DETECTION OF CHROMOSOMAL ABNORMALITIES IN PATIENTS WITH MULTIPLE MYELOMA BY FISH AND THEIR CORRELATION WITH CLINICAL SIGNIFICANCE AND PROGNOSTIC VALUE

Qin Huang, Min Mao, Li An, Yan Li, Xiaoyan Zhang, Ling Fu, Tao Lang, Zengsheng Wang, Yuling Nie, Xiaomin Wang*
Department of Hematology, People's Hospital of Xin jiang Uygur Autonomous Region, Urumqi, PR China

ABSTRACT

Objective*: To detect the chromosomal abnormalities in patients with multiple myeloma (MM) by fluorescence in situ hybridization (FISH) and to explore their correlation with clinical significance and prognostic value.*

Methods: *83 MM patients who were treated in our hospital from April 2015 to January 2017 were selected as the observation group, and 12 patients with non-hematological malignant diseases who provided bone marrow specimens were selected as the control group. The IGH, 1q21, RB1, D13S319, and p53 probes were used to detect FISH in MM patients and observe chromosomal abnormalities. Correlation with clinical data (age, R-ISS stage, bone loss, white blood cells, platelets, hemoglobin, erythrocyte sedimentation, creatinine, globulin, albumin, and C-reactive protein (CRP)), clinical significance, and prognostic value were analyzed.*

Results: *Among 83 patients with MM, 64 (77.11%) had chromosomal abnormalities, and 19 patients had negative test results. Among those with abnormalities, 8 cases (12.50%) had all four of the chromosomal abnormalities tested for via FISH, 17 cases (26.56%) had three kinds of abnormalities, and 16 cases (25.00%) had two kinds of abnormalities. There were 23 cases (35.94%) with only one chromosome abnormality. We found that IGH rearrangement was related to bone damage and albumin, 1q21 amplification was related to CRP, and 13q14 deletion was related to hemoglobin, albumin and globulin levels, and R-ISS stage. 17p13 deletion was significantly correlated with albumin, platelet, albumin, and globulin levels and R-ISS stages. Among the positive and negative FISH test results, the CR, PR, SD, and PD patients were significantly different in the T-VAD group ($P<0.05$), but not significantly different in the PAD group ($P>0.05$). Among the patients with chromosomal abnormalities, the treatment effect was worse than that of patients with negative results. Patients with IGH rearrangement and 17p13 deletion had significantly lower survival time. Patients with normal chromosomes had a significantly negative correlation with prognosis ($P<0.05$), while 1q21 amplification and 13q14 deletion had no significant correlation with patient prognosis. IGH rearrangement and 17p13 deletion are independent risk factors that affect the prognosis of this group of MM patients.*

Conclusions: *Most patients with MM have chromosomal abnormalities, which are related to some specific clinical measurements. FISH test can identify patients with these abnormalities, who have poorer treatment effect and poor prognosis.*

Keywords: *Multiple myeloma, FISH, chromosomal abnormalities, clinical data, clinical significance, prognostic value, correlation.*

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Introduction

Multiple myeloma (MM) is a malignant plasma cell disease that originates from plasma cells in the bone marrow. It mainly manifests as abnormal proliferation of bone marrow plasma cells, with overproduction of monoclonal immunoglobulin, also called M protein[1]. The onset of MM is slow, there are generally no obvious symptoms in the early stages, and the misdiagnosis rate is high[2]. Therefore, finding more effective detection indicators has become an urgent clinical need. Some studies have found that most patients with MM have abnormal chromosomal changes, and that these have certain correlations with clinical data such as disease stage, blood cells, clinical efficacy, and prognosis[3]. Fluorescence in-situ hybridization (FISH) is the use of fluorescently labeled specific nucleic acid probes to hybridize with the corresponding DNA or RNA molecules in the cell, allowing visualization of the mor-

phological distribution of stained cells by observing the fluorescence signal through a fluorescence microscope[4-5]. FISH is highly sensitive to changes in known sequence fragments, which greatly improves the detection rate of chromosomal abnormalities in patients with MM[6].

We used FISH technology to detect chromosomal abnormalities such as IGH rearrangement, 1q21 amplification, and 13q14 deletion in MM patients, and analyzed the correlation between chromosomal abnormalities and specific clinical data, treatment effects, and prognosis of MM patients.

Materials and methods

Materials

Patients with MM who were treated in our hospital from April 2015 to January 2017 (n=83) were selected as the observation group. There were 46 men and 37 women, aged 37 to 72 years, with an average age of 58.62±4.21 years. According to the Revised International Staging System (R-ISS) criteria for multiple myeloma, they were classified as 6 cases of stage I, 22 cases of stage II (10 cases of stage IIa, 12 of stage IIb), and 55 cases of stage III (33 cases of stage IIIa, 22 of stage IIIb). There were 15 cases of Immunoglobulin A (IgA) type, 5 cases of Immunoglobulin D (IgD) type, 41 cases of Immunoglobulin G type, 14 cases of light chain myeloma, and 8 cases of non-secretory myeloma. At the same time, 12 patients with non-hematological malignant diseases who had provided bone marrow samples were selected as the control group.

Equipment and reagents

The low-speed centrifuge (model: DT5-1B) was purchased from Changsha Xiangrui Centrifuge Co., Ltd. The low-temperature high-speed centrifuge (model: 3-18K) was provided by German SIGMA company. The microtome was purchased from Huasu Technology Co., Ltd., Jinhua, Zhejiang. The low-temperature refrigerator (model: BCD-470WD-PG0 was purchased from Qingdao Haier Group. The incubator (specifications: HH-US) was obtained from Shanghai Hetian Scientific Instrument Co., Ltd. Fetal bovine serum (Specification: Z7185FBS-100) was purchased from Shanghai Laichuang Biotechnology Co., Ltd. Fluorescence microscope (specification: XSP-63XDV) was provided by the Shanghai Optical Instrument Factory. FISH probes (IGH, 1q21, RB1, D13S319, p53) were purchased from Anbiqi Biotechnology Co., Ltd.

Fluorescence in situ hybridization

For patients in both the observation group and the control group, 4 ml of bone marrow blood were extracted, anticoagulant was added, 15% fetal bovine serum was added to a culture bottle, and all was cultured in a 37oC incubator for 48 h. After the cells were hypotonic and fixed, they were stored in a refrigerator at 20oC for FISH detection.

FISH detection: After removing the samples from the refrigerator, the suspension air-drying method was used to prepare the slides, which were aged at room temperature overnight. The slides were placed in a freshly-configured RNase A solution at 37oC for 45 minutes, rinsed, dehydrated, and dried at room temperature for deformation hybridization. The slide was heated to 55oC and then denatured in a 75oC denaturing solution (7 μl of hybridization buffer, 1 μl of deionized water, and 2 μl of the probe) for 10 minutes, dehydrated and dried. After 20 minutes in a 45oC water bath, the sample was dripped onto the slide hybridization area, covered immediately with a coverslip and sealed with rubber glue. The slides were placed in a pre-heated 40oC wet box and hybridized overnight. Then the coverslip was removed, and the slide washed, and dried in the dark. Then the slide was counterstained with 10 μl DAPI staining solution, covered with coverslip and place in a dark box for observation with a fluorescence microscope. At least 100 cells were analyzed per specimen, with image analysis performed using FISH software. FISH detection steps were performed strictly in accordance with the instructions. IGH rearrangement was detected using IGH probes (red and green signals), and 1q21 amplification was detected using 1q21 probes (red signals). 13q14 deletion was detected by D13S319 (red signal) and RB1 (green signal).

Clinical treatment

After approval by the hospital ethics committee, 83 MM patients were randomly divided into two groups: one group was treated with thalidomide, vincristine, adriamycin, and dexamethasone (T-VAD), and the other group was treated with bortezomib, adriamycin, and dexamethasone (PAD), as described below.

T-VAD group:

On days 1 to 4, patients received vincristine (Shenzhen Wanle Pharmaceutical Co., Ltd., specifications: 1mg * 10 bottles, production batch number: 151772) 0.5mg, added to 250ml of 5% glucose solution intravenously, once a day. Doxorubicin (It-

aly Marcia, specification: 10mg/bottle, production batch number: 150186) 10 mg was added to 250 ml of 0.9% sodium chloride solution for intravenous infusion, once a day. Dexamethasone (Tianjin Tianyao Pharmaceutical Co., Ltd., specifications: 1ml: 1mg, production batch number: 20153553) 20mg was added intravenously to 500ml 5% glucose solution, once a day; patients were also given thalidomide (Changzhou Pharmaceutical Factory Co., Ltd., specification: 50mg * 20s, production batch number: 148130) 50mg, three times a day.

PAD group:

On days 1, 5, 9, and 12 subjects received bortezomib (Xi'an Janssen Pharmaceutical Co., Ltd., specification: 35mg * 1 bottle/box, production batch number: 20150071) 1mg/m^2. Doxorubicin 10 mg was added to 250 ml of 0.9% sodium chloride solution for intravenous infusion, once a day. On the 1st, 3rd, 5th, 6th, 9th, 10th, 12th, and 13th days, 20 mg of dexamethasone was added to 500 ml of a 5% glucose solution for intravenous infusion, once a day.

Clinical information

All patients' age, R-ISS stage, bone loss, white blood cells, platelets, hemoglobin, erythrocyte sedimentation, creatinine, globulin, albumin, and C-reactive protein (CRP) were collected. The results are shown in Table 1.

Clinical data		n Positive	FISH test	
			Negative	
Age (years)	≥60	53	44 (83.02)	9 (16.98)
	<60	30	20 (66.67)	10 (18.87)
R-ISS stage	I stage	6	2 (33.33)	4 (66.67)
	IIa stage	10	6 (60.00)	4 (40.00)
	IIb stage	12	9 (75.00)	3 (25.00)
	IIIa stage	33	27 (81.82)	6 (18.18)
	IIIb stage	22	20 (90.91)	2 (9.09)
Bone loss	Yes	69	60 (86.96)	9 (13.04)
	No	14	4 (28.57)	10 (71.43)
White blood cells (×10^9/L)	≥4	46	33 (71.74)	13 (28.26)
	<4	37	31 (83.78)	6 (16.22)
Platelets (×10^9/L)	≥150	52	36 (69.23)	16 (30.77)
	<150	31	28 (90.32)	3 (9.68)
Hemoglobin (g/L)	≥110	35	24 (68.57)	11 (31.43)
	<110	48	40 (83.33)	8 (16.67)
Erythrocyte sedimentation (mm/h)	≥15	67	57 (85.07)	10 (14.93)
	<15	16	7 (43.75)	9 (56.25)
Creatinine (μmol/L)	≥133	30	24 (80.00)	6 (20.00)
	<133	53	40 (75.47)	13 (24.53)
Globulin (g/L)	≥30	52	42 (80.77)	10 (19.23)
	<30	31	22 (70.97)	9 (29.03)
Albumin (g/L)	≥50	36	25 (69.44)	11 (30.56)
	<50	47	39 (82.98)	8 (17.02)
CRP (mg/L)	≤10	21	5 (23.81)	16 (76.19)
	>10	62	59 (95.16)	3 (4.84)

Table 1: Clinical data of MM patients, n (%).

FISH test

For the IGH probe, two yellow fusion signals indicate normal cells; IGH rearrangement is indicated when orange-red signals are separated from green signals or only one yellow signal appears. For the 1q21 probe, two orange-red signals appear to indicate normal cells, while more than two indicates 1q21 amplification.

For the D13S319 probe and the RB1 probe, the appearance of two orange-red signals or two green signals indicates normal cells, and only one orange-red signal or green signal indicates 13q14 deletion. For the P53 probe, two green signals indicate normal cells, and fewer than two indicates 17p13 deletion. Examples are shown in Figure 1. Taking the test results of the 12 control samples as the average, the standard deviation (s) was calculated, and the threshold was + 3s. FISH test results greater than this threshold were considered positive, and otherwise are considered negative.

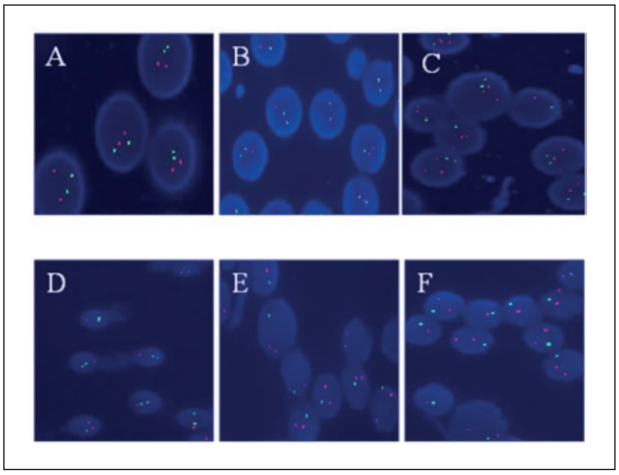

Figure 1: FISH test. A: normal chromosomes; B: IGH rearrangement; C: 1q21 amplification; D: D13S319 deletion; E: RB1 deletion; F: p53 deletion.

Treatment effect

Blood tests, urine tests, immunoglobulins, and bone marrow tests were performed before and after chemotherapy. The evaluation was based on the standard efficacy set by the National Comprehensive Cancer Network (NCCN) International Myeloma Working Group (IMWG)[7]. The therapeutic effects of MM patients were classified as Complete Remission (CR), Partial Remission (PR), Stabilization of Disease (SD), or Progression of Disease (PD).

Prognosis

Follow-up was conducted by telephone and outpatient review. The follow-up period was 24 months after the end of treatment and every 6 months. The

follow-up included routine examinations such as blood routine, urine routine, immunoglobulin and bone marrow.

Statistical methods

The SPSS 23.0 software package was used for statistical data analysis. Qualitative data was expressed as numbers and percentages, and comparison was performed by χ^2 test. Correlation analysis was performed using the Pearson correlation test, and a Cox regression model was used for multivariate survival prognosis analysis. Statistical results were considered statistically significant with a P<0.05.

Results

FISH test results in MM patients

Of 83 patients with MM, 64 (77.11%) had chromosomal abnormalities, and 19 patients had negative test results. Of those with abnormalities, 8 cases (12.50%) with abnormal chromosomes, 17 cases (26.56%) with abnormal chromosomes, 16 cases (25.00%) with abnormal chromosomes, and 23 cases with abnormal chromosomes (35.94%).

Correlation between chromosomal abnormalities and clinical data

The FISH test results of bone marrow specimens from MM patients were analyzed by multiple factors. IGH rearrangement was associated with bone damage and albumin, and 1q21 amplification was associated with increased CRP. The 13q14 deletion was related to the hemoglobin, albumin, globulin, and R-ISS stages. The 17p13 deletion was significantly correlated with the albumin, platelet, albumin, globulin, and R-ISS stages (Table 2).

Chromosomal abnormality	n	Clinical data	*P*
IGH rearrangement	32 (38.55)	bone loss	0.014
		albumin	0.008
1q21 amplification	45 (54.22)	CRP	0.034
13q14 deletion	30 (36.14)	hemoglobin	0.011
		albumin	0.035
		globulin	0.009
		R-ISS stage	0.014
17p13 deletion	47 (56.63)	white blood cell	0.048
		platelets	0.041
		albumin	0.008
		globulin	0.031
		R-ISS stage	0.004

Table 2: Statistically significant correlations between chromosomal abnormalities in MM patients and some clinical data.

Correlation between chromosomal abnormalities and treatment effects

In the T-VAD group there were significant differences in disease progression between those patients who had positive versus negative FISH test results (P<0.05). The differences were not significantly different in the PAD group (P>0.05). Among the patients with positive FISH results, the treatment effect was less than that in patients with negative results (Table 3).

Group		CR	PR	SD	PD
T-VAD	positive	8	13	11	18
	negative	2	4	3	2
P		—	—	—	0.039
PAD	positive	6	5	4	3
	negative	5	2	2	2
P		—	—	—	0.562

Table 3: Correlation between chromosomal abnormalities and treatment effects (n).

Correlation between chromosomal abnormalities and patient prognosis

Kaplan-Meier survival curves[8] were used to assess the relationship between chromosomal abnormalities and survival prognosis of patients with MM. The survival time of patients with IGH rearrangement and 17p13 deletion was significantly lower than that among patients with normal chromosomes, and these abnormalities were significantly negatively correlated with patient prognosis (P<0.05).

Patients with 1q21 amplification and 13q14 deletion had no significant correlation with their prognosis (Figure 2). A Cox regression curve model was created for analysis. The results showed that IGH rearrangement and 17p13 deletion were independent risk factors affecting the prognosis of this group of MM patients (Table 4).

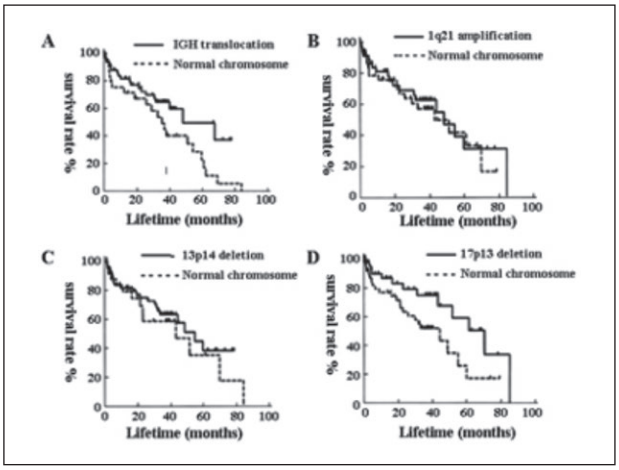

Figure 2: Correlation between chromosomal abnormalities and patient prognosis. A: IGH rearrangement; B: 1q21 amplification; C: 13q14 deletion; D: 17p13 deletion.

Factors	*P*	95% CI	
		Lower limit	Upper limit
IGH rearrangement	0.042	0.009	0.414
1q21 amplification	0.612	0.037	0.292
13q14 deletion	0.466	0.762	3.275
17p13 deletion	0.033	0.671	6.796

Table 4: Cox proportional risk model multivariate analysis results.

Discussion

Monoclonal immunoglobulin can often be detected in blood and urine. This abnormal globulin can be deposited in various organs of the body, eventually leading to organ damage[9]. MM is often accompanied by diseases such as multiple osteolytic damage, hypercalcemia, anemia, and kidney damage; because the production of normal immunoglobulin is inhibited, MM patients are also prone to various bacterial infections[10]. At present, indicators such as molecular cytogenetics, CRP, plasma cell reproduction rate, and bone marrow plasma cell morphology are the most reliable indicators of the development and prognosis of MM. Among these, molecular cytogenetics is the most important. Common molecular cytogenetic changes in MM include chromosomal abnormalities such as IGH rearrangement, 1q21 amplification, 13q14 deletion, and 17p13 deletion. FISH technology is now used to detect chromosomal abnormalities in MM patients. FISH is a non-radioactive in situ hybridization method for detecting DNA sequences by performing DNA hybridization on chromosome, cell, or tissue section specimens with special fluorescein-labeled probes[11]. In this study we use FISH technology to detect chromosomal abnormalities in patients with MM, and explore their relationship with clinical data, treatment effects, and prognosis of MM patients.

In our study, almost all MM patients had chromosomal abnormalities, and these were mostly of complex karyotypes. IGH rearrangement is a non-random molecular change; Mateos et al. found that the IGH gene is located on the 14q32 chromosome. When MM occurs, plasma cells will undergo overfrequency mutations and even class conversions. Therefore, 14q32 rearrangements often occur, resulting in IGH gene break rearrangements[12-13].

We found IGH rearrangement to be significantly correlated with bone damage and albumin levels, and Cox regression curve analysis showed IGH rearrangement to be related to the prognosis of MM patients. 1q21 amplification is a more common chromosomal abnormality in patients with MM. The CKS1B gene is located on chromosome 1q21 and is a member of the Cks/Sucl protein family, which is related to cell cycle regulation[14]. Sitry et al. believe that CKS1B could regulate the degradation of p27 by SKp2. Our study found that 1q21 amplification has a certain correlation with CRP levels, which has an important relationship with the development of MM disease. Though 1q21 amplification was associated with poorer prognosis of MM, we found no correlation with treatment effect. Vacca et al. found that the absence of stained humans in patients with MM is also more common on chromosome 13, and mainly manifests as a loss of the arm part. In this study, two different probes, D13S319 and RB1, were used to detect the deletion of 13q14 fragments in different regions, thereby improving the detection rate of 13q14 mutations[15]. In our study, 13q14 deletion was found to be related to hemoglobin, albumin, and globulin levels and R-ISS stage, but it was not significantly related to the prognosis of MM patients. The 17p13 deletion can lead to the deletion of the p53 gene, and this deletion indicates that MM has developed to an advanced stage. We found 17p13 deletion to be significantly negatively related to the effect of treatment and survival prognosis.

In summary, FISH technology is an important method for detecting chromosomal abnormalities in patients with MM, and its sensitivity is high. Chromosomal abnormalities such as IGH rearrangement, 1q21 amplification, 13q14 deletion, and 17p13 deletion are significantly correlated with specific clinical indicators. The effect of treatment among patients with FISH-identified chromosomal abnormalities was less beneficial than that among patients without them. IGH rearrangement was significantly negatively correlated with patient prognosis, while patients with 1q21 amplification and 13q14 deletion had no significant correlation with their prognosis. However, due to the insufficient sample size and short follow-up time in this study, further analysis of chromosomal abnormalities in MM patients and their clinical significance is needed.

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Corresponding Author:
Xiaomin Wang
Email: ijuu29@163.com
(China)