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EFFECTS OF RETINOIDS IN HUMAN PROSTATE CANCER CELL LINES: INDUCTION OF APOPTOSIS AND INHIBITION OF CELL MOTILITY

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INTRODUCTION AND OBJECTIVES: Retinoids are natural or synthetic derivates of vitamin A. They are known inhibitors of carcinogenesis in epithelial tumors. We investigated the effects of the natural retinoid *13-cis* retinoic acid (13cRA) and the synthetic retinoid 4-hydroxyphenylretinamide (4-HPR) on cell motility and induction of apoptosis in the prostate carcinoma cell lines LNCaP, DU145 and PC3.

METHODS: Cell motility was evaluated in 48-well micro-chemotaxis chambers with polycarbonate membranes (pore size 8 µM) coated with collagen III. Cells were incubated 6 days with 13cRA (1-75 µM) or 4-HPR (0.001-20 µM). Apoptosis was assessed after incubation of cells with retinoids by PI-staining and DNA-flowcytometry as well as by the Annexin-V method.

RESULTS: Apoptosis was induced by both retinoids in all 3 cell lines in a dose- and time-dependent manner yielding an inhibition of cell growth up to 95%. Maximum apoptosis was achieved in LNCaP after 144h, in DU145 and PC3 after 120 h, respectively. In LNCaP, higher values for maximum apoptosis (86±8%) were detected than in DU145 (58±5%) or PC3 (57±5). Regarding cell motility, in LNCaP no motility was observed while DU145 and PC3 showed an active motility. Motility of these two lines was inhibited in a dose-dependent manner by both retinoids:

Retinoid	Cell line	IC50 (µM)	IC100 (µM)
13cRA	DU145	18	30
	PC3	22	75
4-HPR	DU145	0.2	5
	PC3	0.1	5

Compared with 13cRA concentrations of 4-HPR yielding half maximum (IC50) and complete (IC100) inhibition of cell motility were significantly lower.

CONCLUSIONS: Besides their effect on inhibition of cell proliferation, retinoids showed substantial effects on induction of apoptosis and inhibition of cell motility in prostate cancer cell lines. 4-HPR seems to be more effective than 13cRA. Due to the more favourable toxicity profile of 4-HPR, this interesting compound may be tested in clinical trials either alone or in combination therapy approaches.

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Analysis of proapoptotic tumor suppressor function of BARD1 (BRCA1 Associated RING domain protein) in rat ovarian cancer model

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(a) Introduction The BRCA1-associated RING domain protein BARD1 is a putative tumor suppressor of breast and ovarian cancers, thought to act in conjunction with the breast cancer gene BRCA1 (1, 2). While BARD1's accessory role to BRCA1 is consistent with co-expression of both genes, BARD1 expression in cells devoid of BRCA1 is suspicious of a BRCAI independent function of BARD1 (3). ***(b) Experimental design***: To test this hypothesis BARD1 is overexpressed or repressed in vitro, and the cellular response to cellular stress or mutagens in relation to BARD1 expression levels is analyzed. To test the tumor suppressor function of BARD1 in vivo, the tumorigenicity of ovarian cancer cells (4) (NuTu-19) lacking or expressing BARD1 is determined after injection into immuno-competent mice. NuTu-19, when injected intraperitoneal into Fischer rats 344 develop into tumors within three weeks. ***(c) Results***: Overexpression of BARD1 in vitro induces cell death with all features of apoptosis in several cell types. BARD1 repressed cells are defective for the apoptotic response to DNA damaging agents. NuTu-19 cells do not express BARD1 and apoptosis induction upon stress is retarded in this cell line. Exogenous expression of BARD1 partially restores the apoptotic response. It can be expected, based on preliminary experiments, that increasing the expression level of BARD1 in NuTu-19 cells should lead to inhibition, delayed and/or reduced tumor growth. ***(d) Conclusions:*** Several tumor suppressor genes have been identified that when mutated predispose the carrier to breast and ovarian cancer. The functions encoded by these genes affect the cellular defense mechanisms such as DNA repair and apoptosis. BARD1 plays a role in BRCA1 dependent DNA repair and, based on our data, in apoptosis in response to genotoxic insults; high expression levels of BARD1 render cells more susceptible to apoptosis inducing drugs while BARD1 repressed cell become resistant to drug treatment. Therefore the expression of BARD1 should protect from, or lead to delayed tumor formation by NuTu-19 cells. Depending on the outcome of the ongoing study BARD1 could be an important factor in the design of future therapies. (1) Wu, L. C., Wang, Z. W., Tsan, J. T., et al, (1996). Nat Genet 14,430-40. (2) Hao Thai, T., Du, F., Tsan, J.T., et al. (1998) Hum Mol Genet 7:195-202. (3) Irminger-Finger I., Soriano, J.V., Montesano, R., et al. (1998). J Cell Biol. 143, 1329-1339. (4) Major AL, Rose GS et al. (1997)

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EFFECTS OF CELEBREX AND ZYFLO ON LIVER METASTASIS AND LIPIDPEROXIDATION IN PANCREATIC CANCER IN SYRIAN HAMSTERS

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Although selective inhibition of eicosanoid synthesis is supposed to have effects on carcinogenesis it is still unknown, whether pancreatic cancer might be influenced as well. Therefore we evaluated the impact of selective cyclooxygenase-2 inhibitor Celebrex and selective 5-lipoxygenase inhibitor Zyflo on liver metastasis in a solid model of chemically induced pancreatic adenocarcinoma in Syrian hamster. In week 33 animals were sacrified and incidence of pancreatic carcinomas and liver metastases was determined. Furthermore, number and size of liver metastases were measured. Biochemically, activities of antioxidative enzymes and concentration of products of lipidperoxidation were determined in liver metastases and non-metastatic hepatic tissue. The incidence, number and size of liver metastases were decreased by combined therapy of Zyflo and Celebrex. Furthermore activity of antioxidative enzymes was increased and concentration of lipidperoxidation was decreased in non-metastatic hepatic tissue. Accordingly combined therapy increased lipidperoxidation in liver metastases. Thus the combination of Celebrex and Zyflo might be a new concept in advanced pancreatic cancer to decrease tumor growth in liver metastases.

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Differentiation of Promyelocytic Leukemia: Alterations in Fas (CD95/Apo-1) and Fas Ligand Expression

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Prolonged survival of leukemic blasts contributes to the pathological mechanism of acute promyelocytic leukemia (APL). Whilst treatment of APL with retinoic acid (RA) is a model of differentiation therapy, little is known about possible effects of this treatment on the Fas/FasL system. Investigation of APL cells from patients undergoing differentiation therapy with RA and of HL-60 and U-937 cells cultured with RA resulted in a reduction of surface expression of both Fas and its ligand. Accordingly, the sensitivity of the cells to anti-Fas induced apoptosis decreased proportionally and the reduced expression of FasL resulted in a decreased ability of the leukemic cells to induce apoptosis in T cells. Our findings demonstrate that there are significant changes in Fas and FasL expression during RA treatment of APL, which likely have consequences for the interaction between host immune and leukemia cells and thus may be involved in the beneficial effects of differentiation therapy.