

RESEARCH ARTICLE

Effect of combinational therapy on DNA methylation in *BCR-ABL*⁺ALL.

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ABSTRACT

Epigenetic modifications like aberrant DNA methylation are currently considered as fundamental hallmarks of human cancer, playing a pivotal role in hematological malignancies. Moreover, the patients were characterized to develop drug resistance and poor prognosis due to different epigenetic and genetic mechanisms recent studies have indicated that epigenetic alterations (changes in histone deacetylation and DNA methylation) and dysregulation of miRNAs can also lead to leukemogenesis in acute lymphoblastic Leukemia (ALL). The epigenetic alterations are due to the imbalance between protein acetylation and deacetylation which are regulated by histone deacetylases (HDACs). Hence, epigenetic modifying agents like Vorinostat can impact these epigenetic pathways. Thereby, this paper we intend to study about the role of the combinational therapy of penta-O-galloyl-β-d-glucose (PGG) in combination with HDACi Vorinostat in modulating methylation status of miRNA expression on BCR-ABL⁺ cell line SUP-B15 cell line.

KEYWORDS: DNA methylation, miRNA, Combinational therapy**INTRODUCTION**

Methylation is an important mechanism regulating gene expression with hypermethylation of CpG-rich regions which are associated with gene silencing. The methylation occurs at cytosine (C) bases located 5' to guanine (G) in a CpG dinucleotide and known as CpG islands. During methylation, the methyl groups are transferred to the CpG dinucleotide by DNA methyltransferases. Methylation occurs during cancer, it transcriptionally silences the tumor suppressor genes by hypermethylation. This hypermethylation of tumor suppressor genes thereby leads to disruption of key molecular pathways such as apoptosis, DNA repair pathways, cell cycle checkpoints and cell differentiation. Moreover, it also causes activation of metastasis/invasion pathways, drug resistance and proliferation signal transduction [1].

Further, these methylation changes on miRNAs can directly or indirectly regulate cancer progression either by acting as tumor suppressors or oncogenes, respectively. Therefore, aberrant methylation is an important determinant for leukemogenesis in ALL [2,3]. Recent studies have identified that miRNA-203 controls the expression of ABL and is additionally hypermethylated by the CpG island in some cases of acute lymphoblastic leukemia [4,5]. Thus, miRNA-203 expression

controls the expression levels of *BCR-ABL1* translocation. Studies also reported that methylation dependent epigenetic silencing of miR-152 and miR-10b-5p play a crucial role in modulating tumor progression in Multiple Myeloma [6].

The miRNA-221 and miRNA-222 target KIT which is an oncogene, and functions as tumor suppressors in erythroblastic cells and other human solid tumors [7]. Furthermore, the miRNA-29 family can directly regulate the expression of DNMTs and increase expression of DNMT3a and DNMT3b thereby downregulating the methylation of tumor suppressor genes such as FHIT and WWOX [8]. Hence, epigenetic silencing of tumor suppressor miRNAs by promoter hypermethylation and histone deacetylation is known to modulate the expression of tumor-specific translocation proteins like *BCR-ABL* [5,9].

Survival in ALL has improved in clinical trials with treatment modification based on patients' pharmacodynamics and pharmacogenomics. The epigenetic targets have been discovered and are currently under validation for new anticancer therapies like combinational therapies. Growing evidence suggests that bioactive components impact epigenetic processes often involved with reactivation of tumor suppressor genes, activation of cell survival proteins and induction of cellular apoptosis in many types of cancer [10]. Recently, phytochemicals have been

demonstrated to reactivate tumor suppressor genes in lung and breast cancers [11]. It was also demonstrated that polyphenols also contribute to epigenetic changes associated with the fate of cancer cells and have emerged as potential drugs for therapeutic intervention [12]. In another study, it was proposed that the synergistic anticancer effects of Vorinostat and Epigallocatechin-3-Gallate against HuCC-T1 Human Cholangiocarcinoma Cells were demonstrated [13]. Our data from the previous study revealed that by combinational therapy there was a profound effect in upregulation of miRNA-203 re-expression and miRNA-125b downregulation when compared to PGG and Vorinostat used as single agents in *BCR-ABL*⁺ leukemic cell line [14]. Hence, in this study we determined the role of Vorinostat in combination with PGG on DNA methylation status of miRNAs and their target genes in *BCR-ABL*⁺ALL.

Materials and Methods

Chemicals

SUP-B15 is a *BCR-ABL*⁺ALL cell line obtained from The American Type Culture Condition (ATCC). Vorinostat and penta-O-galloyl- β -d-glucose (PGG) were purchased from Sigma Aldrich (St. Louis, MO, USA). Goat anti-rabbit-IgG-HRP and β -actin (internal control) were purchased from Sigma Aldrich (St. Louis, MO, USA). RPMI-1640, penicillin, streptomycin, fetal calf serum (FCS), *BCR-ABL*, p53, survivin and β -actin (Internal control) primers were purchased from sigma chemical company, St. Louis, Missouri, USA.

Cell culture

SUP-B15 cells were cultured in a tissue culture flask. Cells were routinely maintained in RPMI-1640 with phenol red, supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM glutamine, 1% sodium pyruvate, penicillin (100 U/ml), streptomycin sulfate (100 μ g/ml) (0.025 μ g/ml) 0.1 mM modified Eagle's medium non-essential amino acids and 50 μ M 2-mercaptoethanol. The cells were maintained in an atmosphere of amphotericin 5% CO₂ and 95% air in a humidified 37°C incubator. The medium was changed every day by pelleting out the cells in 1500 rpm for 15 min and adding fresh medium.

Drugs used

Penta-O-galloyl- β -d-glucose (PGG) was dissolved in 0.02% of DMSO in various concentrations (20 μ g, 40 μ g, 60 μ g and 80 μ g) /ml of RPMI 1640 medium and used for the analysis.

Standard drug

Vorinostat (SAHA), a known HDACi was used as a standard drug (1 μ M concentration) [15].

Experimental setup

The cultured SUPB15 cell line was divided into four groups:

Group I: Control (SUPB15 cell line)

Group II: Standard drug 1 μ M Voronistat

Group III: PGG treated 40 μ M

Group IV: Combinational therapy (1 μ M Voronistat and 20 μ M of PGG)

Agarose gel electrophoresis for DNA fragmentation

DNA was isolated from SUP-B15 untreated cells and treated cells using DNA/RNA isolation kit (Qiagen) according to the manufacturer's protocol. Agarose gel electrophoresis was carried out for the analysis of DNA fragmentation [16].

Procedure

Briefly, Cells were collected, washed with PBS twice and then lysed in 100 ml of lysis buffer (50 mM Tris (pH 8.0), 10 mM EDTA) for 3 h at 56°C and treated with 0.5 mg/ml Rnase A. Gel loading buffer (50% glycerol, 1 mM EDTA, 0.25% bromophenol blue and 0.25% xylene) and samples were loaded onto a presoldified, 2% (w/v) agarose gel containing 0.1 mg/ml ethidium bromide. Agarose gels were electrophoresed at 50 V for 90 min in TBE buffer. Then the gel was stained with ethidium bromide and viewed under UV-transilluminator and photographed.

Reverse Transcription - Polymerase Chain Reaction (RT-PCR) *BCR/ABL* expression in control and treated cells

RNA was isolated from SUP-B15 untreated cells and treated cells using DNA/RNA isolation kit (Qiagen). The reverse-transcription polymerase chain reaction (RT-PCR) [17]. Briefly, RNA was reverse transcribed to make complementary DNA (cDNA) copies using a commercially available kit (Genei two-step RT-PCR kit). PCR primers specific to the DNA sequences of portions of *BCR/ABL* were used to amplify the cDNA. The PCR products were separated by agarose gel electrophoresis and visualized by UV trans illumination and the relative expression of *BCR-ABL* was determined. The sequences of oligonucleotide primers (Sigma Aldrich Chemicals Pvt. Ltd., Bangalore, India) used in multiplex RT-PCR for *BCR-ABL* fusion transcripts as the target gene are as follows:

Methylation expression profiling using microarray

DNA extraction and sodium-bisulfite modification: Cells were harvested and genomic DNA was extracted using the QIAamp DNA Blood Minikit (Qiagen, Germany). Genomic DNA (1.5 g) was bisulfite treated using the EZ DNA Methylation Kit (Zymo Research, USA). Briefly, each sample was incubated for 15 min at 37°C in a thermal cycler (Gene Pro; BioER, China), mixed with the CT conversion reagent, incubated for 16 h at 50°C, and then stored at 4°C. The bisulfite-modified DNA was purified and eluted with 30 μ l of the provided M-Elution buffer. Finally, 50 ng aliquots were used as templates for PCR.

Microarray and data analysis: Microarrays were performed in triplicates for SUP-B15 cell line. The samples were hybridized on and The Software Used for DNA Analytics was Agilent Genomic Workbench 7.0. The BATMAN ALGORITHM was used to determine the absolute methylation level for CpG dinucleotides that were contained within DNA fragments queried by probes across the microarray. The methylation status detection algorithm was designed for two-color assays, where the green (Cy3) channel was comprised of input DNA, and the red (Cy5) channel was comprised of the affinity- enriched DNA. The microarray was carried out by Genotype Services, Bangalore using Agilent platform.

Bioinformatics prediction of the methylation status of miRNA and their target genes: Target genes, their respective gene

ontologies (GO terms) and pathways were predicted for all the significant differential methylation expression of miRNAs of SUP-B15 using Gene Spring GX version 11.5 software. A Cytoscape imaging tool was used to draw the microRNA and important target gene interactions.

Statistical analysis

All the Real time data analysis was performed using ABI-7500 software version-2.0.1. Data was normalized according to default parameters. Correlation statistics were checked with Graph pad prism version-6. The microarray raw data files were imported to Gene Spring GX software version 11.5 for log₂ transformation. Significant differential miRNAs were obtained by using unpaired Student's t test with p-value cut off 0.05 and 0.01.

Results and Discussion

DNA fragmentation

By electrophoresis, the SUP-B15 cells showed increased DNA fragmentation characteristic of apoptosis on combinational therapy whereas the untreated SUP B15 cells did not show any DNA fragmentation. Growing evidence suggests that bioactive components impact epigenetic processes often involved with reactivation of tumor suppressor genes, activation of cell survival proteins and induction of cellular apoptosis in many types of cancer [10]. Recent studies have reported that HDAC inhibitors are known to induce apoptosis in ALL cells [18]. Induction of apoptosis activates the endonuclease that is involved in the breaking of DNA into oligo nucleosome length fragments of 100 to 150 bp resulting in a typical ladder in DNA electrophoresis [19]. Similarly, in the present study, it was reported that these inhibitors induced apoptosis by DNA fragmentation. Hence, our study reports the cytotoxic effect of the inhibitors which leads to apoptosis.

Inhibition of *BCR-ABL*⁺ transcript on combinational treatment on leukemic cells

By RT-PCR analysis, it was observed that SUP-B15 cells on combinational therapy (Group IV) showed decreased expression of *BCR-ABL*⁺ with a fold change of >2 when compared to untreated cells (Group I). Moreover, on combinational therapy the cells showed a more significant decreased effect when compared to Group II (PGG) and Group III (Vorinostat) treated cells (Figure 1). In accordance with the results of the present study, it was also reported that HDAC inhibitors like SAHA induced apoptosis by decreased protein expression levels of *BCR-ABL* and c-myc in BV-173 cells (leukemic cell line) [20]. Moreover, Depsipeptide another HDACi upregulated IL-3 gene expression of AML1/ETO positive leukemia cell which is essential for normal haematopoiesis [21]. It was also proposed that the synergistic anticancer effects of Vorinostat and Epigallocatechin-3-Gallate against HuCC-T1 Human Cholangiocarcinoma Cells [13]. Phytochemicals have been shown to induce apoptosis and also inhibit the activity of tyrosine kinase in human leukemic cell line K562 [22]. Thus, our study proves that the mRNA expression of *BCR-ABL*⁺ was significantly downregulated on combinational treatment in SUP-B15 cells.

DNA methylation pattern on combinational treatment in sup-B15 cells

The results of differentially methylated genes of miRNAs are shown in Tables 1 and 2. The CpG content of differentially methylated groups in untreated and treated samples were studied using Batman Algorithm. 850 genes were included in the Agilent platform and among them, 68% was hypermethylated with dense CpG islands. By methylation analysis, it was identified that in the group 1 cells(untreated) 159 oncogenic miRNAs were identified to be hypomethylated and 190 tumor suppressor miRNAs were hypermethylated (<56%). On combinational

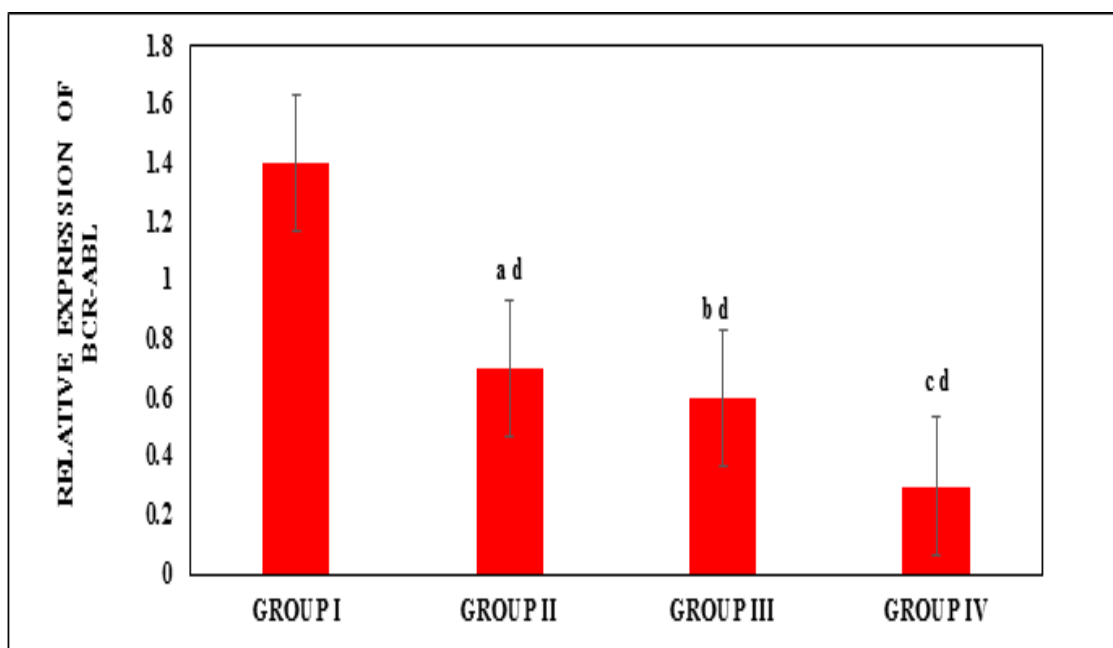


Figure 1. Expression levels of the *BCR-ABL* on combinational treatment in SUP B15 cell line. Values are expressed mean + SD for three independent experiments. Comparisons are made between a. Group I cells vs. Group II cells; b. Group I cells vs. Group III cells; c. Group I cells vs. Group IV cells; d. Group I cells vs. Group II and Group III cells; The symbol a, b, c and d represents statistical significance at $p < 0.05$.

miRNAs	Geo Mean Control	Geo Mean Treated	P_value	CpG Island	Log Ratio	Batman Call
hsa-miR-451a	0.00	-1.52	0.0000	CpG: 153	0.00	0
hsa-miR-144-3p	0.02	-1.45	0.0000	CpG: 257	0.00	0
hsa-miR-10a-5p	0.02	-1.45	0.0000	CpG: 257	0.51	1
hsa-miR-125b-5p	0.05	-1.42	0.0000	CpG: 257	-1.42	-1
hsa-miR-126-5p	0.00	-1.39	0.0000	CpG: 257	-2.11	1
hsa-miR-486-5p	-0.01	-1.35	0.0000	CpG: 257	-0.50	1
hsa-miR-27a-3p	0.00	-0.89	0.0011	CpG: 178	-0.13	1
hsa-miR-101-3p	0.01	-0.88	0.0000	CpG: 178	0.00	0
hsa-miR-424-3p	-0.02	-0.87	0.0000	CpG: 178	0.72	1
hsa-miR-221	0.05	-0.86	0.0000	CpG: 178	-0.97	-1
hsa-miR-614	-0.01	-0.85	0.0000	CpG: 178	0.55	1
hsa-miR-3659	0.01	-0.84	0.0000	CpG: 246	-2.27	1
hsa-miR-4731-3p	-0.07	0.81	0.0000	CpG: 615	1.83	1
hsa-miR-17-92	0.01	-1.35	0.0000	CpG: 615	-0.90	-1
hsa-miR-3150b-3p	0.02	0.89	0.0030	CpG: 615	-0.96	-1
hsa-miR-476-3p	0.00	0.88	0.0000	CpG: 615	0.00	0
hsa-miR-486-3p	0.02	5.08	0.0000	CpG: 615	2.35	1
hsa-miR-335	0.07	-4.93	0.0000	CpG: 615	-1.06	-1
hsa-miR-182-5p	0.00	-4.75	0.0000	CpG: 615	0.93	1
hsa-miR-6516-3p	0.02	-4.45	0.0036	CpG: 615	0.20	1
hsa-miR-4733-5p	0.04	-4.34	0.0253	CpG: 615	-0.03	-1
hsa-miR-3679-3p	0.04	-4.24	0.0000	CpG: 615	1.30	1
Batman Call						
1	Represents methylated states of the probe					
-1	Represents unmethylated states of the probe					
0	Represents unchanged states of the probe					

Table 1. Expression levels of the oncogenic miRNAs on treatment with PGG+HDACI in BCR-ABL⁺ ALL cell line.

miRNAs	Geo Mean Control	Geo Mean Treated	P_value	CpG Island	Log Ratio	Batman Call
hsa-miR-181a-5p	0.19	11.33	0.0000	CpG: 16	0.00	0
hsa-miR-363-3p	0.19	7.71	0.0000	CpG: 159	1.06	-1
hsa-let-7c-5p	0.19	7.16	0.0000	CpG: 159	0.84	1
hsa-miR-98-5p	0.19	6.79	0.0000	CpG: 126	-0.58	-1
hsa-miR-340-5p	0.82	6.78	0.0010	CpG: 45	1.72	1
hsa-miR-326	-0.17	6.71	0.0009	CpG: 45	0.89	1
hsa-miR-7161-5p	0.19	6.30	0.0000	CpG: 187	1.21	1
hsa-miR-181b-3p	0.19	6.14	0.0000	CpG: 187	0.51	1
hsa-miR-3912-5p	0.19	5.92	0.0000	CpG: 78	1.94	1
hsa-miR-155	0.19	5.60	0.0000	CpG: 175	0.99	1
hsa-miR-7155-3p	0.19	5.40	0.0000	CpG: 78	0.00	0
hsa-miR-4753-5p	0.19	5.39	0.0000		0.00	0
hsa-miR-4524b-5p	0.89	5.34	0.0059	CpG: 22	0.00	0
hsa-miR-3689b-3p	0.84	5.29	0.0057	CpG: 23	-0.91	-1
hsa-miR-7-1-3p	0.19	5.04	0.0000		0.00	0
hsa-miR-6871-5p	0.84	5.00	0.0067	CpG: 46	0.19	1
hsa-miR-382-5p	0.81	4.95	0.0063	CpG: 46	0.00	0
hsa-let-7i-5p	-0.88	4.92	0.0019		1.19	1
hsa-miR-4495	0.85	4.88	0.0094	CpG: 18	-0.07	-1
hsa-miR-203	0.19	4.77	0.0000	CpG: 17	1.89	1
hsa-let-7g-5p	-0.05	4.72	0.0000	CpG: 271	1.58	1
hsa-miR-3714	0.85	4.42	0.0149	CpG: 271	0.00	0

hsa-miR-15a-3p	0.19	4.12	0.0000	CpG: 271	0.21	1
hsa-miR-181b-5p	-0.09	4.08	0.0000	CpG: 271	0.87	1
The results are the means \pm SD, $P < 0.01$.						
Batman Call						
1	Represents methylated states of the probe					
-1	Represents unmethylated states of the probe					
0	Represents unchanged states of the probe					

Table 2. Expression levels of the tumor suppressor miRNAs on treatment with PGG + HDACI in BCR-ABL⁺ ALL cell line.

Pathway	miRNA	TOTAL GENES	Log Ratio of the genes	P-value	Genes
Apoptosis	hsa-miR-182-5p, hsa-miR-203, hsa-miR-125b-5p, hsa-miR-17-32, hsa-miR-335, hsa-miR155, hsa-miR-151b	5	0.384025	0.8860	AKT1, PRKACG, RIPK1, PRKAR1B, DFFB
Jak-STAT signaling pathway	hsa-miR-320b, hsa-miR-99b-5p, hsa-miR-182-5p, hsa-miR-203, hsa-miR-125b-5p, hsa-miR-17-32, hsa-miR-335.	6	0.460829	0.9903	LIF, AKT1, IL7, SPRED2, IL15RA, TPO
p53 signaling pathway	hsa-miR-182-5p, hsa-miR-203, hsa-miR-125b-5p, hsa-miR-17-32, hsa-miR-335, hsa-miR155, hsa-miR-151b,	5	0.384025	0.7380	TSC2, RPRM, SFN, GADD45B, GTSE1
PI3 kinase pathway	hsa-miR-602, hsa-miR-27b-3p, hsa-miR-370-3p, hsa-miR-210-3p, hsa-miR-151a-5p	20	1.536098	0.0065	FOXL1, PTGER3, FOXA2, FOXK1, GNA11, SFN, FOXO3, FOXQ1, FOXN3, AKT1, FOXQ1, YWHAG, PDPK1, FOXE1, FOXC2, FOXB2, FOXC1, FOXD4, FOXD3

Table 3. Representation of pathways (Kegg and panther) for the methylated genes.

therapy, 180 oncogenic miRNAs (280CpG islands) were hypermethylated (<68%) and in contrast 42 tumor suppressor miRNAs (220CpG islands) was hypermethylated. The log fold change geo mean cut level of ≥ 0.8 were used for criteria for fold change in methylated and unmethylated treated SUP-B15 cells as compared to the untreated SUP-B15 cells (Tables 1 and 2).

The above results are also consistent with previous observations in patients with BCR-ABL⁺ ALL in which DNA hypermethylation and DNA hypomethylation were observed to be representative epigenetic alterations. Hence, we agree with other study that indicates, gene deregulation through hypermethylation may contribute to the transformation of the hematopoietic cells [23].

Impact of DNA methylation and miRNA dysregulation on functional pathways of genes

The miRNA genes involved in regulating cancer-related pathways are silenced in association with CpG island hypermethylation [24]. Hence, we sought to study the methylation status of these genes in the control and the treated samples using PGG and Vorinostat. Functions and Pathways of Methylated genes obtained using KEGG and PANTHER pathways and the changes in the expression levels (log ratio) of the miRNAs regulated their target genes as described in Table 3.

Role of combinational therapy on DNA methylation status of miRNAs characteristic for BCR-ABL⁺ all expression

In the present study, we further determined the DNA methylation status and miRNAs which were characteristic for BCR-ABL⁺ ALL expression. They include miRNA 221, miRNA 125b, miRNA 335 and miRNA-17-92 were reported to be oncogenic, while miRNA-203, miRNA-155 and miRNA-326 were tumor suppressor genes (Figures 2 and 3). Therefore,

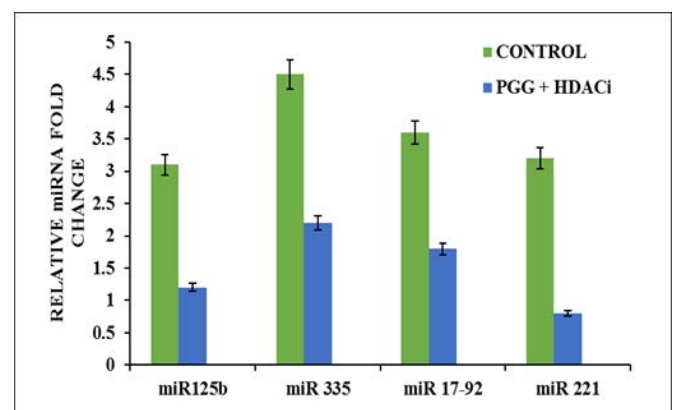


Figure 2. Expression levels of the oncogenic miRNAs on combinational treatment in BCR-ABL⁺ ALL cell line. The results are the means \pm SD. * $P < 0.05$.

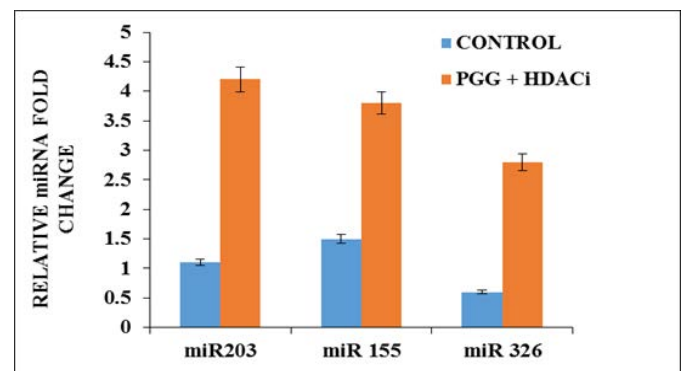


Figure 3. Expression levels of the tumour suppressor miRNAs on combinational treatment in BCR-ABL⁺ ALL cell line. The results are the means \pm SD. * $P < 0.05$.

hypermethylation of tumor-suppressive miRNAs leads to tumorigenesis by targeting oncogenic pathways Hence on

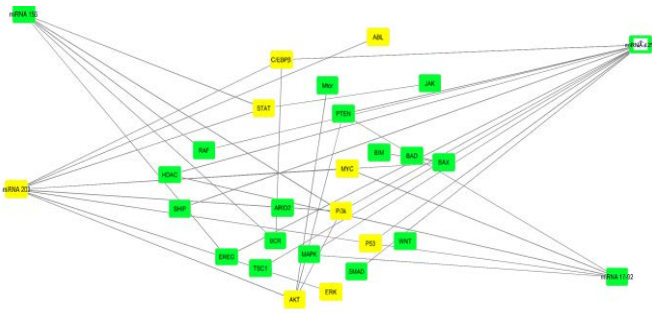


Figure 4. The microRNA (miRNA)-mRNA target network was constructed using Cytoscape. Functions and Pathways of Methylated genes were predicted by KEGG and PANTHER pathways; The green boxes represent differentially regulated miRNAs-targeted genes by combinational therapy; and the yellow box represent miRNA 203 and their target genes involved in the most significantly regulated on combinational therapy enriched Gene Ontology terms.

combinational treatment, it was identified that the promoter of miRNA 221, miRNA 125b, miRNA 335 and miRNA-17-92 were hypermethylated (80 CpG islands) with log ratio <1 on treatment when compared to control samples which leads to its downregulation however, unmethylation were observed in the promoter of miRNA-203, miRNA-155 and miRNA-326 (110 CpG islands) with log ratio <1.5 which lead to upregulation of these miRNAs (Figures 2 and 3). The changes in the expression levels (log ratio) of the miRNAs regulated their target genes as described in Table 3. Recent studies suggest that DNA hypermethylation may lead to gene silencing of tumor suppressor genes, thereby triggering leukemogenesis [25]. Another study showed that miRNAs can be dysregulated by promoter methylation in human malignancies [5].

A significant association was found between the findings of expression pattern of miRNA 203 and *BCR-ABL*. On analysis, we observed a negative correlation between miRNA-203 and *BCR-ABL*⁺ in treated cells when compared to control cells ($r = -0.66$, $p < 0.05$). This correlation study indicates that restoration of miRNA-203 expression by the combinational treatment reduced the proliferation of tumor cells and thereby modulates the expression of tumor specific translocation proteins [5]. Recent studies have identified that miRNA-203 controls the expression of ABL and is additionally hypermethylated by the CpG island in some cases of acute lymphoblastic leukemia [4]. Thus, miRNA-203 expression controls the expression levels of *BCR-ABL1* translocation (Figure 4). Similar to our current study, in our previous study it was identified that miRNA 125b which is an oncogene was significantly downregulated by combinational therapy while the miRNA 203 was upregulated in SUP-B15 cells [14].

Role of combinational therapy DNA methylation status of miRNAs characteristic for *BCR-ABL*⁺ all expression and their pathways

By the KEGG and PANTHER pathways it was found that miRNA-203 controls the expression of ABL and is additionally hypermethylated by the CpG island in which enhances the expression of the *BCR-ABL1* oncogene [4]. Similarly, we found that hypermethylation leads to downregulation of miRNA 326 activates the MDR gene which leads to drug resistance and thereby progression of the disease. miRNA 125b promoted the leukemic cell growth by inhibiting the Bak1 expression which

reduces apoptosis. On combinational therapy the methylation status of these miRNAs and their related pathway were regulated. In our study we also found miRNA-17-92 was upregulated and was identified to be a target of PI3K/AKT pathway was reversed on combinational therapy. MiRNA 221 and miRNA-17-92 plays a major role in downregulation BAX/BAK pathway leading to oncogenesis in *BCR-ABL* cells (Figure 4). Thus, hypermethylation of tumor-suppressive miRNAs plays an important role in leukemogenesis through the activation of target oncogenic pathways. Hence, in this study restoration of tumor-suppressive miRNAs expression by therapeutic intervention like combinational therapy reduced the proliferation of tumor cells in an ABL-1 dependent manner and thereby modulated the expression of tumor specific translocation proteins.

The epigenetic alterations in miRNA are due to the imbalance between protein acetylation and deacetylation which are regulated by histone deacetylases (HDACs). Histone deacetylase inhibitors (HDACi) are a relatively new class of anti-cancer agents that play important roles in epigenetic or non-epigenetic regulation of miRNA expression. Among the HDACi, Suberoylanilide hydroxamate (vorinostat, SAHA), was recently approved for the treatment of cutaneous T cell lymphoma (CTCL). Moreover, HDACi as monotherapy has limited agents are less potential and reported to be toxic. Therefore, better outcomes could be achieved on the rationally designed studies on HDACi like polyphenols which produces effective and safe regimens associated with more durable remissions on treatment. The changes in the miRNA expression are generally influenced by chemical structure of the polyphenols which includes polymerization, esterification, acetylation and methylation [26].

Studies have described that degree of polymerization of the polyphenols has a major role in miRNA expression [27]. Among the chemical constituents, a well-known gallotannin precursor is 1,2,3,4,6-penta-O-galloyl- β -D-glucose (PGG), which consists of a glucose molecule in which five -OH groups are esterified with Gallic Acid, has been shown to suppress tumor growth [12]. The rapid and potent inhibitory activity of PGG which is a naturally occurring gallotannin can restore the activity of the silenced genes by S phase arrest in cell cycle and a downregulation of cyclin D1 for G1 arrest [28]. Studies have the impact of phytochemicals such as gallic acid, catechins and ellagitannins on expression of miRNAs [29,30].

Studies have indicated the clinical benefits of the combinational therapy of vorinostat (HDACi) with 5-azacytidine (DNMTi) in patients with childhood acute lymphoblastic leukemia and acute myeloid leukemia [31,32]. Studies have reported that TSA treatment reduced the genomic DNA methylation level in T24 cells (bladder carcinoma) and MDA-MB-231 cells (breast carcinoma) through an active demethylation process [33]. Moreover, studies have demonstrated that methylated miRNA genes are upregulated by epigenetic drug treatment in cancer cells [34].

Conclusion

Phytochemicals have been demonstrated to inhibit DNMTs and reactivate tumor suppressor genes in lung and breast cancers. In addition to these events, the generation of S-adenosyl L-homocysteine (SAH) at abnormally high concentration levels is an important mechanism of inhibition of DNA methylation. Our results are also consistent with the above study by the demethylating and acetylating activity of PGG and Vorinostat.

Polyphenols can cause an increase in SAH and homocysteine levels thus resulting in inhibition of DNA methylation. Hence, these results indicate that the epigenetic modifying agents, such as PGG and HDACi modulate specific miRNA levels which can lead to the downregulation of target oncogenes and provides a new avenue for the development of therapeutic approaches in treatment of leukemia.

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