Conclusions: The results show low levels of Sulf expression, restricted to the superficial zone in normal articular cartilage. Sulf mRNA and protein levels are increased in aging and OA cartilage. This increased Sulf expression may change the sulfation patterns of heparan sulfate proteoglycans and activities of several important growth factors and thus contribute to abnormal chondrocyte activation and cartilage degradation in OA.

148 CHONDROCYTE APOPTOSIS WITH HEAT STRESS IS INDUCED BY P53 PATHWAY

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Purpose: There are some reports that osteoarthritis (OA) cartilage has a higher number of apoptotic chondrocytes than does normal cartilage. In response to DNA damage and other cellular 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. In the present study, we investigated the responses of chondrocytes to heat stress, and how chondrocytes apoptosis were induced by heat stress.
Methods: To apply heat stress to NHAC-kn cells (human normal chondrocytes), they were cultured in 6well plates for 2 days at 37ºC in 5% CO_2/95% humidified air. And then, these plates were carefully sealed and placed in circulatory hot water bath set at 43ºC or at 37ºC as a control temperature for 30 min, 1 hour, and 2 hours. After the heat stress, the plates were removed from the water bath and immediately changed the new medium and incubated in 5% CO_2/95% humidified air for 0, 12, 24, or 48 hours. Chondrocytes apoptosis were detected by TUNEL staining and western blotting. To explore the function of p53, NHAC-kn cells were pre-treated with 50 μM of pifithrin-alfa (sigma), which is specific inhibitor of p53 mediated apoptosis, for 24 h before induction of heat stress.
Results: Apoptotic cells were significantly increased by heat stress in a time depend manner (Figure 1). The expression levels of p53 were increased gradually after induction of heat stress (Figure 2b). p53AIP1 was not detected in the chodrocytes without heat stress (Figure 2a), however p53AIP1 was expressed and increased by heat stress (Figure 2b). The expression levels of cleaved caspase-9 increased by heat stress (Figure 2b). Apoptotic cells were decreased when chondrocytes were incubated with pifithrin-alfa (Figure 3). The expression levels of p53, p53AIP1, and cleaved caspase-9 were decreased when chondrocytes were incubated with pifithrin-alfa (Figure 3c).
Conclusions: Our results showed that expressions of p53 and p53AIP1 were increased by heat stress, and apoptosis were mostly inhibited when chondrocytes were pre-incubated with pifithrin-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.

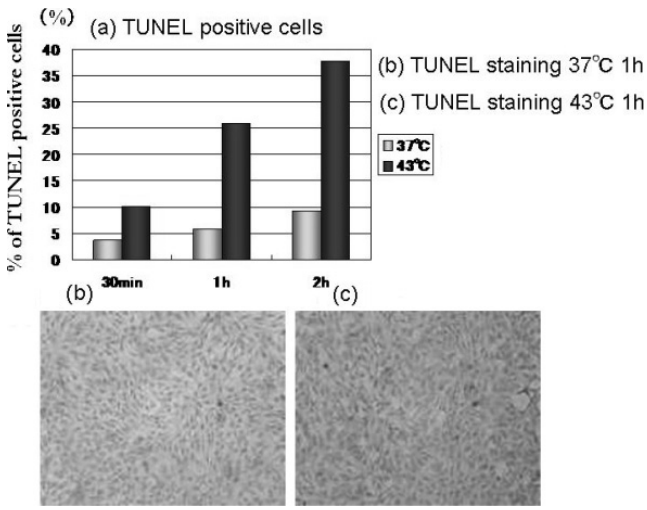
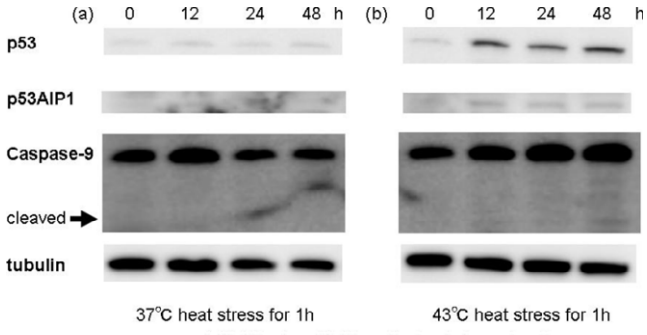
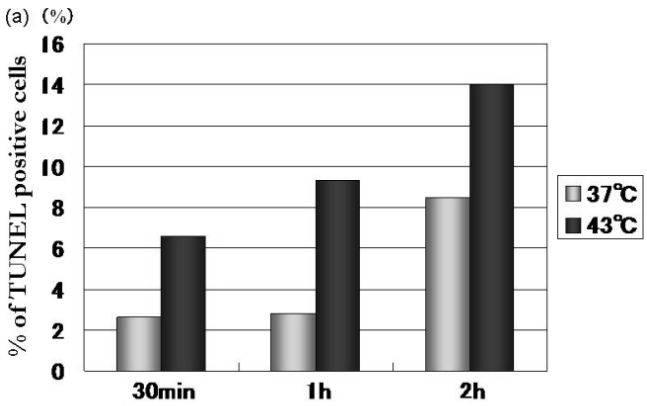


Figure 1.



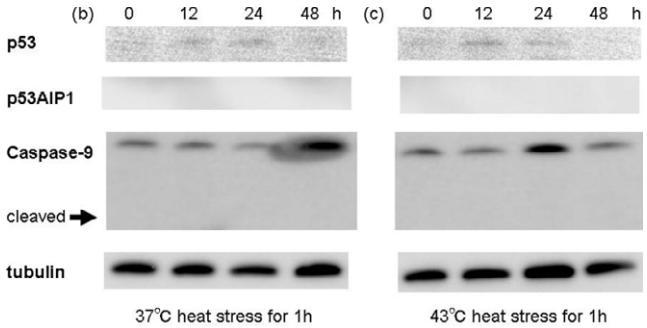
(a)(b)Western blotting after heat stress for 1h.

Figure 2.



(a)TUNEL positive cells with pre-incubated with pifithrin-alfa.

Figure 3a.



(b)(c) Western blotting after heat stress for 1h with pre-incubated with Pifithrin-alfa.

Figure 3b,c.

149 HISTOLOGIC CLASSIFICATION OF LOOSE BODIES IN OSTEOARTHROSIS

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Purpose: Histologically based analyses of the nature and origin of loose bodies occurring in osteoarthrosis have been few, and further study is warranted.
Methods: We histologically examined 84 loose bodies and 9 related lesions (synovial membrane nodules) surgically removed from 24 joints of 24 patients with osteoarthrosis.
Results: The 84 loose bodies included 48 chondral loose bodies (type I), 26 osteochondral loose bodies (type II), and 10 osseous loose bodies (type III). The 26 osteochondral loose bodies (type II) could be subdivided into 8 composed of cartilage with enchondral ossification (type IIa), 11 consisting of mature bone covered by cartilage without enchondral ossification (type IIb), and 7 made up of mature bone and partially articular cartilage or hyaline cartilage (type IIc). Synovial membrane nodules could be also divided into three types in the same manner as loose bodies. Many type IIa, type IIc and type III loose bodies and all