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REVIEW ARTICLES

Novel therapeutic targets in osteoarthritis: Narrative review on knock-out genes involved in disease development in mouse animal models

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Abstract

Osteoarthritis (OA) can affect every joint, especially the knee. Given the complexity of this pathology, OA is difficult to treat with current therapies, which only relieve pain and inflammation and are not capable of restoring tissues once OA has started. Currently, researchers focus on finding a therapeutic strategy that may help to arrest disease progression.

The present narrative review gives an overview of the genes involved in the development and progression of OA, assessing *in vivo* studies performed in knock-out mice affected by OA, to suggest new therapeutic strategies. The article search was performed on the PubMed database and www.webofknowledge.com website with the following keywords: "knee osteoarthritis" AND "knockout mice". The included studies were in English and published from 2005 to 2015. Additional papers were found within the references of the selected articles. In the 55 analyzed *in vivo* studies, genes mainly affected chondrocyte homeostasis, inflammatory processes, extracellular matrix and the relationship between obesity and OA. Genes are defined as inducing, preventing and not influencing OA. This review shows that joint homeostasis depends on a variety of genetic factors, and preventing or restoring the loss of a gene encoding for protective proteins, or inhibiting the expression of proteins that induce OA, might be a potential therapeutic approach. However, conclusions cannot be drawn because of the wide variability concerning the technique used for OA induction, the role of the genes, the method for tissue evaluations and the lack of assessments of all joint tissues.

Key Words: *genes, knee, knock-out, mice, osteoarthritis, therapy*

Introduction

The homeostasis of the extracellular matrix (ECM), which is mainly composed of collagens (COLLs), proteoglycans (PGs) and aggrecan [1], and the balance between anabolic and catabolic activities of chondrocytes [2] are disrupted in the chronic degenerative and slowly progressive pathology of osteoarthritis (OA) [3]. Moreover, both biomechanical and biochemical factors, which are responsible for the initiation of OA [3], also induce sclerosis of subchondral bone (SB), osteophyte formation, synovial inflammation and fibrosis, pathological changes in ligaments, menisci and tendons and joint space narrowing [4–7]. Chondrocyte viability decreases with a concomitant increase in apoptosis, hypertrophy, Col10a1 and Mmp13 expression and procatabolic responses, which are features making chondrocytes unable to maintain and regenerate cartilage [8,9]. The pathogenesis of OA is complex because several risk factors are involved: aging, sex and genetic factors (primary OA) or mechanical, traumatic and metabolic factors, such as the altered signaling pathways involved in cholesterol biosynthesis or thyroid hormone levels in chondrocytes (secondary OA) [10–12]. In addition, a major role of inflammation in OA development is acknowledged, due to the

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increase in the oxidative stress and catabolic microenvironment that concur in PG and COLL degradation [13].

Current therapeutic strategies consist of analgesics, glucocorticoids, nonsteroidal anti-inflammatory drugs (NSAIDs) or hydroxyapatite administration, physical exercise (targeted strength training), diet or arthroplasty in the final stages of the disease [14]. Unfortunately, these strategies are not able to restore the native cartilage physiology or interfere with the progression of OA. These approaches only relieve pain and inflammation and are not considered to be disease-modifying therapies [15]. Recently, tissue engineering techniques employing mesenchymal stromal cells (MSCs) have been evaluated, but they have provided conflicting results and showed impediments related to their unestablished clinical use and lack of standardized protocols [8,16]. For all these reasons, there is an urgent need for therapeutic intervention capable of arresting or reverting disease progression and an appropriate animal model to evaluate new hypotheses, concepts and treatments. *In vivo* OA animal models are useful tools not only to understand the disease mechanism but also to evaluate the effectiveness of a therapy [17]. However, there is no gold standard model to evaluate OA. Although the mouse model does not allow the evaluation of the biomechanical function of the human joints, it is a primary choice for molecular studies. This is because of low maintenance costs for mice, the progress in mouse genetics and the easy availability of knockout (KO) mice, which allow the evaluation of time-dependent changes in OA joints and give the possibility to induce several genetic modifications in these animals [17,18]. Preclinical research on KO mice has increased over the past decade, making the mouse the best candidate model for the study of molecular pathways involved in OA [19,20].

Knee OA has a high prevalence rate compared with other types of OA, and it is present in earlier age groups, particularly in young obese women [16].

The present review aims to give an overview of the genes involved in OA knee development, by analyzing the *in vivo* studies performed over the past decade in KO mice to identify possible therapeutic targets.

The genes examined in the studies here reviewed encode for proteins involved in chondrocyte signaling pathways, inflammatory processes, cartilage ECM and in the relationship between obesity and OA. In addition, these genes are categorized as inducing, preventing and non-influencing.

Methods

In the current review, the article search was performed on the PubMed database using the following MeSH terms: "Osteoarthritis, Knee"(Mesh) AND "Mice, Knockout"(Mesh). The search limits were set for English language and articles published from January 1, 2005, to January 30, 2015. Additional articles were found within the references listed in the selected articles.

A second search was performed on the www.webofknowledge.com website with the following keywords: "knee osteoarthritis" AND "knockout mice", with a 2005–2015 time span and in the English language.

Results

A total of 27 *in vivo* studies were initially found in the PubMed database. Nine studies were not relevant and therefore were not included; these were *in vitro* studies, editorials or those focusing only on pain, rheumatoid arthritis, temporomandibular OA or OA in humans. A further search was performed by screening the reference lists of the 18 selected articles and 9 additional studies were found. Thus, 27 *in vivo* studies from this search were included in this review.

The www.webofknowledge.com database search retrieved 87 articles, 68 of which were not included because they did not concern KO mice or OA, or they were reviews, editorials, *in vitro* studies or considered micro-RNA. In addition, some of the excluded articles were duplicates of the previous PubMed database search [20] or the full-text was not available [8]. Nine additional studies were found by reading the reference lists of the 19 articles selected, and therefore 28 articles from this search were included in this review.

Overall, in the present review a total of 55 *in vivo* studies were analyzed (Figure 1), divided according to the process or compartment affected by gene KO: (i) chondrocytes, (ii) inflammation, (iii) cartilage matrix or (iv) relationship between obesity and OA. In addition, the paragraphs were further grouped into subsections, according to the role of gene KO in OA development: (i) inducing, (ii) preventing or (iii) non-influencing roles (Figure 2). This sub-classification was made depending on the study results, and thus a gene might have been classified in more than one subsection. Moreover, genes are reported by their official symbols, which may vary from the names used in the reviewed papers. The effects of gene KO were compared with responses in wild-type (WT) counterparts unless otherwise specified.

Chondrocytes

Twenty-five of fifty-five studies belong to this group, which includes genes encoding for transcription factors, enzymes, receptors and regulators of intracellular signaling pathways, regulate chondrocyte homeostasis