Primary extramedullary plasmacytoma: similarities with and differences from multiple myeloma revealed by interphase cytogenetics

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ABSTRACT

Primary extramedullary plasmacytoma is an indolent neoplasm that infrequently converts to multiple myeloma. Since cytogenetic data on extramedullary plasmacytoma are lacking, we studied 38 cases of this type of neoplasm by fluorescence *in situ* hybridization. Fourteen cases (37%) contained *IGH* breaks, including six with a t(4;14) translocation. No translocations t(11;14), t(14;16), t(8;14), nor breaks involving *MALT1, BCL6* or *FOXP1* were found. Loss of 13q (40%), as well as chromosomal gains (82%) were common. There was no correlation between chromosomal alterations and clinical features or local relapse. Cytogenetically, extramedullary plasmacytoma and multiple mueloma are closely related. However, the distribution of *IGH* translocation partners, with the notable absence of t(11;14), is different.

Key words: extramedullary plasmacytoma, multiple myeloma, cytogenetics, IGH translocation, fluorescence *in situ* hybridization.

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Introduction

Primary extramedullary plasmacytoma (EMP) is defined as an extraosseous proliferation of neoplastic plasma cells without evidence of bone or bone marrow involvement as evidenced by morphological bone marrow examination and radiographic studies.[1] EMP accounts for about 4% of plasma cell tumors, and more than 80% occur in the upper aerodigestive tract. EMP usually shows an indolent clinical course with good response to local radiotherapy, a tendency to local relapse and infrequent conversion to multiple myeloma (MM).[2-4] Despite these marked differences in clinical presentation and prognosis, infiltrates of EMP are morphologically indistinguishable from MM spreading to extramedullary locations,[5] and there are no well defined parameters predicting which patients with EMP are at risk of developing MM. On the other hand, some authors have speculated that EMP may represent a form of extranodal marginal zone B-cell lymphoma (MALT-type lymphoma) with extreme plasmacytic differentiation.[6]

In recent years, the introduction of interphase fluorescence *in situ* hybridization (FISH) has enabled identification of clinically and prognostically relevant MM subgroups defined by recurrent chromosomal aberrations.[7,8] Fifty to 70% of cases of MM have translocations involving the immunoglobulin heavy chain (*IGH*) gene locus at 14q32. In contrast to most translocations in non-Hodgkin's lymphoma, a large number of different chromosomal partners of 14q32 have been identified in MM, including the t(11;14)(q13;q32) involving the *CCND1* (cyclin D1) locus in 15-25% of patients, and the t(4;14)(p16;q32) involving the fibroblast growth factor receptor 3 (*FGFR3*) locus in 10-15% of cases.[8,9] A frequent numerical aberration is loss of chromosome 13 or parts of its long arm, which occurs in about half of MM patients.[8] Furthermore, about half of MM patients exhibit hyperdiploidy, characterized by non-random chromosomal gains. Based on these data, two main types of MM of prognostic relevance have been defined, namely hyperdiploid cases with lower frequencies of recurrent 14q32 translocations or chromosome 13 abnormalities, and pseudo- or hypodiploid cases with a high incidence of

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recurrent *IGH* translocations and frequent Δ13.[8,10,11] Many of these aberrations can already be identified in monoclonal gammopathy of unknown significance (MGUS), indicating that they represent early events in the pathogenesis of MM.[8,12] In contrast to MGUS, data on cytogenetic alterations in EMP, another possible precursor lesion of MM, are completely lacking. Using interphase FISH on paraffin sections, we studied a series of well-characterized EMP to identify similarities with and differences from MM on the genetic level.

Design and Methods

Patients

Cases with a diagnosis of EMP were retrieved from the archives of the participating institutions. Primary EMP was defined as an extra-osseous, malignant plasma cell proliferation with evidence of light chain restriction, and no radiographic evidence of osteolytic lesions, bone marrow involvement or other features diagnostic of MM at the time of the initial diagnosis or within the following 6 months. The cases were graded according to the criteria proposed by Bartl *et al.*[13] The study was approved by the local ethics committee.

FISH probes

Break-apart probes were used to screen the following chromosomal regions: 14q32 (*IGH*), 8q24 (*C-MYC*), 18q21 (*MALT1*), 3q27 (*BCL6*), and 3p14.1 (*FOXP1*). The LSI *IGH*, LSI *MALT1*, LSI *BCL6* and the LSI *CMYC* Dual Color, Break Apart Rearrangement Probes were obtained from Abbott (Wiesbaden, Germany). The BAC clone for the *FOXP1* region was labeled as described previously.[14] In patients with an *IGH* break, the presence of *IGH* translocations with the partners *CCND1* (11q13), *FGFR3* (4p16), *C-MAF* (16q23) and *C-MYC* (8q24) was studied with the dual color translocation probes LSI *IGH/CCND1*, LSI *IgH/FGFR3* and LSI *IGH/CMAF*, and the LSI *IGH/CMYC*, CEP 8 Tri-Color Probe (Abbott). The D13S319 probe SpectrumOrange™ mapping at 13q14 (RB1) was used to test for 13q deletions. For detection of aneusomy, we employed the LSI D5S23/D5S721, CEP9, CEP15 Multi-Color Probe Panel, SpectrumOrange™ labeled α-satellite probes CEP 1, CEP 3 and CEP 8 and SpectrumGreen™ labeled probe CEP 11 (Abbott).

FISH on paraffin tissue sections and FISH evaluation

Depending on the amount of available tissue, FISH was performed either on single serial paraffin sections or on a tissue microarray. Normal lymphoid tissue, MM and non-Hodgkin's lymphoma samples with known translocations were included as controls. Interphase FISH and signal evaluation was performed as described previously.[15] At least 100 nuclei per sample were analyzed. The cut-offs for all probe sets were determined on ten reactive samples (mean ± 3 standard deviation [SD]). In addition, metaphase spreads, as well as sections of paraffin-embedded cell blocks of the MM cell lines KMS11, KMS18 and OPM2, all carrying a t(4;14) and KMS12, carrying a t(11;14), were used to confirm the specificity of probe hybridization.[15] The threshold of positivity was evaluated for each probe separately on 1,000 nuclei from reactive samples and set at 10.3% (mean ± 3 SD) for *IGH* breaks, and 15.5% for *C-MYC*, *MALT1*, *FOXP1* and *BCL6* breaks. The cut-off values for the translocation dual fusion probes ranged from 10.8 to 20.1%, respectively. Since the use of tissue sections leads to a high background of cells with one 13q14 signal, the cut-off for *RB1* deletions identified with the 13q14 probe was conservatively set at 40%. The threshold for polysomies for chromosomes 1, 3, 5, 8, 9, 11, 15 was set at 12.5% (*Online Supplementary Figure S1*).

Immunohistochemistry

The expression of cyclin D1 (clone SP4, monoclonal rabbit, Lab Vision, Fremont, CA, USA) and cyclin D3 (Novocastra, Newcastle, UK) was investigated as previously described.[15]

Statistical analysis

Fisher's exact test or the χ^2 test was used to identify associations between chromosomal aberrations and clinical features. All tests were two-sided and p values less than 0.05 were considered statistically significant.

Results and Discussion

Of the 41 cases retrieved with a diagnosis of primary EMP, three cases localized in the upper respiratory tract were found to have co-existent MM and were excluded from further analysis. The main clinical findings of the remaining 38 cases are summarized in Table 1. The median age of the 38 patients was 59 years (range, 30-84). Twenty-nine were male and only eight females. In 28 (74%) cases the neoplasm was localized in the upper aerodigestive tract. Detailed clinical follow-up beyond the 6-month period as defined in the inclusion criteria was available for 26 patients, with a median observation time of 41.5 months (range, 8-187 months). None of the patients died of disease-related causes. A single patient showed demineralization of long bones 12 months after the diagnosis of EMP, interpreted as possible progression to MM, but without biopsy confirmation. Seven of 26 (27%) patients suffered local relapses.

The FISH results are summarized in Table 1. Breaks in the 14q32 region were observed in 14/38 (37%) cases of EMP, at a slightly lower rate than in MM. An *IGH/FGFR3* fusion indicating a t(4;14)(p16;q32) translocation was detected in 6/38 (16%) cases. No t(11;14)(q13;q32), t(14;16)(q32;q23) or t(8;14)(q24;q32) translocations were found. One (4%) of 26 evaluable cases showed a break of *C-MYC*, but was negative for the t(8;14) with the *IGH/C-MYC* fusion probe. No breaks were observed with the

Table 1. Clinical and FISH data of the cases of extramedullary plasmacytoma.

	Clinical data			FISH data				
Case	Age	Sex	Localization	Grade	14q32 IGH break	Specific trans-location	Polysomy 1,3,5,8, 9,11 or 15	Del 13q14
1	74	F	lymph node	1	n	n	n	n
2	81	F	lymph node	2	n	n	9, 15	n
3	84	M	pharynx	2	n	n	1, 5, 15	del
4	63	F	oral cavity	1	break	t(4;14)	11, 15	n
5	53	M	pharynx	1	break	n	5, 11	n
6	72	F	skin	1	break	t(4;14)	11, 15	n
7[A]	69	F	pharynx	1	break	t(4;14)	1, 9, 15	del
8	49	F	pharynx	1	break	n	5, 11	n
9	77	M	lymph node	1	n	n	1, 5, 9, 11, 15	del
10[§]	53	M	soft tissues	1	n	n	5	n
11	59	M	tonsil	1	break	t(4;14)	5, 11, 15	n
12[CSH]	57	M	pharynx	1	n	n	5, 15	n
13	45	M	not noted	2	n	n	3,5, 9, 11, 15	del
14[A]	71	F	soft tissues	2	n	n	3,5, 9, 15	n
15[A]	n.n.	n.n.	tonsil	1	break	n	1, 5, 15	del
16	82	M	larynx	1	n	n	1, 5, 9, 11, 15	del
17	33	M	oral cavity	2	n	n	1, 11	n
18	40	M	pharynx	1	n	n	1, 9, 11	n
19	70	M	lymph node	2	break	cmyc break	1, 5, 8, 15	del
20	56	M	lymph node	1	n	n	1, 3, 5, 8, 9, 11, 15	n
21a[+]	31	M	sinus	1	n	n	15	n
21b[+,§]	35	M	sinus	1	n	n	15	n
22	39	M	pharynx	2	n	n	9, 15	n
23	78	M	pharynx	1	break	n	5, 11, 15	del
24	71	M	nose	2	n	n	5, 11, 15	n
25	58	M	pharynx	1	n	n	5, 9, 11	n
26	78	M	nose	2	break	t(4;14)	11, 15	del
27[§]	36	M	lymph node	2	break	t(4;14)	9, 11	del
28	52	M	sinus	2	n	n	n	n
29	58	F	sinus	2	break	n	9, 11	del
30[§]	30	M	skin/pharynx	2	n	n	11	n
31	45	M	nose	2	n	n	n	del
32	60	M	nose	2	n	n	9, 11	n
33[§]	61	M	nose	1	n	n	9, 11	n
34	81	M	larynx	1	break	n	n	del
35	37	M	sinus	2	n	n	n	n
36[§]	78	M	pharynx	1	break	n	n	del
37[§]	64	M	nose	1	n	n	n	n
38	59	M	larynx	1	n	n	9, 11	del

[+]Same case with a 5-year follow-up; A: local amyloid deposition; CSH: crystal storing histiocytosis; §documented local recurrence; n: normal; M: male; F: female; del: deletion.

MALT1, *BCL6* and *FOXP1* probes. Thus, the *IGH* partner chromosome was not identified in 8/14 (57%) cases with a 14q32 break.

The t(4;14) is the second most frequent recurrent translocation in MM.[8] It occurs in about 10-15% of cases and carries a poor prognosis.[7,8] It seems to be less frequent in MGUS than in MM, indicating that it leads to more rapid progression to MM. One of the EMP patients with t(4;14) suffered a local relapse, but did not develop bone marrow infiltration over a period of 7 years. Although the number of patients with t(4;14) is too small to darw firm conclusions, the occurrence of this translocation in EMP could indicate that effective therapy may achieve disease control, or that additional genetic aberrations are required for systemic disease. Of note, the t(4;14) is also found in primary AL amyloidosis, further indicating that this translocation is associated with a range of plasma cell dyscrasias.[16]

The lack of the t(11;14)(q13;q32) translocation, which occurs in 15-25% of MM patients and results in strong overexpression of cyclin D1, in EMP is remarkable.[8,12,15] The absence of this translocation in the EMP cases was confirmed by complete negativity for cyclin D1 by immunohistochemistry. The lack of the t(11;14) points to true biological differences between MM and EMP, and suggests that this translocation is strictly associated with bone marrow involvement. Interestingly, the t(11;14) occurs in MGUS at a similar frequency as in MM, indicating that MGUS, but not primary EMP, represents a precursor lesion for cases of MM with this translocation.[8,11] Of note, two of the EMP cases reclassified as MM because of positive bone marrow carried a t(11;14) translocation, validating the clinical relevance of this observation.[5,8,11]

Other, less frequent recurrent translocations in MM, including the t(14;16) involving *C-MAF* and the t(6;14)(p21;q32) involving *CCND3*, were also absent in our series of EMP, the latter indicated by the lack of cyclin D3 immunostaining.[8,11,17] The single break involving *C-MYC* was not associated with the classical t(8;14) juxtaposing *C-MYC* and *IGH*. In MM, *C-MYC* alterations are considered secondary events and are found at very high frequency in established MM cell lines.[18] The rarity of *C-MYC* breaks in EMP is compatible with a less advanced disease state at the genetic level.

Fifteen of 38 cases (40%) showed loss of chromosome region 13q14, and nine of these 15 cases also had an *IGH* translocation (p=0.01). This is similar to the rate in MM, in which Δ13q occurs in 40-50% of cases and is associated with the recurrent *IGH* translocations.[8,12] However, whereas MM patients with Δ13q are considered to be at intermediate to poor risk, no correlations of Δ13q with clinical features or relapse were identified in our EMP series. Since 13q also occurs in MGUS, one can similarly assume that the presence of this abnormality alone is insufficient to have a negative prognostic impact.[19]

Chromosomal gains were frequent, with 31/38 EMP cases (82%) showing extra copies of one of the examined chromosomes (1, 3, 5, 8, 9, 11 and 15), though with varying percentages of involved tumor cells (Table 1). Hyperdiploidy, defined as gains in at least three of the tested five centromeric probes, was identified in 14/26 (54%) cases with complete results for all examined chromosomes. One case (case 11) showed evidence of tetraploidy. The aneuploidy rate was similar for cases with *IGH* break (50%).

In MM, hyperdiploidy with nonrandom gains involving mainly chromosomes 1, 5, 9, 11, and 15 defines the second largest cytogenetic group; patients in this group have a lower frequency of the common IGH translocations, and better prognosis and therapy response as compared to the non-hyperdiploid group. Again, the results in primary EMP indicate a close relationship with MM and additionally document that there are virtually always detectable chromosomal alterations in EMP, despite the

commonly benign evolution of this neoplasm.[7,8,11,20]

There was no correlation between chromosomal aberrations and clinical features, grading, or development of local relapse for the 26 patients with available follow-up data beyond the 6-month period. Of the seven patients with local relapse, two had both a break in the *IGH* locus and Δ13q, including case 27 with a t(4;14), whereas four of the other five patients had extra copies of at least one chromosome (Table 1).

Another aim of our study was to clarify whether EMP or a subgroup of these cases is related to MALT-type lymphomas with plasmacytic differentiation, as suggested previously.[6] The absence of breaks involving the *MALT1* and the *FOXP1* loci, which are frequently involved in MALT lymphomas, in addition to the presence of chromosomal alterations more typical of MM, speak against this possibility.[21-23] In summary, this first molecular cytogenetic study of EMP demonstrates significant similarities with MM, despite its markedly different clinical presentation and clinical course. However, the different spectrum of chromosomal partners of 14q32, with the absence of the t(11;14) translocation in EMP, indicates that EMP and MM are more than just different tissue manifestations of the same disease process.

Authorship and Disclosures

KB was the primary investigator in this study, analyzed and interpreted the data, and wrote the paper; EH performed FISH analyses, and was involved in the study design and data interpretation; MK, GO, CB-S, LdL, SP and HL contributed to case selection and evaluation of clinical data and were involved in data analysis and interpretation; UJ performed the statistical analysis and interpreted data; PH performed image analysis and was involved in data interpretation; LQ-M and FF designed and supervised the study, were involved in case selection and critically reviewed the data and the manuscript.

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