

Microsatellite instability is a rare phenomenon in transition from chronic to blastic phase chronic myeloid leukemia

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Little is understood about the basic biological mechanisms that underlie the reasons for acute transformation in chronic myeloid leukemia (CML). Previously published reports on the potential role of microsatellite instability (MSI) in the transition from chronic to blastic phase CML, are limited in number and contain controversial results. We aimed to analyse DNA of chronic phase and blastic phase archive material of 13 CML patients for genomic instability identified by the presence of MSI using D1S430, D2S123, D3S1611, D11S29, D14S65, D17S520, BAT 40 markers, the dinucleotide repeat located in ABL, and the trinucleotide repeat located in BCR. Only two out of 13 patients manifest alterations in banding patterns in two or more loci examined and presented MSI (15%). We conclude that MSI may be a late occurring but rare event in CML evolution, and in itself is far from explaining the general instability observed in CML. [Turk J Cancer 2001;31(2):63-71]

Key words: CML, chronic phase, blastic transformation, microsatellite instability

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder of the hematopoietic stem cell, characterised by a triphasic clinical course. Massive expansion of myeloid lineage cells without maturation arrest is observed in the initial chronic phase. The disease progresses into an accelerated phase, followed by blastic crisis resembling acute leukemia. The disease is associated with a cytogenetic marker known as the Philadelphia (Ph) chromosome. The Ph chromosome is nearly always present in CML and is thought to be the primary genetic event in disease pathogenesis (1). Molecularly it is formed by the fusion of the ABL proto-oncogene localised on 9q34; with the BCR gene localised on the 22q11 (2). The resulting fusion gene encodes a 210 kD hybrid Bcr/Abl protein with elevated tyrosine kinase activity when compared to c-Abl (3). Being one of the most extensively studied human diseases, many have been established in elucidating the biological properties of Bcr/Abl expressing hematopoietic cells, but

still much remains to be discovered (4-6). It is of great importance to understand the fundamental causes for genomic instability that results in the accumulation of secondary genetic changes in chronic phase hematopoietic cells, for both effectiveness of therapeutical interventions and patient survival are determined mainly by the duration of chronic phase (4,5). For this reason it is essential to define the molecular events resulting in acute transformation.

Nearly eight years ago, a distinct form of genetic alteration has been reported in sporadic colon cancer and hereditary non-polyposis coli colorectal cancer (HNPCC) patients (7-9). This new class of genetic alteration termed microsatellite instability (MSI) is characterised by allelic shifts of microsatellite DNA in tumour tissue when compared to its matched constitutional tissue of the same patient. MSI has been reported to be caused by mutations in the mismatch repair (MMR) system, resulting in the accumulation of replication errors (10,11). HNPCC patients were found to have germline mutations in the mismatch repair genes in which they proceed through carcinogenesis of the colon via Knudsen's double hit model for tumour suppressor genes. Many investigations were done on a variety of sporadic neoplasms due to the potential role this type of replication errors may have in cancer etiology. Although MSI was detected in approximately 9-15% of sporadic tumours; mutations in the MMR genes could not be detected in most of them, which implies the presence of other components of repair pathways not yet identified (12). Similar results were obtained in reports published on hematopoietic system malignancies (13-15).

In search of mechanisms that may contribute to the transition to blastic phase in CML, this identified pathway of carcinogenesis immediately drew attention. Several reports on the potential role of MSI in the transition from chronic to blastic phase have been published (16-21). Unfortunately reports on this subject are limited in number and contain controversial results. Wada et al (16) reported MSI in at least two of five microsatellite loci in 52.6% of the patients studied and concluded that MSI is strongly associated with acute transformation. In contrast, other reports (in which 10-82 different microsatellite loci were studied) found no evidence for MSI or loss of heterozygosity (LOH) in CML (17,19-21). Additional investigations on the potential role of replication errors are needed to clarify the contradictory results previously reported. For these reasons, we examined chronic and blastic phase archive material of 13 CML patients for MSI, using nine microsatellite markers, in aim to determine the frequency of this phenomenon in transition from chronic to blastic phase CML.

Materials and Methods

Bone marrow samples of blastic and chronic phase CML were obtained from archive material of 13 patients with a confirmed diagnosis. Bone marrow samples were recovered from paraffin blocks or unstained cytological smears. Paraffin embedded bone marrow biopsies were microdissected to remove bone and connective tissue as much as possible before sample recovery. The remaining tissue was scrapped off the slide and washed with xylol twice followed by another washing procedure performed by adding one volume of ethanol. Two hundred μ l Proteinase K, 50 ITET (Tris-EDTA-Tween 20), was added on to the pellet and the mixture was left for overnight incubation at 55°C. Phenol-chloroform extraction was performed in the morning. Cells stripped off unstained cytological

smears were lysed in 100 μ l Proteinase K, 500 μ l ISTE, 1% SDS at 55°C for 3 hours and phenol-chloroform extracted afterwards.

DNA extracted from both chronic and blastic phase bone marrow samples of the same patients were analysed for evidence of genetic instability at 9 microsatellite loci. Primer sequences for D1S430, D2S123, D3S1611, D11S29, D14S65, D17S520 markers; the dinucleotide repeat in the ABL gene, located in the ABL1. PCR1 amplicon (Genome Database Accession ID: GDB119640); and the trinucleotide (CCG) repeat in the BCR gene, located in the 5'UTR region (Genome Database Accession ID: GDB 120562) were all obtained from The Genome Database (GDB- www.gdb.org). The primer sequence for the BAT 40 marker was obtained from previous reports (22). Primers were commercially synthesised by Genosys Biotechnology's Inc.

Polymerase Chain Reaction (PCR) mixture for each marker contained 100 ng of genomic DNA, 20 pmol of each primer, 100 μ M of each dNTP, 10X buffer (100 mmol/L TrisHCL, pH 8.8, 500 mmol/L KCL, 15 mmol/L MgCl₂, 0.01% w/v gelatine), and 1U of Taq Polymerase (Boehringer Mannheim Biochemicals Cat# 1146165) in a 20 μ l volume. Amplification was accomplished by thermal profiles including 7 minutes at 94°C predenaturation in all reactions and 30 cycles at 94°C for 45 seconds, 55°C for 45 seconds, 72°C for 45 seconds and a 7 minutes-94°C extension for D2S123, D11S29 and D14S65 markers; 33 cycles at 94°C for 60 seconds, 55°C for 60 seconds, 72°C for 60 seconds and a 7 minutes-94°C extension for BAT40; 33 cycles at 94°C for 60 seconds, 56°C for 60 seconds, 72°C for 60 seconds and a 7 minutes-94°C extension for the ABL dinucleotide repeat; 30 cycles at 94°C for 45 seconds, 55°C for 60 seconds, 72°C for 60 seconds and a 7 minutes-94°C extension for D1S430; 30 cycles at 94°C for 30 seconds, 49°C for 45 seconds, 72°C for 45 seconds and a 7 minutes-94°C extension for D17S520; 33 cycles at 94°C for 30 seconds, 55°C for 45 seconds, 72°C for 90 seconds and a 7 minutes-94°C extension for D3S1611. Various thermal profiles were used to amplify the trinucleotide repeat in the BCR gene, but amplification could not be succeeded. Two volumes of denaturant gel loading buffer were added on to the amplification products. The samples were heat denatured at 95°C for 7 minutes and run on standard denaturing 8% polyacrilamide gels. Bands were visualised by silver staining using modifications of previously described methods (23).

Results

We aimed to investigate the role of genomic instability identified by the presence of MSI, in the transition from chronic phase to blastic transformation in CML. DNA of chronic phase and blastic phase archive material of 13 CML patients were analysed using D1S430, D2S123, D3S1611, D11S29, D14S65, D17S520, BAT40 markers and, di- and tri- nucleotide repeats localised in the ABL and BCR genes respectively. The representative results of microsatellite alterations are given in Table 1. MSI was identified by random shifts in the electrophoretic mobility of the short nucleotide repeats. Reproducibility was confirmed with triplicate assays, including DNA isolation from slides obtained at the same date, new rounds of PCR amplification and gel electrophoresis.

Table 1
The representative results of microsatellite alterations between
chronic and blastic phase bone marrow specimens of CML patients

No	Blastic Phenotype	D2S123	D14S65	D11S29	D17S520	D1S430	D3S1611	ABL	BAT40	BCR
1	myeloid	N	N	N	N	N	N	N	N	*
2	myeloid	N	N	N	N	N	N	N	N	
3	myeloid	MSI	N	N	N	N	N	N	N	
4	lymphoid	MSI	LOH	MSI	N	N	N	N	N	
5	myeloid	MSI	N	N	N	N	N	N	N	
6	myeloid	N	N	N	N	N	N	N	N	
7	lymphoid	N	LOH	MSI	MSI	N	N	N	N	
8	myeloid	N	N	N	N	N	N	N	N	
9	myeloid	N	N	N	N	N	N	N	N	
10	myeloid	LOH	N	N	N	N	N	N	N	
11	myeloid	N	N	N	N	N	N	N	N	
12	myeloid	N	N	N	N	N	N	LOH	N	
13	myeloid	MSI	N	N	N	N	N	N	N	

*The trinucleotide (CCG)₁₇ repeat in the BCR gene could not be amplified
 N: no alteration detected; MSI: instability detected; LOH: loss of heterozygosity

No alterations in banding patterns were observed for the D1S430, D3S1611 and BAT 40 markers. Four out of 13 patients exhibited MSI (33%) and one patient exhibited loss of heterozygosity (LOH) (7.7%) with D2S123 (Figure 1). Two out of 13 patients exhibited MSI with D11S29 (15%). D17S520 exhibited MSI in only one case (7.7%). MSI was not demonstrated for D14S565, but LOH was observed in 2 cases with this marker (15%), both proceeding through lymphoid blast crisis. One case exhibited LOH for the ABL dinucleotide repeat (7.7%). As a result, only 2 out of 13 patients presented MSI (15%) (Figure 2).

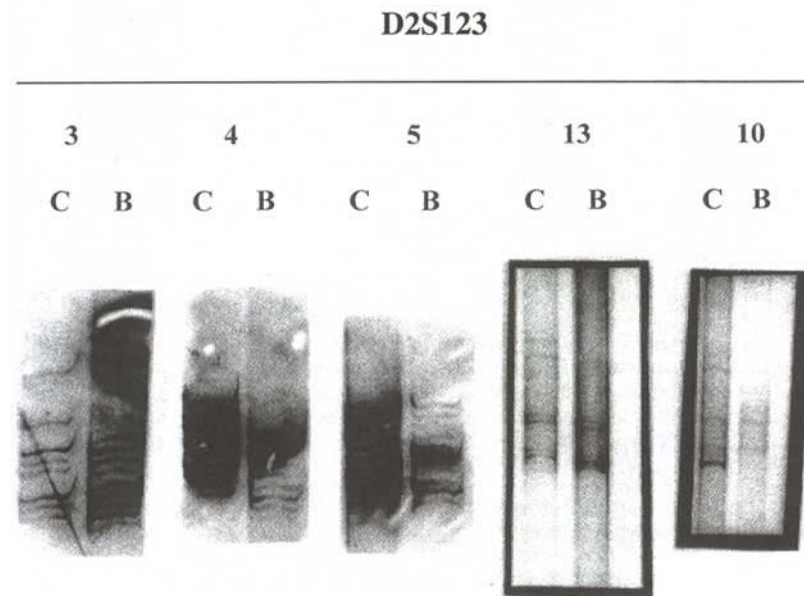


Fig 1. Comparison between chronic and blastic phase CML bone marrow DNA samples at the D2S123 locus. Patients 3, 4, 5, and 13 exhibits MSI. Patient no. 10 exhibits LOH (C: chronic phase bone marrow; B: blastic crisis bone marrow)

The trinucleotide (CCG)₁₇ repeat in the BCR gene is localised in the 5'UTR region and could not be amplified with the specific primers given in GDB. Different thermal profiles and protocols including the use of 7-deaza GTP was tested, but amplification could not be succeeded. Our unsuccessful attempts in amplifying this region are probably due to denaturation problems resulting from the high CG content of the region.

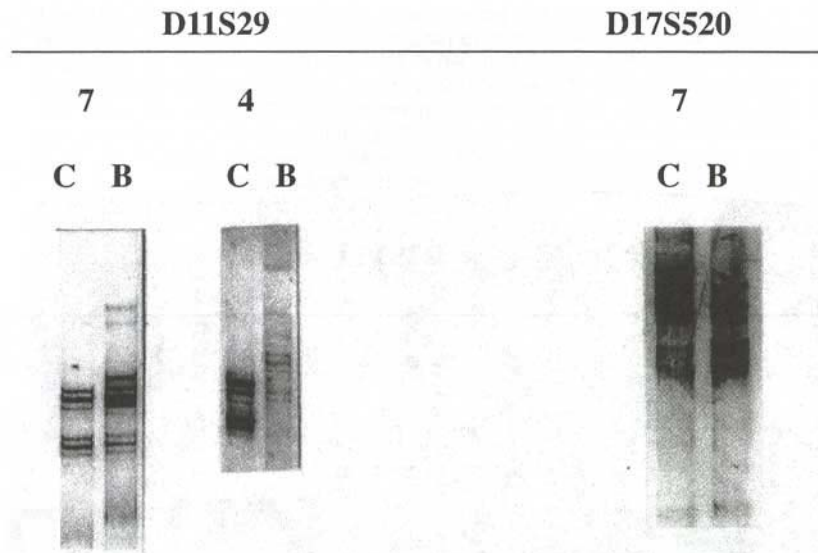


Fig 2. Comparison between chronic and blastic phase CML bone marrow DNA samples exhibiting MSI at the D11S29 (patients no. 4 and 7) and D17S520 (patient no.7) loci respectively (C: chronic phase bone marrow; B: blastic crisis bone marrow)

Discussion

Genomic instability due to defects of the MMR pathway, is now recognised as a mechanism in the etiology of HNPCC and is proposed to have a role in approximately 9-15% of sporadic tumours including colon, prostate, lung, ovarian, gastric and breast cancer (12). This type of genomic instability is characterised by allelic shifts in short tandem repeats termed as microsatellites. MSI was first reported in sporadic colon cancer and tumours of HNPCC patients. HNPCC patients were found to have germline mutations in the mismatch repair genes in which they proceed through carcinogenesis of the colon, via Knudsen's double hit model for tumour suppressor genes (7-9). Investigations have also detected MSI in a variety of sporadic cancers including hematopoietic neoplasms (12-15). Among reports on MSI in human hematopoietic malignancies are those that aim to examine the role of MSI in the transition from chronic to blastic phase CML (16-21). The identification of mechanisms responsible for blastic transformation has extreme importance in both patient evaluation and in establishing better therapeutic strategies. Unfortunately little is understood about the basic biological mechanisms that underlie the reasons for acute transformation of chronic phase hematopoietic cells. Work done by different groups on the role of MSI in this change of course have reported contradictory results.

We studied chronic and blastic phase archive material for MSI of 13 CML patients using nine microsatellite markers. As reported previously (19), we observed that suboptimal amplification conditions and impure DNA samples might

generate spurious PCR bands. For this reason triplicate experiments and new rounds of isolation and amplification were performed on different slides obtained on the same date. As a result, only 2 out of 13 patients were shown to exhibit MSI (15%). Both of these patients preceded through lymphoid blast crisis. To our knowledge, microsatellite instability exhibited within markers D2S123, D11S29 and D17S520, was not reported previously to be associated with lymphoid blast crisis in CML. Unfortunately the number of patients in our study is insufficient to propose a relationship. In addition, our results do not support the view stating MSI may be significantly associated with CML evolution. They also differ from other laboratories reporting only occasional LOH and no MSI (16,19-21). Our study suggests that MSI may occur occasionally in CML. Other possibilities that contribute to the discrepancy between results from different laboratories must also be taken into consideration, such as the choice of microsatellite markers and/or the fact that a consensus on interpretation, methodology or reliability criteria of MSI analysis is not reached.

Secondary clonal chromosomal aberrations and genetic abnormalities observed in time, implicates the existence of genomic instability in chronic phase CML hematopoietic cells. The nature and mechanisms that underlie this instability are still unknown. Whether this genomic instability may be defined as MSI is questionable. CML accumulates secondary genetic abnormalities mainly at the chromosomal level. It is not likely that a linear accumulation of point mutations as observed in MMR deficiencies will produce complex genetic rearrangements, deletions, trisomies, amplifications etc. at the chromosomal or molecular level as detected in CML. The detection of MSI during transition to acute transformation, may just reflect the presence of a yet undefined genomic instability resulting in a defect of the MMR pathway. In this case MSI will be a late occurring, but rare event in CML evolution as previously proposed by Wada *et al* (16).

Colon cancer presenting MSI seem to have a distinctive phenotype including a diploid karyotype, rarely exhibiting chromosomal rearrangements, LOH and aneuploidy (12). On the contrary, CML and other haematological malignancies manifest these gross genetic aberrations. It would be interesting to correlate MSI(+) leukemia with their karyotypes to see the extent of chromosomal level aberrations. Unfortunately we were not able to obtain the karyotypes of the patients in our study.

We believe that the proposed model for colorectal tumorigenesis (24) where MMR deficiency plays a major role, does not accurately represent CML evolution. Despite the various differences in presentation, progression, and biology of the two diseases; the colorectal tumorigenesis model proposes an explanation of how a benign tumour may progress into cancer. Adenomas of the colon do not inevitably proceed into a neoplastic course; actually only a few adenomas will progress through a carcinogenetic pathway. On the contrary, acute transformation and accumulation of secondary genetic aberrations seems to be inevitable in CML. Again this observation implies, a yet to be defined mechanism(s) of instability that may or may not be present at onset of disease. At present, defects in the MMR pathway is the only mechanism with experimental evidence, demonstrating that a single mutation in a cell may result in an accumulation of mutations necessary for the carcinogenesis process. This mechanism in itself is far from explaining the general instability observed in CML, and more research

will be needed to elucidate pathways that contribute to acute transformation of chronic phase hematopoietic cells.

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