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Increased cell senescence is associated with decreased cell proliferation in vivo in the degenerating human annulus

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Abstract

BACKGROUND CONTEXT: During disc degeneration, there is a well-recognized loss of cells. This puts the remaining cell population at high risk for any further decrease in cell function or cell numbers. Cell senescence has recently been shown to be present in the aging/degenerating human disc. Senescent cell are viable, metabolically active, persist, and accumulate over time, but cannot divide. Little is known about the relationship between renewal of the disc cell population via cell proliferation and disc cell senescence.

PURPOSE: To determine the percentage of senescent cells and proliferating cells in the human annulus in vivo.

STUDY DESIGN/SETTING: Human annulus specimens were obtained from surgical subjects and control donors in a study approved by the authors' Human Subjects Institutional Review Board.

PATIENT SAMPLE: One Thompson Grade I disc, 4 Grade II discs, 9 Grade III discs, and 12 Grade IV discs were studied.

OUTCOME MEASURES: The percentages of senescent cells and the percentage of proliferating cells.

METHODS: Immunohistochemistry was used to detect senescent cells using an antisenescence-associated beta-galactosidase antibody, and an antiproliferation antibody (Ki67). An average of 410 cells/specimens was counted to determine the percent senescence, and an average of 229 cells was counted to determine the percent proliferation.

RESULTS: Cell proliferation was low in both surgical and control normal donor annulus tissue (4.09%+1.77 (26), mean+SD (n)). There was no significant difference in the percentage of proliferating cells for more degenerate discs versus healthier discs (4.7%+1.6 (21) for Grades III and IV vs. 5.3%+1.9 (5) for Grades I and II). More degenerated Grades III and IV discs contained significantly greater percentages of senescent annulus cells than did the healthier Grades I and II discs (44.4%+20.0 (21) vs. 18.8%+11.0 (5), respectively; $p=.011$). A significant negative correlation was present between the percentage of senescent cells versus the percentage of proliferating cells, $r=-0.013$, $p=.013$. No correlation was present between age and the percentage of senescent cells or age and the percentage of proliferating cells.

CONCLUSIONS: Because senescent cells cannot divide, senescence may reduce the disc's ability to generate new cells to replace cells lost to necrosis or apoptosis. Senescent cells also accumulate in the disc over time, such that their metabolic patterns may contribute to the pathologic changes seen in degenerating discs. Novel data presented here show a significant negative correlation between the percentage of senescent cells and the percentage of proliferating cells during disc degeneration. Molecular work is underway in our lab to help us determine whether senescent cells in the disc secrete factors that can result in decreased proliferation in neighboring cells.

Keywords: Disc degeneration; Cell proliferation; Cell senescence; Immunohistochemistry

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Introduction

Cell senescence (also termed replicative senescence) occurs when normal cells stop dividing. This phenomenon was initially described more than 40 years ago during studies of cultured human fibroblasts [1]. Senescent cells are viable, but exhibit alterations in phenotype and altered gene expression patterns [2–5]. Senescent cells may have altered responsiveness to external stimuli and may secrete factors, which can influence neighboring cells or their nearby extracellular matrix. There is currently a great deal of interest in the manner in which cell senescence may contribute to age-associated loss of function or age-related pathology in vivo, and molecular studies are directed toward elucidating mechanisms and pathways which activate the senescence program in cells [6].

The current views of cell senescence not only recognize that it is a condition in which cells can no longer respond to mitogenic signals and thus cannot proliferate, but also point out that senescence is associated with alterations in nuclear structure, protein processing, gene expression, and cell metabolism. The senescent state is a response to specific trigger(s) or multiple signaling pathways, including telomere uncapping, oxidative stress, DNA damage, and oncogene activation [3,7,8]. Senescence represents a general cellular response mechanism which, when activated, results in numerous morphologic and functional changes [2].

Our laboratory and two other groups have recently published papers pointing to the importance of senescence in the disc. Roberts et al. have provided evidence that there was a greater proportion of senescent cells in herniated than non-herniated discs, and more senescent cells in the nucleus pulposus compared with the annulus [9]. Our laboratory showed that that the proportion of senescent cells increased significantly with increasing stages of disc degeneration ($p<.0001$) [10]. The third paper on disc cell senescence came from Le Maitre et al. and determined that the senescent disc cell phenotype is associated with increased catabolism involving metalloproteinase 13 and aggrecanase [11]. This finding is important because it links senescence with matrix degradation, one of the major problems in disc degeneration. This study also showed that disc cells exhibit accelerated senescence with decreased telomere length. (Shortening of telomeres [the nucleoprotein complexes at the ends of chromosomes] has been suggested as one of the hypotheses to explain senescence.) An additional publication from our group provided information on senescence using laser capture microdissection and microarray analysis [12]. We identified two senescence-related genes, which were significantly up regulated in more degenerated discs compared with healthier discs: growth arrest-specific 1 gene (GAS) (which inhibits DNA synthesis, inhibits cell cycle progression in vitro, and is expressed in senescent fibroblasts [13,14]), and lysyl oxidase-like 2, which is expressed in senescent human fibroblasts [15].

EVIDENCE & METHODS

Context
Because of the significance of disc degeneration and the loss of disc cells as the degenerative process progresses, it is important to understand the mechanism by which the cells are lost. This is particularly important in the biological treatments and strategies toward disc regeneration or prevention of degeneration in which growth factor or gene transfer strategies are used to target disc cells, or if strategies need to introduce new cells into the disc.

Contribution
The results of this study show that overall there is low cell proliferation in all annulus tissues in control and degenerative discs, showing that this is a very difficult tissue to proliferate and renew itself. There was no significant difference for the percent proliferating cells for the more degenerative discs versus the healthier discs, however, the greater grades of degeneration contain greater percentages of the senescent annulus cells than the healthier grades, respectively. It also appears that as the percent senescent cells goes up, the percent proliferating cells goes down.

Implications
Cell senescence and the factors controlling these metabolic events may be primary factors in disc degeneration. The disc environment is austere and this article allows insight into potential mechanisms where the disc cells are lost and no longer renewing themselves.

– The Editors

In the present study, we examine the percentage of cells in human annulus specimens and investigate the relationship of proliferation to cell senescence.

Methods

Source of disc tissue

Experimental study of disc specimens was approved prospectively by the authors' Human Subjects Institutional Review Board. Patient specimens were derived from surgical discectomy procedures. Surgical specimens were transported to the laboratory (less than 30 minutes after surgical removal) in sterile tissue culture medium and placed in