patients in chronic phase were analyzed by conventional cytogenetic analysis and by FISH experiments with probes specific for ABL and BCR genes. All breakpoints on other chromosomes involved in variant t(9;22) and in additional rearrangements have been characterized by FISH experiments and bioinformatic analyses. Breakpoint regions on other chromosomes involved in variant t(9;22) and additional rearrangements were included in 250 Kb size intervals. Each interval was checked for the presence of interspersed repeats classes (Alu and LINE repeats), segmental duplications (SDs), GC content, gene density, and miRNA. *Results.* The molecular cytogenetic analysis revealed 50 CML cases identifying three main subgroups: i) cases with variant chromosomal rearrangements other than the classic t(9;22)(q34;q11) (9.5%); ii) cases with cryptic insertions of ABL1 into BCR, or vice versa (1.3%); iii) cases bearing additional chromosomal rearrangements concomitant to the t(9;22) (1.1%). Bioinformatic analysis showed that the majority of breakpoints on chromosomes involved in variant or additional chromosomal rearrangements showed a high frequency of Alu repeats. In fact, 41 out of 58 (71%) breakpoints showed an Alu content of more than one whereas the remaining 17 out of 58 (29%) had a content of less than one. Instead, the LINE content was lower than one in 44 out of 58 (76%) breakpoints. Most of the analyzed breakpoints map within gene-rich regions in 45 out of 58 (78%) breakpoints. Moreover, 49 out of 58 (84%) breakpoints revealed a low SDs density. A GC content >1 was detected in 43 out of 58 (74%) breakpoints. The search for miRNAs revealed a different density from the expected value in 33 out of 58 (57%) breakpoint regions. In detail, in 29 (88%) and 4 out of 33 (12%) breakpoints a higher or lower number of miRNA than the expected value was identified, respectively. In the remaining 25 out of 58 (43%) breakpoints no miRNA was revealed in the 4 Mb analyzed intervals. *Conclusions.* This study revealed a high content of Alu repeats, genes density, GC frequency, and miRNAs in the great majority of the analyzed breakpoints, suggesting their potential involvement in the CML pathogenesis. In conclusion, our findings demonstrate that the involvement of chromosomes other than 9 and 22 is not a random event but could depend on specific genomic features.

C069

DECREASED LEVEL OF SHP-1 PROVIDES AN ADDITIVE SURVIVAL ADVANTAGE TO PHILADELPHIA CHROMOSOME-POSITIVE (PH$^+$) CELLS DERIVED FROM PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML) AND ACCOUNT FOR RESISTANCE TO IMATINIB (IMA) TREATMENT

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The majority of patients with CML achieve a deep response to Ima treatment, however, a limited group of them may show or develop resistance to this therapy. While mutations of Bcr/Abl kinase domain as a cause of resistance has been described in around 50% of resistant patients, other mechanisms accounting resistance are still poorly understood. In this study, we investigated the protein-tyrosine phosphatase SHP-1, a protein with a tumour suppressor activity, as a possible determinant for resistance to Ima in an *in vitro model* constituted of the KCL22 Ph$^+$ cell lines (sensitive/KCL22-S and resistant/KCL22-R) and in patients with CML. In particular, the resistant phenotype is associated with low level of SHP-1 expression and lacks of its interaction with the proto-oncogene SHP-2, another protein-tyrosine phosphatase. Thus, consistently with epigenetic regulation of the SHP-1 expression, we found that aberrant methylation of its promoter is fundamental for the down-regulation detected in KCL22-R compared to KCL22-S cell line (0.006±0.004 *vs.* 0.8±0.2 SHP1/ABL copy numbers). Then, immunoprecipitation assay shown that in our model one of the main interactors of SHP-1 is SHP-2, that, differently from SHP-1, acts as a positive regulator of Ras/MAPK pathway. The most likely mechanism for SHP-2 activation is the posphorylation of a single tyrosine residue, Tyr-542, that has been demonstrated to be sufficient for activating MAP kinase pathway. Using western blot analysis we found that Ima exposure reduce SHP-2 Tyr-542 posphorylation only in sensitive KCL22-S but not in resistant KCL22-R cell line, where SHP-1 is expressed at lower levels. Therefore, we assumed that SHP-1 might have a negative role on SHP-2 activation modulating its Tyr-542 phosphorylation. To evaluate the functional role of SHP-1, we forced its expression in KCL22-R and assessed that this phosphatase restores Ima sensitivity, since KCL22-RSHP-1$^+$ ceases to proliferate after Ima exposure and concurrently SHP-2 is detected in the inactivated status. Consistently with this finding, SHP-2 knocking-down in KCL22-R decreases cell viability after Ima exposure and induces a significant reduction of activation status of STAT3 (60%) and ERK1/2 (70%). Both KCL22-RSHP-1$^+$ and KCL22-RSHP-2- treated with 2nd generation of TKIs (Nilotinib and Dasatinib) show a similar response of the parental sensitive KCL22-S treated with Ima, thus confirming that our model system could be even applied to study the resistance to 2nd generation TKIs. The role of SHP-1 as determinant of Ima sensitivity was further corroborated by the expression data in bone marrow (BM) samples of 60 consecutive untreated CML patients. The level of SHP-1 mRNA is significantly lower in patients classified as failure responder according to the ENL criteria, than optimal responders (p less than 0.0001). Thus, our data indicate that SPH-1 in Ph$^+$ cells may modulate their sensitivity to Ima treatment through the regulation of the activity of SHP-2.

C070

BCR-ABL TYROSINE KINASE ACTIVITY MODULATES THE PHOSPHORYLATION, LOCALIZATION AND FUNCTION OF INTERFERON REGULATORY FACTOR 5 (IRF-5) IN CHRONIC MYELOID LEUKEMIA CELLS

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Introduction. Interferon Regulatory Factor 5 (IRF-5), is a nuclear protein which modulates the immune response against viral infections and cancer. IRF-5 is a transcription factor with tumor suppressor-like properties that regulates the expression of several genes including Bak, Bax, p21 and caspase-8. Aim of this research was to study the relationship between IRF-5 and the BCR-ABL oncoprotein of Chronic Myeloid Leukemia (CML). *Methods.* We assessed IRF-5 expression by immunoblot on both primary cells derived from CML patients and three immortalized cell lines (K562, KCL22 and KYO-1). We also investigated the association between IRF-5 and BCR-ABL using immunoprecipitation assays before and after treatment with the kinase inhibitor Imatinib Mesylate (IM). IRF-5 intracellular localization was investigated through fractionation experiments while growth in soft agar or in methylcellulose media was used to determine the transforming activity of CML cells expressing different IRF-5 constructs. *Results.* We found that IRF-5 is expressed in both primary and immortalized CML cells. Co-immunoprecipitation assays demonstrated that IRF-5 associated with BCR-ABL and was a target of the oncoproteins kinase activity. In CML cells, IRF-5 was mostly confined to the cell cytoplasm. However, treatment with IM or with alpha-Interferon (IFN) relocalized IRF-5 to the nucleus suggesting that BCR-ABL modulated its nuclear-cytoplasmic shuttling. Mutagenesis of IRF-5 tyrosine 104 to phenylalanine generated a mutant that displayed reduced levels of tyrosine phosphorylation. In addition, IRF-5 Y104F mainly localized to the cell nucleus, confirming that tyrosine-phosphorylated IRF-5 is preferentially cytoplasmic. Finally, over-expression of IRF-5 Y104F significantly reduced proliferation and foci formation of both CML cell lines and CD34$^+$ cells isolated from four patients in chronic phase and one in blast crisis. *Conclusions.* Our findings demonstrate that BCR-ABL associates with IRF-5 and causes its phosphorylation on tyrosine 104, thereby preventing IRF-5 nuclear localization and transcriptional activity. Pharmacological strategies aimed at disrupting the interaction between BCR-ABL and IRF-5 may represent a novel approach to reduce the proliferation of CML cells.