Osteoblast-specific overexpression of amphiregulin leads to transient increase in cancellous bone mass in mice

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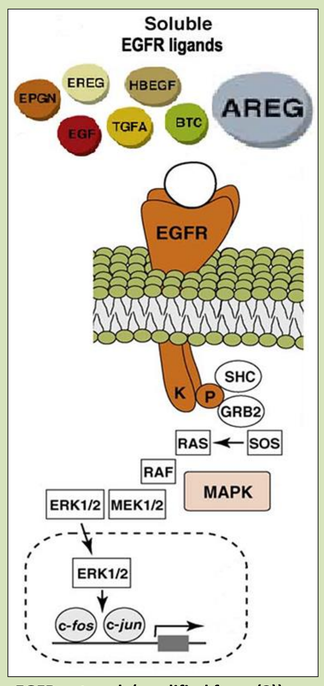
Introduction

The Epidermal Growth Factor (EGF) Receptor family comprises four transmembrane glycoproteins with tyrosine kinase activity, namely, EGFR/ERBB1, ERBB2, ERBB3 and ERBB4, which are expressed in the epithelial, mesenchymal and other cell lineages. These receptors recognize and bind to several peptide ligands (3). Although EGFR and its ligands are known to be physiologically expressed in the skeletal cells, their functions in the bone are poorly defined.

Amongst the various EGFR ligands expressed in the bone, one of the most important is amphiregulin (AREG). AREG is known to stimulate osteoblast proliferation and inhibit their differentiation and mineralization (5). AREG knockout mice show a significantly reduced tibial trabecular bone (5). In rats, it has been published that AREG is a PTH-regulated gene, both in vitro (UMR 106-01 cells) and in vivo (4,5). Several lines of evidence in the literature suggest that the effects of intermittent PTH on bone cells may be mediated, at least in part, via the AREG-EGFR signaling pathway.

AIM

To better understand the role of AREG in bone biology, we aimed to characterize the detailed bone phenotype of a transgenic mouse line overexpressing AREG in skeletal cells.



EGFR network (modified from (2))

Material and Methods

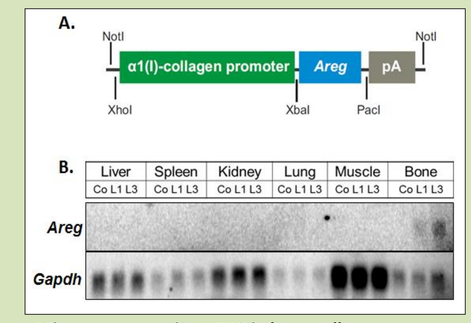
- Transgenic mice overexpressing AREG in bone cells under the osteoblast-specific murine 2.3 kb collagen α1(I) promoter (1) were generated in an inbreed background (FVB/N).
- 4w, 8w, 10w, 5m and 18m-old male AREG-tg mice and their wild-type littermates were employed in the study.
- Bone phenotype of femur and fourth lumbar vertebra was analyzed by pQCT and micro-CT.
- Trabecular histomorphometry of femur and first lumbar vertebra was performed.
- *In vitro* proliferation and differentiation of osteoblasts isolated from neonatal mouse calvariae was studied.

Confirmation of AREG Overexpression in Bone

Northern blot confirmed bone-specific overexpression of AREG in two independent mouse lines (Line 1 and Line 3).

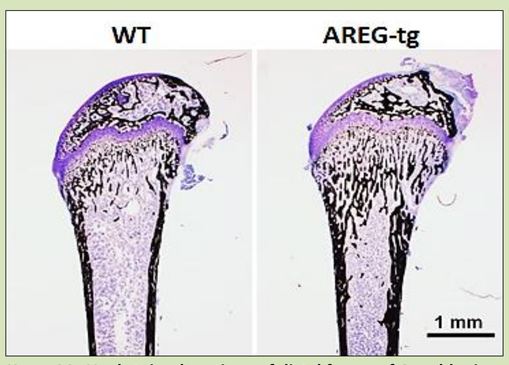
L1 mouse line was used for all experiments described here.

AREG-tg mice did not show any changes in body weight or gross phenotype compared with the WT controls.

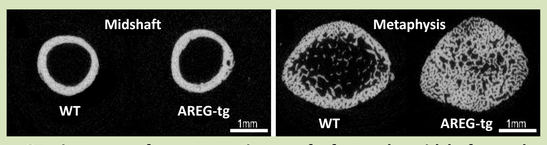


Mice overexpressing AREG in bone cells

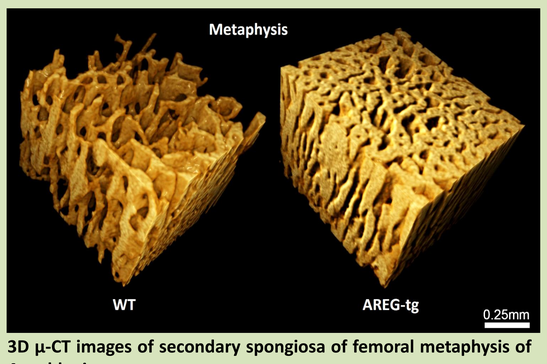
Overexpression of AREG leads to a transient increase in femoral trabecular bone mass



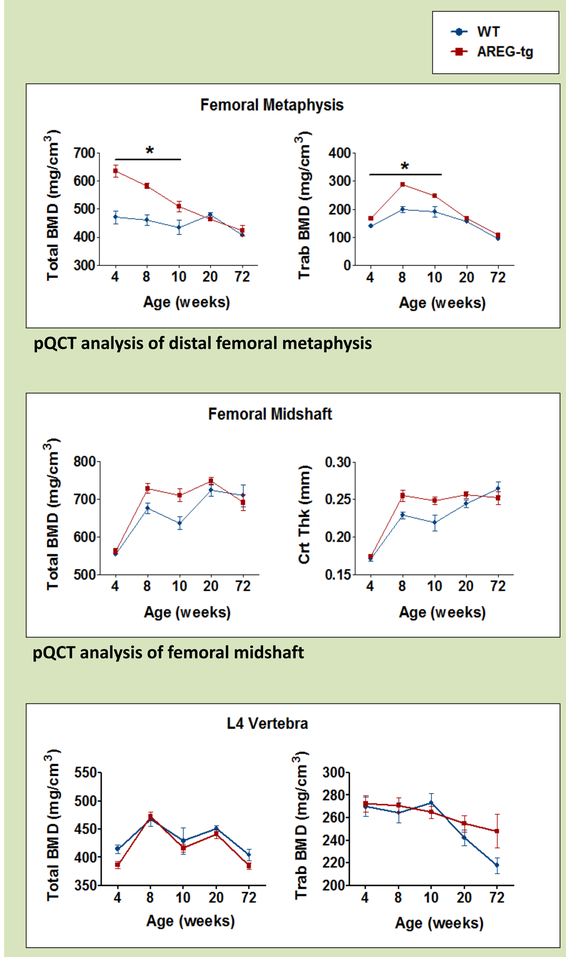
Kossa Mc Neal stained sections of distal femur of 4w-old mice



µ-CT images of cross-sections of femoral midshaft and metaphysis of 4w-old mice



3D µ-CT images of secondary spongiosa of femoral metaphysis of 4w-old mice



pQCT analysis of distal femoral metaphysis

pQCT analysis of femoral midshaft

pQCT analysis of L4 vertebra

Histology of the distal femur showed an increase in trabecular bone in 4w-old AREG-tg mice as compared to the WT controls.

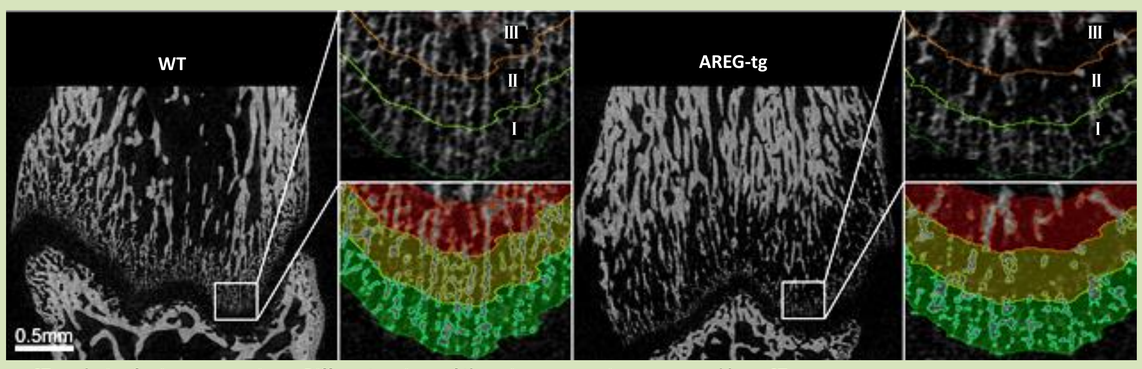
µ-CT analysis confirmed a higher trabecular bone mass and increased trabecular thickness in the distal femoral metaphysis of 4w-old AREG-tg mice.

pQCT analysis of the distal femoral metaphysis showed a significantly higher trabecular volumetric bone mineral density (BMD) in 4w, 8w and 10w-old AREG-tg mice.

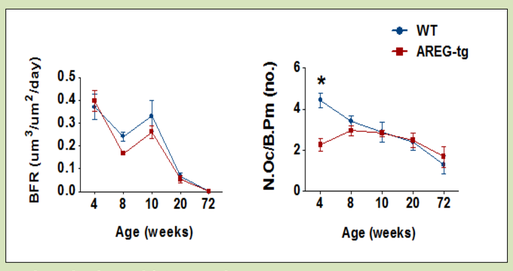
However, the high bone mass phenotype in AREG-tg mice was transient and completely disappeared in 5m and 18m-old mice.

No significant changes were observed in the femoral midshaft and in the L4 vertebrae of AREG-tg mice at all time points.

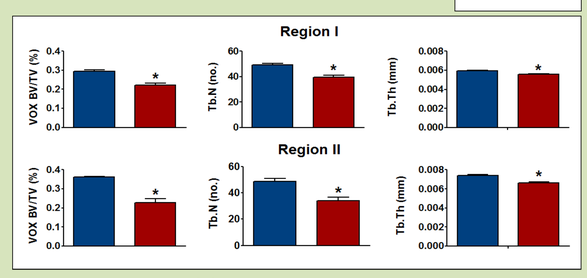
AREG overexpression reduces osteoclast number without affecting bone formation



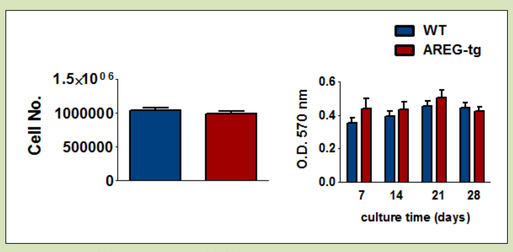
µ-CT analysis of primary spongiosa : Different regions of the primary spongiosa measured by µ-CT



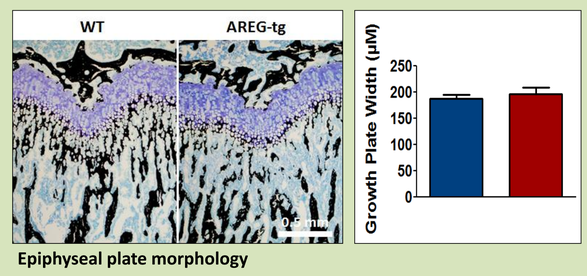
Trabecular bone histomorphometry



µ-CT measurements of the primary spongiosa



In vitro proliferation and differentiation assays



Epiphyseal plate morphology

Trabecular bone histomorphometry in the distal femoral metaphysis showed unchanged bone formation rate (BFR) in AREG-tg mice versus the WT controls. However, osteoclast number (Oc.N) was significantly decreased in 4w- and 8w-old AREG-tg bones. No changes in bone turnover were observed between the genotypes in 10w-, 5m- and 18m-old mice and in L4 vertebra at all time points (data not shown).

In vitro assays showed no differences in proliferation and osteogenic differentiation of AREG-tg osteoblasts, indicating that the phenotype was non-cell autonomous.

No morphological alterations were observed in the epiphyseal plate of the AREG-tg mice. The growth plate width of the AREG-tg mice was comparable to the WT controls.

µ-CT analysis of the primary spongiosa did not provide evidence of changes in number or thickness of the calcified cartilage spicules immediately below the distal femoral growth plate (region I) in AREG-tg mice (data not shown). However, AREG-tg mice had decreased bone volume and lower trabecular number and thickness than the WT controls in the regions II and III at further distances from the growth plate.

Conclusions

Our data suggest that AREG overexpression in osteoblasts leads to a transient increase in trabecular bone mass of the appendicular skeleton by a growth-related, non-cell autonomous mechanism, leading to a positive bone balance with unchanged bone formation and lowered bone resorption. The molecular mechanism underlying the site-specific effect of osteoblastic AREG overexpression on bone mass remains to be clarified.

The authors state that they have no conflicts of interest.

References

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5. Qin L., Tamasi J., Raggatt L., Li X., Feyen J.H., Lee D.C., Dicicco-Bloom E. and Partridge N.C. (2005). J. Biol. Chem. 280, 3974-3981.

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