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Random aneuploidy in neoplastic and pre-neoplastic diseases, multiple myeloma, and monoclonal gammopathy

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Abstract In this study we evaluated the aneuploidy rate of cells from patients considered to have a premalignant condition (monoclonal gammopathy or MGUS) and patients with multiple myeloma, as well as healthy controls. By applying a fluorescence situ hybridization technique, we estimated the random aneuploidy rate of α-satellite (centromeres) probes from chromosomes 9 and 18. The monosomy and total aneuploidy rates were higher in the two study groups compared to the control group. The monosomy rate was significantly higher in the MGUS group compared to the group with chromosome 18 α-satellite probes, a finding that was reported before in preneoplastic conditions. Our results support the cancer aneuploidy theory that carcinogenesis is initiated by a random aneuploidy, which is induced either spontaneously or by a carcinogen. The resulting karyotype instability sets a chain reaction of aneuploidization, which generates even more abnormal and eventually cancer-specific combinations and rearrangements of chromosomes.

1. Introduction

A century ago, Boveri proposed that cancer is caused by aneuploidy because it correlates with cancer and because it generates "pathological" phenotypes in sea urchins [1]. Duesberg and Rasnick performed biochemical and biological analyses of aneuploidy and gene mutations, which indicated that aneuploidy is probably the only mutation that can generate the complex phenotypes of cancer [2]. In view of this, they proposed a coherent two-stage mechanism for all aspects of cancer and carcinogenesis. In the first stage, both genotoxic and nongenotoxic carcinogens cause aneuploidy. In the second stage, aneuploidy generates new and eventually neoplastic phenotypes autocatalytically because aneuploidy destabilizes the karyotype [2–6].

The hypothesis of cancer predicts preneoplastic aneuploidy [7]. Duesberg and Rasnick recently confirmed this prediction by demonstrating that "aneuploidy precedes and segregates with carcinogenesis." According to their theory, neoplastic aneuploidy differs from non-neoplastic aneuploidy quantitatively and qualitatively; i.e., they postulated an as yet poorly defined threshold for neoplastic aneuploidy [7,8–10]. A progression of minor aneuploidies in preneoplastic lesions to major aneuploidies in cancer cells has since been confirmed [2].

Multiple myeloma (MM) occurs as a result of malignant transformation of plasma cells in the bone marrow. The main manifestations of the disease include lytic bone lesions, pancytopenia, and renal failure. In most patients, a monoclonal immunoglobulin is found in the plasma [11]. A similar protein is found in patients with monoclonal gammopathy of unknown significance (MGUS). These patients are otherwise healthy but develop B-cell malignancies at a rate of about 2% per year. Both diseases share some of the common chromosomal abnormalities, such as 13q deletions, immunoglobulin heavy-chain (IGH) translocations, trisomy 8, and deletion of the long arm of chromosome 20 [12–14]. Thus, MGUS is considered a premalignant condition [14]. In a previous study we found that the rate of asynchronous pattern of replication was significantly higher in MM patients

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compared to a control group, and MGUS patients exhibited this pattern at an intermediate rate [15].

The aim of this study was to assess and compare the random aneuploidy rate of a neoplastic state such as multiple meyloma to a preneoplastic state such as MGUS. We also aimed to estimate, based on the rate of aneuploidy, which MGUS patients are at risk for developing malignancy in the future.

We applied a fluorescence in situ hybridization (FISH) technique on leukocytes of patients with MGUS and MM with α-satellite (centromeres) probes for chromosomes 9 and 18, which were used in our previous studies [16,17].

2. Materials and methods

2.1. Patients

Eight patients with MM (ages 50–87 years) and seven patients with MGUS (ages 55–85 years) were included in this study. They underwent cytogenetic evaluation as a part of their diagnostic workup. Eight healthy, age-matched individuals who had normal karyotypes served as controls.

2.2. Probes

Two direct-labeled commercial α-satellite probes from Vysis (Dowers Grove, IL) were used for chromosomes 9 and 18: CEP 18 α-satellite SpectrumGreen (Vysis no. 32-18032) and CEP 9 α-satellite SpectrumOrange (Vysis no. 32-132009).

2.3. FISH

Peripheral blood (2×10^6 cells/mL) was incubated in RPMI 1640 medium with 20% fetal calf serum. Cells were harvested after 3–4 days according to standard cytogenetics procedures.

Fresh slide spreads were denatured for 2 minutes in 70% formamide/2× standard saline citrate (SSC) at 70°C and dehydrated in a graded ethanol series. The probe mix was then applied to air-warmed slides (30 μL, mix sealed under a 24× 50-mm glass coverslip) and hybridized for 18 hours at 37°C in moist chamber. After hybridization, the slides were washed in 50% formamide/2× SSC for 20 minutes at 43°C, rinsed in two changes of 2× SSC at 37°C for 4 minutes each, and placed in 0.05% Tween 20 (Sigma, Rehovot, Israel). For FISH analysis, the slides were counterstained in 4′,6-Diamino-2-phenylindole (DAPI; Sigma) antifade solution and analyzed for simultaneous viewing of FITC (fluorescein isothiocyanate), Texas red, and DAPI with an imaging processing system (Applied Imaging, Santa Clara, CA).

2.4. Cytogenetic evaluation

To determine aneuploidy in each of the nonsynchronized cell samples, we examined 300 interphase cells separately for chromosomes 9 and 18. In each cell, we recorded the number of hybridization signals. The rate of aneuploidy was inferred from the percentage of cells that had one, three, or more hybridization signals per cell.

2.5. Statistical analysis

The two-sample *t*-test and nonparametric test were applied for testing differences between the study groups for quantitative parameters. All tests were two-tailed, and a *P* value of 0.05 or less was considered statistically significant. We used Microsoft Excel software.

3. Results

The mean results of the aneuploidy rate for chromosomes 9 and 18 in the different study groups are detailed in Table 1.

The aneuploidy rate with an α-satellite probe for chromosome 9 was as follows (Table 2): in both study groups (mean of MM = 9.5 and of MGUS = 10.3) the monosomy rate was significantly higher than in the control group (mean = 2.6, $P < 0.001$). The total aneuploidy rate (the finding of one, three, or more signals, mean = 2.8) was significantly higher in both study groups (mean of MM = 10.4 and of MGUS = 10.6) compared to the control group ($P < 0.05$). The difference in the aneuploidy rate with an α-satellite probe for chromosome 18 was as follows (Table 3): in both study groups (mean of MM = 6.1 and of MGUS = 10.2) the monosomy rate was significantly higher than in the control group (mean = 3.0, $P < 0.02$). This rate was significantly higher in the MGUS patients than in patients with MM ($P < 0.05$). Three or more signals were found significantly more in patients with MM (mean = 1.62) compared to controls (mean = 0.17, $P = 0.035$). This rate approached significance when compared to patients with MGUS ($P = 0.059$). The total aneuploidy rate was significantly higher in both study groups (mean of MM = 7.7 and of MGUS = 10.44) compared to the control group (mean = 4.17, $P < 0.05$, Table 2). The proportion of the aneuploidy rate of the different MGUS patients is shown in Table 4.

4. Discussion

We found a higher "random aneuploidy" rate in the groups of MGUS and MM patients compared to healthy

Table 1
The mean rate of aneuploidy in the study and control groups

Group	Probe	One signal	Two signals	Trisomy and more
Control	9	2.62 ± 1.19	97.33 ± 1.15	0.17 ± 0.36
MGUS	9	10.28 ± 2.88	89.4 ± 2.86	0.28 ± 0.49
MM	9	9.45 ± 3.77	89.83 ± 3.47	0.92 ± 0.72
Control	18	2.99 ± 1.48	97.2 ± 1.53	0.166 ± 0.31
MGUS	18	10.23 ± 3.80	89.52 ± 3.80	0.4 ± 0.25
MM	18	6.08 ± 2.89	92.37 ± 3.41	1.62 ± 1.74