LETTER to the EDITOR

Absence of EZH2 Gene Mutation in Chronic Myeloid Leukemia Patients in Blast Crisis

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Dear Editor

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disease consisting of chronic phase (CP), accelerated phase (AP) and blast crisis (BC). In recent years, molecular mechanism of CML- BC has been comprehensively investigated. Based on the DNA sequencing, several somatic gene mutations in patients with CML-BC has been revealed. These gene including RUNX1, ASXL1, IKZF1, WT1, TET2, IDH1, IDH2, NRAS, KRAS, CBL, CBLB, TP53, and GATA2 (Zhang et al., 2008; Grossmann et al., 2011; Makishima et al., 2011).

The histone methyltransferase gene EZH2 is the catalytic subunit of the PRC2 polycomb complex and mediates transcriptional repression through its histone methyltransferase activity (Grossmann et al., 2012). Mutations in the EZH2 gene were recently described in patients with B-cell lymphomas (Morin et al., 2010), chronic myelomonocytic leukemia (CMML) (Jankowska et al., 2011), adult and pediatric acute myeloid leukemia (Makishima et al., 2010; Ernst et al., 2012), myelofibrosis (Guglielmelli et al., 2011), myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN) and the overlap myelodysplastic/myeloproliferative neoplasms (MDS/MPN) (Ernst et al., 2010; Makishima et al., 2010). It was revealed that EZH2 mutations were correlated with poor survival. Among patients with MDS/MPN, patients harboring EZH2 mutations had an inferior survival compared with those without EZH2 mutation (Ernst et al., 2010). In addition, EZH2 mutations were independently associated with shorter survival in patients with primary myelofibrosis (PMF) (Guglielmelli et al., 2011). These results indicate that EZH2 plays an important role in hematological disease. To date, it still remains unclear whether EZH2 mutations are also presented in patients with CML-BC. In the present study, we sought to investigate the prevalence of EZH2 mutation in CML-BC patients.

A total of 55 patients with CML-BC enrolled in Jiangsu Institute of Hematology were retrospectively analyzed in the present study which was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University following Declaration of Helsinki. The median age was 39 years old (12-69 years), and the cohort was all Chinese with the majority of male

Table 1. Clinical Characteristics of Patients with CML-BC

Characteristics	Value (median, range)
Number of patients	55
Clinical features	
Median age, y (range)	39 (12-69)
M/F	38/17
Blast crisis	
Myeloid	43
Lymphoid	12
Cytogenetics	
t (9; 22) (q34; q11)	25
t (9; 22) (q34; q11) and ACA	21
Simple variant/t (v; 22) (BCR-ABL+)	1
Complicated variant/t (v; 9; 22)	1
Karyotypic failure (BCR-ABL+)	7

ACA, additional cytogenetic aberrations; Simple variant/t (v;22): translocation involving 22q11 and a chromosome other than 9q34; Complicated variant/t (v; 9; 22): translocation involving 9q34, 22q11, and one or more other chromosomes; Other: different from above-mentioned karyotype

Table 2. Primer List of EZH2 (Exons 2-20)

EZH2-exon 2-F	AAGTGTTTTAAGGATTTAACCATGCA
EZH2-exon 2-R	CCTTTATATTTAGGGAGGCATTTCTG
EZH2-exon 3-F	TTTCTCCTTTCCTCTCCTTCA
EZH2-exon 3-R	TCCAATAGCATAAACCAAAAGATG
EZH2-exon 4-F	GGCTACAGCTTAAGGTTGTCCT
EZH2-exon 4-R	CTGTCTTGATTCACCTTGACAAT
EZH2-exon 5-F	AAATCTGGAGAACTGGGTAAAGAC
EZH2-exon 5-R	TCATGCCCTATATGCTTCATAAAC
EZH2-exon 6-F	AGGCTATGCCTGTTTTGTCC
EZH2-exon 6-R	AAAAGAGAAAGAAGAAACTAAGCCC
EZH2-exon 7-F	GTAGCAGAGCTGGGAGTAGAA CCTA
EZH2-exon 7-R	GTAATGCAGAGTACCACAAGTACACATG
EZH2-exon 8-F	CATCAAAAGTAACACATGGAAACC
EZH2-exon 8-R	TTGTAATAAATGATAGCACTCTCCAAG
EZH2-exon 9-F	TCCATTAATTGACTTTTCCAGTG
EZH2-exon 9-R	ACCTCCACCAAAGTGCAAAG
EZH2-exon 10-F	TTCTCTTCCATCAAAATGAGTTTTAG
EZH2-exon 10-R	TCCTCACAACACGAACTTTCAC
EZH2-exon 11-F	GAGTTGTCCTCATCTTTTCGC
EZH2-exon 11-R	CCAAGAATTTTCTTTGTTTGGAC
EZH2-exon 12-F	AAGAATGGTTTGCCTAAATAAGAC
EZH2-exon 12-R	CCTTGCCTGCAGTGTCTATC
EZH2-exon 13-F	TCTTGGCTTTAACGCATTCC
EZH2-exon 13-R	CAAATTGGTTTAACATACAGAAGGC
EZH2-exon 14-F	TGATCGTTTCCATCTCCCTG
EZH2-exon 14-R	AGGGAGTGCTCCCATGTTC
EZH2-exon 15-F	GAGAGTCAGTGAGATGCCCAG
EZH2-exon 15-R	TTTGCCCCAGCTAAATCATC
EZH2-exon 16-F	TTTTTGATGATGTGATTGTGTTTT
EZH2-exon 16-R	TGGCAATTCATTTCCAATCA
EZH2-exon 17-F	TTCTGTCAGGCTTGATCACC
EZH2-exon 17-R	CTCGTTTCTGAACACTCGGC
EZH2-exon 18-F	AGGCAAACCCTGAAGAACTG
EZH2-exon 18-R	TTCCAATTCTCACGTCAAAGGTA
EZH2-exon 19-F	CATTCGGTAAATCCAAACTGCT
EZH2-exon 19-R	AATGCTCATGGCAAAGTGACC
EZH2-exon 20-F	ACCCACTATCTTCAGCAGGCTTT
EZH2-exon 20-R	CTTCCACATATTCACAGGCAGTATTAGT

(69.1%). Main characteristics of patient in this study are summarized in Table 1.

Genomic DNA was prepared either from purified fraction of mononuclear cells after Ficoll density centrifugation or methanol/acetic acid-fixed cells routinely prepared for cytogenetic analysis. Then PCR amplification of the entire coding region of EZH2 exons (2-20) followed by direct bidirectional DNA sequencing were performed as previously described. Primers were used for both PCR amplification and Sanger sequencing. Sequences of primers were shown in Table 2. PCR products were sequenced on both strands by using an ABI 3730 XL DNA Analyzer (Applied Biosystems, Foster city, CA, USA).

However, none of EZH2 mutations, including somatic mutations and novel single nucleotide polymorphisms (SNPs), were found in CML-BC patients enrolled in our study.

In conclusion, our study demonstrated that there was no evidence of EZH2 mutations in the 55 cases of CML-BC analyzed and the results was consistent with others' study in which EZH2 mutations were also absent in 40 CML-BC patients (Ernst et al., 2010). It was suggests that the EZH2 mutations might not involved in the leukemogenesis of CML and the progression from CML-CP to CML-BC.

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