DNA METHYLATION: GENE PROFILING IN A CHRONIC MYELOID LEUKEMIA CELL LINE RESISTANT MODEL

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Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by overproduction of immature and mature myeloid cells in the peripheral blood, bone marrow and spleen [Tabarestani S., et al, J Cancer Prev 2016]. Before the advent of imatinib mesylate (IM) therapy, and second-generation tyrosine kinase (TK) inhibitors (dasatinib and nilotinib), the median survival of patients was approximately 6 years. CML cells carry the Philadelphia chromosome (Ph), the result of a mutual translocation between chromosomes 9 and 22, t(9;22)(q34;q11), encoding a novel BCR-ABL1 oncogene [Huang X., et al. Cancer 2012]. This chimeric gene codifies for a TK receptor, which is constitutively activated and directly responsible of the malignant process in CML cells. IM binds to the inactive conformation of BCR-ABL, and prevents conformational changes needed for BCR-ABL activation. While IM resulted in approximately 83% event-free survival and 93% freedom from progression at 6 years, primary and acquired resistances do occur [Maino E., et al. Expert Rev Anticancer Ther, 2014]. Among the known causes, there are mechanisms depending on BCR-ABL, including point mutations especially in domain that codifying for TK or overexpression of the BCR-ABL fusion protein. Among the different possible mechanisms of drug-resistance, there are genetic alterations, as mutations or genic deletions and chromosomal abnormalities, which provoke loss of functionality against tumor suppressor genes and excessive stimulation of oncogenes. In addition to this, also epigenetic modulation may modify gene expression, without change DNA [Groenbaek K., et al. Basic and Clinical Pharmacology & Tossicology, 2006]. Epigenetics includes modifications in gene expression caused by heritable, but potentially reversible, changes in DNA methylation and chromatin structure: the major epigenetic mechanisms are DNA methylation, histone acetylation and methylation, nucleosome positioning and miRNAs. DNA methylation occurs when a methyl group is added to the 5' position of the cytosine ring of CpG (regions of DNA often associated with the transcription start sites of a gene, and also found in gene bodies and intergenic region) dinucleotides [Huang J., et al. Current Drug Targets, Dic 2011]. Based on these forewords, the aim of this study was to evaluate global methylation profile in K562 cells sensitive and resistant to increasing concentrations of IM.

K562 cell line, a human immortalized CML line in blast crisis, was purchased from Istituto Zooprofilattico Sperimentale, and was grown in RPMI 1640 medium supplemented with 10% FBS. To generate resistant sublines, growing cells were exposed to increasing concentrations of IM (0.05, 0.1, 0.2, 0.3, 0.5, 1, and 3 μM/L) over a time period of 9 months. Cells were considered resistant when they re-acquired the ability to grow in presence of the IM concentrations and vitality was close to 100%. We isolated DNA from each subline, and DNA methylation was performed by bisulfite sequencing using EZ DNA Methylation-GoldTM Kit (Zymo Research). The PCR products were purified using High Pure PCR Product Purification Kit (Roche). For the Infinium® MethylationEPIC BeadChip array processing, β values were obtained from genome studio after background subtraction and internal control normalization. Differential methylation analyses

between groups were performed with Limma package and pvalues were adjusted by Bonferroni correction. Data has been analysed using GenomeStudio software, comparing the global methylation level of each sample against untreated cells, as control.

Considering all the methylation data of treated cells against untreated, with a Delta AVG <-0.33 or >0.33 and pvalues <0.01, 38 genes -2 hypomethylated and 36 hypermethylated- resulted significantly demethylated. We designed pyrosequencing primer to validate these preliminary data and validations of the most significant deregulated genes (HARS, PTPRF, TP73, ARHGEF10, FHDC1, DUSP6, PLD6, MIR548H4) are ongoing.

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