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.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>CpG-11</th>
<th>CpG-10</th>
<th>CpG-9</th>
<th>CpG-8</th>
<th>CpG-7</th>
<th>CpG-6</th>
<th>Min</th>
<th>Max</th>
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<tr>
<td>K562</td>
<td>23.2</td>
<td>62.8</td>
<td>76.5</td>
<td>64.1</td>
<td>47.9</td>
<td>52.9</td>
<td>23.2</td>
<td>76.5</td>
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<tr>
<td>K562-R</td>
<td>37.6</td>
<td>45.2</td>
<td>82.6</td>
<td>64.9</td>
<td>62.3</td>
<td>50.5</td>
<td>37.6</td>
<td>82.6</td>
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<tr>
<td>K562-R+5-Aza</td>
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<td>3.4</td>
<td>3.2</td>
<td>5.2</td>
<td>5.4</td>
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</tr>
<tr>
<td>Low Meth Control</td>
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<td>6.9</td>
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<tr>
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<td>92.5</td>
<td>74.5</td>
<td>83.3</td>
<td>93.8</td>
<td>74.5</td>
<td>94.0</td>
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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.

![Box plot](image.png)

**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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References:


