Local Activation of Notch Signaling Enhances Geriatric Bone Regeneration

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Introduction

The elderly have an increased incidence of fragility fractures, which are prone to heal more slowly and develop into mal-unions at a higher rate. In turn, this results in high rates of morbidity. It is therefore important to develop new therapeutics to increase the robustness and speed of geriatric fracture healing. We have recently developed a model of geriatric fracture healing in very old mice. Geriatric mice with closed tibial fractures show reduced callus size and therefore a reduction in callus bone formation, compared to their younger counterparts and provide a suitable model to study mechanisms that influence geriatric fracture healing.[1] Notch signaling has been shown to enhance healing of injured skeletal muscle.[2] Our laboratory has previously shown that Notch pathway components are upregulated during fracture healing and that inhibition of canonical Notch signaling during fracture healing alters bone healing.[3] The objective of this study was to determine whether activation of Notch signaling could increase fracture healing in geriatric mice. The Notch-pathway consists of a number of cell bound ligands and receptors, such that when a ligand binds to a receptor, it triggers proteolysis of the extracellular and transmembrane part of the receptor and 'frees' the Notch Intracellular Domain (NICD), which then translocates into the cell nucleus where it triggers effector genes. We hypothesize that upregulating NICD using localized adenoviral delivery will enhance bone regeneration.

Methods

We used 21 young adult (5-month-old) and 21 very old (25-month-old) mice. We surgically inserted an intramedullary pin in both tibiae, after which we the bones were fractured using the three-point bending apparatus described in previous studies.3 Five days after surgery, mice received a 30 µl injection with an NICDeGFP adenovirus (Notch Intracellular Domain, enhanced Green Fluorescent Protein) in one leg, and a GFP adenovirus (Green Fluorescent Protein) in the other. After 10, 20 and 40 days post-fracture (DPF) we euthanized the mice and harvested the tibiae. All tibiae were scanned using a Scanco 35 MicroCT and were analyzed for bone in the callus by outlining the callus and semi-manually excluding the bone. RNA was harvested for expression analysis and microCT'ed bone was processed for histology. For statistical analysis we used an unpaired, one-sided student's T-test using Excel© and a two-way ANOVA using SPSS14©.

Results

NICD treatment significantly increases callus volume (20% increase) in both young and old 20 DPF mice relative to GFP treatment (young: $p=0.0497$, old: $p=0.028$, see Figure 1A). Furthermore, NICD results in increased bone volume fraction (BVF) (~50% increase) and trabecular thickness in old 40 DPF mice ($p=0.030$ and $p=-0.038$ respectively, see Figure 1B and 1C), and an increased tissue mineral density (10% increase) in young DPF 40 mice ($p=0.01907$, see Figure 1D).

Discussion

These results clearly show a positive effect of Notch activation on both callus size and bone formation, particularly in the geriatric mice.. Consistent with our previous results, the increase in callus size with NICD treatment is phenotypically opposite of what was seen when Notch signaling was inhibited during fracture healing using dominant negative mastermind (dnMAML) over-expressing mice.[4]

Significance

This is the first demonstration that local activation of Notch signaling positively influences bone regeneration. This research represents an important step in developing Notch activation as a therapeutic for enhancing bone regeneration.

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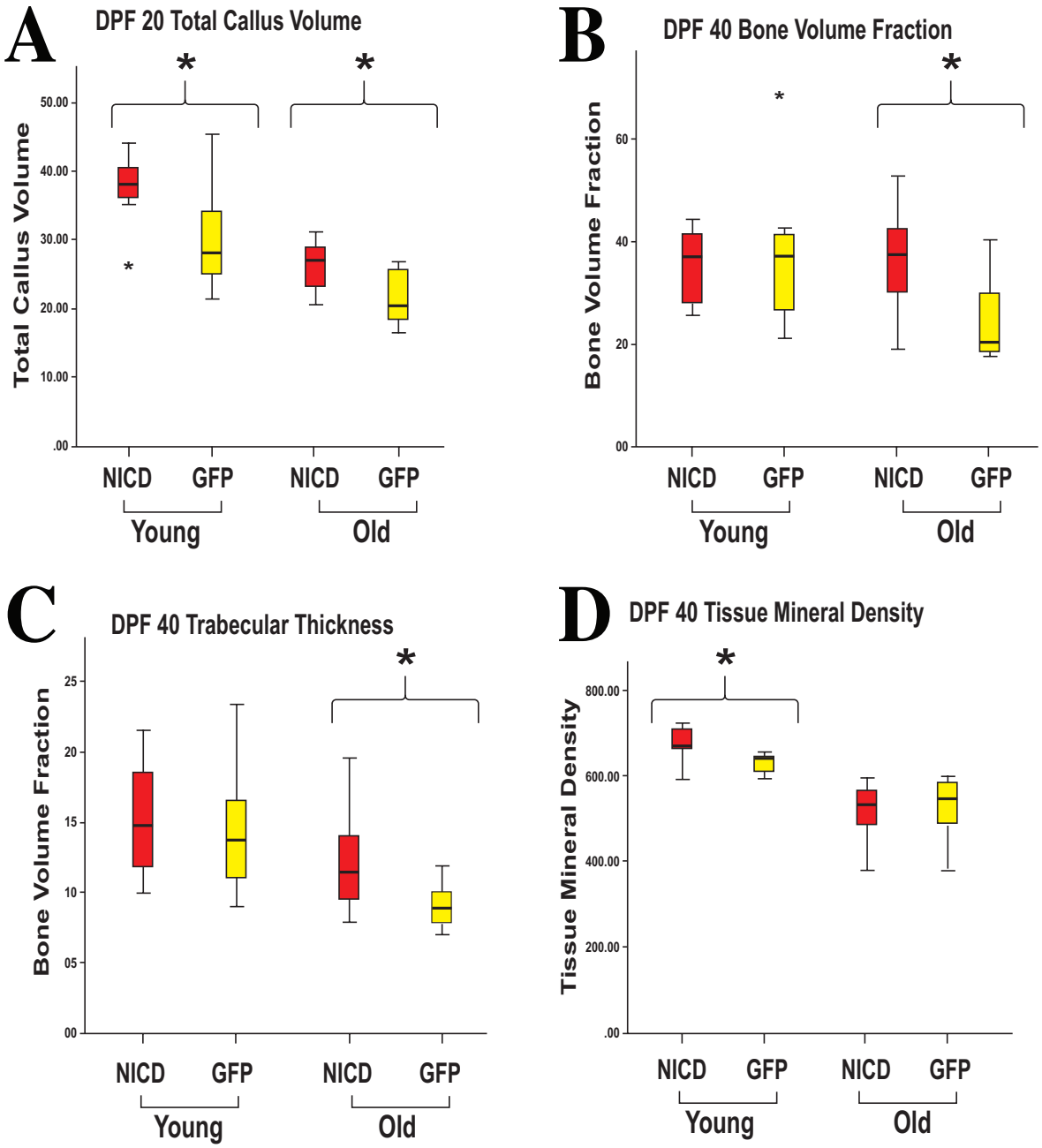

Figure 1. Micro-CT analysis of NICD treated fractures. NICD is red, GFP is yellow. * = significance between groups within brackets. A) *DPF 20 Total callus volume.* Shows significant difference in both young and old mice (p=0.0497 and p=0.028, respectively). B) *DPF 40 Bone volume fraction.* Shows significant difference in old mice (p=0.030). C) *DPF 40 Trabecular thickness.* Shows significant difference in old mice (p=0.038). D) *DPF 40 Tissue mineral density.* Shows significant difference in young mice (p=0.01907).

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