detectable in the medium of vehicle- or NS398-treated cultures. PTH induced *RANKL* expression and formation of osteoclastlike cells in hBMSCs. As seen in murine BMSCs, PTH stimulated OB differentiation only in NS398- or OPG-treated hBMSCs. Genes reported to mediate the anabolic effects of PTH–*IGF1*, *BMP2*, *RUNX2* and *WNT10B*– were elevated by PTH only in OPG- or NS398-treated cultures. The WNT antagonist *DKK1* was inhibited by PTH only in OPG- or NS398-treated cultures. To assess secretion of SAA from preosteoclasts, marrow was expanded with hM-CSF to make bone marrow macrophages (hBMMs), which were treated with RANKL to stimulate osteoclastogenesis. RANKL increased the secretion of SAA1 and SAA2 on days 4-5 of culture prior to the appearance of TRAP+ multinucleated osteoclastlike cells on day 7. RANKL did not increase *SAA1* and *2* expression in NS398-treated cultures. Conditioned medium from RANKL treated hBMMs, but not from RANKL+NS398 treated hBMMs, added to hBMSCs prevented PTH-stimulated OB differentiation. Addition of recombinant SAA1 and SAA2 to hBMSCs in the presence of OPG inhibited PTH-stimulated OB differentiation. We conclude that SAA is a novel means by which preosteoclasts can inhibit PTH-stimulated OB differentiation in hBMSCs. Because SAA proteins can be elevated to high levels during inflammation, they may mediate bone loss associated with inflammation.

***Disclosures: Shilpa Choudhary**, None.*

SU0153

Beneficial Effects of Low Doses of the Phytoestrogen Quercetin on Osteoblastic Cells. Virginia Lezcano*[1], Lilian I Plotkin[2], Susana Morelli[3]. [1]INBIOSUR UNS, Argentina, [2]Department of Anatomy & Cell Biology,Indiana University School of Medicine, Indianapolis, IN, Roudebush Veterans Administration Medical Center, Indianapolis, IN., United states, [3]INBIOSUR (UNS-CONICET) Departamento de Biol., Bioq. y Fcia., Universidad Nacional del Sur, Bahía Blanca, Argentina., Argentina

Currently, there is a global trend to use natural bioactive compounds such as phytoestrogens (PEs), present in a wide variety of foods, for their beneficial biological effects demonstrated *in vitro* and *in vivo* including antioxidant, anti-inflammatory, anticancer, and antidiabetic activities. PEs are plant-derived non-steroidal compounds that bind to estrogen receptors and have estrogen-like activity. Given that the increase in life expectancy of the population has led to bone health becoming a major concern, in this work we investigated the effects of the PE quercetin (QUE) on the estrogen receptor-positive murine osteoblastic cell line MC3T3-E1. A dose dependent effect of QUE was observed on cell viability after 48 h of exposure, determined by MTS assay; with inhibition of cell viability at 20-100 μM concentrations and no change at lower concentrations. In parallel, by trypan blue assay a significant increase in cell number was obtained at 1 μM QUE. The wound healing assay show that low doses of QUE stimulate osteoblastic cell migration, with a significant closure at 12h, which further increases after 24h of treatment. Cell migration and proliferation are specific cell functions that require cell attachment and spreading. Using a cell adhesion assay we found a 60% increase in cellular adhesion when cells were treated with 1 μM of QUE, and no changes were observed with higher concentrations. QUE is generally considered to have strong antioxidant potency and provides protection against oxidative injury in cultured cells. We found that the pretreatment with 1 or 10 μM of QUE for 48h protects against H_2O_2-induced toxicity in MC3T3-E1 cells. Altogether, these results indicate that the beneficial effects of QUE on bone formation cells are observed at low doses while high doses of QUE have shown to be deleterious for MC3T3-E1 cells. Furthermore, QUE at high doses increases Erk1/2 and decreases Akt activation, with the consequent increase in the levels of active pro-apoptotic protein BAD, as assessed by Western blot analysis; and blockade of Erk1/2 activity with PD98059 decreases cell death induced by QUE. Based on these findings, we conclude that QUE has positive effects on migration, proliferation, adhesion and antioxidation of osteoblastic cells when it is used at doses lower than 20 μM; and may be consider a potential natural therapeutic alternative for bone healing repair in osteopathologies.

***Disclosures: Virginia Lezcano**, None.*

SU0154

Decreased bone density and osteoblast activity in Rad-null mice. Catherine Withers*, Jonathan Satin, Douglas Andres. University of Kentucky, United states

Rad GTPase, a member of the RGK (Rem, Rad, Gem/Kir) subfamily of Ras-related small G-proteins, has primarily been studied as a potent negative regulator of L-type calcium channel current in the heart, but the role of Rad in bone has not been evaluated. Rad-null mice are smaller in size than wildtype littermates from birth through adulthood, and Rad loss has no effect on gross skeletal development. Microcomputed tomography analysis indicates that Rad depletion leads to a significant reduction in both cortical and trabecular bone density. The total cross-sectional area and the medullary area of Rad-null femurs are larger than wildtype, while the cortical bone area and cortical thickness are significantly reduced and the cortical porosity is significantly higher in Rad-null femurs. Trabecular bone density is also significantly reduced, with Rad-null femurs exhibiting a decrease in trabecular bone volume fraction, a decrease in trabecular number, and an increase in trabecular spacing. Dynamic histomorphometric analysis of Rad-null femurs indicates that the periosteal bone formation rate is slower and the periosteal mineralizing surface is smaller in the absence of Rad. Tartrate-resistant acid phosphatase staining of osteoclasts is not different in wildtype and Rad-null femur sections, suggesting that the deficits in bone density in Rad-null mice are not due to increased bone resorption. Conversely, expression of osteoblast marker genes is lower in the absence of Rad, suggesting that the bone deficits observed in Rad-null mice may instead be due to depressed bone formation by osteoblasts. In this vein, primary osteoblasts isolated from Rad-null mice exhibit reduced calcium deposition compared to wildtype osteoblasts by alizarin red staining. Together, these data suggest that Rad GTPase is a novel signaling protein involved in osteoblast differentiation and activity.

***Disclosures: Catherine Withers**, None.*

SU0155

Identification of a chemical compound that stimulates osteoblast differentiation and inhibits osteoclast differentiation. Ju Ang Kim*, Young-Ae Choi, Yong Chul Bae, Hong-In Shin, Eui Kyun Park. Kyungpook National University School of Dentistry, Korea, republic of

Through screening to isolate small molecule that controls bone formation and bone resorption, we found that some compounds strongly stimulated osteoblast differentiation and mineral deposition in human bone marrow mesenchymal stem cells (BMSCs), and inhibited osteoclast differentiation of mouse bone marrow cells. Of some positive compounds, KR-34A induced mineral deposition in a dose dependent manner, and expression of osteoblast marker genes such as *ALP*, *COL1*, *ON*, *OPN*, and *OC*. Analysis of transcription factors that control osteoblast differentiation showed that expression of *RUNX2* and *SP7* were induced by KR-34A. KR-34A also induced transcriptional activation and nuclear translocation of Runx2. In addition, KR-34A induced phosphorylation of p38MAP kinase and ERK, and KR-34A-induced mineral deposition was dramatically inhibited by MEK inhibitors, PD98059 and U0126. U0126 also inhibited expression of RUNX2. Thus, KR-34A stimulation of osteoblast differentiation is medicated through expression, nuclear translocation and transcriptional activation of Runx2 by ERK signaling pathway. On the other hand, KR-34A dose-dependently inhibited osteoclast differentiation as assessed by TRAP staining and activity. Expression of osteoclast marker genes was also significantly inhibited by KR-34A in a dose dependent manner.

Taken together, our data demonstrate that KR-34A stimulates osteoblast differentiation through RUNX2, and inhibit osteoclast differentiation. The data presented in this study strongly suggest that KR-34A has both anabolic and antiresorptive activities, and thus potential candidate molecule for intervention of skeletal disease including osteoporosis, arthritis and ostelysis.

***Disclosures: Ju Ang Kim**, None.*

SU0156

Igfbp2, Inhbb and Sema4f are Wnt3a-inducible in Osteoblasts, Independent of Lrp5/6 receptors. Aimy Sebastian*[1], Nicholas R. Hum[2], Deepa K. Murugesh[2], Sarah Hatsell[3], Aris N. Economides[3], Gabriela G. Loots[2]. [1]UC Merced, School of Natural Sciences, United states, [2]Lawrence Livermore National Laboratories, Physical & Life Sciences Directorate, United states, [3]Regeneron Pharmaceuticals, United states

Wnt signaling is a major regulator of bone metabolism yet, very little is known about the target genes regulated by LRP5/6 mediated Wnt signaling in osteoblasts. To identify genes regulated by canonical Wnt signaling, neonatal calvarial osteoblasts (OB) isolated from *C57Bl6* (*WT*), *Lrp5* knockout ($Lrp5^{KO}$) and *Lrp5/6* double knockouts ($Lrp5/6^{KO}$) were treated with Wnt3a for 24 hrs and gene expression changes were quantified by RNA-Seq. *Lrp5/6* deficient OBs were generated by treating $Lrp5^{flox/flox}$; $Lrp6^{flox/flox}$; *UBC-CreERT2* OBs with tamoxifen *in vitro; Lrp5/6* expression was 30% of *WT* levels, in these OBs. In *WT* OB we identified 257 genes including several Wnt pathway members such as *Porcn*, *Fzd1*, *Lef1*, *Tcf7* and *Axin2* as up-regulated and 359 genes including master regulator of chondrogenesis *Sox9* and adipogenesis regulator *Pparg* as down-regulated in response to Wnt3a treatment. Comparison of Wnt3a targets with gene expression data from different stages of osteoblast differentiation suggested that majority of the genes up-regulated by Wnt3a are highly expressed during early stages of osteogenic differentiation and possibly involved in the regulation of osteoblast proliferation/differentiation. Consistent with this, MC3T3 pre-osteoblastic cells treated with Wnt3a displayed a significant increase in proliferation. By comparing $Lrp5^{KO}$ to *WT* OB we identified 146 genes up and 498 genes down-regulated in $Lrp5^{KO}$ OB. Only 15 genes identified as Wnt3a targets in *WT* OB showed >1.5 fold up or down-regulation in $Lrp5^{KO}$ OB relative to *WT* OB. Next, we compared Wnt3a treated $Lrp5^{KO}$ OBs to sham treated *WT* OBs and found that 76% (469/616) of the Wnt3a targets were >1.5 fold up or down-regulated in $Lrp5^{KO}$ OBs in response to Wnt3a treatment, suggesting that Wnt3a mediated signaling is largely unaffected by the loss of Lrp5 in osteoblasts. We identified 162 genes differentially regulated between $Lrp5/6^{KO}$ OB and *WT* controls. These genes include 33 Wnt3a targets including *Porcn*, *Tcf7* and *Axin2*. Only 34 genes were found to be >1.5 fold up or down-regulated between Wnt3a treated and sham treated $Lrp5/6^{KO}$ OBs. Interestingly, Wnt3a treatment differentially regulated 15 Wnt3a targets including *Igfbp2*, *Inhbb*, and *Sema4f* in $Lrp5/6^{KO}$ OB possibly through an Lrp5/6 independent mechanism, which requires further validation. Altogether, this study has identified several novel Wnt target genes and provides some new insights into Lrp5/6 dependent Wnt signaling in osteoblasts.

***Disclosures: Aimy Sebastian**, None.*