

Tenascin-C, a novel target to inhibit new bone formation in axial spondyloarthritis, linked with inflammation, mechanical strain and tissue damage

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Axial spondyloarthritis is a chronic inflammatory musculoskeletal disease hallmarked by the paradoxical co-occurrence of inflammation, trabecular bone loss in the vertebrae and new bone formation with syndesmophyte growth potentially leading to spinal fusion or ankylosis. All these features can contribute to the burden of disease: pain, fatigue and loss of mobility and function.¹ State of the art effective treatment strategies such as tumour necrosis factor (TNF) and interleukin (IL)-17 inhibitors focus on barring inflammation, but whether these approaches suffice to halt the bone remodelling aspects of the disease that determine the ultimate prognosis of patients is still debated.¹ Long-term studies indicate that sustained suppression of inflammation impacts structural disease progression.² Yet, the high individual variability in the ankylosis process suggests that there is an unmet need for early direct intervention in high risk or rapidly progressing patients.³ Anatomy and imaging studies demonstrate that spinal ankylosis originates from pathological changes in the enthesis, the insertion sites of ligaments and tendons onto the bone. Former studies highlighted how growth factor pathways that are essential in skeletal development and growth, are inappropriately reactivated in the ankylosis process.^{4,5} However, targeting signalling systems such as the bone morphogenetic protein and Wnt cascades comes with important considerations about impact on other tissues and organs.

More attractive therapeutic targets may be found in earlier and specific disease processes, an area that remains largely

unexplored. In this journal, Li *et al* identify tenascin-C (TNC) as a key driver of new bone formation originating from the enthesis, and mechanistic experiments intriguingly position this extracellular matrix molecule as a converging node between inflammation, mechanical strain, tissue damage and new bone formation.⁶ TNC is a glycoprotein with a number of remarkable features as extensively discussed by Midwood *et al*.⁷ The founding father of the tenascin family, TNC is abundantly found in extra-cellular matrix during development. The molecule's name is based on its abundance in tendons (ten-) from embryos or *nasci*, Latin for 'to be born'. An alternative early name was cytotactin, now reflected in the C epithet and defining its function as a cell adhesion molecule. TNC has a multimodular structure allowing it to bind a wide variety of ligands both on the cell surface as well as in the extracellular matrix.⁷ During development it is typically found at sites of motile cells, during branching processes and in tissues associated with locomotion: bone, tendons and ligaments. In adult life, its minute levels increase on injury and inflammation, suggesting a role in coordinating repair. Applying the concept that ankylosis in spondyloarthritis is an inadequate repair or remodelling response to counter damage or mechanical instability,⁸ would suggest that TNC levels may rise in disease-affected entheses but direct evidence for a presence and role of TNC in this particular disease context was missing.

In a whole transcriptome analysis approach using an amazing collection of spinal ligament tissues from axial spondyloarthritis patients and from controls with primarily orthopaedic issues, TNC and genes associated with increased levels of TNC were found to be upregulated in diseased tissue compared with the controls.⁶ Subsequently, the authors demonstrate how absence of the *Tnc* gene

in genetically modified mice as well as anti-TNC antibody treatment inhibit the development of joint ankylosis in dedicated animal models of arthritis. They further unravel the underlying molecular mechanism by extensive *in vitro* and *in vivo* work: presence of TNC decreases the adhesion force of the extracellular matrix. This likely means that the mechanical interactions between the extracellular matrix and the cells within it are altered. In a connective tissue built to withstand mechanical force such as the enthesis, this will affect mechanosensing by the cell and mechano-transduction onto and into the cell. Optimal sensing and transduction of mechanical forces can be considered part of the homeostatic response. Hence, these changes, linked to the presence of TNC, will alter enthesal cell biology.

Effectively, by decreasing the adhesion force, the Hippo/YAP signalling pathway is activated, leading in its turn to increased chondrogenesis, a critical early step in the process of endochondral ossification that is leading to new bone formation and ankylosis. As proof of concept, selective targeting of the Hippo/YAP pathway abrogates new bone formation in murine arthritis. Single cell sequencing data further reveal that TNC is predominantly secreted by fibroblasts in the enthesis. In line with earlier observations that TNC expression is induced by inflammation, TNF α , IL-17A and IL-22 are increasing TNC levels in human fibroblasts isolated from ligamentous tissue. Hence, TNC can be linked to the concepts of abnormal mechanical stress, inflammation and a molecular shift within the fibroblasts towards chondrogenesis.

Whereas the paper by Li *et al* offers an exciting view into a novel mechanism that likely contributes to the ankylosis process in axial spondyloarthritis, it also triggers a number of new questions and topics for further research. The preclinical data using anti-TNC antibodies are impressive and suggest that targeting an extra-cellular matrix molecule within a connective tissue such as the enthesis by antibodies is possible. However, it is still unclear whether such an approach would work in patients and whether associated toxicity would be acceptable. Although mice with a genetic deletion of *Tnc* are born without striking abnormalities, Li and colleagues report an observed altered neurological behaviour in adult mice, confirming earlier data.⁹ This and other potential effects such as defects in the wound healing process would require careful attention.¹⁰ Thus, whether TNC is a better target than bone morphogenetic

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proteins and Wnt proteins remains to be demonstrated.

TNC is a complex multimodular protein suggesting that different domains within the molecule may have different functions.⁷ Hence, specific targeting of different domains could be further evaluated in a pre-clinical setting to assess the efficacy and safety of different in depth targeted approaches, potentially identifying antibodies that selectively inhibit the change in adhesion force. TNC has the ability to form multimers including hexamers. It remains unclear under what form of TNC has the observed effect in the *in vitro* and *in vivo* models discussed by Li *et al*, another important consideration when developing a targeted strategy in humans. Similarly, there are a number of different splice variants of TNC⁷ and it remains unclear which form(s) play the observed key role in the models of axial spondyloarthritis.

Furthermore, for a deeper understanding of what drives the bone formation process, other triggers, beyond inflammation, should be considered as being able to trigger upregulation of TNC. Such triggers could include mechanical tissue damage or some biomechanical forces, in particular in a genetically susceptible individual. Although the current results, with human data derived from the axial skeleton, and murine data from the peripheral skeleton, point towards one overarching principle, some nuances are likely to depend on the anatomic location and function of the ligamentous tissue.¹¹ In addition, understanding exactly how TNC is regulated, may identify alternative targeted approaches, bypassing the need to completely interrupt TNC signalling and diminishing safety concerns related to other tissues and organs.

Lastly, the question remains which patients will benefit from targeting TNC. The multiple effective anti-inflammatory agents that are currently available seem to have the potential to halt new bone formation when given early and in a sustained way.^{12–14} Despite all recent advances in training of physicians and in imaging, a diagnostic delay remains a concern for patients with axial spondyloarthritis.¹⁵ A TNC-targeting approach might offer an escape route for those patients in whom disease activity has been already too high

for too long, or in whom the process of bone remodelling already has started and where solely stopping inflammation will not suffice anymore. Of note, TNC has been suggested as a biomarker for axial spondyloarthritis in different studies, although that the effect is not specific as increased levels have also been seen in patients with rheumatoid arthritis.^{16–18} The current data suggest that the value of TNC levels as predictive factor for radiographic progression need to be urgently studied.

In summary, the discovery that targeting TNC in mouse models of disease inhibits the progression of new bone formation is novel and important. Translation of this concept into clinical practice comes with challenges, but appears to be worth ample consideration and active investigation.

Handling editor Josef S Smolen

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests Leuven Research and Development, the technology transfer office of KU Leuven, has received consultancy, speaker fees or research grants on behalf of Rik Lories from Abbvie, Amgen (formerly Celgene), Boehringer-Ingelheim, Eli-Lilly, Galapagos, Janssen, Kabi-Fresenius, MSD, Novartis, Pfizer, Biosplice Therapeutic (formerly Samumed), Sandoz and UCB.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Provenance and peer review Commissioned; externally peer reviewed.

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To cite Van Mechelen M, Lories R. *Ann Rheum Dis* 2021;**80**:823–824.

Received 4 May 2021
Accepted 6 May 2021
Published Online First 14 May 2021



► <http://dx.doi.org/10.1136/annrheumdis-2021-220002>

Ann Rheum Dis 2021;**80**:823–824.
doi:10.1136/annrheumdis-2021-220443

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