Editorial

The burning questions of heterotopic ossification

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Heterotopic ossification (HO) is a pathologic process of ectopic bone formation in soft tissues. HO could be a costly clinical complication, or a rare life threatening disorder. Recent findings from different labs have tremendously improved our understanding of this pathologic process, but many burning questions remain. For example: (I) how far do we still need to go to achieve a fairly complete understanding of this disorder? And where are we now? (II) What is/are the best way(s) to efficiently control this debilitating, yet heterogeneous disorder? Levi's group (1) used a burn model to show us some novel intriguing insights of this pathologic process, and their finding, among others, actually raised more questions, which will be the major focus of this editorial. Indeed, even though it is our intention to put their finding in a right prospective, highlight the implications for both basic researches and clinical applications, identify the potential limitations and caveats, and discuss how to further address the potential issues in the future, the immediate goal of this editorial is raising awareness.

The most significant and intriguing finding that has direct clinical implication is that Levi's group identified another novel druggable target, i.e., the remote application of apyrase (ATP hydrolyzing agent) in the burn site decreased HO formation and mitigated functional impairment later. Equally interesting for the basic research is that, mechanistically, burn site apyrase treatment not only decreased extracellular ATP and increased intracellular cAMP, but also decreased phosphorylation of SMAD1/5/8 in MSCs *in vitro*.

They first surveyed a repository of adipose tissues from 244 burn patients, looking at a subset of genes related to BMP-mediated canonical SMAD signaling that is known to play a role in HO. This was actually an risky launch pad for the subsequent MSC study, since the MSC is really a negligible subpopulation of the harvested heterogeneous cell masses from burn patients, therefore, any finding comes out of this survey wouldn't automatically reflect the changes of MSCs, and to make the matter worse, additional variations, such as that of the anatomic location, could potentially further dilute the real MSC signals.

With that in mind, even though the survey data itself (*Figure 1*) was not strong by any standard, the up-regulation of the canonical SMAD signaling pathway in MSC must be extremely robust, if this data only reflects the changes of the MSC subpopulation. In fact, we argue that the changes of the MSC could not theoretically possible to account for the overall changes observed in *Figure 1*, i.e., other subpopulation must have contributed to the observed changes. Nevertheless, this primary indication was subsequently reinforced with more relevant hMSCs study that directly probed the osteogenic differentiation of hMSCs from control and burn patients (*Figure 2*). This set up a stage for the following up mechanistic or prove-of-concept preclinical trial in mice.

For the mechanistic study, instead of looking at the commonly investigated pathways, Levi's group interrogated an underappreciated extracellular nucleotide processing pathway for the potential underlying mechanisms (2). It is known that MSCs possess a significant display of extracellular nucleotides receptors, such as adenosine receptors (A2A and A2B), and nucleotide processing ectoenzymes, such as CD39, an endogenous apyrase enzyme, and CD73, an ecto-5' nucleotidase (3,4). More

interestingly, defect in this pathway has already associated with different types of osteogenic disorders. For example, defects in CD73 is known to lead to the calcification of joints and arteries (5), while mice lacking the adenosine A2B receptor display reduced osteoblast activity, osteopenia, and delayed fracture healing (6). It was against this background, the authors treated the burn injured mice with apyrase, an ATP hydrolyzing agent, which leaded to the above-mentioned significant finding.

The data are indeed very interesting, but to appropriately interpret the data is by no means easy, and the challenges come from at least four fronts:

(I) Mechanistically, it is difficult to establish a logical and internally consistent working model to explain all the data. For example, it is easy to understand that apyrase, an ATP hydrolyzing agent, decreased the extracellular ATP, but it is not easy to explain the observed high intracellular cAMP, and it is even harder to understand how apyrase cause reduced phosphorylation of SMAD1/5/8. Directly? Or indirectly? Or both?

One particular challenging aspect is that mMSCs with the observed expression changes *in vitro* were actually tempo-spatially separated from original mMSCs that were directly exposed to the apyrase treatment *in vivo*. How does the treatment applied on the burning site change the behavior of the progenies of remote mMSCs (located at the inguinal fat pads, far from burning sites), days or weeks later?

(II) Another challenge is to reconcile current data externally with the literatures. Since the adenosine receptor A2B has been associated with increased osteogenesis (6), which suggests that the burn-induced reduction of adenosine A2B receptor levels would have the desirable effect of inhibiting, rather than promoting, HO formation. And similarly, the apyrase administration should have the undesirable effect of promoting osteogenesis, by encouraging adenosine receptor expression.

(III) What are the specific implications for the future clinical applications? From the available data, it seems clear that topical application of apyrase in the context of burn injury is a promising prevention strategy of later HO. However, to translate this finding successfully to the treatment of human patients who, unlike the lab mice, usually don't live in sterile environments, there is a very delicate balance to keep, i.e., to keep the desirable inflammation response strong enough to prevent the infection and encourage healing, while still limit the unwanted and exaggerated inflammation response that can lead to ectopic bone.

Furthermore, for HO associated with other clinical contexts, such as in the context of brain or spinal cord injuries, there will be more hurdles to be circumvented. For example, in most of these contexts, topical application is likely either impractical, or insufficient, or both. In this case, other more appropriate routes or paradigms have to be established first. To do that, investigators have to address the concern that whether apyrase treatment can be safely administered through other, such as through systematical, routes.

(IV) What are the implications of current finding to basic research? Currently, it is commonly accepted that three key factors are necessary for the ectopic bone formation, i.e., osteo-potent cells (usually multi-potent stem/progenitor cells), a permissive niche, and an inflammatory insult. How does apyrase treatment affect these key factors? This study provided some clues that: (I) apyrase treatment inhibited the classic BMP signaling pathway, therefore it could potentially affect both the osteo-potent cells, and the permissive niche directly; and (II) previous studies also suggested that apyrase treatment reduces the overall inflammatory response (7), thereby likely inhibits the HO indirectly. However, the available data by no means delineate the exact underlying molecular or cellular mechanisms.

Overall, this is an interesting study which provided some valuable insights of HO. However, future detailed studies will be urgently needed to delineate the exact molecular mechanisms and answers all these burning questions.

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