Bone Reports 13 (2020) 100620

Contents lists available at ScienceDirect

Bone Reports

journal homepage: www.elsevier.com/locate/bonr

Concurrent Oral Presentations

Concurrent Oral Presentations 1: Basic/Translational: Mechanisms of osteoporosis

COP07

TGF-β induced senescence is a novel therapeutic target for treating osteoporosis in Gerodermia Osteodysplastica

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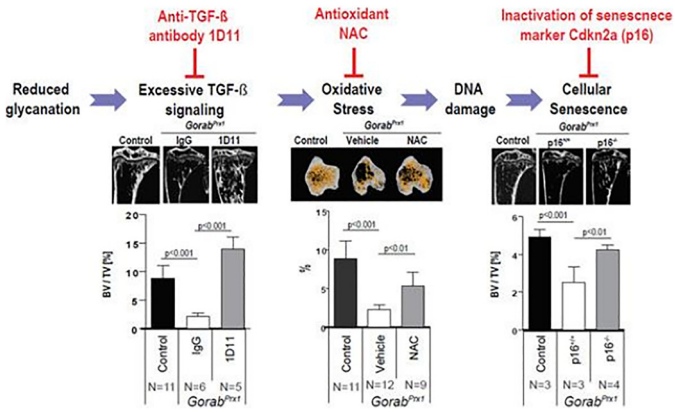
Gerodermia osteodysplastica (GO) is a rare hereditary disorder characterized by lax skin and osteoporosis. GO is caused by loss of function mutations in the gene *GORAB*, which is essential in mediating the COPI-mediated retrieval of trans-Golgi enzymes. Loss of Gorab caused defective protein glycosylation and reduced glycosaminoglycan contents. This leads to immature collagen network and elevated activation of TGF-β signaling. In this study, we used patient fibroblasts and a conditional mouse model in which *Gorab* is inactivated in the long bones (*Gorab*^{Prx1}) to study the role of overactivated TGF-β signaling in the pathomechanism of GO. We report that excessive TGF-β signaling elevated Nox4 expression in *Gorab*^{Prx1} (2.00 ± 0.23 fold, N=6, p< 0.01) and in patient fibroblast (2.05 ± 0.07 fold, N=4, p< 0.01), resulting in increased mitochondrial superoxide (1.74 ± 0.14 fold, N=4, p< 0.01 in *Gorab*^{Prx1}; 1.20±0.06 fold in patient fibroblast, N=6, p< 0.01). The increased oxidative stress is correlated with DNA damage accumulation in *Gorab*^{Prx1} (2.35±0.24 fold increased γH2AX level, N=3, p=0.015) and in patient fibroblast (1.52±0.05 fold increase in cells with 53BP1 nuclear foci; N=3, p< 0.01). This led to significant upregulation of the cell cycle inhibitor Cdkn2a (p16) expression (4.02±0.50 fold in *Gorab*^{Prx1}, N=5, p< 0.001; 6.32± 1.03 in patient fibroblasts, N=4, p< 0.01) and cellular senescence. Targeting this TGF-β induced senescence axis with either anti-TGF-β antibody 1D11, the antioxidant N-acetylcysteine (NAC), or inactivation of Cdkn2a ameliorated the osteoporosis phenotype. These results demonstrated the crucial role and therapeutic potential of targeting TGF-β-induced senescence in GO.



Summary Figure

doi:10.1016/j.bonr.2020.100621

COP08

Protective effect of *Saccharomyces boulardii* CNCM I-745 on the development of inflammatory osteoclasts and inflammatory bone destruction

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Pathological bone destruction is characterized by the emergence of inflammatory osteoclasts (i-OCLs) that differ from steady-state tolerogenic osteoclasts (t-OCLs) by their progenitors and opposite immune functions. However, the mechanisms controlling their emergence are poorly understood. Recent findings showed that bone loss in osteoporosis strongly correlates with gut microbiota dysbiosis, through inflammatory response modulation. Thus, our aim was to better characterize these two OCL subsets and identify new potential specific targets for i-OCLs focusing on gut eubiosis restoration.

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Comparative RNA-sequencing of purified i-OCLs and t-OCLs revealed an upregulation of pattern recognition receptors (PRRs) involved in anti-fungal responses including Dectin-1 (p=0.07; log2FC -1.19), Mincle (p=0.06; log2FC -0.87) and TLR2 (p< 0.0001; log2FC -1.86) in i-OCLs. *In vitro,* PRR-specific agonists significantly reduced i-OCLs formation (p< 0.0001) without affecting t-OCLs or the phagocytic capacity and viability of OCLs. Similar results were observed *in vitro* in the context of osteoporosis where agonists blocked OCL differentiation from ovariectomized (OVX), but not SHAM control mice (p< 0.0001). Thus, to modulate i-OCLs *in vivo*, we used *Saccharomyces boulardii* CNCM I-745 (*Sb*), a well-studied probiotic known for its anti-inflammatory effects. *Sb* administration in OVX-mice significantly reduced bone destruction compared to untreated mice (BV/TV,p< 0.0001) (Fig. 1) and the proportion of i-OCLs (p< 0.0001) without affecting t-OCLs.

Our results demonstrate that specific i-OCL inhibition is highly promising to combat inflammatory bone destruction. Moreover, our findings highlight the protective effect of *Saccharomyces boulardii* CNCM I-745 on bone destruction and provide novel regulatory mechanisms as new therapeutic targets for inflammatory bone loss.

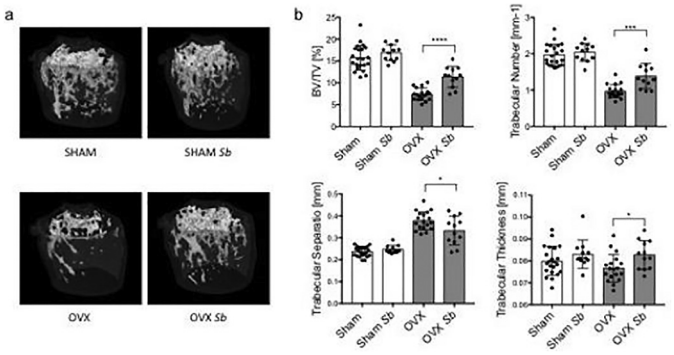

Fig. 1. Saccharomyces boulardii CNCM I-745 has a protective effect on osteoporosis. MicroCT analysis of OVX and SHAM control mice treated with the probiotic yeast Saccharomyces boulardii (Sb). **a** Representative μCT images of femurs from SHAM OVX mice treated with Sb. **b** Histograms indicate mean ± S.D. of bone volume fraction (BV/TV), trabecular number, thickness and separation (n = 12.). *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; n.s. no significant difference.

doi:10.1016/j.bonr.2020.100622

COP09

Enpp1 enzyme replacement restores bone mass in murine model of Enpp1 associated osteoporosis

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Human heterozygous ecto-nucleotide pyrophosphatase/phosphodiesterase (ENPP1) deficiency results in mild elevation of FGF23, mild phosphate wasting, intermediate levels of plasma pyrophosphate, and early onset osteoporosis (EOOP), a phenotype recapitulated in homozygous Enpp1 deficient mice (*Enpp1*$^{asj/asj}$). To determine whether Enpp1 enzyme replacement would affect bone mass, we compared the skeletal phenotype of WT and *Enpp1*$^{asj/asj}$ mice treated with Enpp1-Fc or vehicle on a low magnesium, high phosphate diet to exacerbate the mineralization deficiency ('acceleration diet'). We found that, like *Enpp1*$^{asj/asj}$ mice fed normal chow for 10 weeks, *Enpp1*$^{asj/asj}$ mice fed the acceleration diet for 5 weeks exhibit decreased trabecular bone mass (Tb. BV/TV 70%, Tb.Sp 125%, and TbN 82% of WT) and reduced cortical bone mass (Ct. BV/TV 90% and Ct. Thickness 72% of WT). Also similar to 10-week *Enpp1*$^{asj/asj}$ mice fed normal chow, tibias of 5-week *Enpp1*$^{asj/asj}$ mice fed acceleration diet exhibited less favorable biomechanics (maximum load, stiffness, and total work of 52%, 45%, and 27% of WT, respectively). Treating the *Enpp1*$^{asj/asj}$ mice between weeks 2-5 with Enpp1-Fc normalized trabecular bone mass parameters, and normalized or improved bone biomechanical properties (normalized maximum load compared to WT, improved stiffness to 74% and total work to 63% of WT) and cortical bone mass (improved Ct. BV/TV to 92% and Ct.Th to 81% of WT). Furthermore, qBEI showed no significant differences in BMDD or osteocyte lacunae of mice on 'acceleration diet' (treated or untreated). Finally, a consequence of the acceleration diet was renal failure due to tissue mineralization in untreated *Enpp1*$^{asj/asj}$ mice, which appeared after week 4 and was prevented with ENPP1-Fc. However, no histological findings specific for renal osteodystrophy, including histomorphometry and qBEI, were detected, suggesting that renal osteodystrophy did not develop in the untreated mice. Our results suggest that bone mass in Enpp1 deficiency may respond to Enpp1 enzyme replacement therapy.

doi:10.1016/j.bonr.2020.100623

COP10

Loss of glucocorticoid rhythm induces an osteoporotic phenotype in mice

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Glucocorticoid (GC)-induced osteoporosis is a widespread health problem that is accompanied with increased fracture risk. Detrimental effects of GC therapy on bone have been ascribed to the excess in GC exposure, but it is unknown whether disruption of the endogenous GC rhythm inherent to GC therapy also plays a role. To investigate this, we subcutaneously implanted female C57Bl/6J mice with either vehicle pellets or slow-releasing corticosterone (CORT) pellets to blunt CORT rhythm without inducing hypercortisolism (n=10 mice/group). This experiment was approved by the Central Animal Experiments Committee. Flattening of the CORT rhythm for 7 weeks reduced cortical and trabecular bone volume (-8.1%, P=0.0009 and -25.5%, P=0.017 respectively), cortical and trabecular