Autocrine motility factor regulates the cell survival in human chondrocytes

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Introduction:
Autocrine motility factor (AMF) is a housekeeping gene product present in all cells, is an essential enzyme of catabolic glycolysis and anabolic gluconeogenesis, and regulates tumor cell growth and metastasis. It was reported that AMF-high secretion tumor cells have a resistance against the drug-induced apoptosis, with suppression of mitochondrial pathway. Overexpression of AMF induces the down-regulation of Apaf-1 and caspase-9 gene expression, and leads to the apoptotic resistant phenotype in malignant cells [1].

Osteoarthritis (OA) is the most common degenerative disease of human articular cartilage. It is characterized by extracellular matrix damage and an important loss in tissue cellularity. Apoptosis may also have a role in diseases involving articular cartilage degeneration, such as OA. The present study was undertaken to investigate PGI/AMF function in chondrocyte apoptosis with shear stress.

Materials and Methods: Cartilage tissues were obtained during total joint replacement surgery with OA. Normal cartilage tissues were obtained during surgery of femoral neck fracture. Chondrocytes were isolated and cultured from cartilage tissues. Expressions of AMF in chondrocytes were analyzed by western blotting. To determine the chondrocyte apoptosis and AMF, western blot analysis for caspases-9 and AMF was performed after loading shear stress with erythrose 4-phosphate (E4P) which is a specific inhibitor of AMF.

Human normal chondrocytes were transfected with AMF plasmid with lipofection method. Expressions of AMF, caspase-9, and Apaf-1 in chondrocytes were analyzed by RT-PCR. To explore the anti-apoptotic ability of AMF, shear stress was introduced in human chondrocytes after transfection of AMF. Chondrocyte apoptosis was detected by DNA fragmentation and western blotting with the antibody of caspase-9.

To determine the apoptosis pathways affected by AMF overexpression, PD98059 (MAPK inhibitor) and LY294002 (PI3K inhibitor) were used. After incubation with PD98059 or LY294002 expression of caspase-9 and Apaf-1 were analyzed by RT-PCR and chondrocyte apoptosis with shear stress was detected by DNA fragmentation. To investigate the intracellular signaling of AMF, the activation of PI3K/Akt and MAPK pathways were analyzed by western blotting with phosphospecific antibodies of Akt and MAPK after induction of shear stress. Finally, the pathway from the activation of MAPK and PI3K to the apoptosis through mitochondria was analyzed by western blotting with antibody of Bcl-xL, which is one of the Bcl-2 family members.

Results: AMF was expressed in OA and normal chondrocytes with western blot analysis (Fig.1a). After treatment with E4P, expression of AMF significantly decreased, and cleaved caspases-9 increased with loading shear stress. And expression of AMF decreased in a time dependent manner in both groups (Fig.1b).

Figure 1

![Figure 1](image1.jpg)

Expression level of AMF increased significantly in AMF transfected NHAC-kn, compared with the control (Fig.2a,b). Expression levels of caspase-9 and Apaf-1 decreased significantly in AMF transfected cells (Fig.2c). DNA fragmentation occurred in NHAC-kn and vector only-transfected cells after shear stress induction for 6 hours. In contrast, DNA fragmentation could not be detected in AMF transfected cells (Fig.2d), and cleaved caspase-9 was not expressed in AMF transfected cells (Fig.2e).

When AMF transfected cells were treated with PD98059 and LY290042, expression of caspase-9 and Apaf-1 were fully recovered by RT-PCR (Fig.3a), and DNA fragmentation could be detected (Fig.3b). MAPK phosphorylation levels were increased in AMF transfected cells compared with NHAC-kn and vector only-transfected cells, and decreased by inhibition of MAPK with PD98059 (Fig.3c). Akt phosphorylation levels were increased in AMF transfected cells compared with NHAC-kn and vector only-transfected cells, and decreased by inhibition of PI3K with LY294002 (Fig.3d). Finally, expression levels of Bcl-xL were increased AMF transfected cells compared with NHAC-kn and vector only-transfected cells, and decreased by inhibition of MAPK and PI3K (Fig.3c,d).

Discussion: In recent years, many authors have shown that the rate of chondrocyte apoptosis is increased in OA cartilage and speculated on the role of apoptosis in the pathogenesis of OA. In this study, we confirmed that AMF is expressed in OA and normal chondrocytes, and expression of AMF decreased after loading shear stress. And chondrocyte apoptosis increased after treatment with E4P. So, we suspected that AMF plays an important role for cell survival and apoptosis in human chondrocyte.

To directly investigate the functional role of AMF on anti-apoptosis, we transfected the AMF plasmid into NHAC-kn and established the cells overexpressing AMF. These cells did not express caspase-9 and Apaf-1, and were resistant to shear stress induced apoptosis. Treatment with MAPK or PI3K inhibitors suppressed these anti-apoptotic characters. Western blot analysis using phosphospecific antibody against MAPK and Akt revealed that these phosphorylation levels were increased only in AMF transfected cells and not in controls. These phosphorylation levels were abrogated by inhibition of MAPK and PI3K. From the results of western blot analysis with Bcl-xL antibody, the anti-apoptotic effect of AMF in human normal chondrocytes was related to the phosphorylation of MAPK and PI3K, and mitochondrial pathway.

In conclusion, we demonstrated for the first time that AMF plays an important role in the apoptosis of human normal chondrocytes, and regulates expression of caspase-9 and Apaf-1 via complex pathway mainly through the activation of MAPK and PI3K signaling pathway.

References