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DEL1 protects against chondrocyte apoptosis through integrin binding

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ABSTRACT

Background: Osteoarthritis (OA) is a debilitating disease process, affecting mobility and overall health of millions. Current treatment is for symptomatic relief and discovery of approaches to halt or reverse damage is imperative. Deletion of *developmental endothelial locus-1* (*Del1*) has been shown to increase severity of OA in knockout mice. We examined the intracellular pathways involved in the ability of DEL1 to protect chondrocytes from apoptosis and anoikis and hypothesized that it functioned via integrin signaling.

Materials and methods: Primary human chondrocytes were treated with various inducers of apoptosis, including anoikis, in the presence of added DEL1 or bovine serum albumin as control. Various inhibitors of integrin binding were examined for their effect on DEL1 activity. Downstream signaling pathway components were detected by immunoblotting.

Results: The addition of DEL1 protected chondrocytes from multiple inducers of apoptosis as measured by cell survival, terminal deoxynucleotidyl transferase dUTP nick end labeling and caspase 3/7 assays ($P < 0.05$). The effect of DEL1 was blocked by RGD peptides and by antibodies directed to integrin $\alpha_V\beta_3$, but not by controls or antibody to integrin $\alpha 1$ ($P < 0.05$). Treatment with DEL1 promoted ERK and AKT activation when cells were attached, but only AKT activation under conditions of anoikis.

Conclusions: DEL1 protected chondrocytes from apoptosis in response to activators of either the intrinsic or extrinsic pathways, and to anoikis. This effect was mediated primarily through integrin $\alpha_V\beta_3$. This represents a therapeutic target for therapies to prevent cartilage degeneration in OA.

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Introduction

Osteoarthritis (OA) is the most common joint disorder in the United States. About 85% of people over age 75 y have some evidence of OA, and 10% of men and 13% of women have symptomatic OA of the knees.[1,2] The clinical manifestations of OA begin with pain in the joints that can progress to the point where mobility is significantly impaired. The disability resulting from OA can lead to cardiovascular and overall health decline due to diminished mobility and ability to exercise.

The most common forms of degenerative OA seen in the population is thought to occur secondary to trauma to the articular surface due to mechanical injury; however, genetic risk factors have also been identified.[3] OA starts insidiously through repeated trauma to the joint surfaces from athletic or work injury, or simply from obesity. Trauma stimulates the release of a number of factors, including metalloproteinases and TNF-α, contributing to degradation of the highly specialized cartilage extracellular matrix (ECM) and apoptosis of articular chondrocytes.[4] Chondrocytes have very limited ability to regenerate, and their loss leads to erosions in the articular joint surface, eventually exposing bone. The articular surface serves the important function of providing a smooth, frictionless surface to enable motion through the joint. Once the protective articular cartilage is gone, bones grind against each other, and this manifests as pain and decreased range of motion over time.

Treatment for OA involves, paradoxically, exercise to maintain strength and range of motion in the joint.[5] Symptomatic relief can be obtained through the use of nonsteroidal anti-inflammatory drugs. Nutriceuticals in the form of chondroitin and glucosamine have been touted to relieve symptoms, but larger controlled studies show minimal impact.[6,7] Neither the nonsteroidal anti-inflammatory drugs nor the nutriceuticals have been shown to prevent or slow progression of the disease.[8,9]

Microfracture surgery has gained popularity as an attempt to repair defects in the articular cartilage. Small holes are drilled into the affected articular cartilage, and new cartilage formation is seen. However, it is now believed that the cartilage formed in response to microfracture is fibrous cartilage rather than true hyaline cartilage.[10,11] Fibrous cartilage does not have the same durability and mechanical properties as hyaline cartilage, and microfracture surgery is therefore seen as a temporizing measure.[12] When the joint surface is completely degraded and the pain becomes severe enough, joint replacement becomes the only option available to restore mobility and limit pain.[13,14] Despite the large number of people impacted by OA, there are no other options for treatment, and a tremendous need exists for identifying novel treatments.

We have recently demonstrated that mice with a deletion of the *developmental endothelial locus-1* (*Del1*) gene, encoding a secreted matricellular protein, develop more severe OA in a model of traumatic OA.[15] Examination of the joints revealed increased apoptosis in the articular chondrocytes. *In vitro*, DEL1 protected chondrocytes from apoptosis, and chondrocytes from knockout (KO) mice were more susceptible to apoptosis.[15]

Del1 was originally identified using an enhancer trap technique looking for genes that might be important in vasculogenesis.[16] The DEL1 protein has a modular structure typical of matricellular proteins characterized by three epidermal growth factor-like repeats and two discoidin-like domains. An RGD motif is present in the second epidermal growth factor-like repeat. This RGD motif has been shown to be important in stimulating angiogenesis *in vitro* through binding of integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$.[17] However, it is not clear that DEL1 has a significant role in either vasculogenesis or angiogenesis *in vivo*. *Del1* KO mice show no evidence of defects in vasculogenesis or angiogenesis.

DEL1 has also been shown to have a role in the inflammatory response. DEL1 aided macrophage clearance of apoptotic cells. The RGD motif in DEL1 bound integrin on the macrophage while the discoidin-like domain bound phosphatidylserine on the cell membranes of apoptotic cells bring them in proximity to allow engulfment.[18,19] More recently, DEL1 was shown to inhibit leukocyte recruitment by competing with endothelial intercellular adhesion molecule-1 for binding to integrin LFA-1 ($\alpha_L\beta_2$).[20]

We hypothesized that DEL1 functions in chondrocytes to prevent apoptosis through its ability to bind integrins. Because integrin signaling can vary based on the cell type and the integrin involved, we also looked at potential-specific integrins that might be serving as the receptor. We show here that DEL1 prevented apoptosis in chondrocytes from both intrinsic and extrinsic triggers of apoptosis, and in anoikis. DEL1 was similar in its effect to fibronectin (FN) and vitronectin (VN), and the effect was mediated through integrin binding. We propose that integrin-binding proteins may have a role in treatment of OA.

Materials and methods

Cell culture

Normal human articular chondrocytes (NHACs) (Lonza, Walkersville, MD) were cultured in standard conditions with chondrocyte growth medium (CGM) supplemented with 5% fetal bovine serum (FBS), human epidermal growth factor, hFGF-2, transferrin, R3-IGF-I, and ascorbic acid (Lonza). Low passage NHACs (passages 3 to 4) were used for this experiment. Before treatment, all NHACs were exposed to low serum (1% FBS) CGM with no other supplements for 24 h to induce quiescence.

In vitro *apoptosis assays*

NHACs were seeded at a density of 5×10^3 cells/100 μL in DEL1-coated or bovine serum albumin (BSA)–coated 96-well plates. Apoptosis of attached NHACs was induced with 200 μM etoposide (Calbiochem, San Diego, CA), or TNFα/actinomycin D (Sigma–Aldrich, St Louis, MO) 10 ng/mL each. For anoikis, 1×10^4 cells/100 μL NHACs were placed on poly-HEMA–coated plates prepared by coating with 1 μL/mm^2 of 12 mg/mL poly-HEMA (Sigma–Aldrich) in 95% ethanol and then allowed to dry. NHACs were cultured in CGM with 1% FBS in suspension with the addition of 250 ng DEL1 or BSA, and 0.5% methyl