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The value of fluorescence *in situ* hybridization for the detection of 11q in multiple myeloma

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A B S T R A C T

Background and Objectives. A large number of chromosomal abnormalities have been detected in multiple myeloma (MM). The most frequent are chromosome 13q deletions and translocations affecting the immunoglobulin heavy chain gene (*IGH*). Recent studies using comparative genomic hybridization (CGH) have shown that gains of 11q represent one of the most frequent genomic changes in MM. However CGH is not generally used in routine clinical laboratories.

Design and Methods. In the present study, efficiency of fluorescent *in situ* analysis (FIS)H analysis in the detection of 11q abnormalities in MM patients was investigated. Cytogenetic and FISH studies with three different specific probes for the regions containing the genes *BCL1* (11q13), ATM (11q22) and MLL (11q23) were simultaneously performed in 52 patients: 9 cases with 11q abnormalities detected by conventional cytogenetics and 43 cases without 11q abnormalities. FISH analysis identified 11q aberrations that were undetected by cytogenetics in 16 out the 43 cases (37%).

Results. Gains on 11q were present in 13 cases (30%) while rearrangements on 11q were observed in the remaining 3 cases. No losses were found. All 11q gains involved the three regions analyzed (*BCL1*, *ATM* and *MLL* genes) while only rearrangements of *BCL1* were observed. In all control cases the 11q alterations were confirmed by FISH. A good overall correlation between CGH and FISH was observed. Nevertheless gains on *BCL1*, *ATM* and *MLL* genes were observed in 3 cases displaying a normal CGH.

Interpretation and Conclusions. In summary, chromosomal abnormalities on 11q are frequent in MM. FISH studies demonstrate a high sensitivity at detecting this abnormality and should be used in the routine evaluation of MM.

Key words: multiple myeloma, cytogenetics, fluoresecence *in situ* hybridization, comparative genomic hybridization, 11q

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Conventional cytogenetic analyses show the presence of karyotypic abnormalities in 20-50% of patients with multiple myeloma (MM).[1-4] Studies with interphase fluorescence *in situ* hybridization (FISH) demonstrate that the incidence of chromosomal abnormalities in MM is higher than previously suspected.[5-7] The most frequent genetic abnormalities in MM are chromosome 13q deletions and translocations affecting the immunoglobulin heavy chain (*IGH*) gene. With the exception of t(11;14), these have been associated with an adverse outcome.[8-12] In addition, comparative genomic hybridization (CGH) is a useful technique for identifying gains and losses of DNA sequences in tumors with a low proliferative index, such as MM. Thus, recent studies using CGH have shown that chromosomal imbalances are present in most of MM patients and gains of 11q represent one of the most frequent genomic changes.[13-16] By contrast, the reported incidence of this abnormality in cytogenetic studies is rather low. Since CGH is not generally used in routine clinical laboratories and cytogenetic analysis underestimates 11q abnormalities we decided to investigate the efficiency of FISH analysis in the detection of 11q abnormalities in MM patients and to gain insight into the characterization of the specific 11q regions involved in these cases.

Design and Methods

Patients

A total of 52 MM patients were included in the study and divided into two cohorts: 9 cases with 11q abnormalities detected by conventional cytogenetics (positive control

group for FISH analysis) and 43 cases without 11q abnormalities. Within this second group we included both cases with normal karyotype (15 patients) and patients with abnormal cytogenetics other than 11q (28 cases). Patients showing a t(11;14) were excluded, except for case no. 50 with t(11;14) and gains on 11q (Table 1).

Conventional cytogenetics

Samples were processed according to the methodology previously described.[17] Chromosomes were identified by G-banding and karyotypes were described according to the International System for Cytogenetic Nomenclature (ISCN).[18] A karyotype was considered to be normal when no clonal chromosomal abnormalities were detected among 20 metaphases examined.

Fluorescence in situ hybridization

Dual color FISH studies were performed with three different specific probes for the regions containing the genes *BCL1* (11q13.3) (LSI IGH/CCND1, dual color, dual fusion translocation probe), *ATM* (11q22.3) (LSI ATM probe) and *MLL* (11q23.3) (LSI MLL, dual color, break apart rearrangement probe) (Vysis, Downers Grove, IL, USA) as previously described.[19] In addition CEP 11, subtelomeric probe (Telvysion 11p) and painting probe (WPC11) (Vysis) were used. Briefly, chromosome spreads were treated with pepsin (0.1 mg/mL). After washing with 0.5XSSC NP 40 for 30' at 37°C, slides were denatured in 70% formamide/2XSSC solution for 2' at 73°C. The probe was denatured at 75°C for 5'. Hybridization was performed overnight at 37°C in a moist chamber. Post-hybridization washing was carried out in 0.1XSSC NP 40 for 5´ at 65°C first and then with phosphate buffer solution for 5´ at room temperature. Slides were counterstained with DAPI. Hybridization was carried out according to the manufacturer's instructions. Slides were analyzed on an Olympus BX60 coupled to a Cytovision Ultra system (Applied Imaging, Sunderland, UK), using a cooled, charge-coupled camera. A total of 500 interphase nuclei were analyzed using Vysis scoring criteria. Based on the results using these probes in ten normal controls, the cut-off point for the identification of alteration was set at >5% cells with an abnormal signal.

Comparative genomic hybridization

CGH studies were performed according to previously reported procedures.[16] Calculation of the tumor DNA to normal DNA fluorescent ratios along the length of each chromosome was performed using an automated CGH software package (Cytovision, Applied Imaging). Ratio values obtained from at least 10 metaphase cells were averaged. Ratio values above 1.25 and below 0.75 were considered to represent chromosomal gain and loss, respectively. Over-representations were defined as high-level amplifications when the profiles exceeded the cut-off value of 1.5. Negative control experiments were performed using differentially labeled male versus male and female versus female DNA. Additional control experiments included the interchange of the digoxigenin-dUTP and biotin-dUTP labels between normal and tumor DNA.

Results

FISH studies demonstrated that the percentage of 11q alterations is higher than that shown by cytogenetics (Table 1). Thus, FISH techniques detected abnormalities on 11q in 16 out of the total 43 samples with normal 11q by conventional cytogenetic analyses (37%): 13 of the abnormalities were gains (cases 1-13) and 3 were rearrangements which consisted of 2 ins(11;14) (cases 14 and 15) and one t(11;14) (case 16). The assessment of the two insertions was performed in metaphases showing a fusion signal into an apparently normal chromosome 11. All gains involved the three different regions analyzed (*BCL1*, *ATM* and *MLL*). In 10 out of these 13 cases with gain on 11q, a gain of both centromeric and subtelomeric 11p regions was confirmed by FISH. This indicates that although the majority of 11q gains results from trisomy 11 (10 out of the 13 patients), there are some cases (3 patients) with only gain on specific regions on 11q. No losses were found (Table 1). Among the 43 patients without 11q abnormalities by conventional cytogenetics, we had included both cases with a normal karyotype (15 cases) and patients with chromosomal abnormalities other than 11q (28 cases). Our hypothesis was that this second cohort of patients might have occult 11q abnormalities more frequently and that these would be revealed by FISH analysis. However the results showed a similar incidence of 11q abnormalities in both subgroups (40% vs 36%).

The control group was formed of nine patients with 11q abnormalities detected by conventional cytogenetics. In the seven cases with an extra chromosome 11q FISH studies confirmed the gain on 11q (cases 44-50). In the two other cases (cases 51 and 52), with a translocation involving chromosome 11q, FISH analysis failed to demonstrate involvement of the three genes analyzed (*BCL1*, *ATM* and *MLL*). Nevertheless, additional FISH studies with chromosome 11 painting probe on metaphase cells confirmed that cases 51 and 52 had 11q abnormalities but with the breakpoint telomeric to the *MLL* gene. Case 50 was of particular interest since cytogenetics had detected the co-existence of both t(11;14) and a gain on 11q13-qter; duplication of 11q13-qter is the result of a der(14)t(11;14)

Table 1. Cytogenetics, FISH and CGH results in 52 patients with MM.

C	Cytogenetics	CGH	BCL1	ATM	MLL	CEP 11	11p
1	46,XY [21]	normal	+	+	+	+	+
2	46,XY[20]	normal	+	+	+	+	+
3	46,XX[20]	normal	+	+	+	+	+
4	46,XX,-4,+9,add(10)(q21),del(17)(p11)[5]/46,XX[15]	nd	+	+	+	+	+
5	45,XY,-13[3]/46,XY[17]	nd	+	+	+	+	+
6	46,XX,del(3)(p21)[2]/46,XX[18]	nd	+	+	+	+	+
7	47,XX,+9[3]/46,XX[17]	nd	+	+	+	+	+
8	50,XY,+2,+5,del(6)(q16q25),+9,-13,-14,-15,+18, +19,+2mar[2]/46,XX[12]	nd	+	+	+	+	+
		+	+	+	+	+	
9	45,XY,-13[3]/46,XY[19]	nd	+	+	+	+	+
10	45,XY,-13[3]/46,XY[18]	nd	+	+	+	+	+
11	53,XY,+1,+1,+3,+4,+5,+15,+mar[2]/46,XY[18]	nd	+	+	+	normal	normal
12	46,XY,dup(4)(q28q35)[3]/46,XY,add(1)(p36) [3]/46,XY [14]11q14-q22	amplification	+	+	+	+	normal
		+	+	+	+	normal	
13	47,XY,+mar[6]/46,XY[14]	gain 11q13-q25	+	+	+	+	normal
14	46,XY[20]	abnormal (11q not affected)	ins (11;14)	normal	normal	normal	nd
15	46,XX[24]	abnormal (11q not affected)	ins (11;14)	normal	normal	normal	nd
16	46,XY[20]	normal	t(11;14)	normal	normal	normal	nd
17	46,XX[20]	abnormal (11q not affected)	normal	normal	normal	normal	nd
18	46,XY[20]	abnormal (11q not affected)	normal	normal	normal	normal	nd
19	46,XX [21]	abnormal (11q not affected)	normal	normal	normal	normal	nd
20	46,XY[22]	abnormal (11q not affected)	normal	normal	normal	normal	nd
21	46,XX[24]	abnormal (11q not affected)	normal	normal	normal	normal	nd
22	46,XY[21]	abnormal (11q not affected)	normal	normal	normal	normal	nd
23	46,XY[20]	abnormal (11q not affected)	normal	normal	normal	normal	nd
24	46,XY[23]	normal	normal	normal	normal	normal	nd
25	46,XX[20]	gain 11q11-q13	normal	normal	normal	normal	nd
26	46,XX,-2,add(11)(p13),+mar[3]/46,XX[17]	nd	normal	normal	normal	normal	nd
27	47,XX,-13,+2mar[2]/46,XX[18]	nd	normal	normal	normal	normal	nd
28	45,XX,-13[2]/46,XX[18]	nd	normal	normal	normal	normal	nd
29	50,XX,+5,+9,+10,+21[2]/46,XX[19]	nd	normal	normal	normal	normal	nd
30	46,XY,dic(2;12)(p21;p13)[3]/46,XY[18]	nd	normal	normal			

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C	Cytogenetics	CGH	BCL1	ATM	MLL	CEP 11	11p
31	46,XY,del(13)(q13q14)[4]/ 46,XY[16]	nd	normal	normal	normal	normal	nd
32	46,XX,add(3)(p14)[2]/46,XX[18]	abnormal (11q not affected)					
33	45,XY,-13[3]/46,XY[18]43,XY,-1, der(4)t(1;4)(q31;p16),add(5)(q34),+6, add(12)(p13),-14,der(16)t(1;16)(q31;q13),-17,	abnormal (11q not affected)	normal	normal	normal	normal	nd
34	-22[16]/46,XY [4]	abnormal (11q not affected)	normal	normal	normal	normal	nd
35	45,XY,der(12)t(1;12)(q21;p13),-13[5]/46,XY[16]	nd	normal	normal	normal	normal	nd
36	45,XX,-5[4]/45,XX,-13[2]/46,XX[14]	nd	normal	normal	normal	normal	nd
37	47,XX,+3[3]/46,XX[19]	nd	normal	normal	normal	normal	nd
38	46,XY,add(11)(p12)[5]/46,XY[15]	nd	normal	normal	normal	normal	nd
39	41<45,XY,-1,-2,-14,-21[cp6]/46,XY[14]	nd	normal	normal	normal	normal	nd
40	51,XY,+3,+5,+3mar[3]/46,XY[18]	nd	normal	normal	normal	normal	nd
41	46,XY[20]	nd	normal	normal	normal	normal	nd
42	50,XY,+5,+9,+10,-13,+2mar[3]/47,X,-Y,del(2)(q21), +5,-6,+9,+10,-13,add(19)(p13),+mar[3]/46,XY[13]	nd	normal	normal	normal	normal	nd
43	52,XX,+3,+5,+6,+9,add(14)(q32),+2mar[3]/46,XX[18]	nd	normal	normal	normal	normal	nd
44	47,XY,del(1)(q21q42),-2,+3,+5,-8,+9,-10,+11, der(15)t(1;15)(q21;q26)[7]/92,XXYY[2]/46,XY[15]	+11	+	+	+	+	nd
45	61,X,-X,del(1)(q31),+del(1)(p31),+2,+3,+5,+6,+7,+9, +11 +11,+15,+16,+17,+18,+19,+20,+21,+22[12]/46,XX[10]	+11	+	+	+	+	nd
46	55,XY,+1,+del(1)(p21p36),+dic(2;11)(p23;p11), +3,+9,+11,-13,+15,+add(17)(q25),+19,+20[6]/46,XY[14]	+11	+	+	+	+	nd
47	53,XX,+5,+7,+9,+11,+16,+21,+22[4]/46,XX[14]	nd	+	+	+	+	nd
48	46,XY,+1,+1,+3,+5,-8,-9,-9,+11,-13,-20[3]/ 46,XY[15]	nd	+	+	+	+	nd
49	51,XY,der(1)t(1;18)(p36;q12),+3,+9,+11,+21, +22[6]/46,XY[9]	nd	+	+	+	+	nd
50	44,XX,add(3)(q26),dic(5;9)(q13;p24),-9, t(11;14)(q13;q32),add(12)(q24),-13,+der(14) t(11;14)(q13;q32),add(19)(p13),-20 [cp15]	gain 11q13-qter	t(11;14)x2	+	+	normal	nd
51	39,Y,-X,del(1)(q21q42),-4,del(9)(q22q33),add(11) (q23),-13,-15,-19,-20,-21,-22,+mar[10]/46,XY[5]	normal	normal	normal	normal	normal	nd
52	47,XX,i(1)(q10),+5,der(11)t(1;11)(p31;q23), del(14)(q13q32)[12],46,XX[7]	nd	normal	normal	norma	normal	nd

C, cases; +, three probe signals; nd, not data; 11p, subtelomeric 11p probe.

duplication. FISH results confirmed the rearrangement as well as *BCL1*, *ATM* and *MLL* gains (Table 1). Finally, CGH studies were performed in 25 out of the 52 patients. Seven of these 25 cases showed abnormalities on 11q by CGH (28%): one amplification (case 12) and 6 gains (cases 13, 25, 44-46 and 50). CGH identified 11q abnormalities that had gone undetected by conventional cytogenetics in three cases (no. 12, 13 and 25). On the other hand, FISH studies confirmed the presence of gains on 11q detected by CGH in all but one case (no. 25). Moreover, FISH detected gains in 11q in 3 cases with normal CGH (cases 1-3) (Table 1).

Discussion

Conventional cytogenetic studies have shown that t(11;14) and trisomy 11 are the most recurrent abnormalities of chromosome 11 in MM patients.[1-4] In addition, CGH studies in MM have revealed a high incidence of gains on 11q.[13-16] However information regarding FISH studies on 11q, other than t(11;14), is scanty.[20,21] In this study we found a high incidence of abnormalities on the chromosome arm 11q. Interestingly, a high percentage of patients (37%) with an apparently normal 11q by cytogenetics displayed aberrations in 11q once FISH studies were applied, the abnormalities being most frequently trisomies of chromosome arm 11q, but also t(11;14) and cryptic insertions of *IGH* into chromosome 11 that could not be identified by cytogenetics. The inability of cytogenetic techniques to detect 11q abnormalities might be attributed to the low proliferative index of plasma cells as well as to the poor quality of chromosomes. Thus, the subclonal abnormal populations could be undetected in metaphase cell studies and FISH analysis in interphase cells is necessary.[22] By contrast, all cases with trisomy of 11q by cytogenetics showed gains in 11q by FISH. Breakpoints in cases with der(11q) were found to be telomeric to *MLL* in both cytogenetic and FISH analyses. Only rearrangements of *BCL1* were observed. In all but one case the trisomies of chromosome arm 11q comprised all three genes studied (*BCL1*, *ATM*, and *MLL*) suggesting that there is not a preferential region involved in gains on 11q. Thus, FISH analysis with the IGH/CCND1 probe is a cheaper and quicker option, providing the same information as the application of all three 11q probes.

Comparative genomic hybridization is a useful technique for identifying gains and losses of DNA sequences in tumors with a low proliferative index, such as MM.[13-16] A good overall correlation between the CGH and FISH techniques was observed in the present series. The explanation for the three cases with normal CGH and three *BCL1*, *ATM* and *MLL* copies would be the low number of plasma cells with these abnormalities (11-35%).[23] The only case with gain on 11q detected by CGH but not identified by FISH had the extra region on q11-q13, therefore centromeric to *BCL1*. In summary, chromosomal abnormalities on 11q are frequent in MM. The FISH technique shows a high sensitivity for detecting this abnormality and should be prospectively investigated in MM in order to elucidate its potential prognostic influence.

MBG: conception and design and fluorescence in situ hybridization experiments, drafting of the article; JMH: conception and analysis of the data, final approval of the manuscript; JLG: cytogenetics and comparative genomic hybridization experiments; EL: comparative genomic hybridization experiments; MC: fluorescence in situ hybridization experiments; JH: collection of clinical data and follow-up of the patients; FJFC: collection of clinical data and follow-up of the patients; NCG: analysis of data and critical revision of the paper; JFSM: participated in the design of the study, critical revision of the paper and final approval.

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