specific enzymatic assay. The localization of polyP in the myeloma cell lines was determined by confocal microscopy. The U266 myeloma cell line was used to study whether extracellular polyP affects Ig secretion and survival. Different human cell lines were used to test the specificity of polyP in viability. We analyzed Ig secretion of PC form Bone Marrow and Peripheral Blood after polyP addition. A conventional tetanus toxoid booster immunization was used to increase PC proportion in order to examine the apoptotic effects of polyP. Ig secretion and Apoptosis was determined by ELISA and FACS respectively. *Results*. Micromolar levels of polyP that is present principally as polymers of 75 phosphate units have been found in the U266 and IM9 myeloma cell lines. PolyP is accumulated in intracellular vacuoles similar to the previously reported platelet dense granules and acidocalcisomes of the unicellular eukaryotes. Addition of polyP to human PC produces an unexpected inhibition of Ig secretion and a stimulation of apoptosis. PolyP generates apoptosis specifically in PC, myeloma (malignant PC) cell lines, and B lymphoid cell lines. Normal B cells, T cells, total blood mononuclear cells, and non-lymphoid cell lines are not affected by polyP. In U266 myeloma cell line, polyP induces the externalization of phosphatidylserine, the activation of caspase-3, and the arrest of the cell cycle. Protective effects of IL-6 do not overcome the polyP-induced apoptosis. *Summary/conclusions*. Taken together, our results and suggest for the first time the relevance of polyP for the humoral immune response and open prospects for polyP as a novel therapy for myeloma.

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METHYLATION STATUS OF THE P57KIP2 GENE IN PATIENTS WITH PLASMA CELL DYSCRASIAS

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Background. Oncogenesis is related to cell cycle deregulation. Aberrant DNA methylation, leading to silencing of regulatory genes, has emerged as one of the most frequent molecular changes in haematological malignancies. The p57KIP2 is a tumor suppressor gene that belongs to the CIP/KIP family of cyclin dependent kinase inhibitors that negatively regulate cell cycle progression. *Aim*. We have studied the methylation status of the promoter region of p57KIP2 gene in patients with multiple myeloma (MM) and Waldenström's macroglobulinemia (WM) in order to correlate the methylation pattern with the disease's phenotype. *Patients and Methods*. We have studied bone marrow and paired peripheral blood samples from 12 consecutive MM patients (9 male, 3 female, age range 50-83, median 59) and 2 consecutive WM patients (1 male and 1 female, age 75 and 47 years).

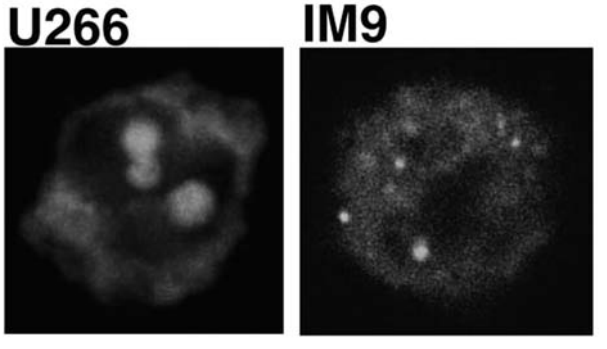

Figure 1. Localization of polyP on U266 and IM9 cells.

Samples from 9/12 MM patients and 2/2 WM patients were taken at diagnosis whereas the remaining 3/12 samples were taken during the course of the disease. Genomic DNA was extracted using standard protocols (Quiamp DNA mini kit). After bisulfite treatment procedure the DNA was PCR amplified with primers specific for the methylated and the unmethylated alleles of the gene. The PCR products were separated on 2% agarose gel. Bone marrow DNA from healthy donors served as negative control. We have also used human male genomic DNA universally methylated for all genes (Intergen Company, Purchase, NY) as positive control. *Results*. Two patients had stage IA disease and did not receive any treatment, five MM patients had stage IIA or more advanced disease and started on VAD chemotherapy, two patients were started on oral melphalan and methylprednisolone, one patient was on plateau, and two patients had progressive disease after having received VAD and were started on bortezomib therapy. One patient with WM was started on cyclophosphamide, dexamethasone and rituximab and the other patient did not receive any treatment. Classical cytogentic analysis was available on 5/12 MM and 1/2 WM patients and the karyotype was reported as normal. All patient samples showed no band corresponding to the methylated allele of the p57KIP2 gene. The band corresponding to the unmethylated allele was clearly visible in all samples. *Conclusion*. To our knowledge this is the first report on p57KIP2 methylation status in patients with plasma cell dyscrasias. Our data show that methylation of p57KIP2 gene is not a frequent event in the patients studied. Further studies are needed to confirm the above results.

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EVALUATION OF THE RELATION BETWEEN ANGIOGENIC CYTOKINES, SELECTED BIOLOGICAL PARAMETERS AND PROGNOSTIC FACTORS IN MULTIPLE MYELOMA

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Background. Multiple myeloma is an unusually heterogenous disease with individually different course, response to therapy and prognosis. Up-to-date diagnostic and stratification systems have, however, an important limitation in their insufficient absorption of those parameters, that express intrinsic biological properties of myeloma cells and the microenvironment of the bone marrow. The aim of this study was to evaluate the relation of 10 biological parameters to 6 substantial prognostic factors in multiple myeloma. *Methods*. The analysed group consisted of 66 persons evaluated at the time of diagnosis, before the start of chemotherapy. For the assessment of serum levels of examinated molecules were used following Methods. REA, RIA, ELISA and the technique of sandwich enzymatic immunoassay, for the assessment of proliferative and apoptotic properties were used propidium iodide (PC-PI) and annexin V (PC-AI) indices evaluated with the help of flow-cytometry. Statistical analysis was carried out using Pearson and Spearman test and/or using U-test according to Mann-Whitney. *Results*. High occurence of abnormal serum level of evaluated parameter was found in the case of S-β-2-microglobulin (95,5%), S-thymidinekinase (57,5%), S-sVCAM-1 (78,5%), S-ICTP (87,0%), S-soluble osteoprotegerin (sOPG 76,5%) , S-sSyndecan-1 (56,5%) and low index of apoptosis of plasma cells (PC-AI, 78%). Correlation analysis (Pearson test) revealed a mutual relationship between serum levels of β-2-microglobulin to sVCAM-1 (r=0,39, *p*=0,002), sICAM-1 (r=0,33, *p*=0,011), sOPG (r=0,53, *p*=0,001), sHGF (r=0,34, p=0,006), sSyndecan-1 (r=0,38, *p*=0,003) and sFas (r=0,42, *p*=0,001); of S-albumin to sVCAM-1 (r=-0,29, *p*=0,036), ICTP (r=-0,33, *p*=0,016), sOPG (r=-0,63, p=0,000), sHGF (r=-0,39, p=0,003) and sSyndecan-1 (r=-0,29, *p*=0,042; of S-thymidinekinase to sSyndecan-1 (r=0,46, *p*=0,000) and sFas (r=0,29, *p*=0,019). In neither of the cases was found the relation of PINP and VEGF to any of the evaluated prognostic factors. There was no relation found between any of the analysed parameters and PC-PI or PC-AI. With the use of U-test there was found a relationship of serum levels of sIL-6R (< > 100IU/l) to β-2-microglobulin (*p*=0,001), albumin (p=0,002) and to PC-PI (*p*=0,046). *Conclusion*: The above study established the possibility to enrich the traditional algorithms used in clinical practice for individual characteristics of MM with the parameters sOPG, sHGF, sSyndecan-1 and sFas.

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COMPARISON OF SERUM LEVELS OF BIOLOGICAL PARAMETERS IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND MULTIPLE MYELOMA

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Background. The presented work is focused upon the evaluation of the differences between serum levels of selected biological parameters in monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM), especially from the point of view of their potential benefit for clinical practice. *Methods*. Analysed group of 96 patients (30 patients with MGUS and 66 patients with MM) was assessed at the time of diagnosis before the start of therapy. For the