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## In Focus

# Applying Proteomics to Study Crosstalk at the Cartilage-Subchondral Bone Interface in Osteoarthritis: Current Status and Future Directions

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Osteoarthritis (OA) is the most common age-related degenerative joint disorder. It is considered a disease of the entire joint, which is not simply a result of attrition but rather abnormal remodelling and coalescent failure of multiple tissues. Despite the prevalence of OA, therapeutic strategies are currently limited to the treatment of pain and inflammation. New disease-modifying agents that slow progression and diagnostic biomarkers are urgently required. Given the lack of treatment options to repair degradation of articular cartilage, anti-resorptive agents and anabolics, such as bisphosphonates, are candidate treatments for OA (Karsdal et al., 2014). An optimal treatment for OA will likely target at least two joint tissues, yet the factors that govern the bone-cartilage interactions during pathogenesis remain largely unknown.

The bone-cartilage interface is an important synergistic unit consisting of the area between the deep layers of articular cartilage and the underlying subchondral bone (Yuan et al., 2014). The close physical association between subchondral bone and cartilage allows interaction and suggests biochemical and molecular crosstalk may contribute to OA pathology (Goldring, 2012). Crosstalk is elevated at the osteoarthritic bone-cartilage juncture due to osteochondral angiogenesis and bone remodelling (Fig. 1). Vascular invasion from bone into cartilage and development of microcracks and fissures provide pathways for communication (Li et al., 2013). Alterations in either bone or carti-

lage can modulate signalling pathways including HIF-2 $\alpha$ , OPG/RANK/RANKL, TGF- $\beta$  and Wnt $\beta$ -catenin; these pathways may alter homeostasis of neighbouring tissues and affect the function of the joint unit as a whole (Yuan et al., 2014). The biomarker CTX-II has been detected at the bone-cartilage interface and is associated with osteophyte formation (Bay-Jensen et al., 2008). It has also been suggested that changes in the uCTX-II level may reflect the functional bone-cartilage unit, indicating the importance of crosstalk in OA pathophysiology (Lories and Luyten, 2011). The role of the crosstalk in the progression of OA requires additional investigation using genomics, epigenetics, proteomics and metabolomics approaches.

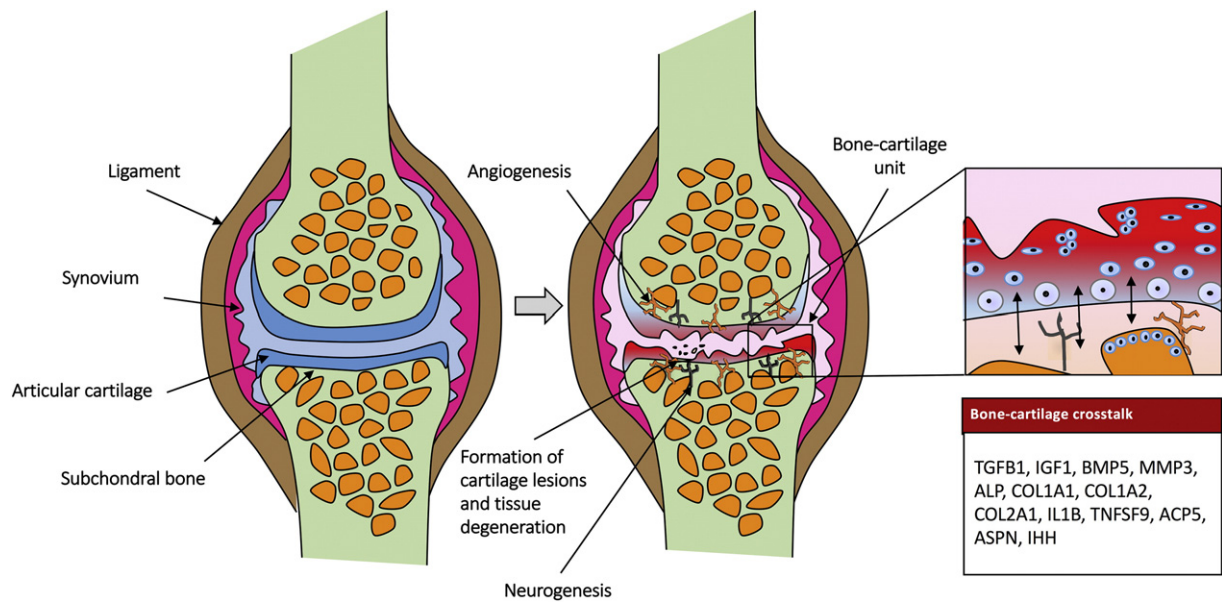
Proteomic technologies make it possible to identify factors involved in OA progression and build a library of mediators. Studies have revealed numerous cytokines, proteases, and matrix fragments in serum, synovial fluid, and articular cartilage of OA patients. However, little information is currently available regarding the protein profiles of disturbances in subchondral bone or bone-cartilage crosstalk (Boris Chan et al., 2015). Gene expression profiles of subchondral bone isolated from early experimental OA have identified dysregulated genes with roles in bone and cartilage development, remodelling and degeneration (Zhang et al., 2012). That study indicates the importance of further investigations into the subchondral bone using high-throughput protein analyses. Additionally, transcriptome-proteome combined study of OA synovial tissue revealed that gene expression changes do not always coincide with protein levels, thus again demonstrates the need for comprehensive proteome studies of subchondral bone to validate transcriptomics (Lorenz et al., 2003).

It is technically challenging to extract proteins of interest from bone due to the abundance of collagens and minerals. A pilot study using HCl/Urea extraction identified 119 proteins in OA subchondral bone with only 7 proteins shared between the low and high damage regions. This data suggests that signalling pathways in the corresponding subchondral bone region may relate to OA severity (Boris Chan et al., 2015). However, validation is required with the authors acknowledging that the regional variations could be due to experimental error.

Bone-cartilage communication models are needed to elucidate soluble mediators. Previously we have employed a cartilage explant model system to investigate the secretome of cartilage in response to inflammatory stimuli (Williams et al., 2013). A similar model system is required using osteochondral co-cultured explants to identify novel

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**Fig. 1.** Molecules involved in crosstalk at the bone–cartilage interface during osteoarthritis (OA) development and progression. Genes with altered expression profiles in an early experimental OA model include transforming growth factor  $\beta$ 1 (TGF $\beta$ 1), insulin-like growth factor-1 (IGF1), bone morphogenic protein-5 (BMP5), matrix metalloproteinase-3 (MMP3), alkaline phosphatase (ALP), collagen type 1 (Col1a1) and type 2 (Col2a1), interleukin-1 $\beta$  (IL1 $\beta$ ) and Indian hedgehog (Ihh). Note that the list is not exhaustive.

soluble factors released in response to inflammation or loading when the bone–cartilage juncture is intact. Recently an *in vitro* three-dimensional microsystem that models the osteochondral unit was developed. Osteogenic and chondrogenic tissues were produced using mesenchymal stem cells seeded within biomaterial scaffolds in a multichamber bioreactor (Lin et al., 2014). Tissue-specific gene expression, matrix production and a basophilic developing tidemark were detected. Introduction of interleukin-1 $\beta$  (IL-1 $\beta$ ) to either the chondral or osseous medium induced strong degradative responses both locally and in the opposing tissue type. IL-1 $\beta$  treatment of the osseous compartment resulted in a stronger catabolic response in the chondral layer than direct IL-1 $\beta$  application to the chondral component. This study provides evidence for active biochemical communication across the bone–cartilage interface and supports the osteochondral nature of OA (Lin et al., 2014).

Development of cell based model systems will allow investigation into the release of soluble factors by stimulated osteoblasts/osteocytes that can induce a pro-catabolic phenotype in chondrocytes. An iTRAQ secretome analysis revealed that a soluble protein (14-3-3 $\epsilon$ ) was differentially present when a mechanical stress was applied on osteoblasts and that these secreted mediators could activate chondrocytes to produce metalloproteinases (Priam et al., 2013).

In conclusion, molecular studies of crosstalk at the bone–cartilage interface will aid our understanding of the pathophysiology of OA and advance therapeutic approaches. This will facilitate the development of novel drugs and agents that specifically block mechanisms of crosstalk responsible for the structural changes in OA and provide a novel system for biomarker discovery.

### Conflict of Interest Statement

The authors wrote this paper within the scope of their research positions. The authors declare no conflict of interests.

### Competing Interests

The authors declare no competing interests.

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