Bone Histological Response to anti-Myostatin and anti-Activin A antibodies in an *Osteogenesis Imperfecta* Murine Model

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Introduction

Osteogenesis imperfecta (OI), also known as brittle bone disease, is a heritable connective tissue disorder caused by structural or quantitative anomalies in the genes that encode type I collagen and is phenotypically manifested in type I collagen-containing tissues; particularly bone (2). To study OI in the lab, we employ the G610C murine model. Heterozygote G610C mice have a G-to-T nucleotide transversion, changing a glycine codon to a cysteine codon in the pro-α2(I) chain of type I collagen, and exhibit phenotypic and biochemical features typical of moderate, nonlethal forms of type I and IV human OI (Fig. 1). There is no cure for OI, and current treatment options can provide benefit but may also result in side effects in patients. Thus, there is a need for substitutive treatment methods to improve bone strength and quality in OI.

Bone, a mechanosensing organ, responds to high mechanical loads by stimulating new bone formation and altering bone geometry to withstand increased forces, typically from increased muscle mass (3). Myostatin (mstn), a member of the TGF-β superfamily, is a negative regulator of muscle growth. Mice deficient in myostatin experience muscle hypertrophy and hyperplasia (7).

In previous studies, G610C mice treated with a soluble activin receptor type IIB-mFc (sActRIIB-mFc) fusion protein to inhibit myostatin presented with increased hind-limb muscle weights (4). The exact mechanism of sActRIIB-mFc remains unknown and negative side effects in humans have been noted, likely due to the receptor's ability to bind multiple targets in addition to myostatin. Of sActRIIB-mFc targets, myostatin and activin-A (ActA) are predicted to play the biggest role in bone and muscle (Fig. 2).

Bone is composed of three major cell types: osteoblasts, osteoclasts, and osteocytes. Osteoblasts secrete matrix for bone formation, osteoclasts absorb bone tissue, and osteocytes are osteoblast derived cells which act as mechanosensors. To determine the effects of inhibiting myostatin and activin on these cell types and numbers, we have been performing histological examination. The focus of this study is to evaluate osteoclast number, size, and activity.

Here, we treated male wildtype mice with anti-myostatin or anti-activin-A specific antibodies to investigate their roles in bone health (5). **We hypothesize that postnatal inhibition of myostatin or activin-A will reduce osteoclast number and activity in G610C mice, though more data is needed to determine full impact on bone.**

Figure 1: OI classification

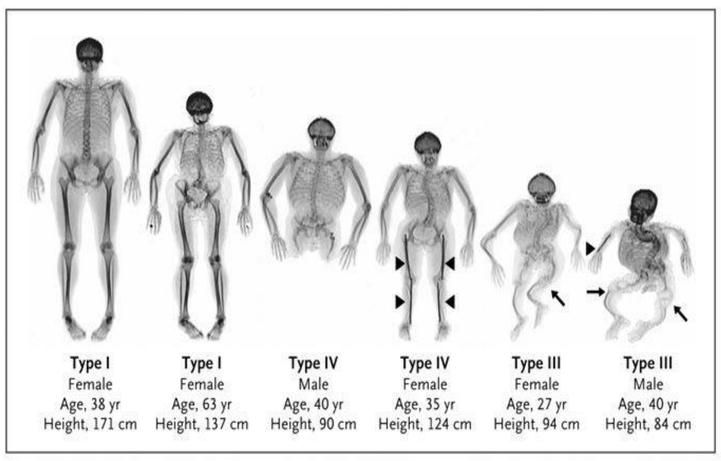

Figure 1: The Sillence classification of osteogenesis imperfecta. The Sillence classification system identifies four main types of OI ranging from mild to severe with type I being mild; type II, perinatally lethal; type III, severely deforming, and type IV moderately deforming (8).

Figure 2: Signaling pathways of Myostatin and Activin A

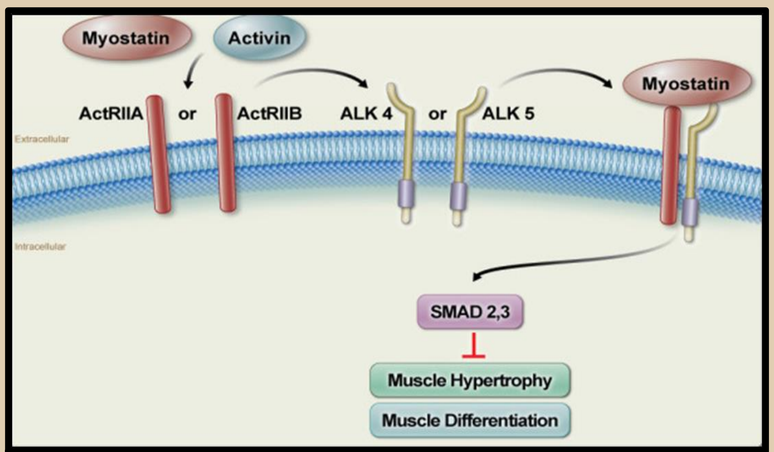

Figure 2: Both myostatin and Activin A signal via the ActRIIB receptor
Lee, S. J., & Glass, D. J. (2011). Treating cancer cachexia to treat cancer. *Skeletal muscle*, *1*(1), 2. doi:10.1186/2044-5040-1-2

Methods

Drug Administration – Male WT mice were randomly assigned to antibody groups: anti-activin-A (ActA), anti-myostatin (Mstn), or control (Ctrl). The animals were treated twice a week with 10 mg/kg of the respective antibodies by intraperitoneal (IP) injection beginning at 5 weeks of age (24 injections total). At 4 months of age, mice were anesthetized to evaluate muscle contractility, and euthanized to harvest their muscles and bones for further analyses.

Histology –Tibias were embedded in methyl methacrylate (5). Using the HM355S microtome (Thermo Fisher Scientific, Waltham, MA, USA), 5 µm thin undecalcified sections were then longitudinally obtained. Three non-adjacent sections per animal were de-plasticized and stained either with tartrate resistant acid phosphatase (TRAP) for histomorphometric analyses of osteoclasts. The Zeiss Axiovert 200M (Zeiss, Oberkochen, Germany) and the MetaMorph software (Molecular Devices, Sunnyvale, CA, USA) were used to obtain series of images at 20x magnification which were then compiled into one. Histomorphometric analyses of the static parameters were performed using ImageJ (Image J2) in accordance with Egan and colleagues protocol (1).

Figure 3: Bone cell types

Figure 3: Bone includes multiple cell types, all with different functions
https://bio.libretexts.org/Bookshelves/Introductory_and_General_Biology/Book%3A_General_Biology_(Boundless)/38%3A_The_Musculoskeletal_System/38.2%3A_Bone/38.2B%3A_Cell_Types_in_Bones

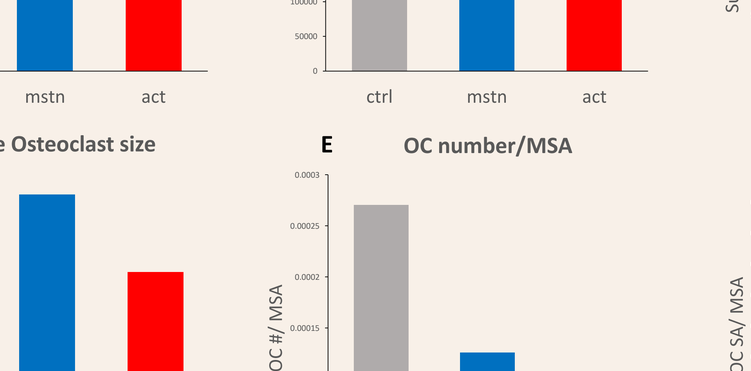

Figure 4: Osteoclast Histology

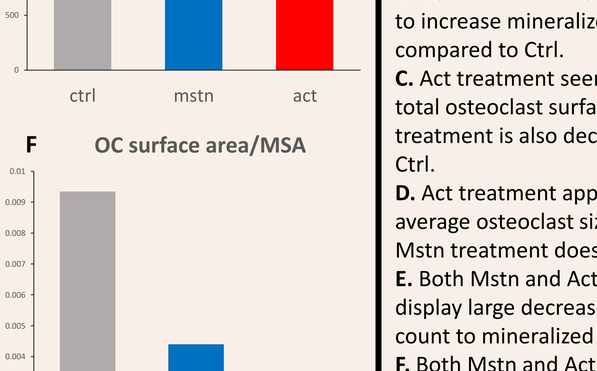

Figure 4: Osteoclasts become visible through TRAP staining where they appear as multinucleated cells lining mineralized bone surface areas.
Original lab photo: sample 14G5257

Figure 4: Preliminary Osteoclast Findings

ctrl mstn act

Tibias of anti-activin A (act) and anti-myostatin (mstn) specific antibody treatments show preliminary decreases in osteoclast size, count, and activity.

A. Both Mstn and Act treatments appear to exhibit decreased osteoclast numbers relative to the Ctrl.
B. Both Mstn and Act treatments appear to increase mineralized surface area compared to Ctrl.
C. Act treatment seems to exhibit lowest total osteoclast surface area. Mstn treatment is also decreased relative to Ctrl.
D. Act treatment appear to decrease average osteoclast size relative to Ctrl. Mstn treatment does not.
E. Both Mstn and Act treatments seem to display large decreases in osteoclast count to mineralized surface area ratios.
F. Both Mstn and Act treatments seem to display large decreases in osteoclast surface area to mineralized surface area ratios.

n= 2 for all treatment types

Conclusions

With a sample size of 2 for each treatment category, no final conclusions may be drawn. Preliminary data suggests that both anti-myostatin and anti-activin A antibody treatments reduce osteoclast size, count, and total surface area. The results also suggest that mineralized surface area increases through both anti-myostatin and anti-activin A antibody treatments. Current preliminary data supports our hypothesis. Osteoclast count and size decrease may also reduce activity, though more samples and data are necessary to draw final conclusions.

Future work

- Increase sample sizing to 5 per each treatment category.
- Evaluate finalized data for values of significance.
- Evaluate identical statistics for osteoblasts to better understand the relationship between bone absorption and creation in each treatment category
- Evaluate identical statistics in female G610C mice.
- Replicate experiment protocol for our homozygous *oim* mouse model which models human OI type III.

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Acknowledgements

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