Correspondence on ‘Mechanical overloading promotes chondrocyte senescence and osteoarthritis development through downregulating FBXW7’ by Zhang et al

The recent publication in the Annals of Rheumatic Diseases by Zhang et al1 ‘Mechanical overloading promotes chondrocyte senescence and osteoarthritis development through downregulating FBXW7’ includes conclusions that ‘inhibition of JNK activity ameliorated chondrocyte senescence and cartilage degeneration’ and ‘this study suggests that targeting FBXW7–MKK7–JNK signalling may be a novel therapeutic approach for osteoarthritis (OA) treatment’. We were surprised to see these conclusions since we found very different results in a recent study2 examining the role of JNK signalling in OA. In that study, rather than using a chemical inhibitor that can have off-target effects, as was done in the Zhang et al study,1 we evaluated JNK knockout mice in both the DMM model of OA performed at 12 weeks of age and in the model of naturally occurring age-related OA at 18 months of age. We conducted a well-powered study with 15 mice per experimental group and extensive, blinded, histologic assessment of OA with outcome measures of cartilage damage, osteophytes and synovial hyperplasia, as well as histomorphometric measures of cartilage and subchondral bone. We did not find any reduction in OA severity in JNK1, JNK2 or JNK1/2 double knockout mice in the DMM model, while in 18-month old mice with spontaneous OA, we noted more severe OA in JNK1 and JNK2 single knockouts (we did not study double knockouts in the ageing study). The increase in OA severity in the aged JNK knockouts was associated with an increase in the cell senescence marker p16

The conflicting evidence that JNK signalling promoted senescence provided in the Zhang et al1 paper was based solely on the use of a compound, DTP3, that the authors claimed to be an ‘MKK7 inhibitor’. MKK7 and MKK4 are the upstream kinases that phosphorylate and thus activate JNK. However, DTP3 does not directly inhibit MKK7 kinase activity but rather it causes GADD45β, a protein that binds to and inhibits MKK7, to disassociate from MKK7, which in cancer cells promotes MKK7 kinase activity resulting in JNK activation rather than inhibition.6 7 DTP3 is being developed as a therapeutic for multiple myeloma where the release of MKK7 from its inhibitor GADD45β results in apoptotic cell death through JNK activation.8

The results shown in the manuscript by Zang et al1 did not examine if DTP3 treatment inhibited JNK activity, as they proposed, by inhibition of MKK7-mediated JNK phosphorylation. This would require immunoblotting cell and/or tissue lysates with anti-phospho-JNK antibodies, a standard method used to verify inhibitors. Instead, the data presented in the manuscript examined phospho-JNK by immunofluorescence in isolated cells and tissue sections from mice after treatment with DTP3. Immunofluorescence can be quite subjective, is dependent on which cells are chosen for images and lacks the ability to confirm which specific protein is being visualised. A known problem with the phospho-JNK antibody is that it can cross react with phospho-ERK due to the similarities around the phosphorylation sites in the two MAP kinase family members. Without confirmation of the proteins by immunoblotting where the expected molecular weights can be verified, it is not certain which protein is being detected by immunofluorescence.

We decided to test DTP3 in human articular chondrocytes to determine if it was indeed able to inhibit JNK phosphorylation induced by two different stimuli, IL-1β at 10 ng/mL which is well known to activate JNK phosphorylation and by 1 μM purified recombinant fibronectin fragment that has also been shown to activate JNK in chondrocytes by our lab9 and others.10 DTP3 has been shown to cause cell death of multiple myeloma cells with an IC50 of 28 nM but not in non-malignant cells. For chondrocytes, we tested a range of doses between 0.01 μM and 100 μM (0.01, 1, 10 and 100 μM). We did not observe cell death at these doses and DTP3 did not have any effect on JNK phosphorylation at any of the doses tested with either stimulus (figure 1). Our results do not support the ability of DTP3 to inhibit JNK signalling in chondrocytes, consistent with its known mechanism of action.

Thus, based on the published literature concerning both the mechanism of action of DTP3 and the role of JNK in cellular senescence, and our own experiments, there is a lack of evidence to support the conclusions made by Zhang et al1 that the effects of DTP3 on OA development in mice that they observed are due to inhibition of JNK activation or that JNK promotes senescence.

Figure 1  Effects of DTP3 on JNK phosphorylation in response to catabolic stimuli. Primary cultures of human articular chondrocytes were changed to serum-free media and pretreated with DTP3 dissolved in DMSO (MedChem Express) for 30 min at the indicated concentrations. Cultures were then treated with either 1 μM purified human recombinant 42 kD fibronectin fragment (FN-f), 10 ng/mL IL-1β, or PBS for 30 mins. Cells were lysed and then lysates with equal total protein measured using the BCA assay were immunoblotted using an antibody to phospho-(p)-JNK (Thr183/Tyr 185) (rabbit mAB #4668 from cell signalling). The blots were stripped and reprobed for total (t)-JNK2 (rabbit mAB #9258 from cell signalling). The experiment was repeated with cells from an independent donor with 60 mins of FN-f, IL-1β or PBS stimulation with similar results demonstrating DTP3 does not inhibit activation of JNK phosphorylation by FN-f or IL-1β at any DTP3 dose tested.
Possible explanations for the observed effects of DTP3 could be due to release of GADD45β from MKK7, which perhaps has an effect independent of MKK7 and JNK or an off-target effect of DTP3 in chondrocytes where another pathway is being altered.

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