

A role for TGF- β in osteoarthritis-related pain?

“...This underlines the two faces of TGF- β in OA: good for young healthy cartilage, bad for synovial tissue, osteophytes and now also: pain.”

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Pain in osteoarthritis: where does it come from?

Osteoarthritis (OA) is a joint disease hallmarked by the progressive destruction of cartilage. For patients, the degrading cartilage by itself does not become a relevant issue unless they lose the function of their joint. Their main problem and reason to visit their physician is pain. When searching for a cause of pain, inflammation is usually directly in view. In general, there is a clear and obvious link between inflammation and pain. In OA, it has become clear that inflammation can indeed be involved in the disease to some extent, but the severity and duration varies from patient to patient. Moreover, in a subset of OA patients, there is pain without any sign of an active inflammation, which clearly points toward an alternative source of pain that has remained under the radar [1].

The usual suspects when it comes to pain are inflammatory factors like TNF- α and IL-1, which in turn can induce NGF [2]. NGF acts as a survival factor for neurons and a sensitizer of nociceptors [3]. It has gained much attention due to its very clear involvement in OA pain [4,5]. Bannwarth *et al.* showed that there were significant differences in responsiveness to anti-NGF agents in chronic pain disorders, but the most promising efficacy data were found in patients with symptomatic OA. NGF inhibition was more efficient than placebo and more efficient than regular drugs like oxycodon, celcoxib and naproxen on both pain and joint function in Phase III trials [5,6].

There is an obvious link between NGF and inflammation as NGF can be induced by inflammatory factors. However, when

inflammatory factors were inhibited in animal studies, this only resulted in pain alleviation during inflammatory phases and not during noninflammatory phases. In contrast, blocking NGF resulted in pain prevention both in inflammatory as well as in noninflammatory phases [7]. This underlines that there are sources of NGF other than inflammation alone.

Cartilage as a source of pain?

Until now, the general perception on cartilage damage and pain is that these are distinct, nonrelated features. This is dominated by the fact that cartilage is not innervated as well as the obvious link between pain and inflammation. Intuitively cartilage itself is not linked to pain. Therefore, investigations on a potential functional interplay between cartilage damage and pain are very scarce.

In 2005, for a different purpose, we published a study where we investigated the counter-regulation of IL-1 effects on gene expression by TGF- β making use of an expression array [8]. This study showed that nearly all IL-1-regulated genes were counteracted by TGF- β . However, there were six genes that were upregulated by TGF- β as well as IL-1. Strikingly one of these genes was NGF. In 2010 Kapetanakis *et al.* linked cartilage damage to TGF- β levels in OA patients and to pain [9]. These observations inspired us to postulate that there was more to cartilage than just progressive degradation. In healthy cartilage, the extracellular matrix is loaded with very high amounts of TGF- β [10]. As soon as the cartilage is degraded, this TGF- β will be released from the cartilage



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extracellular matrix. Iannone *et al.* showed that chondrocytes can produce NGF, but that this production is low in normal chondrocytes [3]. However, upon damage, NGF production increased and was even further enhanced in severely damaged cartilage. Therefore, we hypothesized that TGF- β released from damaged cartilage could stimulate chondrocytes to produce NGF. As Pecchi *et al.* found, similar to our study in 2005, that IL-1 could lead to NGF expression in chondrocytes [11], we decided to test whether TGF- β exposure indeed led to NGF expression comparable to IL-1 in a chondrocyte cell line. As it turned out, TGF- β could induce NGF, but to even higher levels than the inflammatory factor IL-1. We repeated these experiments in isolated primary chondrocytes and cartilage explants from mice, cows and humans and even in human OA, each with consistent findings: TGF- β induces NGF in higher levels than IL-1 [12]. This showed that factors other than inflammatory mediators could be involved in OA pain. Moreover, an unexpected player came into view for pain in OA: TGF- β .

Initially we expected that NGF-induction by TGF- β would run via TAK1 as both IL-1 and TGF- β can signal via TAK1. IL-1 indeed showed to be dependent on TAK1 for NGF induction in chondrocytes, but when we inhibited TAK1 during TGF- β stimulation, only a slight reduction in NGF levels could be observed, indicating alternate signaling pathways to be involved [12]. We then identified that blocking ALK5/Smad2/3 signaling could completely abrogate NGF induction by TGF- β .

TGF- β in OA pain

The knowledge on TGF- β involvement in OA-pain is very limited. From studies in other fields we can conclude that TGF- β can be involved in diverse pain-related processes. TGF- β can regulate factors involved in nociception, sensitization and Ca^{2+} influx, like Cdk-5 and TRPV1 [13,14]. In addition, it is a protective factor for nerves and can help to regenerate nerves after injury [15]. In one other cell type TGF- β was found to induce NGF expression using p38 MAPK and JNK [16]. Whether or

not TGF- β is involved in growth of new nerves as can be found during OA and whether or not this runs via NGF is not known yet. Walsh and colleagues however, recently suggested that it might not be a matter of more nerves in OA, but rather that nerves in OA are more sensitive causing more pain in OA [17].

We found that TGF- β induction of NGF in chondrocytes could explain noninflammatory OA pain. However, this does not rule out an additional role of TGF- β in pain during inflammatory phases of OA. Many cell types produce TGF- β in response to inflammatory factors [18]. Therefore, the other cell types that line the joint cavity will also encounter TGF- β , and in turn could also produce NGF themselves. Whether TGF- β -induced NGF production occurs in these cells and whether this is achieved by using the same or alternative TGF- β signaling pathways as compared with chondrocytes remains to be elucidated.

The fact that TGF- β can induce NGF in chondrocytes is a crucial finding especially since active TGF- β is abundantly present during OA and plays a crucial role in the common pathological features of OA, like synovial fibrosis and osteophyte formation [19,20]. Given our recent data, it seems we can now add pain to this list. Although blocking TGF- β seems evident to overcome all of these problems, this would only make matters worse, as without active TGF- β signaling cartilage cannot survive. TGF- β signaling via Smad3 is crucial to prevent chondrocytes from undergoing hypertrophy and OA [21]. This underlines the two faces of TGF- β in OA: good for young healthy cartilage, bad for synovial tissue, osteophytes and now also: pain.

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