TARGETING OF FOCAL ADHESION KINASE BY SMALL-INTERFERING RNAs REDUCES TYPE II COLLAGEN EXPRESSION NOT AGGREGCAN IN HUMAN CHONDROCYTES

*Kim, Y H; +**Lee, J W
+Brain Korea 21 Project for Medical Science, +**Department of Orthopedic Surgery, Yonsei University College of Medicine, Seoul, Korea ljwos@yumc.yonsei.ac.kr

INTRODUCTION

Many adapter proteins including Shc, Crk, p130Cas, paxillin, talin, vinculin, caveolin, focal adhesion kinase (FAK) and pyk2 were recruited by integrin signaling. These proteins and others interact with the integrin when integrins are bound to ECM ligands which induce a conformational change in the receptor subunits. It has been reported that above proteins played a role in intracellular signaling by extracellular matrix proteins such as type I collagen or laminin. Less is known about intracellular signaling, such as FAK-related signaling, in interactions between type II collagen and chondrocytes. Therefore, this study demonstrated the molecular events contributing to FAK in chondrocytes-type II collagen interactions and changes of type II collagen expression by suppressing FAK protein synthesis using small-interfering RNA (siRNA)-mediated depletion.

METHODS

Cell culture: Human MSCs from bone marrow aspirates were serially subcultured every 8 day up to 5 passages. Porcine or human articular chondrocytes were isolated by digestion with 0.1 % collagenase and 0.065 % hyaluronidase for 6 hrs. For alginate bead culture, cell pellet was encapsulated in 1.2 % alginate solution at a density of 2x10⁶ cells/ml. The coating of a culture dish was done with 1 µg/ml type II collagen (Chondrex) for 12 hrs at 4°C. Cell suspension (1.5 x 10⁵ cells) was placed in each coated dish and allowed to incubate for 5 days at 37°C. Western blot analysis: The cell lysates were separated on 10 % SDS-PAGE and transferred to a PVDF membrane followed by immunoblotting with p-ERK, ERK (santa cruz) and FAK (upstate biotechnology). GAPDH (RDI) was used as internal control to normalize protein quantification.

RT-PCR: Total RNA was isolated from cell pellets by the RNeasy kit (Qiagen). 1 µg of total RNA was reverse-transcribed with use of the Omniscript kit (Qiagen). cDNA was amplified in a total volume of 50 µl containing 1X PCR buffer, 0.2 µM of each primer, and 1U of Taq DNA polymerase. siRNA: siRNA construct was designed to target against human FAK (GenBank accession number NM_153831 and NM_005607), cells were plated at a density of 4x10⁵ cells/well in 24 well plates before transfection. Cells were transfected with 0.2 pmole/µl of siRNA construct using lipofectamine Reagent (Invitrogen). Transfection efficacy was measured by transfecting Fluorescein-labeled siRNA (Bioneer, South Korea) and counting fluorescence-positive cells by using a fluorescent microscope.

RESULTS

FAK was strongly expressed in chondrogenesis of human MSCs from 10 hr to 14 day and fresh or early passaged chondrocytes. But, the expression was declined in differentiated chondrocytes. This result suggested that signaling pathway to maintain or induce chondrogenic phenotype might be mediated at least by FAK (Fig. 1).

DISCUSSION

Chondrocytes grown in monolayer are changed their specific gene expression such as type II collagen and aggrecan whereas alginate-cultured chondrocytes maintain their differentiated phenotype for months. Our study shows that FAK plays a critical role in type II collagen expression to maintain chondrocytes phenotypes and in vitro chondrogenesis. It was proven that depletion of FAK did interfere with type II collagen expression but not aggrecan. Therefore, FAK-dependent downstream signaling, such as ERK pathway, might regulate type II collagen expression during chondrogenesis or maintenance of chondrocytes phenotypes.

ACKNOWLEDGEMENT

This research was supported by a grant (code: SC3020) from the Stem Cell Research Center of the 21st Century Frontier Research Program funded by the Ministry of Science and Technology, Republic of Korea.

53rd Annual Meeting of the Orthopaedic Research Society
Paper No: 0003