Leukemia Research 59 (2017) 32–40

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

MicroRNAs that affect the Fanconi Anemia/BRCA pathway are downregulated in imatinib-resistant chronic myeloid leukemia patients without detectable *BCR-ABL* kinase domain mutations

E Yap[a,*], ZA Norziha[a], A Simbun[b], NR Tumian[a], SK Cheong[c], CF Leong[c], CL Wong[a,d,e]

[a] *Haematology Unit, Department of Medicine, National University of Malaysia Medical Centre, Kuala Lumpur, Malaysia*
[b] *Bioeasy Sdn Bhd, Shah Alam, Selangor, Malaysia*
[c] *Haematology Unit, Department of Pathology, National University of Malaysia Medical Centre, Kuala Lumpur, Malaysia*
[d] *Haematology Unit, Department of Medicine, Sunway Medical Centre, Kuala Lumpur, Malaysia*
[e] *Sunway Institute for Healthcare Development, Sunway University, Kuala Lumpur, Malaysia*

ARTICLE INFO

Keywords:
Chronic myeloid leukemia
Imatinib resistance
miRNA-seq
Fanconi anemia
BRCA pathway

ABSTRACT

Chronic myeloid leukemia (CML) patients who do not achieve landmark responses following treatment with imatinib mesylate (IM) are considered IM-resistant. Although IM-resistance can be due to *BCR-ABL* kinase domain (KD) mutations, many IM-resistant patients do not have detectable *BCR-ABL* KD mutations. MicroRNAs (miRNAs) are short non-coding RNAs that control gene expression. To investigate the role of miRNAs in IM-resistance, we recruited 8 chronic phase CML patients with IM-resistance who tested negative for *BCR-ABL* KD mutations and 2 healthy normal controls. Using miRNA sequencing, we identified 54 differentially expressed miRNAs; 43 of them downregulated. The 3 most differentially downregulated miRNAs were miR-146a-5p, miR-99b-5p and miR-151a-5p. Using real-time quantitative reverse transcriptase-polymerase chain reaction, the expression patterns of the 3 miRNAs were validated on the same cohort of 8 patients in addition to 3 other IM-resistant CML patients. *In-silico* analysis showed that the predicted gene targets are *ATRIP, ATR, WDR48, RAD51C* and *FANCA* genes which are involved in the Fanconi Anemia/BRCA pathway. This pathway regulates DNA damage response (DDR) and influences disease response to chemotherapy. Thus it is conceivable that DDR constitutes a key component in IM-resistance. Further research is needed to elucidate miRNA modulation of the predicted gene targets.

1. Introduction

Chronic myeloid leukemia (CML) is a myeloid neoplasm caused by the *BCR-ABL* fusion gene, a product of the chromosomal translocation t (9:22) which results in the Philadelphia (Ph) chromosome. This fusion gene encodes the BCR-ABL oncoprotein, a constitutively active tyrosine kinase that causes dysregulated cellular proliferation and apoptosis resistance via interference in downstream signalling pathways. Current standard management of patients who have CML is with *BCR-ABL* inhibition with tyrosine kinase inhibitors (TKIs) such as imatinib mesylate (IM). Resistance to IM is an emerging issue [1]. Failure to achieve established landmark responses despite IM therapy constitutes treatment failure and the patient is deemed resistant to IM. When this occurs, the patient is tested for possible kinase domain (KD) mutations on the *BCR-ABL* fusion gene and possibly be treated with an alternative TKI. KD mutations of *BCR-ABL* are responsible for approximately 40% of all cases of resistance [2]. Thus, in the majority of patients who failed TKIs, no KD mutations are detected. Patients who are resistant to all commercially-available TKIs with no detectable *BCR-ABL* KD mutations have poor prognosis. Due to the increasing prevalence of CML and the availability of more potent TKIs, this group of patients represents an unmet medical need.

MicroRNAs (miRNAs) belong to one of the classes of non-coding RNAs which are functional RNAs that do not translate into protein. miRNAs are in the range of 19–22 nucleotides long and transcribed from pri-miRNAs which are first processed into pre-miRNAs by Drosha, an intranuclearRNase III enzyme. Pre-miRNAs are then exported to the cytoplasm where Dicer, another RNAse III enzyme cleaves them to give rise to mature miRNAs. MiRNAs play an important role in hemato-poeisis from apoptosis to cell differentiation regulation [3]. The role of miRNAs in the pathogenesis of CML is established, and has expanded the role of *BCR-ABL* [4–7]. It has also been shown that miRNA profile

* Corresponding author.
E-mail address: ernie.yap@gmail.com (E. Yap).

http://dx.doi.org/10.1016/j.leukres.2017.05.015
Received 28 February 2017; Received in revised form 13 May 2017; Accepted 16 May 2017
Available online 18 May 2017

Table 1
Patient Characteristics.

Patient	Age	Sex	Switched to Nilotinib	Duration of Imatinib (months)	Duration of Nilotinib (months)	Time to Hematologic Remission (months)	Best Cytogenetic Response	Time to Best Cytogenetic Response (months)	Sokal Score at diagnosis
1	24	M	N	61.5	–	2.5	Minor	36.57	1.52
2	55	M	Y	56.94	11.73	1.41	Partial	53.55	0.67
3	65	F	N	21.09	–	10.97	Minor	34.96	1.37
4	55	M	Y	26.35	10.58	0.66	Complete	36.90	1.12
5	28	M	N	120.05	–	3.22	Complete	21.62	0.48
6	52	F	N	28.88	–	2.3	Minor	10.15	1.3
7	44	M	Y	54.31	26.45	2.04	Complete	56.61	1.57
8	30	F	Y	89.26	53.16	39.79	Partial	134.93	0.55

Complete cytogenetic response (CCyR) − no Ph-positive metaphases.
Partial cytogenetic response (PCyR) − 1–34% of cells have Ph-positive metaphases.
Major cytogenetic response (MCyR)- 0–35% of cells have Ph-positive metaphases (Complete + Partial).
Minor cytogenetic response- > 35% Ph-positive metaphases.

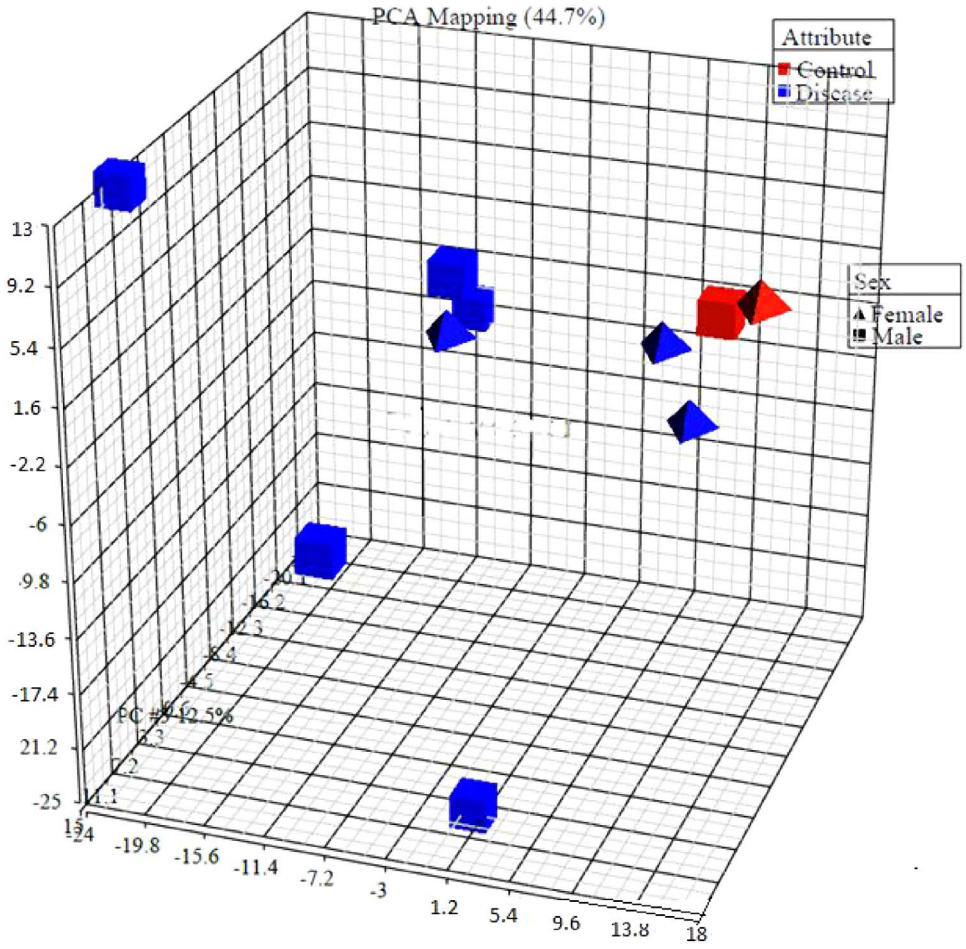

Fig. 1. 3-Dimensional Principal Component Analysis (PCA) mapping showing distinct clustering of IM-resistant CML and normal controls.

undergoes dynamic changes following IM therapy [8,9].

We hypothesize that miRNA dysregulation also plays a role in IM-resistance in which no *BCR-ABL* KD mutations are identified. Next generation sequencing (NGS) can detect the expression levels of each miRNA in the miRNome, and is thus an effective and accurate approach for evaluation of global miRNA expression levels. To the best of our knowledge, NGS of the miRNome of CML patients who are IM-resistant has not previously been performed, and such an analysis may provide insight into the molecular mechanisms of this phenomenon.

2. Materials and methods

We enrolled 8 patients (5 Malays, 2 Chinese and 1 Indian) from the National University of Malaysia Medical Centre (UKMMC) diagnosed with CML in chronic phase, demonstrated primary resistance to IM as described in the National Comprehensive Cancer Network (NCCN) 2015 guidelines [10] and tested negative for *BCR-ABL* KD mutations. Briefly, mutation analyses were undertaken using denaturing high performance liquid chromatography (dHPLC). The PCR products of samples that showed altered dHPLC profile were then directly sequenced with both forward and reverse primers after purification.