



The role of superficial layer cells in maintenance of articular cartilage surface integrity

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Background

The superficial layers (surface and sub-surface) of human articular cartilage represent specialized joint tissues with cellular and matrix compositions different to the deeper layers. The distinct properties of the superficial layers confer specialized functions and also provide a frontline barrier to degenerative insult (cellular, soluble, biomechanical) from the joint space. The design of interventions to protect/regenerate the superficial layers in the earliest stages of joint pathology will require that the details of cellular phenotype and matrix turnover in this location are more fully understood. In normal human joints (Grade 0), single cells in the surface layer, and the next 2–3 cell layers (down to about 20 μm) appear fibroblast-like and flattened parallel to the surface, unlike deeper cells (20–200 μm from surface), which are more oval and can be present in small groups. Collagen typing of normal samples (1) suggests the presence of a collagen I/III-rich superficial “membrane” (about 5 μm or a single cell in depth) covering the articular surface. This layer is also very clearly seen in sections stained with Safranin O/Fast Green and viewed at high magnification under polarized light (2). In very early OA (Grade 1) the surface layer is roughened, the sub-surface loses Safo staining (3), and in some areas changes can include a 1–3 cell deep fibrous cellular pannus (4) including cells with processes up to 30 μm in length. While studies in mice, such as that by Zhang *et al*. (5), continue to provide insight, it is clear that murine knee joints are a relatively poor mimic of the human joint, particularly in terms of the cell phenotype/structure/function relationships for the articular cartilage. This is underlined by the fact that the

20 μm surface layer in humans represents less than 1% of the full cartilage depth, whereas in mice it represents about 10%. Also, and very relevant to the paper being discussed here (5), the calcified region accounts for only about 3% of the cartilage depth in humans but 25% or more in mice, meaning that the contribution of the superficial and calcified regions to overall tissue properties will be much greater in mice than humans. Nonetheless, it appears that studies comparing wild-type and genetically-modified mice can provide important insights into the basic mechanisms involved in cartilage degeneration *in vivo* (6).

Objective, technology and summary of major findings

A major objective of the work being discussed here (5) was to examine the effect of surface layer chondrocyte death on murine cartilage *in vivo*. This was achieved technically by breeding mice with a tamoxifen-inducible DNA insertion linking lubricin (Prg4) expression to synthesis of the cytotoxic agent Diphtheria Toxin A. The technology was used to determine the effects of induced surface chondrocyte death on the outcome of joint injury via meniscal ligament resection (the so-called DMM model of murine OA). Evaluation of cell death was with a novel confocal methodology for quantifying the number and concentration of DAPI-stainable chondrocytes in 10 μm sections from the surface down to 50 μm in murine articular cartilages. Cartilage deterioration was evaluated by H&E/Safo histology and cell division by Edu staining.

As viewed by this commentator, the major findings can be

summarized as follows:

- (I) When DTA expression was induced at 21–30 days in normal mice [the second figure from (5)], the cartilage at 40 days had significantly fewer surface layer (0–20 μm) chondrocytes compared with no-DTA controls; moreover, the cell killing did not cause gross deterioration or fragmentation of the stainable cartilage, suggesting that the cells killed by the DTA were not required for cartilage homeostasis under these conditions. This finding is rather unexpected since it is widely held that the surface layer is maintained by production of matrix components (such as Prg4) by the local cells, and high cell death in this layer might be expected to result in some evidence of tissue degradation;
- (II) H&E stained sections of the DMM cartilage from no-DTA and DTA joints were markedly different at 12 weeks after surgery. With no-DTA the DMM resulted in surface roughening on both femur [the fifth figure from (5), lower panel, column 1] and tibia [the fifth figure from (5), lower panel, column 3] but with DTA the equivalent surfaces were smooth [the fifth figure from (5), lower panels, columns 2 and 4], which is consistent with a DTA-mediated surface protection. However, the surface-protected DMM-DTA samples were also very different histologically to the no-DMM/no-DTA and the no-DMM/DTA controls from both tibia and femur [the fifth figure from (5), upper panels]. The most marked abnormality seen in DMM-DTA samples [the fifth figure from (5), lower panels, columns 2 (tibia) and 4 (femur)] and not found in no-DMM controls was the paucity of H&E staining of both cells and matrix in the calcified cartilage, which was also seen to some extent in the articular zone. Overall, these findings indicate that the production of DTA by Prg4+ve cells may have protected the surface layer from DMM-induced roughening, but it also appeared to have a more generalized effect which resulted in a decrease in cell numbers and loss of matrix staining in the DMM calcified zone;
- (III) Other evidence for a broader effect of DTA on joint tissues was the profound changes it induced in the histological features of normal murine cartilages and fibrous tissues at 6 weeks, 3 and 9 months. This included what appears to be an inhibition of fibrous overgrowth at all ages [the

fifth figure in the Supplemental from (5)] and a loss of ligament integrity at 6 months [the sixth figure in the Supplemental from (5)].

Conclusion and comments

The authors concluded that DTA-induced death of cells in the cartilage surface layer protects the tissue from DMM-induced surface roughening. While reasonable, this conclusion appears premature since the DTA treatment had effects on other zones of the cartilage, and apparently on other Prg-4+ve joint tissues, such as fibrous overgrowth. It therefore seems possible that the protection was a result of cell death in other tissue types instead of, or in addition to, superficial chondrocytes. Indeed our current understanding of cartilage matrix turnover is that the death of cells results in a loss of both biosynthesis and degradation (collectively termed turnover) of matrix components, so that DTA-mediated cell death will result in a local deficiency in both processes (7).

In summary, the paper provides new information on the effects of diphtheria toxin A synthesis (linked to Prg4 expression) on murine joint cartilages in normal and DMM-treated animals. While DTA clearly kills cells in the cartilage surface zones, its effects appear to be widespread in the joint organ so that more work will be needed to delineate the role of superficial layer cells per se in cartilage catabolic damage in this murine model. Further, extrapolating from murine to human cartilages in this context appears problematic given the wide disparity between species in the proportion of the total tissue depth occupied by the superficial and calcified regions.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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