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Editorial
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Differential Regulation of Tumor Suppressor Gene: *MIG-6* in Human Arthritis

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Editoral

Human Osteoarthritis (OA) exhibits early symptoms of the diseases in the joints and cartilage during the process of aging [1]. The articular chondrocytes in normal cartilage maintain a dynamic equilibrium between synthesis and degradation of extracellular matrix (ECM) components [1-3]. Osteoarthritis can be caused and/or driven by an imbalance in cartilage metabolism, inflammation and mechanical injury [1-4]. However, in all the three cases, OA-affected cartilage (as compared to non-OA and healthy cartilage) is characterized by covert inflammation, induction of Damage Induced Molecular Proteins (DAMPS), clonal expansion of hypertrophic chondrocytes and apoptosis with increased levels of nitric oxide and DNA damage. These pathophysiological changes result in disruption of matrix equilibrium, progressive loss of cartilage and joint pain [1-4]. Recently, the application of "omic" technologies in OA hadnot only shed light on molecular mechanisms of OA [3-6] but resulted ingeneratingsubsets of OA-patients with distinct molecular, clinical and epidemiological profiles. For example, a cluster of genes defined as an "IL-1β signature" was observed in the blood of a subset of OA-patients who exhibited higher pain score, impaired function of joints, and an increased risk of radiological progression as compared to OA-affected individuals without an "IL-1β signature" [7]. Osteoarthritis also hasa strong genetic component, (influenced by environmental factors, gender, and lifestyle) whichinvolves multiple genes, which may follow the classical Mendelian or non-mendelian inheritance or both [5]. For example, there is 40% probability of heritability of OA of the knee and a 65% likelihood of inheritability of OA of the hand and hip. Female Hip-OA showed possible linkages to chromosome 2q, 4q; whereas the early onset of OA was associated with 6q, 11q; and the common form of OA was linked to 16p and 16q. Thus gender, pathology, and risk factors contribute to the geneticcomplexity of OA [5,8].

Pritykin et al. [9] have identified 2517 multifunctional genes in *Homo sapiens*. Recent studies have shown stress, hormones, and growth factors can induce a multifunctional and broadly distributed Mitogen-Inducible Gene 6 (*MIG*-6 or *ERRFI*1) [10]. The expression of *MIG*-6 is observed in various tissuesand may function through several signaling pathways such as EGF-Receptor, MET, SPRY2, SAPK, and JNK. These *MIG*-6$^{-/-}$ mice exhibited an increase in DNA damage, with a surgein adenocarcinomas and uncontrolled proliferation of cells in the gallbladder, bile duct, and lung. Thus *MIG*-6 also exhibits properties of a candidate tumor-suppressor gene in several neoplastic cells [10]. *MIG*-6$^{-/-}$ mice induced pathogenic hypertrophy, which augments other complex diseases such as insulin resistance, diabetes and related physiological effects, which includehypertension, cholesterol metabolism, atherosclerosis, decreased organ functions and ultimately multi-organ failures [11-15]. Multifunctional genes like *MIG*-6 are interwoven in multiple biological processes resulting in distinct physicochemical properties as described above; These multifunctional genes are evolutionarily conserved, broadly expressed, essential in protein interaction networks, and likely involved in human disorders [9,10]. Likewise, *MIG*-6 is a multifunctional gene (with properties of a tumor-suppressor gene) that could also influence the homeostasis of cartilage. For example, ubiquitous deletion of *MIG*-6 in mice (*MIG*-6$^{-/-}$) developed osteoarthritis-like symptoms in multiple joints [16]. Furthermore, cartilage-specific deletion of *MIG*-6 augmented proliferation of chondrocytes similar to hypertrophic chondrocytes in OA and preferentially affected the weight-bearing knee cartilage, but rarely other joints [1,17]. The early-onset of the degenerative joint disease in down-regulated *MIG*-6 was accompaniedwith aberrant proliferation of precursor mesenchymal cells in the deep zone of the cartilage [16,17]. Furthermore, injury to ligaments and meniscus can independently augment

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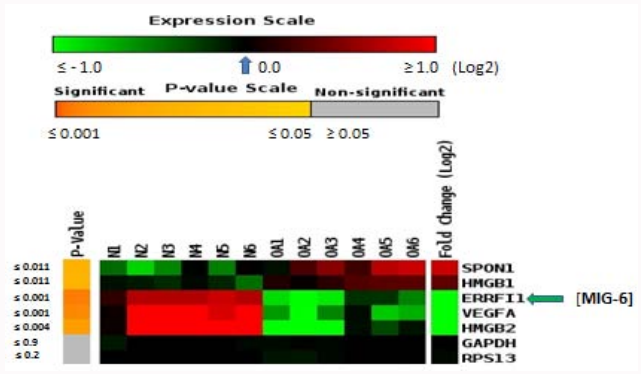

Figure 1: Gene expression of *MIG*-6 in normal and OA-affected cartilage.

joint damage similar to OA in *MIG*-6$^{-/-}$ mice [18]. Although germline disruption or decrease in *MIG*-6 expression is associated with human tumors, (breast, pancreas and ovarian cancers); in skin, it develops epithelial hyperplasia and in joints it causes hyperproliferation of cells leading to degenerative joint diseases such as OA [10,16,17]. These studies, suggest that down-regulation of *MIG*-6 not only incites but also renders joints susceptible to OA. Given these observations, we examined the mRNA expression of *MIG*-6 from human cartilage obtained from normal and OA-affected cartilage.

Human cartilage from knee was used for this study [3,4]. The cartilage slices were procured from individual undergoing knee replacement surgery for OA based on the American College Rheumatologists (ACR) symptomatic criteria. The non-arthritic knee cartilage from donors with fractures or after knee amputations of accident victims. We also received healthy non-OA cartilage from National Development and Research Institute (NDRI, Philadelphia, PA, USA). All the tissues were made available after permission from the Institution Review Board for the use of human tissues in clinical research from New York University's Hospital for Joint Diseases. The average demographics of non-OA normal cartilage were 63% females and 37% males while it was 72% females and 28% males for the OA-affected cartilage donors. The normal cartilage donors represented 34%, 25% Caucasians, African Americans, 21% Hispanics and 20% Asians with an average donor age of 24-75. The OA-affected individual donors were 51% Hispanics, 21% Caucasians, 17% African Americans, and 11% Asians with an average donor age of 60-85. All the clinical samples were administered via the Pathology laboratory at HJD. OA-affected cartilage samples with a range of an OARSI score of 3-5 were used in the study. Similarly, normal cartilage samples in a range of OARSI scores of 0-1 were used in the study [2,3].

Gene expression array of *MIG*-6 (*ERRFI*1) together with other markers is shown in normal and OA-affected cartilage. Normal (N) and OA-affected cartilage (OA) was pulverized under liquid nitrogen, to extract total RNA equivalent amounts of total RNA were pooled into six different cohorts (designated as N1 to N6) of normal and (OA1 to OA6) of OA respectively. Each cohort of normal or OA-affected cartilage groups consisted of RNA that represented a minimum of 5 and a maximum of 12 donors as recently reported [2,3]. Affymetrix U133A and B gene chips were hybridized, and the background was normalized and corrected using the bio conductor package "affy" with 'RMA' method. The differential expression and statistical analysis were performed using Linear Models as described [3,4]. A Biomart database was utilized to annotate the probe to Ensembl genes (version 70). Significances of differentially expressed genes were considered if the multiple tests corrected (FDR) p-value was ≤ 0.05. The heat map was produced on median centered expression value using Gitools [3,4]. Stable housekeeping genes (GAPDH and ribosomal protein (RPS 13)) were used as internal controls in these analyses.

Normal and OA-affected cartilage from human knees was procured under the guidelines of Institutional Review Board for the use of clinical tissues at NYU-HJD School of Medicine as previously reported [2-4]. The non-OA controls and OA-affected cartilage were subjected to definitions described by OARSI and ACR (Figure 1) [2-4]. Given the heterogeneity of the OA in different joints [1,5,8], we focused on individuals with knee OA. Since the susceptibility of OA varies across different demographics, (including males, females, age, source, and ethnic backgrounds) we used a combination of donors. We randomly pooled the RNA from each donor in equal amounts to further stratify the data before procuring the p-values for significant changes in gene expression arrays. Non-OA healthy controls and OA-affected knee cartilage were pulverized in liquid nitrogen to extract total mRNA for gene expression arrays and or RT-PCR [2-4]. We have previously reported that numerous transcripts were significantly modulated in OA-affected cartilage as compared to normal cartilage [2-6]. Several of these transcripts were further validated using RT-PCR and or immunostaining using a separate set of clinical samples [2-6]. In the present study, we selected four reference genes (*SPON*1, *HMGB*1, *HMGB*2, and *VEGFA*) that are differentially expressed in normal and OA-affected cartilage. The optimum expression of each of these genes was not only essential for balanced homeostasis in normal cartilage, but their differential expression from normal cartilage has been reported to have profound effects on cartilage and/or bone homeostasis [3,4]. The f-spondin (*SPON*1) and *HMGB*1 transcripts were significantly up-regulated in human OA-affected cartilage [3]. F-spondin and *HMGB*1 regulate terminal differentiation of chondrocytes and endochondral bone formation in normal cartilage [3,19]. Similarly, the mRNA expressions of *HMGB*2 and *VEGFA* are down-regulated in human OA-affected cartilage as compared to normal cartilage (Figure 1) [3]. *HMGB2*$^{-/-}$ mice indicated that *HMGB*2 was essential for chondrocyte and mesenchymal stem cell differentiation in cartilage [20], whereas *VEGFA*$^{-/-}$ mice showed reduced angiogenesis, which was accompaniedby impaired bone development and removal of terminally differentiated hypertrophic chondrocytes [21]. These four differential expressiongenes in OA-affected cartilage share many of the characteristics in the corresponding transgenic or knockout mice [2,4,19-21].

The same set of gene expression array data ware utilized to represent the expression of *MIG*-6 as shown in Figure 1. Like *HMGB*1 and f-spond [3,19], the mRNA of *MIG*-6 showed a significant ($p \leq 0.001$) increase in all the cohorts of normal human cartilage. Like the normal human cartilage, a high level of *MIG*-6 expression was evident in the kidney and liver of both humans and rats [10]. In contrast, *MIG*-6 was significantly down-regulated (like *HMGB*2 and *VEGFA*) in all the cohortsof human OA-affected cartilage as compared to normal cartilage (Figure 1). This observation now validates the clinical observations in man and the OA-like disorder in *MIG*-6$^{-/-}$ mice [5,16-18]. *MIG*-6 is a negative regulator of IL-1β, TNFα, and collagen biosynthesis. Indeed the absence or limiting amounts of *MIG*-6 in OA-affected cartilage may contribute to an uncontrolled increase in IL-1β, TNFα, and collagen biosynthesis in OA-affected cartilage [1]. *MIG*-6 is known to bind to the kinase active domain of EGF-Receptor and functions as an inhibitor of EGF-Receptor [5]. EGF-Receptor is

a multifunctional receptor involved in cellular signaling associated with cell proliferation, apoptosis, cell survival and cell migration [10]. Some recent studies have also suggested the possible mechanism of *MIG*-6 in the destruction of cartilage via the EGF-Receptor [22]. The loss of *MIG*-6 activity or EGF-Receptor signaling results in: excessive chondrocyte proliferation, ECM-degrading proteases such as MMP13 and ADAMTS5, with increased osteophytes and bony outgrowths in the joints [5,16-18, 22]. Most human OA-affected cells in cartilage are highly proliferative, hypertrophic and "tumor-like" in nature with a significant amount of molecular inflammation, MMP13, ADAMTS5 activity, increased osteophytes and bony outgrowths [1,2,22]. These observations highlight the significance of *MIG*-6: a multifunctional tumor suppressor gene in homeostasis of human cartilage and perhaps uncontrolled chondrocyte proliferation and hypertrophy in human OA-affected cartilage.The high expression of *MIG*-6 in non-OA and healthy cartilage may counterbalance the mechanical stress and proliferative activity induced by mechanical forces, and thus *MIG*-6 may assist in sustaining normal cartilage homeostasis [5,16-18]. Genetic disruption (*MIG*-6$^{-/-}$) in mice may be a useful model for further understanding the mechanism of OA and therapeutic intervention. The molecular mechanism by which the *MIG*-6 mRNA is down-regulation in human OA-affected cartilage requires future investigations. These observations open the door for the modulators of EGF-Receptor-related drugs for the treatment of OA considering the inflammatory and molecular events associated with OA reside in the joints and also in the OA-affected PBMCs in the blood [2,3,7,16-18,22].

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