Int J Hum Genet, 19(2): 66-69 (2019)
PRINT: ISSN 0972-3757 ONLINE: ISSN 2456-6330 DOI: 10.31901/24566330.2019/19.02.720

Duplication of Isoderivative Ph Chromosome with Tp53 Deletion in a Case of Imatinib Resistant CML

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KEYWORDS Isoderivative Philadelphia Chromosome. Tp53 Deletion

ABSTRACT Philadelphia chromosome, a consequence of BCR/ABL1 fusion on chromosome 22, is present in ninety percent chronic myeloid leukemia cases and corresponds to good response to first-line Imatinib Mesylate, regardless of the disease phase. There still remains a subset of patients failing to respond to Imatinib. These patients often harbor cytogenetic abnormalities like BCR/ABL1 gene amplification and Tp53 deletion that are strongly associated with resistance. ABL1 domain mutation is another major cause of resistance. The researchers' report a case of CML, displaying resistance to Imatinib- the current standard of care, due to duplication of isoderivative Philadelphia chromosome, translocation t(17;22)(p11.2;q11.2) and Tp53 deletion. This case additionally also showed ABL1 domain mutation. Chromosomal aberrations in this patient were identified by conventional karyotype and FISH, ABL domain mutation by RQ-PCR and BCR/ABL1 fusion gene copy number by real time PCR. Identifying such chromosomal aberrations can help predict clinical outcome and modify treatment.

INTRODUCTION

Chronic Myeloid Leukemia (CML), a result of t(9;22)(q34.13;q11.23) (BCR/ABL1- gene fusion), is characterized by constitutively expressed tyrosine kinase. Imatinib, the standard of care, complexes with this BCR/ABL1 fusion gene and inactivates it (Al-Achkar et al. 2013). Despite being highly efficient, fifteen to twenty five percent patients display resistance to Imatinib (Ramachandran et al. 2016). This is thought to occur predominantly due to BCR/ABL1 gene amplification (Milojkovic et al. 2009). Isoderivative Ph chromosome is likewise associated with resistance (Whang-Peng et al. 1973; Kovacs et al. 1986; Szych et al. 2007; Vundinti et al. 2014). Deletion of Tp53 gene (Kokate et al. 2015), a secondary event in CML although infrequent is blatantly linked with disease progression and poorer prognosis (Otero et al. 2005). Literature also states mutations in kinase activating domain of BCR/ABL1 fusion gene to be associated with resistance to Tyrosine Kinase Inhibitor (TKI) (Ramachandran et al. 2016).

The researchers describe a case of Imatinib resistant CML, where resistance was a consequence of duplicated isoderivative Philadelphia chromosome and Tp53 deletion. Additionally, presence of ABL1 domain mutation worsened prognosis even further. To the best of the researchers' knowledge, such a CML case has not been previously reported.

METHODOLOGY

Fluorescence In-situ Hybridization was performed using LSI BCR/ABL1 dual colour dual fusion (DCDF) (Zytovision, Germany) probe. This probe was a mixture of BCR gene specific sequence tagged with a green fluorophore and an ABL1 gene specific sequence tagged with a red fluorophore. Metaphase FISH (same probes) was used to confirm duplication of isoderivative Philadelphia chromosome. GTG banding (karyotyping) was performed according to laboratory protocol (McGowan-Jordan et al. 2016). Tp53 deletion was confirmed using Tp53 dual

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colour deletion probe (Tp53 tagged red and centromere 17 tagged green) (Zytovision, Germany).

CASE HISTORY

The 46-year-old male patient, diagnosed with CML in 2009 (with baseline ABL1 translocation ratio- 15%), was referred to the researchers' institute in April 2018 (now deceased). The patient denied *gutka* or alcohol addiction. Assessment of liver did not indicate liver decompensation or jaundice. Blood cell count revealed white blood cells (WBC) 14.60× 10^9/L with eight percent blast cells, hemoglobin (Hb) 6.60g/dl and platelets 243× 10^9/L. Biochemical investigation informed high levels of serum globulin (4 g/dL), serum alkaline phosphatase (153 U/L), serum LDH (326 U/L) and low levels of serum sodium (133 mmol/L). Histopathology indicated scanty marrow particle showing edematous and fibrotic (grade II) marrow. Morphology of bone marrow aspirate revealed reduced cellularity with dysmyelopoiesis. Microbiology analysis by chemiluminiscent microparticle immunoassay showed reactive HBsAg.

Molecular cytogenetics (FISH) showed a signal pattern of 5F1R1G in ninety five percent interphase cells (Fig.2a), indicating presence of BCR/ABL1 amplification (5F) and one normal chromosome 9 (1R) and 22 (1G) each. Metaphase FISH confirmed four fusions to be attributable to duplicated isoderivative Philadelphia chromosomes, however surprisingly, the green signal (1G) was present on chromosome 17 indicating t(17;22) (Fig.1a). GTG-banding, showed a karyotype 46,XY,t(9;22)(q34;q11.2),+ ider(22)t(9;22)x2,der(17)t(17;22)(p11.2;q11.2),-22 (Fig.1b) thus confirming translocation of BCR gene on chromosome 17 (p-arm). Tp53 deletion due to t(17;22)(p11.2;q11.2) was confirmed by 1R2G FISH signal pattern (Fig.2b).

Molecular department confirmed BCR/ABL1 gene transcript level using real time quantitative PCR. BCR/ABL1 copy number was observed to be 767900 (ABL copy number- 423800, IS conversion factor- 1.41, IS normalized copy number- 255.483%,). The specimen was then tested for mutations in the ABL kinase domain which showed a heterozygous 185bp deletion- p.R362fs*21, involving exon 7 within the tyrosine kinase domain of the BCR/ABL1 fusion product.

At the researchers' centre, the patient was primarily started on standard 400 mg Imatinib therapy but due to incompliance (high blood counts), 800mg Imatinib was prescribed for 15 days. Subsequent blood count showed low hemoglobin and high WBC. Following this, 600 mg Imatinib was started for a week. However soon after, the patient died. This indicated that the patient was unresponsive/ resistant to Imatinib Mesylate, even at higher doses.

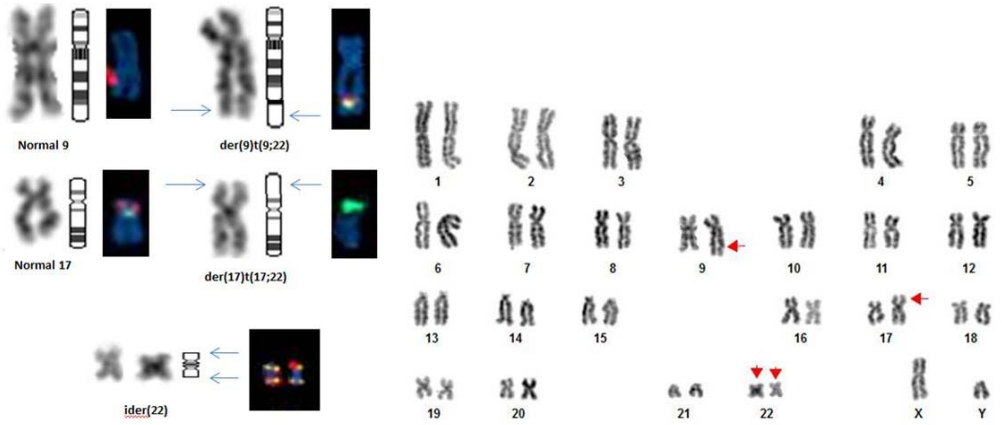
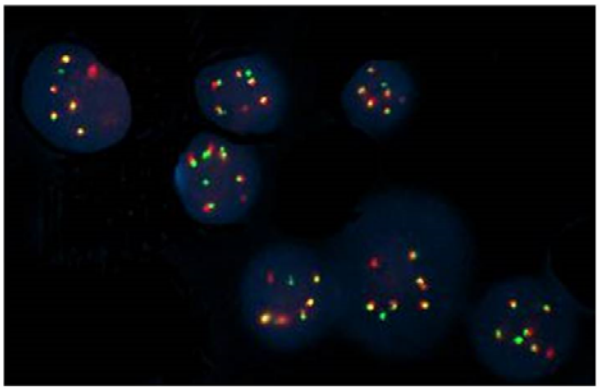


Fig.1. A) Partial karyotype, Idiogram and metaphase FISH (BCR/ABL1 and Tp53 probes) images showing normal 9 with 1R, der 9 with 1F (ABL1/BCR fusion), normal 17 with 1R1G (R-Tp53; G-CEP), derivative 17 with 2G due to CEP 17 and t(17;22) and 2 copies of isoderivative 22 (4F)
Fig.1. B) Karyotype-46,XY,t(9;22)(q34;q11.2),+ider(22)t(9;22)x2,der(17)t(17;22)(p11.2;q11.2),-22



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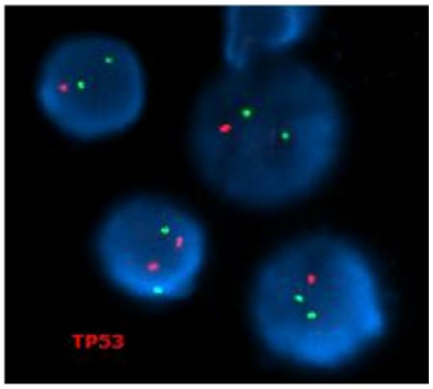

B

Fig.2. A) 5F1R1G showed 5 copies of BCR/ABL1 (5F), normal chromosome 9 (1R) and 22 (1G) using BCR/ABL1 probe

Fig. 2. B) 1R2G confirming Tp53 deletion (R-Tp53 gene G-CEP 17)

DISCUSSION

The researchers confirm that resistance to TKI was most certainly due to amplified BCR/ABL1 oncogene and Tp53 deletion. This was also congruent to published literature that suggested elevated expression of BCR/ABL1 oncogene to be a major cause of resistance (Al-Achkar et al. 2013) and Tp53 gene deletion to worsen response to TKI therapy/ promote resistance. The researchers deduce that the duplication of mutated oncogene caused impaired drug binding capacity or efflux of anti-tyrosine kinase drugs and Tp53 deletion inhibited apoptosis. A critical review of literature showed dissimilar opinions when associating ABL domain mutations to resistance. Awidi et al. (2012) suggest ABL domain mutations to be a major cause of resistance but Abdullaev et al. (2017) reported 15 out of 32 cases with p.R362fs*21 mutation to be sensitive to TKI therapy. Tp53 gene deletion, on the contrary, has always been linked to poor clinical response.

CONCLUSION

The researchers conclude that, in their case, amplification of BCR/ABL1 fusion gene, deletion in Tp53 gene and additional secondary aberrations led to TKI resistance. Mutation of the ABL1 kinase could also be an added factor to the unfortunate outcome. A comprehensive study in cases resistant to TKI is required to understand clinical outcomes in such genetic aberrations.

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Paper received for publication in January 2019
Paper accepted for publication in May 2019