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Editorial

Insight into the function of DIO2, a susceptibility gene in human osteoarthritis, as an inducer of cartilage damage in a rat model: is there a role for chondrocyte hypertrophy?

The detection of the relevant susceptibility genes has the promise to provide insight into underlying mechanisms of the development and progression of osteoarthritis (OA), and to point to new therapeutic strategies[1]. Early genetic studies on OA have provided evidence that several genes harbor OA susceptibility alleles including the growth differentiation factor 5 gene (GDF5)[2,3], SMAD family member 3 (SMAD3)[4], and the deiodinase iodothyronine type 2 and 3 genes (DIO2 and DIO3)[5,6]. More recently, Arthritis Research UK Osteoarthritis Genetics (arcOGEN), Translational REsearch in Europe Applied Technologies for OsteoArthritis (TREAT-OA), RAAK (Research Arthritis and Articular Cartilage), and other consortia have identified several OA-related quantitative trait loci by compiling genome wide association study (GWAS) data[7,8,9,10,11]. Since many of the OA susceptibility genes found by GWAS of blood cell samples are known to be involved in early skeletal development, the possibility that alterations in their expression or activity in the adult, and particularly in cartilage and bone where OA manifestation occurs, was proposed[12].

The subject of a recent report by Nagase *et al.*[13], the OA susceptibility gene DIO2, encodes the deiodinase type 2 protein (D2), which is responsible for catalyzing the conversion of intracellular inactive thyroid hormone (T4) to its active form (T3). During normal growth plate development, active T3 subsequently signals the terminal maturation of the chondrocytes leading to cell hypertrophy, degradation and mineralization of the cartilage matrix, and bone formation[14,15]. DIO2 is among a number of OA susceptibility genes, including GDF5 and DOT1L, which are active in pathways during pre- and post-natal joint development leading to endochondral ossification[12]. Such findings have led to the notion that chondrocyte signaling events that take place in hypertrophic chondrocyte differentiation in the growth plate may reappear in the adult and lead to the development of OA in articular cartilage[16], although this is controversial. When first identified, the DIO2 polymorphism was proposed to cause a deficiency of DIO2 and decreased thyroid hormone availability, impacting on cartilage matrix integrity, chondrocyte viability, and osteophyte formation[5]. Subsequent functional genomic studies have shown increased *DIO2* gene expression and higher levels of protein in cartilage obtained from OA-affected compared to healthy joints[17,18]. These consequences were proposed to be due to imbalanced expression of the DIO2 risk allele at a 30% higher rate than the reference allele in OA compared to healthy cartilage[17]. Furthermore, the *DIO2* susceptibility single nucleotide polymorphisms (SNPs) showed an association with hip joint geometry and OA susceptibility[19], suggesting that variation in local T3 bioavailability in the growth plate may contribute to subtle variations in joint shape, which subsequently could influence biomechanical stability of the articular cartilage in aging individuals. In the context of skeletal development, it is of interest that Hedgehog-inducible WD repeat and SOCS box-containing protein 1 (WSB-1) modulates thyroid hormone activation and parathyroid hormone related protein (PTHrP) secretion[20]. Altered DIO2 activity in articular cartilage, therefore, may disrupt homeostasis by promoting hypertrophic chondrocyte differentiation and other adverse events that lead eventually to OA onset or progression[5,12].

In the studies of Nagase *et al.*[13], microarray analyses of human cartilage showed that *DIO2* was expressed at levels of more than 2-fold higher in OA samples compared to healthy controls, whereas *DIO1* was not detected and *DIO3* was not significantly different. Also, they showed that *Dio2* was expressed at higher levels than other deiodinase genes in articular cartilage of 8-week-old rats and that T3 treatment of cultured chondrocytes and cartilage explants isolated from these rats increased the gene expression of markers associated with chondrocyte hypertrophy and endochondral ossification, including alkaline phosphatase, type X collagen, osteocalcin, and Runx2, as reported previously by others[21–23]. T3 also increased the expression of several cartilage matrix-degrading proteinases and enhanced the effects of interleukin (IL)-1α. The *DIO2* gene is known to be upregulated by the pro-inflammatory nuclear factor (NF)-κB signaling pathway[24], and the siRNA-mediated suppression of DIO2 was shown to increase IL-1β-induced expression of inflammatory mediators such as cyclooxygenase 2 (COX2) and IL-1β itself[25]. Thus, the consequences of the imbalanced deiodinase expression and activity will likely depend upon the availability of other upstream and downstream signals associated with inflammation and mechanotransduction. Also, discrepancies in findings of various studies may be due to differences among species, tissue sites, and age, as well as the models used.

The initial microarray and *in vitro* data presented by Nagase *et al.*[13] provided the rationale for generating cartilage-specific *Dio2* transgenic rats by *Col2a1*-driven expression of human (h) *DIO2* in a bacterial artificial chromosome (BAC) construct. Since *Dio2*-deficient mice display no growth plate abnormality, the severe cartilage degradation due to surgically induced OA in rats with forced transgenic expression of h*DIO2* in articular cartilage may reflect the pathological changes associated with upregulated expression in certain OA patients harboring the risk allele. Furthermore, this transgenic rat model will be a useful tool for further following up mechanisms associated with other factors involved in chondrocyte hypertrophy and OA, including HIF-2α, which the

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authors suggest may be regulated by T3, as well as those genes reflecting increased anabolism in late-stage OA, such as both type I collagen (*COL1A1*) and type II collagen (*COL2A1*) collagen genes, observed by Nagase *et al.*[13] and in other microarray studies of human OA cartilage[26,27].

In conclusion, the findings of the study by Nagase *et al.*[13], by analyses in human and rat cartilage and in various culture models, take us a step closer to understanding the potential connection between *DIO2* expression and chondrocyte hypertrophy in OA. The novel rat model, in which *Col2a1*-driven *Dio2* overexpression resulted in enhanced cartilage degradation when the animals were challenged with surgically induced post-traumatic OA, however, did not define chondrocyte hypertrophy as the responsible event. Rather, the overexpression of *Dio2* was associated with enhanced catabolic events consistent with the *in vitro* data showing that T3 induces several proteinase genes and enhances IL-1-induced gene expression. Whatever the precise mechanism, it is clear from this and other studies that imbalanced deiodinase regulation and activity in articular cartilage may lead to impaired cartilage tissue homeostasis and enhance the probability of development of osteoarthritis due to biomechanical trauma or aging.

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