

THE ROLE OF SNORD116 IN CARTILAGE AGEING AND OSTEOARTHRITIS

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Abstract:

Purpose: Osteoarthritis (OA) presents as a change in the chondrocyte phenotype and an imbalance between anabolic and catabolic processes. Factors such as age affect onset and progression. Classically small nucleolar RNAs (snoRNAs) direct chemical modification of RNA substrates and enable endoribonucleolytic pre-rRNA processing. However, characterization of diverse non-canonical snoRNA functions are increasingly becoming elucidated; regulation of chromatin structure, mediators of oxidative stress and microRNA-like functions. The loss of two gene clusters encoding the non-canonical snoRNAs SNORD115 and SNORD116 causes Prader-Willi syndrome (PWS). They are also involved in alternative splicing of specific target genes. These orphan snoRNAs do not target RNA substrates. PWS is characterized by hyperphagia, short stature, hormonal imbalances and reduced muscle mass. Additionally diminished bone mineral density and osteoporosis are common at a young age. Snord 116 knockout mice show reduced bone formation indicating a link between the PW critical region and osteoporosis. Overexpression or knockdown (PWS) of SNORD116 in HEK cells changed the expression of 200 mRNA genes, whilst in the hypothalamus from PWS versus normal patients 12 gene expression levels were validated as influenced by SNORD116. We have previously identified, following transcriptomic analysis of cartilage, a reduction in SNORD116 in OA and ageing. Therefore we hypothesize SNORD116 has a role in OA.

Methods: Total RNA was extracted from cartilage following total knee arthroplasty from the medial (old normal; protected (P); n=10) and lateral (old OA; unprotected (U); n=10) femoral condyles from donors aged 62.6 ± 7.3 (mean \pm standard deviation) years and the medial side of the lateral femoral condyle following anterior cruciate ligament repair surgery from young donors; 23.7 ± 3.8 years. Microarray analysis was undertaken using Affymetrix miRNA 4.0 arrays. Differentially expressed snoRNAs were defined with a FDR<0.05. OA severity was based on Kellgren and Lawrence scoring and histologically grading. Expression changes in SNORD116 were validated in an independent cohort (n=8 per condition) using qRT-PCR. Additionally qRT-PCR determined the effect of tissue culture conditions, mechanosensitivity and passage on snord116 expression using equine chondrocytes and cartilage. Changes in expression of SNORD116 in OA chondrocytes and OA-like conditions were studied in chondrocytes using interleukin-1 β (IL-1 β) and healthy or OA synovial fluid. SNORD116 knockdown was undertaken in immature murine articular chondrocytes (iMACs) derived from a snord116Prx1Cre mouse line. iMACs derived from male pups (n=3 wild type; n=3 snordprx1Cre) were exposed in triplicate technical replicates to IL-1 β or TNF α for 24h and hypertrophic, chondrogenic and inflammatory gene expression measured using qRT-PCR.

Results: Microarray results of cartilage identified an increase in SNORD116 in OA (P versus U; FDR<0.04) and a reduction in age (young versus U; FDR<0.04). These findings were validated in an independent cartilage cohort using qRT-PCR; p<0.03 and p<0.01 respectively. The expression of SNORD116 was not affected by oxygen tension, low serum or passage (up to P4) in chondrocyte culture conditions. Expression of snord116 was not dependent on the area of the joint the cartilage was taken from (high or low load areas). There was also an increase in SNORD116 in OA chondrocytes (p<0.04). SNORD116 expression responded to both OA and healthy synovial fluid

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