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MEETING REPORT

Non-coding RNAs and posttranscriptional regulation in cartilage and bone

Jennifer J Westendorf[1] and Mary B Goldring[2]

[1]Departments of Orthopedic Surgery and Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA. [2]Research Division, Hospital for Special Surgery, and Department of Cell and Developmental Biology, Weill Cornell Medical College, New York, NY, USA.

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Meeting Report from the 2012 Annual Meeting of the Orthopedic Research Society, San Francisco, CA, USA, 4–7 February 2012

The latest advances in osteoarthritis research were once again on display at the 2012 Orthopedic Research Society meeting. This year, non-coding RNAs as disease biomarkers and crucial regulators of cartilage and bone biology were major topics of discussion as two Spotlight presentations and multiple abstracts were presented on this epigenetic mechanism of posttranscriptional gene expression.

Introduction

Osteoarthritis (OA) is a progressive and complex degenerative joint disease characterized by articular cartilage damage, erosion of the cartilage extracellular matrix and thickening of the subchondral bone.[1] OA can sometimes be traced to an incidence of joint trauma, but in many idiopathic cases it is assigned to progressive overuse of a biomechanically imperfect joint. Whether the tissue damage is severe or minor, it stimulates biological responses that change the structural and mechanical properties of joint tissues. Over time, the damage leads to debilitating pain that is only relieved by joint replacement. The ability to predict and monitor OA progression in patients is a major clinical challenge. Sophisticated imaging research is attempting to identify early morphological signs of joint damage, however, there are also efforts in biomarker discovery because serum factors are more sensitive and inexpensive indicators of disease progression.

Non-coding RNAs, microRNAs (miRNAs) and Epigenetic Mechanisms in Cartilage and Other Joint Tissues

Gary Gibson, PhD (Henry Ford Hospital) delivered a Spotlight presentation on Saturday 4 February 2012 entitled 'Serum Non-coding RNAs as Biomarkers for Osteoarthritis'. The detection of non-coding RNAs, particularly miRNAs, in serum from cancer patients[2] inspired Dr Gibson and his team to search for these molecules in blood from post-traumatic OA patients, in whom the date of onset can be accurately predicted and disease progression is relatively rapid and pathologically homogeneous. RNA was isolated from the blood of patients with whole-organ magnetic resonance imaging scores (WORMS[3]) ranging from 0 to 35 and processed for miRNA expression analysis. In this initial search, a single-serum non-coding RNA directly correlated with OA disease and cartilage damage. Surprisingly, this non-coding RNA was not a miRNA, but a small nucleolar RNA (snoRNA), U48, which is commonly included as a normalization control in TaqMan arrays. The group subsequently identified another snoRNA, U38, whose presence correlated well with WORMS scores of >4. In animal models of joint disease, U38 and U48 were increased in joints that were destabilized by surgical transection of ligaments, but were decreased in antigen-induced inflammatory models. The source of U38 and U48 remains unknown, however, nucleolar microautophagy (originally described in *Saccharomyces cerevisiae*[4]) was identified as a potential mechanism for their release. The data suggest that snoRNAs are released if cell autophagy is compromised, as observed in OA tissues. These compelling results require further validation, but the groundwork is clearly in place to study these new potential biomarkers over time in OA patients and animal models.

Other presentations in this Spotlight session described investigations of the potential roles of microRNA cartilage and other joint tissues in the context of OA. Mature miRNAs are only 20–22 nucleotides in length. They bind to 3′-untranslated regions (UTRs) of mRNAs in a sequence-directed fashion and inhibit protein synthesis by sequestering mRNA, promoting mRNA degradation or repressing translation. Ishizuka *et al.* from the Nagoya University reported that miR-27b and miR-23b help to maintain articular cartilage homeostasis by downregulating HIF2α and its responsive downstream genes, MMP13, VEGF and RUNX2, which have roles in chondrocyte hypertrophy and endochondral ossification during growth plate development.[5] This and other studies reporting a role for HIF2α in OA[6] were also supported by a presentation by Hashimoto *et al.*, Hospital for Special Surgery, New York, NY, USA.[7] Differences in methylation status of catabolic gene promoters have been reported in chondrocytes from OA patients compared with healthy individuals.[8]

This study showed that HIF2α-driven MMP13 transcription depends upon the CpG methylation status of the promoter.[7] Matsukawa *et al.*, also from Naoki Ishiguro's group at Nagoya University, reported that miR-125b is decreased in OA compared with normal cartilage and identified a potential mechanism by which miR-125b may prevent cartilage degradation by binding to the 3′-UTR of ADAMTS4 mRNA and inhibiting its translation.[9] Okuhara *et al.* from Hiroshima University reported that several miRNAs are expressed at higher levels in the peripheral blood mononuclear cells of OA patients than in healthy subjects, namely miR-146a, miR-155 and miR-223 at early stage OA and miR-155 at the later stage.[10] In a poster presented by Dr Paul Fanning from the University of Massachusetts Medical School, differential expression patterns of miRNAs were revealed in cartilage samples from spared vs non-spared compartment of varus knees of OA patients.[11]

As with many of the miRNAs mentioned above, miR-210 has been reported to be involved in normal tissue homeostasis, and Shoji *et al.* from Hiroshima University reported that intra-articular injection of non-functional double-stranded miR-210 accelerated ligament healing by promoting angiogenesis in a rat model of partial anterior cruciate ligament injury.[12] The Hiroshima group of Mitsuo Ochi (Ujigo *et al.*) also reported that administration of miR-210 to mice with spinal cord injury promotes regeneration.[13] Furthermore, a microarray analysis reported by Dr CT Chen from the University of Texas Southwestern showed that a number of miRNAs are downregulated, associated with upregulation of inflammatory and catabolic response genes, in rotator cuffs with full-thickness tears, as well as in the synovium.[14]

MicroRNAs in Skeletal Development and Maintenance

Jane Lian, Ph.D. (University of Massachusetts Medical School) delivered a comprehensive review of how miRNAs control bone formation on Sunday, 5 February, in a Spotlight presentation titled 'MicroRNA Functions in the Skeletal Landscape'. Dr Lian elegantly summarized the incredible redundancy in miRNA biology, as each miRNA can target many genes and each gene can be regulated by many miRNAs.[15] Moreover, miRNAs operate on multiple components of the biological systems and pathways, and thus manage regulatory networks through both feed-forward and feedback mechanisms. The importance of miRNAs in skeletal development was revealed in animal models, in which mature miRNAs cannot be produced because of the inactivation of Dicer, a ubiquitous enzyme that cleaves precursor microRNAs to the mature form. Dicer deletion in collagen II-expressing chondrocytes causes abnormal growth plate development,[16] whereas its inactivation in mature, osteocalcin-expressing osteoblasts increases bone mass during aging.[17] In osteoblasts, miRNAs regulate bone mass by fine-tuning the activation of the major pathways (for example, Wnt and Bmp2 signaling) and the expression of crucial molecules (for example, Runx2 and collagen).[18–21]

Numerous abstracts also described the roles of specific miRNAs in normal and pathological bone formation. For example, Ji *et al.* from Dr Leon Nesti's group at the National Institute of Arthritis, Musculoskeletal and Skin Diseases described miRNAs that are dysregulated in traumatized muscle tissues that develop heterotopic ossification, including miR1 and miR-206 that are known to have roles in satellite cell differentiation and muscle injury.[22] miR-146b expression in HO tissues suggests that the cells are stressed due to chronic inflammation. Wang *et al.* from Beijing and Hong Kong reported that miR-214 inhibits bone formation in mice and humans possibly through effects on bone differentiation.[23] Two additional studies from the same groups presented by Dr Ge Zhang further explored the therapeutic effects of targeting miR-214 with antagomirs, showing promotion of bone formation in ovariectomized mice with established osteoporosis and in osteoporotic mice due to hind-limb suspension.[24] In mice overexpressing miR-365, first identified as a mechano-responsive miRNA on microarrays,[25] post-natal growth plate development is severely retarded, as reported by Yang *et al.* from Dr Qian Chen's lab at the Brown University.[26] Thus, non-coding RNAs are emerging as important and powerful regulators of skeletal development and cartilage and bone homeostasis. Their stability in serum makes them novel and potentially useful predictors of early skeletal disease.

Conflict of Interest

The authors declare no conflict of interest.

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