Induction of apoptosis in the synovium of autoantibody-mediated arthritis in mice by intra-articular injection of double-stranded microRNA-15a

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Objective. MicroRNA (miRNA) is a family of non-coding RNAs which exhibits tissue-specific or developmental stage-specific expression patterns and is associated with human diseases [1]. MiRNA-15a (miR-15a) is reported to induce cell apoptosis by negatively regulating the expression of B cell lymphoma 2 (BCL2) which suppresses the apoptotic processes [2]. An anti-apoptotic protein, particularly in BCL2, is reported to be highly up-regulated in rheumatoid arthritis (RA) synovial fibroblasts in comparison to osteoarthritis, and the apoptotic process in the RA synovial fibroblasts is described to may be suppressed by the overexpression of BCL2 [3]. On the other hand, there was no report that made an attempt of injection of atelocollagen-mediated miRNA into the joint. The purpose of this study was to investigate whether double-stranded (ds) miR-15a administered by intra-articular injection could be uptaken in cells and induce dysfunction of BCL2 and cell apoptosis in the synovium of arthritis mice in vivo.

Methods. Male DBA/1J mice were induced to develop arthritis by an arthritogenic cocktail of 4 monoclonal antibodies to type II collagen combined with lipopolysaccharide stimulation. The mice in the experimental group were injected with ds miR-15a labeled with FAM/atelocollagen complex into the knee joint. In the control group, control siRNA/atelocollagen complex was used. The expression level of miR-15a in the synovium of the injected joint knee was analyzed by quantitative polymerase chain reaction (PCR). The expression of FAM in the synovium was observed by fluorescent microscopy. In the synovium, the expression of BCL2 was examined by Western blotting and the expressions of BCL2 and caspase3 were observed by immunohistochemistry.

Results. To confirm the expression of the endogenous miR-15a, quantitative RT-PCR was performed. Mmu-miR-15a was significantly down-regulated in arthritis mice compared to non-arthritis mice (data not shown). Also, up regulation of BCL2 protein in arthritis mice in comparison with non arthritis mice was confirmed by western blotting(data not shown). The expression level of miR-15a in the synovium of the injected joint knee was analyzed by quantitative polymerase chain reaction (PCR). The expression of FAM in the synovium was observed by fluorescent microscopy. In the synovium, the expression of BCL2 was examined by Western blotting and the expressions of BCL2 and caspase3 were observed by immunohistochemistry.

Discussion. These results indicated the induction of cell apoptosis after the intra articular injection of ds mir-15a in the synovium 24hr after the control siRNA/atelocollagen complex injection into the joint (a, b). The expression of green fluorescence in the synovial cells 24 hr after FAM labeled ds miR-15a/atelocollagen complex injection into the joint (c, d). a, e, High bright microscopic field, b, f; High power field of FAM labeled ds miR-15a/atelocollagen complex injected joint (c; FAM, d, FAM, g; Merge, original magnification 400×, bar = 100μm). h; High power field of red rounded area of figure g, * = synovium. In immunohistochemistry, the expression of caspase3 indicating the number of apoptotic cells to have increased in the synovium of the experimental group (b), in comparison to the caspase3 expression in the control group (a). Original magnification 400×, bar = 100μm. * = joint cavity.

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