Identification of new molecular and cellular mechanisms involved in bone diseases
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OBJECTIVES
Gorham-Stout disease (GSD) is a very rare disorder characterized by extensive angiomatous proliferation and progressive osteolysis without new bone formation. Only ~200 patients were reported. The quality of life is very poor since patients display pain, fractures, functional impairment and swelling of the affected regions. The etiology of GSD is unknown and diagnosis is performed by exclusion criteria. Unfortunately, there are no set therapeutic approaches for patients. We aim to investigate the bone phenotype and to identify molecular and cellular alterations in GSD patients.

METHODS
Eight patients were recruited for this study. Bone biopsy analysis was performed. Bone turnover markers were measured by ELISA assay. In vitro osteoclast and osteoblast cultures were performed to evaluate alterations of differentiation, morphology and activity. Gene expression was evaluated by transcriptomic and Real-Time RT-PCR analysis. Moreover the effects of GSD sera on bone cells were investigated to understand the involvement of systemic factors.

RESULTS
Bone biopsy analysis revealed in patients fibrous tissue, increase of osteoclast number, vessels and osteocyte lacunae area. In patient sera high levels of ICTP, VEGF-A, IL-6 and Sclerostin were revealed. Patient's osteoclast precursors showed a 2-fold increased ability to differentiate into osteoclasts, with more nuclei per cell. About 75% of GSD osteoclasts displayed a more motile phenotype. A 2-fold increased ability to resorb bone was observed in GSD osteoclast cultures compared to control. Transcriptomic analysis revealed an enrichment of PI3 kinase, EGF receptor and beta-arrestin pathways. To investigate the involvement of systemic factors in GSD, Healthy Donor (HD)-PBMC were treated with GSD sera and showed increased osteoclastogenesis compared to control sera-treated cells. Bone Marrow Mesenchymal Stem Cells isolated from a patient revealed a defect of osteogenic differentiation, as shown by reduced ALP activity and expression compared to HD-MSC. Affected osteoblasts displayed reduced ability to form mineralized nodules. Transcriptome analysis revealed in GSD osteoblasts a modulation of pathways involved in bone morphogenesis and ossification and an increase of osteoclastogenic potential. Moreover, HD-osteoblasts treated with GSD sera showed decreased expression of ALP and COL1a2 and increased RANKL/OPG ratio.

CONCLUSIONS
These results suggest that in Gorham-Stout disease the alteration of bone remodeling activity is related to bone cell autonomous defects and systemic factors. Understanding the molecular and cellular defects in GSD patients will allow to have a correct diagnosis and new therapeutic options for this rare disease.
Pubblicazioni Rossi Michela

List of publications of the applicant


