Clinical significance of cytogenetics and interphase fluorescence in situ hybridization analysis in newly diagnosed multiple myeloma in Taiwan

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Background: The incidence of multiple myeloma (MM) is lower in Asia than in Western countries. However, it is not known whether cytogenetic abnormalities (CA) characteristic of MM in Asia differ from those documented in the West.

Patients and methods: We analyzed CA by conventional cytogenetics (CG) and/or fluorescence in situ hybridization (FISH), assessed their clinical significance in 150 Chinese MM patients and compared our data with that derived from Western countries.

Results: CA were detected by CG (CG_CA) in 44 (29.3%) of the 150 patients and by FISH (FISH_CA) in 59 (67%) of the 88 patients studied. Presence of either CG_CA or FISH_CA was associated with a poor prognosis. Patients with CG_CA and hyperdiploid chromosomes, always associated with several trisomies, had a longer survival (median 25 months versus 12 months; \( P = 0.025 \)) in comparison with those with non-hyperdiploid chromosomes, usually associated with a monosomy 13/partial deletion of 13q (\( D_{13} \)) and a rearrangement of 14q32. A novel recurrent CG_CA, add(19)(p13), was found in four patients: all males with immunoglobulin G/lambda isotypes, extramedullary myeloma at diagnosis and a poor prognosis. Three groups of patients with significantly different survival, CG_\( D_{13} \), FISH_\( D_{13} \) but without CG_\( D_{13} \), and neither CG_\( D_{13} \) nor FISH_\( D_{13} \) (median 9 versus 15 versus 32 months; \( P = 0.013 \)) were identified.

Conclusions: We conclude that MM CA in our patients are similar to those noted in Western countries, and that combined CG and FISH analysis can predict prognosis. The clinical significance of add(19)(p13) needs to be further investigated.

Key words: cytogenetics, fluorescence in situ hybridization, multiple myeloma, prognosis

Introduction

Multiple myeloma (MM) is a genetically unstable malignancy of postgerminal center B-lineage cells [1]. Using conventional cytogenetics (CG) and interphase fluorescence in situ hybridization (FISH), with other modern cytogenetic techniques, it has now been established that aneuploidy with chromosome abnormalities (CA) is almost universal in MM [2–4]. Almost all CA in MM involve complex numeric and structural changes [2, 5]. Common numeric changes in MM are trisomies involving chromosomes 3, 5, 7, 9, 11, 15, 19 and 21, and monosomies involving chromosomes 8, 13, 16, 17, 22 and Y [2, 5, 6]. Common structural changes in CA include rearrangements of 14q32, partial deletion of chromosome 13, duplications of 1q and deletions of 1p, 6q, 11q and 17p [1, 5, 7]. Some CA in MM are not randomly distributed, but are tightly associated and may be responsible for the variability of MM with respect to the natural history, morphological and immunological features of myeloma cells and clinical status [5, 6, 8, 9]. Two aneuploidy groups of MM, hyperdiploid and non-hyperdiploid MM, have been proposed based on chromosome numbers [10, 11]. These two ploidy subcategories are associated with distinct clinical presentation and outcome [10–12].

Analogous to acute and chronic leukemias, such CA of MM represent not only the biological basis of the clinical heterogeneity, but also the prognostic parameters [13]. Hypodiploidy and deletion of chromosome 13, including monosomy 13 and partial deletion of 13q (\( \Delta 13 \)), could be used as negative prognostic predictors for MM patients receiving either conventional chemotherapy [12, 14–16] or high-dose chemotherapy followed by autologous hematopoietic stem cell transplantation (auto-HSCT) [17–21]. Other recurrent CA in
MM, such as t(4;14), t(14;16), deletion of 17p13, abnormalities of 11q and 22q, monosomies of 2, 3, 14 and 19, and 1p deletions, are associated with a poor prognosis [6, 14, 16, 22–24]. Analysis of CA in MM patients is required to elucidate the pathogenesis of the disease, predict treatment outcome and possibly identify distinct groups of MM patients who could benefit from aggressive or novel therapies.

Uneven geographical distribution of non-random CA in malignant disorders has been reported previously [25, 26]. It was suggested that the heterogeneity in the incidence of non-random CA in various areas was a reflection of ethnic differences or environmental factors [27]. We found that CA in Chinese lymphoma patients, in Taiwan, were different from those noted in lymphoma patients in Western countries [28]. Indeed, the incidence of MM in Asia is much lower than that recorded in Western countries [29]. It is not clear whether this disparity in CA between Asia and Western countries also extends to CA characteristic of MM. Therefore, we have used conventional cytogenetics and FISH to analyze CA and their clinical significance in 150 Chinese patients with newly diagnosed MM in Taiwan and compared our findings with those derived from Western countries.

Patients and methods

Patients

Between June 1986 and December 2003, 150 newly diagnosed and untreated MM patients with accessible cytogenetic results at our hospital were enrolled. In addition to the CG analysis, 88 patients had FISH performed simultaneously. The work was approved by our institutional human ethics committee that oversees research involving human subjects. Written informed consent was obtained from all patients.

Conventional cytogenetics

Bone marrow (BM) samples were aspirated into heparinized syringes and CG with G-banding method was performed on BM cells after 1–3 days of unstimulated culture as described previously [26]. CA and ploidy levels were defined according to International System for Human Cytogenetic Nomenclature [30]. For comparison with the results in other areas [10], patients were divided into two groups: one with hyperdiploid MM (47–57 chromosomes) and the other with non-hyperdiploid MM including the hypodiploid (35–45 chromosomes), pseudodiploid (46 chromosomes) and tri-/tetraploid MM (58–103 chromosomes). Complex CA was defined by more than two cytogenetic changes.

Fluorescence in situ hybridization

FISH analysis was performed as previously described [31]. Deletion of 13q14 was determined by combined use of a retinoblastoma gene-1 probe LSIRB1 (Vysis, Downers Grove, IL, USA) and a reference chromosome 10 centromeric probe LPE010G (Cytocell Ltd, Banbury, UK) in dual-color. Deletion of 17p13, where the p53 gene was located, was determined by use of a LSp53 probe (Vysis) combined with a chromosome 17 centromeric probe (Cytocell Ltd). Several other centromeric probes, LPE003R, LPE007G, LPE011R and LPE018G (Cytocell Ltd), were used to detect trisomies/monosomies of chromosomes 3, 7, 11 and 18, respectively. To improve the specificity of the FISH analysis, we combined the FISH technique with immunofluorescence staining of the cytoplasmic light chain of plasma cell (PC) (clg-FISH; anti-human kappa and anti-human lambda probes, conjugated with 7-amino-4-methylcoumarin-3-acetic acid; Vector Laboratories, Burlingame, CA, USA) as described previously [32]. BM cells from five transplantation donors were used as normal controls. Threshold levels for gain or loss of signals for each probe in clg-FISH, which were set at the mean of normal controls plus three standard deviations, were as follows: 13q14 deletion, 1.8%; 17p13 deletion, 2.4%; +3, 0.7%; +7, 2.0%; +11, 1.9%; and +18, 1.9%. However, we only regarded the findings of any abnormal signals in more than 10% of 100–300 scored PC as true evidence for CA on the clg-FISH analysis, in order to avoid false positives [11].

Treatment

A total of 131 (87.3%) received chemotherapy with MP (melphalan 9 mg/m² and prednisolone 60 mg/m² daily orally on days 1–4) regimen every 4–6 weeks until plateau phase was achieved or the disease was refractory to the treatment. Owing to poor clinical status or hesitation, 19 patients (12.7%) did not receive chemotherapy. A total of 71 patients (47.3%) with high tumor burden were treated with two to four cycles of combination chemotherapy of VAD [vincristine 0.4 mg/m², continuous intravenous infusion (CIVF), days 1–4; doxorubicin, 9 mg/m², CIVF, days 1–4; dexamethasone, 40 mg, CIVF, days 1–4 and 8–11] at an interval of 4 weeks. High-dose chemotherapy (melphalan 200 mg/m²) followed by auto-HSCT was administered to 10 patients.

Treatment response criteria were as described previously [33, 34]. In brief, responders included patients who had achieved a complete response, partial response or minimal response. Non-responders included the patients who had no response or had progressive disease. Overall survival (OS) was defined as the time period from the date of diagnosis to the date of death, regardless of cause.

Statistical analysis

χ² or Fisher’s exact tests were used for between-group comparison of the discrete variables. Two-sample t-test was used for between-group comparison of the means. Kaplan–Meier survival curves were used for estimation of OS. Log-rank test was used to test for differences in OS between groups. Several salient clinical and laboratory variables, including age, sex, disease stage, BM plasmacytosis, M-component isotype, levels of hemoglobin (Hb), white blood cell (WBC), platelet (PLA), lactate dehydrogenase (LDH), calcium (Ca), creatinine (Cr), C-reactive protein (CRP) and β2-microglobulin (β2M) were assessed in all patients at diagnosis to determine its possible association with CA. All the variables and the CA were examined for their prognostic values on OS. Those factors with statistically prognostic significance from univariate analysis were tested by multivariate analysis with the Cox proportional hazards regression model using forward stepwise selection. In these prognostic analyses, continuous variables were categorized by the cutoff values as follows: age ≥60 years, Durie–Salmon stage ≥III, BM plasmacytosis ≥30%, IgA isotype, Hb <10 g/dl, WBC <4 × 10⁹/l, PLA <1.5 × 10¹¹/l, LDH >465 IU/l, Ca ≥2.4 μmol/l, Cr ≥2 mg/dl, CRP ≥4 mg/dl and β2M ≥2.5 mg/l, as set up in previous reports [18, 20]. All directional P values were two-tailed, with a P value of ≤0.05 considered significant for all tests. All analyses were performed using SPSS 8.0 software (SPSS, Inc., Chicago, IL, USA).
Results

Patient characteristics

There were 98 males and 52 females, with a median age of 62 years (range 24–88). The main bioclinical features are summarized in Table 1. Up to December 2004, the median follow-up time for the patients was 62 months. The median OS for all patients was 27 months.

Prevalence and details of CA detected by CG

Among the 150 newly diagnosed MM patients, CA were detected by CG (CG_CA) in 44 patients (29.3%; Table 2). The prevalence of CA increased with progressing disease stage, being 14.3%, 29.6% and 31.2% in the patients with Durie–Salmon stage I, II and III diseases, respectively. Of the 44 patients with CG_CA, 19 (43.2%) showed hyperdiploidy and 25 (56.8%) non-hyperdiploidy; the latter included 11 (25%) hypodiploidy, 11 (25%) pseudodiploidy and three (6.8%) tri-/tetraploidy (Table 2).

A total of five patients had numeric abnormalities only, nine had structural abnormalities only and the remaining 30 patients exhibited both numeric and structural abnormalities. Complex CA were found in 35 patients (79.5%) with a median of 12 changes per patient (range four to 24). Common numerical changes were trisomies 9 (+ 9, 18/44 or 40.9%), 15 (34.1%), 3 (25%), 7 (25%) and 19 (25%), and the monosomy 13 (−13; 31.8%). At least one trisomy was found in 24 patients (24/44; 54.5%); trisomies tended to occur in clusters, and 18 of these 24 patients had multiple trisomies (more than three trisomies) (Table 2). The incidence of multiple trisomies was higher in hyperdiploid than non-hyperdiploid MM (84.2%...
Table 2. Cytogenetic data from 150 multiple myeloma patients

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Number of patients</th>
<th>Incidence in patients with CA (%)</th>
<th>Incidence in MM patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal</td>
<td>44</td>
<td>100</td>
<td>29.3</td>
</tr>
<tr>
<td>Hyperdiploidy</td>
<td>19</td>
<td>43.2</td>
<td>12.7</td>
</tr>
<tr>
<td>Hypodiploidy</td>
<td>11</td>
<td>25.0</td>
<td>7.4</td>
</tr>
<tr>
<td>Pseudodiploidy</td>
<td>11</td>
<td>25.0</td>
<td>7.4</td>
</tr>
<tr>
<td>Tri-tetraploidy</td>
<td>3</td>
<td>6.8</td>
<td>2.0</td>
</tr>
<tr>
<td>Multiple trisomies (≥3)</td>
<td>18</td>
<td>40.9</td>
<td>12.0</td>
</tr>
<tr>
<td>−13/13q14</td>
<td>19</td>
<td>43.2</td>
<td>12.7</td>
</tr>
<tr>
<td>1q duplication</td>
<td>16</td>
<td>36.4</td>
<td>10.7</td>
</tr>
<tr>
<td>14q32</td>
<td>13</td>
<td>29.5</td>
<td>8.7</td>
</tr>
<tr>
<td>1p−</td>
<td>12</td>
<td>27.3</td>
<td>8.0</td>
</tr>
<tr>
<td>11q13 or 23</td>
<td>12</td>
<td>27.3</td>
<td>8.0</td>
</tr>
<tr>
<td>−17/17p−</td>
<td>8c</td>
<td>18.2</td>
<td>5.3</td>
</tr>
<tr>
<td>8q24</td>
<td>7</td>
<td>15.9</td>
<td>4.7</td>
</tr>
<tr>
<td>6q−</td>
<td>7</td>
<td>15.9</td>
<td>4.7</td>
</tr>
</tbody>
</table>

*aWith a median of seven trisomies (range three to 11); 13 patients had four or more trisomies.

*bIncluding 14 patients with −13 and five with deletion of long arm of chromosome 13 involving band q14; 10 of them had simultaneously 1q duplication, seven each had 14q32 rearrangements and 1p− and six each had 11q13 or 23 rearrangements and −17/17p−.

*cIncluding four patients with −17 and four with 17p−.

CA, chromosome abnormality; MM, multiple myeloma.

versus 8.0%, \( P<0.001 \). The most frequent structural aberrations were duplications of 1q (16/44, 36.4%), followed by rearrangements involving 14q32 (29.5%), deletions of 1p (1p−, 27.3%), rearrangements involving 11q13 (15.9%) or 11q23 (11.4%), abnormalities involving 8q24 (15.9%) and interstitial deletions of 6q (15.9%). CA involving chromosome regions where immunoglobulin light-chain genes were located, 2p11 (κ) and 22q11 (λ), were noted in only one patient (2.3%) each.

CA of chromosome 13 were observed in 23 patients, including −13 in 14 patients and partial deletion involving 13q14 in five patients; thus, a total of 19 patients showed loss of chromosome 13q14 (Table 2).

With regards to abnormalities usually accompanied with other structural aberrations, 10 of the 19 patients had simultaneous 1q duplication, seven each had 14q32 rearrangements and 1p−, and six each had 11q rearrangements and −17/17p13−. Interestingly, the incidence of −13 or partial deletion of 13q (Δ13) detected by the CG (CG_Δ13) was more common in the non-hyperdiploid MM than in the hyperdiploid MM (56% versus 26.3%; \( P=0.049 \)). 14q32 rearrangements detected by the CG (CG_14q32) were observed in 13 patients (13/44; 29.5%), including four with t(11;14)(q13;32), one with t(6;14)(p21;q32) and eight with add(14)(q32), in which the translocation partners of 14q32 could not be identified. Similar to the CG_Δ13, the incidence of CG_14q32 was also higher in the non-hyperdiploid MM than in the hyperdiploid MM (40% versus 15.8%; \( P=0.081 \)).

FISH is more sensitive than CG in detecting specific CA

Data from the comparisons between CG and FISH in the detection of specific CA in the 88 patients who had both techniques simultaneously performed is shown in Table 3. CG_CA were noted in 23 patients (26.1%) and CA detected by FISH (FISH_CA) were noted in 59 patients (67%). FISH_CA from three representative patients are shown in Figure 1. Interestingly, 40 of the 65 patients who did not have CG_CA showed FISH_CA. Deletion of 13q14 on FISH analysis (FISH_Δ13) occurred in 30 patients. Notably, the FISH_Δ13 was all monoallelic and present in all but one patient with CG_Δ13.

Biolclinical features of patients with the CA

The salient characteristics in the patients with CG_CA and those with normal karyotypes were compared and the results are presented in Table 1. Significantly, the patients with CG_CA had more plasma cells in the BM, lower PLA levels and higher incidence of extramedullary myeloma (extra-MM) at diagnosis.

Among the 44 patients with CG_CA, the patients with CG_Δ13 had lower Hb and PLA levels in comparison to other patients in this group (median 7.2 versus 9 g/dl, \( P=0.010 \); and 1.2 \( \times \)10^11/l versus 1.7 \( \times \)10^11/l, \( P=0.048 \), respectively). The incidence of this abnormality was higher in patients with a λ subtype MM than in those with a κ subtype (83.3% versus 12%; \( P<0.001 \)). Patients with CA involving 8q also had lower platelet levels but higher calcium levels in comparison with patients without this CA (median 0.9 \( \times \)10^11/l versus 1.6 \( \times \)10^11/l, \( P=0.007 \); and 2.8 versus 2.4 μmol/l, \( P=0.035 \), respectively). Patients with CA involving 1p were more likely to have soft tissue plasmacytomas in comparison with patients without this CA (38.9% versus 7.7%; \( P=0.021 \)). Interestingly, all four patients with 19p13 rearrangements, add(19)(p13),

Table 3. Comparison of CG and FISH data

<table>
<thead>
<tr>
<th>Chromosome abnormality</th>
<th>No. of patients with the abnormality (%)</th>
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<tbody>
<tr>
<td></td>
<td>CG (26.1%)</td>
</tr>
<tr>
<td></td>
<td>FISH (67.0%)</td>
</tr>
<tr>
<td>del(13q14)(a)</td>
<td>8 (9.1)</td>
</tr>
<tr>
<td></td>
<td>30 (34.1)</td>
</tr>
<tr>
<td>del(17p13)(b)</td>
<td>3 (3.4)</td>
</tr>
<tr>
<td></td>
<td>6 (6.8)</td>
</tr>
<tr>
<td>+3</td>
<td>8 (9.1)</td>
</tr>
<tr>
<td></td>
<td>23 (26.1)</td>
</tr>
<tr>
<td>+7</td>
<td>6 (6.8)</td>
</tr>
<tr>
<td></td>
<td>19 (21.6)</td>
</tr>
<tr>
<td>+11</td>
<td>5 (5.7)</td>
</tr>
<tr>
<td></td>
<td>18 (20.5)</td>
</tr>
<tr>
<td>+18</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td></td>
<td>14 (15.9)</td>
</tr>
</tbody>
</table>

\(a\)Including −13/13q14−.

\(b\)Including −17/17p13−.

CG, conventional cytogenetics; FISH, fluorescence in situ hybridization.
Axioplan, Zeiss, Germany).

Chromosome 11 (LPE011R) and two green signals for the centromeres of chromosome 18 showing two red signals for the centromeres of chromosome 11 from a MM patient without abnormality of chromosomes 11 and 18. Chromosome 10 but only one red signal for 13q14. (LSIRB1). The surrounding non-plasma cells do not have blue cytoplasm staining. (A) A plasma cell from a normal control showing two green signals for the centromeres of chromosome 10 (LPE010G) and two red signals for 13q14 (LSIRB1). The plasma cell was distinguishable by the intense blue fluorescence of the cytoplasm. (B) A plasma cell from a multiple myeloma (MM) patient with monosomy 13/partial deletion of chromosome 13 showing two green signals for the centromeres of chromosome 10 but only one red signal for 13q14. (C) A plasma cell from a MM patient without abnormality of chromosomes 11 and 18 showing two red signals for the centromeres of chromosome 11 (LPE011R) and two green signals for the centromeres of chromosome 18 (LPE018G). (D) A plasma cell from a MM patient with trisomy 11 showing two green signals for chromosome 18 but three red signals for chromosome 11 (×600 magnification; fluorescence microscope from Axioplan, Zeiss, Germany).

were males. The add(19)(p13) was the sole chromosome abnormality in one patient. These patients all had monoclonal immunoglobulin of IgG isotype and showed extra-MM at diagnosis, compared with 15% and 27.5%, respectively, in those patients with non-add(19)(p13) CA in this study (P = 0.037 and 0.01, respectively).

Of the 88 patients who underwent FISH analysis, the differences of the bioclinical characteristics between the patients with the specific FISH_CA and those without were also compared. Patients with FISH_Δ13 had a higher incidence of extra-MM (P = 0.02) at diagnosis. Additionally, the incidence of trisomy 11 in patients associated with IgG MM (P = 0.042).

**Association between CG_CA and clinical outcome**

Notably, the patients with CG_CA had a significantly shorter median OS than the patients with normal karyotypes (18 ± 4.2 versus 28 ± 5.1 months; P = 0.029) (Figure 2A). In univariate analysis, other clinical and laboratory variables that were associated with shorter OS were stage III disease (P = 0.009), BM plasmacytosis ≥30% (P = 0.006), Hb <10 g/dl (P = 0.006), PLA <1.5 × 10¹¹/l (P < 0.001), LDH ≥465 IU/l (P = 0.032), Cr ≥2 mg/dl (P < 0.001), CRP ≥4 mg/dl (P = 0.006) and β₂M ≥2.5 mg/l (P = 0.012). In multivariate analysis, only the CG_CA [relative risk (RR) of death 1.9; 95% confidence interval (CI) 1.2–3.2; P = 0.009], PLA <1.5 × 10¹¹/l (RR of death 2.1; 95% CI 1.3–3.4; P = 0.001) and Cr ≥2 mg/dl (RR of death 2.5; 95% CI 1.5–4; P < 0.001) were the independent prognostic factors for OS. CG_CA remained as an independent prognostic factor on OS only if the patients who had received chemotherapy were analyzed and the response to chemotherapy, as a bimodal covariate (responders versus non-responders), was also included in the multivariate analysis (data not shown).

Among the 44 patients with the CG_CA, the median OS for patients with hyperdiploidy, pseudodiploidy, hypodiploidy and tri-/tetraploidy was 25 ± 12.8, 18 ± 8.1, 9 ± 5.2 and 7 ± 0 months, respectively (P = 0.05). Patients with hyperdiploidy MM had a significantly longer median survival in comparison with non-hyperdiploid MM patients (25 ± 12.8 versus 12 ± 4 months; P = 0.025) (Figure 2B). Furthermore, among the patients with CG_CA, patients with CG_Δ13 had a significantly shorter median survival in comparison with patients without this CA (9 ± 3.5 versus 22 ± 13.5 months; P = 0.047) (Figure 2C). In addition, the four patients with add(19)(p13) were found to have much shorter OS than the patients with other CA (2.5 ± 2.5 versus 18 ± 5.8 months; P = 0.032) (Figure 2D). No other recurrent CG_CA showed a significant impact on OS among patients with CG_CA. To determine the most important factor contributing to shortened survival in patients with CG_CA, the ploidy status (hyperdiploidy versus non-hyperdiploidy), CG_Δ13, add(19)(p13), as well as IgA isotype, PLA <1.5 × 10¹¹/l and Cr ≥2 mg/dl that were associated with shorter OS by univariate analysis, were subsequently tested by multivariate analysis. Our data showed that only add(19)(p13), Cr ≥2 mg/dl and PLA <1.5 × 10¹¹/l were independent prognostic factors, and the associated RRs of death were 7.2 (95% CI 2–25.8), 3.4 (95% CI 1.3–8.7) and 2.9 (95% CI 1.3–6.5), respectively (P = 0.002, 0.011 and 0.012, respectively).

**Association between FISH_CA and clinical outcome**

Among the 88 patients who had undertaken the CG and FISH analysis simultaneously, patients with FISH_Δ13 had a shorter OS in comparison with patients without this CA (13 ± 2.2 versus 32 ± 9 months; P = 0.014) (Figure 2E). FISH_Δ13, BM plasmacytosis ≥30%, LDH ≥465 IU/l, Cr ≥2 mg/dl, CRP ≥4 mg/dl and response to chemotherapy chosen from univariate analysis were tested in subsequent multivariate analysis. The CA of FISH_Δ13 was the best independent predictor of a shorter OS (RR of death 3.7; 95% CI 2–7; P < 0.001). Another prognostic factor that remained significant in the multivariate analysis was Cr ≥2 mg/dl (RR of death 2.6; 95% CI 1.4–4.9; P = 0.004). Interestingly, significant differences in OS could also be demonstrated among the three groups of patients with
Figure 2. Kaplan–Meier survival curves of the multiple myeloma (MM) patients showing (A) the overall survival in the patients with normal karyotypes is longer than those with chromosomal abnormalities (CA) detected by conventional cytogenetics (CG_CA); (B) among the 44 patients with CG_CA, the overall survival in the hyperdiploid group is longer than in the non-hyperdiploid group; (C) patients with CA other than CA_D13 had better prognosis in comparison with patients with CA_D13; (D) the patients with CA other than add(19)(p13) had better overall survival in comparison to patients with add(19)(p13); (E) in the subgroup analysis of 88 patients who had undertaken CG and FISH analyses simultaneously, the overall survival of patients without FISH_D13 was longer than that of patients with FISH_D13; (F) significant differences in survival were noted between patients with CG_D13, patients with FISH_D13 only but without CG_D13, and other patients without CG_D13 or FISH_D13.
CGΔ13, FISHΔ13 only without CGΔ13, and other karyotypes with neither CGΔ13 nor FISHΔ13 (9 ± 7.8, 15 ± 2.8 and 32 ± 8.9 months, respectively; P = 0.013) (Figure 2F).

Discussion

The prevalence of CG_CA was 29.3% in the cohort of 150 Chinese patients with newly diagnosed MM. The majority of CG_CA cases showed complex structural and numerical changes, and involved hyperdiploid chromosomes. In general, the incidence of CG_CA and the frequent non-random abnormalities in our study were comparable to those from Western countries [2, 5, 6, 10–12, 20, 22]. In line with the findings that patients with CG_CA had a larger tumor burden, a higher labeling index and Ki-67 growth fraction of PC when compared with the patients without CG_CA [6, 7, 35, 36], we found that the prevalence of CG_CA was higher in stage III than in stage I disease, and the patients with CG_CA had significantly higher BM plasmacytosis, lower levels of platelets and higher incidence of extra-MM than the patients with normal karyotypes.

We demonstrated that the CG_CA in MM was associated with a shorter OS. Although CG_CA was associated with a high proliferative index of PC and advanced tumor stage, it was still an independent prognostic factor on multivariate analysis. In addition, there were distinct differences in clinical and laboratory features between hyperdiploid and non-hyperdiploid MM. The former was more frequently associated with CG_CA of multiple trisomies, whereas the latter was more associated with the CGΔ13 and CG_14q32. Importantly, the median OS was significantly shorter in the patients with non-hyperdiploid MM than in those with hyperdiploid MM (12 versus 25 months), especially in those with hypodiploid (9 months) and tri-/tetraploid chromosomes (7 months), which were considered as the same diploidy status [12]. Neither CGΔ13 nor CG_14q32 could be used to further separate non-hyperdiploidy MM into different prognostic categories. These findings are similar to reports from Western countries [10–12], and suggest that the two distinct groups of MM, delineated by the chromosome ploidy and patterns of CG_CA, truly exist without ethnic or geographical variations. However, the pathogenesis and mechanism for the survival differences between the two groups are still obscure. Unfortunately, further studies are partly hampered by lack of suitable cell lines derived from the hyperdiploid MM [11]. We recently developed a new SCID mouse/human chimeric animal model, which can sustain the viability and growth of primary MM cells and might be beneficial in facilitating further studies [37].

Owing to the low proliferative index of PC, CG used alone underestimates the prevalence of CA in MM [2, 11]. FISH enables identification of CA without requiring metaphases within the PC. However, FISH can only provide information for specific target regions, which is of limited value in the interpretation of highly complex karyotypes in MM. In contrast, CG gives a complete overview of CA present in the malignant clone. Therefore, combining the two methods is particularly useful in the analysis of clinically relevant CA in MM. In this study, patients with FISHΔ13 had a shorter median survival than those without this CA, and FISHΔ13 remained the best independent prognostic factor on the multivariate analysis. Furthermore, there was a significant difference in the OS among patients with CGΔ13, patients with FISHΔ13 but without CGΔ13, and patients with neither CGΔ13 nor FISHΔ13 (median 9 versus 15 versus 32 months, respectively; P = 0.013). It is likely that the net effect of Δ13 on prognosis is greater when Δ13 is detected by CG than when it is detected by FISH [21, 36]. This is because of the additive effects of a large tumor burden and a high proliferation rate of tumor cells, which usually accompany CG_CA, on the prognosis [11].

To the best of our knowledge, this is the first report to show that MM with add(19)(p13) is a distinct cytogenetic entity that is associated with the male gender, IgG/λ isotype, presence of extra-MM and a poor prognosis. To further support the notion, two other previously treated MM patients, not included in this study, who had complex chromosome changes with add(19)(p13) at the time of referral from other hospitals were also male, had IgG/λ isotype and showed extra-MM at that time (personal communication).

From a review of available literature, non-random chromosomal rearrangements of 19p13 have been reported in four of 21 MM patients (19%) from Japan [38]. One of these patients had add(19)(p13) and the other three had translocations involving 19p13. Additionally, all four patients had comparable clinical features to our patients, all were males with extra-MM and two of them had IgG/λ isotypes [38]. Notably, among the patients with CG_CA, the patients with add(19)(p13) were likely to have much shorter OS than the patients without this CA. Rather than the CGΔ13 and non-hyperdiploidy, the presence of add(19)(p13) became the most powerful prognostic factor associated with short OS on multivariate analysis in the patients with CG_CA. However, the prognostic impact of add(19)(p13) in MM needs to be confirmed by further studies involving a larger number of patients. The genetic changes associated with the chromosomal abnormalities remain to be elucidated. Several known genes mapped to the band 19p13.3, such as the E2A gene, which encodes the enhancer-binding protein E12/E47 and is rearranged in most cases of acute lymphoblastic leukemia with translocation t(1;19)(q23;p13.3) [39], and the basigin gene, which is a member of the Ig superfamily and plays a role in intercellular recognition [40], are potential candidates. Recently, a novel cryptic translocation involving 19p13.3 and IgH was found in chronic B-cell lymphocytic leukemia and large B-cell lymphoma [41], which hinted at the involvement of other novel genes.

In summary, CG and FISH analyses identified several chromosomal abnormalities highly associated with the clinical features and prognosis of newly diagnosed MM patients. Combined CG and FISH studies separated MM patients into three groups with significantly different OS; one with CGΔ13,
one with FISH_Δ13 but not CG_Δ13, and the remaining one with neither CG_Δ13 nor FISH_Δ13. The presence of add(19)(p13) as the sole chromosomal abnormality in one patient suggested that this CA plays a role in pathogenesis for a subset of MM patients and demonstrated the existence of a specific cytogenetic entity.

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References

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