Evidence for Reactive Oxygen Species induced Apoptosis in Anteromedial Gonarthrosis

Rout, R; 1Mcdonnell, S M; 1Xia, Z; 1Kendrick, B J L; 1Price, A J; +1Hulley, P A
+1Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Science, University of Oxford philippa.hulley@ndorms.ox.ac.uk

INTRODUCTION
Anteromedial gonarthrosis (AMG) is a distinct phenotype of knee osteoarthritis (OA), with a specific pattern of disease. There is full thickness cartilage loss anteromedially, progressing to an area of damaged cartilage, and then to an area of macroscopically and histologically normal cartilage posteriorly. This reproducible pattern of disease can be considered to be a spatial model of OA progression (Figure 1).

Figure 1 – Anteromedial Gonarthrosis

Apoptosis, or chondrocyte cell death, has been shown to be a feature of OA cartilage, however the triggers are poorly understood; similarly, reactive oxygen species (ROS) have been implicated in OA. They have never been studied in a replicable topographical model of OA. This study characterises the regional levels of cell death and implicated ROS in AMG using a number of immunohistochemical studies.

METHOD
Ten tibial resection specimens were obtained from patients undergoing unicompartmental knee arthroplasty. Ten above knee amputations (from patients with peripheral vascular disease) were used as age matched controls.

Cross sections taken through all regions were paraffin embedded. Regions were marked as T1 (most damaged) to T3 (least damaged) and N (histologically normal). Routine histology (Haematoxylin and Eosin, and Safranin-O) was performed and immunohistochemical studies were conducted for Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), Active Caspase 3, Cytochrome C, Active Bax, Bim, 3-Nitrotyrosine and Forkhead box O3A (FOXO 3A).

RESULTS
Cell death, as detected by TUNEL appeared predominantly in the surface layer of chondrocytes of damaged cartilage (p<0.001). Median values were 23% in superficial cartilage (range 0 – 51) compared to 0% in deeper cartilage (range 0 – 15). There was a significant difference in TUNEL staining between regions (p=0.001). This ranged from 26% (most damaged) to 4% (undamaged) (Figure 2).

There was a good correlation with degree of cartilage damage (p=0.66, p<0.001) as defined by histological grade and TUNEL was significantly higher (p<0.001) in AMG compared to the control samples which showed an average of 2% TUNEL overall.

Upstream markers of apoptosis (Active Caspase 3, Cytochrome C, Active Bax), assessed qualitatively, were present in a similar distribution to that of TUNEL staining.

Figure 2 – 3-Nitrotyrosine and TUNEL by zone

3-Nitrotyrosine was also shown to be a predominantly surface phenomenon. There was a significant difference (p<0.001) between regions, ranging from 58% (most damaged) to 10% (undamaged) (figure 2). Again, this was significantly higher that the control samples (p<0.001).

In line with indicators of ROS mediated damage, Bim and FOXO3A were also detected (Figure 3).

Figure 3 – FOXO3A Positive cytosol staining
(200x magnification)

DISCUSSION
The mechanism of apoptosis in OA cartilage has not been studied in depth, and understanding the biochemical and molecular responses of ‘stressed’ chondrocytes may provide invaluable information about the specific causes of cell death. Such cellular responses may provide targets for disease modification, thus delaying or preventing the need for joint arthroplasty.

We conclude that AMG is a phenotype demonstrating cartilage at progressive stages of disease. Apoptosis involves the intrinsic mitochondrial pathway and ROS appear to be implicated. Further work is needed to provide evidence of what lies further upstream of markers demonstrated in this study.