Chondrocyte apoptosis with heat stress is induced by p53 pathway
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Introduction
Osteoarthritis (OA) is a progressive disorder associated with
degeneration and destruction of articular cartilage. There are some
reports that OA cartilage has a higher number of apoptotic chondrocytes
than does normal cartilage[1]. In response to DNA damage and other
acellular stresses, the cellular levels of p53 protein are greatly increased.
And then the elevation of p53 induces the cell cycle arrest or apoptosis.
We reported that apoptosis by shear stress in chondrocytes were
dependent on p53 pathway. In patients with OA, the intra-articular
temperature possibly elevates to further higher degree due to local
inflammation and aberrant frictional force induced by nonphysiological
mechanical loading. It was reported that heat stress on chondrocytes
were induced apoptosis, but it is still clearly unknown how chondrocytes
apoptosis were induced by heat stress[2]. In the present study, we
investigated the responses of chondrocytes to heat stress, and how
chondrocytes apoptosis were induced by heat stress.

Materials and Methods
Cell culture
NHAC-kn cells (human normal chondrocytes) were grown(37°C in 5% CO2) in Dulbecco’s modified Eagle’s medium(Sigma, St. Louis, MO)
supplemented with 10% fetal bovine serum and 100 units/ml of
penicillin/streptomycin.
To explore the function of p53, NHAC-kn cells were pre-treated with 50
µM of pifithirin-alfa(sigma), which is specific inhibitor of p53 mediated
apoptosis, for 24h before induction of heat stress.
Heat stress
To apply heat stress to NHAC-kn cells, they were cultured in 6well
plates for 1 day at 37°C in 5% CO2/95% humidified air. And then, these
plates were carefully sealed and placed in circulatory hot water bath set
at 43°C or at 37°C as a control temperature for 30minutes, 1hour, and
2hours. After the heat stress, the plates were removed from the water
bath and immediately changed the new medium and incubated in 5%
CO2/95% humidified air for 0, 6, 12, 24, or 48 hours.
TUNEL assay
NHAC-kn(3×105) were cultured in 8-well chamber slides. After heat
stress, the cultured NHAC-kn were fixed for 10 minutes with 4% neutral
buffered formalin, and apoptotic cells were determined using a TUNEL
assay kit, according to the protocol of the manufacturer.
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.
Heat stress induced apoptotic signals were confirmed by detection of
full-length and cleaved caspase 9. Expression of full-length and cleaved
caspase 9 was detected using mouse anti-human caspase 9 monoclonal
antibody.
Results
A large number of TUNEL-positive chondrocytes were detected in the
heat stress groups(Figure1c). In contrast, a few TUNEL-positive
chondrocytes were detected in the control groups(Figure1b). TUNEL-
positive chondrocytes were significantly increased by heat stress in a
time depend manner(Figure1a). The expression levels of p53 were
increased gradually after induction of heatstress(Figure2b). p53AIP1
was not detected in the chondrocytes without heat stress(Figure2a),
however p53AIP1 was expressed and increased by heat stress(Figure2b).
The expression levels of cleaved caspase 9 increased by heat
stress(Figure2b). TUNEL-positive chondrocytes were decreased when
chondrocytes were incubated with pifithirin-alfa(Figure3a). The
expression levels of p53, p53AIP1, and cleaved caspase 9 were
decreased when chondrocytes were incubated with pifithirin-
alfa(Figure3c).

Discussions:
It is said hyperthermia induced damage to articular cartilage and
fibrillated articular cartilage was susceptible to thermal challenge
compared with normal cartilage. Heat stress is suggested to result in
acceleration of the cartilage degeneration. Heat stress induced articular
chondrocyte apoptosis, but the effect of heat on articular cartilage had
not yet been fully investigated. Our results showed that expressions of
p53 were increased by heat stress, and apoptosis were mostly inhibited
when chondrocytes were pre-incubated with pifithirin-alfa, which
was isolated for its ability reduce p53-mediated apoptosis. These indicated
that most of apoptosis by heat stress in chondrocytes were dependent on
p53 pathway.

References
heat stress and NO-induced apoptosis with HSP70 expression.
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