Acute lymphoblastic leukemia in a patient with fragile X syndrome: cytogenetic and molecular features

Malignancies in patients with fragile X syndrome are rarely reported. A 42-year-old man with fragile X syndrome presented with precursor B-cell acute lymphoblastic leukemia (ALL). Cytogenetic analysis showed a stemline 46, XY, t(9;22)(q34;q11) and a sideline 46,XY, t(8;14)(q24;q11), t(9;22)(q34;q11). Molecular analysis of the *FMR1* gene showed a neoplastic leukemic clone possessing a full expansion of the CGG repeat, with associated aberrant methylation of the promoter CpG islands. However, analysis during morphologic remission showed that the promoter CpG island was apparently unmethylated in the regenerating normal hematopoietic cells. During subsequent relapses, the *FMR1* CGG repeat was unstable, with the appearance of multiple leukemic subclones possessing different repeat expansions. Our case suggested that deregulation of the FMR1 gene might have contributed to leukemogenesis in our case.

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Fragile X syndrome is the most frequent form of inherited mental retardation in males.[1] It is transmitted as an X-linked dominant disorder with reduced penetrance. Affected males show a variable degree of mental retardation, mild dysmorphic features, and macroorchidism. Cytogenetically, it is marked by the presence of an inducibe fragile site at chromosome Xq27.3.[2] Molecularly, expansion and methylation of trinucleotide repeats of CGG at the FRAXA site is the commonest abnormality, and results in down-regulation of the FMR1 gene, which carries the hypermutable CGG repeat in the 5' untranslated portion of its first exon.[3-5] The level of expression of the FMR1 gene correlates with the clinical severity of the fragile X syndrome. Interestingly, instability of nucleotide repeats and aberrant CpG methylation, a *sine qua non* in the abnormal *FMR1* gene in fragile X syndrome, are also commonly seen in many different neoplastic disorders as microsatellite instability and aberrant gene promoter methylation respectively.[6,7] However, only very few cases of malignancies have been described in patients with fragile X syndrome. We report a unique case of acute lymphoblastic leukemia (ALL) in a patient with fragile X syndrome. A 42-year-old man with mild mental retardation and fragile X syndrome diagnosed since his teens presented with spontaneous bruising. Physical examination was unremarkable. A complete blood count showed hemoglobin (Hb): 5.9 g/dL, white cell count (WCC): 62.1×10^9/L (82% blasts), and platelet count: 9×10^9/L. A bone marrow aspiration confirmed the diagnosis of common ALL, with leukemic blasts shown to be Tdt$^+$, HLA-DR$^+$, CD10$^+$, CD19$^+$, CD79a$^+$, CD34$^+$, and CD13$^+$ on immunophenotyping. There was no expression of cytoplasmic or surface immunoglobulin. He was treated with combination chemotherapy (prednisolone, vincristine and adriamycin), and achieved a morphologic remission. However, several weeks later the leukemia relapsed again, and the patient died subsequently from severe sepsis. Cytogenetic analysis of the blasts at diagnosis showed a stemline 46, XY, t(9;22)(q34;q11) and a sideline 46,XY, t(8;14)(q24;q11), t(9;22)(q34;q11) [cp13] (Figure 1A). Reverse transcription polymerase chain reaction (RT-PCR) confirmed the presence of the *m-BCR/ABL* chimeric transcript typical of Philadelphia chromosome positive ALL (data not shown). To confirm the diagnosis of fragile X syndrome, a 72-hour culture of the peripheral blood lymphocytes under folate restriction was performed. This showed a fragile site at Xq27.3 (Figure 1B). After the initial chemotherapy, the marrow was in morphologic remission. Cytogenetic analysis of the remission marrow showed normal metaphases only. However, RT-PCR still showed *m-BCR/ABL*, indicating the presence of occult leukemic blasts at morphologic / cytogenetic remission. High molecular weight DNA of the patient's marrow at diagnosis, morphologic remission and subsequent relapses was digested overnight to completion with *Eco*RI and *Eag*I, Southern transferred to nitrocellulose filters, and hybridized with the *FMR1* probe StB12.3. Figure 1C shows the restriction map of the FMR1 gene. The 5.2 kb *Eco*RI fragment contains the promoter (CpG island, where the *Eag*I site is located) and exon 1 (with the CGG repeats ranging in normal people from 5 to 52, average 30) of the *FMR1* gene. In normal females, digestion with *Eco*RI and *Eag*I results in a 2.4 kb fragment and another 2.8 kb fragment (labeled by the StB12.3 probe) from the *FMR*1 gene on the active X chromosome, since the *Eag*I site is not methylated. However, digestion of the FMR1 gene on the inactive X chromosome only results in a 5.2 kb fragment, as the *Eag*I is methylated and insensitive to *Eag*I digestion (lane F, Figure 1E). In normal males, where the X chromosome is active, only the 2.8 kb fragment is seen on *Eco*RI/*Eag*I digestion (lane M, Figure 1D). In patients with the fragile X syndrome, the CGG repeat is markedly expanded (>200 repeats), and is associated with aberrant methylation of the promoter CpG islands (with therefore abolition of the EagI site). Therefore, digestion with EcoRI / EagI gives a fragment larger than 5.2 kb (typically >6 kb). The CGG expansion may be different in different cell populations, so that several bands or a smear pattern of larger than 5.2 kb may also be observed. In our patient at diagnosis, a single 6.5 kb fragment was observed (lane Dx, Figure 1E). This was expected, as the leukemic blasts were derived from a single mutated cell, which carried one particular CGG expansion, which gave one predominant band on Southern hybridization. At morphologic and cytogenetic remission (lane Rem, Figure 1E), in addition to the 6.5 kb band that indicated occult leukemia (which had also been shown by positive RT-PCR for m-BCR/ABL), two more bands of 6.1 and 4.8 kb were observed. Since the 6.1 kb band was only a minor band, it could have conceivably arisen from somatic mutation of the leukemic clone. More interesting was the 4.8 kb band, which was the major band and therefore represented the FMR1 gene fragment coming from the non-leukemic hematopoietic cells that had regenerated after chemotherapy. The location of the band in this position could be explained by the presence of a fully expanded mutation (>600 repeats) that was associated with minimal methylation (thus an unmethylated EagI site), a phenomenon uncommonly seen but well reported in fragile X males. This would mean that the CGG expansion of the regenerating hematopoietic cells would be of the order of 2 kb. To verify this, hybridisation of StB12.3 with an EcoRI digest of the remission marrow sample would be useful. Unfortunately, not enough sample was left for this experiment. At subsequent relapses (Rel_1 and Rel_2, Figure 1E), an additional 7.0 kb band was seen, together with the 6.5 and 6.1 kb bands, suggesting that the CGG expansion was unstable in the leukemic clone during clonal evolution. To investigate if the unstable CGG expansion in the leukemic blasts was but a reflection of a generalized microsatellite

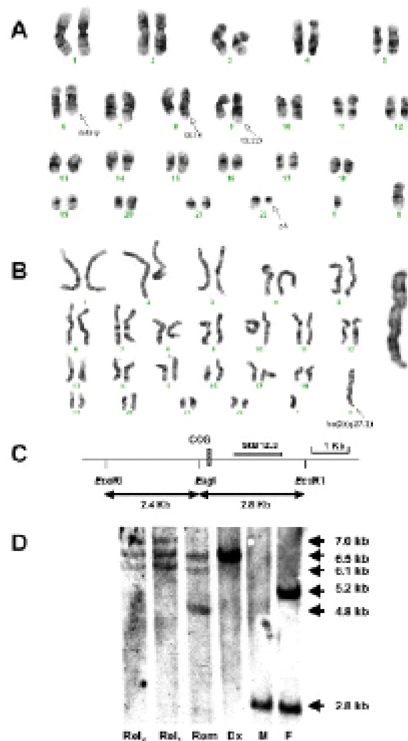

Figure 1A. Karyogram showing 46,XY, del(6)(q25), t(8;14)(q24;q11), t(9;22)(q34;q11). Figure 1B. Karyogram of lymphocyte culture, showing the fragile site fra(X)(q27.3). The chromosome X is enlarged in the insert to show the fragile site. Figure 1C. Restriction map of the FMR1 gene, showing the localization of the probe StB12.3. Figure 1D. Southern blot analysis of the FMR1 gene with the StB12.3 probe. Lane F: normal control female showing two bands of 2.8 kb and 5.2 kb; lane M: normal control male showing only a single band of 2.8 kb, lane Dx: sample at leukemic diagnosis, showing a predominant band of 6.5 kb corresponding to the leukemic clone; lane Rem: morphologic remission marrow sample, showing a predominant band of 4.8 kb corresponding to the regenerating non-leukemic hematopoietic cells, a 6.5 kb band indicating residual leukemia, and a 6.1 kb band arising from leukemic clonal evolution; lanes Rel1 and Rel2: samples from subsequent relapses, showing the predominant 6.5 kb leukemic band, and the 6.1 kb and 7.0 kb bands arising from leukemic clonal evolution.

instability, polymerase chain reaction amplification of several polymorphic bi/tri-nucleotide microsatellite markers was performed. No evidence of microsatellite instability could be observed (data not shown). This case illustrated some informative features of malignancies complicating fragile X syndrome. Firstly, although fragile X syndrome is not uncommon, malignancies have rarely been described in these patients. Only about 20 patients with cancers of the brain, lung, colon, prostate, testis, pancreas, urinary bladder, uterus and nasopharynx; and hematologic malignancies including ALL and myelodysplastic syndrome,[9] have been reported. Although some of these malignancies might be mere coincidences, rare cancers such as testicular seminoma, gliomas and blood cancers seemed to be over-represented in the few cases of neoplasm reported. This is intriguing, as the testis, brain and the hematolymphoid system are sites where the *FMR1* gene product, the fragile X mental retardation protein (FMRP), is expressed in normal people.[8] This implies that non-expression of the FMRP protein in tissues where it is normally expressed may be associated with neoplastic transformation of these tissues; suggesting that FMRP might have tumor suppressing properties. Our current case of leukemia also lends support to such a contention. Furthermore, aberrant methylation of the *FMR1* gene occurred preferentially in the leukemic clone in our case. This differential methylation of the *FMR1* between tumor and non-tumorous tissues had been observed in patients with fragile X syndrome,[10] suggesting aberrant *FMR1* gene methylation might confer clonal advantage to the neoplastic cells. Moreover, the CGG repeat expansion appeared to be unstable, with the development of subclones with different expansion sizes occurring in subsequent relapses; further implying that deregulation of the *FMR1* gene might be involved in leukemic evolution/clonal progression. In conclusion, we have presented a rare case of leukemia complicating fragile X syndrome. Our observations suggest that suppressed/abnormal FMRP expression might be contributory to leukemogenesis. The FMRP possesses two KH motifs and one RGG box, which interact with RNA and polyribosomes.[1] It remains to be elucidated if perturbations of these biochemical processes might contribute to carcinogenesis. Future studies of the differential deregulation of the *FMR1* gene in tumorous and non-tumorous tissues in patients with fragile X syndrome are required to address these issues.

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