Background: The Paget's disease of Bone (PDB) is characterized by a chronic and focal disorder of bone remodeling. PDB is currently considered a complex and multifactorial disease, as a result of a synergistic association of genetic variants with environmental risk factors. The genetic component would explain certain epidemiological traits such as the predisposition to develop in certain ethnic groups and the strong tendency to family aggregation. The most important susceptibility gene for PDB is the Sequestosome-1 (SQSTM1), and encodes a 440-amino-acid protein called p62 protein. SQSTM1 gene occur in between 20 and 40% of patients with a family history of PDB, and 5 and 10% of patients with 'sporadic' disease, according to the series studied. Thus far, 29 SQSTM1 mutations have been identified in patients with PDB.

Objectives: To analyze the importance of SQSTM1 gene (p62) variants in the susceptibility to develop PDB, as well as to evaluate the genotype-phenotype correlation in our PDB population.

Methods: The molecular study was carried out by sequencing the SQSTM1 gene (p62) in a population of 200 PDB affected patients and 200 hypernormal controls. An "in silico" functional analysis of the different genetic variants was performed using the information provided by the splicing prediction algorithms, the miRNA target prediction algorithms, the gene-specific algorithms for predicting pathogenicity (SIFT, PolyPhen, PMut) and the interspecific evolutionary conservation study. The statistical analysis was performed using SPSS statistical software Windows (v23) and the R software version 2.15.0 for Windows. The study was approved by the CEIC (Hospital del Mar-Parc de Salut Mar (2007/2885/I)) and all participants signed an informed consent agreeing to participate in the study.

Results: The 27% of the PDB affected patients were carriers of different gene SQSTM1 mutations, being the most frequent identified the p.P392L, described in the 20% of cases. In terms of genotype-phenotype relation, being a carrier of gene SQSTM1 mutations is associated to a more severe phenotype based on a greater disease activity and a greater extent at diagnosis as well as higher complications during the course of it, without the presence of an associated family history positive. In our patient cohort 17 mutations have been identified in coding regions of the SQSTM1, with six newly described "missense" genetic variants of new description, associated all of them to a higher risk of developing PDB and distributed in different structural and functional domains of the p62 protein, being able to cause dysfunctions in the different cell signalling processes where intervenes p62 like apoptosis, autophagy, pro-otic degradation through the ubiquitin-proteasome system, the regulation of antioxidant response and the cell survival mediated by RANK-TRAF6- NF-kB signaling pathway), implicated in the pathogenesis of Paget's disease of bone.

Conclusion: In the molecular study of the SQSTM1 gene, seventeen mutations were identified in the coding regions of the gene, being six "missense" genetic variants of new description, associated all of them to a higher risk of developing PDB and distributed in different structural and functional domains of the p62 protein.

REFERENCES:

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POS0347
CHARACTERIZATION OF THE TRANSCRIPTOMIC SIGNATURE OF BONE MARROW CELLS IN OSTEOPOROSIS ASSOCIATED WITH SYSTEMIC MASTOCYTOSIS

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Background: Systemic mastocytosis (SM) is a rare mononclonal mast cell disease, associated with vertebral osteoporosis (OP) in ~30% of the patients. This OP frequently leads to multiple vertebral fractures, in particular in young or premenopausal patients. Soluble mediators secreted by the pathogenic mast cells and subsequent activation of osteoclasts are thought to explain the development of such OP. However, data about the nature and the regulation of the pro-osteoporotic factors is limited. We hypothesize that a transcriptomic approach, assessing bone marrow cells involved in this pathological condition, can identify the specific determinants of fragility fractures in SM.

Objectives: To identify a bone marrow transcriptomic profile associated with osteoporosis in SM.

Methods: We analyzed clinical data and bone marrow samples collected at diagnosis in SM patients from our Reference Centre for Mucocutaneous mastocytosis (CEREMAST) and fulfilling the SM WHO criteria. All patients signed an informed consent. Twelve SM patients with OP and fragility fracture (OP group) were age- and gender-matched with 12 SM patients without OP or fragility fracture (non-OP group). We used a Nanostring nCounter approach to compare the bone marrow mRNA profile. We used a predefined panel of 800 transcripts relevant for an osteo-immunological analysis. Genes expressions were normalized and compared by a Wilcoxon test with R software. We performed a pathway analysis with C5, C7, KEGG and Reactome databases.

Results: Both OP and non-OP groups included 8 women and 4 men, with similar average age (OP 56.1 +/- SD 8.7 vs non-OP 57.1 +/- 9.3). The 12 patients from the OP group had at least 1 vertebral fracture (VF) with an average number of VF of 4.9 +/- 2.6. Lumbar and hip bone mineral density was significantly lower in the OP group. The transcriptomic analysis revealed a specific profile associated with osteoporotic vertebral fractures in SM, with 26 differentially expressed genes (see Figure 1). The analysis highlighted an involvement of NECTIN2 and of IL6/STAT3. Pathway analysis suggested a role for genes involved in cell cycle regulation, T cell activation, protein kinase activity and differentiation and activation of monocyctic cells.

Figure 1. Heatmap of the differentially expressed genes in bone marrow from SM patients with osteoporosis and fragility fracture versus without osteoporosis.

Conclusion: Our results highlight that a transcriptomic analysis of bone marrow is relevant to identify biomarkers associated with OP in SM. In addition to the pathogenic role of clonal mast cells and of monocyctic lineage, this condition may involve non-clonal immune cells such as T cells.

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POS0348
DEVELOPING A WHOLE MOUNT FLUORESCENT OSTEOCLAST ACTIVITY ASSAY USING THE ELF97 PHOSPHATASE SUBSTRATE TO VISUALISE AND QUANTIFY IN SITU OSTEOCLAST ACTIVITY IN ZEBRAFISH (DANO RERIO)

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Background: Developmental osteoclasts play a critical role in the development of bone. The pathogenesis of bone diseases is greatly affected by the balance between osteoclast formation and activity. Osteoclasts are the bone-resorbing cells that secrete phosphatase enzyme, which hydrolyses the bone. The fluorescence substrate ELF97 is a synthetic phosphatase reaction substrate that is composed of a fluorescent dye and a phosphatase substrate. The ELF97 phosphatase reaction substrate can be used as a fluorescent probe to monitor osteoclast activity in different stages of osteoclastogenesis.

Objectives: To develop a novel whole mount fluorescent assay using the ELF97 phosphatase substrate to visualise and quantify osteoclast activity in zebrafish (Danio rerio) at different stages of osteoclastogenesis.

Methods: Zebrafish were exposed to ELF97 and then incubated with the ELF97 phosphatase reaction substrate. The zebrafish were observed under a fluorescence microscope to visualise the osteoclast activity. The ELF97 fluorescence intensity was quantified using ImageJ software. The ELF97 fluorescence intensity was compared between control and treated zebrafish.

Results: The ELF97 fluorescence intensity was significantly higher in treated zebrafish compared to control zebrafish. The ELF97 fluorescence intensity was highest in the osteoclasts at the late stages of osteoclastogenesis.

Conclusion: The ELF97 phosphatase substrate can be used as a novel whole mount fluorescent assay to visualise and quantify osteoclast activity in zebrafish. This assay can be used to study the role of osteoclasts in the development of bone diseases.