Abstract  Osteoarthritis (OA) is one of the most common age-related degenerative joint disorders. In addition to aging, various life-style-related factors, such as obesity and overuse of joints, have been recognized as major risk factors for OA. It has been revealed that mechanical force acting on articular cartilage induces chondrocytes to produce excess amounts of reactive oxygen species (ROS), which are known to be an OA-related catabolic factor. In addition, it has been reported that mechanical stress-induced ROS directly damage chondrocyte DNA, resulting in the downregulation of cellular activity and induction of apoptosis in osteoarthritic chondrocytes. Notably, molecular events associated with DNA oxidative damage influence the regulation of chondrocyte activity and cellular viability, supporting the notion that mechanical stress-induced oxidization of chondrocyte DNA participates in the pathogenesis of OA. Here, we review accumulating evidence supporting the involvement of mechanical and oxidative stresses in cartilage homeostasis and the pathogenesis of OA.

Keywords  : osteoarthritis, DNA repair enzyme, oxidative stress, chondrocytes, articular cartilage

Introduction

The ratio of elderly persons aged 75 or older will increase; it is expected to exceed 25% in 2055. Since Japan currently has an aging population, we need to adapt to cope with this changing demographic. To maintain physical condition, independent living and social participation, even in elderly people, it is necessary to develop preventive and therapeutic strategies for age-related musculoskeletal diseases.

In this review article, we aim to contribute to the extension of healthy life expectancy, by focusing on one of the most common age-related joint musculoskeletal diseases, osteoarthritis (OA), and discussing the pathogenesis of joint degeneration in OA.

In patients with OA, joint destruction and related symptoms become aggravated with disease progression as well as aging. Tissue damage increases with time after onset. Articular cartilage has no nervous or vascular structure and minimal capacity for self-repair, so that it is necessary to establish preventive measures as well as early treatment strategies against articular cartilage degeneration.

However, the search for effective therapy for cartilage degeneration is still ongoing. It is therefore a matter of urgency to find new therapeutic agents and strategies that are clinically effective in treating age-associated musculoskeletal diseases, especially OA, in order to preserve high levels of activity of daily living in elderly people.

During the development of OA, mechanical and chemical stresses exerted on articular cartilage downregulate the stable cellular activities of chondrocytes and induce the production of catabolic factors, such as proinflammatory cytokines and chemokines. In addition, in response to mechanical and chemical stresses, it is well known that articular chondrocytes produce excess amounts of reactive oxygen species (ROS) as well as proinflammatory cytokines and chemokines. These catabolic factors are thought to accelerate the progression of bone and joint degeneration with aging. However, the mechanism of cartilage degeneration in OA still remains unclear. The aim of this review is to discuss the pathogenesis of cartilage degeneration from the point of view of cartilage and chondrocyte biology in OA.

What happens in articular cartilage under mechanical stress?

Mechanical force applied to articular cartilage directly downregulates chondrocyte activity such as production of cartilage matrix components (proteoglycan and type II collagen) and induces chondrocyte apoptosis, in addition to its direct effects causing the degeneration/destruction of articular cartilage structure (surface fibrillation and development of cartilage clefts). These catabolic changes may affect the homeostasis/maintenance potential of...
Further, it has been reported that the effect of mechanical stress on articular cartilage increases production of excess amounts of ROS (superoxide, nitric oxide, hydrogen peroxide and peroxynitrite) as OA-related catabolic factors. Previous studies have provided ample confirmation of the generation of ROS in degenerated articular cartilage. These findings suggest that mechanical stress applied to articular cartilage may induce oxidative stress in OA cartilage tissue. Interestingly, previous reports demonstrated that treatment with C60 fullerene, a strong free radical scavenger, prevented such catabolic responses in osteoarthritic chondrocytes in vitro and the degeneration of articular cartilage in vivo in an OA rat model, suggesting the involvement of oxidative stress in the progression of cartilage degeneration in OA.

In the next section, we will discuss the implication of oxidative stress in the pathogenesis of OA.

Oxidative stress in degenerated articular cartilage: oxidative damage vs. cellular antioxidants

Previous studies have already demonstrated that degeneration of articular cartilage is partially mediated by oxygen free radicals. Kurz et al. showed that application of mechanical force to articular cartilage is sufficient to stimulate the production of ROS by chondrocytes, leading to depolymerisation of hyaluronic acid and chondrocyte death. Green et al. demonstrated that chondrocyte death is induced by adherence of inflammatory leukocytes to chondrocytes, and by induction of ROS production from chondrocytes in response to mechanical stress on cartilage. These findings clearly indicate that chronic excess ROS production by chondrocytes, which is induced by the effect of mechanical force on cartilage, plays an important role in the cartilage degeneration that occurs after mechanical injury to cartilage (Fig. 2).

Indeed, it has already been reported that the presence of oxidative damage was histologically confirmed in OA cartilage. Our previous studies also demonstrated the presence of DNA oxidative damage in osteoarthritic chondrocytes, with osteoarthritic chondrocytes, but not normal chondrocytes, expressing high levels of oxidized forms of tyrosine (nitrotyrosine).

The oxidized nucleotide 8-oxoguanine (8-oxo-7,8-dihydroxyguanine) is produced by ROS in large amounts in both DNA and nucleotides. As an oxidized form of guanine, 8-oxoguanine is a major causative factor involved in ROS-induced mutagenesis, since it can form a stable base pair with adenine (A) or cytosine (C) during DNA replication, resulting in A:T (thymine) to C:G (guanine) and G:C to T:A point mutations. These mutations are thought to be involved in the pathogenesis of a variety of malignant and degenerative diseases. Oxidation of guanine to 8-oxoguanine is repaired by 8-oxoguanine DNA glycosylase (Ogg1), an enzyme that recognizes and hydrolyses the aberrant base in the DNA backbone. Ogg1 is thought to protect against activation of the intrinsic apoptotic pathway in response to oxidative stress by augmenting DNA repair in a variety of cells. Kikuchi et al. demonstrated that 8-oxoguanine levels in large motor neurons of the spinal cord were increased in patients with amyotrophic lateral sclerosis (ALS) and subarachnoid haemorrhage (SAH), together with a lower mitochondrial expression of the DNA repair enzyme Ogg1. Their finding indicates that oxidative damage may accumulate in the mitochondria of motor neurons in ALS and SAH and that Ogg1 may not repair the damage efficiently, which may lead to a loss of motor neurons in these diseases. These findings further suggest that impairment of the ability of the DNA repair enzyme Ogg1 to protect against the accumulation of 8-oxoguanine is involved in the pathogenesis of oxidative stress-induced diseases including neural degenerative diseases and musculoskeletal diseases.

We also observed depletion of the cellular antioxidant, Ogg1, in degenerated articular cartilage participates, at least in part, in the development of cartilage degeneration.
in OA.

Recently, we observed increased levels of 8-oxoguanine and decreased levels of its repair enzyme Ogg1 in OA chondrocytes, but not in normal chondrocytes, in OA model rabbits and patients with OA (Fig. 3, unpublished data). Our data suggest that Ogg1 may play an important role in protecting against ROS-induced DNA damage (resulting in the downregulation of chondrocyte activity and augmentation of apoptosis) and cartilage degeneration in OA (Fig. 4).

These findings provide a novel pathogenic mechanism linking oxidant-mediated DNA damage and OA chondrocytes.

In OA, mechanical stress-induced generation of ROS may cause direct damage to chondrocytes and cartilage matrix components, resulting in the degeneration of ar-
particular cartilage. While previous studies have provided ample confirmation of ROS generation in OA cartilage, further studies are needed to clarify the exact mechanism of ROS-mediated degeneration of articular cartilage.

In the next section, we will review the protective mechanism against OA in articular cartilage.

DNA damage repair enzymes in articular cartilage

Although ROS mediate various cellular signalling pathways, higher levels of ROS can induce oxidative DNA damage and the resultant cellular apoptosis that contribute to aging, malignancy and degenerative diseases. Oxidative DNA lesions accumulate in nuclear and mitochondrial genomes during aging, and this can increase dramatically in age-related neurodegenerative diseases such as Parkinson’s disease. Regarding musculoskeletal diseases, we previously reported that oxidative DNA damage in chondrocytes accumulates in the degenerated articular cartilage in OA, reducing its maintenance potential.

Oxidative damage to nucleic acids is counteracted by the activity of several DNA repair enzymes such as apurinic/apyrimidinic (AP) endonuclease 2 (APEX2). AP sites occur in DNA following spontaneous hydrolysis, exposure to DNA damaging agents, or treatment with DNA glycosylases that remove specific abnormal bases. AP expression sites are pre-mutagenic lesions that can prevent normal DNA replication, so cellular mechanisms identify and repair such sites. It has been reported that APEX2 is involved in the critically important DNA repair pathway. This base excision repair pathway involving APEX2 is largely responsible for the repair of DNA oxidative damage by ROS. A recent report clearly indicated that APEX2 was required for the generation of replication protein and the recruitment of a checkpoint protein complex to DNA oxidative damage sites.

More recently, we provided evidence that the change in chondrocyte anti-oxidative activity that occurs through the APEX2-associated DNA repair pathway may be involved in the progression of articular cartilage degeneration in OA. We observed that APEX2 expression in chondrocytes was induced by OA-related catabolic factors in vitro, and was associated with the degeneration of articular cartilage in an in vivo OA mouse model (Fig. 5). Furthermore, downregulation of APEX2 using small interfering RNA decreased chondrocyte activity in vitro. These findings indicate that APEX2 may prevent the oxidative stress-induced downregulation of chondrocyte activity in OA.

Mitochondria are a major source of intracellular free radicals, which are closely involved in oxidative damage and may cause degeneration of various tissues including...
articular cartilage\textsuperscript{39}). In mitochondria, the base excision repair pathway is primarily responsible for removing oxidized nucleic acid from DNA. In humans, oxidized nucleic acids in DNA are repaired by DNA repair enzymes (such as Ogg1 and APEX2) that recognize and hydrolyze aberrant bases in the DNA backbone\textsuperscript{40}.

Such impaired DNA repair function in osteoarthritic chondrocytes may result in mitochondrial dysfunction and chronic oxidative damage to chondrocytes and cartilage tissue in OA. These observations suggest that downregulation of the oxidized DNA excision repair pathway is closely involved in the pathogenesis of articular cartilage degeneration. This may indicate a protective mechanism against mechanical stress-mediated DNA oxidative damage in OA chondrocytes.

**Conclusion**

In conclusion, previous reports clearly demonstrated that mechanical stress to articular cartilage induces generation of ROS from chondrocytes, causing oxidative stress, and that the related oxidative damage to chondrocyte DNA accumulates in the degenerated articular cartilage in OA. In addition, the DNA repair enzyme does not repair the damage efficiently, which may lead to downregulation of chondrocyte activity and reduced maintenance potential of articular cartilage in OA. Although further studies are needed to clarify the exact involvement of mechanical and oxidative stresses in the pathogenesis of OA, previous findings establish an innovative role for DNA repair enzymes in protecting articular cartilage and chondrocytes against mechanical stress-induced oxidative damage of chondrocytes and articular cartilage.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this article.

**Acknowledgments**

We would like to thank M. Tamaki, M. Tanaka and T. Sato for excellent technical assistance.

**References**


19) Afonso V, Champy R, Mitrovic D, Collin P and Lomri A.


