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Centrosome amplification as a possible marker of mitotic disruptions and cellular carcinogenesis in multiple myeloma

E. Dementyeva [a], P. Nemec [a], F. Kryukov [a], K.R. Muthu Raja [a], J. Smetana [a], R. Zaoralova [a], H. Greslikova [a], R. Kupska [a], P. Kuglik [a,b], R. Hajek [a,c,*]

[a] University Research Centre, Czech Myeloma Group, Babak Research Institute, Masaryk University, Brno, Czech Republic
[b] Laboratory of Molecular Cytogenetics, Department of Genetics and Molecular Biology, Institute of Experimental Biology, Masaryk University, Brno, Czech Republic
[c] Department of Internal Medicine and Hematooncology, The Faculty Hospital Brno, Brno, Czech Republic

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ABSTRACT

Centrosome amplification (CA) as a potential marker of mitotic disruptions in multiple myeloma (MM) was investigated in two populations of B-cell lineage: B-cells and plasma cells (PCs). Using immunofluorescent staining, it was shown that CA in B-cells is present in $3.2 \pm 2.5\%$ in healthy donors versus $9.9 \pm 7.9\%$ in MM patients ($p < 0.0001$). Based on the calculated threshold value of CA in B-cells, 37% (14/38) of MM patients were positive. There was no significant correlation between CA-positive MM cases (based on PC samples evaluation) and the occurrence of cytogenetic abnormalities in PCs, including del(13)(q14), del(17)(p13), gain(1)(q21) and hyperdiploidy.

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1. Introduction

Centrosomes are small cell organelles composed of two cylindrically shaped centrioles surrounded by pericentriolar material in a normal mitotic cell. The centrosome function is to direct mitotic bipolar spindles in a process that is essential for accurate chromosome segregation during mitosis [1,2]. Centrosomes duplicate once per cell cycle, and each daughter cell receives one centrosome upon cytokinesis [3].

The presence of multiple centrosomes in tumor cells is associated with the formation of multipolar mitotic spindles and faulty chromosome segregation which usually results in aneuploidy of both daughter cells [3]. Genomic instability contributes to the pathogenesis of tumors as it causes gains/amplifications of oncogenes or losses of tumor suppressor genes [4], e.g. multiple myeloma (MM) loss of TP53 on chromosome 17, and loss of RB1 gene on chromosome 13. Centrosome amplification (CA) has been detected in a variety of solid tumors and hematological malignancies including MM [5–10]. Recent studies have shown the presence of CA in all stages of monoclonal gammopathies. Moreover, the centrosome index (CI), which is strongly correlated with the percentage of clonal cells with centrosome amplification, increased significantly from MGUS to MM. It is likely that CA contributes to the accumulation of genomic abnormalities in tumor cells during disease progression in MM [10].

Normal plasma cells (PCs) are terminally differentiated B-cells, and their centrosomes usually cannot be visualized using immunofluorescence labeling [10]. Since PCs are considered to be non-dividing terminally differentiated cells, centrosome duplication or amplification would not be expected to occur under physiological circumstances [11]. We hypothesize that in myeloma cells CA also occurs in an earlier stage of plasma cell development, i.e. B-cells with CA as aberrant precursors of PCs develop into clonal mature malignant plasma cells carrying the same CA.

The objective of our study was to evaluate the presence of CA in two populations of B-cell lineage – including B-cells and PCs – from MM patients and to assess whether it is associated with established prognostic factors, such as chromosomal abnormalities.

2. Materials and methods

2.1. Patients

A total of 70 MM patients treated at the University Hospital, Brno, Czech Republic, were included in the study. Their baseline characteristics are summarized in Table 1. Bone marrow and peripheral blood of MM patients were obtained during routine diagnostic procedures. Seventy patients were evaluated for CA, and 18 of them were evaluated for both B-cells and PCs. For 50 patients, PCs were eval-

* Corresponding author at: University Research Centre, Czech Myeloma Group, Babak Research Institute, Masaryk University, Kamenice 5, Building A3, 62500 Brno, Czech Republic. Tel.: +420 728854226; fax: +420 549498480.
E-mail address: rom.hajek@seznam.cz (R. Hajek).

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Table 1
Clinical and biological characteristics of the studied MM patients.

I. Patients characteristic	n (%)
Sex (n = 70)	
Male	34 (49%)
Female	36 (51%)
Ig isotype (n = 61)	
IgG isotype	32 (53%)
IgA isotype	19 (31%)
IgM isotype	2 (3%)
Free light chains	8 (13%)
ISS stage (n = 52)	
I stage	26 (50%)
II stage	12 (33%)
III stage	9 (17%)
Durie–Salmon stage (n = 57)	
I stage	4 (7%)
II stage	17 (30%)
III stage	36 (63%)
II. Patients characteristic	**Median (range)**
Age (years)	65 (40–84)
Hemoglobin (g/l)	11.1 (7.1–15.7)
β2-microglobulin (mg/l)	2.94(1.02–38.20)
Albumin (g/l)	40.45(22.1–51.50)
C-reactive protein (mg/l)	4.35(0.00–104.40)
Calcium (mmol/l)	2.35(1.98–3.64)
LDH (μcat/l)	3.49(1.79–9.08)
Monoclonal Ig (g/l)	28.65 (0.0–79.8)

uated and for 38 patients B-cells were evaluated for CA. Percentages of PCs and B-cells in bone marrow were 8.6% (0.2–86.1%) and 10.35% (1.0–49.0%) respectively [median (range)]. As a negative control and for the calculation of B-cells CA positivity threshold, CA was evaluated in B-cells from peripheral blood of 20 healthy donors. All healthy donors had normal parameters of peripheral blood. All patients and healthy donors signed informed consent which was approved by the Hospital Ethical Committee.

2.2. Immunofluorescent labeling and image analysis

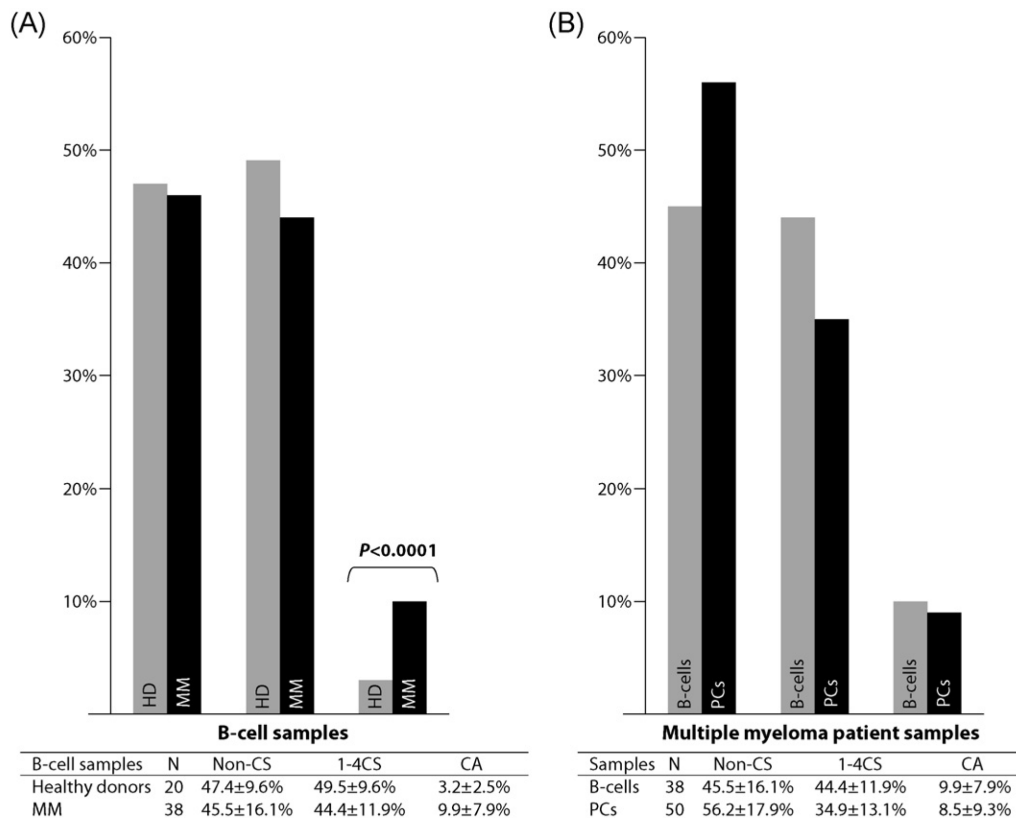
Bone marrow mononuclear cells (BMMC) were isolated using gradient density centrifugation on Histopaque 1077 (Sigma–Aldrich, USA). Cytospin slides for immunolabeling detection of CA in PCs were prepared as follows: approximately 100,000 BMMC were placed on a slide and air dried for 24 h at room temperature. In case of infiltration of PCs in BMMC less than 5%, CD138+ cells were sorted directly on microscope slides by fluorescence-activated cell sorting (FACS) using anti-CD138 fluorescence-labeled antibody (Beckman Coulter, Inc., CA, USA). PCs were then fixed in methanol for 5 min at room temperature. B-cells were isolated from BMMC after CD138+ cells depletion using magnetic cell separation (MACS) utilizing magnetic labeled anti-CD138 antibodies (Miltenyi Biotec, Germany). Early stages of B-cells (CD19+) were sorted directly on microscope slides from CD138-cell fraction by FACS using anti-CD19 fluorescence-labeled antibody (Beckman Coulter, Inc., CA, USA).

Immunofluorescent staining of centrin in B-cells and PCs was performed as previously described with required modification for commercially available antibodies [10]. Centrin, an integral centrosome protein, was selected as the target for determination of centrosome copy number in B-cells and PCs. For PC visualization, immunoglobulin light chain staining (cIg) was used as described previously [12].

One hundred cells were scored per each slide. Up to four centrin signals (representing four centrioles of two centrosomes) can be present in a normal cell depending on the phase of cell cycle. Thus, the presence of more than four centrin signals was chosen as a criterion for CA [13]. According to the centrin copy number, we were able to identify three cell subpopulations: (1) no centrin signal (non-CS), (2) 1–4 centrin signals (1–4CS) or (3) more than four signals of centrin. Samples with more than 10% of PCs with >4 signals of centrin were considered as CA-positive [10]. For B-cells, the threshold for CA positivity was calculated as the mean of the percentage of CA-positive cells detected in healthy donors plus three standard deviations.

2.3. cIg FISH analysis

Interphase FISH with cytoplasmic immunoglobulin light chain staining (cIg FISH) was performed as described previously [12]. Assessment of chromosome abnormalities by FISH was done as previously described by our colleagues [14]. Hyperdiploidy was done by using Multi-Color Probe Panel (LSI D5S23/D5S721, CEP



B-cell samples	N	Non-CS	1-4CS	CA
Healthy donors	20	47.4±9.6%	49.5±9.6%	3.2±2.5%
MM	38	45.5±16.1%	44.4±11.9%	9.9±7.9%

Samples	N	Non-CS	1-4CS	CA
B-cells	38	45.5±16.1%	44.4±11.9%	9.9±7.9%
PCs	50	56.2±17.9%	34.9±13.1%	8.5±9.3%

Fig. 1. Centrin staining and centrosome amplification in (A) B-cells of healthy donors and MM patients and (B) B- and plasma cells of MM patients [mean ± SD].

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