1-1-2014

NFATs are good for your cartilage!

F. Beier
Schulich School of Medicine & Dentistry, fbeier@uwo.ca

Osteoarthritis and Cartilage 22 (2014) 893–895

Osteoarthritis and Cartilage

Editorial

NFATs are good for your cartilage!

Keywords:
Osteoarthritis
NFAT
Mouse models
Articular cartilage

While osteoarthritis is now considered a whole joint disease[1], breakdown of articular cartilage remains one of its hallmarks. Thus, there is continuing need for a better understanding of the mechanisms responsible for maintaining cartilage homeostasis during natural aging. Greenblatt and colleagues now expand on earlier studies[2,3] by demonstrating essential functions of NFAT (nuclear factor of activated T cells) transcription factors in articular cartilage health[4].

NFATs are a family of highly regulated transcription factors that have the potential to link many extracellular signals to the nuclear transcriptional machinery[5]. Most notably, subcellular localization of the four calcium-regulated NFAT proteins (NFATc1-4) is regulated by multiple pathways. Phosphorylation of the proteins by a number of different kinases such as GSK-3 leads to retention in the cytosol, whereas activation of the phosphatase calcineurin by Ca^{2+} ions results in nuclear translocalization and regulation of transcription by NFATs. Of note, a fifth family member (NFAT5) is not regulated by calcium signaling but by osmotic mechanisms instead; NFAT5 will not be discussed here.

The first NFAT to be implicated in cartilage biology was NFATc2 (also called NFAT1 and NFATp). Conventional knockout mice for this gene develop extra-articular cartilage nodules that undergo endochondral ossification[2]. This study also showed that NFATc2 deficiency increases chondrocyte proliferation and concluded that this particular NFAT is a suppressor of chondrogenesis. Interestingly, a subsequent publication showed many signs of osteoarthritis, including cartilage degeneration, osteophyte formation and subchondral bone changes, in *Nfatc2* KO mice, due to altered expression of catabolic and anabolic genes in chondrocytes[3]. These authors argue that the ectopic cartilage masses formed outside the joints are a result of attempts to repair the damage to articular cartilage. It is difficult to decipher (at least in the conventional KO model) whether this model is correct or whether the two phenotypes are independent of one another. Nevertheless, the fact that the same mutation causes loss of articular cartilage on one hand and ectopic formation of cartilage in the joint periphery on the other hand is intriguing. Together with the expression patterns of NFAT family members in cartilage discussed below (especially NFATc1), these data suggest that the NFAT pathway might be involved in distinguishing articular from growth plate chondrocytes, an important process in joint biology that is not well understood[6]. Further indication that this might be the case came from a follow-up study[7] demonstrating that lentiviral shRNA-mediated knockdown of *Nfatc2* in articular chondrocytes *in vitro* results in reduced expression of articular chondrocyte markers (aggrecan, collagen II etc.), but increased expression of hypertrophic markers such as collagen X, along with matrix-digesting enzymes and pro-inflammatory cytokines[7].

Since the original study by Glimcher's group had shown that all four calcium-regulated NFAT proteins are expressed in cartilage[2], Greenblatt and colleagues now expanded these studies to include NFATc1 (also called NFAT2) and NFATc3 (or NFAT4)[4]. Intriguingly, NFATc1 protein expression is restricted to the superficial zone of the articular cartilage, somewhat resembling the pattern of lubricin expression. Equally interesting, *NFATc1* mRNA expression is reduced in lesional osteoarthritic cartilage in human patients. Because of the lethality of total *Nfatc1* KO mice, the authors then generated cartilage-specific KO mice for this gene, as well as for *Nfatc3*. *Nfatc1* mutant mice did not display any differences to wild type mice either during normal development or in the DMM (destabilization of the medial meniscus) model of post-traumatic osteoarthritis. However, in the context of the *Nfatc2* KO described above[2,3], cartilage-specific loss of NFATc1 greatly accelerated the onset of cartilage degeneration and other markers of osteoarthritis, such as joint subluxations, osteophyte formation and subchondral bone sclerosis. At the molecular level, these double mutant mice exhibited increased expression of genes encoding many proteases involved in degradation of the cartilage extracellular matrix, such as MMP13, ADAMTS-5 and HTRA1, along with the hypertrophic chondrocyte marker collagen X. At the same time, expression of Sox9 and lubricin were reduced in mutants (although collagen II transcript levels were slightly increased). Collectively, these data provide very strong evidence that loss of NFAT signaling in chondrocytes promotes a catabolic and hypertrophic phenotype. Of note, the severe cartilage degeneration in these double KO mice appears to be caused largely by abnormal joint development (rather than defects in adult articular cartilage *per se*), but nevertheless these studies point to an essential role of NFAT signaling in cartilage and joints.

While the double mutants showed accelerated cartilage degeneration compared to the *Nfatc2* KO mice, the aforementioned

http://dx.doi.org/10.1016/j.joca.2014.04.011

formation of ectopic cartilage in the joint periphery was not different from the *Nfatc2* KO mice. This might be simply due to the tissue-specificity of the Col2-Cre driver line used to inactivate *Nfatc1*, but could also be interpreted as evidence that articular cartilage degeneration and formation of ectopic cartilage are independent events. Further studies are required to resolve this question. In addition, the current study also demonstrates that cartilage-specific deletion of *Nfatc3* does not cause skeletal phenotypes by itself or enhance the phenotype of *Nfatc2* KO mice (the later in contrast to *Nfatc1*)[4]. This suggests that NFATc3 is less important than its cousins in cartilage homeostasis, but other recent studies suggest that it might have some roles in chondrogenesis[8]. Analyses of triple mutant mice, or even quadruple when considering *Nfatc4* (NFAT3) which has been implicated in the control of chondrocyte gene expression[9], might be required to reveal minor roles of these additional family members. The basis for the differences in phenotypes between the various mutant lines will be important to decipher – are these differences simply due to different expression patterns in the joint, or do they reflect different molecular mechanisms, interacting partners and/or target genes?

The current manuscript clearly provides compelling evidence for an important protective role of NFAT signaling in cartilage. However, a number of earlier *in vitro* studies suggest that NFAT signaling can induce catabolic genes (e.g., ADAMTS4 and 9) in chondrogenic cells[10,11], which is opposite to the protective roles *in vivo* discussed here. This discrepancy needs to be resolved, although the results from the more physiological *in vivo* models appear more relevant to cartilage health in patients. Moreover, the paper by Greenblatt and colleagues, and the earlier *in vivo* studies discussed here, also raise many novel and exciting questions. For example, NFAT proteins have been shown to be key regulators of both osteoblast and osteoclast physiology[5]. Given the ever increasing evidence for a fundamental role of bone remodeling in osteoarthritis pathogenesis[1,12], roles of NFAT proteins in these cells need to be considered in the context of osteoarthritis, especially when using conventional KO mice as in the case of *Nfatc2*. Additional joint tissues such as the synovium also need to be included in future studies on NFAT contribution to osteoarthritis.

Since NFAT activity is so tightly regulated by upstream signaling pathways, the phenotypes described in the discussed studies also raise questions on which extracellular signals connect to these transcription factors. Both activators (calcineurin) and inhibitors (for example GSK-3) of NFAT can link to a large number of mechanical and biochemical stimuli that could all act through this protein family, but few of the extracellular regulators of NFAT activity in chondrocytes have been identified. Recent studies suggest that NFAT activity and expression in chondrocytes is regulated by both Notch signaling and Wnt5a, but these results were largely obtained using *in vitro* models of growth plate chondrocytes and it is not clear how much they apply to articular cartilage *in vivo*[13,14]. With regards to intracellular regulators of NFAT signaling, cartilage-specific KO mice for *Gsk3b* (encoding GSK-3β) do not show a major skeletal phenotype during development[15] or aging (Gillespie and Beier, unpublished), likely because of compensation by GSK-3α. However, pharmacological inhibition of GSK-3 signaling causes increased cartilage degeneration in rats[16]. This would be at odds with the protective role of NFAT discussed here, since GSK-3 inhibition should result in increased NFAT activity. However, GSK-3 signaling controls many intracellular signaling pathways, including canonical Wnt signaling, which might be responsible for the effects observed by Miclea and colleagues[16]. Moreover, contribution of other pathways, such as casein kinases and DYRK kinases, to NFAT inactivation might compensate for the effects of GSK-3 inhibition on NFAT localization. Inhibition of calcineurin by cyclosporine A has been shown to decrease the severity of osteoarthritis in mouse models[17], again not in line with the expected decreased NFAT activity under these conditions. But, as discussed for the GSK-3 inhibitors, it is not clear whether these effects are due to altered NFAT activity or other pathways affected by cyclosporine A. Clearly, lots of work remains to be done to link NFAT activity to specific intra- and extracellular upstream pathways.

A related, similarly important question is the regulation of NFAT gene and protein expression in chondrocytes. As discussed above, NFATc1 shows a very specific expression in the superficial zone of cartilage. Among the few transcription factors with a similar restricted expression is the Ets family member Erg[18,19] – it will be interesting to see whether a regulator relationship exists between NFATc1 and Erg. NFATc2 does not show the same restricted expression pattern as NFATc1 in the joint, but its expression in articular cartilage increases in young adult mice (compared to developmental stages)[7]. A recent abstract demonstrated reduced expression of NFATc2 in articular cartilage after mice reached 1 year of age, in parallel to reductions in proteoglycan staining and cartilage ECM gene expression[20]. Overall, these expression data suggest dynamic control of NFATc2 expression in articular cartilage that is closely associated with cartilage health. Rodova and colleagues identified a number of histone modifications associated with *Nfatc2* transcription, as well as candidate histone demethylases responsible for these modifications[7]. These data provide a strong basis for future studies into the epigenetic control of NFAT gene expression in articular cartilage.

Finally, since NFAT proteins are transcription factors, identification of their direct target genes in cartilage is crucial for a better understanding of their role in cartilage homeostasis and osteoarthritis. Genome-wide approaches such as ChIP sequencing and RNA sequencing will be required to obtain a comprehensive view of both protein-coding genes and non-coding RNAs regulated by this pathway.

In conclusion, *in vivo* and *in vitro* data from both mice and humans make a compelling case for a crucial role of NFAT family members in maintaining cartilage and joint health. However, much more work is required to elucidate both upstream and downstream components of this pathway and to determine whether it is a potential therapeutic target in osteoarthritis.

Author contributions

FB wrote the article.

Conflict of interest

The author declares no conflict of interest.

Acknowledgments

FB is supported by a salary award from the Canada Research Chairs Program, and research in his laboratory is supported by operating grants from the Canadian Institutes of Health Research. The funding sources had no role in the preparation of this article.

References

1. Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. Arthritis Rheum 2012;64:1697–707.
2. Ranger AM, Gerstenfeld LC, Wang J, Kon T, Bae H, Gravallese EM, *et al.* The nuclear factor of activated T cells (NFAT) transcription factor NFATp (NFATc2) is a repressor of chondrogenesis. J Exp Med 2000;191:9–22.

3. Wang J, Gardner BM, Lu Q, Rodova M, Woodbury BG, Yost JG, *et al*. Transcription factor Nfat1 deficiency causes osteoarthritis through dysfunction of adult articular chondrocytes. J Pathol 2009;219:163–72.
4. Greenblatt MB, Ritter SY, Wright J, Tsang K, Hu D, Glimcher LH, *et al*. NFATc1 and NFATc2 repress spontaneous osteoarthritis. Proc Natl Acad Sci USA 2013;110:19914–9.
5. Sitara D, Aliprantis AO. Transcriptional regulation of bone and joint remodeling by NFAT. Immunol Rev 2010;233:286–300.
6. Pitsillides AA, Beier F. Cartilage biology in osteoarthritis–lessons from developmental biology. Nat Rev Rheumatol 2011;7:654–63.
7. Rodova M, Lu Q, Li Y, Woodbury BG, Crist JD, Gardner BM, *et al*. Nfat1 regulates adult articular chondrocyte function through its age-dependent expression mediated by epigenetic histone methylation. J Bone Miner Res 2011;26:1974–86.
8. Tomita M, Reinhold MI, Molkentin JD, Naski MC. Calcineurin and NFAT4 induce chondrogenesis. J Biol Chem 2002;277:42214–8.
9. Tardif G, Pelletier JP, Fahmi H, Hum D, Zhang Y, Kapoor M, *et al*. NFAT3 and TGF-beta/SMAD3 regulate the expression of miR-140 in osteoarthritis. Arthritis Res Ther 2013;15:R197.
10. Yaykasli KO, Oohashi T, Hirohata S, Hatipoglu OF, Inagawa K, Demircan K, *et al*. ADAMTS9 activation by interleukin 1 beta via NFATc1 in OUMS-27 chondrosarcoma cells and in human chondrocytes. Mol Cell Biochem 2009;323:69–79.
11. Thirunavukkarasu K, Pei Y, Moore TL, Wang H, Yu XP, Geiser AG, *et al*. Regulation of the human ADAMTS-4 promoter by transcription factors and cytokines. Biochem Biophys Res Commun 2006;345:197–204.
12. Henrotin Y, Pesesse L, Sanchez C. Subchondral bone and osteoarthritis: biological and cellular aspects. Osteoporos Int 2012;23(Suppl 8):S847–51.
13. Zanotti S, Canalis E. Notch suppresses nuclear factor of activated T cells (NFAT) transactivation and Nfatc1 expression in chondrocytes. Endocrinology 2013;154:762–72.
14. Bradley EW, Drissi MH. WNT5A regulates chondrocyte differentiation through differential use of the CaN/NFAT and IKK/NF-kappaB pathways. Mol Endocrinol 2010;24:1581–93.
15. Gillespie JR, Ulici V, Dupuis H, Higgs A, Dimattia A, Patel S, *et al*. Deletion of glycogen synthase kinase-3beta in cartilage results in up-regulation of glycogen synthase kinase-3alpha protein expression. Endocrinology 2011;152:1755–66.
16. Miclea RL, Siebelt M, Finos L, Goeman JJ, Lowik CW, Oostdijk W, *et al*. Inhibition of Gsk3beta in cartilage induces osteoarthritic features through activation of the canonical Wnt signaling pathway. Osteoarthritis Cartilage 2011;19:1363–72.
17. Yoo SA, Park BH, Yoon HJ, Lee JY, Song JH, Kim HA, *et al*. Calcineurin modulates the catabolic and anabolic activity of chondrocytes and participates in the progression of experimental osteoarthritis. Arthritis Rheum 2007;56:2299–311.
18. Iwamoto M, Tamamura Y, Koyama E, Komori T, Takeshita N, Williams JA, *et al*. Transcription factor ERG and joint and articular cartilage formation during mouse limb and spine skeletogenesis. Dev Biol 2007;305:40–51.
19. Iwamoto M, Higuchi Y, Koyama E, Enomoto-Iwamoto M, Kurisu K, Yeh H, *et al*. Transcription factor ERG variants and functional diversification of chondrocytes during limb long bone development. J Cell Biol 2000;150:27–40.
20. Zhang M, Lu Q, Caldwell K, Crist J, Theleman C, Wang J. Decreased Nfat1 expression contributes to dysfunction of articular cartilage in aging mice. J Bone Miner Res 2013;28(Suppl 1). Available at: http://www.asbmr.org/education/AbstractDetail?aid=16f1a293-57cc-4b5b-8f1e-02a53ecd09a0;. Accessed April 8, 2014.

F. Beier*
Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario N6A 5C1, Canada

* Address correspondence and reprint requests to: F. Beier, Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario N6A 5C1, Canada. Tel: 1-519-661-2111x85344.
E-mail address: fbeier@uwo.ca.