Glutamine metabolism underpins osteoclastogenesis and bone resorption

Guoli Hu, Yilin Yu, Robert Tower, Courtney M. Karner
University of Texas Southwestern Medical Center

Disclosures: Guoli Hu (N), Yilin Yu (N), Robert Tower (N), Courtney M. Karner (N).

ABSTRACT INTRODUCTION: Osteoclasts are bone resorbing cells that are essential to maintain skeletal integrity and function. Excessive osteoclast activity causes both age-related and pathological bone loss associated with several diseases including osteoporosis. While many of the growth factors and molecular signals that govern osteoclastogenesis are well studied, a large gap in our knowledge exists about the role and regulation of cellular metabolism during osteoclastogenesis. Osteoclast differentiation is associated with increased glucose uptake and glycolysis, increased fatty acid oxidation and increased mitochondrial biogenesis. Despite this, little is known about how the intracellular metabolome changes during osteoclast differentiation and how the metabolism of individual nutrients impacts the osteoclast metabolome. Advances in our understanding of differentiation associated metabolic changes will aid in identifying metabolic vulnerabilities that can be exploited therapeutically to reduce bone resorption and pathological bone loss.

METHODS: Cell culture experiments were performed in primary bone marrow macrophages (BMM). BMMs were isolated from 2-4 month old mice and expanded in αMEM containing 25ng/mL M-CSF, and then cultured with 25ng/mL M-CSF and 40ng/mL RANKL for up to 5 days to undergo osteoclastogenesis. TRAP staining was performed with an acid phosphatase leukocyte diagnostic kit. Protein and mRNA expression were evaluated by Western blot or qPCR respectively. Bone resorption was analyzed using Pit assay where BMM were differentiated on bovine bone slices for 6 days. Bones slices were fixed with 2.5% glutaraldehyde and stained with 1% toluidine blue. Glutamine metabolism was analyzed in BMMs induced to differentiate for 0, 2 or 4 days by incubating cells in either 2 mM [$^{13}C_5$]-glutamine or 2mM [$^{15}N_\alpha$]-glutamine for 6 hours followed by GC/MS. Stable isotopomer distributions were measured and corrected for natural abundance. Metabolite concentrations were quantified using LC-MS/MS and normalized with DNA concentration. Mice with a floxed *Gls* allele (*Gls*^{fl}) were crossed to either the *LysMCre* or *Csfr1CreERT2* deleter strains to conditionally ablate *Gls* in myeloid lineage or committed osteoclast precursors respectively. Bone phenotypes were analyzed using μCT and histological analysis. Ovariectomy (OVX) or sham surgery were performed in10-week-old female mice. Statistical significance was determined by paired 2-tailed Students *t* test or Ordinary one-way ANOVA with Tukey's multiple comparisons. $p<0.05$ was considered statistically significant. The animal studies committee at the University of Texas Southwestern Medical Center approved all mouse procedures.

RESULTS: Osteoclast differentiation is characterized by broad metabolic changes including increased abundance of amino acid and nucleotide metabolites and reduced abundance of metabolites in energetic. Maintenance of the osteoclast metabolic signature is governed by the increased consumption and metabolism of glutamine. Inhibiting glutamine metabolism specifically in mature osteoclasts resulted in a complete loss of the osteoclast metabolic signature and inhibited bone resorption in the pit assays. Highlighting the specific requirement for glutamine metabolism to maintain the osteoclast metabolic signature, inhibiting either glycolysis or fatty acid oxidation did not affect metabolite abundance in mature osteoclasts. Mechanistically, osteoclasts rely on glutamate transamination to couple the anabolic utilization of glutamine carbon and nitrogen for amino acid and nucleotide biosynthesis to support osteoclast differentiation and bone resorption. Importantly, genetic ablation of glutamine metabolism in either myeloid lineage cells or osteoclast precursors inhibited osteoclastogenesis and bone resorption resulting in increased bone mass. Conversely, genetically increasing glutamine metabolism in myeloid cells enhanced osteoclastogenesis and resulted in accelerated bone loss. Highlighting the therapeutic implications of these findings, genetically inhibiting glutamine metabolism prevented ovariectomy induced bone loss in mice.

DISCUSSION: Here we present data that glutamine metabolism is a metabolic lynch pin in osteoclasts and is essential to maintain homeostatic concentrations of many metabolites and is essential for both osteoclastogenesis and bone resorption. Our data suggest that osteoclasts are like cancer cells which have increased reliance on glutamine metabolism. Targeting glutamine dependency using Telaglenastat is currently being developed as a potential cancer treatment. It may be possible to repurpose these strategies to develop new pharmacological agents to treat diseases associated with excessive bone resorption.

SIGNIFICANCE: Genetic inhibition of GLS dependent glutamine metabolism reduces osteoclast differentiation and bone resorption and prevents ovariectomy induced bone loss. Thus, our data strongly suggest that manipulation of glutamine metabolism may provide a novel therapeutic approach to limit excessive or pathological bone resorption.

ACKNOWLEDGEMENTS: This work was supported by NIH R01 grant AR071967.

IMAGES:

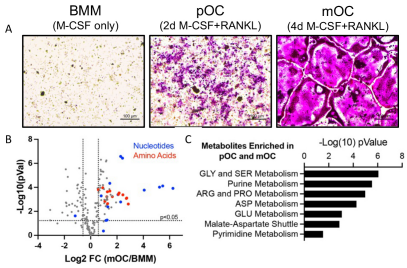

Figure 1: Intracellular amino acids and nucleotides increase during osteoclast differentiation. (A) TRAP staining of osteoclast differentiation *in vitro*. **(B)** Volcano plot of all metabolites detected in primary bone marrow macrophage (BMM) and mature Osteoclast (mOC). The x-axis is the mean ratio and y-axis is the *p* value between mOC and BMM. **(C)** Graph showing metabolic pathways that are significantly enriched in both pOC and mOC compared to BMM. Data represent the mean from n=4 independent experiments. FDR≤0.05.

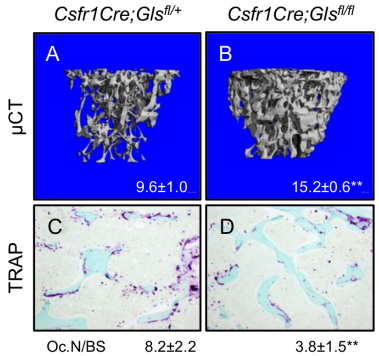

Figure 2: *Gls* is required cell autonomously in osteoclast precursors to regulate bone mass in mice. Representative μCT images **(A-B)** or TRAP staining **(C-D)** of distal femur trabecular bone in 2-month-old *Csfr1CreERT2;Gls*$^{fl/+}$ (Wild Type) **(A,C)** or *Csfr1CreERT2;Gls*$^{fl/fl}$ **(B,D)** female mice (N=7). Similar results were observed in male mice. Mice were injected with tamoxifen once daily five times beginning at 1-month of age and bone phenotypes were evaluated 1 month later. **$p\leq0.01$.

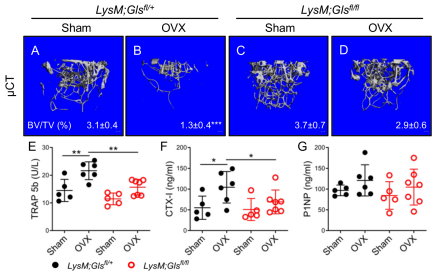

Figure 3: Knockout of GLS in osteoclasts prevents ovariectomy induced bone loss. Ovariectomy (OVX) performed on 10-week-old female mice and bone phenotypes were analyzed 5 weeks later. **(A-D)** Representative μCT images of distal femur trabecular bone in *LysMCre;Gls*$^{fl/+}$ (Wild Type) **(A-B)** or *LysMCre;Gls*$^{fl/fl}$ **(C-D)** female mice (N=6). **(E-G)** Quantification of serum ELISA for markers of osteoclasts (TRAP5b), bone resorption (CTX-1) or bone formation (P1NP). * $p<0.05$, ** $p<0.01$, *** $p<0.001$.