A potential role of homeobox transcription factors in osteoarthritis

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Abstract: When a healthy joint progressively becomes osteoarthritic, the structures of the affected cartilage, bone and synovia undergo an initial phase of rearrangement. The exact molecular and cellular events occurring in this early osteoarthritic transition phase still remain elusive. Homeobox (*Hox*) genes encode for transcription factors that typically regulate limb morphogenesis and skeletal formation during development. More recently they were shown to be required for tissue remodelling and homeostasis in adults and to be modulated in a variety of pathologies. Here we present and discuss the hypothesis that dysregulation of specific *Hox* genes is associated with the onset and development of osteoarthritis (OA). Discovering mechanisms modulating *Hox* gene expression could not only provide important information in understanding OA pathology and its initiation, but also help to identify biomarkers reflecting the state of early OA. This knowledge would allow anticipating the time window for clinical treatment of the affected cartilage and assist in the development of innovative strategies to restore joint homeostasis, e.g., by cell or gene therapy.

Keywords: Osteoarthritis (OA); cartilage; hox; homeobox; neural-crest; nasal chondrocytes; regenerative medicine

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Hox genes in adult tissues

The highly conserved Homeobox (abbreviations: Hox for general reference; HOX specifically for the human species) transcription factors play an unquestioned crucial role as master regulators during embryonic vertebrate development and morphogenesis by controlling the three dimensional body plan organisation (1,2). In addition to these known functions in embryonic models, more recently an important role has been ascribed to these transcription factors during remodelling and homeostasis of adult tissues (3,4). By regulating their downstream targets (e.g., matrix-degrading proteinases, extracellular matrix proteins or adhesion molecules), *Hox* gene products were shown to influence physiological tissue remodelling processes e.g., during wound healing, mainly by coordinating angiogenesis (5). Due to their regulatory functions on tissue synthesis and degradation, it is not surprising that *Hox* genes have also been identified to be differentially expressed in several diseases, such as atherosclerosis (6), diabetes (7) or cancer (8,9), reflecting pathophysiological conditions characterized by an aberrant metabolism and concomitant with locally atypical vascularization.

Osteoarthritis (OA) pathogenesis and need of early markers

Disrupted metabolism and enhanced vascularisation have been described to be hallmarks of OA. Being the most prevalent degenerative joint disorder leading to pain and disability of millions of people worldwide, OA has become a major burden for patients and healthcare institutions. In the etiology of OA, several key factors have been identified, including mechanical stress (caused mainly by overload, malalignment or obesity), previous joint injuries and aging (10). Being in the focus of research since decades, substantial efforts have been undertaken to unravel mechanisms leading to degradation of the cartilage matrix and ultimately resulting in abnormal remodelling of the affected joint (11). It seems that complex events resembling endochondral bone formation as seen during embryonic development are initiated in OA, leading to cartilage/

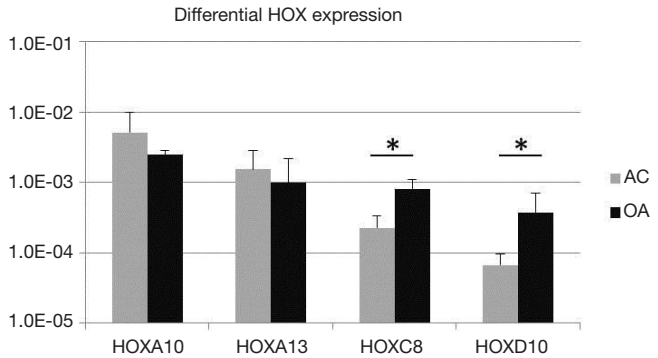

Figure 1 Differential *HOX* gene expression profile of human osteoarthritic chondrocytes (OA; n=5) as compared to articular chondrocytes isolated from healthy cartilage (AC; n=9) was assessed by quantitative real-time RT-PCR (mean ± standard deviation). Statistically significant differences of P≤0.05 (non-parametric Mann-Whitney-U analysis) are indicated by an asterisk. OA, osteoarthritis.

joint degeneration (12). But still the mechanisms and factors priming these events, as well as markers associated with the onset of early stages of OA, are far from being comprehensively defined.

Early stages of OA are often not accompanied by pain and thus onset of the disease regularly appears unnoticed. Only when degeneration has progressed to a non-revertible, painful stage and structural changes of the joint are visible by MRI, patients are diagnosed for OA. At this stage, treatment actions are not likely to succeed due to the largely damaged state of the joint. As no disease modifying drugs are available, actions are limited to palliation of symptoms—and in many cases terminated by replacement of the joint with a prosthetic implant. Identification of markers to define a stage prior to the onset of irreversible joint degeneration would thus be crucial to anticipate the window for potential clinical treatment, possibly increasing the likelihood of successful repair of the cartilage.

Hox genes in chondrogenesis and OA

Among the factors known to be important during skeletal development, *Hox11* genes (such as *Hoxa11* and *Hoxd11*) are known to regulate chondrocyte differentiation (13,14), whereas genes of the *Hox8* group are essential during bone development (15,16). Dysregulation of such *Hox* genes that are essential during joint formation are consequently believed to be involved in several cartilage/skeletal disorders in adults and might thus lead to OA development with time (17).

The onset of another pathology leading to cartilage degradation, namely rheumatoid arthritis, has also been connected with *Hox* gene regulation. In particular, the inflammation-induced overexpression of Ezh2 (histone methyl transferase, functioning as the catabolic subunit of polycomb repressor and whose targets include *Hox* genes), correlating with rheumatoid arthritis, was shown to repress the expression of a number of *Hox* genes (18,19).

Unpublished data from our research group indicate differential expression of selected *HOX* genes in articular chondrocytes (AC; n=9) isolated from healthy cartilage as compared to chondrocytes retrieved from osteoarthritic (OA, n=5) joints. Whereas *HOXA10* and *HOXA13* tended to be lower expressed in OA chondrocytes, *HOXC8* and *HOXD10* were significantly up-regulated in the pathological tissues (*Figure 1*) and correlated with an upregulation of more than 100-fold of VEGF in OA chondrocytes (data not shown), as previously also described by others (20). In tumors and wound healing, HOXD10 was shown to have an anti-angiogenic effect via reduction of VEGF expression (21,22). In an osteoarthritic environment, that is characterized by an atypically increased vascularization, unexpected upregulation of *HOXD10* could reflect an attempt of cells to react and attenuate blood vessel ingrowth, thus underlining the tissue-specific and context-dependent function of *Hox* genes (5).

Potential regulation of *Hox* gene expression in OA by microRNAs (miRNA)

In the search of applicable and predictive markers of early OA, miRNAs have been suggested (23) as they are differentially expressed in OA as compared to healthy joints (24,25). MiRNAs are small non-coding RNAs that function as translational and post-transcriptional regulators of gene expression (26). Interestingly, at least two miRNA families are located within the *HOX* clusters regulating their expression (27), further indicating a potential connection between OA and *HOX* gene dysregulation. Those miRNAs located within the *HOX* gene cluster were also shown to be implicated in Huntington's disease pathogenesis (28). Moreover, dysregulation of miRNAs has recently been linked to age-related human neurodegenerative diseases (29). Together, these findings indicate a potential role of differentially expressed specific miRNAs in the regulation of *HOX* genes in OA.

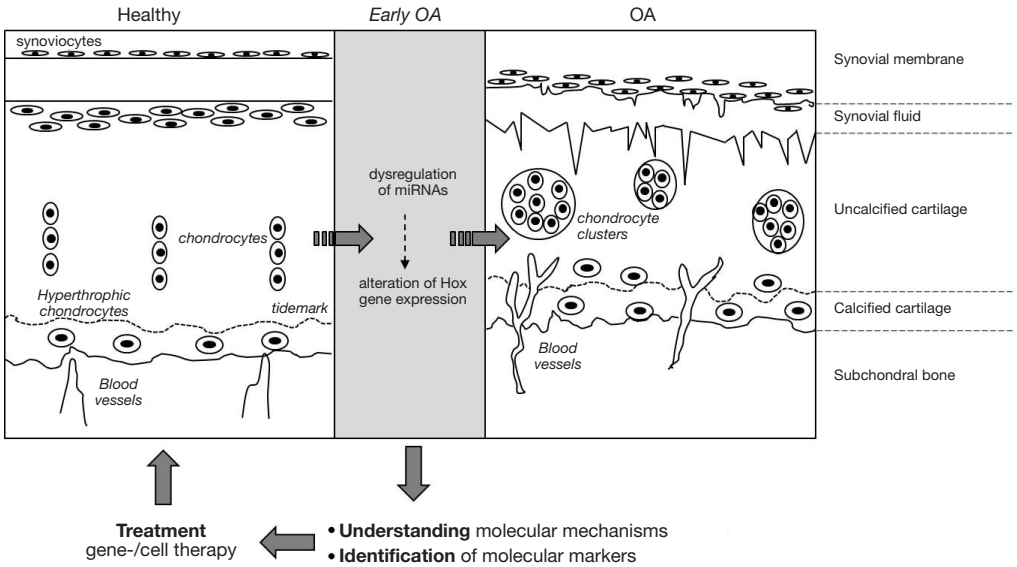

Figure 2 Schematic overview of the potential role of *HOX* genes in the early phases (early OA) and development of OA. Changes in specific microRNA (miRNA) expression were identified under pathological (OA) conditions and have been suggested to occur in the early stages of OA, potentially leading to dis-regulation of specific *HOX* genes. Unravelling the mechanisms modulating *HOX* gene expression could provide important information in understanding the pathology and initiation of OA. Identification of markers reflecting the state of early OA would further allow anticipating a clinically promising time window for treatment by established cell based cartilage repair techniques, possibly introducing alternative cell sources, and also by further improved methods based on gene therapy approaches. OA, osteoarthritis.

Perspective

Involvement of *HOX* gene regulation in a wide variety of pathologies and our preliminary results indicate a potential link between OA and HOX transcription factors. Unravelling the mechanisms modulating their differential expression in the development of OA could provide relevant information to better understand this pathology and its initiation. Furthermore *HOX* genes and/or their upstream regulators should be considered to possibly identify markers of early stages of OA. Defining those early stages and thereby a time window for potential clinical treatment at the beginning of the disease (*Figure 2*) would open new possibilities in the development of new strategies for treatment of OA before irreversible destruction of the knee joint, and thus diminish the need for total joint replacement by artificial prostheses.

Comprehension of the role of *HOX* genes in OA could also enhance successful implementation of cell-therapies for its treatment. Some reports have described the use of autologous AC as cell suspension (ACT; autologous chondrocyte transplantation) (30) or assisted with a collagen-based matrix (31) not only for repair of fresh and confined cartilage injuries, but also for the repair of early stage osteoarthritic lesions. The clinical outcome in this more complex setting was inferior in quality and accompanied by a higher failure rate as compared to non-osteoarthritic cartilage defects (30,31). Still, the success rate was acceptable in the reported cases, justifying the treatment of patients with early signs of OA using conventional/matrix assisted ACT in order to avoid or delay prosthetic knee replacement. To improve the quality and success rate of cartilage repair in early osteoarthritic conditions, different cell sources with improved cartilage forming capacities should be considered. Several progenitor-like cell types including mesenchymal stromal cells from different sources (32) and (inducible) pluripotent stem cells (33,34), but also more restricted/differentiated cells originating from other sources of cartilage, such as nasal chondrocytes (35), have been proposed and investigated for their compatibility in cartilage repair. Whether these alternative cell types will also be molecularly and physiologically compatible in a more complex pre-osteoarthritic environment is yet to be investigated and defined. In this context, *Hox* gene expression could be used to investigate such compatibility and to predict interaction of the implanted cells/tissue with resident cells (35).

Hox genes may also represent the target of a gene therapy approach to OA. *Hox* gene therapy has already been shown to reverse dysfunctional mesenchymal progenitor cells in diabetic mice by gene transfer of *Hoxd3* (36) or *Hoxa3* (7). This approach of restoring a "healthy" expression level of Hox as master transcription factor(s) should also be considered in the search for potential means to treat OA. Importantly, given that *Hox* genes typically regulate cell function as a constructed circuit, activation (or de-activation) of different *Hox* genes in defined patterns may be necessary to instruct a specific cell behaviour, also depending on the targeted cell type and site of repair.

Modulation of *HOX* genes was identified during aging in mesenchymal stromal/stem cells (37) and in particular HOXB7 was indicated as a master regulator of age related behavioural changes in progenitor cells (38). In fact, elderly people are more susceptible to diseases with known dis-regulation of HOX, such as cancer, diabetes or atherosclerosis—and also OA is typically associated with aging. Future studies investigating *HOX* gene modulation could therefore generate more fundamental understanding of the onset and development of a variety of age-related, degenerative diseases, and potentially lead to improved therapeutic strategies applicable in different pathological settings.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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