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THE ROLE OF HSP72 IN VIVO - A SCID MOUSE MODEL

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We demonstrated that an HSP72 cell surface expression is inducible by physical (heat) as well as by chemical (etherlipids) means on sarcoma and on leukemic cells. Some carcinoma cells exhibit an HSP72 cell surface expression under physiological conditions. The inducible and non-inducible HSP72 cell surface expression on tumor cells correlates to an increased sensitivity to lysis mediated by non-MHC restricted natural killer (NK) cells.
In an effort to study the role of HSP72 as a tumor-specific recognition structure in vivo we generated autologous carcinoma sublines by cell-sorting. These tumor sublines exhibit an identical MHC and adhesion molecule expression pattern and differ only with respect to the capacity to express HSP72 on the plasma membrane. Since this cell surface expression pattern remains stable for all further cell passages these autologous tumor-sublines provide an ideal tool to test the tumorigenicity in SCID mice. Characterization of tumor biopsy material derived from SCID mice that were injected with HSP72 expressing and non-expressing tumor sublines revealed that the tumorgrowth was comparable and the cell surface expression pattern remained stable, in vivo. Adoptive intraperitoneal transfer of different human NK sublines into tumor-bearing mice are in progress to study the role of HSP72 as a tumor recognition structure in vivo.

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BIOCHEMICAL MECHANISMS REALIZATION OF ANTITUMOR ACTIVITY OF UKRAIN

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We have studied the biochemical mechanisms realization of antitumor activity of Ukrain on 280 male and female Vistar rats with implanted tumors (W-256, sarcomas M-1 or M-45, hepatocellular cancer) . The administration of the drug was started after 3-7 days following the subcutaneous inoculation at a dose of 0,02 mg/kg i.p. or i.v. for 7-14 consecutive days depending on the type of the tumors. The results have been evaluated the tumor weight, survival rate, histochemical and electron microscopy examinations, biochemical alterations (effects of Ukrain on formation of pool of free amino acids in liver, blood plasma and tumors, on the protein synthesis, glycolysis and glycogenesis, on the activities of the processes governing activity in the tricarboxylic acid cycle, rates of substrates, coenzymes).

The above presented observations indicate that Ukrain, beside a local malignotoxic effect, may influence positively the development and regulation of amino acids and protein metabolism, contrary to other chemotherapeutics and radiotherapy that are destructive. This observation from the biochemical point of view corresponds to an enhanced proteolysis. The application of Ukrain also decreased the NAD/NADH ratio, an activation of glycogenesis and proteolysis in tumor tissue as well as a favorable effect of the drug on amino acid pool formation in blood plasma of rats.

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Generation and immortalisation of a tumor specific cytotoxic T cell line from a healthy donor

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Recently, several human tumor antigens have been identified which can be recognized CTL. The glycoprotein mucin, encoded by the gene MUC1, is such a tumor antigen expressed on breast and pancreatic carcinoma cells. The high density of this molecule on the cell surface and its perfect tandem repeat structure can lead to a recognition of mucin by human cytotoxic T cells (CTL) in an MHC unrestricted manner. To study this special mucin-CTL interaction we established a cytotoxic T cell line. For this purpose, B cell lines were generated by infection of primary human tonsillar B cells with a mini-EBV-MUC1 plasmid packaged into an EBV coat. After treatment with a glycosylation inhibitor, these cells were used to stimulate peripheral blood leucocytes (PBL) from healthy donors. The PBL were stimulated eight times in intervals of 7-10 days. We were able to establish a cytotoxic T cell line from a healthy donor demonstrating the possibility to prime naive T cells against tumor cells by this approach. The T cells are either CD4+ or CD8+, and ab TCR+. They lyse different mucin expressing tumor cell lines independent of their HLA-phenotype. This effect could be blocked with mucin core peptide specific antibodies. These T cells have been immortalized by infection with Herpesvirus saimiri. Currently, the T cell line is used to analyse the T cell receptor repertoire.

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Liposome-mediated gene transfer into human dendritic cells

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Dendritic cells transfected with cDNA of tumor antigens are candidates for tumor vaccines to induce a cellular immune response. An efficient gene transfer into dendritic cells which is easy to handle is a prerequisite for the use of such kinds of vaccines in clinical trials. We evaluated gene transfer methods into human dendritic cells using different kinds of liposomes. As an example for a tumor antigen we used the glycoprotein mucin, encoded by the gene MUC1. Due to an underglycosylation of tumor cells mucin core protein epitopes are exposed to the immune system and can be recognized by cytotoxic T-cells in an MHC unrestricted manner. Dendritic cells were obtained from human peripheral blood using IL-4 and GM-CSF. We evaluated the following cationic liposome preparations for their toxicity and transfer efficacy: Lipofectin, DOTAP and DMRIE/DOPE. Lipofectin consistently gave the best results. After treating the transfected cells with a glycosylation inhibitor they exposed high levels of the relevant mucin epitopes. The transfection rate, as determined by flow cytometry using three different mucin core protein specific antibodies, ranged from 30 to 60%. The expression of the epitopes was stable for at least 96 hours. Ou results show that cationic liposomes are a useful mean to efficiently transfect human dendritic cells.