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UP-REGULATION OF SURFACE CD95 FOLLOWING γ-IRRADIATION OF $p53^{WILDTYPE}$ TUMOR CELLS, BUT NOT $p53^{MUTANT}$ OR $p53^{(-/-)}$ CELLS.

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Signals received through CD95 can induce apoptosis in sensitive cells. Surface expression of CD95 can be enhanced by treating cells with interferon-γ or tumor necrosis factor-α. However, given the potential utility of this molecule in inducing tumor cell death, a more comprehensive understanding of the mechanisms regulating CD95 expression is needed. Our goal is to investigate the induction of surface CD95 expression by human tumor cells. Since the p53 tumor suppressor gene product is reported to have transcription-enhancing activity for the *cd95* gene, we have studied cell surface expression of CD95 on γ-irradiated tumor cell lines expressing either wildtype, mutant, or no p53. All but one of the cell lines studied expressed low to intermediate levels of CD95 constitutively. Two $p53^{wildtype}$ lines responded to γ-irradiation by up-regulating surface levels of CD95. Up-regulation occurred coincidentally with a G1 cell cycle arrest. Conversely, all of the seven examined $p53^{(-/-)}$ and $p53^{mutant}$ cell lines failed both to up-regulate surface CD95 expression and to arrest at G1. These findings support the hypothesis that wildtype p53 activity is neccessary for up-regulation of CD95 following γ-irradiation, and suggest that mutations in the *p53* gene may eliminate the propensity of tumor cells to up-regulate CD95 expression after treatment with ionizing radiation. Given that: i) tumor-specific CTL and NK cells are able to deliver signals through surface CD95, ii) p53 is often mutated in tumor cells, and iii) clinical regression of tumors after radiotherapy is sometimes a prolonged event, it is possible that disruption of p53 activity may contribute to a radiotherapy-resistant phenotype by eliminating irradiation-induced up-regulation of surface CD95.

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OVEREXPRESSION OF erbB2/neu ONCOPROTEIN AND FAS LIGAND DEATH SIGNALS IN BREAST CANCER.

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Data are despatched in these days about the possibility that Fas Ligand (CD95L/FasL)-bearing tumor cells may prompt activated Fas(+) T cells to commit suicide(apoptosis) (1). In their counterattack, tumors are safe from their own weapons either because they do not recieve FasL death signals (i.e tumor cells express little or not Fas) (2) (3) or because their Fas receptor signalling pathway is unresponsive to FasL cross-linking (4). Here, we investigated the modulation of Fas/FasL system in a transgenic(erbB2/neu overexpressing) model of breast cancer. Immunohistochemistry showed that wherease mice tumor-free breast expressed comparable amounts of both Fas and Fas L antigens, a Fas unbalanced-FasL expression was clearly discerned in malignant lesions. In vitro apoptosis of Fas-sensitive L1210 cells occurred either upon plating with carcinoma cryosections or with tumorogenic cell lines isolated from the same tumor. The possibility that erbB2 upregulated FasL was substained by Western blotting of transgenic cancer cells and of mouse fibroblasts transformed with an intact human-erbB2 protooncogene. Thus, beside to cause malignity, we propose that erbB2 overexpression may contribute to the immunoprivilege of breast cancer.

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STUDY OF MECHANISM OF APOPTOSIS INDUCTION BY UKRAIN *IN VITRO*

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Aim: To study the ability of Ukrain to induce apoptosis and affect protein secretion in different cell lines, the influence of Ukrain *in vitro* on DNA-nicking enzymes of human lymphocytes (Me^{2+}-dependent endodeoxy-ribonucleases and topoisomerases I and II) and thrombocyte function. Methods: Several cell lines of different origin were used: chinese hamster ovarian cell line (CHO), several hybridoma lines, murine mammary gland sarcoma cells nonmetastatic (CSML) and metastatic (CSML-100), and some other. Cells were treated with Ukrain as well as with Etoposide in a range of 1-100μg/ml. For the detection of the activity level of apoptosis enzymes in lymphocytes of cancer patients PCR-EIA technique was used. The reaction of thrombocytes release was employed for the study of human thrombocyte function.
Results: Ukrain possesses strong cytostatic activity and causes cell death via apoptosis within the same concentration range as Etoposide. An unusual feature of Ukrain action is an inhibition of protein secretion by cells within first hours of their exposition to the drug. Ukrain inhibited topoisomerases I and II and activated Me^{2+}-dependent endodeoxyribonucleases of lymphocytes of cancer patients. Ukrain was non-toxic for thrombocytes in contrast to Etoposide. The combined treatment of cells with Ukrain and Etoposide demonstrates synergistic effect at lower concentration of both agents. Conclusion: Ukrain induces apoptosis in vitro in number of cell lines. Its efficiency is higher towards all sensitive cells than that of Etoposide. The mechanism of apoptosis induction by Ukrain is inhibition topoisomerases I and II and activation Me^{2+}-dependent endodeoxyri-bonucleases. Due to observed synergistic effect of Ukrain and Etoposide composition of both drugs could be recommended to decrease effective doses for cancer treatment.