CHARACTERIZATION OF MOLECULAR MECHANISM RESPONSIBLE FOR *FLT3* GENE OVEREXPRESSION IN ACUTE LEUKEMIAS

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Introduction. *FLT3* overexpression is a recurrent event in many high-risk subtypes of acute leukemia. *FLT3* activating mutations (*FLT3*-AM) explain only 35% of acute myeloid leukemia (AML) and 12.5% of acute lymphoblastic leukemia (ALL) cases with *FLT3* overexpression, supporting the idea that there are other regulatory mechanisms responsible for this aberrant profile. Recent studies have described that somatically acquired mutations in non-coding regulatory regions can create neomorphic enhancers leading to an aberrant expression of critical oncogenes. Therefore, we hypothesized that neomorphic enhancers could be a possible molecular mechanism responsible for *FLT3* overexpression in both ALL and AML cases lacking *FLT3*-AM. **Methods.** We first characterized *FLT3* expression in AML, B and T-ALL cell lines using RNA-seq data available on the CCLE website. These cell lines were then grouped according to the presence or absence of *FLT3*-AM based on mutation data available on the same portal. Subsequently, we used ChIP-seq data for H3K27ac, a mark of active chromatin, of leukemia cell lines available on the GEO repository to evaluate potential enhancer regions associated with *FLT3* overexpression. By combining all these data, we classified the cell lines in three groups: 1) *FLT3* overexpression with *FLT3*-AM, 2) *FLT3* overexpression without *FLT3*-AM; and 3) *FLT3* normal or low expression. **Results.** We have characterized 35 AML, 15 B-ALL and 15 T-ALL cell lines according to *FLT3* expression. Only 3 AML cell lines showed *FLT3*-AM (MOLM13, MV-4;11, PL-21) and these mutations were not found in other leukemia subtypes. Based on data availability, 5 AML, 4 B-ALL and 6 T-ALL cell lines were evaluated for H3K27ac ChIP-seq. Our results demonstrated active chromatin regions upstream of *FLT3* transcriptional start site in two B-ALL cell lines with *FLT3* overexpression (SEM, RS-4;11), which are not present in normal CD34+ hematopoietic stem cells (HSCs). We also observed the presence of aberrant H3K27ac marks in MOLM13 and MV-4;11 within the *FLT3* promoter region when compared to normal CD34+ HSCs or compared to other AML cell lines with *FLT3* overexpression but without any *FLT3*-AM. This mark was not observed in T-ALL, nor in normal thymic cells. For AML groups, we identified 10581 differential peaks present in group 2 (versus group 1 and group 3), which 45% is located in distal intergenic regions. Among these peaks, we observed 5 regions that co-occur with variants in group 2 cell lines. **Conclusion.** Our data show the presence of potential neomorphic enhancers regions in cell lines with *FLT3* overexpression, however, further analyzes are required to confirm these initial findings. We will search for alterations with potential neomorphic enhancers formation in these non-coding regulatory regions in both cell lines and patient samples. Then, the results will be validated by in vitro assays in order to demonstrate the association between the neomorphic enhancers and *FLT3* overexpression.

Keywords: Acute leukemia; *FLT3* overexpression; molecular mechanisms

METHODS

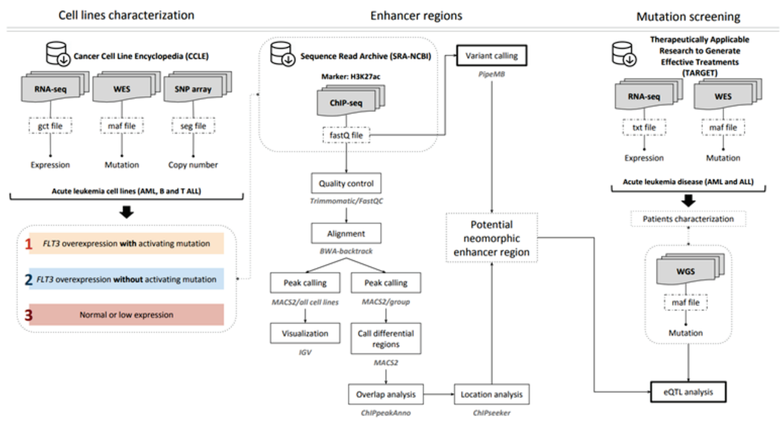

Figure 1. Study design and workflow.

RESULTS

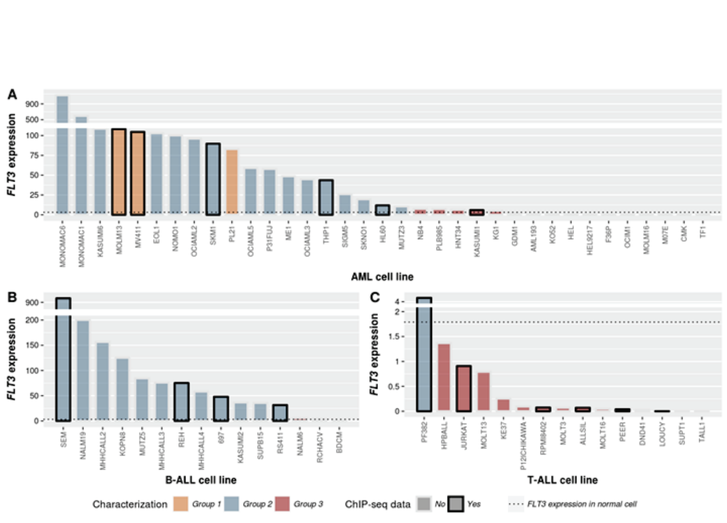

Figure 2. Characterization of leukemia cell lines. Bar graphs demonstrating the *FLT3* expression in AML **(A)**, B-ALL **(B)** and T-ALL **(C)** cell lines. Group 1: cell lines with *FLT3* overexpression and activating mutation (orange); Group 2: cell lines with *FLT3* overexpression but without an activating mutation (blue); Group 3: cell lines with normal or low *FLT3* expression (red). Healthy bone marrow (for AML and B-ALL) or thymus (for T-ALL) samples were used as a calibrator for determination of *FLT3* overexpression. Due to data availability, six AML, four B-ALL and six T-ALL cell lines were evaluated for H3K27ac Chip-Seq (black border).

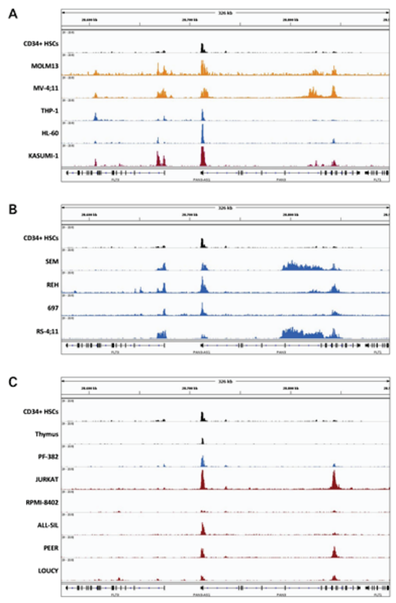

Figure 3. ChIP-Seq Analysis. IGV snapshot showing ChIP-seq peaks profile for H3K27ac at the *FLT3* and adjacent PAN3 gene locus in AML **(A)**, B-ALL **(B)** and T-ALL **(C)** cell lines. Cell lines overexpressing *FLT3* with activating mutations are shown in orange or without alterations in blue, normal or low expression were represented in red. We used human normal hematopoietic stem cell (CD34+ HSCs) and thymus cells as control.

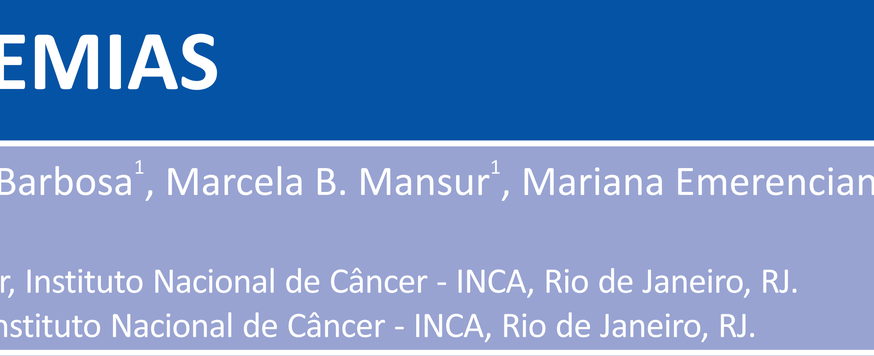

Figure 4. Differential peaks among AML groups. Venn diagram representing the differential peaks among AML groups *versus* human normal hematopoietic stem cell (CD34+ HSCs), highlighting peaks exclusively observed in group 2. In the right, the pie plot shows the frequency of peaks annotated regarding their location in different genomic regions. Group 1: cell lines with *FLT3* overexpression and activating mutation (orange); Group 2: cell lines with *FLT3* overexpression but without an activating mutation (blue); Group 3: cell lines with *FLT3* normal or low expression (red).

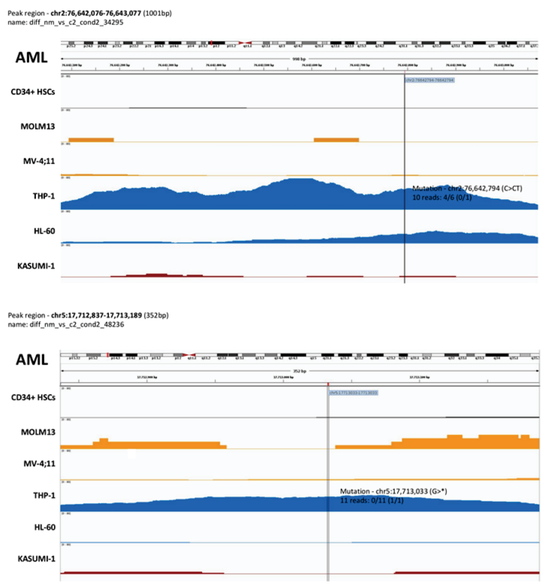

Figure 5. Potential neomorphic enhancer regions in AML. Two examples of non-coding regions where both super enhancer markers and mutation were identified, one on chromosome 2 and one on chromosome 5. The figure represents ChIP-seq peaks profile for H3K27ac in *FLT3* overexpression with activating mutations (orange), *FLT3* overexpression without activating mutations (blue) and *FLT3* normal expression (red) cell lines.

CONCLUSION

Our data show the presence of potential neomorphic enhancers regions in cell lines with *FLT3* overexpression, however, further analyzes are required to confirm these initial findings. We will search for alterations with potential neomorphic enhancers formation in these non-coding regulatory regions in both cell lines and patient samples. Then, the results will be validated by in vitro assays in order to demonstrate the association between the neomorphic enhancers and *FLT3* overexpression.

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