Effect of HDAC9 and HDAC11 on Osteoclast Differentiation and Activity

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Abstract

DNA is packaged tightly creating an environment unconducive to transcriptional activation. Chromatin modifications must be made in order to facilitate transcription in eukaryotes. Histone deacetylases (HDACs) have become an important focus in research due to their role in many fundamental processes including differentiation, growth arrest and apoptosis. Previous research has shown that HDACs can influence transcription factors and play a crucial role in bone homeostasis. This study aims to understand the role of HDAC9 and HDAC11 on osteoclast differentiation and activity. While few studies have been able to identify HDAC11's role in osteoclastogenesis, it has been shown in previous studies that overexpression of HDAC9 inhibits osteoclast differentiation while conversely loss of HDAC9 expression leads to enhanced osteoclastogenesis. To investigate the effects of HDACs 9 and 11, I conducted a series of experiments and found loss of HDAC9 or HDAC11 expression leads to enhanced osteoclast differentiation and elevated bone resorption.

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Introduction

Histone Deacteylase

Gene expression of eukaryotes begins with the conversion of deoxyribonucleic acid (DNA) to ribonucleic acid (RNA) in a process called transcription. One mechanism to regulate transcription is to control the accessibility of DNA by winding the DNA tightly around proteins or histones. Histones keep the DNA in a condensed form, which makes it less accessible to transcription factors. Histone deacetylases (HDACs) are enzymes that deacetylate or modify histones and/or other cellular proteins to negatively regulate gene expression. In contrast, histone acetylases (HATs) are proteins that acetylate histones, which increases accessibility of the DNA for transcription. Acetylation then leads to increased expression of certain genes that either promote or inhibit cell differentiation.

HDACs are divided into two classes based on their protein sequence. Class I consists of HDACs 1, 2, 3, and 8. Class I HDACs reside exclusively in the nucleus of all cell types and are similar in protein sequence. Early experiments show HDACs 1, 2, 3, and 8 are grouped together because they repress transcription through their deacetylase activity. Class II consists of HDACs 4, 5, 6, 7, 9, and 10. Class II HDACs are located in the cytoplasm as well as the nucleus and are expressed in a more restricted tissue pattern indicating they may be involved in cell differentiation and development. Class II HDAC's have also been shown to repress transcription, but their mechanism of inhibition is less clear as they have the ability to act independently of their deacteylase activity[2].

Since acetylation plays a key role in the regulation of gene expression, it has inspired research to focus on HDACs in relation to the aberrant gene expression. HDACs play a role in the maintenance of bone. Others have shown HDACs can regulate osteoblast and chondrocyte differentiation which can be reviewed in Bradley, et al.[2] The focus of my research is on their regulation of osteoclastic gene expression.

Bone Homeostasis

Bone is essential for makeup of the human skeleton and undergoes cyclical changes of bone formation and bone resorption throughout life. Osteoclasts are key regulators in bone homeostasis and function to resorb bone. Bone resorption is balanced by osteoblasts which synthesize new mineralized bone. In certain cases, the amount of bone resorption does not equal the amount of bone deposition. When bone homeostasis is altered, it can manifest in a variety of pathological conditions such as osteoporosis.

Osteoblasts and osteoclasts activity determine whether bone homeostasis is maintained. It begins with an osteoclast precursor being recruited to the surface of bone, fusing into a multinuclear cell to become a mature osteoclast. Osteoclasts form a ruffled border as they contact the bony surface and form a resorptive pit referred to as a Howship's lacunae. In this resorptive pit, osteoclasts secrete hydrogen ions creating an acidic environment to resorb the organic bony matrix[12]. After osteoclasts have completed their function, osteoblasts move into the

resorptive site and begin to lay down new bone. It is important to understand that there is a third cell involved in bone homeostasis, an osteocyte. Osteocytes are terminally differentiated osteoblasts that have become embedded in the bone matrix. Recently it was shown that osteocytes express Receptor activator of nuclear factor kappa-B ligand (RANKL) and Osteoprotegrin (OPG) to regulate osteoclast differentiation[19].

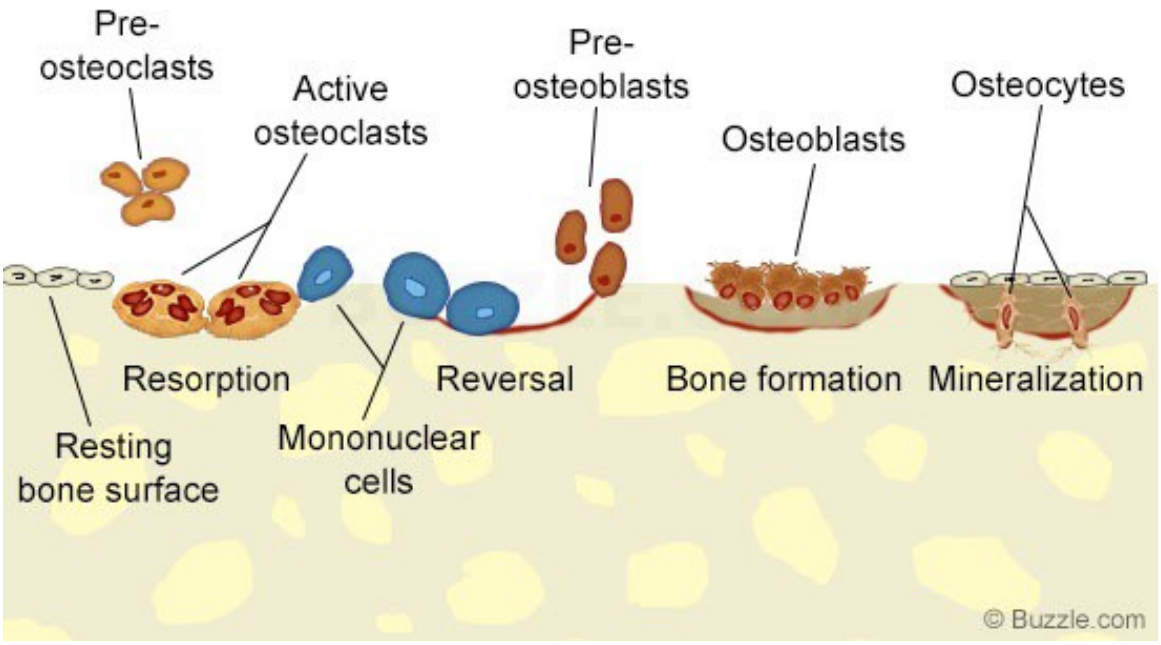


Figure 1: Bone remodeling showing resorption by osteoclasts, formation by osteoblasts, and mineralization by osteocytes.
www.umich.edu/news/Releases/2005/Feb05/bone.html

Osteoclasts

Osteoclasts are multinucleated cells that originate from hematopoietic stem cells. Specifically osteoclasts belong to the monocyte/ macrophage lineage and eventually differentiate into osteoclast precursor cells with help from macrophage colony stimulating factor (M-CSF) and RANKL. M-CSF is a cytokine secreted

by osteoblasts and signals the hematopoietic stem cells to differentiate into monocytes/osteoclast precursors[16]. Osteoclast precursors exhibit the receptor RANK on its surface. RANK binds to RANKL expressed on the surface of either osteoblasts or osteocytes leading to the osteoclast precursor differentiating into a mature osteoclast.

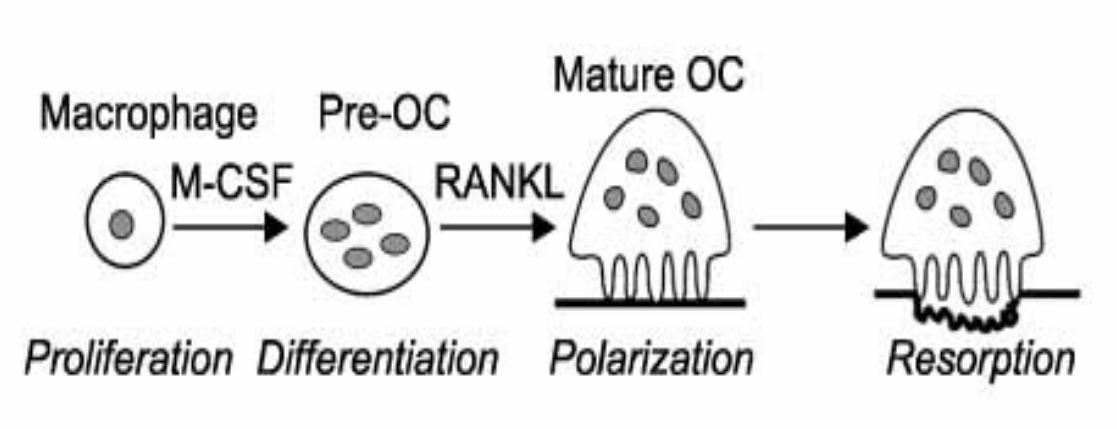


Figure 2: Osteoclast formation. J Korean Orthop Assoc. 2009 Apr;44(2):151-158. https://synapse.koreamed.org/ViewImage.php?Type=F&aid=421001&id=F1&afn=43_JKOA_44_2_151&fn=jkoa-44-151-g001_0043JKOA

RANKL is a member of the tumor necrosis factor (TNF) cytokine family and is essential for osteoclastogenesis. It has also been found to be critical for lymph node organogenesis, lymphophoiesis, and immune regulation[20]. Over expression of RANKL has been linked to several degenerative bone diseases including rheumatoid arthritis and psoriatic arthritis. Mice null for RANKL expression do not undergo osteoclastogenesis which manifests as an osteopetrotic phenotype[19].

The mechanism by which osteoclast production is inhibited is through binding of

Osteoprotegrin (OPG) to RANKL. This inhibits RANK-mediated nuclear kappa factor B (NF-κB) from being activated. RANK-mediated NF-κB serves as a key regulator for osteoclast differentiation. OPG binding to RANKL prevents RANK from binding to RANKL and thus inhibits osteoclast precursor cells from further differentiating to mature osteoclasts. As a result, excess OPG leads to increased bone density and could be a target for future research. This could help further the treatment modalities for treating diseases with deficient bone density such as osteoporosis.

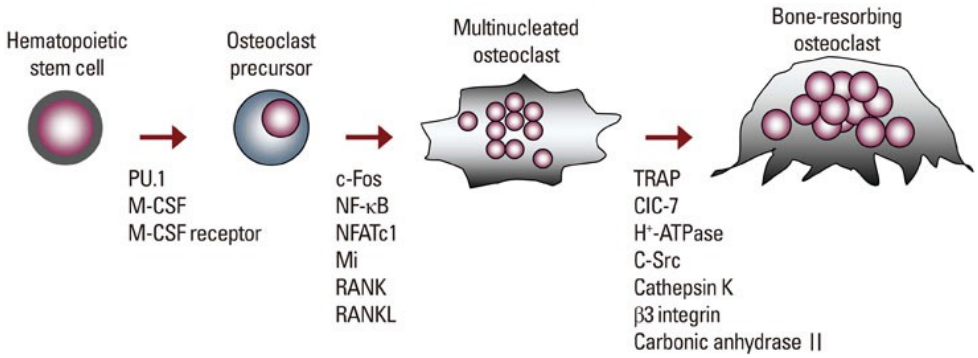


Figure 3: Detailed schematic diagram with key players in osteoclast formation. Image modified from Endocrinol Metab. 2010 Dec;25(4):264-269. English. Published online Dec 31, 2010. http://eenm.org/search.php?where=aview&id=10.3803/EnM.2010.25.4.264&code=2008ENM&vmode=PUBREADER#!po=40.9091

HDACs and Bone

HDACs interact with transcription factors to effect differentiation of osteoblasts and osteoclasts. For example, HDACs can inhibit Runx2, a transcription factor that when inhibited leads to decreased osteoblast differentiation and mineralization. Research is being conducted to find a more definitive answer to how HDACs effect

osteoclast differentiation. In 2011, Jensen and Mansky et al. found that in vitro loss of HDAC7 accelerated osteoclastogenesis and enhanced osteoclast formation. HDAC7 represses the transcription factor MITF, which is necessary for osteoclast differentiation. RANKL and
M-CSF are no longer able to activate the MITF and induce genes necessary for osteoclast differentiation[10].

HDAC9 is a member of the Class II HDACs due to its protein makeup and similarities with HDACs 4, 5 and 7. It has three known splice variants, HDAC 9a/ HDAC9b/HDAC9c. The focus of my study will be on HDAC9c and HDRP. The Mansky lab has determined that only HDAC9c and HDRP are expressed in osteoclasts. HDAC9c or HDRP lacks a catalytic domain necessary for deacetylase activity. To remedy this deficiency, HDRP recruits HDAC3, thus allowing it to now have deacteylase activity necessary for repression of transcriptional activity[6].

A study conducted by Jin, et al. found HDAC9 to be a negative regulator of osteoclastogenesis. Deletion of HDAC9 in their study lead to enhanced osteoclast differentiation and elevated bone resorption ultimately leading to lower bone mass. Osteoclastogenesis can be enhanced by other signaling pathways such as nuclear receptor transcription factor PPARγ. Interestingly, HDAC9 is able to inhibit PPARγ in synergy with SMRT/NCoR co-repressors. PPARγ, on the other hand, can combine with NF-kB and inhibit HDAC9. These interactions are important to understand when examining the relationship between HDAC9 and

osteoclastogenesis. The authors concluded that the antiosteoclastogenic effect is mediated by suppression of HDAC9 and PPARγ/RANKL signaling. In the absence of RANKL, HDAC9 impedes PPARγ's ability to function[6]. However, the study by Jin et al. did not determine which isoform of HDAC9 was disrupted in their mouse model.

A review of the literature provides little information is known about HDAC11. A study published by Gao et al. in 2002 described HDAC11 as a novel and unique member of the histone deacetylase family that may have distinct physiologic roles from other known HDACs. Sequence analysis of HDAC11 protein shows that it contains conservative residues at the catalytic core regions shared by both Class I and II HDACs[5]. HDAC11 was found in the study to be the smallest member in the family and the most closely related to HDAC3 and HDAC8, which suggests it may be more closely related to class I than class II HDACs. However, the classification of HDAC11 has yet to be determined. While epitope tagged HDAC11 protein localizes primarily to the nucleus (similar to Class I), it also may be present in protein complexes that contain HDAC6 in the cytoplasm according to co-immunoprecipitation experiments by the same authors[5].

Previous studies have shown that HDAC6 decreases adhesion of osteoclasts through its ability to deacetylate microtubules[4]. Further experiments need to be done to help clarify HDAC11's role in osteoclastogenesis.

HDAC Inhibitors

Currently, HDAC inhibitors are used to treat epilepsy and certain cancers[7,8,9]. FDA

approved Valproate is a medication used to treat certain cancers, but also has been shown with long term use to decrease bone mineral density in humans[1,18]. Other in vitro studies have shown HDAC inhibitors such as Trichostatin A (TSA) can suppress osteoclast differentiation and enhance osteoblast differentiation[13,14]. TSA and sodium butyrate are considered broad spectrum HDAC inhibitors which can inhibit all Class I and Class II HDACs[10].

Osteoclasts in Orthodontics

Orthodontic forces alter the bone homeostasis and induce remodeling. Osteoblast and osteoclast activity determine the fate of bone as orthodontists move teeth within the mandible and maxilla. Chemical messengers lead to remodeling of alveolar bone and tooth movement. Studies have demonstrated increases in the concentrations of RANKL and OPG during orthodontic tooth movement, which suggests that stress created by applying an orthodontic force may induce the formation of osteoclasts through up regulation of RANKL[11]. Tooth movement typically begins two days after orthodontic forces are initiated beginning as osteoblasts and osteoclasts remodel the bony socket.

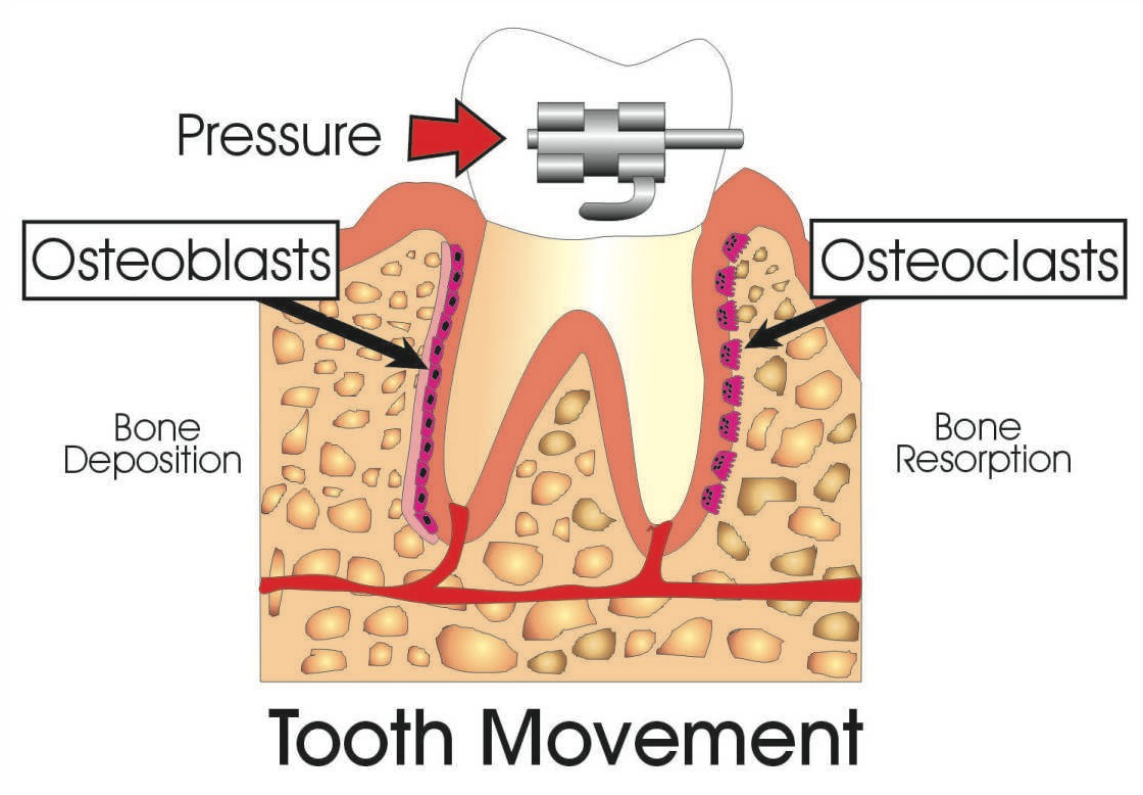


Figure 4: Osteoclasts are formed to remove bone on the right side (compression side). Osteoblasts are formed to add new bone and remodel resorbed areas on the left side (tension side). The tooth is the diagram has orthodontic forces moving it to the right as indicated by the arrow. http://omnidentalgroup-austin.blogspot.com/2014/08/drinking-coffee-accelerates-orthodontic.html

HDACs could prove be clinically significant to the field of orthodontics as well as many other fields that deal with treatment or manipulation of bony structures. These experiments will help to enhance our understanding of HDAC11 and HDAC9, and hopefully contribute to a larger goal of understanding how HDACs can regulate osteoclast differentiation, skeletal formation, and the field of orthodontics.

Hypotheses

1. My first hypothesis is that expression of HDAC9 inhibits osteoclast differentiation. I expect that knockdown of HDAC9 will increase osteoclastogensis. I expected both an increase in size as well as increased resorption compared to the control.
2. My second hypothesis is that HDAC11 also inhibits osteoclast differentiation. For this hypothesis to be correct, I expect that knockdown of HDAC11 will increase osteoclastogenesis. This would produce increased size of osteoclasts after TRAP stain and increased areas of resorption on the calcium phosphate resorption plates compared to the control.

Specific Aims

The specific aims of this study are to determine whether reduced expression of HDAC9 and HDAC11 affect osteoclast differentiation and activity. This will be done by infecting osteoclasts with lentiviruses expressing shRNAs targeting HDAC9 or HDAC11.

Analysis will be done to evaluate the size and number of osteoclasts after fixing the cells and performing a TRAP stain. Further analysis will be conducted to observe the pit number, average pit size, and percent area resorbed on the calcium phosphate resorption plates.

Materials and Methods

Harvesting bone marrow and osteoclasts

Bone marrow macrophages were harvested from the femurs and tibiae of wild type mice. The ends of the bones were cut and the marrow flushed. Red blood cells were lysed from the bone marrow tissue with red blood cell lysis buffer, and the remaining cells plated on 100mM plates and cultured overnight in osteoclast media (phenol red-free alpha-MEM (Gibco) with 5% heat-inactivated fetal bovine serum (Hyclone), 25 unites/mL penicillin/ streptomycin (Invitrogen), 400 mM L-Glutamine (Invitrogen), and supplemented with 0.1% CMG 14-12 conditioned media containing M-CSF). The non-adherent cell population, including osteoclast precursor cells, were then separated and re-plated at approximately 100,000 cells per well in the presence of 0.1% CMG 14-12. After 48 hours plated cells are stimulated with M-CSF and RANKL to begin osteoclast differentiation. From this point on, the cells in culture are osteoclasts. Two dishes with 24 wells were used in each round of the experiment. One dish was used for TRAP staining and the other, a calcium phosphate coated plate, to analyze demineralization.

Lentiviral Transfection

Lentiviral vectors were used encoding shRNAs against HDAC9 or HDAC11 or a control shRNA. Lentiviral vectors were used to produce replication defective lentiviruses according to the manufacturer's protocols. Twenty-four hours after plating, lentivirus was added to the osteoclasts at 37°C in the presence of 0.1% CMG 14-12 conditioned media. Two distinct lentiviruses were used in this study:

each virus was added to 3 wells on each plate. Lentivirus numbered 029 (shRNA #1) and 224 (shRNA #2) express an shRNA against HDAC11. Lentivirus numbered 592 (shRNA #1) and 594 (shRNA #2) express a shRNA against HDAC9. We verified that the shRNA knocked down expression of HDAC11 or HDAC9 (confirmed by qPCR). The same pattern for addition of virus was used in each round of experiment. A control virus that expresses a scrambled shRNA that should not affect expression of any HDAC was added to 3 wells to demonstrate the effect of virus infection on the osteoclasts. The next day lentivirus was removed from the wells and cells were fed with 0.1% CMG 14-12 conditioned media and RANKL. Cells were fed every other day with RANKL and 0.1% CMG 14-12 conditioned media. After 5 days, the cells were fixed with 4% paraformaldehyde.

TRAP Stain

The fixed cells were stained for tartrate resistant acid phosphatase (TRAP) expression with tartrate (5 mg), Napthol AS-MX phosphate, 0.5 mL M, M-Dimethyl formamide 50 mL acetic acid buffer (1 mL acetic acid, 6.8 g sodium acetate trihydrate, 11.5 g
sodium tartrate in 1 L water), and 25 mg Fast Violet LB salt. The cells were incubated for approximately 20 minutes and then images were captured with light microscopy on the 4x objective. Images had to be converted into binary format on NIH Image J for measurements of size and number to be recorded.

Resorption Plates

Bone marrow macrophages (BMMs) were plated on OsteoAssay plates (Corning) in 0.1% CMG 14-12 conditioned media for 2 days. After 2 days BMMs were given 0.1% CMG 14-12 conditioned media and RANKL to stimulate differentiation. Once cells had differentiated into multinuclear osteoclasts the media was aspirated and 100 uL of 10% bleach was added to each well and incubated at room temperature for 5 minutes. Resorption occurred for 5 days. The wells were then washed twice with H_2O, allowed to air-dry. Images were captured with light microscopy on 4x objective. Images needed to be converted into binary format on NIH Image J for measurements of size, number, and percent of area resorbed to be recorded.

Statistical Analysis

All experiments were run at least three times. Results show the mean ± standard deviation. ANOVA analysis followed by a Tukey's multiple comparison test was used to compare data utilizing the computer program Prism version 5.

Results

Knockdown of HDAC9 Expression Enhances Osteoclast Differentiation

The study conducted by Jin et al. concluded that the class II HDAC9 is a physiologically relevant modulator of bone remodeling and skeletal homeostasis. Their study found HDAC9 controls bone turnover by suppressing osteoclast differentiation and bone resorption[6]. Our present study attempts to further investigate the role of HDAC9 in osteoclast differentiation and activity by infecting wild type mouse cells with two different viruses expressing a shRNA against HDAC9 or a control shRNA virus.

Cultures were given RANKL until multicellular osteoclasts appeared. Cells were fixed and TRAP stained. Both HDAC9 shRNA infected cells showed a significant decrease in number of multicellular osteoclasts compared to the control infected cells. The average number of osteoclasts was found to be 312 for the control group, 225 for shRNA #1, and 25 for shRNA#2. The average size of the osteoclasts increased in the HDAC9 shRNA infected cells compared to the control. The average size of the osteoclasts was 0.004

mm^2 for the control, 0.007 mm^2 for shRNA #1, and 0.006 mm^2 for shRNA #2.

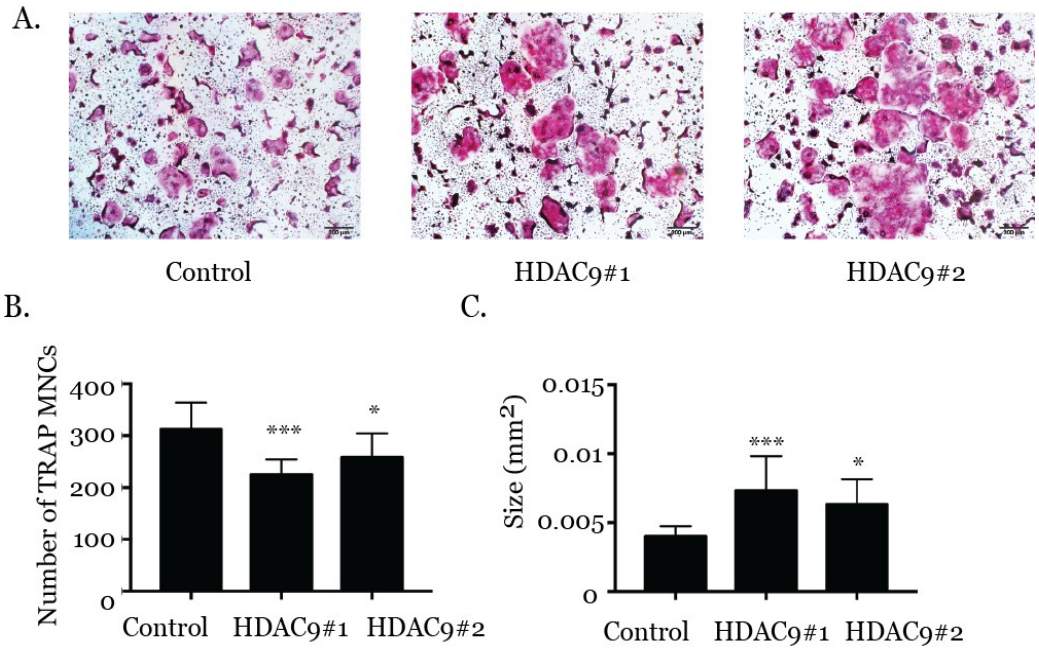


Figure 4. Knockdown of HDAC9 increases osteoclast differentiation. (A). Representative images of TRAP stained osteoclasts. Quantification of (B) Number of TRAP positive cells containing three or more nuclei (C) Size of TRAP positive cells containing three or more nuclei. *** denotes $p \leq 0.001$, * denotes $p \leq 0.05$.

Osteoclasts with decreased HDAC9 Expression have increased activity

Wild type bone marrow macrophages were infected with either a HDAC9 shRNA or control shRNA virus (as described above) and plated on calcium phosphate coated plates. We examined pit number, average pit size, and total percent area resorbed.

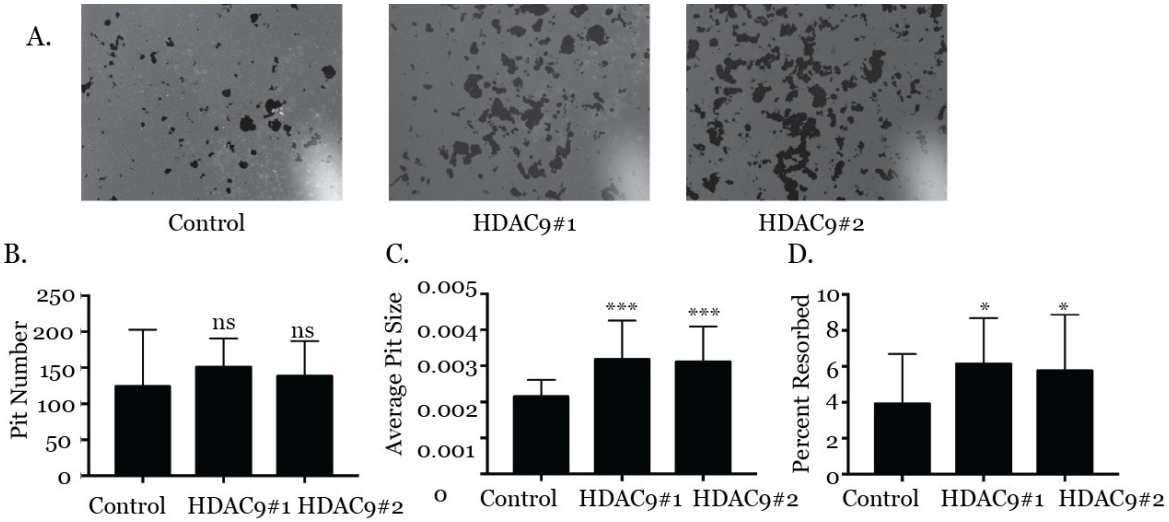
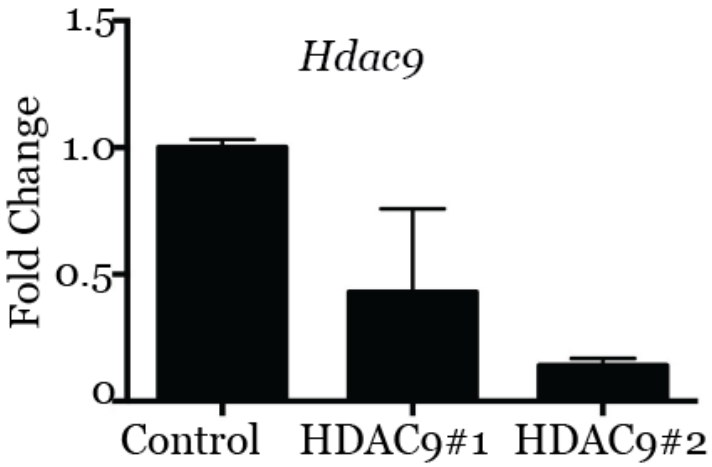


Figure 5. Knockdown of HDAC 9 increases resorption. (A) Representative images of calcium phosphate coated wells. Quantification of (B) pit number, (C) average pit size,
(D) percent area resorbed. ns denotes no statistical significance, * denotes $p<.05$, *** denotes $p \leq 0.001$.

Pit number for HDAC 9 shRNA #1 was 150 compared to 124 for control while pit number for HDAC9 shRNA #2 was 138. Neither were statistically significant. The average pit size was .002 mm^2 for the control and .003 mm^2 for shRNA #1 and for shRNA #2. The average percent area resorbed for the control was 3.9% for the control, 6.1% for shRNA #1, and 5.7% for shRNA #2.



Graph demonstrating knock down in expression of HDAC9

Osteoclast precursors from WT mice were infected with either a lentivirus expressing a scrambled (control) shRNA or lentiviruses expressing two distinct shRNAs targeting *HDAC9*. Real time PCR was used to measure *HDAC9* expression 48 hours after infection by the lentivirus. I detected a significant reduction in *HDAC9* in the shRNA-infected cells compared to the control-infected cells.

Osteoclasts with reduced HDAC11 expression are enhanced in differentiation

The study by Gau et al. determined HDAC11 is a unique deacetylase displaying characteristics from Class I and Class II HDAC family members. Their study found HDAC11 resides predominantly in the nucleus, a characteristic shared by Class I HDACs. However, HDAC11 mRNA is found in specific tissues and may be present on protein complexes containing HDAC6, a known Class II HDAC. Our study attempted to gain more understanding of HDAC11's role in osteoclast differentiation and activity. We infected wild type bone marrow macrophages with either a shRNA targeting HDAC11 or a control shRNA. Cultures were given RANKL until multicellular osteoclasts appeared. Cells were fixed and TRAP stained. The HDAC11 shRNA infected cells showed a decrease in the number of osteoclasts when compared to the control (although not statistically significant). The average number of osteoclasts was found to be 315 for the control group, 277 for shRNA #1, and 268 for shRNA#2. The average size of the osteoclasts was 0.003 mm^2 for the control, 0.0065 mm^2 for HDAC11 shRNA #1, and 0.007 mm^2 for HDAC11 shRNA #2.

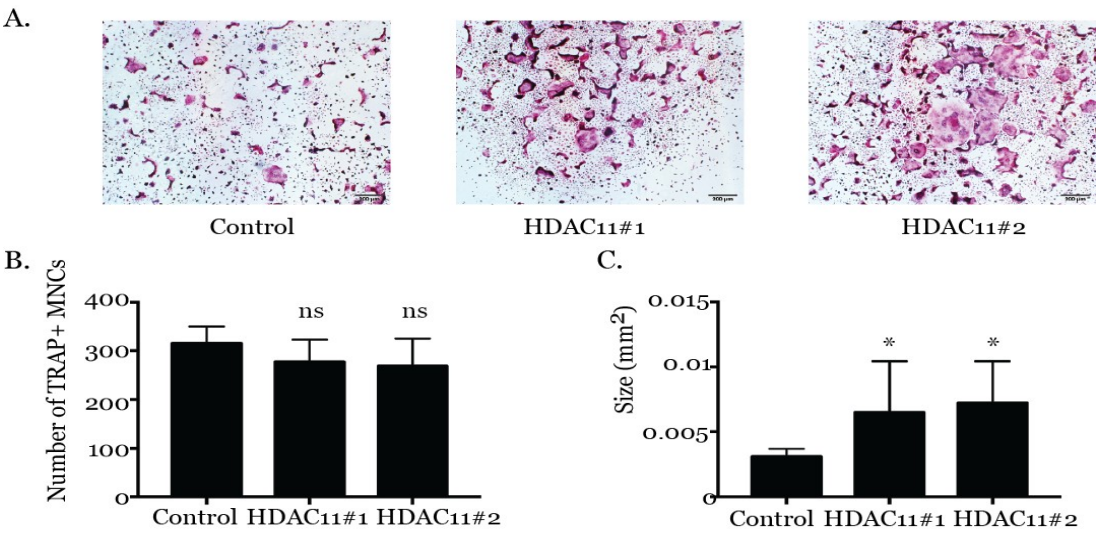


Figure 6. Knockdown of HDAC11 increases osteoclast differentiation. (A). Representative TRAP stained images of osteoclasts. Quantification of (B) Number of TRAP positive cells containing three or more nuclei (C) Size of TRAP positive cells containing three or more nuclei. ns denotes no statistical significance, * denotes $p \leq 0.05$.

Osteoclasts with reduced expression of HDAC 11 shRNA have increased activity

Bone marrow macrophages were infected with either a HDAC11 shRNA or a control shRNA and plated on calcium phosphate coated plates. We examined pit number, average pit size, and total percent area resorbed.

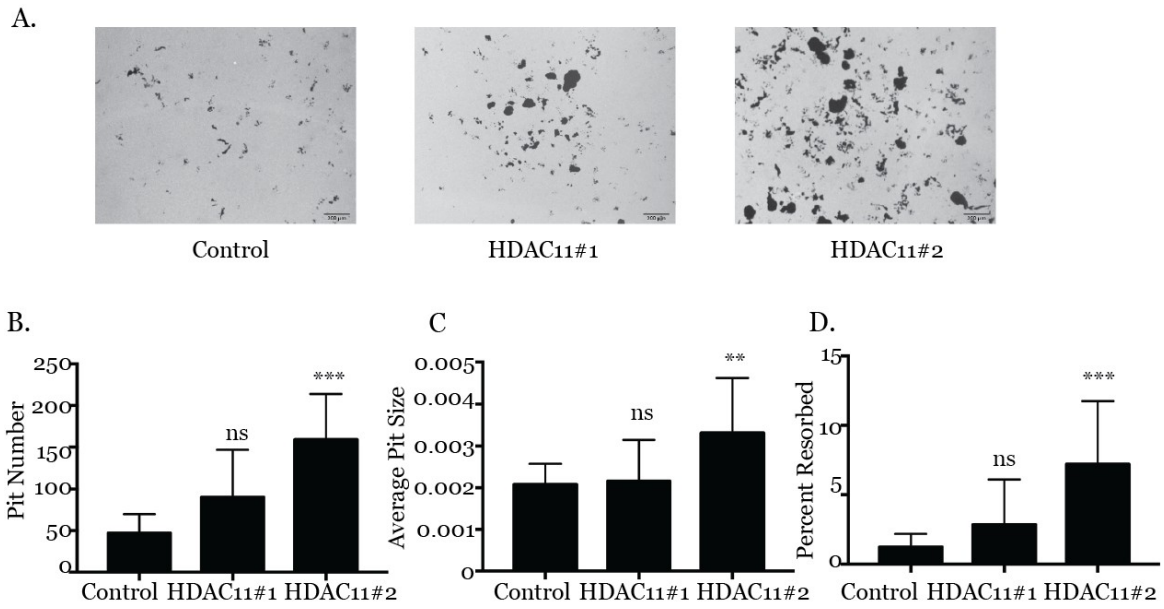
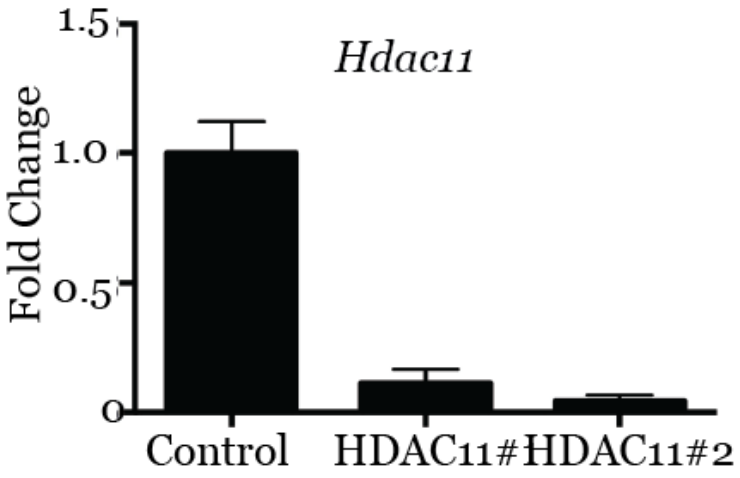


Figure 7. Knockdown of HDAC11 increases resorption. (A) Representative images of calcium phosphate coated wells. Quantification of (B) pit number, (C) average pit size,
(D) percent area resorbed. ns denotes no statistical significance, * denotes $p<.05$, *** denotes $p \leq 0.001$.

The pit number was 47.38 for the control, 90.15 for HDAC 11 shRNA #1 and 159.23 for HDAC11 shRNA #2. The average pit size was 0.002 mm^2 for the control, 0.002 mm^2 for shRNA #1, and 0.003 mm^2 for shRNA #2. The average percent area resorbed for the control was 1.25%, 2.87% for shRNA #1, and 7.21% for shRNA #2.



Graph demonstrating knock down in expression of HDAC11

Osteoclast precursors from WT mice were infected with either a lentivirus expressing a scrambled (control) shRNA or lentiviruses expressing two distinct shRNAs targeting *HDAC11*. Real time PCR was used to measure *HDAC11* expression 48 hours after infection by the lentivirus. I detected a significant reduction in *HDAC11* in the shRNA-infected cells compared to the control-infected cells.

Discussion

A previous review of the HDAC family by de Ruijter el al. found HDACs to be key enzymes in the regulation of gene expression. Inhibition of HDACs can lead to a variety of processes including apoptosis, necrosis, differentiation, inhibition of proliferation and cytostasis[4]. Our study examined the effect of inhibiting HDAC9 and HDAC11 on osteoclast differentiation and activity.

Our results showed that when shRNAs were used to knockdown HDAC9, osteoclasts decreased in number by approximately 1.2-1.4 fold and increased in size by 1.2-1.5 fold. The increase in size is due to increased osteoclast fusion as evidenced by decrease in the number of TRAP positive multinuclear cells. Osteoclasts with decreased HDAC9 expression have increased activity manifested as a 1.5 fold increase in percent area resorbed when treated with HDAC9 shRNA. These findings agree with the previous study by Jin et al. that found decreased HDAC9 expression leads to increased osteoclast differentiation and increased bone resorption. Jin et al. demonstrated that the antiosteoclastogenic effect of HDAC9 is mediated by suppression of the PPARγ/RANK signaling pathway. There are two forms of HDAC9: one full length HDAC9 and one HDRP which lacks the catalytic domain[4]. The study by Jin et al. did not demonstrate which forms of HDAC9 are inhibitors of osteoclast differentiation. While both forms are known to be expressed in osteoclasts, no study has distinguished between the two forms. HDAC11 shRNA infected osteoclasts demonstrated a decrease in osteoclast number (though not statistically significant). The osteoclasts

did show a statistically significant increase in osteoclast size compared to the control. This is the first study to demonstrate the role of HDAC11 in regulating osteoclast differentiation and activity. HDAC11 is a unique HDAC that shares characteristics common with both class I and II. The Mansky lab has preliminary data demonstrating that expression of HDAC11 was highest at day 2- 3 which may indicate a role in osteoclast fusion since fusion of osteoclast precursors occurs at days 2-3. Other studies were able to show expression of HDAC11 in other cells such as the kidney, heart, brain, skeletal muscle, and testis[5]. This suggests that although HDAC11's function is unknown at this time, its function may be tissue specific. Future studies will help us understand the transcription factors in osteoclasts that interact with HDAC11, where HDAC11 is expressed (nucleus and/or cytoplasm), and if there is a shuttling function associated with HDAC11.

Previous work from the Mansky lab demonstrated that suppression of HDAC7, a class IIa HDAC, promoted osteoclastogenesis and increased osteoclast size, indicating HDAC7 is a repressor of OCL differentiation. Interestingly, suppression of HDAC3, a class I HDAC, had the opposite effect of HDAC7 suppression, leading to reduced osteoclast number and size. Additionally, another class IIa HDAC, HDAC9, has also been shown to represses osteoclast differentiation through a mechanism distinct from HDAC7's role in OCL differentiation, indicating that the remaining class II HDACs may also play separate and pivotal roles in osteoclastogenesis.

The field of orthodontics applies forces to teeth in order to move them within bone. Once

force is applied, fluid is expressed from the periodontal ligament (pdl) causing the tooth to displace within the pdl space compressing the ligament in some areas and stretching it in others. During this time, there is an increase in RANKL and osteoprotegrin (OPG) in the gingival crevicular fluid surrounding teeth. This suggests the pdl cells under stress induce formation of osteoclasts via upregulation of RANKL[11]. Advances in technology have lead to several new methods geared toward shortening treatment time by accelerating orthodontic tooth movement. Orthodontists may look to bone biologists for answers in the future about the use of HDACs to impact the bone remodeling process.

This study is able to conclude that osteoclasts with reduced HDAC9 expression are enhanced in differentiation and have increased activity. Additionally, osteoclasts with reduced HDAC11 expression are enhanced in differentiation and have increased activity. Further research will help us better understand the mechanism by which HDAC9 and HDAC11 inhibit osteoclast differentiation. Our experiments used cultured cells in vitro and are thus not able to definitively state HDAC 9 and 11's role in osteoclast differentiation and activity. It has already been shown by others that loss of HDAC9 in a mouse model leads to enhanced osteoclast differentiation; however, neither my study nor the animal model was able to determine if HDRP or HDAC9 is the critical protein necessary in regulating osteoclast differentiation. To conclusively determine HDAC 11's role, it would require engineering a mouse species null for HDAC 11 expression in osteoclasts. Experiments are still ongoing in the Mansky lab to help us answer these questions.

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