Analysis of G banded karyotypes in myeloma cells

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SUMMARY Karyotypes of bone marrow cells from 24 patients with multiple myeloma (MM) and two patients with de novo plasma cell leukaemia (PCL) were analysed by Giemsa banding (G banding). Chromosome aberrations were found in 13 patients with MM and both patients with PCL. Hyperdiploid and hypodiploid lines were present in eight and five of the patients with MM, respectively. Marker chromosomes derived from structural rearrangements were present in all eight cases of MM with hyperdiploid lines, although markers of uncertain origin were rare in those patients with hypodiploid lines. Chromosome 1 participated most often, and chromosomes 5 and 9 often played a part in the structural rearrangements. Chromosomes 3, 5, 7, 9, 11, 15, 19, and 21 were subject to numerical aberrations. In the two patients with PCL one had a hypodiploid line with a 14q+ marker derived from a t(11;14) and the other a hyperdiploid line. The breakpoints on the chromosomes participating in the structural rearrangements in myeloma showed a good correlation with known fragile sites and oncogene locations on the corresponding chromosomes.

Multiple myeloma is a neoplasm of the B cell line characterised by a monoclonal proliferation of plasma cells which, with rare exceptions, secrete whole immunoglobulins or immunoglobulin light chains. The bone marrow is commonly affected. Cytogenetic studies on the myeloma cells show that chromosomal abnormalities have a role in the development of the disease. In prebanding studies 25% of patients with myeloma were found to have abnormal cell lines in their bone marrows.¹ Hyperdiploidy was observed more commonly than hypodiploidy and pseudodiploidy. Large metacentric, submetacentric, and acrocentric markers were often described, usually with no convincing consistency in their morphology from patient to patient.^{1–7}

The application of chromosome banding techniques has facilitated the analysis of the various marker chromosomes in myeloma cells. Chromosomal aberrations are found to entail the activity of most of the autosomes in the genome. There is extensive participation of chromosome 1 and considerable participation of chromosome 3 in the structural rearrangements.^{8–11} Several vulnerable break points are located on these chromosomes as well as on chromosomes 5, 6, 7, 9, 11, and 22. The rearrangements between these chromosomes give rise to the morphologically various markers in myeloma. Chromosome 14 is also subject to marker formation. The 14q+ marker, often described in myeloma and PCL,

is, however, very consistent and seems to be independent of other markers affecting the break points of chromosomes 1, 3, and other autosomes.^{8–10 12 13} The presence of extra chromosomes 3, 5, 9, 11, 15 and absence or presence of chromosomes 7, 8, 13, and 16 commonly occurs in the aneuploid cells of myeloma.

Generally speaking, chromosomal participation in the aberrations in myeloma is complicated. It seems, however, to have an organised pattern, and there are specific vulnerable break points on a few chromosomes. The number of cases studied by G banding is low. Analysis on more cases is needed to evaluate the importance of these findings with respect to their role in the development of the disease. We report here the analysis of G banded chromosome preparations of bone marrow cells from 24 cases of multiple myeloma (MM) and two cases of plasma cell leukaemia (PCL).

Material and methods

Bone marrow aspirates from 24 patients with confirmed MM and two patients with de novo PCL were collected for cytogenetic studies between May 1984 and May 1985. Table 1 summarises the clinical data and cytogenetic findings. Cases 1 to 24 were MM and cases 25 and 26 were PCL. Five patients (cases 9, 13, 15, 16, and 19) had had some treatment before the first sample was taken. Samples were taken more than once from three patients with MM (1, 2, and 15) and from one patient (25) with PCL. One patient (case 5) had a very short survival of four days after diagnosis.

G banded karyotypes in myeloma cells

Table 1 Clinical data and cytogenetic findings of 24 patients with MM and two with PCL

Case No	Age and sex	Abnormal* protein class	Plasma cells in marrow (%)	Skeletal disease	Treatment†	Survival after diagnosis (months)	Cytogenetic examination				
							Date of sampling	Chromosome counts§	No of cells	Abnormal cells (%)	
1	F/87	k	37	Yes	M.P.	15+	8.4.84	46XXN/45XON	28/4		
2	M/59	IgGλ	15	No	M.	7	7.3.85	45-47	3	8.6	
							2.5.84	46XXN	27	0	
								46XYN/45XON	40/3		
3	F/73	IgGk	40	Yes	None	8+	26.9.84	45/42	4/4	4.1	
							31.8.84	46XYN/45XON	> 25	0	
								46XXN	93		
4	F/53	IgGk	19	Yes	M.	17+	13.9.84	52-53	22	19	
5	F/61	IgAλ	60	Yes	M.	4d	24.10.84	46XXN	50	47.3	
6	F/72	λ	30	Yes	A.B.C.M.	7+	13.11.84	74-84	45		
								46XXN	10		
								43	23	69.7	
7	F/74	IgGk	20	Yes	A.B.C.M.	6+	7.12.84	46XXN	50		
8	F/74	IgA	47	Yes	M.P.	6+	18.1.85	48	3	5.7	
								46XXN	14		
								45/54	4/3	33.3	
‡9	F/71	IgGk	30	Yes	P.M.C.	55+	24.1.85	46XXN	16		
								47/55	1	5.9	
								46XXN	44		
10	M/75	k	24	Yes	A.B.	15+	11.4.85	46XY(ab)	2/1	6.4	
11	M/75	IgG	12	Yes	None	6	6.8.84	56-59	37	(100)	
								46XYN/45XON	2	5.1	
								45	43/5		
12	M/69	IgGk	4	Yes	M.P.	9+	23.8.84	46XYN/45XON	2	4.0	
13	M/71	IgGλ	27	Yes	M.	23+	20.12.84	45	30/2	13.5	
								46XYN	5		
									33	8.3	
14	F/53	λ	65	Yes	A.B.C.M.	5+	22.1.85	45	3		
								46XXN	19		
								42-45	9	32.1	
‡15	F/36	IgGk	20	No	CVP, M.	19+	28.10.83	46XXN	11		
								11.5.84	46XXN/(+ DM)	> 50/(3)	
								13.12.84	46XXN/(+ DM)	33/1	0
‡16	M/41	IgGk	43	Yes	A.M.C.M.	24+	17.5.84	46XYN/45XON	11/1	0	
								21.9.84	46XXN	26	0
								12.11.84	46XYN	35	0
17	F/72	IgGk	20	No	None	8+					
18	M/62	IgGk	30	Yes	A.B.C.M.	6					
‡19	M/53	IgG	13	Yes	C.M.P.	24+	23.1.85	46XYN/45XON	16/2	0	
20	M/72	IgGλ	65	No	V.A.D.	2+	3.5.85	46XYN/45XON	23/2	0	
21	M/67	IgA	70	No	M.P.	8	23.8.84	46XYN	1	*	
22	F/86	IgAλ	55	Yes	M.VAD.	7+	26.10.84	46XXN	5		
23	F/72	IgM	50	Yes	M	6+	14.11.84	45	1	*	
								46XXN	6	*	
								46XYN	5	*	
24	M/54	IgG	60	Yes	M.P.	7+	7.1.85				
25	M/57	IgG	23	Yes	C.A.V.P. V.A.D.	9+	7.9.84	46XYN	38		
								49-52	20	34.5	
								46XYN	23		
							27.9.84	50-53	16	41.0	
								46XYN	26		
								53	8	23.5	
							5.3.85	46XYN	7		
								51-53	13	65.0	
								46XYN	8		
26	M/57	k	56	Yes	AVP X 2	1	21.9.84	38-42	29	78.4	

*The immunoglobulin light chain proteins are not typed in all the patients.

†M = Melphalan; P = Prednisolone; A = Adriamycin; B = BiCNU; C = Cyclophosphamide; V = Vincristine; D = Dexamethasone (+): still alive.

‡Patients who received chemotherapy before cytogenetic studies.

§N = normal; ab = abnormal; DM = double minute.

Bone marrow cells were cultured for one to four days in tissue culture media without the addition of mitogens. Three kinds of media were used—McCoy's (Gibco), Iscove's (Gibco), and modified Iscove's (Imperial Laboratories, Salisbury). Mitotic cells were harvested, and chromosome preparations were G banded by standard 2xSSC/trypsin-Giemsa method.

Results

Table 1 shows the chromosome counts in the 24 patients with MM and the two with PCL. A sufficient number of metaphase cells analysed by G banding was obtained in the 20 cases of MM and both cases of PCL. Cells with karyotypic abnormalities were scored in 13 cases of MM (1 to 13). Case 14 had aneuploid

Fig. 1 G banded partial karyotypes of cases of MM with structural rearrangements illustrated by marker chromosomes as designated in Table 2.

cells with random loss of chromosomes, which did not show clonality. In case 15 bone marrow was examined on three occasions. On the second and third occasions double minute extra chromosomes (DM) were found in some otherwise karyotypically normal cells, which were not found in the first sample. Extra chromosomal double minutes are one of the cytogenic manifestations of gene amplification,¹⁴ which could have resulted from chemotherapy given to this patient. The karyotypes were normal in the five other patients (16 to 20). In four cases (21 to 24) very few mitotic cells were available for analysis and they showed normal chromosome counts. The results were not conclusive. High percentages of aneuploid cells were present in the bone marrow of both patients with PCL.

Table 2 gives the designation of abnormal karyotypes and marker chromosomes as determined by G banding in the 13 patients with MM and the two with PCL. Fig. 1 also shows the marker chromosomes. Hypodiploidy was found in five patients with myeloma. Three patients (11, 12, and 13) had abnormal lines of 45 chromosomes with monosomy of either chromosome 21 or 22. Two types of abnormal

lines were found in case 2. One had a 45 chromosome count, missing one of the chromosomes 18 or 21. The other had a 42 chromosome count, missing several chromosomes accompanied by structural rearrangements between chromosomes 12, 15, 19, and 21. Case 5 had predominantly hypodiploid cells of 43 chromosome count. The stem line had a complicated karyotype with structural rearrangements between chromosomes 1, 5, 6, 9, 12, 14, and 20.

Eight of the patients with MM had hyperdiploid lines in the bone marrow. Cases 4 and 8 had high chromosome counts of 74–84 and 63. The karyotypes (Table 2) indicated that these cells were derived from tetraploid cells following the loss of some chromosomes from their tetraploid genomes. In case 4 further structural changes occurred between the additional chromosomes, which gave rise to variable numbers of markers of uncertain origin in the abnormal cells. In this patient's marrow about half of the mitotic cells were hyperdiploid with consistent reciprocal translocation between chromosomes 11 and 14 and an extra 14q+ marker. Four patients (3, 7, 9, and 10) had hyperdiploid lines with chromosome counts around 52 to 55. Extra chromosomes 3, 5, 7, 9, 11, 15, 19, and

Table 2 Designation of modal and subline abnormal karyotypes and markers as determined by G banding in 13 patients with MM and two patients with PCL

Case No	Karyotype designation of abnormal cells	Marker designation
1	47XX (-7, ?9, i(14q), +2 mar)	i(14)(qter → cen → qter) + mar
2	46XO (+mar) 46XX (-10, +mar) 45XY (-18) 45XY (-21) 42OY (-11, -12, -13, -14, -18, -19, -X, 6q-, 7p+, 15q+, 21q+, +t(12;19), +t(12;?), +r(?)) 42OY (-8, -12, -13, -17, -18, -19, -X, 6q-, 15q+, 21q+, +t(12;19), +t(12;?), +r(?))	del(6)(pter → q21:) t(7;?) (7qter → 7p15:?) t(15;?) (15pter → 15q24:?) t(21;?) (21pter → 21q22:?) t(12;19) (12qter → 12q11::19qter → 19pter) t(12;?) (12pter → 12q13:?) r(?)
3	53XX (+3, +5, +9, +9, +15, +19, +21) 52XO (+3, +5, +9, +19, +21, -X, +t(1;9), +mar 22q-) 53XX (+3, +5, +9, +9, +15, +19, +21, t(1;7))	recp(1;9) (1pter → 1q12::9q34 → 9qter; 9pter → 9q34::1q12 → 1qter) del(22)(pter → q13:) +mar recp(1;7) (1qter → 1p13::7p22 → 7pter; 7qter → 7p22::1p13 → 1pter) t(1;7) (1pter → 1q11:?)
4	75XXXXX (+1, +2, +3, +4, +5, +6, +6, +7, +8, +8, +9, +12, +15, +16, +17, +19, +21, +21, +22, +1q-, +2t(3;19), +t(11;14), t(11;14), 14q+, +4mar)	recp(3;19) (3qter → 3p13::19p13 → 19qter; 19qter → 19q13::3p13 → 3pter) recp(11;14) (11pter → 11q13::14q32 → 14qter; 14pter → 14q32::11q13 → 11qter) t(14;?) (14pter → 14q32:?) +4mar
5	43XO (-13, -15, 1p+, 5q-, inv(6), t(1;9), 12p+, 14q+, 20q-)	dup(1p) (1qter → 1q12::?:1p13 → 1qter) t(1;9) (1qter → 1q13::9q32 → 9pter) del(5)(pter → q23::q33 → qter) t(14;?) (pter → 14q32:?) inv(6)(pter → p21::q21 → p21::q21 → qter) recp(12;20) (12qter → 12p13::20q12 → 20qter; 20pter → 20q12::12p13 → 12pter)
6	48XX (+5, -14, -16, -18, 1p-, +t(1;?) +3 mar)	t(1;?) (1qter → 1p13:?) t(1;?) (1pter → 1p13:?) +3mar
7	54XX (+3, +5, +5, +7, -8, +11, +15, +15, +19, 1p-, +9q+)	t(1;?) (1qter → 1p13:?) dup(9q) (pter → q12::q12 → q34::q12 → qter) +mar
8	63XX (+1, +1, +2, +2, +3, +4, +5, +6, +6, +13, +13, +14, +19, +19, +21, +22, +mar)	
9	47XX (+18) 55XX (-1, +3, +7, +9, +9, +13, +15, +18, +5q+, +2 mar)	t(5;?) (5pter → 5q35:?) +2mar
10	46XY (9q+) 46XY (9q+, t(2;7))	dup(9q) (pter → q12::q12 → q22::q12 → qter) t dic(2;7) (2qter → 2p25::7p11 → 7qter), ace(7pter → 7p11:) t(2;?) (2qter → 2p25:?)
11	55XY (+3, +5, +7, +9, +9, +11, +16, +21, +2p+)	
12	45XY (-22)	
13	45XY (-21)	
25	45XY (-22)	
26	52XY (-6, -7, -8, +9, +9, -10, -11, -13, -15, -16, -17, +19, -20, t(1;5), 2q+, +t(2;11), +t(3;5), 5p+, +i(6p), +i(7q), +i(8p), +i(8q), +t(11p+;?) +t(2;11), +11q+, t(16;21), +t(16;20;21) +mar)	recp(1;5) (1qter → 1p12::5q35 → 5qter; 5pter → 5q35::1p12 → 1pter) recp(2;11) (2pter → 2q11::11p13 → 11pter; 11qter → 11p13::2q11 → 2qter) t(2;?) (2pter → 2q37:?) t(3;5) (3qter → 3p12::5p13 → 5pter) t(5;?) (5qter → 5p13:?) i(6)(pter → cen → pter) i(7)(qter → cen → qter) i(8)(pter → cen → pter) i(8)(qter → cen → qter) t(11;?) (11qter → 11p11:?) t(11;?) (11pter → 11q25:?) t(16;20;21) (16pter → 16q13::20p12 → 20pter; 20qter → 20p12::21p11 → 21pter; 21qter → 21p11::16q13 → 16qter) +mar t(11;14) (11qter → 11q13::14q32 → 14pter)

21 were present in these hyperdiploid cells. Derived structural changes were common in the side line abnormal cells, with chromosomes 1, 5, and 9 participating often. Marker chromosomes of uncertain origin were common in these hyperdiploid cases. The

diploid cells of case 10 had an abnormal karyotype in which there was an extra band on the long arm of one chromosome 9 (Fig. 1). One of the cells examined had an additional translocation between chromosome 2 and 7, which gave rise to an acrocentric and a

Fig. 2 Diagrammatic representation of break points (black circles) on chromosomes participating in structural rearrangements in 13 cases of MM as designated in Table 2. Known fragile sites (*), oncogenes (○), and break points of neoplastic disease (arrows) of corresponding chromosomal regions are also plotted and indicated below chromosomes. AML = Acute myeloid leukaemia; CML = chronic myeloid leukaemia; B CLL = B cell chronic lymphocytic leukaemia; pre-B-ALL = pre-B cell-type acute lymphocytic leukaemia; NHL = Non-Hodgkin's lymphoma; T-Lym = T cell lymphoma; BL = Burkitt's lymphoma; FL = follicular lymphoma; ES = Ewing sarcoma; Ova Ca = ovarian cancer; Neu = neuroepithelioma.

dicentric chromosome marker (Fig. 1). The acrocentric chromosome was likely to be lost in further cell divisions whereas the dicentric chromosome would easily misdivide and give rise to markers in the derived cells. Cases 1 and 6 had chromosome counts of 47 and 48. An extra marker chromosome of the size of chromosome 21 or 22 was present in all the abnormal cells in case 1. In case 6 the hyperdiploid cells had trisomy of chromosome 5 and monosomy of chromosomes 14, 16, and 18, plus structural changes on chromosome 1, and three extra markers.

In the two patients with PCL, the bone marrow samples of case 25 were examined four times during the year, and a considerable number of hyperdiploid cells were present in the marrow. The modal hyperdiploid karyotype showed very complicated structural rearrangements entailing the participation of chromosomes 1, 2, 3, 5, 6, 7, 8, 11, 16, 20, and 21. Case 26 had predominantly hypodiploid cells in his marrow, with a modal number of 40. A consistent 14q+ marker was present that was derived from a translocation between chromosomes 11 and 14.

Break points on the chromosomes occurring in the structural rearrangements found in the 13 patients with MM are plotted on the diagrammatic representation of chromosome bands as given by the Paris Conference¹⁵ (Fig. 2). The fragile sites, oncogene sites, and several break points resulting from chromosome rearrangements associated with some leukaemias and lymphomas are also indicated on these chromosomes.

Discussion

In this series 13 of the 20 patients with MM, whose bone marrow cells were adequately analysed by G banding, showed chromosomal abnormalities. The abnormal chromosome composition varied from case to case and among cells in the same patient. Among the eight cases of MM and the one case of PCL (25) with hyperdiploid lines the karyotypes of hyperdiploid cells in cases 4 and 8 were derived most likely from a tetraploid cell, which originally appeared in the marrow, followed by chromosome loss from the

tetraploid genome. In the rest of the cases the hyperdiploid clones would have been derived most likely from the chromosome gain from the diploid cells. Several chromosomes seemed to be preferentially preserved by the hyperdiploid cells with growth advantage in the marrow. Structural rearrangements often occurred between the duplicated chromosomes in the hyperdiploid cells and subsequently gave rise to various markers. As was reflected in the patients' survival time, the effect of chromosomal duplication followed by further changes between the additional chromosomes seemed to be less drastic than that of the abnormalities that entail chromosomal rearrangements in a euploid complement of a cell. Two of the five cases of MM (2 and 5) and the one case of PCL (26) with hypodiploid lines all had a high percentage of abnormal cells, in which chromosome loss accompanied by structural changes occurred. The subsequent overgrowth of these hypodiploid cells in the marrow caused deleterious effects in the patients. These patients did not survive long.

A 14q+ marker chromosome, which is commonly associated with B lymphocyte neoplasia, was found in only one of the 13 patients with MM with abnormal lines. It has been suggested that the appearance of the 14q+ marker may be associated with the development of a leukaemic phase in myeloma.⁹⁻¹² On the other hand, the banding studies in patients with PCL indicate that 14q+ seems to be a consistent marker for PCL.^{9,10} One of the patients with PCL in this study had a 14q+ consistent marker derived from a t(11;14) in the hypodiploid line.

Despite the heterogeneity in the chromosome composition of myeloma cells there were non-random vulnerable regions of breakage on a few chromosomes. When these break points were plotted and compared with the known chromosomal fragile sites and oncogene regions, as defined by Yunis and Soreng,¹⁶ the coincidence was striking. Eleven break points were located at or near the fragile sites. Six break points coincided with the oncogene sites. Nine break points occurred at the vulnerable spots that play a part in specific translocations found in acute leukaemias and lymphomas (Fig. 2). Studies in different haematological diseases have shown that chromosome break points of several specific translocations coincide with the fragile sites and oncogene locations.¹⁷ These may be important in oncogenesis—for example, in Burkitt's lymphoma, in which a translocation acts by joining the c-myc oncogene on chromosome 8 close to one of the immunoglobulin loci normally active in B lymphocytes, resulting in a constitutive activation of the oncogene.¹⁸

A neoplastic phenotype develops by a multistep process. The heterogeneity of the chromosome aberrations found in myeloma cells reflects the complexity

of the regulation of plasma cell development. As in other haematological disorders, the genetic mechanisms of chromosomal rearrangements, in association with the fragile sites and oncogenes, may apply to myeloma as well as to other malignant conditions.

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