Pogostost in sopojavljanje ponavljajočih se kromosomskih sprememb pri bolnikih s plazmocitomom

Frequency and coexistence of recurrent chromosomal aberrations in multiple myeloma patients

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Izvleček

Izhodišča: Pri bolnikih z diseminiranim plazmocitomom (DP) najdemo tako številčne kot strukturne kromosomske spremembe. Poleg znanih kromosomskih sprememb, ki jih določamo redno, se v literaturi pojavljajo podatki o napovednem pomenu delecije kromosoma 1p, amplifikacije kromosoma 1q, delecije kromosoma 6q in amplifikacije kromosoma 15q, ki jih trenutno rutinsko še ne določamo. V okviru naše raziskave smo hoteli preveriti pojavnost navedenih novejših kromosomskih sprememb pri 68 na novo diagnosticiranih bolnikih z DP. Zanimalo nas je tudi, kakšna je sopojavnost vseh kromosomskih sprememb, tako novejših kot tudi tistih, ki jih določamo redno.

Metode: Izbrane kromosomske spremembe smo določali s komercialno dostopnimi DNA-sondami s fluorescentno in situ hibridizacijo (FISH).

Rezultati: Novejše kromosomske spremembe smo našli pri 69 % bolnikov, od tega delecijo kromosoma 1p pri 10 % bolnikov, amplifikacijo kromosoma 1q pri 40 % bolnikov, delecijo kromosoma 6q pri 10 % bolnikov in amplifikacijo kromosoma 15q pri 47 % bolnikov. Ugotovili smo sopojavnost amplifikacije kromosoma 1q z nekaterimi kromosomskimi spremembami, ki jih že dlje določamo rutinsko. Dokazali smo povezanost med amplifikacijo kromosoma 1q in del(13)(q14.3), t(4;14)(p16.3;q32) ter del(17)(p13.1). Poleg tega smo dokazali, da obstaja povezava med amplifikacijo kromosoma 15q in del(17)(p13.1) ter med amplifikacijo kromosoma 15q in delecijo kromosoma 6q.

Zaključki: V literaturi je potrjen napovedni pomen nekaterih novejših kromosomskih sprememb pri DP. V naši raziskavi smo ugotovili, da so te spremembe pogoste tudi pri naših bolnikih, zato bi bilo smiselno njihovo določanje z naborom DNA-sond pri DP.

Abstract

Background: The genome of myeloma plasma cells is characterized by marked instability comprising both complex numeric and structural abnormalities. Recent data in the literature suggest that multiple myeloma (MM) is also associated with deletion of 1p, amplification of 1q, deletion of 6q and amplification of 15q. These chromosomal aberrations have an impact on MM prognosis. We have looked for above mentioned chromosomal abnormalities in 68 newly diagnosed MM patients. Furthermore, our aim was to establish whether certain chromosomal abnormalities occur together with the others or exclusively autonomously.

Methods: Chromosomal aberrations were detected using commercially available FISH DNA probes.

Results: Deletion of 1p, amplification of 1q, deletion of 6q and amplification of 15q were present in 10 %, 40 %, 10 % and 47 % of patients, respectively. Our results confirm that amplification of 1q and del(13)(q14.3) are highly associated. We also detected an association between amplification of 1q and t(4;14)(p16.3;q32), and del(17)(p13.1). Additionally, the association between amplification of 15q and del(17)(p13.1), and del(6q) was statistically confirmed.

Conclusions: Literature data confirm the prognostic significance of newly tested chromosomal abnormalities in MM patients. Since they are frequent in our group of MM patients, the addition of DNA-probes into MM FISH probe panel has a substantial meaning for their detection .

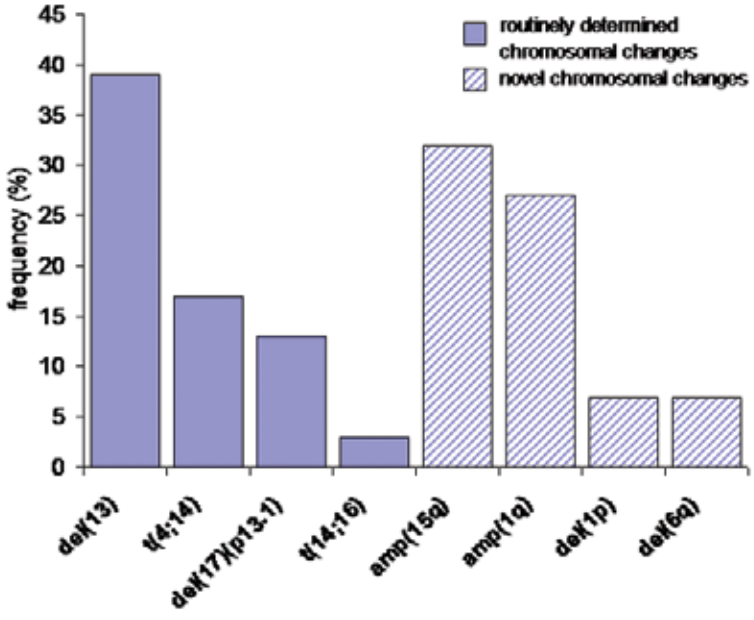

Figure 1: Frequency of routinely determined chromosomal changes and novel chromosomal changes.

Introduction

Multiple myeloma (MM) is a clonal B-cell disorder characterized by an excess of monotypic plasma cells in the bone marrow secreting monoclonal immunoglobulins in the serum and/or urine, with a concomitant decrease in normal immunoglobulins. Symptoms include anemia, immunosuppression, bone destruction and renal failure.[1] MM, which accounts for approximately 10 % of all hematologic cancers, is an incurable disease with a heterogeneous clinical course, and survivals ranging from a few months to longer than 10 years.[2]

Standard cytogenetic investigations of MM patients have proven to be technically very difficult due to relatively low proliferation rate of malignant plasma cells in cell cultures. Moreover, due to a heterogeneous distribution of plasma cells in the bone marrow, the percentage of plasma cells in the obtained sample may significantly vary depending on the site of sample aspiration. Thus, contamination of plasma cell cultures with normal bone marrow cells represents a constant problem. From this point of view fluorescence *in situ* hybridization (FISH) represents superior technique since both, metaphase and interphase cells are analyzed.[1,3] The genome of myeloma plasma cells is characterized by marked instability comprising both complex numeric and structural abnormalities. MM patients with chromosomal aberrations are more likely to present with features of aggressive disease and worse prognosis in comparison with patients without chromosomal aberrations. Numeric abnormalities encompass monosomies and trisomies of chromosomes. Accordingly, MM aneuploidy can be broadly categorized into hyperdiploid and non-hyperdiploid, the latter comprising hypodiploidy, pseudohypodiploidy and near-tetraploidy. MM patients with hyperdiploid karyotypes have a better outcome, whereas patients with non-hyperdiploid karyotypes have a dismal prognosis. The most frequent recurrent structural abnormalities include partial deletion of chromosome 13 (13q14), partial deletion of chromosome 17 (17p13.1) and chromosomal translocations involving the immunoglobulin heavy chain (IgH) locus. The major partner genes include cyclin D1 (11q13), cyclin D3 (6p21), *FGFR3-MMSET* (4p16.3), c-maf (16q23) and mafB (20q11).[4-9]

Recently emerging data suggest that MM is also associated with deletions of 1p (del(1p)) and 6q (del(6q)) and amplifications of 1q (amp(1q)) and 15q (amp(15q)). These chromosomal abnormalities have an impact on MM prognosis. Amp(1q) and del(1p) are associated with poor prognosis.[5,6,10-12] Patients with newly diagnosed MM with amp(1q) have an inferior overall survival, compared with those lacking amp(1q).[13] It is also associated with tumour progression[10,13] and drug resistance.[12,14] Due to frequent del(1p) and amp(1q) in MM patients, it is assumed that del(1p) could lead to hemizygosity of at least 1 tumour suppressor gene, and amp(1q) could induce overexpression of 1 or more oncogenes.[15] Amplification of 15q as part of hyperdiploid MM is associated with a favourable prognosis. Hence, we have looked for above mentioned chromosomal abnormalities in newly diagnosed MM patients. Furthermore, our aim was to establish whether certain chromosomal abnormalities occur together with the others or exclusively autonomously.

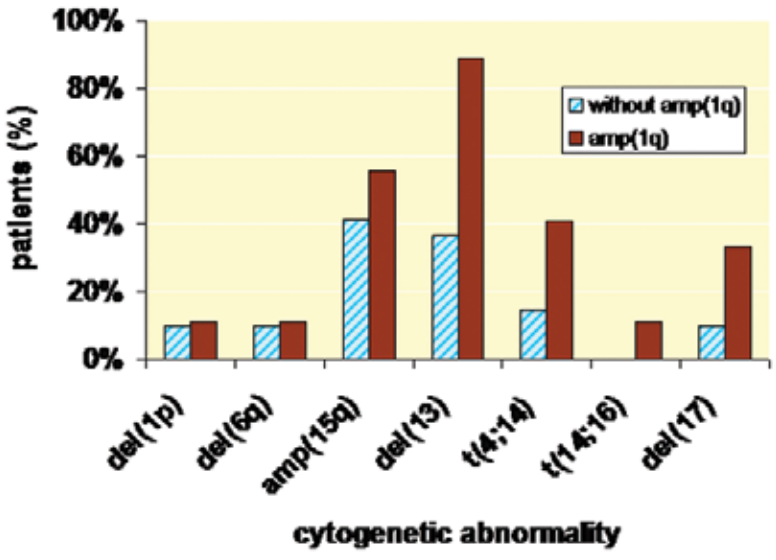

Figure 2: Comparison of frequency of cytogenetic abnormalities between 27 patients with amp(1q) and 41 patients without amp(1q).

Methods

Our group consisted of 68 newly diagnosed MM patients. The study was performed on bone marrow samples obtained at diagnosis after the patient's informed consent in accordance with ethics provisions for research on humans.

All patients have been routinely tested for the presence of recurrent aberrations del(13)(q14.3), del(17)(p13.1), t(4;14)(p16.3;q32), and t(14;16)(q32;q23) using commercially available DNA probes LSI D13S319 (Vysis), ON p53(17p13)/SE17 (Kreatech), LSI IGH/FGFR3 (Vysis) and LSI IGH/MAF (Vysis), respectively. Additional chromosomal abnormalities were detected by FISH DNA probes (Kreatech), namely ON 1q21/SRD(1p36) and ON MM 15q22/6q21. Blood samples of 10 healthy individuals were used to determine cut-off values for each tested probe. The cut-off values were set at 6 % for 1q21, and 15q22 and 4 % for 1p36, and 6q21.

FISH procedure: Bone marrow cells were cultured for 24 hours (37° C, 5 % CO_2) in Marrowmax (Gibco) and Bone marrow karyotyping medium (Biochrom). After colcemid exposure, cells were harvested and prepeared for FISH analysis according to standard procedures.[16,17] For each patient 400 cells were scored by two analysts. Result was given as an average of all scored cells. A positive result was each one above the previously established cut-off value for a particular probe tested.

Statistical analysis: Statistical evaluation was conducted using χ^2-test. When contingency table had 5 or less expected observations in one or more cells, Fisher's exact test was applied. Results were considered significant if the p value was less than or equal to 0.05. The statistical analysis was performed using SigmaPlot®11.

Results

Recurrent chromosomal changes were detected in 84 % of MM patients. In 11 patients we did not detect any abnormality, 14 patients had 1 abnormality, 19 patients had 2 abnormalities, 10 patients had 3 abnormalities, 7 patients had 4 abnormalities, 6 patients had 5 abnormalities and 1 patient had 6 out of 8 abnormalities. The clone size ranged between 5–100 % (Table 1). Del(13)(q14.3), t(4;14)(p16.3;q32), t(14;16)(q32;q23), and del(17)(p13.1) that have been routinely determined for the last four years were detected in 66 % of patients while the frequency of newly tested del(1p), amp(1q), del(6q), and amp(15q) was even higher (69 %). The most frequent aberration detected in our group was del(13) followed by amp(15q) and amp(1q). t(14;16)(q32;q23) was confirmed in only 3 patients (Table 1) (Figure 1).

With the exception of del(1p), the tested aberrations are rarely found as a single abnormality (Table 1). Furthermore, three of them were never seen as the only change. The coincidence of one of them, namely amp(1q), with the remaining tested aberrations is shown in Figure 2. Amp(1q) was detected in 27 patients. 89 % (24) of patients with amp(1q) had concomitant del(13)(q14.3). 41 % (11) of patients with amp(1q) had also t(4;14)(p16.3;q32), and 33 % (9) of patients with amp(1q) had concomitant del(17)(p13.1). Statistical analysis confirmed that amp(1q) is associated with del(13)(q14.3) ($p< 0.001$), t(4;14)(p16.3;q32) ($p=0.032$), and del(17)(p13.1) ($p=0.035$). All three patients with detected t(14;16)(q32;q23) also had amp(1q). However, statistically this association was not significant (p=0,058), likewise

Table 1: Frequency of cytogenetic abnormalities and clone size (number of patients was 68).

	Number of patients (%)	Number of patients with a sole aberration[a] (%)	Clone size (%)
All aberrations	57 (84)	14 (25)	/
Novel chromosomal aberrations	47 (69)	8 (17)	/
amp(15q)	32 (47)	5 (16)	7–92
amp(1q)	27 (40)	1 (4)	8–85
del(1p)	7 (10)	2 (29)	6–21
del(6q)	7 (10)	0	11–89
Previously determined aberrations	45(66)	6 (13)	/
del(13)(q14.3)	39 (57)	5 (13)	5–95
t(4;14)(p16.3;q32)	17 (25)	1 (6)	5–100
del(17)(p13.1)	13 (19)	0	5–66
t(14;16)(q32;q23)	3 (4)	0	20–88

[a] *of all patients with a particular aberration*

the association between del(6q) and t(14;16)(q32;q23) (p=0.282).

Near 43 % (3 out of 7) of patients with del(1p) had concomitant t(4;14)(p16.3;q32) About 31 % (10 out of 32) of patients with amp(15q) had concomitant del(17)(p13.1) and 19 % (6 out of 32) of patients with amp(15q) had concomitant del(6q). Statistics confirmed an association between amp(15q) and del(17)(p13.1) (p=0.037), and del(6q) (p=0.046).

Discussion

Four recurrent chromosomal changes are in our laboratory routinely determined in MM patients by FISH: del(13)(q14.3), del(17)(p13.1), t(4;14)(p16.3;q32), and t(14;16)(q32;q23). They all have a negative prognostic meaning that has been changing to some extent due to new therapeutic options during the last years. Some new aberrations were also found that will probably become important in diagnostic workup of MM patients in the future. Their role has not been well established yet, therefore we compared their frequency in our population with literature data in an attempt to detect similarities and/or differences between our group and other multiple myeloma patients.

The novel aberrations tested were detected in 47 patients (Table 1), which is even higher than the number of patients with routinely determined aberrations (45). In 12 out of 68 patients only newly tested aberrations were detected. By application of the newly tested probes, in about 18 % of newly diagnosed MM patients that were negative for routinely determined chromosomal changes a cytogenetic marker with an established prognostic meaning can be found. Moreover, previously considered as patients with good prognosis these 12 patients actually belong to the group with poor prognosis.

The frequencies of del(1p), amp(1q) and del(6q) in our group of MM patients are comparable to literature data.[18,19,20,21] Amp(15q) was found to be the most frequent (47 % of patients), which is in great agreement with others[20] although some other studies detected amp(15q) to a lesser extent.[22]

Previous studies showed high association between deletions 13/13q and abnormalities of 1p and/or 1q.[4] Recurrent chromosomal changes were rarely found as a sole anomaly (Table 1). Their coexistence can be clearly seen from Figure 2 for amp(1q). We were able to confirm association of amp(1q) with del(13)(q14.3), and t(4;14)(p16.3;q32), and del(17)(p13.1) also by statistical analysis. All associations with t(14;16)(q32;q23) did not reach statistical significance (p> 0.05), most likely due to a low number of patients. In fu-

ture it is necessary to include more patients with del(1p), del(6q), and t(14;16)(q32;q23) to establish the association between tested chromosomal abnormalities.

Conclusions

With the addition of four new DNA-probes into MM FISH probe panel in considerable number of newly diagnosed MM patients a specific cytogenetic marker with defined prognostic meaning can be determined. We also confirmed that amp(1q) is highly associated with del(13)(q14.3), t(4;14)(p16.3;q32), and del(17)(p13.1). Additionally, the association between amp(15q) and del(17)(p13.1), and del(6q) was statistically confirmed.

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