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Cytogenetic and interphase fluorescence in-situ hybridization (FISH) analysis of multiple myeloma and its clinical correlation in Taiwan – a preliminary result

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SUMMARY

From June 1987 to October 2003, bone marrow (BM) cells obtained from 160 patients with previously untreated multiple myeloma (MM) were analyzed by conventional cytogenetic (CC) method and 57 of the BM samples were also analyzed by interphase DNA fluorescence in situ hybridization (FISH) using multiple probes specific for chromosome 13q14, 17p13, centromeric 3, 7, 11, and 18. We aimed at (1) studying the frequency of cytogenetic aberrations in our MM patients, (2) investigating the independent significance of CC and FISH results in clinical ground, in terms of response to conventional chemotherapy, duration of response and overall survival (OS).

Among those 160 patients, 109 (68.1%) were men, 51 (31.9%) were women, the median age at diagnosis was 62 years (range, 24 to 88). Fifteen (10.0%) of them were Durie-Salmon stage I, 32 (21.3%) were stage II, 50 (33.3%) were stage IIIa and 53 (35.3%) were stage IIIb. By CC analysis, cytogenetic aberrations were detected in 40 patients (25.6%). Patients with stage IIIb had higher incidence of cytogenetic aberrations than those with stage I disease, 29.4% vs. 13.3%, respectively. Among the 39 patients, complex cytogenetic aberrations occurred in 29 patients (74.4%), and 14 patients (50%) had hyperdiploidy, 6 patients (21.4%) had hypodiploidy, 2 patients (7.2%) had triploidy. The most common numeric change was trisomy 9, followed by

trisomy 15 and monosomy 13. The most common structural change was 1q duplication, followed by 14q32 abnormalities, including three with t(11;14)(q13;q32) and one with t(6;14)(p21;q32). Intriguingly, add (19)(p13) was a non-random chromosome aberration, which occurred in 3 patients who all had extramedullary plasmacytomas at diagnosis. In this study, there was no significant difference in the treatment response rate (65% vs. 62.5%, p=0.846), duration of response (median, 8±4.6 vs. 12±2 months, p=0.276) or OS (median, 11±3.2 vs. 28±6.4 months, p=0.067) between those patients with cytogenetic aberrations and those without. On univariate analysis, chromosome 1q duplication was significantly associated with short OS (median, 9±2.6 vs. 27±5 months, p=0.022), so as partial or complete loss of chromosome 13 and/or abnormalities involving chromosome 11q (12±2.5 months vs. 28±7.1, p=0.028). However, on Cox stepwise regression analysis including conventional prognostic factors and the cytogenetic aberrations, only stage (p=0.003) was a independent prognostic factor for OS.

In the 57 BM samples analyzed by FISH, at least one of the non-random chromosome aberrations were detected in 33 samples (57.9%), including 13q deletion in 30% of samples, 17p deletion in 5.5%, trisomy 3 in 22.2%, trisomy 7 in 24.1%, trisomy 11 in 22.2%, and trisomy 18 in 13%. Of note, FISH had higher sensitivity than CC in detecting chromosome aberrations in MM; for example, 13q & 17p

deletions were found only in 11.3% and 1.9% of the patients respectively, by CC. Although 13q deletion was associated with poor response to conventional chemotherapy (46.7% vs. 72.4%, p=0.092) and short median OS (24±13 vs. 28±10.5 months, p=0.16), these differences were below the level of statistical significance, which was probably resulted from limited number of samples. In this study, trisomy 18 was associated with short duration of response (3 months vs. 14±4 months, p=0.03).

In summary, 25% of our MM patients have cytogenetic aberrations by CC method. FISH revealed higher sensitivity than CC in detecting the non-random aberrations. Chromosome 1q, partial or complete loss of chromosome 13q and/or chromosome 11q abnormalities might be associated with poor clinical outcome in MM, but need to be confirmed by further studies on more patients.

INTRODUCTION

By analyzing conventional cytogenetics (CCs) and, recently, interphase fluorescence *in situ* hybridization (FISH), it is now known that the chromosome aberrations (CAs) are nearly universal in multiple myeloma (MM) [Avet-Loiseau et al, 2002; Boersma-Vreugdenhil et al, 2003; Hallek et al, 1998; Zandecki et al, 1996]. Several non-random CAs were found to have significant impact on the biology and

prognosis of MM. Generally, aneuploidy is ubiquitous [Zandecki et al, 1996], and translocations involving the immunoglobulin heavy-chain (IgH) gene locus in the chromosome 14q32 region have been implicated in the origin of MM [Bergsagel et al, 1996]. Although there are yet consistent changes, some of the recurrent CAs, such as hypodiploidy and chromosome 13 deletion, are also served as independent prognostic predictors for MM patients [Fonseca et al, 2003; Konigsberg et al, 2000; Smadja et al, 2001]. Continuous absence of metaphase-defined chromosome 13 and hypodiploidy could ensue long-term survival in MM patients treated with melphalan-based tandem autotransplantations [Shaughnessy et al, 2003]. Likewise, deletion of 17p13.1, the genomic locus of the *p53* tumor suppressor gene, have been associated with an adverse patient outcome [Drach et al, 1998; Ortega et al, 2003]. The MM patients could also stratified as different risk groups by their CAs and levels of β_2-microglobulin (β_2M) [Konigsberg et al, 2000], and therefore the treatment strategies could be decided. High- risk group patients, with unfavorable CAs and/or β_2M >4 mg/l, could have more favorable outcome from high-dose chemotherapy than from standard-dose chemotherapy [Kaufmann et al, 2003]. In addition, several investigators suggest that the observed clinical heterogeneity among MM patients is also likely to be related to the underlying CAs [Avet-Loiseau et al, 2003; Hallek et al, 1998; Fonseca et al, 1999; Garand et al, 2003]. Analyzing the CAs of MM

nevertheless becomes an important issue to elucidate the pathogenesis, to predict treatment outcome, and possibly to classify distinct groups and design different treatment strategies of MM.

Although there have been many works done on this field, a large-scaled study on CAs of MM outside the Western countries was relatively lack. We report our results on CAs of 160 Chinese patients with newly diagnosed MM by using conventional cytogenetic and 57 of them have FISH analyses simutaneously. The correlation between CAs and clinical presentations, as well as survival is especially focused.

MATERIALS AND METHODS

Patient characteristics and bone marrow samples. From June 1987 to October 2003, 160 newly diagnosed and previously untreated MM patients at our hospital were recruited. Bone marrow (BM) samples were aspirated into heparinized syringes and bone marrow mononuclear cells (BMNCs) were enriched by Ficoll-Hypaque (Lymphocyte Separation Medium; Organon Teknika, Durham, NC) gradient centrifugation. All patients have the conventional karyotype analysis at the time of study entry and 57 patients have the interphase FISH analysis simultaneously. The clinical and demographic characteristics of the patients were listed in Table 1, and the median follow-up for these patients was 20 months (range, 1 to 150 months). Written

informed consents were obtained from all patients and were kept in the records.

Cytogenetics. Conventional G-band cytogenetics were performed on BM samples obtained from all of our patients enrolled as described previously [Huang et al, 1999].

Fluorescence *in situ* hybridization. The fluorescence *in situ* hybridization (FISH) analysis was performed as previously described [Tien et al]. We used several sets of centromeric probes to detect either the deletions or translocations of the chromosome 3, 7, 9, 11, 13, 17 and 18. We also used the 14q32 probe to detect any translocation involved the chromosome region. To improve on the specificity of the scoring process we combined interphase FISH with immune-fluorescence detection of the cytoplasmic light –chain (immuno-FISH). Briefly, For the purpose of this analysis, FISH abnormal required the presence of one signal (heterozygous deletion) in at least 20% of about 200 scored light chain-restricted clonal plasma cells. The threshold value of 20% for definition of chromosome 13 deletion by FISH was selected by adding three standard deviations to the median probe deletion value of normal subjects (median, standard deviation,)

Treatment. One hundred and thirty three patients (86.4%) received chemotherapy with either CP (cyclophosphamide 100mg/m^2 and prednisolone 60mg/m^2 daily orally on days 1~4) or MP (melphalan 9mg/m^2 and prednisolone

60mg/m^2 daily orally on days 1~4) regimen every 4 to 6 weeks until plateau phase was achieved or the disease was refractory to the treatment. Seventy patients (47.3%) with high tumor burden at diagnosis were treated with two to four cycles of combination chemotherapy of VAD at an interval of 4 weeks [vincristine, 0.4mg/m^2, continuous intravenous infusion (CIVF), d1~4; doxorubicin, 9mg/m^2, CIVF, d1~4; dexamethasone, 40mg, CIVF, d1~4 & d8~11] which was followed by either CP or MP. patients did not receive chemotherapy because of their hesitation or poor clinical status. One patient received high-dose therapy with autologous hematopoietic stem cell transplantations (autoHSCT).

Treatment response. The criteria for treatment response were described previously [Huang et al, 1999]. In short, the patients were considered to achieve complete response (**CR**) when M-proteins in serum and urine were absent on immunofixation electropheresis (IFE), plasma cells were less that 5% in BM, soft tissue plasmacytomas disappeared if initially present, and there was no increase of lytic bone lesions for at least 6 weeks. Partial response (**PR**) was defined as more than 50% and 90% reduction in the levels of M protein in serum and urine respectively, more than 50% reduction in the size of soft tissue plasmacytomas if present and no increase of lytic bone lesions, minimal response (**MR**) as 25~49% and 50~89% reduction in the levels of the M protein in serum and urine respectively, 25~49%

reduction in the size of soft tissue plasmacytomas if present and no increase in the size or number of lytic bone lesions, and progressive disease (**PD**) as an increase of 25% in the serum or urine M protein levels after two cycles of the initial chemotherapy, more than 25% increase of the plasma cells in the BM, increase in the size of existing bone lesions or soft tissue plasmacytomas, development of new bone lesions or soft tissue plasmacytomas, or development of hypercalcemia not attributable to any other cause. No response (**NR**) was defined as a response that did not meet the criteria of either MR or PD. Relapse was indicated by the presence of one or more of the following findings at two successive evaluations: an increase of 25% in the serum or urinary M protein level above the nadir, reappearance of the M protein in serum or urine by IFE, and an increase in the size or number of lytic bone lesions or plasmacytomas if existed. Progression-free survival (**PS**) was defined as the period from the initial attainment of MR in patients finally achieving CR, PR or MR to the time of relapse. Overall survival (**OS**) was defined as the time period from the date of diagnosis to the date of death for any reasons.

Statistical analysis. The Chi-square or Fisher's exact tests were used for between-group (with specific abnormalities vs. without specific abnormalities) comparison of the discrete variables. The two-sample *t* test was used for between-group comparison of the means. The Kaplan-Meier survival curves were

used for estimation of PS and OS. The log-rank rest was used to test for differences in survival between groups. Several salient clinical and laboratory variables at the time of study entry, including age, disease stage, platelet count, and levels of lactate dehydrogenase (LDH), alkaline phosphatase (ALP), creatinine, c-reactive protein (CRP), β_2-microglobulin (β_2m) were assessed to determine possible association with cytogenetic aberrations or FISH abnormalities tested. Variables determined statistically significant from univariate analysis of this response were subsequently tested using multivariate logistic regression. All directional *p* values were two-tailed, with a *p* value of 0.05 or less considered significant for all tests. All analyses were performed using SPSS (Version 8.0) software.

RESULTS

Prevalence of cytogenetic aberrations and its clinical characters.

By CCs analysis, CAs were observed in 40 patients (25.6%). Normal karyotype and no metaphases of the cytogenetics were observed in 95 (60.9%) and 21 (13.5%) patients respectively. The salient characteristics between the patients who had CAs and those who had normal or no metaphases were also compared in Table 1.

According to the chromosome number: were hypodiploid, were pseudodiploid, were hyperdiploid, and were near-tetraploid. The distribution of total chromosome

number among patients with CAs was shown in Figure 1.

Among these CAs, there were numeric and structural abnormalities respectively, which were listed in Table 2. The most common numerical changes were .Trisomies present in at least 10% of patients involved chromosomes, 3, 5, 7, 9, 11, 15, 19 and 21. Monosomies and deletions, usually of the q arm, affected predominantly chromosome 6, 13, 16, and 22. CAs at diagnosis, also had del(13) and had any abnormalities of chromosome 13, including del(13), del(13q), and t(13q).

Structural anomalies of chromosome 1 were most frequent that of patients had duplications in the q-arm and had translocations involving the p-arm. Other noteworthy structural anomalies included deletion of chromosome 6q in of patients and translocations involving 6q in of patients. Translocation involving 14q32 (IgH locus) were observed in of patients. Translocation involving 16q were observed in of patients. The t(11;14)(q13;q32) was observed in 3 () patients. Structural chromosomal anomalies were significantly more common among patients with the hypodiploid form of MM.

Correlation between the abnormalities and the clinical outcome.

Among patients evaluable for response, those with detected by either CC and/or FISH had a lower likelihood of an objective response than those without the abnormality. Otherwise there were no major differences noted.

Prognostic features of the chromosome abnormalities. (Analyzing…….Figure 1)

DISCUSSION

Based on CCs analysis, the MM patients in this study could be divided into three groups of normal karyotype (60.9%), abnormal karyotypes (25.6%), and no mitosis (13.5%), which would possibly correlate with their clinical outcomes. It is known that CAs detected by such traditional technique can be found in 18% to 35% of MM patients at diagnosis, 40% to 60% with aggressive disease and up to 85% of patients with plasma cell leukemia. Clearly, the potential of conventional cytogenetic studies to detect an abnormal clone in myeloma is associated with aggressiveness of disease [Rajkumar et al, 1999; Sawyer et al, 2001].

Interestingly, in of our patients who underwent simultaneously conventional cytogenetic and FISH analysis, not unexpectedly the FISH was more sensitive than conventional cytogenetics in detection of CAs if any, for some CAs in MM are cryptic to CCs, while other CAs in MM are subtle and easily overlooked by CCs. However, it is quite expensive to perform the global survey of CAs by using FISH, not only the probes but also high technique level. Therefore, it is quite important to select the most clinical relevant probe, like chromosome 13q.

We also find subgroups of MM patients classified according to their underlying

CAs if any and show that these abnormalities alone can establish prognostic categories. At first, the negative effect on prognosis of the hypodiploid state was found in this study, which was similar to others [Debes-Marun et al, 2003; Smadja et al, 2001]. On the contrary, the hyperdiploid variant of MM, and trisomies, are associated with a favorable prognosis compared to those with hypodiploid myeloma. It was known that even among patients with abnormal karyotypes, specific CAs can impart biologic variability in MM [Debes-Marun et al, 2003]. MM patients with the t(4;14)(p16;q32), t(14;16)(q32;q23), and deletion of 17p13.1 have been reported to have worse prognosis than the ones with other CAs [Fonseca et al].

Clustering of those CAs was also demonstrated in this study, similar as others [Smadja et al, 2003], 14q32 translocations in this study is found to be a very rare event in hyperdiploid cases. Whether the CAs demonstrated in this study were primary or secondary cytogenetic changes were unclear

Interestingly, one of the recurrent CAs, 19P was found to correlate with formation of extramedullary plasmacytomas in our patients, which seemed to be a novel finding. and certain specific CAs are known to be associated with adverse outcome.

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Figure 1