Regulation of p38 MAPK phosphorylation inhibited chondrocytes apoptosis in response to heat stress and mechanical stress

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Objective

Activation of p38 MAPK has traditionally been associated with the stress response and some apoptotic processes. However, the function of p38 MAPK in chondrocytes is still not clearly understood. We have shown that mechanical stress induced chondrocyte apoptosis, and inhibition p53 activation prevented chondrocyte apoptosis induced by mechanical stress [1]. In this study, we analyzed the expression of p38 and phosphorylated p38 in chondrocytes of osteoarthritis (OA) cartilage and normal cartilage. We induced chondrocyte apoptosis by heat stress or mechanical stress, and then investigated the relationship between chondrocytes apoptosis and phosphorylation of p38 MAPK.

Methods

OA cartilage samples were obtained from patients undergoing total knee replacement surgery, and normal cartilage samples were obtained from patients undergoing surgery for femoral neck fracture. Chondrocytes were isolated from the human cartilage and cultured. Expression of p38 and phosphorylated p38 in OA cartilage and normal cartilage were analyzed by immunohistochemistry and Western blotting. Heat stress or mechanical stress was introduced in normal human knee chondrocytes. In order to evaluate the function of p38, normal knee chondrocytes were pre-treated with p38 small interfering RNA (siRNA) before induction of heat stress or mechanical stress. Chondrocyte apoptosis was detected by TUNEL staining and Western blotting. The expression of p38 and phosphorylated p38 were detected by Western blotting.

Results

Immunohistochemistry showed that phosphorylated p38 was expressed at the superficial zone in OA cartilage(Fig 1A, a). However, phosphorylated p38 was not expressed at the superficial zone in normal cartilage (Figure 1A, b). Phosphorylated p38 was expressed at the hypertrophic zone in both OA and normal cartilage (Figure 1A, a, c). The expression of phosphorylated p38 in OA chondrocytes were significantly higher than in normal chondrocytes (Fig 1B).

The percentage of TUNEL-positive apoptotic cells significantly increased in a time-dependent manner after exposure to 39°C heat stress (Fig 2A). Western blotting showed that phosphorylation of p38 increased after exposure to 39°C heat stress for 1, 3, and 5 hours (Fig 2B). Expression of cleaved caspase 9 increased in a time-dependent manner after exposure to 39°C heat stress (Fig 2B). The percentage of TUNEL-positive apoptotic cells significantly increased after exposure to 10% shear stress (Fig 2C). Western blotting showed that phosphorylation of p38 increased after exposure to 10% shear stress for 12 hours (Fig 2D). Expression of cleaved caspase 9 increased after exposure to 10% shear stress (Fig 2D).

The percentage of TUNEL-positive apoptotic cells induced by heat stress was significantly decreased when p38 siRNA was transfected in comparison with that of apoptotic cells when p38 siRNA was not transfected (Fig 3A). Western blotting confirmed that p38 and phosphorylated p38 expression were inhibited when p38 siRNA was transfected decreased (Fig 3B). Cleaved caspase 9 expression was not detected when siRNA was transfected (Fig 3B). Similarly, the percentage of TUNEL-positive apoptotic cells increased by 10% shear stress. However, those apoptotic cells induced by shear stress was significantly inhibited when p38 siRNA was transfected in comparison with that of apoptotic cells when p38 siRNA was transfected (Fig 3C). Western blotting confirmed that p38 phosphorylated p38 expression were inhibited when p38 siRNA was transfected (Fig 3D). Cleaved caspase 9 expression was also decreased when p38 siRNA was transfected (Fig 3D).

Conclusion

We demonstrated that heat stress and mechanical stress increased chondrocyte apoptosis via phosphorylation of p38. Stress-induced chondrocyte apoptosis decreased due to inhibition of p38 MAPK activation when chondrocytes were incubated with p38 specific siRNA transfection. In contrast, the phosphorylation of p38 MAPK was increased in OA chondrocytes. Our results showed down regulation of p38 MAPK activation inhibited chondrocyte death induced by heat stress and mechanical stress. Therefore, evaluation of the interaction between phosphorylation of p38 and chondrocyte apoptosis could be one of the keys to elucidating OA etiology.

References