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RESEARCH

Study of the role of miRNA in mesenchymal stem cells isolated from osteoarthritis patients☆

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KEYWORDS
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Abstract
Objective: miRNAs act as gene silencers that are involved in the regulation of essential cell functions. miR-335 is involved in regulating cell differentiation processes in progenitor cells. Mesenchymal stem cells (MSCs) are progenitor cells of chondrocytes and osteoblasts responsible for homeostatic maintenance of cartilage and bone. The aim of this study was to determine a possible relationship between the expression of miR-335 and osteoarthritis.
Methods: MSCs obtained from the bone marrow of 3 osteoarthritic patients and 3 controls with no clinical signs of osteoarthritis or osteoporosis were cultured and phenotypically and functionally characterized in a 3-step culture. Expression levels of miR-335 and the mesoderm-specific transcript gene – *MEST* – that controls its expression were determined by quantitative PCR.
Results: Differences in the expression levels of miR-335 and *MEST* (median [interquartile range]: 1.69 [0.85–1.74], and 3.85 [3.20–5.67] were detected between MSCs isolated from patients with osteoarthritis and controls. Although the differences detected did not reach statistical significance ($P = .1$), a clear trend toward lower expression of miR-335 in osteoarthritis MSCs was observed.
Conclusions: Given that miR-335 has the different genes involved in the Wnt signaling pathway as potential targets, the observed trend may help to ascertain, at least partially, some of the alterations which determine the onset or progression of osteoarthritis, and can therefore serve for the design of future therapeutic targets for the treatment of this disease.

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PALABRAS CLAVE
Células madre mesenquimales;
Microácidos ribonucleicos;
Artrosis

Estudio del papel de los miARN en células madre mesenquimales aisladas de pacientes artrósicos

Resumen
Objetivo: Los miARN actúan como silenciadores génicos que están implicados en la regulación de funciones celulares esenciales. El miR-335 participa regulando los procesos de diferenciación celular en células progenitoras. Las células madre mesenquimales (MSC) son células progenitoras de los condrocitos y osteoblastos encargados del mantenimiento homeostático del cartílago y hueso. El objetivo de este estudio era determinar una posible asociación entre la expresión de miR-335 y la enfermedad artrósica.
Metodología: Las MSC obtenidas de la médula ósea de 3 pacientes artrósicos y 3 controles sin signos clínicos de artrosis ni osteoporosis se cultivaron y caracterizaron fenotípica y funcionalmente en el pase 3 de cultivo. Así mismo, mediante PCR cuantitativa se determinaron los niveles de expresión de miR-335 y del gen mesoderm-specific transcript –*MEST*–, que controla su expresión.
Resultados: Se detectaron diferencias entre las MSC aisladas de pacientes con artrosis y los controles en los niveles de expresión de miR-335 y de *MEST* (mediana [rango intercuartílico]: 1,69 [0,85-1,74]; 3,85 [3,20-5,67]). Aunque las diferencias detectadas no alcanzaron una significación estadística (p = 0,1), sí se apreció una clara tendencia a una menor expresión de miR-335 en las MSC de pacientes artrósicos.
Conclusiones: Teniendo en cuenta que miR-335 tiene como dianas potenciales diferentes genes que participan en la vía de señalización de la Wnt, la tendencia observada podría determinar, al menos en parte, algunas de las alteraciones que determinan el inicio o progresión de la artrosis, y puede, por lo tanto, servir en el diseño de futuras dianas terapéuticas para el tratamiento de esta enfermedad.

Introduction

Osteoarthritis (or arthrosis) is the most prevalent rheumatic condition, particularly affecting individuals of advanced age.[1] It is characterized by chronic and progressive joint damage, inflammation, pain and, in advanced stages, loss of function of the affected joints. At a tissue level, the main characteristic of osteoarthritis is sclerosis of the subchondral bone and, secondarily, progressive loss of the joint cartilage.[2] While the alteration of articular homeostasis, resulting in a net loss of cartilage and formation of osteophytes, is concomitant with the disease, its root causes are unknown. At present, the multifactorial etiology of osteoarthritis is generally accepted, as is its association with metabolic, structural, genetic and environmental factors.[3–6] Due to the evident physiological changes which take place in cartilage, for many years it has been assumed that this is the starting point of the disease. However, research conducted in recent years has highlighted the important role of the bone underlying the cartilage (subchondral bone) in the development of the disease.[7–10] In this context, our group has previously described the existence of an association between a genetic polymorphism linking type X collagen and its reduced expression in mesenchymal stem cells (MSCs) with an osteoarthritic origin. These experimental findings revealed that the mechanism responsible for the correct regeneration of cartilage in osteoarthritis had its origins in both deficient formation of the extracellular matrix and possible alterations in the differentiation potential of MSCs into hypertrophic chondrocytes.[11]

The role of MSCs in osteoarthritis is not trivial, as these are the parent cells of osteoblasts and chondrocytes, which, in turn, are responsible for synthesizing the cartilage and bone extracellular matrix and, therefore, are essential for the formation and regeneration of bone and cartilage.[12]

In light of this evidence, it is highly probable that an alteration of the differentiation process of these cells could lead to an abnormal development and tissue homeostasis, contributing to the etiopathogenesis of osteoarthritis. In this regard, our group recently described the existence of alterations in the signal pathway of Wnt/β-catenin[11] in osteoarthritic MSCs. This pathway is essential for the control of key processes, such as cell proliferation, migration and differentiation.[13–15] Gene expression is regulated by different mechanisms which allow or prevent their transcription and, ultimately, their function through the proteins they encode. One of the most recently studied regulatory mechanisms is that exerted through miRNAs. These are a family of non-coding RNAs, with an approximate size of between 18 and 24 nucleotides in length. miRNAs act as gene silencers, repressing mRNA translation or inducing their degradation.[16] Thus, depending on the biological context, they are potentially capable of modulating the signaling activity of a cell signal transduction pathway, for example, one involved in the differentiation of MSCs, by selectively suppressing or activating specific components.[17]

Recently, due to the increasing interest in the study of miRNA regarding regulation of tissue differentiation, several miRNAs have been identified whose expression is increased during the differentiation of MSCs into different mesenchymal phenotypes. Thus, miR-26b[18] and miR-337[19] regulate