IMPACT OF ASXL1 MUTATIONS IN HOXA GENE EXPRESSION IN MYELOFIBROSIS

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INTRODUCTION

Primary myelofibrosis (PMF) is a myeloproliferative neoplasm, clonal hematopoietic disorder characterized by somatic mutations in the JAK/STAT pathway. PMF patients can also harbor mutations in genes involved in epigenetic regulation, such as TET2 and ASXL1.

ASXL1 belongs to the group of ETP genes (Enhancer of trithorax and Polycomb), which encode proteins necessary for the maintenance of activation and repression of target genes. In murine models and human leukemic cell lines it has been shown that ASXL1 mutations lead to the overall reduction of H3K27me3 and increased expression of genes involved in leukemogenesis, such as the HOXA genes (Figure 1)

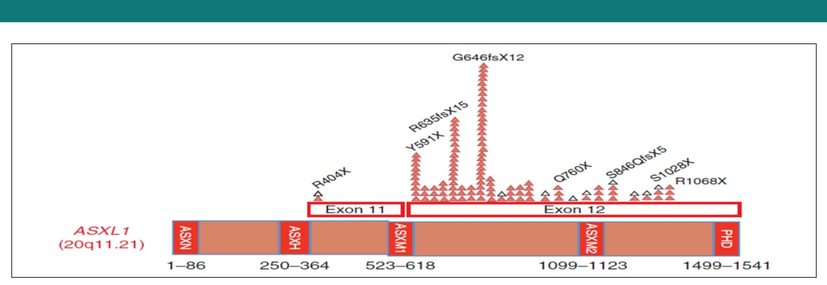

Figure 1. Protein domain structure and location of amino acids affected by mutations in ASXL1

OBJECTIVE

- Evaluate the impact of ASXL1 mutations on HOXA gene expression in PMF,
- Evaluate the impact of ASXL1 mutations in the epigenetic regulation of pluripotency maintenance and hematopoietic differentiation.
- Study promoter methylation, gene expression and protein along the in vitro differentiation in granulocytes from CD34+ cells
- Study of promoter methylation and gene expression of ASXL1 target genes in ASXL1 WT and mutated iPSC and iPS-derived granulocytes

METHODS AND RESULTS

Patients samples and Nucleic acid obtention

Previous work from our group identified *ASXL1* mutations in 8/46 PMF patients in our cohort (ASXL1mut). RNA samples was extracted using TRIZOL and were obtained from granulocytes from 7 ASXL1mut PMF patients, 19 ASXL1WT PMF patients and 11 healthy donors (HD). cDNA was synthetized using SuperScriptII reverse transcriptase and random hexamer primers

Gene Expression

The normalization of quantitative real-time RT-PCR data was performed from the analysis with GenEx software that use both algoritms , NormFinder and GeNorm, to calculate a gene expression normalization factor for each sample based on the geometric mean as described by Vandersompele et al. The PPIA gene was selected like most stable reference genes (Figure 2)
A dilution series of know template concentration of cDNA of diferent linages cells were used in order to create a standard curve for calculation of relative gene expression levels and to evaluate reaction efficiency (Figure 3)
Gene expression was evaluated by quantitative real-time PCR for a panel of *HOXA* genes (*HOXA6,7,9,10,11* and *13*). Was analyzed in groups of PMF patients (ASXL1 WT or MT) by quantitative real-time PCR using SYBR Green and specific primers.
The Mann-Whitney test was used to compare the variables and $p<0.05$ was considered significant.
For *HOXA10*, a significant difference was observed when comparing HD with ASXL1mut and ASXL1WT samples (Figure 4)

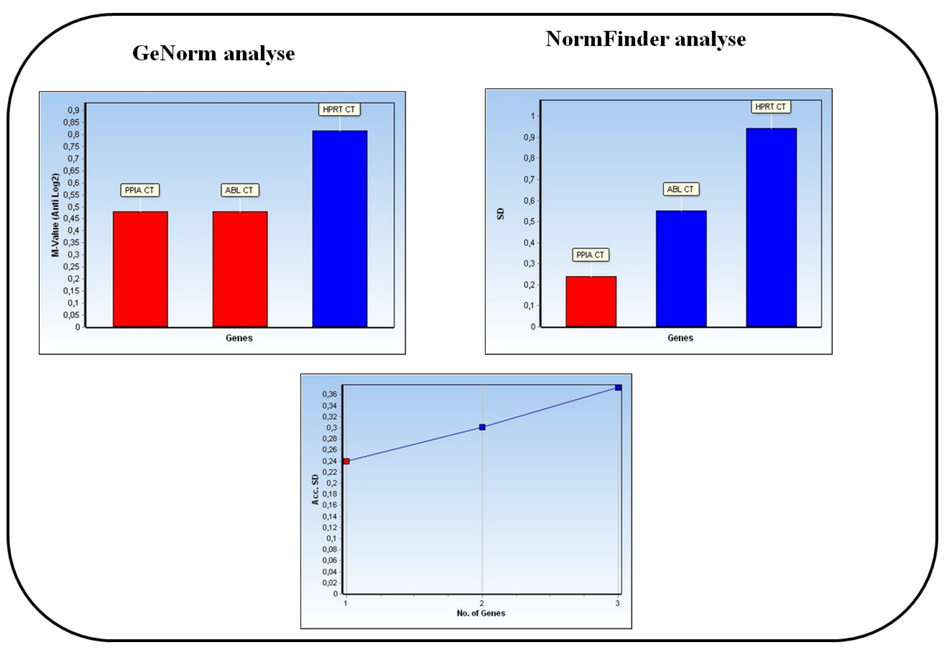

Figure 2. Normalization analyse with GenEx software

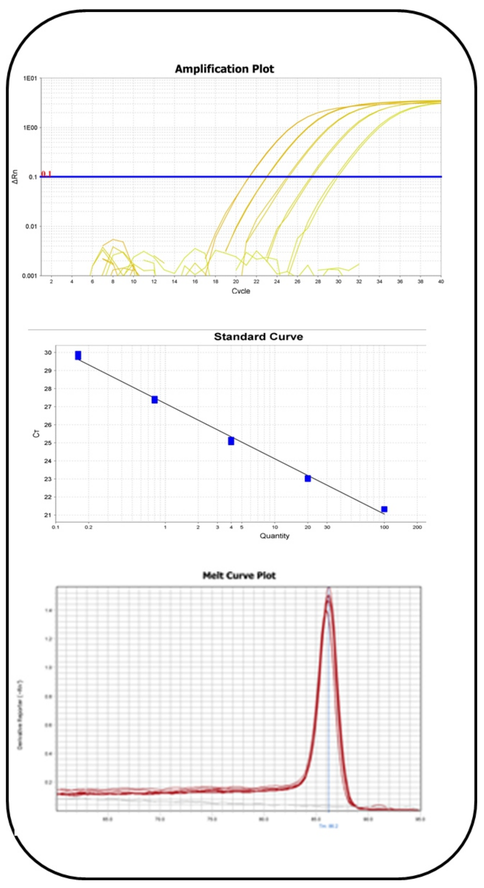

Figure 3. Standard curve and efficiency analysis of the reactions

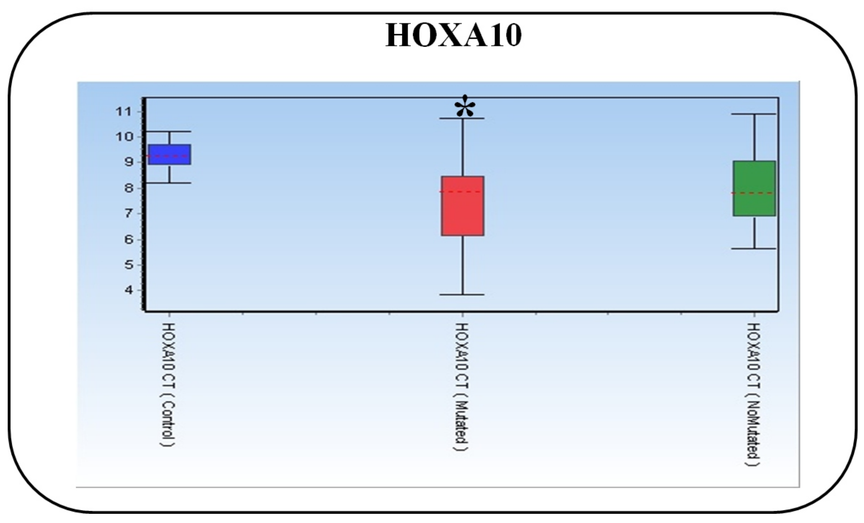

Figure 4. Comparative plot between the groups of patients ASXL1 WT, MT and controls.

For the other *HOXA* genes analyzed, no difference was observed between groups. To further understand the impact of *ASXL1* mutations on *HOXA* gene expression, promoter methylation status will be evaluated. Promoter regions were analyzed for identification of CpG islands and primers were designed using Methyl Primer Express software. After conversion of the DNA samples with sodium bisulfite, the regions of interest was amplified by PCR and willbe analyzed by direct sequencing.

In vitro granulocytic differentiation from HSC is currently being performed. Subsequently, we will also evaluate *HOXA* gene expression during IPSC *in vitro* differentiation.)

CONCLUSIONS

Tthe study of promoter methylation and gene expression of ASXL1 target genes in ASXL1 WT and mutated iPSC and iPS-derived granulocytes will contribute to understand the impact of these somatic mutations in the epigenetic regulation of pluripotency state and myeloid differentiation.

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA

MINISTÉRIO DA SAÚDE