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Chromosomal aberrations in 130 patients with multiple myeloma studied by interphase FISH: diagnostic and prognostic relevance

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Abstract The study described the molecular cytogenetic characterization of myeloma cells in 130 patients via interphase fluorescence in situ hybridization. Nine repetitive DNA probes (for chromosomes 3, 7, 9, 11, 15, 17, 18, X, and Y) as well as seven single-copy DNA probes (for chromosomes 13, 17, 21, and two each for chromosomes 5 and 22) were used for the hybridizations. Using this panel of probes, we were able to show aberrations in 86% of patients. Most of them had one to three aberrations. There was a distinct correlation between the number of aberrations per patient and the tumor stage. Thus, the proportion of patients with 8–12 aberrations increased from 16% in stage II to 26% in stage III. There were marked differences among the chromosomes with respect to the prevalence of genomic losses and gains and deletions of gene loci. Chromosomes 3, 5, 7, 9, 11, 15, and 21 showed a preference for genomic gains. Losses were most often found for chromosomes 13 and 17 (locus specific) as well as for the X and Y chromosomes. The frequency of monosomies and trisomies were approximately the same for chromosomes 15 and 18, which indicates a skewed pattern of distribution. We found two specific aberrations that caused distinct changes in the survival rates of the patients: deletion 13q14 (28% of patients) and translocation of the IGH locus 14q32 (79% of 39 patients who were analyzed separately). The results obtained in this study yielded data of extremely relevant prognostic value.

1. Introduction

Investigations in recent years have shown that the diagnosis of specific cytogenetic alterations in patients with acute leukemia can represent an important risk factor in terms of the prognosis. The correlation between specific cytogenetic aberrations and their possible prognostic relevance in patients with multiple myeloma has not yet been investigated as closely. One reason for this is that the detection of aberrations is much more difficult in these patients. Even in extensive studies, only 20–50% of patients presented with identifiable numeric and/or structural chromosomal aberrations. Analysis of the DNA content of plasma cells revealed that aneuploidies occur frequently in patients with multiple myeloma: 61–68% of these cases showed hyperdiploid clones, 9–20% were pseudodiploid clones, and 10–30% were hypodiploid clones [1–3]. Furthermore, two studies based on flow cytometry showed that the percentage of multiple myeloma patients possessing an aberrant karyotype far exceeds the 30–40% assumed thus far. In fact, practically all patients possessed aneuploid karyotypes [4,5]. At this time, it seems very likely that a considerably higher number of patients have chromosomal aberrations than had been assumed previously.

Standard cytogenetic investigations of patients with multiple myeloma have hitherto proven to be technically very difficult, one reason being the relatively low proliferation rate of the malignant plasma cells retrieved from bone marrow [6]. Furthermore, contamination of these cultures with normal bone marrow cells poses a constant problem. Estimations of the chromosomal status of malignant plasma cells in previous studies may well have been skewed by the inclusion of an unknown percentage of normal cells [7,8].

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Studies based on fluorescence in situ hybridization (FISH), a technique in which both metaphase and interphase cells are analyzed, have shown considerably higher rates of chromosomal aberrations in these patients [9]. Using FISH, researchers have demonstrated that chromosomal aneuploidies occur independently of disease stage and previous therapy, whereas studies based on standard cytogenetic methods had established distinct links among therapeutic measures, the stage of the disease, and the frequency of chromosomal aberrations. Moreover, it is assumed that advanced stages of multiple myeloma may be associated with a higher proliferation rate, which would promote the detection of an abnormal karyotype [10]. It is not surprising, therefore, that an abnormal karyotype has usually been equated with an unfavorable prognosis.

This study aims to establish the correlation between tumor stage and cytogenetic findings via interphase FISH in a collective of 130 patients with multiple myeloma to make quantitative assessments regarding tumor-specific aberrations and to specify prognostic factors.

2. Materials and methods

2.1. Patients

The current study was carried out between November 2001 and September 2002 on bone marrow aspirates from 130 patients diagnosed with multiple myeloma. Most of the patients were receiving chemotherapeutic treatment when the first or second aspiration was performed. Five patients had a monoclonal gammopathy of undetermined significance, and two patients were recurrent.

All patients were registered with regard to other risk factors such as age, stage, isotype, c-reactive protein, ß2-microglobulin, morphology, remission status, number of previous chemotherapy sessions, and so on. Records were kept of the success of each patient's therapy. Interphase FISH was carried out on samples from 130 patients, which included 71 men and 59 women. The patients' ages at diagnosis ranged from 40 to 88 years, with the average age of 63 years. Seventy-two percent of the patients were in stage III, 16% were in stage II, and 12% were in stage I of the disease.

Of the 25 aspirates of patients with 0–25% plasma cells in the bone marrow, 6 aspirates were within the range of normal plasma cell infiltration (up to 10%). The average rate of plasma cell infiltration in the bone marrow at the time of investigation amounted to 50%.

2.2. Methods of investigation

Single-copy probes as well as repetitive DNA probes were used for the FISH performed in this investigation. The latter type of probe included α-satellite and classic satellite DNA probes, which target the centromeres and heterochromatin near the centromeres. All probes were directly labeled. Seven repetitive probes for the autosomes 3, 7, 9, 11, 15, 17, and 18 were selected, as well as probes for the chromosomes X and Y. In addition, the following eight specific loci on six autosomes were targeted, which have been found to show increased aberrations in several studies: single-copy probes 5p15.2, 5q31, 13q14, 14q32, 17p13, 21q22.13~21q22.2, 22q11, and 22q13 were used (Vysis/Abbott GmbH, Wiesbaden, Germany). Before studies were started, the hybridization efficiency for all probes was confirmed to be higher than 97%.

Hybridization was performed according to the supplier's protocols. Microscopic analysis was carried out using a Diaplan fluorescence microscope (BH 2-RFCA, Olympus and Axioskop 2; Carl Zeiss AG, Oberkochen, Germany) fitted with single-band filters for four colors. Two-hundred cells were analyzed for each case.

3. Results

3.1. Aberrations per patient and tumor stage

Determination of the number of aberrations per patient was based upon the total data derived from all probes. Fig. 1 shows the number of aberrations per patient relative to the percentage of all patients (n = 130). In all, 13.8% of patients did not exhibit any aberrations for the whole spectrum of probes tested. The highest percentages of patients were found for the sets of one to three aberrations per patient (12.3, 10.8, and 13.1%, respectively). The lowest percentage of patients was found for the sets of 10–12 aberrations per patient (0.8, 3.1, and 1.5%, respectively).

Furthermore, we found a correlation between the number of aberrations per patient and the tumor stage. Findings for alterations per patient were subdivided into three groups: 0–3 aberrations, 4–7 aberrations, and 8–12 aberrations. A comparison of these three groups with the tumor stages of the patients revealed a characteristic distribution (Table 1).

Patients in stage I of the disease had 0–3 aberrations in more than 50% of cases, while 4–7 aberrations were slightly less frequent (about 47%) and the subset of 8–12

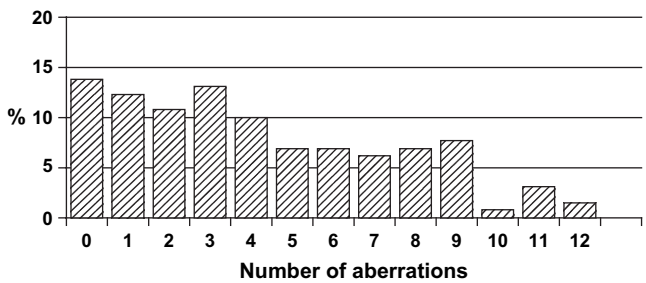

Fig. 1. Number of aberrations per patient relative to the percentage of all patients (n = 130).