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Cytogenetics of Multiple Myeloma

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A B S T R A C T

Great studies of multiple myeloma (MM) strongly suggested that specific chromosomal changes are of prognostic significance in patients with MM[1]. We have performed cytogenetic analysis and recently fluorescent in situ hybridization (FISH) on 43 cases of MM. Clonal chromosomal changes were present in 24 (56%) cases. Hyperdiploid karyotype was found in 12 (50%) cases, hypodiploid in 8 (33%) cases, and 4 (17%) cases had a pseudodiploid karyotype. The most common numerical abnormalities were gains of whole chromosomes 15, 11, 3 and 6. Whole chromosome losses were also frequent involving chromosomes X, 13, 14, and 8. Most cases showed also structural rearrangements 71% (n=17): del(1p), dup(1q), del(5q), del(13q), del(17p) and t(11;14)(q13;q32) (n=4, 17%). Chromosome –13/13q deletion was found in 42% (n=10) cases; complete loss of 13 was observed in 67% (n=7) cases, whereas 33% (n=3) had interstitial deletions. In the majority of the cases there was a mixture of abnormal and normal metaphases.

***Key words**: bone marrow, multiple myeloma, cytogenetics, DMSO*

Introduction

Multiple myeloma (MM) is a clonal B-cell malignancy characterized by proliferation of malignant plasma cells that accumulate within bone marrow and usually secrete paraprotein. MM is associated with lytic bone lesions or diffuse osteoporosis and normal Ig production is impaired by immuneparesis (<1% cases are non-secretor)[2]. The annual incidence of MM is ~30/1,000,000 patients, usually incurable, within a median survival of 3 years, and 10% of patients survive more than 10 years[3]. Although it occurs in 10% of hematological malignancies, MM represents less than 1% of the reported malignancies with chromosomal abnormalities[4].

The low mitotic index of plasma cells in vivo and as well in vitro causes chromosomal changes in multiple myeloma and related disorders to be not well-defined. On the basis of banded chromosomes, abnormal karyotypes were found in 30–50% of cases; more often in advanced stages than in newly diagnosed patients. A number of studies have demonstrated that MM have highly complex karyotype in the majority of patients[5]. Cytogenetic results revealed hyperdiploid clone characterized by a distinct pattern of chromosome gains, and hypodiploid clone often accompanied by –13/13q deletion[6]. Recent advances in molecular cytogenetic techniques fluorescent in situ hybridization (FISH) and comparative genomic hybridization (CGH) have revealed that chromosome aberrations can be found in the majority of MM cases. Survival studies have shown that hypodiploidy and missing or partial deletion 13, and abnormalities of 11q and 22q have been significantly associated with worse prognosis[4]. IgH (14q32) translocations may be primary genetic events but some variants will likely be progression events (secondary translocations)[7].

In this study we present 43 cases MM analyzed by classical and molecular cytogenetics, in the time of diagnosis. Two of these cases are archived samples.

Patients and Methods

Bone marrow aspirates were collected from 43 patients at the time of diagnosis. The age of the patients

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ranged from 49 to 83 years, with a median of 63 years. Among 43 patients, 24 were women and 19 were men. For conventional cytogentics, bone marrow aspirates were processed using 24 hr and 48 hr culture with stimulation. Two samples previously archived in dimethyl sulfoxide (DMSO) were washed three times in RPMI 1640.

FISH was performed using specific DNA probes (Kreatech and Vyses) according manufacture instructions. To detect aneuploidy centromere probes were used and for detection 13q14 deletions RB1 and for t(11;14) IGH/CCND1 specific probes on interphases, mainly 200 cells and three metaphases. Results were abnormal when the percent of cells with any given chromosome abnormality exceeded the normal cut-off value. All FISH probes were validated using negative or positive controls.

Results

Among 43 successfully analyzed samples clonal chromosomal abnormalities by GTG- banding (Figure 1) and FISH was detected in 24 (56%) cases. Two samples archived in DMSO over year were with satisfactory dividing index. MM has also been successfully studied by interphone FISH that can be done in nondividing cells (Figure 2). FISH has been applied for the study of trisomies/monosomies, deletion 13, 17p13.1 and translocations involving 14q32 (IgH) locus. Karyotypes were classified according to the International System for Human Cytogenetic Nomenclature (2005) (ISCN 2005)[8] into three categories (Table 1). Hyperdiploid with >46 chromosomes in 12 cases (50%), two of them with incomplete karyotype, hypodyploid <45 in 8 cases (33%), and pseudodiploid with 46 chromosomes with structural and numerical aberrations in 4 cases (17%). Abnormal karyotypes were usually complex with multiple numerical and structural changes. In 7 cases there were only numerical abnormalities without structural abnormalities. In the majority of the cases with an abnormal karyotype, there was a mixture of normal and abnormal metaphases, and only in three were exclusively abnormal cells.

The most common whole chromosome gains were; +15 (n=5, 21%), +11 (n=5, 21%), +3 (n=4, 17%), +6 (n=4, 17%), +19 (n=4, 17%) and +21 (n=4, 17%), there were no gains of chromosomes 2, 8, 16, 22 and Y.

Losses of whole chromosomes were; –13 (n=7, 30%), –X (n=6, 25%), –14 (n=6, 25%), –8 (n=5, 21%), and –16 (n=4, 17%), there were no losses of chromosomes: 3, 10, 11, 15 and 19.

Structural chromosomal abnormalities were detected in 71% (n=17) cases. Most frequent deletion was deletion of chromosomes 1p/q, 5q and 13q. Chromosome –13/13q

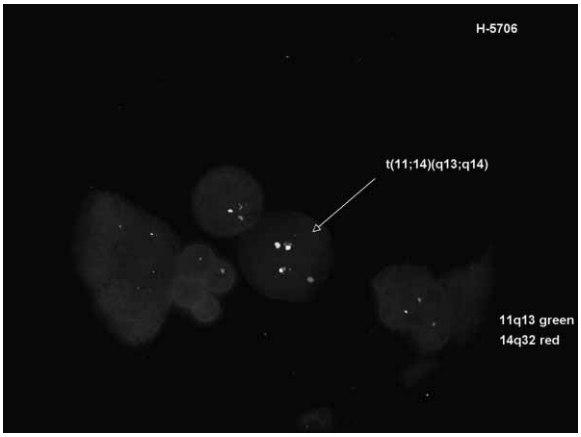

Fig. 2. Interphase FISH analysis for detection of t(11;14) (q13;q32). Red hybridization signals represent 11q13 (cyclin D-1) and green signals represent the 14q32.3 region. The presence of a t(11;14) is indicated by the appearance of a yellow fusion signal that results from colocalization of both probes.

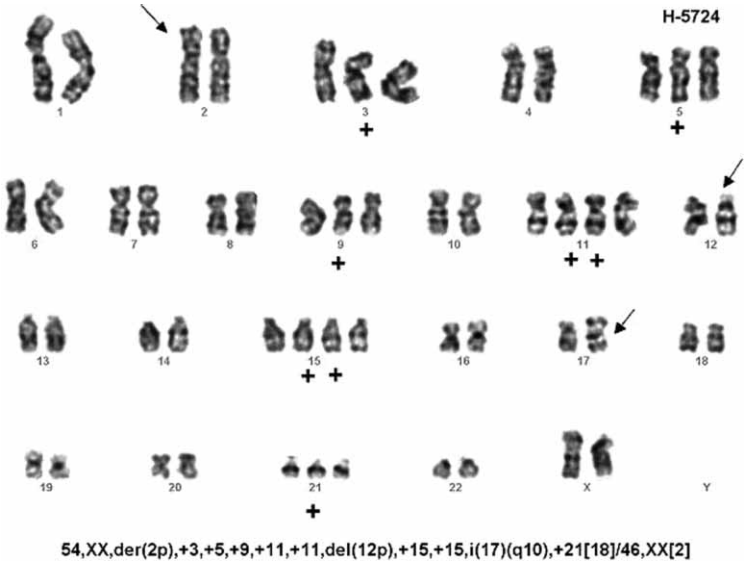

Fig. 1. Hyperdiploid karyotype (GTG banded) with numerical and structural changes.

TABLE 1
ABNORMAL KARYOTYPES OF 24 CASES WITH MULTIPLE MYELOMA

	Age/sex	Karyotype
Hyperdyploidy		
1.	79M	60~90,XY,+1,+6,+….inc[5]/46,XY[25]
2.	49M	53,XY,+6,+7,+…. inc[5]/46,XY[20]
3.	68F	46~48,XX,+del(5)(q13),del(5q23q31),+9,+der(11)t(11p;?),+12,der(19)t(19p;?),der(22)t(22q;?) [cp25]
4.	51F	47,XX,+9,dm[2]/46,XX[12]
5.	?M	47,XY,+19[3]/46,XY[10]
6.	?F	49,XX,+3,del(6q),t(11;14)(q13;q32),+der(14)t(14q;?),+15[8]/46,XX[6]
7.	58F	54,XX,der(2p),+3,+5,+11,+11,der(12p),+15,+15,i(17)(q10),+21[cp18]/46,XX[6]
8.	69F	59~61,X,–X,–X,der(3q)t(1p;3q),–4,del(7)(q22),–8,i(8)(q10),–13,–14,+15,–16,+18,+19,–20,+21, –22[9] /46,XX [13]
9.	49M	50–52,XY,+3,+6,+7,del(13q),+19,+21[cp3]/46,XY[15]
10.	68M	47,XY,i(1)(q10),t(1p;7pq;10q),i(10)(q10),+mar[6]/46,XY[3]
11.**	?F	72–74<3n>,XX,del(1q),t(2;14)(q?;q32),+del(5q),+del(5q),t(5q;10q)–8,add(8q),–9,+11,–13,–14,–14,+3–5 mar[cp5]/46,XX[7] *
12.**	76F	52,–X,–X,del(1q),+3,+4,+7,+11,–13,der(14)t(14q;?),+17,+18,+19, der(22)t(1p;22q) [cp4]/46,XX[1] *
Hypodiploidy		
13.	77F	44~45,XX,t(1;22)(p32;q11),del(3q),der(5)t(5q;?),–7,del(11p),–13,–13,der(14)t(14q;?),–17,der(17)t(17p;?),+18,del(20q),+del(20q),+mar2[cp25]/46,XX[12]
14.	77 M	45,X,–Y[3]/46,XY[30]
15.	66F	43–46,XX,+X,+X,–1,–2,–2,–4,+6,–7,–8,+11,+13,+14,+15,–16,–17,–18,–18,+20,+20,–21,–21, +mar[cp3]/46,XX[25]
16.	75M	45,X,–Y[3]/46,XY[20]
17.	64M	42–43,XY,–4,–5,–6,–7,–22[cp3]/ 46,XY[18]
18.	57F	45,X,–X[20]
19.	73F	44–45,XX,del(1p),t(5;8)(q35;q11q22),t(6q;10q),–12,–13,–14,–14,+2mar[cp8]
20.	65F	41–42,X,–X,t(1;11)(p13;q13),der(2q?),del(4)(q?27),–8,t(9;22)(q12;q13),t(11;14)(q13;q32),–12,–13,–14,–16,der(17),t(12;17)(q12;p13)[cp7]/46,XX[30]
Near-diploidy		
21.	75M	46,XY,inv(3)(q21q26),?der(15q)[cp3]/ 46,XY[18]
22.	64M	46,XX,t(15;16)(q22;p13?3) [5]/46,XX[20]
23.	73F	46,XX,–8,del(13(q14),+21[cp3]/46,XX[23]
24.	65F	46,XX,del(13)(q14),1–2dmin[2]/46,XX[10]

* Bone marrow samples archived in DMSO

deletion was found in 42% (n=10) of cases; complete loss of 13 was observed in 67% (n=7) of cases, whereas 33% (n=3) had interstitial deletions. The fifteen translocations were mostly complex, chromosomes involved in rearrangements were 1p/q in 5, 14q in 4 cases (17%) two t(11;14)(q13;q32), t(2;14)(q?;q32), and der(14)t(14q32;?). Isochromosomes of long arms chromosomes: 1, 8, 10 and 17 were present in 4 cases (17%). Addition material of unknown origin affected chromosomes 14q (n=2.8%) and 17p (n=2.8%).

Discussion

Cytogenetic analysis of MM have been limited by the low proliferate activity of plasma cells in culture. Despite that, chromosome analysis provides a wide array of chro-

mosome aberrations in proliferating plasma cells from patients with MM[9]. On the basis of classical cytogenetic technique (GTG-banding), abnormal karyotypes were found in 30–50% of cases[10]. Interphase FISH studies with centromeric probes have shown aneuploidy in 80% to 90% of cases, suggesting that clonal chromosomal abnormalities are frequent in those disordes[11]. Several studies have shown that aneuploidy has a significant impact on the prognosis of disease and choice of treatment. In our study of 43 MM in 56% cases with chromosomal aberrations according to their chromosome number three groups were identified. Hyperdiploid group in 50% cases with chromosome number greater than 46, chromosome gains were; +15 (21%), +11 (21%), +3 (17%), +6 (17%), +19 (17%) and +21 (17%). In some studies, the presence of certain trisomies was associated with an improved survival[13]. Second group 33% cases had hypodiploid karyotype with 41–45 chromosomes, losses of chromosomes were; –13 (30%), –X (25%), –14 (25%), –8 (21%), and –16 (17%). Hypodiploid MM is associated with a shorter survival. Hypodiploidy has been found to be associated with deletion 13 as a prognostic marker[13]. Third group was pseudodiplod with structural and numerical chromosomal aberrations in 17% cases. Chromosome –13/13q deletion was found in 42% cases; complete loss of 13 was observed in 67%, whereas 33% had interstitial deletions. The biological consequences of t(11;14)(q13;q32) remain unknown, although it has been shown that the MMs presenting this translocation are unexpectedly less proliferative than others[1]. Rearrangements of 14q32 were present in 4 cases (n=4, 17%) two t(11;14)(q13;q32), t(2;14)(q?;q32), and der(14)t(14q32;?).

Taken together, the results from both the metaphase and interphase cytogenetic studies support the hypothesis that specific chromosomal aberrations are of major prognostic relevance in MM. With banded chromosomes and FISH specific chromosomal abnormalities can be detected easily, thus providing a valuable data. Combinations of recently proposed prognostic factors such as cytogenetics and international scoring system (ISS) could readily predict prognosis[14].

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CITOGENETSKI REZULTATI 24 SLUČAJA MULTIPLOG MIELOMA

SAŽETAK

Velika ispitivanja multiplog mieloma (MM) naglašavaju da su određene kromosomske promjene od indikativnog značaja za pacijente s MM. Izvršili smo citogenetičke analize, a i u novije vrijeme i florescentnu in situ hibridizaciju (FISH) na 43 slučaja sa MM. Klonske kromosomske promjene bile su prisutne kod 24 (56%) slučaja. Hiperdiploidni kariotip je nađen kod 12 (50%) slučaja, hipodiploidni kod 8 (33%) slučaja, i 4 (17%) slučaja imalo je pseudodiploidni kariotip. Najčešće brojčane nepravilnosti bile su suvišci kromosoma 15, 11, 3 i 6. Manjak čitavih kromosoma bili su česti kod kromosoma X, 13, 14, i 8. Većina slučajeva također je pokazala strukturalne preuredbe 71% (n=17): del(1p), dup(1q), del(5q), del(13q), del(17p) and t (11;14)(q13;q32) (n=4,17%). Kromosom – 13/13q delecija nađen je u 42% (n=10) slučaja, potpuni gubitak kromosoma 13 uočen je kod 67% (n=7) slučaja, dok je 33% (n=3) bilo s intersticijskom delecijom. U većini slučajeva bile su uz abnormalne prisutne i normalne metafaze.