine monophosphate (cAMP) is known to inhibit phosphorylation of SMAD1/5/8 and thereby prevent osteogenesis (6). Increasing cAMP activity intracellularly has also been seen with phosphodiesterase inhibitors which suppress osteogenic differentiation of MSCs (7,8).

While delineating the precise mechanistic details between extracellular ATP hydrolysis and intracellular cAMP signaling was not a goal of our study, one link was provided by the increase in signaling through adenosine receptors A2A and A2B. These receptors are important for purine metabolism and also for the inflammatory response in general (9). In regards to osteogenic differentiation of MSCs, the A2B receptor has been shown to increase osteogenic tendency. Knockout mice lacking A2B develop osteopenia with poor osteoblast activity (10). Conversely the A2A receptor promotes adipogenesis and blockade of A2A causes increases osteogenicity (11). We saw a reduction in both A2A and A2B in MSCs from mice that had suffered a burn injury, with restitution of these levels following apyrase treatment at the burn site. Clearly there is impetus for further exploration of the effects of these and the other two types of adenosine receptors (A1 and A3) and their differential roles in osteogenicity and ectopic bone formation. In this respect our findings raise more questions than answers as to the specific role of adenosine receptors, however a clinically relevant connection may be found.

While the exact mechanism whereby apyrase increases cAMP levels intracellularly are not fully delineated, the terminal effect resulting in decreased osteogenesis through reduction in SMAD phosphorylation is clear. One of the exciting themes of recent HO research is the developing body of work showing that trauma induced HO has many histologic and mechanistic similarities to genetic forms of HO including fibrodysplasia ossificans progressive (FOP). It has been established that trauma induced HO develops in an endochondral fashion, as is the case in FOP. Much research has been completed showing that ectopic bone in FOP is caused by a point mutation (R206H) in the type 1 BMP receptor ALK2 causing it to be constitutively active (12,13). Similarly, BMP signaling is critical in trauma induced HO. We saw a striking reduction in ectopic bone formation with the application of a small molecule inhibitor of BMP type 1 receptors ALK2 and ALK3-LDN-193189. This compound, which is a derivative of dorsomorphin, was originally developed as a potential treatment for FOP (14). In a similar vein, much of the well-delineated mechanistic details of ectopic bone formation in FOP may be similar to the pathways in trauma-induced HO. Future translational research in reactive bone formation may benefit greatly by taking advantage of the robust body of work already completed on FOP.

There are several major questions of critical importance that still remain to be answered including the identification of the progenitor cell line(s) responsible for HO, understanding the signaling mechanisms responsible for progenitor cell differentiation which link inflammation and bone formation, and understanding how diverse injuries create the permissive niche that induces ectopic bone formation. Some leading hypotheses of progenitor cell etiology include endothelial cells; bone marrow derived mesenchymal cells locally or circulating, macrophages, and circulating hematopoietic cells (15-19).

Elegant studies using tracers for Tie-2 have suggested that at least 50% of HO is derived from vasculogenic precursors, which may be explained by other findings that an endothelial to mesenchymal transition can produce HO (20). Local vasculogenic factors including vascular endothelial growth factor (VEGF), and platelet derived growth factor (PDGF) are crucial components of the permissive niche for HO (16,21). There are also important connections between these vasculogenic cytokines and BMP signaling which seems to be the final common pathway through which vasculogenic, osteogenic, and purine signaling functions. Future directions may also benefit from a focus on chondrogenesis which has consistently been shown to be the first step in ectopic bone formation. Given the endochondral nature of HO development, preventing cartilage formation may logically be the step at which prophylactic intervention would be the most efficacious.

Clinically, there are many promising developments on the horizon. Because calcified bone is a highly specialized tissue for which the body does not have native capability to quickly dismantle and resorb, current treatment for HO relies on surgical excision. These procedures are difficult and carry high rates of morbidity and recurrence (22,23). For these reasons, much of the current translational research on HO focuses on prophylaxis to prevent ectopic

bone formation and early diagnostic modalities.

Current prophylactic regimens rely on NSAIDs (e.g., indomethacin), radiation, or bisphosphonates; all of which have their own divers efficacies and drawbacks (5). While these systemic treatments have shown general efficacy and are used clinically, there may be great benefit obtained from more localized treatment with substances such as apyrase, or from more targeted inhibitors of osteogenesis or chondrogenesis. Indeed, one of the most surprising and promising results of our findings on apyrase was the future effects of an initial application, which we have also seen improves wound healing (24). This type of targeted therapy could be very useful in burn wounds, trauma repair, and arthroplasty where the general location of potential ectopic bone formation is known. However, apyrase application may impractical for HO resulting from TBI, poly-trauma, and other inflammatory states stemming from more global insults.

We certainly agree that it would be prudent and of potential great benefit to further study the efficacy and mechanistic details of apyrase use at both a basic science and clinical level (13). Furthermore we believe it would be wise to continue to include advancements in both the fields of HO and FOP in future projects and hypotheses given the general concordance in results thus far. Ultimately it will require a multifaceted approach for these advances in translational medicine to make a difference in the clinical treatment of HO.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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