Connexin 43 in Osteocytes but not Osteoblasts Regulates Osteoclast Formation and Bone Resorption via the RANKL Pathway

Zhao, Y.; Paul, E.M.; Grigoryeva, L; Donahue, H. J.
Division of Musculoskeletal Sciences, Department of Orthopaedics and Rehabilitation, Penn State College of Medicine, Hershey, PA 17033.
yzhangle@psu.edu

INTRODUCTION:
Normal remodeling of bone requires synchronized activity between bone resorbing osteoclasts and bone forming osteoblasts. Emerging data suggest that osteocytes coordinate osteoclast and osteoblastic activity via connexins (Cx) and gap junctions. Gap junctions are membrane spanning channels that allow passage of ions and signaling molecules, less than 1KD in size, between two adjacent cells. Each gap junction is composed of 2 hemichannels or connexons and each connexon is comprised of 6 connexins. The predominant form of connexin in osteoblasts, osteoclasts and osteocytes is Cx43. To study the in vivo function of Cx43, we bred osteoblasts/osteocytes specific Cx43 deficient mice. Our previous results showed that Cx43 knockout (KO) mice have osteopenic bones in the presence of increased bone resorption. This suggests that Cx43 deficiency in mouse osteoblasts or osteocytes increases osteoclastic bone resorption. To address this we examined the hypothesis that osteocytes from Cx43 deficient mice have an altered RANKL/OPG expression ratio that results in altered osteoclastic resorption.

METHODS:
Cx43 knockout mice and morphology studies: Osteoblast/osteocyte specific Cx43 knockout (KO) mice were generated by breeding mice expressing Cre recombinase under the control of the human osteocalcin promoter (OC-Cre) with mice in which the Cx43 gene is flanked by two loxP sites (Cx43fl/fl). Femoral midshaft density was obtained by microCT (Scanco VivACT 40). Serum bone formation and resorption markers PINP and CTX were measured with ELISA kits. For histological studies, mice femoral sections were stained with TRAP and counter stained with methyl green. TRAP positive cells with more than three nuclei were counted as osteoclasts.

Cell culture and gene expression: Osteoblasts were obtained by culturing chips from WT or KO mouse long bones in αMEM with 15% FBS and ascorbic acid. Osteocytic MLO-Y4 cells were cultured in αMEM with 5% FBS and 5% CBS. Cx43 mRNA levels were decreased in MLO-Y4 cells with Cx43 siRNA and 48 hrs after siRNA transfection, total RNA was collected and Cx43, RANKL and OPG gene expression quantified by real time RT-PCR.

RESULTS:
Our breeding strategy resulted in litters with Cx43 deleted from osteoblasts/osteocytes (Cx43 KO), and wild type (WT) mice. Previously, we reported that microCT of humeri revealed that bones from KO mice were osteopenic, relative to WT. KO mice displayed significant periostal expansion, cortical thinning, increased porosity, and reduction in bone mineral density and bone volume over total volume. However, dynamic histomorphometry showed KO mice have a moderate but not statistically significant increase in bone formation and bone mineral apposition rates, at least 8 weeks of age. Interestingly, TRAP staining revealed that KO mice have more osteoclasts in distal and cortical femurs than WT mice (Figure 1).

KO mice also displayed increased serum levels of the bone resorption marker CTX, but no significant change in levels of the bone formation marker PINP, relative to WT mice. These data suggest that ablation of Cx43 in mouse bone cells leads to osteopenic bone due to increased bone resorption. To investigate the mechanism underlying the phenotype of Cx43 KO mice, we examined whether osteocytic Cx43 gap junctions may affect osteoclastogenesis. Our data revealed that decreasing Cx43 expression in MLO-Y4 cells with Cx43 siRNA increases the MLO-Y4 RANKL/OPG expression ratio (figure3). Interestingly, RANKL and OPG expression ratios were not changed in primary osteoblasts cultured from KO mice compared to osteoblasts from WT mice, suggesting that Cx43 in osteocytes but not osteoblasts regulates osteoclast formation and bone resorption via the RANKL pathway (figure 2).

Figure 2. Osteoclasts from KO mice showed decreased Cx43 mRNA (A) but not RANKL/OPG mRNA ratios (B). However, decreasing Cx43 in osteocytic MLO-Y4 cells (C), increased RANKL/OPG expression ratio (D). N=10. * p<0.05.

DISCUSSION:
Our group previously demonstrated that shear stress stimulated MLO-Y4 cells to increase osteoclastic hFOB differentiation via gap junctional intercellular communication (GJIC). Here we show that Cx43 in osteocytes regulates osteoclast formation and bone resorption by altering the osteocytic RANKL/OPG expression ratio. Thus the effect of MLO-Y4 on osteoclastogenesis occurs independent of GJIC between osteocytes and osteoclasts as the change in RANKL/OPG expression ratio occurred in MLO-Y4 cells in monolayer culture. More importantly, our data show decreasing Cx43 in osteoblasts does not change RANKL/OPG expression, which suggests that Cx43 in osteoblasts regulate osteoclasts formation through pathways other than RANKL or may not regulate osteoclastic formation at all. It has been well accepted that osteocytes are the mechanosensors in bone and they can regulate osteoblast differentiation upon mechanical stimulation. Previous data suggest that fluid flow increases Cx43 phosphorylation and GJIC in MLO-Y4 cells, and decreases MLO-Y4 cell induced osteoclastogenesis by increasing soluble RANKL/OPG ratios. Taken together, these data suggest that Cx43 in osteocytes but not osteoblasts regulates both bone formation and resorption via RANKL pathway.

ACKNOWLEDGEMENT: This work was supported by the Pennsylvania State Tobacco Settlement Formula Fund, NIH AG13087-11 to HJD and NIH 1R03AR057546-01 to YZ.

REFERENCES: