Azacytidine Enhances Sensitivity Response to Imatinib in BCR/ABL positive CML Cell Line

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Azacytidine (5-Aza) is a chemotherapeutic drug that has been known to restore the expression of Tumour suppressor genes by de-methylation and shown clinical efficacy in Myelodysplastic syndrome (MDS) [1-3]. Currently, 5-Aza is being used in UK for the treatment of some adults with MDS, chronic myelocytic leukemia (CML) and acute myelocytic leukemia (AML) [4]. Majority of CML patients treated with imatinib, a BCR/ABL inhibitor would develop resistance under prolonged therapy. Signal transducer and activator of transcription 3 (STAT3) is an oncogenic transcription factor that is constitutively activated in various human cancers including hematological malignancies. Activation of STAT3 represents an important mechanism of imatinib resistant [5]. Methylation of *SHP-1* is involved in the constitutive activation of STAT3 [6], and a low level of *SHP-1* is not sufficient to inhibit activated STAT3 [7]. Epigenetic silencing of *SHP-1* also plays a role in the development of resistance to imatinib in BCR/ABL positive CML cells [8].

Here we evaluated the expression of *SHP-1* gene and its methylation status with sensitivity response of resistant CML cell lines to imatinib before and after treatment with 5-Aza. For this purpose, BCR/ABL positive CML cell lines, K562 and K562-R, an imatinib resistant cell lines were treated with 5-Aza. Cytotoxicity of imatinib and apoptosis were determined by MTS and Annexin-V, respectively. Gene expression analysis was detected by real time-PCR, STATs activity using Western blot and methylation status of *SHP-1* gene by pyrosequencing analysis. There was a significant hypomethylation of *SHP-1* gene in K562-R+5-Aza cells compared to other cells ($p=0.003$), Table 1.

Table 1: Pyrosequencing analysis results showing a significant hypomethylation (p=0,003) in 6 CpG islands of SHP-1 gene in K562-R+5-Aza cells compared to other cells.

Sample ID	CpG-11	CpG-10	CpG-9	CpG-8	CpG-7	CpG-6	Min	Max
K562	23.2	62.8	76.5	64.1	47.9	52.9	23.2	76.5
K562-R	37.6	45.2	82.6	64.9	62.3	50.5	37.6	82.6
K562-R+5-Aza	5.0	3.4	3.2	5.2	5.4	0.0	0.0	5.4
Low Meth Control	6.8	6.9	2.7	10.5	8.3	5.9	2.7	10.5
High Meth Control	93.7	94.0	92.5	74.5	83.3	93.8	74.5	94.0

Gene expression analysis indicates a significant re-expression of *SHP-1* gene (p=0.001) after treatment of K562-R cells with 5-Aza (K562-R+5-Aza cells), Fig 1. Interestingly, the re-expression of *SHP-1* in K562-R+5-Aza cell lines, was associated with STAT3 inactivation and higher sensitivity to imatinib. In conclusion, 5-Aza could enhance efficacy of imatinib on BCR/ABL CML cells through re-expression of *SHP-1* gene and inhibition of STAT3 signaling that could be a new target in cancers treatment.

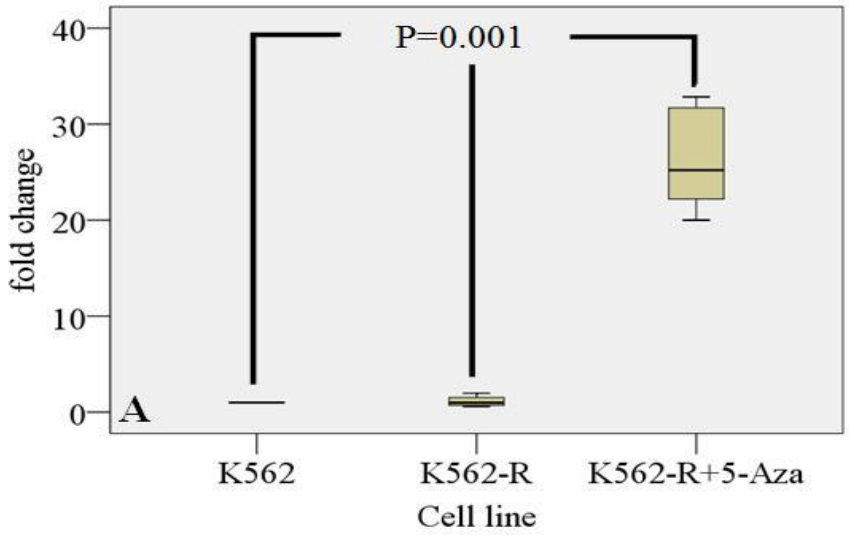

Fig. 1: Box plot depicts the results of real time-PCR, it shows a significant re-expression of *SHP-1* gene (p=0.001) in K562-R+5-Aza cells compared to other cells.

Keywords: 5-Aza, Resistance, *SHP-1*, STAT3

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