Characteristics and prognostic value of extramedullary chromosomal abnormalities in extramedullary myeloma

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To the Editor: Multiple myeloma (MM), the second most common hematological malignancy, is caused by clonal proliferation of plasma cells and presents characteristics including anemia, bone pain, renal insufficiency, and hypercalcemia. The adhesion of myeloma cells to the bone marrow (BM) microenvironment results in the mainly intramedullary involvement of MM, and sometimes, however, plasma cells can disseminate and infiltrate into extramedullary (EM) sites, called extramedullary disease (EMD). The prevalence of EMD in MM is 6% to 8% at initial diagnosis and can be as high as 10% to 30% during disease progression/relapse.[1] An increasing incidence of EMD has been reported over the decades, possibly owing to advances in imaging as well as the growing secondary EMD resulting from prolonged survival. EMD is a highly aggressive disease entity with a poor response to treatment even in the era of novel agents; hence the exploration of pathogenesis and characteristics of extramedullary myeloma (EMM) is of significance.[2]

The EM invasion of MM cells reflects the altered biology of MM cells, particularly the ability to grow independently of the BM microenvironment. These alterations may be triggered by genomic instability. Previous studies revealed that *P53* deletion, *RAS* mutation, and *FAK* upregulation may account for the EM migration of MM cells.[2] Since cytogenetic aberrations are relevant to the biological nature of tumor cells, they will ultimately determine the clinical outcome of MM. The revised International Staging System (R-ISS) and Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART), both widely used for prognostic categorizing in MM, take cytogenetic aberrations as predictive markers for prognosis. Nevertheless, current studies on EMM mainly investigated the chromosomal abnormalities in BM rather than EM lesions, and little is known about the cytogenetic differences between medullary and EM lesions. Accordingly, we analyzed the cytogenetic characteristics of lesions from different locations and of different types in patients with EMM and further explored the role of cytogenetic aberration in the pathogenesis and prognosis of EMM in this work. To the best of our knowledge, this is one of the most extensive studies comparing chromosomal abnormalities on paired samples in EMM.

To reveal the chromosomal abnormalities of EMD, 1q21 gain/amplification, *P53* deletion, and immunoglobulin heavy chain (*IgH)* gene translocation were detected by fluorescence *in situ* hybridization (FISH) in paired samples of BM and EM lesions from EMM patients. A total of 30 patients diagnosed with EMM from May 2017 to November 2020 in the First Affiliated Hospital of Nanjing Medical University were enrolled, and this study was conducted in accordance with the *Declaration of Helsinki* and was approved by the institutional review boards of the First Affiliated Hospital of Nanjing Medical University Ethics Committee (No. 2020-SR-589). Informed consent was obtained from all patients before enrollment. In all cases, standard tissue FISH was performed on paraffin-embedded sections as follows: paraffin-embedded sections were dewaxed, rehydrated, heat-treated, and digested in pepsin solution, followed by incubating with respective FISH probes (Vysis 1q21 *CKS1B* SpectrumOrange/1p32 *CDKN2C* SpectrumGreen FISH Probe Kit, Vysis *P53*/CEP17 FISH Probe Kit, LSI *IGH* Dual Color Break Apart Rearrangement Probe, Vysis LSI *IGH/FGFR3* Dual Color Dual Fusion Probes, Vysis LSI *IGH/CCND1* Dual Color Dual Fusion Probes and Vysis LSI *IGH/MAF* Dual Color Dual Fusion Probes [all purchased from Abbott Molecular Inc., IL, USA], *MAFB/IGH* Fusion Probes [purchased from Guangzhou LBP Medicine Science & Technology Co., Ltd., Guangzhou, China]) in the hybridization oven overnight. Chromosomal abnormalities were

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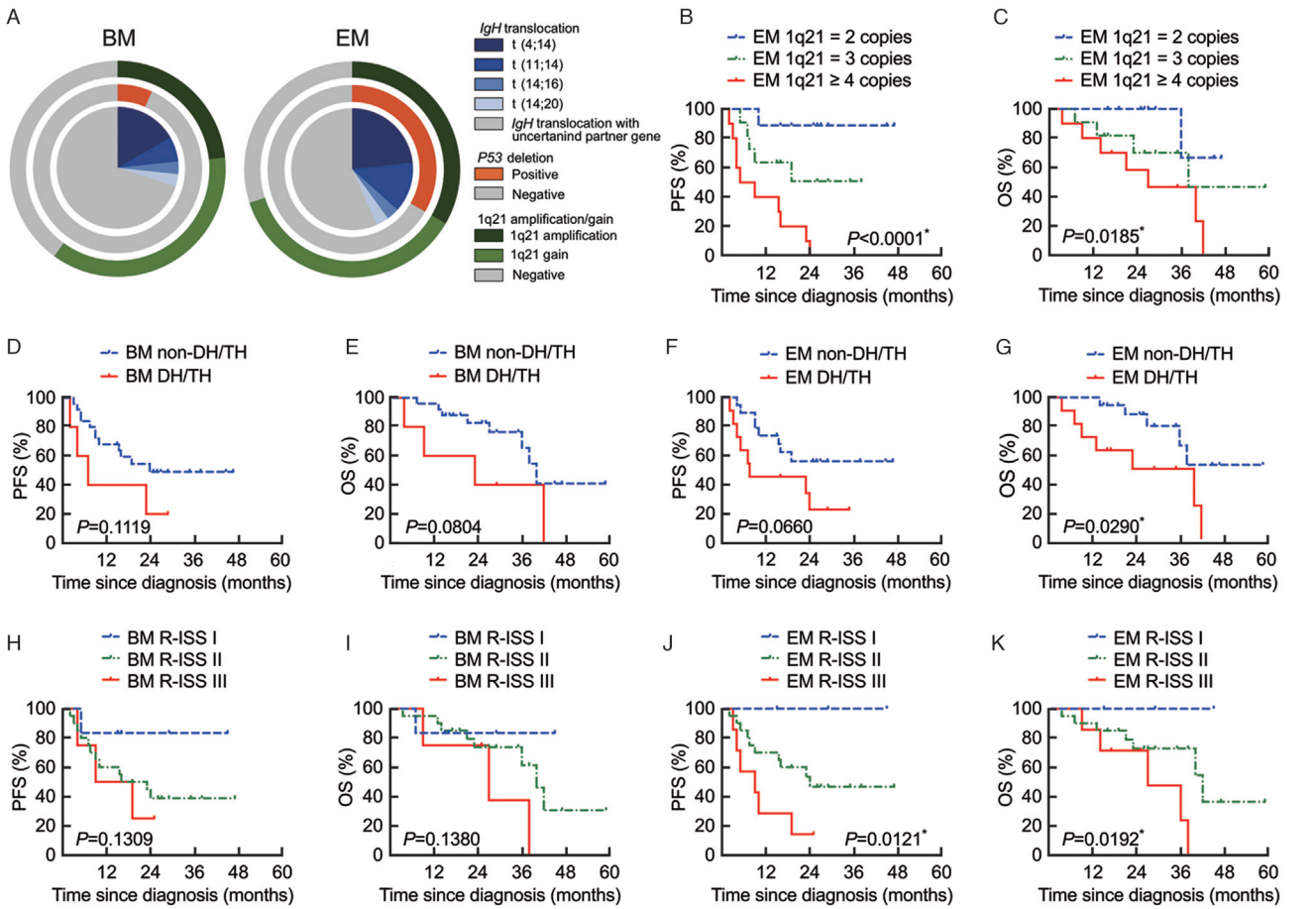

Figure 1: Incidence and prognostic impact of medullary and EM chromosome abnormalities in patients with EM myeloma. (A) Incidence of chromosomal abnormalities in BM and EM lesions. Pie chart depicts the proportional incidence of 1q21 amplification (outer donut), *P53* deletion (interim donut), and *IgH* translocation (inner pie). (B, C) Kaplan-Meier survival curves for PFS (B) and OS (C) regarding different EM 1q21 copy numbers. (D, E) Kaplan-Meier survival curves for PFS (D) and OS (E) of DH/TH and non-DH/TH patients based on medullary chromosomal abnormalities in BM lesions. (F, G) Kaplan-Meier survival curves for PFS (F) and OS (G) of DH/TH and non-DH/TH patients based on EM chromosomal abnormalities in EM lesions. (H, I) Kaplan-Meier survival curves for PFS (H) and OS (I) regarding different R-ISS stages stratified by medullary chromosomal abnormalities in BM lesions. (J, K) Kaplan-Meier survival curves for PFS (J) and OS (K) regarding different R-ISS stages stratified by medullary chromosomal abnormalities in EM lesions. $^{*}P < 0.05$. BM: Bone marrow; DH: Double-hit; EM: Extramedullary; *IgH*: Immunoglobulin heavy chain gene; OS: Overall survival; PFS: Progression-free survival; R-ISS: Revised International Staging System; TH: Triple-hit.

identified by counting 100 non-overlapping tumor cells. The cut-off used for *IgH* break apart or translocation was 10%, and 30% for deletion or gain/amplification. The presence of three copies of 1q21 was defined as 1q21 gain, while the presence of four or more copies was 1q21 amplification.

Among the 30 patients, including 13 males and 17 females, with a median age of 56 years (ranged from 39–78 years), 26 patients were diagnosed with EMD at the onset of MM, and four patients developed EMD during progression. FISH analysis showed a higher frequency of chromosomal abnormalities in EM sites than BM (93.33% *vs.* 70.00%, $P = 0.0195$). It is noteworthy that 13 patients presented additional abnormalities in EM lesions compared with their BM counterparts, of which 11 patients had one additional abnormality (one with 1q21 amplification, seven with *P53* deletion, and three with *IgH* translocation), and two patients possessed two additional aberrations (both got 1q21 amplification, accompanied with *P53* deletion or *IgH* translocation). As a result, the incidence of 1q21 amplification (33.33% [10/30] *vs.* 23.33% [7/30], $P = 0.3901$), *P53* deletion (33.33% [10] *vs.* 6.67% [2], $P = 0.0098$), and *IgH* translocation (43.33% [13] *vs.* 30.00% [9], $P = 0.2839$) in EM lesions was higher than those in BM [Figure 1A and Supplementary Table 1, http://links.lww.com/CM9/B258].

Clinically, EMD is divided into two categories. One is extramedullary-bone associated (EM-B), in which plasma cells in BM extend into contiguous soft tissues via disruption of cortical bones. The other one is extramedullary-extraosseous (EM-E), referring to extraosseous soft tissue masses resulting from hematogenous dissemination and involves almost all systems throughout the body, with an even worse outcome than EM-B.[2] To find out the cytogenetic differences between EM-B and EM-E lesions, we compared the chromosomal abnormalities according to the type of lesions (19 with EM-B and 11 with EM-E). It tended to show a higher incidence of 1q21 amplification (45.45% [5] *vs.* 26.32% [5], $P = 0.4253$) in EM-E than those in EM-B, yet no statistical differences were reached. The incidences of *P53* deletion (27.27% [3] *vs.* 36.84% [7], $P = 0.7020$) and *IgH* translocation (45.45% [5] *vs.* 42.11% [8], $P = 1.0000$) were similar in the two groups [Supplementary Table 2, http://links.lww.com/CM9/B258].

Chromosomal abnormalities are common in MM and abundant studies have revealed the critical role of *P53*, 1q21, and *IgH* in the outcome of MM as well as EMM. Since little is known about the prognostic implications of EM chromosomal abnormalities in EMM, we followed up all the patients with a median follow-up time of 35 months (ranged from 3.5–59 months) for survival analysis. We have noticed that the increase of 1q21 copy number detected in EM lesions retained negative effects on both progression-free survival (PFS) ($P < 0.0001$) and overall survival (OS) ($P = 0.0185$) [Figures 1B and 1C]. There seemed to be a trend for worse outcome of patients with EM *P53* deletion, but the differences were not statistically significant. No significant differences were found in survival between patients with and without EM *IgH* translocation [Supplementary Figure 1, http://links.lww.com/CM9/B258].

Aberration of chromosome 1q21 is the most frequent chromosomal abnormalities in our cohort. Previous studies showed that 1q21 gain and amplification account for approximately 35% and 10% in MM, respectively, both of which suggest a poor outcome, especially for 1q21 amplification.[3] One of our previous studies discovered that 1q21 amplification occurs more frequently in EMM patients.[4] In the current work, the frequency of 1q21 amplification is higher in EM lesions than that in BM and higher in EM-E than EM-B. Therefore, it is possible that 1q21 amplification is a contributing factor to EMD development. We also noted that the increase of EM 1q21 copy number negatively affected both PFS and OS, reaffirming the predictive value of EM chromosomal abnormalities in EMM patients. The deletion of the *P53* gene, which is located at chromosome 17p13 and plays a critical role in suppressing tumorigenesis, occurs in about 7% of MM patients and indicates a high risk of progression and death. It has been reported that EMM patients are more likely to have *P53* deletion than those without EMD, and that *P53* deletion may dampen the adhesion of MM cells to the BM stroma and augment the invasion of tumor cells.[2,5] In the present study, we have observed a statistically higher incidence of *P53* deletion in EM lesions as compared with their BM counterparts in EMM, further illustrating a potential role of *P53* deletion in facilitating the EM invasion of myeloma cells. The *IgH* gene is on chromosome 14q32, and the translocation of *IgH* is observed in about half of MM patients, frequently involving the genes of 11q13 (*CCND1*), 4p16 (*MMSET/FGFR3*), 16q23 (*MAF*), and 20q11 (*MAFB*).[2] Notably, although *IgH* translocations are mainly considered to be the initial or primary genetic events in MM, it accumulates with disease progression. The higher percentage of *IgH* translocation in EM lesions compared to BM suggests the acquisition of mutations during disease progression. These acquired chromosomal abnormalities may alter the biological properties of myeloma cells, thereby driving their migration from the BM to EM organs, where EMD occurs.

To further investigate the prognostic value of EM cytogenetics, we introduced EM chromosomal abnormalities into the existing R-ISS and mSMART systems. According to the latest mSMART, the presence of any two high-risk factors involving t (4;14), t (14;16), t (14;20), del (17p), and gain (1q) is double-hit (DH) myeloma, whereas the presence of three or more high-risk factors is triple-hit (TH) myeloma. While dividing patients according to the presence of DH/TH based on intra- or EM chromosomal abnormalities, DH/TH tended to confer a worse survival, yet no statistical differences were reached between groups except for OS based on EM chromosomal abnormalities ($P = 0.0290$) [Figures 1D–1G]. When it comes to the R-ISS system, we were excited to notice that R-ISS stages based on EM chromosomal abnormalities demonstrating a robust discrimination for the prognosis of EMM ($P = 0.0121$ for PFS and $P = 0.0192$ for OS), whereas no significant differences were found between stages based on medullary cytogenetics [Figures 1H–1K]. Therefore, for EMM patients, EM chromosomal abnormalities showed significant predictive implications, possibly due to the direct regulation of tumor cells by cytogenetic alterations in EM plasmacytomas.

In conclusion, this study demonstrates EM lesions harbored more chromosomal abnormalities than their BM counterparts in patients with EMM and the EM chromosomal abnormalities possess significant prognostic implications. The introduction of EM cytogenetics into the existing R-ISS and mSMART systems allows for more accurate risk stratification for EMM.

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Conflicts of interest

None.

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