Cytogenetic abnormalities and their lack of relationship to the Asp816Val c-kit mutation in the pathogenesis of mastocytosis

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The etiology of mastocytosis remains unclear.[1,2] The point mutation Asp816Val in the c-kit receptor has been reported in PBMCs of patients with mastocytosis in association with more aggressive forms of disease, such as mastocytosis with an associated hematologic disorder (category II) and more severe forms of indolent mastocytosis (category Ib).[2,3] To date, several chromosomal abnormalities have also been reported as detected by routine cytogenetic testing of patients with mastocytosis, but their significance remains unknown, particularly in light of the abnormalities in c-kit in this disease. To explore this issue, we examined the cytogenetic abnormalities in patients with mastocytosis and determined their relationship to the occurrence of the Asp816Val mutation.

METHODS

Cytogenetic testing with either bone marrow aspirates or peripheral blood taken from 17 patients with different categories of mastocytosis was performed by using the standard karyotype technique of Giemsa staining of metaphase spreads. This technique used 1-day cultures of the bone marrow aspirate or PBMCs, followed by banding analysis of 20 metaphase chromosome spreads.

Reverse transcriptase PCR mutation analysis was performed as described[3] in PBMCs from 12 patients with mastocytosis followed up at the National Institutes of Health Clinical Center enrolled in protocol #93-I-0136 and after informed consent (the remaining 5 patients either expired or were lost to follow-up). Results were then correlated with the patients' clinical category of mastocytosis[2] and cytogenetic results.

RESULTS

Correlation of routine cytogenetic testing with restriction endonuclease mutation analysis for the Asp816Val point mutation in PBMCs of patients with mastocytosis is summarized in Table I.

Seven patients were examined who had indolent mastocytosis (category Ia and Ib). None were found to have chromosomal abnormalities with either method of genetic analysis. Conversely, of the 9 patients with mastocytosis with an associated hematologic disorder, 7 of whom were tested for and found to have the Asp816Val mutation, 4 patients (44% of category II mastocytosis) were found to have gross chromosomal abnormalities by means of karyotyping. No consistent pattern of cytogenetic abnormalities was seen in these 4 patients. The affected chromosomal regions in these 4 patients did not correspond to the known location of the human gene for the c-kit receptor, its respective ligand, stem cell factor, or other genes potentially implicated in the etiology of mastocytosis, such as IL-4, IL-6, or IL-9.

Clinically, the 4 patients harboring gross chromosomal abnormalities manifested advanced disease with diffuse infiltration of the bone marrow by mast cells and other dysplastic cell types, predominantly of the mononuclear lineage. Two of these 4 patients had additional cytogenetic abnormalities on retesting as their disease advanced (Table I, patients number 15 and 16).

One patient with aggressive mastocytosis did not have a detectable genetic abnormality as determined by either of these 2 diagnostic techniques.

DISCUSSION

Results from this study of 17 patients with mastocytosis revealed no specific pattern of chromosomal abnormality. We did, however, note that patients with category II mastocytosis had a greater tendency to have detectable chromosomal changes on routine karyotyping. This is consistent with 2 case series performed by 2 independent groups, one using routine cytogenetics[4] and the other using fluorescence in situ hybridization.[5] Neither group found a uniform chromosomal pattern in patients with mastocytosis. In the first study, which had used standard karyotyping techniques, 5 of 13 patients (36%; 2 of whom had urticaria pigmentosa only and 3 of whom had category Ib systemic mastocytosis) were found to have chromosomal abnormalities that consisted of the following aberrations: 5q-, 11q-, -16, 9p+, and +13.[4] In the second study 5 patients had trisomy 9, and 1 patient had trisomy 8; both are common in myeloproliferative disorders.

In our study all patients with a chromosomal abnormality were found to have the Asp816Val mutation on restriction endonuclease digest analysis of PBMC

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TABLE I. Correlation between mastocytosis category, c-kit mutation status and presence of chromosomal abnormalities on cytogenetic testing in 17 patients with mastocytosis

Patient	Mastocytosis category	Asp816Val mutation status	Chromosomal abnormality through cytogenetic testing
1	Ia	(–)	None
2	Ia	(–)	None
3	Ia	(–)	None
4	Ib	NA	None
5	Ib	NA	None
6	Ib	NA	None
7	Ib	(–)	None
8	II (+ MPS)	(+)	None
9	II (+ MPS)	(+)	None
10	II (+ CMML)	(+)	None
11	II (+ *P vera*)	(+)	None
12	II (+ CMML)	NA	Deletion of long arm of chromosome 4
13	II (+ HES)	(+)	Normal variant: 46 XY, inv (9) (P11Q13)
14	II (+ CMML)	(+)	Pericentric inversion of chromosome 5 (p13→q13)
15	II (+ RA)	(+)	20q deletion in 3 of 20 cells in metaphase spread
16	II (+ MPS/HES)	NA	40% of karyotypes missing chromosome 7, 40% of banded chromosomes showing an extra chromosome 21 and 22
17	III	(–)	None

NA, Not available; *MPS*, myeloproliferative syndrome; *CMML*, chronic myelomonocytic leukemia; *P vera*, *Polycythemia vera*; *HES*, hypereosinophilic syndrome; *RA*, refractory anemia.

cDNA. The observation of increased chromosomal breaks in patients with mastocytosis where proliferation may be enhanced is consistent with the current hypothesis that dysmyelopoiesis is related to the c-kit mutation and suggests that the relationship between c-kit–mediated signal transduction and occurrence of chromosomal abnormalities is perhaps due to a defective repair mechanism or mechanisms and should be examined.

Furthermore, these data and that of previous studies support the conclusion that routine Giemsa staining of metaphase chromosomes is of limited routine value because it does not appear helpful in diagnosing mastocytosis and offers no advantage over current methods of assigning prognosis on the basis of clinical and pathologic findings.

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