extending so the cell survival and favour the acquisition and accumulation of genetic alterations; p-53 is a tumor suppressor protein altered in more than 30% of breast cancers; c-ERB-2 oncoprotein overexpression is associated with a poor prognostic in breast cancers. In this context our study tried to establish some correlations between them in order to evaluate the tumor progression.We studied 98 cases with invasive ductal breast carcinomas with tumors T1and T2, immunohistochemical with MoAb.

Material and methods: We studied 98 patients with T1and T2 DICs obtained from two surgical departments between 1995-1999.We used MoAb for Bcl-2 (clone124 Dako A/S,Denmark), p53 protein (clone DO-7 Dako A/S,Denmark) and anti- c-ERB2 (from Boehringer Mannheim), on paraffin embedded tissues. Each case was independently analysed and the number of positive cells was measured in 20 different fields. The cases were evaluated on a specific scale which contain at least three degrees of intensity (except p53 were we measured the IR score).

Results: 65% from tumors with histological grade I expressed Bcl-2 positive cells, and only 12% of them expressed p53.Tumors with histological grade III expressed 34% Bcl-2 positive cells and 56% p53. From the total number of tumors 43,5% overexpressed c-ERB2, 21% from those which were already positive for Bcl-2, and only 43% for p53.

Conclusions: We consider that all these three proins are very important in evaluating the tumour progression. We noticed a good inverse correlation between tumours which expressed bcl-2 and p53. Anyhow we can observe that c-ERB2 staining is more frequently registered in those tumours which are already altered in p53 expression. Bcl-2 expression is more significant in tumors with histological grades I and II as well as p53. We consider that those two proteins are involved in early events prior to other genetic alterations like c-ERB2 overexpression which is a later step in tumour progression.

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Significance modulation of cell membrane molecules for cell death in hematological cell lines

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Programmed cell death (apoptosis) is essential for normal development and for maintenance of cellular homeostasis in multi-cellular organisms. Apoptosis is regulated tightly by a number of gene products that promote of cell death or extend cell survival, but cytokines produced from NK cell and activated cytotoxic lymphocytes have possibility to induced apoptosis in target tumor cells. TNF as a one from these produced cytokines represents a major mediator of immunlogycal and pathophysiological reactions after engaging appropriated receptors. TNF-α in vitro, either suppresses the growth of some myeloid cell lines or stimulates the growth of some lymphoid cells. Since TNF-α make diverse effects on hematopoietic cells, evaluation of role of some cell membrane surface associated antigen expression on target cells was very important for understanding TNF-α effects following cell death or cell activation process.

In this work we analyzed in vitro TNF-α effects on K-562 (eritroleukemic), Raji (malignant B cell line) and PC (originally developed cell line at Institute of Oncology in Sremska Kamenica) cell line prelabeled with CD45 and CD95 monoclonal antibodies in comparison to untreated cells. Cell were incubated with and without TNF-α in final concentration of 500 and 1000 pg/ml of culture medium for short time period (30 min) and apoptotic and necrotic form of cell death were determined after 2, 4, 6, and 24 h by Flow cytometry (Becton Dickinson). Before and after treatment cell membrane antigen expression were detected on gated cell population. Cell viability was determined by SRB assay simultaneously.

Our results showed that in comparison with untreated cells, TNF-α induced significantly increase in apoptotic and necrotic forms of cell death in Raji and PC cells. The results for apoptotic form of cell death, induced by TNF-α on PC cells pre-labeled with anti CD95 MoAb, correlated with TNF-α effects alone at the same points, while cell death were significantly decreased after 24 h contrary to its effects on Raji cells. In contrast to this, TNF-α induced necrotic forms of cell death between 6 h and 8 h on PC and Raji cells incubated with anti CD45 MoAb, which is a significant increase compared with the effect of TNF-α alone. Results also shows that TNF-α in dose dependent manner significantly decrease cell membrane expression on K-562, Raji and PC cells for evaluated antigens, for more then 2 folds, calculated from basal values expression. Further analyses shows that antigen expression did not correlated with cell death process in all examined cell lines. Decrease of some antigen expression, which partly TNF receptor superfamily members, after TNF-α treatment suggested their in-effectively for induction of apoptotic process but more for activation of the necrotic process. Since modulations of activity TNF-α receptor superfamily was involved in regulation of cell proliferation, cellular activation and differentiation, including control of cell survival or death by apoptosis or necrosis we concluded that CD45 and CD95 participated in transduction of signals from cell surface but with different effects in examined hematopoietic cells.