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Gene expression analysis of Slow-Cycling and Leukemia Stem Cells phenotypes panels in Acute Myeloid Leukemia.

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Introduction

One of the greatest obstacles in the treatment of Acute Myeloid Leukemia (AML) is relapse, occurring in 40-50% of younger patients and most elderly patients. The Leukemia Stem Cells (LSCs) model suggests that only a small population of tumor cells is responsible for treatment resistance and recurrence. As LSCs, the slow-cycling cell (SCCs) phenotype is characterized by cells capable of reconstituting the different neoplastic populations, however, its main characteristic is presenting a slow-cycling rate, resembling a state of quiescence. The aim of this study was to find differentially expressed genes, associated with SCC and LSC phenotypes, between recurrent and primary marrow samples of AML patients. Also, to analyze gene expression levels of proposed genetic markers for SCCs and LSCs in bone marrow samples from AML patients.

Methods

Data were acquired from The Cancer Genome Atlas platform (https://portal.gdc.cancer.gov/projects) using the TCGAbiolinks R package. The dataset of a pediatric AML cohort from the TARGET initiative (ID phs000465.v21.p8) was used. The comparative groups used for differential gene expression analysis were recurrent marrow vs. primary marrow, primary marrow vs. primary peripheral blood, and recurrent marrow vs. primary peripheral blood. Over-representation analysis, which analyzes pathways enriched with differentially expressed genes, was performed on Consensus Pathway DB. Bone marrow aspirate samples collected at the time of diagnosis (DX) and at the time of detection of Minimal Residual Disease (MRD) from patients with AML treated at the Hematology Service of Hospital de Clínicas de Porto Alegre (HCPA) were analyzed (CAAE 33989120.1.0000.5327). Total RNA was isolated using TRIzol, and cDNA synthesis was performed using the M-MLV Reverse Transcriptase enzyme. Real-time PCR was performed in triplicate on the StepOne Plus™ PCR system using the GoTaq® qPCR Master Mix.

Results

Figure 1. Data from 185 TCGA samples were analyzed. We observed a different expression profile on the comparisons with recurrent bone marrow samples, both for a list of 43 genes associated with SCCs and for a list of 68 genes associated with LSCs. Of these, we can highlight SALL4, MS4A1, ZEB1, CD34, PROM1, ABCB1, PTCH1, ALCAM, MSI2, SETDB2, KDM5A, EGF, and KDM5B, more expressed in recurrent samples and associated with SCCs (a). CD34, CD96, KIAA0125, HLF, GPR56, CDK6, BMI1, AKR1C3, RAB8B, CRLF3, LPAR6, CPEB4, CD82, ERG, SMIM24, MMRN1, and PPFIBP1 were genes that showed higher expression in recurrent samples and are associated with LSCs (b). We also analyzed the expression of a gene set composed of genes associated with the SCC and LSC phenotypes, including genes related to the metabolic characteristics shown by these cells, which were later analyzed in patient samples as well (c). Regarding this gene set, we only observed lower expression of MYC, GAPDH, and SDHB in the recurrent samples, in comparison to primary samples. The other six genes analyzed presented higher expression in recurrent samples, indicating the possible SCC and/or LSC enrichment happening in these samples.

Figure 2. The overrepresentation analysis resulted in 363 enriched pathways, of which 18 were selected to assess the overlap of the differentially expressed genes contained in these pathways. Of these 18 pathways, two sets, one of five pathways (a) and one of six pathways (b), presented the highest number of shared genes.

Figure 3. In the HCPA patient samples, we observed differential expression patterns of a selected set of genes between the bone marrow samples collected at the time of diagnosis and the first MRD (day 28). The TET2 gene (a) had its expression proportionally increased in all patient samples at MRD compared to DX. The same pattern was observed for the JARID1B gene (b). However, initially, in DX, the expression values were more similar between patients, and later, in MRD, the proportion of the increase in expression was varied between patients. Two patients, diagnosed with immature AML, had increased CPT1a expression (c) after treatment. The same was observed for the CPT2 gene (d). Regarding the expression of the ACAA2 and ACAD10 β-oxidation pathway genes (e,f), both patients diagnosed with immature AML showed increased expression at MRD in relation to DX, and differences were more expressive for the ACAA2 gene. However, the patient diagnosed with the M5 subtype of AML showed a decrease in the expression of ACAA2 and ACAD10 at the time of DRM in relation to DX. Similarly, there was an increase in expression of the SDHB and GAPDH genes (g,i) in MRD in relation to DX in patients diagnosed with immature AML. The MYC gene (h) was the only one in which all patient samples showed lower expression than in the U937 cell line, however, all MRD samples showed increased expression when compared to their DX counterparts.

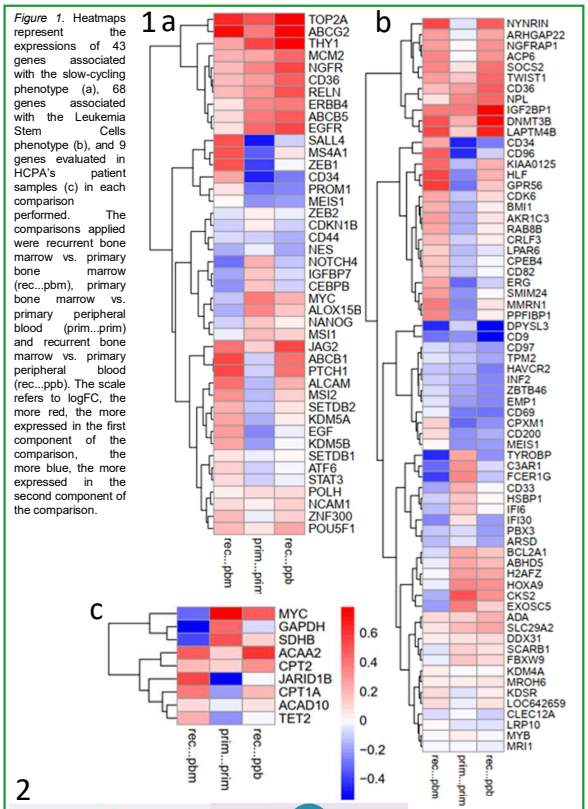

Figure 1. Heatmaps represent the expressions of 43 genes associated with the slow-cycling phenotype (a), 68 genes associated with the Leukemia Stem Cells phenotype (b), and 9 genes evaluated in HCPA's patient samples (c) in each comparison performed. The comparisons applied were recurrent bone marrow vs. primary bone marrow (rec...pbm), primary bone marrow vs. primary peripheral blood (prim...prim) and recurrent bone marrow vs. primary peripheral blood (rec...ppb). The scale refers to logFC, the more red, the more expressed in the first component of the comparison, the more blue, the more expressed in the second component of the comparison.

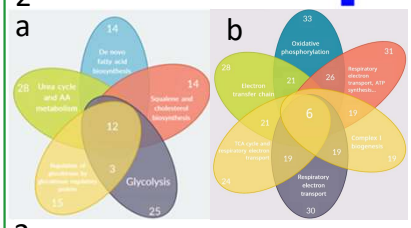

Figure 2. Venn diagrams represent the number of shared genes between selected pathways. These pathway sets were selected due to being enriched in the DEGs of the comparison rec..pbm, representing value to the purpose of this work and presenting the first (a) and second (b) highest numbers of shared genes from a pre-selected sample of 18 pathways.

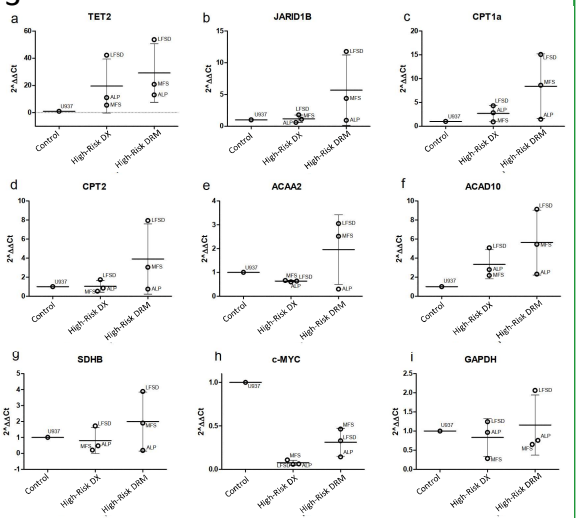

Figure 3. Gene expression results of selected targets in patient samples (acronyms of equal letters indicate the same patient) of Acute Myeloid Leukemia, diagnosis (DX) and minimal residual disease (MRD), in relation to the expression of each gene in the U937 cell line, 2^ΔΔCt values: (a) TET2 gene, (b) JARID1B(KDM5B) gene, (c) CPT1a gene, (d) CPT2 gene, (e) ACCA2 gene, (f) ACAD10 gene, (g)) SDHB gene, (h) MYC gene and (i) GAPDH gene.

Conclusion

Observing the panels of genes associated with LSCs and SSCs in LMA TCGA and HCPA samples, we saw that most genes presented a differentiated expression profile between recurrent and primary samples. This highlights the LSCs and SSCs enrichment that may be occurring due to treatment, and the importance that these phenotypes represent to MRD detection and recurrence.

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