The Growth Factor Nell-1 Promotes Cartilage Regeneration in a Rabbit In Vivo Model

Siu, R K; Zara, J N; Hou, Y; Kwak, J; Zhang, X; James, A W; Nguyen, A; Wu, B M; Ting, K; Soo, C; Lee, M

+1University of California, Los Angeles, CA

leemin@ucla.edu

SIGNIFICANCE:
Osteoarthritis, a degenerative disease of cartilage and a leading cause of pain and debility, affects about 43 million American adults, and is the most prevalent chronic condition among Americans 15 years and older. Development of improved tissue engineering-based methods of cartilage repair and regeneration will advance the progress of new clinical products that will significantly improve quality of life for patients suffering from osteoarthritis and other cartilage disorders.

INTRODUCTION:
Repair of cartilage due to joint trauma remains challenging due to the poor healing capacity of cartilage, adverse effects related to current growth factor-based strategies, and the propensity of chondrocytes to dedifferentiate in vitro, which limits autologous chondrocyte transplantation approaches. Nell-1 (Nel-like molecule-1), a growth factor we first characterized in the context of premature cranial suture fusion, plays important roles in normal chondrogenesis and cartilage organization during development. Translational studies with purified Nell-1 protein support the hypothesis that Nell-1 accelerates differentiation along the osteochondral lineage. Importantly, Nell-1 is a direct transcriptional target of Runx2, and moreover, Nell-1 downregulates Osterix, a regulator specifically required for osteoblastic differentiation, suggesting that Nell-1 may preferentially direct chondrogenic differentiation of bipotential osteochondral progenitors. We previously demonstrated the ability of Nell-1 protein to maintain the cartilaginous phenotype of explanted rabbit chondrocytes in vitro, as assessed by proteoglycan deposition and type II collagen expression. Our objective in the present study is to determine whether Nell-1 can affect endogenous chondrocytes in an in vivo cartilage defect model.

METHODS:
To achieve controlled Nell-1 release and provide a biocompatible, biodegradable scaffold for tissue repair, Nell-1 was incorporated into chitosan nanoparticles and embedded into alginate hydrogels. These implants were press-fit into critical-sized, 3-mm circular osteochondral defects created in the femoral trochlear cartilage of 3-month-old New Zealand White rabbits (n=10). Controls included unfilled defects (n=8) and defects filled with alginate containing PBS-loaded nanoparticles (n=8). Rabbits were sacrificed 3 months post implantation and the trochlear cartilage tissue harvested and processed for histological analysis. Hematoxylin/eosin staining was performed to assess general tissue organization, and Alcian Blue and Safranin-O staining was performed to assess glycosaminoglycan synthesis and proteoglycan deposition respectively. Additionally, immunohistochemistry for type II collagen, PCNA, and type X collagen were used to visualize mature, proliferating, and hypertrophic cartilage tissue respectively, and immunohistochemistry for type I collagen and Runx2 were used to identify bone tissue.

RESULTS:
Defects filled with alginate containing Nell-1 loaded nanoparticles demonstrated significantly improved cartilage regeneration. Remarkably, histology of Nell-1 treated defects closely resembled that of native cartilage, including stronger Alcian blue and Safranin-O staining (Figure 1). Immunohistochemistry of Nell-1 treated defects revealed increased deposition of type II collagen, with negative staining for PCNA and type X collagen, consistent with a mature, stable hyaline cartilage phenotype. Importantly, type I collagen and Runx2 expression was absent in Nell-1 treated defects, suggesting that Nell-1 treatment did not induce formation of osteophytes or other ectopic bone tissue (Figure 2).

DISCUSSION:
Our results suggest that Nell-1 may produce functional cartilage with properties similar to native cartilage and appropriate distributions of extracellular matrix molecules, and is an exciting candidate for novel tissue engineering-based approaches for treating osteoarthritis and other diverse pathologies of cartilage defects and degeneration. Since osteoarthritis frequently involves large, chronic lesions that may exceed the reparative capacity of local cells, future studies will incorporate exogenous cells, including autologous and allogeneic chondrocytes, and perivascular, mesenchymal, and embryonic stem cells into our delivery system to augment repair of more extensive cartilage damage. Finally, Nell-1 and stem cells may be incorporated into next-generation scaffold biomaterials which can conform to the surfaces of the large, irregular cartilage defects characteristic of osteoarthritis.

Figure 1. Basic histology of cartilage defects. Defects were treated with no implant, alginate gels containing PBS-loaded chitosan (Ch/PBS), or alginate gels containing Nell-1 loaded chitosan (Ch/Nell-1). From top to bottom: gross histology; hematoxylin/eosin staining; Alcian blue staining; Safranin-O staining. Dashed circle indicates approximate boundary of defect. Scale bar: 125 µm.

Figure 2. Immunohistochemistry for cartilage and bone markers. Dashed lines indicate the approximate tidemarks separating the upper cartilaginous layer and the underlying subchondral bone. Original magnification: 200x. Scale bar: 125 µm.