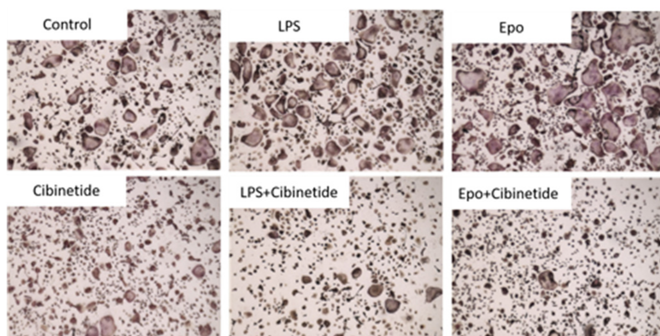
In vitro, LPS as well as Epo administration to bone marrow derived macrophages (BMDM) enhanced osteoclastogenesis, whereas cibinetide administration had the opposite effect in a dose dependent manner. Combining cibinetide with either LPS or Epo treatment inhibited osteoclastogenesis in BMDM, suggesting that cibinetide overrides the pro-osteoclastogenic effect of LPS and Epo (Figure 1).

Our findings demonstrate the increasing complexity of EpoR signaling in bone, and pave the way for clinical translation through potential combination therapy of Epo and cibinetide in anemic patients in an attempt to preserve the erythropoietic actions of Epo, while preventing/attenuating the associated bone loss.

Keywords: Erythropoietin, Cibinetide, Osteoclasts, BMDM.



Cibinetide counteracts the pro-osteoclastogenic effect of LPS and Epo *in vitro*.
BMDMs were treated with RANKL (50ng/ml) along with Epo (10U/ml), Cibinetide (150nM) or Epo+ Cibinetide. After 48 hours LPS (10ng/ml) was introduced to the RANKL or RANKL+ Cibinetide stimulated cultures. On the 4th day multinucleated osteoclasts were stained for TRAP.

Fig. 1.

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Noggin as a regulator of bone remodelling

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Bone Morphogenetic Protein 2 (BMP2) is used in orthopaedic surgery to promote bone healing. The endogenous synthesis of BMP-2 antagonist family members, however, may limit the efficacy of exogenous BMP2. Noggin is one of these inhibitors that blocks the effects of BMP on the differentiation and activation of osteoblast (OB) *in vitro* and *in vivo* and inhibits OB-mediated osteoclast (OC) development. Furthermore, Noggin was found to modulate osteoclastogenesis through a direct effect on OC lineage cells. The present study aimed at elucidating the underlying mechanisms of these effects. Direct (conventional culture dishes) and indirect (transwell culture dishes) co-cultures of murine OB/OPC (Osteoclast Progenitor Cells) and cultures of OPC alone were supplemented with combinations of Noggin, BMP2, L51P (engineered, inactive variant of BMP2) and DMH1 (BMP receptor 1 inhibitor). In cultures of OPC, Noggin but not DMH1 caused an increase in the number of OC by a factor of 3 ($p< 0.01$). This effect could not be reversed by BMP2 and L51P, respectively. In contrast, in co-cultures of OB/OPC, exposure to Noggin attenuated OC development. In direct co-cultures, this inhibitory effect of Noggin was blocked by BMP2 and L51P. In both direct and indirect co-culture systems, exposure to Noggin induced the release of GM-CSF, a potent inhibitor of osteoclastogenesis, by a factor of 6 and 4, respectively ($p< 0.01$). Treatment of the cultures with αGM-CSF Ab, however, restored OC development in the indirect co-culture system only. The data suggests a previously unknown function of Noggin directly acting pro-differentiation on OC lineage cells independently of BMP signalling. In co-cultures, besides GM-CSF, cell-cell contact between OB and OPC is required for mediation of the maximal inhibitory effects of Noggin on OC development. The nature of potential interaction partners for Noggin, however, remains to be elucidated.

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Bone remodeling mechanisms in osteoporotic TgRANKL transgenic mouse models

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Osteoporosis is a multifactorial metabolic disease which is characterized by low bone density, reduced bone quality, and increased risk of fractures. We have recently established genetic osteoporosis models by expression of human RANKL in transgenic mice (TgRANKL). To investigate the pathogenic mechanisms in modeled osteoporosis, we performed RNA sequencing analysis in flushed femurs from TgRANKL mice and wild-type (WT) controls. Our analysis identified 7464 differentially expressed genes and among them 4042 were upregulated and 3422 downregulated in TgRANKL mice compared to WT. Enrichment analysis showed that up-regulated genes were clustered in pathways involving skeletal system development, bone resorption, osteoclast and osteoblast differentiation, Wnt signaling pathway, while downregulated genes were mainly involved in mitochondrial activity and muscle structure. Selected genes from each category were validated using qPCR.

A common characteristic in TgRANKL mice is the progressive development of bone marrow adiposity (BMA). Histological analysis and micro-computed tomography demonstrated that BMA expanded progressively close to resorbed areas. Expression analysis and proteomics in enriched bone marrow mesenchymal stromal cell (BMSC) cultures showed modified metabolic processes including fatty acid metabolism and mitochondrial proteins between TgRANKL and WT mice. Moreover, TgRANKL BMSCs displayed increased adipogenic and decreased osteogenic potential upon differentiation. Furthermore, the effectiveness of an anti-osteoporosis treatment in BMA development was investigated upon treatment of TgRANKL models with alendronate. Notably, alendronate not only improved bone mass (BV/TV, WT: 11.81 ± 1.58 vs Tg5519: 2.00 ± 0.68 vs Tg5519+ALN: 11.40 ± 3.37, $p< 0.05$) but also attenuated BMA expansion at metaphysis (WT: 0.31 ± 0.22 vs Tg5519: 44.41 ± 2.52 vs Tg5519+ALN: 8.27 ± 1.75, $p< 0.001$), indicating a possible involvement of osteoclasts and bone resorption in BMA development. Conclusively, our analysis identified specific markers and deregulated pathways in femurs from osteoporotic TgRANKL models, while also we correlated BMA with BMSC metabolism and osteoclast activity.

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