Short Communication

Combined Morphological and Interphase Fluorescence *in Situ* Hybridization Study in Multiple Myeloma of Chinese Patients

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To gain insight into the real incidence of the numeric chromosomal aberrations and the cell lineage involvement of the neoplastic process in multiple myeloma (MM), we examined 18 Chinese MM patients by May-Grunwald-Giemsa (MGG) staining and fluorescence *in situ* hybridization using three DNA centromeric probes specific for chromosomes 3, 7, and 9. In this investigation, cytogenetic abnormalities were detected in plasma cells (PCs), myeloid cells (MCs), and lymphoid cells (LCs) in all of the MM patients studied. This is the first demonstration of the cytogenetic aberration involved in the myeloid series. Furthermore, the MCs and PCs of 16 MM patients had the same aneuploidies in one or more of the chromosomes analyzed. These data suggest that the neoplastic transformation of MM may occur early in the hematopoietic development. Chromosomal aberrations involving mainly subclones and considerable cellular heterogeneity with gain of a variety of copy numbers of the same chromosome were demonstrated within PCs, which may possibly be the result of an underlying defect of PCs in the control of their number of chromosomes. Whereas PCs showed evidence suggestive of increased polyploidization, MCs and LCs, which exhibited similar chromosomal patterns as the former, rarely did. Thus, the clonal evolution from LC to PC, if that happens in MM, is characterized by chromosomal instability favoring growth of tumor cells with polysomies and polyploidies. (*Am J Pathol* 1999, 154:15–22)

Multiple myeloma (MM) has been regarded as the neoplastic disease of the terminally differentiated B cell with elusive oncogenesis. Although the majority of the tumor cells found in the bone marrow can be recognized by their typical plasma cell morphology, they are the progeny of a yet unidentified myeloma progenitor cell. Suggested possibilities of this clonogenic cell include peripheral blood B-lymphoid cell, pre-B cell, and hematopoietic stem cell.¹ Thus far, knowledge on the nature and development of the clonogenic cell has been lacking. This shortcoming has been an obstacle to rational intervention and contributed to a high fatality of MM from ineffective treatment.

The low proliferative rate of the tumor cells in this malignancy has hampered the conventional cytogenetic analysis. An abnormal karyotype, often a complex mixture of numeric and structural changes, is found in ~50% of patients.² Recent data have revealed that 55% of MM patients had three or more trisomies involving chromosomes 3, 5, 7, 9, 11, 15, 19, and 21.³ The significance of these chromosomal aberrations in MM pathogenesis remains obscure. As a large number of proliferating or nondividing cells can be examined, use of fluorescence *in situ* hybridization (FISH) has improved the detection of cytogenetic abnormalities in MM. In addition, by combination with morphological assessment of the cells studied, the lineage of cells that are involved in the neoplastic transformation can be elucidated. Using FISH and 10 α -satellite DNA probes, Drach et al⁴ showed that 88.9% of MMs were aneuploid for at least one chromosome examined and 66% had aberrations in three or more chromosomes. In the same study, mature myeloid cells evaluated showed no abnormality.⁴ After FISH procedure, cytomorphological details are so obscured that it would be very difficult to ascertain the cell types being assessed. Using combined morphological and FISH

Accepted for publication September 22, 1998.

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(May-Grunwald-Giemsa (MGG)/FISH) technique, more reliable information on the cytogenetics of MM can be obtained, irrespective of the percentages of the plasma cell infiltration. Moreover, myeloid and lymphoid cells can be evaluated in the primary MM samples for involvement of chromosomal aberration. To gain insight into the real incidence of the numeric chromosomal aberrations and the nature of the MM progenitor cell, we examined 18 Chinese MM patients by MGG/FISH using three DNA centromeric probes specific for chromosomes 3, 7, and 9, which frequently showed aberrations in MM.³⁻⁶ In addition, in our experience, these probes work out very well with strong and discrete signals.

Materials and Methods

Patients and Normal Controls

Bone marrow (BM) aspirate samples collected in EDTA bottles from 18 Chinese patients diagnosed to have MM in Prince of Wales Hospital, the Chinese University of Hong Kong, from June 1995 to January 1997 were recruited for the study. The diagnosis and staging were made according to the criteria of Durie and Salmon.^{7,8} Peripheral blood (PB) samples from five healthy volunteer staff and BM aspirates from four healthy BM donors were analyzed for determination of the cutoffs (mean + 3.291 SD).

MGG/FISH

Mononuclear cells from nine patient EDTA BM aspirate and five normal control PB samples were separated by Lymphoprep (Nycomed Pharma, Oslo, Norway) according to the manufacturer's protocol. The cytospin slides prepared were air dried thoroughly overnight and then stored at -70°C until use. All of the BM or PB cytospins and smears from the normal control and patients were fixed in absolute methanol and stained with MGG by standard procedure. The stained slides were then air dried and analyzed by FISH within 1 week after staining. The MGG-stained cytospins and BM smears were viewed under light microscope (OPTIPHOT-2, Nikon, Tokyo, Japan) at $\times 400$ magnification. Fields of interest on the slides were photographed with Kodak Gold III 100 (GA 135-36 Eastman Kodak, Rochester, NY) films. The coordinates on the stage of the microscope were recorded for every shot taken.

Interphase FISH was performed using digoxigenin-labeled α -satellite DNA probes (Oncor, Gaithersburg, MD) specific for chromosomes 3 and 7 and digoxigenin-labeled Classical Satellite DNA probe (Oncor) specific for chromosome 9. BM cytospins or smears were destained in Carnoy's fixative (methanol/glacial acetic acid, 3:1, v/v), and procedures followed then were according to Oncor's FISH protocol with minor modification on post-hybridization wash (the slides were washed in $0.25\times$ SSC (pH 7.0) for 8 minutes at 68°C without agitation). Finally,

the slides were put up for detection of hybridization signals. The cells were evaluated under the fluorescence microscope (OPTIPHOT-2, Nikon) equipped with an oil immersion $100\times$ objective and an fluorescein isothiocyanate filter. One MGG-stained control PB cytospin was hybridized together with each set of sample slides subjected to the FISH procedure. A total of 600 nuclei were analyzed each time under the fluorescence microscope as described previously. Hybridization results of the samples were considered satisfactory if the hybridization signals in the control slide were reasonably strong and the frequency of nuclei showing numeric aberrations was statistically insignificant. All sets of samples were satisfactorily hybridized by these criteria, and the sample slides were then scored. Fields previously photographed were relocated on the stage of the microscope using the reference coordinates recorded. The same cells, plasma cells (PCs), myeloid cells (MCs), and lymphoid cells (LCs), identified by morphological assessment previously, were re-evaluated under the fluorescence microscope for the number of fluorescence signal(s) per nucleus, which was recorded on the LM microphotographs. Unhybridized nuclei were not counted and did not contribute to our data. Only regions with more than 90% of cells showing hybridization signals were analyzed. Only those well separated signals with similar intensity, size, and shape were counted. Cells with ambiguous morphology precluding accurate identification of cell types were excluded from analysis. The fluorescence images were photographed with Kodak Ektapress Plus 1600 color film (5PJC 135-36, Eastman Kodak).

Cutoff Levels

The MGG-stained PB cytospins and BM smears from control donors were subjected to interphase FISH using the probes for chromosomes 3, 7, and 9. A total of 600 per nuclei were analyzed and scored in each slide. The mean percentage of trisomic/monosomic cells in these control samples for the chromosomes assessed was as follows: chromosome 3, $0.9 \pm 0.7/5.4 \pm 2.7$ (cytospin (C)) and $0.8 \pm 0.4/7.1 \pm 2.5$ (smear data (S)); chromosome 7, $0.4 \pm 0.3/6.3 \pm 2.2$ (C) and $0.4 \pm 0.5/5.8 \pm 1.5$ (S); chromosome 9, $1.0 \pm 0.6/6.9 \pm 2.4$ (C) and $0.4 \pm 0.3/8.2 \pm 2.5$ (S). The cutoff levels for the significant numeric changes in the patient samples were defined as any values higher than the mean percentages of aneuploid nuclei plus 3.291SD ($P = 0.001$ by χ^2 test) for the corresponding chromosome in the control samples. As not a single cell from 600 cells studied in the normal controls showed four or more signals per nucleus, it had been determined that the cutoff level for significant tetra- or polysomies were at $\geq 0.3\%$ of cells showing four or more fluorescence signals per nucleus. As only single-probe interphase FISH was performed, monosomies, which had not been verified by dual-probe FISH analysis, were not presented in this paper.

Table 1. Clinical Data and FISH Results of 18 Chinese MM Patients

Patient	Sample type	Age (years)		Immuno-paresis	Osteolytic lesions	Renal function	Calcium level	Alb	BMPC (%)	Para-protein	Treatment (time before test)	Chromosomal changes			Outcome (month number)
		Sex	Stage									PCs	MCs	LCs	
1	C	68 M	III	P	P	I	N	N	52	IgA λ	MP	+3+7+9	+3+7+9	a,+7+9	A(17)
2*	C	80 F	III	P	P	I	N	L	90	IgG κ	RT+Dex	+3 ⁴ +7 ⁴ +9	+7	+7+9	D(03)
3*	C	43 F	III	P	P	N	N	L	80	IgG λ	CEVAD	+3+7 ⁴ +9	+3		D(22)
4*	S	68 F	III	P	P	I	H	L	90	IgA λ	MP, RT	+3+7+9	+3+7+9	+3+7+9	D(15)
5	S	25 M	III	P	P	I	H	L	90	IgG κ	CEVAD	+3+7+9	+3+7+9	a,b,+9	A(10)
6*	S	81 M	III	P	P	I	N	L	80	IgA κ	MP	+3 ⁴ +7 ⁵ +9 ⁵	+3+7+9	+3+7+9	D(05)
7*	S	75 F	III	P	P	N	N	L	30	IgG κ	MP, RT	+3+7+9	+9	+7+9	A(07)
8 [†]	C	49 M	II	A	A	N	N	L	20	IgA λ	MP	+3 ⁴ +7 ⁴ +9 ⁵	+3+7	+3+7+9	A(06)
9	S	51 F	III	P	P	N	N	L	50	IgG λ	MP	+3 ⁴ +7+9	+9	b,+9	D(07)
10	S	60 M	III	A	P	I	N	L	36	IgG λ	NIL	+3+7+9	+9	a,+9	L
11	S	65 F	III	P	A	I	N	N	70	BJP λ	DEX	a,+7 ⁴	a,b,+9	a,b,+9	A(03)
12	S	80 M	III	P	P	N	N	L	75	IgG κ	MP	+7 ⁴ +9	+9	a,+9	A(04)
13 [‡]	C	52 M	III	P	P	N	N	L	65	IgG λ	MP CEVAD (3M)	+7+9	+9	+3+7+9	A(23)
14 [§]	C	41 F	II	P	A	N	N	L	40	IgG κ	CEVAD (1M)	+3 ⁴ +7 ⁴ +9	+3+7 ⁴ +9	a,+7 ⁴ +9	A(43)
15 [‡]	C	62 F	III	P	P	N	N	L	70	IgA κ	MP, CEVAD (12M)	+3	+3 ⁴	+3	A(21)
16 [‡]	C	62 M	II	P	P	N	N	N	20	BJP κ	MP/CEVAD, RT (3M)	+3+7+9	+7+9 ⁴	a,+7+9	D(16)
17 [‡]	C	48 M	III	P	P	N	N	L	80	IgG λ	MP (3M)	+3 ⁴ +7+9	+3+9	+7+9	A(28)
18 [‡]	S	63 F	III	P	P	N	N	L	50	IgA λ	MP, RT (22M)	+3 ⁴ +7+9	+3	+3,b	A(12)

Chromosomal changes identified with a superscript number indicates polysomy; all others are trisomy only. Bold print indicates mixed trisomy and polysomy, with trisomy the predominant clone. Bold with superscript indicates mixed trisomy and polysomy, with polysomy the dominant clone.

BMPC, bone marrow plasma cell infiltration; C, cytospin; S, BM smear; P, present; A, absent; I, impaired; N, normal; H, high; L, low; BJP, Bence Jones protein; M, Melphalen; P, prednisone; RT, radiotherapy; DEX, dexamethasone; CEVAD, cyclophosphamide plus etoposide plus vincristine plus adriamycin plus dexamethasone; +, gain in chromosome; a and b, insufficient cells for analysis of chromosomes 3 and 7, respectively; for outcome results: A, alive; D, dead; L, lost to follow-up.

*Patients with complete FISH data.

[†]Case included because of high percentage of hyperdiploid cells despite low percentage of PCs evaluated.

[‡]Previously treated cases.

[§]Relapse disease.

Results

Patient Characteristics

Table 1 shows the clinical and FISH data of the 18 Chinese MM patients (12 pretreated and 6 previously treated) studied. The male-to-female ratio was 1:1, with a median age of 62.5 years. It is noteworthy that one patient (patient 5) was diagnosed at 25 years of age, which is extremely unusual for MM. Our patient group included five patients with IgG κ , five with IgG λ , two with IgA κ , four with IgA λ , one with BJP κ , and one with BJP λ MM. Fifteen patients were at stage III and three at stage II disease. Patient 14 was diagnosed as a relapse disease. The median follow-up duration was 12 months from the first consultation at Prince of Wales Hospital to the time of data analysis. The median treatment duration before the FISH evaluation for the previously treated cases was 3 months. The complete blood counts revealed a mean hemoglobin level of 9.0 g/dl (range, 6.9 to 11.9 g/dl), white blood cell count of $5.8 \times 10^9/L$ (range, 2.9×10^9 to $7.1 \times 10^9/L$), and platelet count of $224 \times 10^9/L$ (range, 52×10^9 to $432 \times 10^9/L$). Six patients died, and eleven were alive at the time of data analysis. One patient (patient 10) was lost to follow-up (Table 1).

Chromosomal Aberrations of MM Studied by MGG/FISH

Eighteen BM aspirates were analyzed using DNA probes for chromosomes 3, 7, and 9. Except for one patient

(patient 8), the results were considered acceptable for documentation of chromosomal abnormalities, as arbitrarily defined, when the number of cells counted was >100 for PCs, >50 for MCs, and >15 for LCs. This could be achieved in most of the patient samples as indicated in Table 1. The number of cells studied was 266 ± 110 for PCs (range, 104 to 628), 215 ± 131 for MCs (range, 54 to 546), and 54 ± 46 for LCs (range, 18 to 271).

Plasma Cells

FISH data were available for all except one patient (patient 11) for the analysis of chromosome 3. Aneuploidies from one or more chromosomes were found in all of the 18 patients. Multiple polysomies of all three chromosomes were observed in 14 patients. The cytogenetic aberration was also complex with multiple aneuploids (Table 1). Table 2 shows the frequency, range, and mean percentage (clone size) of cells involved in the different chromosomal aberrations. Polysomies 3 (82.4%), 7 (83.3%), and 9 (83.3%) and trisomies 3 (64.7%), 7 (66.7%), and 9 (88.9%) were observed in the PCs. Trisomy 9 was the most common aberration seen, in fact in all cell series. Incidences of the various combinations of chromosomal aberrations in the three cell series are illustrated in Figure 1. Numeric chromosomal changes were commonly derived from a spectrum of variable copy numbers of the three chromosomes in different cells, particularly in PCs (Figure 2, A–H).

Figure 1. Incidence of individual chromosomal abnormalities in multiple myeloma as determined by FISH.

Myeloid Cells

MCs recruited for FISH analysis included erythroblasts and intermediate and mature granulocytic cells. FISH data on MCs were obtained for all except one patient (patient 11) for the analysis of chromosomes 3 and 7. Again, all of the MM patients demonstrated multiple chromosomal aberrations in MCs, which exhibited similar patterns as observed in PCs (Table 1). Almost all aneuploids found in MCs could be demonstrated in PCs except in the two cases (patients 11 and 18), in which trisomies 9 and 3 were observed, respectively, in MCs but not PCs. However, in contrast to PCs, several exceptional features were noted. First, MCs did not show any polysomies beyond tetrasomies (more than four signals/nucleus) (Figure 2, I–L). Second, as shown in Table 2, the trisomic/tetrasomic clones, if found, were also very small (0.5% to 5.5%). Third, numeric aberrations found in PCs were observed less frequently in MCs. Fourth, MCs showed a smaller number of aneuploids than PCs in each MM case (Table 1).

Lymphoid Cells

Analysis of the lymphoid cells was frequently hampered by inadequate samples, which occurred in nine cases (five patients with chromosome 3, two with chromosome 7, and two with both chromosomes). Multiple

cytogenetic aberrations were found in LCs in all except one (patient 3) of the MM patients studied (Table 1). Like MCs, LCs did not demonstrate polysomies beyond tetrasomies, and the trisomic/tetrasomic clones (2.6% to 8.4%), although slightly larger than those of MCs, were also much smaller than those of PCs (Table 2). In three patients (11, 13, and 18), trisomies were found in LCs but not PCs.

Similarities and Differences of Aneuploidies among PCs, MCs, and LCs

For all three cell series, FISH data on chromosomes 9, 7, and 3 were assessed for 18, 14, and 11 patients, respectively. Identical aneuploids with chromosomes 9 (12/18), 7 (6/14), or 3 (4/11) were commonly seen across the three cell series. FISH data on chromosomes 9, 7, and 3 for all three cell series were evaluated in nine patients. Two (patients 4 and 6) of these nine patients showed gains in the same copy numbers of all three chromosomes in PCs, MCs, and LCs (Table 1). Trisomies or tetrasomies found in MCs or LCs could almost always be demonstrated in PCs with few exceptions. The trend of expanded clones of increased polyploidization (multiple polysomies of chromosomes 3, 7, and 9) with multiple gains of various high (three to six) copy numbers of different chromo-

Figure 2. MGG-stained morphology $\times 800$ and FISH preparation $\times 1000$. P, plasma cell; P1, mononucleated PC; P2, binucleated PC; P4, tetranucleated PC; M, myeloid cell; L, lymphoid cell. **A to F:** From patient 6 and with probe for chromosome 9. **A:** MGG-stained BM smear showing two PCs. **B:** The same area of the film, treated by the FISH technique; three (left) and six (right) hybridization signals are seen in each nucleus of the two PCs. **C:** MGG-stained BM smear showing two mononucleated (P1) and one tetranucleated (P4) PC, one lymphocyte (L), and one erythroblast (M). **D:** The same area after FISH; three hybridization signals in one P1 (lower) and six hybridization signals in the other P1 (upper) are noted. A single signal is seen in the nucleus of the lymphocyte. The erythroblast shows two hybridization signals in the nucleus. **E:** MGG-stained BM film showing two mature MCs (M). **F:** The same cells after FISH show two signals in one MC (upper) and three in the other (lower). **G:** MGG-stained BM smear from patient 17 showing three PCs (P) and one MC (M). **H:** FISH analysis of the BM smear in **G** with probe for chromosome 3. Three signals are observed in each of the nuclei of the MC and one PC (center). One PC (upper left) shows a single hybridization signal whereas the other one (upper right) shows two signals in their nuclei. **I:** MGG-stained BM smear from patient 4 showing four PCs (P) and one MC (M). **J:** FISH analysis in the same cells with probe for chromosome 9 showing four signals in the nucleus of the MC, three signals in that of one PC (left), and two signals each in the other two PCs. **K:** MGG-stained cytospin from patient 3 showing one lymphocyte (L), one mononucleated PC, and one binucleated PC (P2). **L:** FISH analysis with probe for chromosome 9. The lymphocyte and the mononucleated PC show two signals in each of their nuclei whereas the nucleus of the MC reveals three signals. The binucleated PC (P2) demonstrates two signals in one and three in the other nucleus.

Table 2. Frequency, Range and Mean Percentage of Cells of MM Cases Involved in Chromosomal Aberrations of PC, MC, and LC

Chromosome	Plasma cell			Myeloid cell			Lymphoid cell		
	Frequency	Range (%)	Mean % of cells $\pm$ SD	Frequency	Range (%)	Mean % of cells $\pm$ SD	Frequency	Range (%)	Mean % of cells $\pm$ SD
+3	64.7	3.4–55.9	20.2 $\pm$ 16.4	53.8	2.5–5.8	4.2 $\pm$ 1.2	54.5	3.7–14.8	6.6 $\pm$ 4.1
+3 ^{4*}	82.4	0.4–38.8	6.2 $\pm$ 12.1	23.5	0.8–1.7	1.0 $\pm$ 0.5	27.3	3.7–6.3	4.6 $\pm$ 1.5
+7	66.7	3.0–49.1	18.6 $\pm$ 19.1	41.2	2.3–5.9	4.7 $\pm$ 3.2	64.3	2.6–6.1	4.1 $\pm$ 1.4
+7 ^{4*}	83.3	0.4–68.0	10.0 $\pm$ 21.3	11.8	0.4–2.2	1.3 $\pm$ 1.3	7.1		3.6
+9	88.9	4.1–62.8	26.6 $\pm$ 19.6	68.8	1.5–11.2	5.5 $\pm$ 2.9	83.3	1.6–8.9	8.4 $\pm$ 8.6
+9 ^{4*}	83.3	0.3–68.5	11.7 $\pm$ 20.3	11.1	0.3–0.7	0.5 $\pm$ 0.3	22.2	1.1–3.6	2.6 $\pm$ 1.1

Chromosomes with superscript 4 indicate polysomy (four or more signals per nucleus); all others are trisomy (three signals per nucleus).

*Cutoff level for tetrasomy, polysomy, etc. was $\geq 0.3\%$.

Table 3. Heterogeneity of Aneuploidy in Plasma Cells of 18 Chinese MM Patients

Patient	Number of hybridization signals/nucleus (% of cells)											
	Chromosome 3				Chromosome 7				Chromosome 9			
	<i>n</i>	3	4	≥ 5	<i>n</i>	3	4	≥ 5	<i>n</i>	3	4	≥ 5
1	304	37.2	0.7		123	45.5	0.8		280	51.8	8.2	1.1
2	247		0.4		266		0.8		345	6.7	0.9	0.6
3	209	3.3	0.5		208		0.5		312	5.8	1.0	3.5
4	205	17.1	1.0		208	11.1	0.5		414	62.8	1.2	
5	304	0.7			452	49.1	0.9		466	32.6	1.3	
6*	177	20.3	36.7	2.2	227	4.8	20.3	48.5	225	17.8	18.7	49.8
7	136	55.9	2.2		277	29.2	0.4		106	40.6	6.6	
8*	67	9.0	25.4	3.0	33	3.0	24.2	30.3	82	12.2	7.3	45.1
9	236		0.4		164	3.0	0.6		328	7.3		
10	181	14.4			299	3.7			104	38.5	2.9	
11	IS	IS	IS	IS	191		0.5		328			
12	152				127		0.8		314	4.1	0.3	
13	178				314	3.2			453	34.2	2.6	
14*	196	5.1	9.7	0.5	173		6.4	0.6	160	12.5	1.9	0.6
15	628	8.9	0.6		397				494			
16 [†]	123	13.8		0.8	307	22.2	6.8	3.6	225	44.4	11.1	3.1
17	271		0.4		304	2.3	1.0	0.3	346	6.1	2.9	0.6
18 [‡]	222		1.4		310	46.5	2.6		270	47.8	4.8	0.4

Percentages of PCs involved with trisomies or polysomies are listed. *n*, total number of PCs counted for the chromosome assessed; IS, insufficient cells analyzed.

*Patients with detectable hexasomic PCs.

[†]Patient with a detectable octasomic PC.

[‡]Patient with a PC showing 11 fluorescence signals.

somes in PCs was in striking contrast to the much smaller trisomic/tetrasomic clones in LCs and MCs.

Heterogeneity of Aneuploidies

The myeloma cells (PCs) displayed a wide spectrum of the number of hybridization signals (range, 1 to 11, but mostly 2 to 6) (Figure 2, A–D, G, and H). In a few cases where multinucleated myeloma cells were present, the progeny nuclei also displayed different combination of variable numbers of hybridization signals (Figure 2, K and L). As illustrated in Table 3, the percentage of myeloma cells affected by each chromosomal aberration was variable in the same MM cases, suggesting that there were probably multiple subclones carrying different numeric cytogenetic abnormalities. For example, in case 4, the percentages of trisomic 3 and 7 cells were, respectively, 17.1%, and 11.1%, whereas trisomic 9 was 62.8%. It is possible that there was a triploid clone making up for ~11% of MM cells whereas ~46% of MM cells may

harbor trisomy 9 alone, with 6% showing trisomies 3 and 9. There is much less cellular heterogeneity among myeloid and lymphoid cells in terms of polysomies.

Correlation of Chromosome Changes with Clinical Parameters

The patterns of chromosomal aberrations were not significantly different between the previously treated and untreated groups. Numeric changes of chromosomes were not found significantly correlated with any of the clinical parameters as listed in Table 1. The only case with gain in chromosome 3 as the sole numeric abnormality was found in a treated IgA MM.

Discussion

Using combined morphological and FISH technique, we showed that all 18 primary Chinese MM cases (100%)

were cytogenetically abnormal. Previous studies by FISH without morphological correlation and conventional cytogenetics reported incidences of aneuploidy from 80% to 96.6% and 50%, respectively.^{2,4-6} Consistent with the cytogenetic data in the literature, high incidences of chromosomal gains were found in PCs of our patients.⁴⁻⁶ Gains of the same copy numbers of all three chromosomes suggested the presence of triploid, tetraploid, and mixed polyploid subclones. More importantly, the myeloid series was found involved in chromosomal aberrations in this study, albeit having only small aneuploid clones.

The MCs in MM have been assumed or found to be cytogenetically normal previously. In the FISH study on MM by Drach et al, only mature myeloid cells with distinct nuclear outlines were analyzed and reported to have no chromosomal changes.⁴ In their other FISH study on monoclonal gammopathy of undetermined significance (MGUS) patients, abnormality of MCs was not observed.⁹ Our finding thus represents the first description of involvement of chromosomal aberration of MCs in MM.

The MM clonogenic cell has been a myth despite recent advances in MM research. The simultaneous occurrence of MM and another hematopoietic disorder in the same patient has been well known in MM. Early sporadic case reports of simultaneous occurrence of acute myeloid leukemia and MM may be the first evidence suggesting that there might be a potential link in the pathogenesis of the two disease entities.¹⁰⁻¹² In the study by Rosner et al,¹³ among 58 acute-leukemia-associated MM patients, 11 cases had either simultaneous development of the two diseases or within several months of each other. Thus, it raised the possibility that acute leukemia may occur as part of the natural history of MM.¹³ In the last two decades, phenotypic and cell culture studies in MM cells have demonstrated a high frequency of expression of multiple-lineage-associated surface antigens (eg, myelomonocytic), similar *in vitro* growth requirements for interleukin (IL)-3 and IL-6 as early hematopoietic progenitors, and the presence of a typical Philadelphia chromosome in short-term culture and a cell line from cases of disseminated MM or plasma cell leukemia.¹⁴⁻¹⁸ These findings provide strong evidence that malignant transformation of MM may start early in the hematopoietic development.

Our current data support that both lymphoid-plasma cell and myeloid lineages carrying similar chromosomal aberrations were possibly pathogenetically related and affected by a common source of genetic defect(s). However, we could not rule out the possibility that these chromosomal abnormalities arose from pathogenetically unrelated causes. One of the possibilities of the former scenario is that the MM precursor cell may be derived from the pluripotent progenitor cell capable of differentiation into myeloid and lymphoid cell lineages. Thus, this may lend further support to the postulation that the neoplastic transformation may occur early in the hematopoietic development.

An alternative explanation for the presence of chromosomal abnormalities in MCs may be that they were acquired genetic damages from aging unrelated to MM

pathogenesis. To test this possibility, we evaluated the myeloid and lymphoid cells in the peripheral blood of two male and one female healthy elderly (70 to 80 years old) subjects for chromosomal aberrations. Abnormalities found in the disease group were not observed. Thus, we believe that these abnormal micro-populations of MCs were genuine abnormalities, which were probably pathogenetically related to the MM development.

Reactive plasma cells (found usually in 2% to 5% in normal subjects), shown to be disomic by previous studies, were not distinguished morphologically from MM tumor cells in this investigation. As the number of the cytogenetically abnormal PCs was much smaller than the total plasma cell population, it is reasonable to assume that many of the MM cells were disomic (Figure 2, I and J). However, the highly heterogeneous and complex pattern of numeric chromosomal aberrations involving mainly subclones in MM suggest that these may be secondary events resulting from genomic instability (GIN), which may occur when there is error in cell cycle control and DNA repair mechanism. However, alterations of the *p53* gene, which are associated with GIN, are infrequent in MM, and a late event in the disease.^{19,20} This discrepancy may be explained by the recent finding in MM of the presence of overexpression of *mdm-2*, an inhibitor of *p53* protein.²¹ Furthermore, the presence of tumor cells with gain of a wide variety of different copy numbers of the same chromosome (Figure 2) may imply a loss of control of chromosome numbers, which is possibly a result from an underlying defect of chromosomal segregation during cell division. A similar phenomenon has also been observed in colorectal cancers.²² This is also supported by our observation on chromosomal aberrations in binucleated PCs in which one nucleus showed a higher number of the same chromosome than the other. However, the problem looks more complex because in some situations, the sum of the chromosome copy numbers from the progeny nuclei was not an even number (Figure 2, K and L). This may imply that other mechanisms, such as defects in DNA replication or translocation, may be involved to produce this unbalanced chromosomal status. Consistent with our observation, a similar postulation of an incapacity of PCs to control their number of chromosomes was made by Zandecki et al²³ in their recent study on MGUS where they identified heterogeneous BMPC clones differing only in the number of chromosomal abnormalities exemplified.

Although the pathogenesis of MM remains unclear, it can be postulated that MM transformation is a multistep process²⁴ with cryptic mutations, including those associated with GIN, possibly occurring early in the hematopoietic development. This abnormal early progenitor cell may subsequently differentiate into the myeloid and lymphoid precursors through clonal evolution with additional mutations. Abnormal lymphoid cells with increased polysomies or polyploidies might have significant growth advantages for clonal expansion and development into the myeloma cell pool. On the other hand, tiny subpopulations of the very early myeloid precursors, with further mutations, may enter the aberrant pathway to produce tumor cells retaining the myeloid antigen expression. This

may create a mechanism for the continuous expansion and development of the neoplastic clone(s), which becomes more heterogeneous both genotypically and phenotypically as the disease progresses. Other genetically unstable myeloid precursors may develop into acute leukemia with other critical mutations particularly driven by chemotherapy.

Previous studies have shown that gain of chromosome 3 was significantly correlated with IgA paraprotein and decrease in renal function.^{4,25} Our observation of trisomy 3 as the sole numeric chromosomal abnormality in one treated IgA MM patient may also suggest its biological significance in this MM subtype, which needs to be confirmed by a larger study.

The results presented may possibly imply that one of the earliest genetic lesions in MM may occur before the commitment to myeloid lineage development. The abnormality may be potentially cryptic and manifested as chromosomal instability. However, this hypothesis remains speculative and needs to be confirmed by further study of clonality of different lineages in MM.

Acknowledgments

We thank Richard Chan for his administrative assistance and the Film Bench of Haematology Laboratory, Prince of Wales Hospital, for the MGG staining.

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