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Molecular mechanisms of cartilage destruction in osteoarthritis

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Brief overview of the biology of OA

Destruction and loss of the articular cartilage is a central feature of osteoarthritis (OA). But cartilage is clearly not the only joint tissue affected by this chronic disease of aging. The pain and disability that result from OA appear to be due to processes that affect multiple tissues including the subchondral bone, the synovium, ligaments and tendons, muscle and, in the knee, the menisci. Unfortunately, the most widely used treatments for OA are mainly directed at reducing pain (which they don't do very well). There is a complete lack of treatments for OA that might slow or stop the disease progression. The development of disease modifying therapy (structure modifying osteoarthritis drugs) requires a better understanding of the basic mechanisms responsible for joint destruction in OA.

The bony changes in OA have been known for many years but their role in the disease process is still not completely understood. Synovial inflammation in OA is not as prominent as in the classic forms of inflammatory arthritis (such as RA), but it does appear to be significant in about a third of patients with advanced disease¹. Recent MRI studies reveal a high frequency of meniscal lesions in OA joints and meniscal as well as ligament damage are clear risk factors for development of OA and for disease progression^{2,3}. However, right or wrong, the most studied tissue and the best characterized in regards to OA is still the articular cartilage, which will be the focus of this report.

The concept of OA as a "degenerative joint disease" is giv-

ing way to the concept of OA as an inflammatory form of arthritis where the inflammation is present at the molecular level within the articular cartilage. There is mounting evidence that the cartilage destruction in OA is the result of the activity of cytokines, chemokines, and other inflammatory mediators⁴. These inflammatory factors are commonly produced by immune cells and synovial cells in the classic forms of inflammatory arthritis but in OA, they are produced by the chondrocytes. The inflammatory mediators produced by chondrocytes include cytokines and chemokines such as IL-1, IL-6, IL-7, IL-8, IL-17, IL-18, MCP-1, LIF, GRO, and oncostatin M; reactive oxygen species such as nitric oxide, superoxide, hydrogen peroxide and peroxynitrite; and lipid-derived inflammatory mediators such as prostaglandins and leukotrienes. These mediators act in an autocrine and paracrine fashion to stimulate the chondrocyte to produce proteolytic enzymes, including aggrecanases and matrix metalloproteinases that contribute to destruction of the cartilage matrix.

In addition to stimulating production of catabolic factors, the inflammatory mediators produced by chondrocytes also contribute to cartilage loss through the inhibition of matrix synthesis. As OA develops, the chondrocytes respond to the damage and loss of matrix by proliferating (resulting in the formation of chondrocyte clusters or "clones") and by attempting to produce matrix including production of matrix proteins more commonly found during development (such as type IIA procollagen). But an imbalance between synthesis and degradation is present resulting in net loss of matrix. The changes observed in OA may be due at least in part to a phenotypic switch where chondrocytes in the articular cartilage assume some of the characteristics of cells in the hypertrophic zone of the growth plate including production of type X collagen and the collagenase MMP-13.

The initiators of the OA process are not entirely clear and most likely are multifactorial². Clearly, abnormal joint loading, due to altered biomechanics (from injury, congenital anatomic defects or obesity), is important, as are genetic factors and factors associated with aging. No matter the initia-

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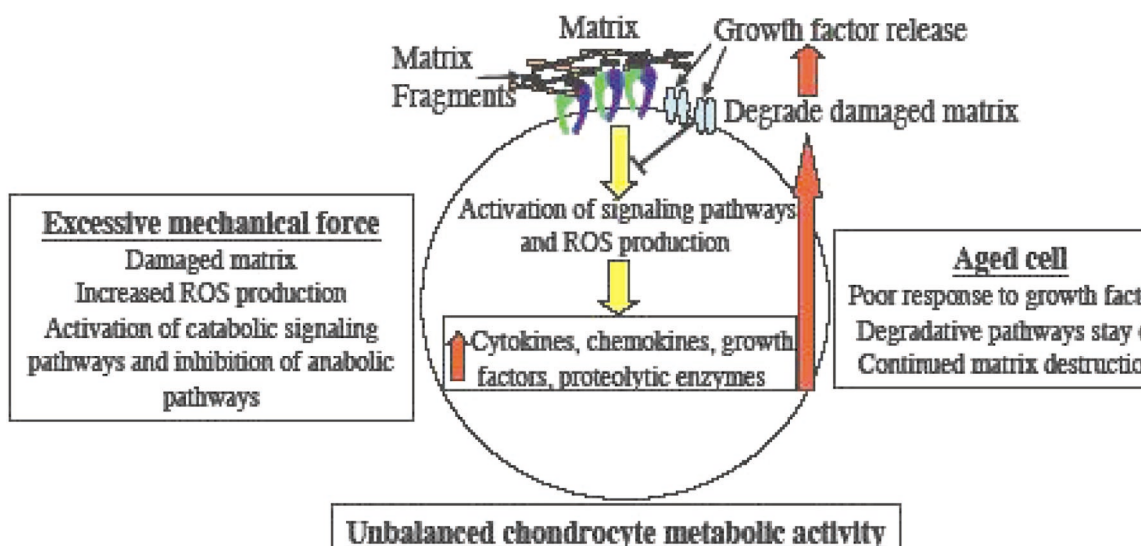


Figure 1. Theoretical model for pathways involved in cartilage destruction during the development of osteoarthritis. Published in *Arthritis Rheum* 2006;54(5):1357-60.

tor, matrix damage results in the production of matrix fragments, including fragments of fibronectin, collagen, hyaluronic acid and other cartilage matrix proteins, which may be important contributors to disease progression. These matrix fragments stimulate the chondrocyte to produce the same inflammatory mediators and proteolytic enzymes that have been found in OA cartilage and listed above⁵⁻⁸.

Normally, when a tissue is damaged the local cells sense a change in the matrix and react with a repair response. Part of the repair response is the removal of the damaged matrix proteins, which may account for the initial production of proteolytic enzymes by chondrocytes as OA develops. Once the damaged proteins are removed, the proteolytic process needs to be turned off so that the cells can synthesize new matrix. A host of growth factors, including BMP-2, BMP-7, CDMs, IGF-I, and TGF- β , are produced by chondrocytes and are stored in the cartilage bound to matrix proteins. These growth factors are released when the matrix is degraded and should serve to both shut down production of catabolic factors and stimulate synthesis of new matrix. But in the OA joint, this anabolic phase of matrix remodeling is insufficient or defective.

Aging may be an important factor contributing to an imbalance in cartilage remodeling in OA. Age-related changes in the chondrocyte result in a cell that is less responsive to growth factor stimulation⁹. If sufficient growth factor activity is not present in aged cartilage, then the tissue will lack generation of the signals necessary to turn-off production of catabolic factors and turn-on matrix synthesis (Figure 1). This could result in a continued cycle of unchecked matrix degradation in response to mechanical forces and continued damage to the matrix.

The potential role of reactive oxygen species in OA

One mechanism that could contribute to an imbalance in anabolic and catabolic activity in cartilage is an age-related increase in cellular levels of reactive oxygen species that result in oxidative stress. The production of ROS, as the result of normal cellular metabolism as well as from environmental insults such as ionizing radiation, has been hypothesized since the 1950s to contribute to cell and tissue aging¹⁰. Relevant to OA, excessive mechanical stimulation can increase chondrocyte production of ROS in sufficient quantities to depolymerize hyaluronic acid¹¹ or even kill chondrocytes¹². We have provided evidence for oxidative damage in aging and in OA cartilage using the oxidative marker nitrotyrosine¹³ and evidence for oxidative stress in chondrocytes by measuring the ratio of oxidized to reduced glutathione¹⁴. We have also shown that chondrocyte production of ROS is required for the production of MMPs in response to stimulation of the $\alpha 5 \beta 1$ integrin by fibronectin fragments¹⁵.

ROS have been known for years to mediate matrix damage in many tissues affected by chronic diseases of aging, including cartilage¹⁶. But a more recent concept in redox biology is the role that ROS play in regulating the activity of specific intracellular signaling pathways¹⁷. Signaling pathways that utilize ROS as "secondary messengers" include those generated by activation of numerous cytokine and growth factor receptors as well as by integrins. Intracellular signaling molecules shown to be regulated by ROS that are relevant to our studies include receptor tyrosine kinases, the MAP kinases (ERK1/2, JNK, p38), lipid pathways (phospholipases, PKC, and the PI3-kinase/Akt pathway), phosphatases, and transcription factors (NF- κ B, p53, and AP-1)^{17,18}. These signaling proteins and transcription fac-

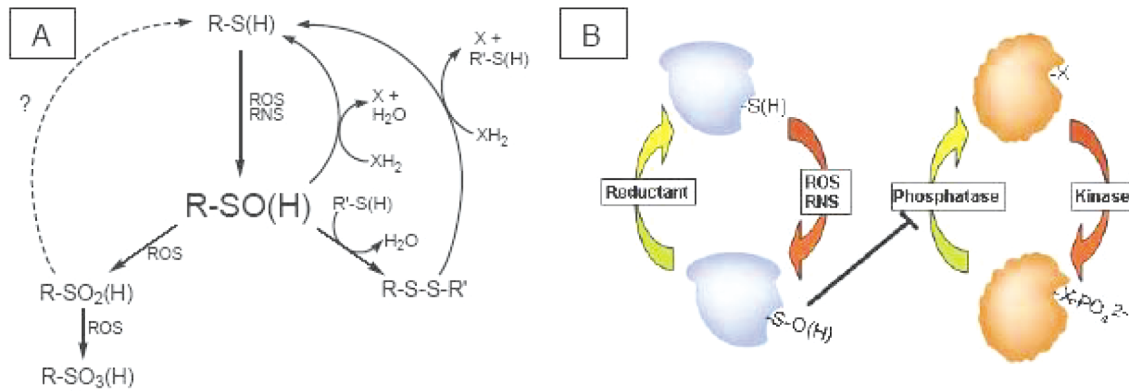


Figure 2. Biological fates of cysteine sulfenic acids. **A.** A susceptible protein thiol (R-SH) is oxidized to sulfenic acid (R-SOH) which can be reduced back to R-SH. XH₂ refers to chemical or biological reductants such as dithiothreitol, glutathione, or reduced, thiol-containing proteins such as thioredoxin and glutaredoxin. R-SOH can react with a nearby R'-SH to form a disulfide R-S-S-R'. In the presence of excessive ROS the R-SOH can be further oxidized to sulfinic (R-SO₂H) and sulfonic (R-SO₃H) acid forms which may or may not be reduced back to R-SH (Poole, 2004). **B.** The reversible oxidation of specific Cys residues to Cys sulfenic acid (S-OH) can alter protein function in a similar fashion as the reversible phosphorylation of specific Ser, Thr, or Tyr residues. Cross-talk between these signaling mechanisms can be mediated by phosphatase inactivation occurring through sulfenic acid formation. (Adapted from Finkel, 2003).

tors have all been shown to be involved in signaling networks that regulate cartilage matrix synthesis and degradation.

A major mechanism for redox signaling is the formation of cysteine sulfenic acid residues (Cys-SOH), which occurs when an ROS, typically hydrogen peroxide (H₂O₂), reacts with a protein thiol (Cys-SH)¹⁹. Because they can be readily reduced from Cys-SOH back to Cys-SH, the oxidation/reduction of protein thiols represents a reversible intermediate similar to the classic signaling intermediates created by phosphorylation/dephosphorylation of tyrosine, serine, or threonine residues (Figure 2). Not all Cys-SH groups are equally susceptible to oxidation. Specificity is generated by both the ionization state and protein microenvironment of a particular protein thiol, which affects how readily it will be oxidized, as well as by the proximity of the protein to where the particular ROS is generated. Susceptible Cys-SH groups have a pKa near or below physiological pH in order to be primarily in the deprotonated thiolate form and reactive with ROS such as H₂O₂.

The formation of sulfenic acid can directly regulate the activity of signaling molecules. For example, in certain isoforms of PKC, reduced disulfides (i.e., Cys-SH groups) in the regulatory domain hold the protein in an inactive state by blocking the catalytic domain. Thiol oxidation results in a conformational change, which relieves the inhibition and allows PKC to be active until reduced again²⁰. An indirect mechanism of signaling activation is the reversible inactivation of phosphatases by oxidation of cysteine residues present in the active site^{17,18,21}. Since phosphatases are necessary to inactivate signaling proteins that are active when in the phosphorylated state, ROS can promote the extended activity of certain signaling through reversible inactivation of specific phosphatases²².

Both redox signaling mechanisms, kinase activation and

phosphatase inactivation, likely participate in growth factor, cytokine, and integrin signaling in chondrocytes. We have recently discovered that redox signaling mediated by sulfenic acid formation is required for MMP-13 production mediated by fibronectin fragment stimulation of $\alpha 5 \beta 1$ signaling in chondrocytes (unpublished results). Using an activity based proteomics approach, we have identified the MAP kinase JNK-2 as one of several chondrocyte proteins that contain sulfenic acid after fibronectin fragment stimulation. We provide evidence for regulation of JNK-2 activity through oxidation of a specific cysteine (Cys222), which results in increased JNK-2 activity.

We have also found that ROS can inhibit IGF-1 signaling in chondrocytes. IGF-1 stimulation of the PI-3kinase-Akt pathway but not the MEK-ERK pathway is necessary for proteoglycan synthesis²³. Treatment of chondrocytes with H₂O₂ activates the ERK MAP kinase while inhibiting the IRS-1-PI-3kinase-Akt pathway. Similarly, when comparing chondrocytes isolated from OA cartilage to those from normal cartilage, we have noted that OA cells have an increase in basal ERK phosphorylation but a lack of IRS-1 and Akt phosphorylation in response to IGF-1. The addition of the anti-oxidant MnTBAP is able to improve the IGF-1 response in explant cultures of OA cartilage and in IGF-1-resistant cartilage from older donors.

These studies provide evidence linking levels of ROS in chondrocytes to activity of both a catabolic pathway (integrin stimulation by fibronectin fragments) and an anabolic pathway (IGF-1 stimulation) where increased ROS activate catabolic signaling and inhibit anabolic signaling. Because levels of ROS appear to increase with aging, these findings suggest a mechanism whereby aging can contribute to the imbalance in anabolic and catabolic activity that contributes to the progression of OA.

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