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COMMENTARY

Mef2c does more than regulate Sost in osteocytes: distinct gender effects

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Sclerostin, the product of the *Sost* gene, has proved to be a potent inhibitor of bone formation, and neutralizing antibody to this protein is proving to permit impressive bone formation. Neutralizing antibody not only has the potential to treat diseases of bone loss, such as osteoporosis, but also has orthopedic applications to reverse the effect of immobilization/disuse on bone mass, promote acceleration of bone fracture healing and increase fixation of implants and screws.[1] Recent human phase 1 trials showed a gain in bone mineral density greater than teriparatide.[2] Neutralizing antibody to sclerostin has been shown to act by directly stimulating bone formation, modeling, whereas other anabolics such as PTH stimulate remodeling.[3] Therefore, discovery and identification of regulators of *Sost* gene expression in addition to regulation of sclerostin protein expression/activity could have potential with regards to generation of additional therapeutics for prevention and treatment of bone loss/bone healing.

In a search for regulators of *Sost* gene expression, it was discovered that myocyte enhancer factor 2, Mef2c, transcription factor, bound to an enhancer of the *Sost* gene and that siRNA-mediated knockdown of the Mef2 family reduced *Sost* expression in UMR106 cells.[4] Of the Mef transcription factors, Mef2c was found to be most highly expressed in osteocytes followed by Mef2a and Mef2d, whereas Mef2b was not detectable. The Mef2c loci was identified as one of the 20 loci important in regulating bone mineral density.[5] As Mef2c appeared to be a significant inducer of sclerostin expression, an obvious question was whether reduction of Mef2c can also be a strategy to increase bone mass through the reduction of *Sost* gene expression.

A series of elegant *in vivo* experiments were performed by Kramer *et al.*[6] to answer this question. In this study, the investigators compared two mouse models; heterozygotes with global deletion of one *Sost* allele and a model of targeted deletion of *Mef2c* in late osteoblasts/early osteocytes using the Dmp1-Cre mouse. Adult mice from 3 to 6 months of age were characterized. As expected, reduction in *Mef2c* lead to a reduction in *Sost* expression in osteocytes, and as expected both models displayed increased bone mass and density. In the *Sost* heterozygotes, an increased osteoblast mineral apposition rate with an unchanged osteoclast surface was observed similar to but to a lesser extent than shown previously with *Sost* homozygous nulls.[7] In contrast, no differences were observed in mineral apposition rate in the *Mef2c*-deficient mice but a significant decrease in bone resorption parameters was observed. Further examination showed an increase of Sfrp2, Sfrp3 and osteoprotegerin (OPG) in the *Mef2c*-deficient mice that was not observed in the *Sost* heterozygotes where these factors remained at normal levels. Therefore, the molecular mechanisms responsible for the increase in bone mass were distinctly different in each model.

The obvious question is why did the reduction in *Sost* expression in the *Mef2c* deficient model not result in an increased mineral apposition rate as observed in *Sost* null animals? The Mef2c could be regulating a *Sost* antagonist responsible for neutralizing the effect of downregulation of Sost on mineral apposition rate. A decrease in the numbers of osteoclasts was observed in the targeted deletion of *Mef2c* model. The decrease in parameters of osteoclast activity could be explained by Mef2c acting as a transcription factor for genes that target/regulate osteoclast activity. Also, the *Mef2c* was targeted for deletion in osteocytes whereas the *Sost* deficiency was global. As Sost expression has also been identified in hypertrophic chondrocytes,[8] could this cell type be having a major role in regulating bone formation? To test this hypothesis, targeted deletion of *Sost* would need to be performed using an osteocyte- and a chondrocyte-specific Cre crossed with floxed *Sost*. Regardless of whether a major target of *Mef2c* is *Sost*, this study opens the door to other potential means to regulate bone mass through targeting expression in the late osteoblast/osteocyte.

Another unexpected observation in the present study was distinct gender differences, not only in the *Mef2c*-deficient mice but also in the *Sost*-deficient mice. Surprisingly, *Mef2c*-deficient males had greater increases in bone mass compared with females, but the opposite was observed in the *Sost*-deficient animals. A gender effect had also been previously described in *Sost* homozygous nulls (see figures 3 and 4 in Li *et al.*[7]) and was also observed and validated in the *Sost* heterozygotes in the present study. It was suggested that the gender-specific differences could be partially due to

OPG expression as no upregulation of OPG could be observed in female *Mef2c*-deficient mice compared with male mice. The investigators could not rule out the differences in OPG expression with the 20% reduced expression of *Mef2c* in females as compared with males. In contrast, OPG expression was reduced in female *Sost* hets, but not in males. Regardless of the mechanism, these studies suggest that the late osteoblast/osteocyte may have a role in gender differences in bone mass.

In summary, Mef2c function is obviously important in regulating bone mass, even if regulation of the *Sost* gene does not appear to be a major target. Second, both genes have gender effects, but through potentially different mechanisms. Third, gender-specific differences in bone mass may be regulated by osteocytes. It will be important to address these observations, especially with regards to development of potential therapeutics with gender-specific effects. Clearly, reduction of Mef2c can be a strategy to increase bone mass, but through other mechanisms than reduction of *Sost* gene expression.

Conflict of Interest

The author declares no conflict of interest.

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