in age-related phenotypes. We have identified two lines which develop osteoarthritis, both with pleiotropic effects, and are characterising the pathogenic pathways in detail. In addition to characterising disease phenotypes we are now beginning to screen for advantageous or healthy ageing phenotypes.

12 COMPREHENSIVE TRANSCRIPTOME ANALYSIS OF AGING-RELATED GENE EXPRESSION IN EARLY PHASE OF POST-TRAUMATIC OSTEOARTHRITIS

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Purpose: Risk factors for OA include age and a history of joint trauma. Age-related changes in the joint occur independent of osteoarthritis, but these underlying differences may affect OA development after injury. The age-related differences in the response to joint injury and post-traumatic OA (PTOA) development remain largely unknown, especially during the early phase of injury. The aim of this study is to elucidate the aging-related changes in early gene expression in response to knee injury using a comprehensive transcriptome analysis.

Methods:
- Animal model of joint injury: Male C57BL/6 mice (12-weeks old as young) and (54-weeks old as old age group) were used (n=8 per group). The right knees were injured with a single mechanical compression, which causes a transient anterior subluxation of the tibia leading to PTOA within 8 weeks. The knees were harvested 7 days after injury and RNA was extracted.
- Microarray analysis: Total RNA was hybridized to an Affymetrix GeneChip® Mouse Gene 1.0 ST Array. For analysis, GeneSpring software was used with all gene expression values for unsupervised clustering of the arrays and principal components analysis. Genes that were differentially expressed (p<0.05) were selected and shown in Fig2B. Of the 273 differentially regulated genes, 188 were up-regulated in young mice, and 30 genes down-regulated by injury in young mice. In summary, our results suggest that the capacity to respond to injury is larger in old mice, perhaps to compensate for their lower basal expression. However, despite an increased injury response in old mice, the absolute levels of these anabolic genes are still lower than in young mice. In summary, our results suggest that the capacity to mount an anabolic response to injury is diminished in old age perhaps due to age-related reduction in the basal level of anabolic genes.

Results:
- Comparative analysis between old and young injured knees: Hierarchical clustering of genes differentially regulated with age are shown in Fig2A. Of the 273 differentially regulated genes, 188 were up-regulated and 85 were down-regulated in YU vs YI. (Fig2B) The top 5 genes with the highest absolute fold change in expression (Fig2B) included anabolic genes that were down-regulated by aging, and genes related to signal transduction that were up-regulated by aging. The top 10 genes associated with these differentially regulated genes are shown in Fig2C.
- Injury response in old and young mice: Comparative analysis between injured knees vs. uninjured knees in old and young mice identified 511 genes that were up-regulated and 28 genes down-regulated by injury in old mice, and 323 genes up-regulated and 30 genes down-regulated in young mice (Fig1B). 218 and 34 genes were regulated by injury specifically in old and young mice, respectively (Fig3). The top 5 genes up- or down-regulated differentially in only old or young mice are listed (Fig 3).

Conclusions: A comparison of old and young uninjured knees shows that the up-regulated genes in old mice are mostly related to intra-cellular signal transduction, while up-regulated genes in young mice include the expression of extracellular matrix components such as collagen. This suggests that young mice have higher basal anabolic activity. In response to injury, these anabolic genes were up-regulated more in the old injured mice when calculated as a fold-change with respect to the contralateral uninjured knee. This indicates that the anabolic response to injury is larger in old mice, perhaps to compensate for their lower basal expression. However, despite an increased injury response in old mice, the absolute levels of these anabolic genes are still lower than in young mice. In summary, our results suggest that the capacity to mount an anabolic response to injury is diminished in old age perhaps due to age-related reduction in the basal level of anabolic genes.

13 MITOCHONDRIAL reactive oxygen species (ROS) PROMOTE HYPEROXIDATION OF PEROXIREDOXINS AND INHIBIT PRO-SURVIVAL IGF-1 SIGNALING IN HUMAN ARTICULAR CHONDROCYTES

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Purpose: Mitochondrial dysfunction resulting in overproduction of ROS is implicated in age-related conditions, including osteoarthritis, although the mechanisms responsible are incompletely understood. The intra-cellular redox balance is maintained by antioxidant enzymes that include the peroxiredoxins (PRXs). Oxidative stress occurs when an imbalance in ROS production and removal disrupts normal cell signaling. ROS inhibition of IGF-1 mediated phosphorylation of the pro-survival protein Akt can result in cell death. The purpose of this study was to examine if mitochondrial ROS overproduction reduced chondrocyte cell viability by altering chondrocyte PRX redox status and IGF-1-Akt signaling.

Methods: Primary chondrocytes isolated from normal human articular cartilage from donors aged 27 to 70 yrs were cultured in monolayer and made serum-free prior to experiments. Chondrocytes were treated with 25 μM menadione to induce mitochondrial ROS and were transduced with an adenoviral vector encoding catalase targeted to the mitochondria (MCAT) to reduce levels of mitochondrial ROS or an empty vector control. Cell viability was assessed using the Live/Dead cell assay.
Phosphorylation of proteins pertinent to IGF-1 and MAPK signaling pathways were analyzed by immunoblotting. Hyperoxidized PRXs resulting from excessive ROS were detected using an antibody that reacts with any PRX family member when it is in the PRX-SO2/SO3 state. Reduced and disulfide oxidized PRX2 (cytosolic) and PRX3 (mitochondrial) were measured by separating the reduced monomers and oxidized dimers using non-reducing SDS-PAGE followed by immunoblotting with isoform-specific antibodies as described by Cox et al, 2010.

**Results:** In time course studies, cell death after menadione treatment was first seen at 3 hrs (38% cell death) with 72% of the cells dead by 9 hrs. Cell death was completely inhibited when MCAT was overexpressed, thus confirming that the stimuli inducing cell death was mitochondrial in origin. Chondrocytes from donors of different ages were treated with menadione for 0-60 minutes in order to examine redox events leading to cell death. Hyperoxidized PRXs were observed with greater amounts seen in cells from older donors (Fig. 1). We further examined specific isoforms of PRX and determined if chondrocytes from older adults were more susceptible to induction of oxidative stress. A significant difference in PRX oxidation was noted between older (avg 63yrs) and younger (avg 38yrs) donors for PRX2 and PRX3 (Fig. 2). Conversely, menadione treatment induced a sustained and significant increase in phosphorylation of the MAP kinases p38 (Fig. 2) and ERK. MCAT blocked menadione induced inhibition of Akt phosphorylation and reduced phosphorylation of catabolic p38.

Conclusions: Mitochondrial generated ROS resulted in hyperoxidation of chondrocyte PRXs which inhibits their ability to serve as antioxidants. Cells from older donors were more sensitive to menadione consistent with a basal state of oxidative stress. PRX oxidation was accompanied by activation of the p38 and ERK MAP kinases and inhibition of the pro-survival protein Akt followed by cell death. These results highlight the ability of mitochondrial ROS to alter redox balance and compromise pro-anabolic cell signaling pathways suggesting a mechanism by which oxidative stress may be a contributing factor to age related decline in chondrocyte function and osteoarthritis progression.

Figure 1. Effect of age on chondrocyte PRX hyperoxidation. Old (avg 61.3±5.9yrs) and young (avg 38.3±6.1yrs) chondrocytes were exposed to menadione (25 μM) for 0-60 minutes and PRX oxidation was evaluated. Immunoblots shown are representative of three independent experiments. Data is presented as mean±SEM, *P<0.05.

Figure 2. Effect of oxidative stress on Akt and P38 phosphorylation. Human articular chondrocytes were treated with menadione (25 μM), IGF-1 (50 ng/ml) or both for 0-90 minutes and phosphorylation was analyzed by immunoblotting for phosphorylated Akt and P38. Blots were then stripped and re-probed for total Akt and total P38. Data shown is densitometric analysis of three independent experiments with phosphoproteins normalized to totals (mean±SEM, *P<0.05).