Molecular cytogenetic characterization of an acquired minute supernumerary marker chromosome as the sole abnormality in a case clinically diagnosed as atypical Philadelphia-negative chronic myelogenous leukaemia

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Summary. A case of chronic myelogenous leukaemia (CML) in a 48-year-old man is reported. To the best of our knowledge, this is the first report of a Philadelphia-negative CML with an acquired small supernumerary marker chromosome (SMC) 11 as the sole abnormality. The derivative chromosome 11 was studied in detail using molecular cytogenetic methods: fluorescence in situ hybridization (FISH) using centromere- and region-specific probes for chromosome 11, microdissection, micro-comparative genomic hybridization (micro-CGH) and the recently developed multicolour banding (MCB) technique. The acquired SMC was determined to be a ring chromosome that can be described as r(11)(p11.2->q13.1::q14.1).

Keywords: CML, Philadelphia negative, multicolour banding (MCB), micro-CGH, FISH.

Chronic myelogenous leukaemia (CML) is a stem cell disorder associated with the presence of the Philadelphia (Ph) chromosome in the bone marrow cells in more than 90% of patients. The Ph chromosome is the result of a chromosomal translocation t(9;22)(q34;q11) leading to a BCR-ABL chimaeric gene, essential in CML pathogenesis. The BCR-ABL rearrangement can be detected in 40% (Thijssen et al. 1999) to 80% (Rerknamnuaychok & et al. 2000) of Ph-negative CML cases by means of reverse transcription polymerase chain reaction (RT-PCR) techniques, fluorescence in situ hybridization (FISH) or genomic Southern blotting (for review see Thijssen et al. 1999). Responses to therapy and prognosis in CML cases without any detectable BCR-ABL rearrangement have been shown to be poorer and structural karyotypic changes are more frequent than in cases with the Ph-rearrangement (e.g. Kurzrock et al. 1990; Aurich et al. 1998). According to Kurzrock et al (1990), in 3% of their patients, for whom they could not detect any changes in the BCR or c-ABL genes, disease phenotype was a morphological facsimile of classic chronic-phase CML. Treatment with hydroxyurea successfully controlled elevated white blood cell (WBC) counts in a manner similar to that expected in BCR-positive counterparts. The remission rate to interferon alfa appeared less than generally achieved in BCR-positive diseases, but the small number of subjects treated in this study precluded a statistically valid comparison. The 50% probability of survival was similar in both groups, while BCR-negative individuals were older (mean age 60 years) than those with Ph-positive CML (mean age 46 years) at presentation. The BCR-negative CML study patients had a low propensity for blastoid transformation (25–50%). In these patients, disease progression was characterized by increasing leukaemia burden with leucocytosis, pronounced organomegaly and eventual bone marrow failure.

We report a case of atypical Ph-negative, BCR-ABL-negative CML without any karyotypic changes apart from an acquired supernumerary small marker chromosome as small as, or even smaller than, half of 18p.

CASE REPORT

In February 1999, a 48-year-old man presented with leucocytosis and splenomegaly. He had lost 6 kg of weight
under calorie-restricted dietary food and reported sporadic nocturnal sweat. Ten days before admittance to the hospital, the patient had undergone dental extraction of two lower incisors and suffered from permanent trickle bleeding. Haematological examination of the peripheral blood revealed a white blood count of 33.6 x 10^3/l with 10% myelocytes, 2% promyelocytes and 8% promyelocytes, a platelet count of 80 x 10^3/l and a haemoglobin concentration of 13.04 g/dl. Abdominal ultrasound showed hepatosplenomegaly and multiple lymphomas in the region of the liver and splenic hilus with a maximum diameter of 20 mm. Clinical examination excluded peripheral lymphadenopathy. Histopathological evaluation of the bone marrow aspirate led to the diagnosis of an atypical CML, without blast excess or increased fibroblast concentration according to the Hanover-classification. Cyto genetics, FISH using the BCR-ABL ES probe (Vysis), and RT-PCR capable of detecting the major fusion transcripts b2a3 and b3a3 (Harrison et al. 1990) could not detect the presence of a Philadelphia chromosome or a BCR-ABL rearrangement. As interphase FISH with the Vysis ES probe is a sensitive method for the detection all types of BCR-ABL rearrangements, including variant and hidden translocations, we suggest real BCR-ABL negativity (van der Plas et al. 1991). The patient was treated with oral administration of hydroxyurea. Under this cytotpressive regimen, the physical condition of the patient improved markedly and the white blood count returned to normal values while platelets increased to values around 130 x 10^3/l. Based on these results, a curative therapy with allogeneic peripheral bone marrow stem cell transplantation was planned. After successful identification of a human leucocyte antigen (HLA)-compatible unrelated stem cell donor, the patient was transplanted in March 2000. Unfortunately, 1 week after the transplantation, the patient developed therapy-resistant sepsis and died 8 d later from multiorgan failure.

As mentioned above, the sole karyotypic abnormality detected by means of conventional cytogenetics in the bone marrow of the patient was a minor supernumerary marker chromosome (SMC). The small SMC showed slight CGB- but no NOR-positive staining and was present in 18 out of 20 metaphase spreads analysed (Fig 1A). The presence of the SMC was excluded in peripheral blood lymphocytes and, thus, it was an acquired, disease-associated alteration. Extended molecular cytogenetic studies were performed to characterize the SMC in detail. Generation of a SMC-specific DNA library (Senger et al. 1997) and reverse painting revealed that it consisted of centromeric and pericentromeric parts of chromosome 11 (11p11.2-11q12) plus a small part of the band 11q14 (Fig 1B). To exclude the possibility that the small signal in the 11q14 band was as a result of a cross hybridization of sequences derived from the 11p11.2-11q12 region, a microdissection library specific for 11p11.2-11q12 and 11q14 has been created and hybridized on normal metaphase spreads and patient’s chromosomes. Both libraries painted the SMC (Fig 1C) but no cross-hybridization was detected between the 11p11.2-11q12 and 11q14 regions of normal chromosomes (Figs 1C-1). This confirmed the origin of SMC from 11p11.2-11q12 and the part of 11q14. According to these data, involvement of well-known oncogenes int2 and cyclin D in the rearrangements that led to SMC formation could be expected. This was checked with FISH using single copy probes for int2 and cyclin D (Oncor). As shown in Fig 1D, no specific signals were detected on the derivative chromosome 11. The SMC has been characterized further using a centromeric-specific probe for chromosome 11 (cep 11: Vysis). As shown in Fig 1D, the derivative chromosome was a ring chromosome, monochromic in 28 out of 30 (Fig 1D, left) and dicentric in 2 out of 30 metaphase spreads [redup(11)] (Fig 1D, right). Interphase analysis using the cep 11 probe

![Fig 1.](image)

Cytogenetic (A) and molecular cytogenetic (B-F) characterization of a small supernumerary marker chromosome (SMC) detected as the sole cytogenetic abnormality in a case of Ph-negative CML. The SMC is marked with arrowheads in (A, B and D). Images were captured on a Zeiss Axioskop microscope (Zeiss, Jena, Germany) with the IKAROS and ISIS digital FISH imaging system (MetaSystems, Altlussheim, Germany) using a X772 CCD camera with on-chip integration (Sony). (A) The acquired SMC was detected by conventional cytogenetics in the bone marrow of the patient in 18 out of 20 metaphase spreads. (B) Results of microdissection reverse painting (according to Senger et al. 1997). Left: a metaphase spread of the patient hybridized with the microdissection library of the marker (red signals) is shown; strong signals on the centromeric region of chromosome 11 and on the completely stained marker are visible. In a greater magnification and on more decondensed chromosomes (right), additional but weaker signals in 11q14 can be detected. (C) Two microdissection libraries, one derived from 11p11.2-11q12 (C-1) and one from 11q14 (C-2) were generated and hybridized on normal metaphase spreads. With the first one, it could be excluded that the small band in 11q14 was as a result of a cross hybridization of centromere near sequences. Signals of both microdissection libraries were detected on the SMC (C-3) and, thus, the reverse painting results were proven. (D) Two-colour FISH using single copy probes for the oncopgenes cyclin D and int2 (Oncor, red signals) – both mapping to 11q14 – detected no specific signals on the derivative chromosome 11. However, using the centromere-specific probe for chromosome 11 (Vysis, green signals), the SMC turned out to be a ring chromosome, monochromatic in 28 out of 30 (right) and dicentric in 2 out of 30 metaphase spreads [redup(11)] (left). (E) Comparative genomic hybridization using the micro-CGH technique (according to Heller et al. 2000) was performed to clarify if a gene amplification of the region 11q14 was hidden within the small SMC. As visible in the upper part, a weak gain of copy number is exclusively detectable in 11p11.1-11q12. Thus, an ampiclon of the 11q14 region was less plausible. The gain in the pericentromeric region of #11 was not as a result of an artifact, as all three chromosomes with larger portions of heterochromatin #1, #9 and #16 show an artificial loss of copy number in the pericentromeric region, no gain (lower part). (F) Using the recently described multicolour banding (MCB) technique (according to Chudoba et al. 1999) applying an MCB probe set for chromosome 11, the SMC could be described as a derivative ring chromosome 11: r11(1p12.2-11q14;1q14:1p12.2). In F-1, the localization of the chromosome 11 region-specific partial chromosome paints and the corresponding fluorochromes with which they have been labeled are shown. In F-2, an overlay of all five fluorochromes and the corresponding fluorescence intensity profiles along chromosome 11 are shown. In F-3, two apparently normal chromosomes 11 in their MCB-pattern and the minor ring of the presented case are depicted. On the right normal chromosome 11, the four breakpoint regions leading to the SMC are marked with arrowheads and in F-4, a schematic drawing of the ring chromosome is presented.
revealed that the supernumerary ring chromosome was present in 124 out of 150 (83%) bone marrow cells. Comparative genomic hybridization (CGH) using the micro-CGH technique (Heller et al. 2000) was performed to clarify if a gene amplification of the region 11q14 was hidden within the small SMC. Only a minor gain of copy number of the pericentric region homologous to the SMC was detectable and none in 11q14 (Fig 1E), which probably was as a result of the small size of the latter area. Finally, the multicolour banding (MCB) technique (Chudoba et al. 1999) using an MCB probe set for chromosome 11 (see Fig 1F) characterized the SMC as a derivative ring chromosome 11: r(11)(p11.2;q13.1;q14.).

CONCLUSIONS

To the best of our knowledge, this is the first described case with such a minor SMC derived from chromosome 11 in Ph-negative CML. A similar molecular cytogenetics study for the characterization of a SMC derived from chromosome 20 has been performed to date in only one case of malignant fibrous histiocytoma (Meloni-Ehrig et al. 1999). Alterations of the long arm of chromosome 11 are rarely reported in CML. Involvement of the band 11q13 in a complex Ph-translocation has been described by Koduru et al. (1993). Moreover, deletions in 11q14 are found in approximately 7% of myelodysplastic syndrome (MDS) cases (Rooney et al. 1994). As an amplification of known oncogenes in 11q14 (int2 and cyclin D) has been excluded, the molecular basis for this complex ring chromosome formation with at least four break events in chromosome 11 still remains to be determined.

Clinical relevance and frequency of such minor SMCs in CML and other leukemic disorders have to be evaluated by further studies. Actually, it cannot be determined: (i) if they have not been reported more frequently before, as they are very rare events, (ii) if they have not been detectable because of low quality metaphase spreads in tumour cytogenetic preparations, or (iii) if detected, they have not been reported, as standard molecular cytogenetic techniques have not been sufficient for their characterization.

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