Expression of p53R2 in chondrocytes is regulated by shear stress.
+1Kohei Kawakita; 1Takayuki Nishiyama; 1Takaaki Fujishiro; 1Shinya Hayashi
1Noriyuki Kanzaki; 1Kenjiro Iwasa; 1Masahiro Kurosaka;
+1Department of Orthopaedic Surgery, Kobe University Graduate School of Medicine, Kobe Japan

e-mail address: nishiyama@med.kobe-u.ac.jp

Purpose:
Chondrocytes apoptosis plays an important role in cartilage degeneration in osteoarthritis(OA), and mechanical injury to cartilage induce chondrocyte apoptosis[1]. In response to DNA damage, p53 expression is up-regulated and regulates the p53-regulated apoptosis-inducing protein 1(p53AIP1). We previously showed that mechanical stress induced chondrocytes apoptosis via p53 and p53AIP1 pathway[2]. While, p53R2 expression is regulated in response to DNA damage. However, p53R2 repairs damaged DNA, and it protects from catabolism of chondrocytes. In this study, we evaluated the p53R2 expression of OA and normal chondrocytes. And we evaluated p53 and p53R2 expression of OA chondrocytes in response to shear stress.

Material and Methods

Immunohistochemistry
OA cartilage samples were obtained from total knee replacement surgery, and normal cartilage samples were from femoral neck fracture. p53R2 expression in chondrocytes of normal and OA cartilage was analyzed by immunohistochemistry.

Cell culture and shear stress
Chondrocytes were isolated from OA and normal cartilage and grown(37°C in 5% CO₂) in Dulbecco’s modified Eagles’s medium(Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum and 100 units/ml of penicillin/streptomycin. To apply shear stress to OA chondrocytes, they were incubated 6well plates with rubber bottom for 1 day at 37°C in 5% CO₂/95% humified air. And then, two%(mild), five%(mild) and ten%(excessive) shear stress was introduced to OA chondrocytes for 12hours by using Flexer cell system.

Western blotting
Cytoplasmic proteins and concentrated supernatants were quantified with protein assay reagent by Bradford method, and diluted to equal concentrations with hypotonic buffer. After isolating proteins in cytoplasm, proteins were separated under reducing condition by electrophoreses on 7.5-15% polyacrylamide gradient gels, and transblotted electrically onto the blotting membrane. Expression of p53 protein was detected using mouse anti-human p53 monoclonal antibody. Expression of p53R2 protein was detected using gout anti-human p53R2 polyclonal antibody.

Real-time PCR
Total RNA was extracted from chondrocytes using RNasey kit(QIAGEN) and reverse transcribed. Quantitative real-time RT-PCR was performed with a Biosystem 7300 sequencer (Applied Biosystems)

Results:
p53R2 was expressed in OA chondrocytes about five thousand times as much as normal chondrocytes by real-time PCR (Figure1a).p53 was well expressed in OA chondrocytes in comparison with normal chondrocytes by western blotting(Figure1b). And p53R2 was expressed in superficial zone in comparison with deep zone in OA cartilage by immunohistochemistry.(Figure2a,b)

The expression of p53 in OA chondrocytes was increased by 2.5 and 10 shear stress and depend on the strength of shear stress. On the other hand, the expression of p53R2 in OA chondrocytes was increased by 2 and 5% shear stress but decreased by 10% shear stress in comparison with control (non stress). (Figure3a,b)

Conclusions:
In response to varied cell stress signal, the p53 tumor-suppressor protein activates a multitude of genes encoding proteins with functions in cell-cycle arrest, DNA repair and apoptosis. When the DNA damage is not so severe, the Ser 15 and Ser 20 residues of p53 are phosphorylated and p53R2 is induced to repair DNA damage. But, if DNA damage is so severe as to be nonrepairable, the Ser 46 residue is phosphorylated and p53AIP1 is induced to cause apoptosis. In our studies, when two and five%(mild) shear stress was applied to chondrocytes, p53R2 was increased. So it is supposed that p53R2 is expressed to repair DNA damage and maintain homeostasis under mild mechanical stress. But when ten%(excessive) shear stress was applied, p53R2 is decreased and p53AIP1 is increased. So DNA repair mechanism is not performed under excessive mechanical stress and catabolic change is occurred. We consider that regulation of p53R2 may lead to strategy of OA treatment. We are doing further investigation to analyze p53R2 expression in chondrocytes.

References