# Intervertebral Disc Aging, Degeneration, and Associated Potential Molecular Mechanisms

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## Abstract

Intervertebral disc degeneration is a major cause of neck and back pain, a very common clinical problem. However, no effective treatment is available, which is largely due to the lack of understanding of molecular mechanisms underlying disc degeneration. Here, we briefly described the process of intervertebral disc aging and degeneration and summarized major findings in molecular signaling pathways implicated in disc aging and degeneration.

## Keywords

Intervertebral disc degeneration; aging; senescence; neck and back pain

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An intervertebral disc consists of an annulus fibrosus ring, a nucleus pulposus core, and two cartilaginous superior and inferior endplates. The outer annulus is made up of highly ordered collagen lamellae in which type I collagen fibers are aligned with elongated fibroblasts (1,2). Relative to the outer annulus, the inner annulus is more like cartilage, containing spherical chondrocyte-like cells, and greater amount of type II collagen and proteoglycans (3). The central nucleus, a highly hydrated gelatinous tissue, is predominantly composed of proteoglycans produced by large notochordal cells (4). The annulus, the nucleus, and the endplates are interconnected to form the most important part of the motion segment of the spine, allowing the intervertebral disc to function as a shock absorber and to resist tensile and torsional forces. Human disc degeneration starts during childhood. As notochordal cells diminish rapidly after birth and are gradually replaced by much smaller chondrocytes, the nucleus becomes dehydrated and cartilage-like by adulthood (5). In the early stage of disc degeneration, clefts and tears occur in the nucleus and the inner annulus, and chondrocyte-like cells in the inner annulus proliferate (cloning) and produce matrix in the vicinity of the structural defects (6). However, the regenerated tissue cannot withstand the daily loading of the spine, leading to structural defect progression. As disc degeneration advances, clefts/tears extend into the outer annulus, and are filled with granular material; fibroblasts in the outer annulus differentiate into chondrocyte-like cells, and deposit matrix; chondrocyte-like cells in the inner annulus and endplates form large clones and migrate into the nucleus (6,7). In the late stage of disc degeneration, collagen content and cross linking increase throughout

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the disc; the distinction between the anatomic regions is no longer possible; and the entire disc becomes fibrotic and scar-like (6,8).

As described above, disc degeneration is an age-related process. Thus, it is difficult to distinguish the physiologic process of disc aging from that of disc degeneration. In general, when a disc with structural failure is combined with accelerated or advanced signs of aging, it is considered to be a degenerate disc (8,9). Given that the process of disc aging is affected by many risk factors such as genetic inheritance, excessive mechanical loading, obesity, trauma, nutrition, smoking, and inflammation, as well as catabolic cytokines and proteases, disc degeneration occurs in every population worldwide (7). It affects almost all individuals by sixth and seventh decade of life. As disc degeneration is a major cause of neck and back pain, a leading cause of disability in people aged less than 45 years, an effective treatment is required (10,11). Currently, this disease is firstly treated with conservative measures for pain relief. If pain persists, surgical therapies include decompression, spinal fusion and disc replacement will be performed. However, all these treatment methods are not curative because none of them can prevent, reverse or slow down the process of disc generation. The lack of drugs that can effectively treat the neck and back pain patients beyond pain relief is largely due to the lack of understanding of the molecular mechanisms underlying disc degeneration.

Senescent cell accumulation in discs plays a central role in disc aging and degeneration, because most risk factors are senescence-inducing stresses and some are consequences of senescent cells (12,13). Senescent cells cease proliferation, but remain metabolically active and exhibit altered gene expression (14). Since in human adult discs, blood vessels are normally restricted to the outmost layers of the annulus, and the inner annulus and entire nucleus are avascular tissue, disc cells resident in these regions experience a limited nutrition supply, hypoxia, anaerobic metabolism, and associated increase in acidity. Accumulating evidence supports the view that disc cells can tolerate this condition, otherwise the cells die or become senescent. For example, when rat or bovine disc cells were cultured at low oxygen (0-5% $O_2$) levels, the cells were viable, underwent proliferation and produced significant amount of proteoglycans, whereas the normoxia (20-21% $O_2$) level caused decreased cell survival rate, reduced proteoglycan synthesis, and enhanced expression of matrix metalloproteinases (MMPs) (15,16). Disc cells are more sensitive to the concentrations of nutrients than $O_2$. Bovine disc cells would die or underwent senescence without glucose, but enhanced proliferation and matrix synthesis in low glucose cultures (15,17). However, if the cells were cultured under high glucose, a glucose-mediated oxidative stress was generated and induced senescence (18). Although permeability and metabolite transport decrease in an aging disc due to low water content in the nucleus and fibrotic feature of entire disc, they increase again when the aging disc is herniated or injured due to trauma or repetitive over-loading (19), which presumably leads to an aberrant increase in concentrations of nutrients in the microenvironment adjacent to the structural defects, because cell cloning, senescent cells, and structural defect extension are frequently detected in the areas adjacent to structural defects (20-22; 9). These phenotypic changes imply a correlation between cell proliferation, cell senescence, and matrix breakdown during disc degeneration progression. Consistently, senescent cell number in human degenerative

discs increases with advancing disc degenerative grade and positively correlates with the expression levels of matrix-degrading enzyme MMP-13 and aggrecanase ADAMTS-5 (23).

Cell senescence transition in human discs is most likely induced via p53-p21-Rb pathway. Several lines of evidence suggest that with advancing disc degenerative grade, senescent cell number is increased, telomere lengthen is shortened, and p53-p21-Rb pathway is actively maintained (24,21,23). When disc cells were cultured *in vitro*, p16-Rb pathway was activated once the cells entered senescence program (25). Although the risk factors for disc degeneration such as excessive loading, trauma, nutrition, and smoking, etc. often induce acute senescence transition in *in vitro* an *in vivo* models via p16-Rb pathway (26,18,15,16), they may exert an effect individually or cumulatively on disc cells in human beings via affecting the telomere-shortening-rate.

Smad ubiquitin regulatory factor (Smurf) 2, an E3 ubiquitin ligase, was highly detected in human degenerated articular cartilage, and overexpression of Smurf2 under the control of type II collagen alpha 1 promoter (*Col2a1*) induces osteoarthritis in *Col2a1-Smurf2* transgenic mice (27). We have recently shown that *Col2a1-Smurf2* transgenic mice also exhibit accelerated age-related intervertebral disc degeneration (9). During development of the disc degeneration in these transgenic mice, many phenotypic changes such as fibroblast-to-chondrocyte differentiation, chondrocyte-like cell cloning, migration, and fibrosis, were similar to those occurring in humans and reflected connective tissue growth factor (CTGF) function during wound healing and scleroderma (28). Indeed, CTGF expression and secretion is increased in the chondrocyte-like cells that are prone to degenerate in *Col2a1-Smurf2* transgenic mouse discs, indicating that Smurf2-mediated disc degeneration is via upregulation of CTGF (9). Because discs possess a limited ability to repair when they are disrupted, tears/clefts in discs are never healed and could cause a persistence of CTGF expression by the cells adjacent to the structural defects due to continuous production and release of TGF-β, an inducer of CTGF expression, by these cells as a cellular response to repetitive excessive deformation of disrupted matrix (29,8,30) (Wu et al., unpublished data). Notably, TGF-β induces Smurf2 expression in chondrocytes *in vitro* (31). Thus, it is possible that in an aging disc, TGF-β activity is increased in the microenvironment adjacent to structural defects, activates Smurf2 gene expression by the local cells. Smurf2, in turn, induces disc generation via upregulation of CTGF.

While Smurf2 was originally found to be an E3 ubiquitin ligase, which targets the TGF-β receptor and receptor-regulated Smads for ubiquitination and proteasomal degradation (32,33), it was reported to induce cell senescence in cultured proliferating fibroblasts via activation of p53 pathway (34). As the senescence associated secretory phenotype accompanies disc aging and degeneration, we are testing a hypothesis that in *Col2a1-Smurf2* transgenic mice, the disc chondrocyte-like cells that overexpress Smurf2 could become senescent, and secrete CTGF, leading to disc degeneration and progression.

## Acknowledgments

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