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INTERMITTENT DHT ADMINISTRATION ENHANCES EFFECT OF DOCETAXEL IN A XENOGRAFT MODEL BY MODULATION OF ERβ, AR AND NEK2

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Introduction & Objectives: Estrogen signalling in advanced prostate cancer is still unclear. Estradiol (E2) and metabolites of DHT can selectively activate ERβ , an estrogen receptor which proposed functions also include antiproliferative action. The aim of this xenograft model was to elucidate the function of ERβ in a chemotherapy regimen with docetaxel and Estradiol E2 (high and low dose – on/ off schedule) and DHT and its effect on tumour growth , expression of steroid receptors and a mitotic kinase, Nek2.

Material & Methods: Tumours were induced in 6 weeks old NMRI nude mice by injection of LnCaP cells (2x10 ^{6} million cells) in both flanks of the animals. Mice bearing tumours of > 200 mm^3 were castrated, randomized in 5 groups and treatment with Docetaxel ip (40 mg/kg), Docetaxel and Estradiol in on-off schedule (High-dose: 1mg/kg or low-dose: 0.1mg/kg s.c., one week on, one week off) or Docetaxel and DHT (1mg/kg – on schedule weekly) started when tumours were considered as androgen independent. Mice were sacrificed when they were compromised by excessive tumour load. Tumours were harvestd and immunohistochemical staining for ERβ, AR, PSA, Ki-67 and Nek2 performed

Results: Tumour growth was 2 fold increased in the high- dose E2 and low -dose E2 treated group compared to the control group. Interestingly, combination therapy of DHT and docetaxel caused a significant reduction of tumour volume (30-50 %) and tumours < 250 mm^3 disappeared. Tumour size in docetaxel treated mice could only be stabilized over 2 cycles. Immunohistochemical staining showed a correlation of ERβ expression , expression of Nek2 and AR in high- dose E2 treated mice and DHT treated mice.

Conclusions: Combination of docetaxel and intermittent DHT application reduced tumour volume significantly in this animal model. DHT might promote chemosensitivity to docetaxel by complex modulation of ERβ, AR and mitotic kinases like Nek2.

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INVOLVEMENT OF THE ESTROGEN RECEPTORβ IN GENISTEIN-INDUCED EXPRESSION OF P21WAF1/CIP1 IN PC-3 PROSTATE CANCER CELLS

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Introduction & Objectives: Dietary genistein, a phytoestrogen derived from soybean, has been suggested as a chemo preventive agent for prostate cancer. Genistein has been reported to exert its anticancer effects via a variety of functional pathways, but the upstream signalling of molecules regulated by genistein remains unclear. In this study, we investigated whether ERβ was involved in genistein-induced expression of cell cycle inhibitors in PC-3 prostate cancer cells.

Material & Methods: Cell proliferation of PC-3 exposed to genistein was measured by WST-1 proliferation assay. The expression of p21, p27 and ERβ in PC-3 cells was assessed by quantitative real-time reverse transcription-PCR. ERβ silencing was performed using a small interfering RNA (siRNA). The transcriotional activity of p21 promoter was determined by the luciferase reporter assay.

Results: Genistein caused marked inhibition of proliferative activity and induced the expression of p21 and ERβ in PC-3 cells. The siRNA against ERβsuppressed the genistein-induced expression of p21 and reduced the transactivation activity of p21 promoter induced by genistein.

Conclusions: ERβ was involved in genistein-induced expression of p21 in PC-3 cells.

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BENEFICIAL EFFECTS ON LNCAP PROSTATE CANCER CELLS DERIVED FROM HISTONE DEACETYLASE INHIBITOR TREATMENTS

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Introduction & Objectives: The benefit of histone deacetylase inhibitors (HDACi) in oncology is currently under intense investigation and already has been subjected to several clinical trials. We recently introduced the HDACi valproic acid as an useful drug in prostate cancer treatment. Our ongoing research focuses on the identification of HDACi eligible for future clinical use. The aim of this study was to present the effects of carbama-zepine (CBZ) on the expression of genes relevant in tumour cell proliferation, apoptosis and invasion.

Material & Methods: Expression analyses in CBZ-treated LNCaP prostate cancer cells were performed by real time RT-PCR (Bio-Rad iCycler and software). Tumour cell viability and proliferation were assessed by AlamarBlue- and BrdU-assays.

Results: Treatments with CBZ depending on concentration induced the expression of several genes which has been silenced in LNCaP prostate cancer cells. mRNA expression of estrogen receptor-β and IGFBP-3 were up regulated. Concomitantly, down-regulated expression was observed for the androgen receptor co-activator PDEF, for PSA and DD3. In addition CBZ decreased viability and proliferation of LNCaP cells.

Conclusions: HDACi CBZ at moderate concentrations affects the expression of highly relevant factors, rectifying their aberrant expression status in prostate cancer. The estrogen receptor-β, a putative tumour supressor and IGFBP-3, an inhibitory factor of the IGF-axis are increased in expression. In contrast, the expression of PDEF is down-regulated after treatment with CBZ which consequently affects markers of prostate cancer PSA and most probably DD3. These beneficial effects of CBZ widen the scope for HDACi-treatments of prostate cancer adding another established drug with known and manageable side-effects.

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AN EXPERIMENTAL STUDY IN NUDE MICE ON THE ELECTROMAGNETIC DIAGNOSIS OF TUMOURS

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Introduction & Objectives: Tissue resonance interference is a new technique for the electromagnetic diagnosis of human neoplasm. Preliminary data were published on the diagnostic accuracy of the TRIMprob in the prostate and breast tumours. No experimental data on any animal model are available. Following to a preliminary feasibility study conducted in June 2006, aim our research was to confirm the diagnostic capacity of the TRIMprob in the mouse model of prostate and breast cancer. Primary objective was to measure signal intensity at 465, 930 and 1395 MHz in control animals and in mice implanted with breast and prostate cancer at different stages of development.

Material & Methods: Thirty nude mouse (15 males and 15 females) (Charles River, Wilmington, MA, USA) were stabilised in our animal facility for a week. Mice were then marked for further identification and examined with the TRIMprob. All measures were performed in triplicate. Every week, for 3 weeks, a group of 5 male mice were then injected with 10^5 cells of PC3 and 5 female mice CG5 cell lines suspended in HBSS (50 microL) in both the left tight (intramuscular) and in the right shoulder (subcutaneous). Four weeks later all animals were anaesthetised and were examined again with the TRIMprob. Tumour size was measured in all animals using a calliper. At week 5, subcutaneous tumours were excised and electromagnetic test performed again. All animals were then sacrificed, all tumours were excised, fixed and processed for histology.

Results: Comparison of TRIMprob data in mice with prostate cancer and breast neoplasm showed no difference between the two tumours so data of the two neoplasms were pooled together. Comparison of paired data obtained in each mouse before and after intramuscular tumour implant showed a significant reduction of signal intensity was measured at 465, increase of signal intensity was observed at 930 and 1395 MHz (*paired Student's t-test).

Frequency (MHz)	Signal intensity (0-255)			*p<
Baseline	3 weeks after implant	Difference		
465	154.33 ± 14.04	64.20 ± 36.83	-90.13	0.000
930	95.90 ± 35.78	137.23 ± 21.90	.41.33	0.000
1395	128.43 ± 16.32	117.73 ± 20.44	-10.7	0.052

Similar results were obtained in the analysis of subcutaneously implanted tumours. In the prostate cancer model, significant correlation was found between size of the tumour implanted i.m. and both the signal intensity at 465 MHz (correlation coefficient 0.682, p< 0.005) and signal reduction from baseline to 4 weeks (0.634, p<0.011). Analysis of TRIMprob signal in control mice showed no change over the study period due to aging

Conclusions: These data provide the first experimental evidence that electromagnetic diagnosis of cancerous tissue by tissue reference interaction with TRIMprob is feasible in the mouse model. Our data confirm data previously published by our group and other in the peer review literature, foster new collaborative research effort in this area and open a new perspective in cancer diagnosis.