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Altered expression of heat shock protein 110 (HSP110), hypoxia up-regulated 1 (HYOU1) and translationally controled tumor protein (TCTP) during colorectal cancer progression

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Prognosis of the colorectal cancer (CRC) patients depends on the extend of disease and possibility of curative surgical intervention which is feasible only for patients with disease limited on primary tumor and regional lymph nodes. Spread of the cancer to the lymph nodes is crutial factor for progression and therapeutic management of the disease. We suppose, that analysis of gene expression profiles of CRC patiens by low-density cancer-focused oligonucleotide arrays enable us to identify a new predictive markers of the extend of disease and lead to better understanding of CRC progression. Forty patients who had histologically confirmed colon adenocarcinoma with a volume fraction showing at least 70% of malignant tumor cells were included. Only patients with no prior chemotherapy or radiotherapy were eligible for the study. Total RNA was extracted from each frozen tumor and paired non-tumoral adjacent mucosa stored in RNAlater immediately after surgical resection. Relative expression levels of 440 genes known to be involved in cancer biology were obtained by low-density oligonucleotide microarrays from 22 samples (11 cases of IUCC stage II, 11 cases of IUCC stage III) with the highest RIN (RNA integrity number). Analysis of gene expression data based on SAM (Significance Analysis of Microarrays) and t-test methods identified 3 genes (HSP110, HYOU1, TCTP) significantly up-regulated in primary tumors of patients who developed lymph nodes metastasis. HSP110 and HYOU1 are molecular chaperones and play role in adaptation of neoplastic cells to hypoxia-induced stress connected with imperfect vascularity of the tumors. We have shown, for the first time, that up-regulation HSP110 and HYOU1 expression is associated with lymph nodes involvement in CRC. We validated differences in expression of HSP110 on the group of forty patients in all clinical stages by more precise TaqMan technology and Real-Time PCR method. We identified highly significant up-regulation (mean 50x) of HSP110 expression in colorectal tumors compared to adjacent non-tumoral mucosa ($p<0,0001$). We observed also correlation of HSP110 and tumor stage but this association was not significant. At the moment, validation of the rest of identified changes in gene expression is undergoing in our lab by more precise quantification method on the mRNA level and also on protein level by immunohistochemistry. Our preliminary data suggest important role of HSP110 in colorectal cancer pathogenesis. Supported by IGA MZ CR NR/9076 – 4 and project MZ0MOU2005

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A dietary phytochemical cocktail impedes prostate cancer growth in vitro and in vivo

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Background: Phytochemicals have been reported to interfere with specific stages of the carcinogenic process [1]. Some of these phytochemicals like curcumin induce apoptosis and cell cycle arrest in prostate cancer cells [2] while green tea has been shown to inhibit prostate cancer development and distant site metastasis in TRAMP mice [3]. Similarly, resveratrol has also been associated with inhibition of various cancers. Based on the premise that a combination of phytochemicals in their dietary form is likely to be as effective as individual molecules in reducing cancer cell proliferation, we investigated the in vitro and in vivo effects of a dietary cocktail, Blueberry Punch (BBP; Dr Red Nutraceuticals Pty Ltd, Queensland, Australia), that incorporates the above mentioned phytoconstituents, on prostate cancer growth.

Materials and Methods: BBP ingredients include fruit juice concentrate (blueberry, red grapes, raspberry and elderberry), grape seed and skin extract, citrus skin extracts, green tea extract, olive leaf/olive pulp extracts, tarragon, turmeric and ginger. Cell growth and DNA synthesis were assessed by MTS and ^{3}H-thymidine incorporation assay respectively. Protein levels were evaluated using Western blotting and immunohistochemistry. For in vivo studies, immunodeficient nude Balb/C mice were inoculated with 5 x 10^5 prostate cancer cells (PC3) and treatment commenced when the tumors reached between 150-200mm^3. Tumor volumes and body weights were monitored twice per week.

Results: Prostate cancer cell (PC3, LNCaP) growth showed a dose-dependent reduction compared with untreated cells after 72 hours of exposure to increasing concentrations (0.08% - 5%) of BBP and this reduction in cell growth was due to decreased DNA synthesis. Non-cancer prostate epithelial cells (PrEC) exposed to same concentrations of BBP demonstrated resistance in terms of cell growth reduction. Exposing cancer cells to varying concentrations of mannitol indicated that the observed effects on cancer cell growth were independent of osmolarity. PC3 cells exposed to BBP showed decreased levels of COX-2, phospho-cytosolic phospholipase A2 and Cyclin D1 protein. Tumor bearing mice (n = 8/group) were administered BBP (10%) in drinking water for two weeks. At two weeks of treatment the tumor size showed a significant decrease (p = 0.004, by two-way ANOVA with repeated measures) compared with mice (n = 8) that were administered regular tap water. Immunostaining for Cyclin D1 indicated decreased protein in xenografts from mice administered BBP.

Conclusions: BBP shows suppressive effects on prostate cancer cell growth both in vitro and in vivo. Further studies to determine the mechanistic pathways involved in the inhibition of cancer cell growth are in progress.

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Characterization of dose-dependent metabolic changes in melanoma cells after irradiation in vitro and in vivo

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Background: Although irradiation is a powerful tool for the therapy of solid tumors, single cells often elude this treatment and constitute a basis for recurrence of the primary tumor and formation of metastases. This rises the question if irradiation-dependent metabolic changes could be responsible for a predisposition of certain cells to show enhanced survival and migratory activity. The aim of our study was to investigate metabolic properties of irradiated melanoma cells and thus to develop and validate appropriate in vitro and in vivo models to characterize new radiopharmaceuticals for diagnosis and therapy of metastases and solid tumors.

Materials and methods: We applied different single-dose X-ray irradiation (1, 2, 5, 7, 10, and 20 Gy) to murine B16-F10 melanoma cells. At particular times we analyzed cell viability, growth properties, clonogenic regrowth capability, cellular proliferation, and expression of cell cycle markers. Furthermore, we analyzed the cellular uptake of the radiotracers 2-[^{18}F]Fluor-2-desoxy-D-glucose and 3-O-Methyl-[^{18}F]fluor-L-DOPA, providing information about the glucose and amino acid metabolism before and after irradiation. Additionally, we performed in vivo studies in a syngeneic mouse model to analyze the capability of untreated and irradiated cells to form lung metastases.

Results: In a dose-dependent manner we detected a decrease in the viability, growth properties and tracer uptake of the melanoma cells. These findings appeared particularly in the period 3 to 6 days after irradiation. In contrast, already one day after irradiation cell cycle analyses showed an increase in the number of G2/M phase cells and the expression of G2-phase markers in irradiated compared to untreated cells. Further we demonstrated an influence of irradiation according to the ability to form lung metastases in the mouse.

Conclusions: Our results indicate that the combination of different in vitro and in vivo approaches is useful for a detailed characterization of metabolic changes in melanoma cells after irradiation. Additionally, the presented approach gives information about dose-dependent effects. These models enable us to characterize new radiotracers and furthermore, to investigate metabolic effects of applied radiopharmaceuticals in combination with experimental radiation therapy.

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Molecular changes during transdifferentiation of androgen-dependent prostate cancer cells involve overexpression of mithocondrial superoxide dismutase

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Prostate cancer therapies have proved to be very ineffective in androgen-independent tumor status. During the last decade some groups have pointed out the possibility that NE cells could play a key role in the transition to androgen independence and in highly resistance to conventional treatment. Among other factors this cellular resistance can also be related with an overexpression of antioxidant cellular defences. Based on this hypothesis we have tried to establish a connection between superoxide dismutase activity, which could explain the high apoptosis resistance shown by NE cells, and the transdifferentiation of androgen-dependent LNCaP cells to NE-like cells. NE phenotype was induced by mean of the endogenous antioxidant melatonin, di-butiryl cyclic adenosine-monophoshate (db-cAMP) addition or by androgen withdrawal (FBSc) for 7 days in LNCaP cells. db-cAMPc is an extended stimulus able to cause NE differentiation in several cell types including LNCaP cells, while androgen withdrawal is the main treatment strategy to avoid prostate cancer growth. Citosolic superoxide dismutase (SOD1), superoxide dismutase mithocondrial (SOD2) and two neuroendocrine markers were detected by western blot. Inmunocitochemistry of culture cells was also used to confirm western blot results. Total SOD cellular activity was determined spectrophotometrically. We observed a rise in the amount of SOD2 protein in all NE cells as compared to control cells. Variations but no significant changes were observed in SOD1. Synaptophisine and Neuron Specific Enolase analysis indicated differential patterns of NE markers according to the NE inductor. By enzymatic assay we could observe that melatonin and FBSc-induced NE cells shared a significative increment in SOD activity. All together these data indicate a heterogeneity among the NE cells observed in prostate cancer. These results could shed some light on the controversy between prostate cancer progression and neuroendocrine differentiation. This work was supported by “Plan Regional de Investigacion (FICYT IB05-126)”.

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Impact of portal triad clamping (Pringle Maneuver)on hepatic function in a hepatocellular carcinoma murine model

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Background: Intraoperative blood loss and consequent transfusion needs are major factors influencing morbidity and mortality following partial hepatectomy (PH). Hepatic vascular inflow occlusion by Pringle maneuver (PM) is often used to prevent bleeding during PH. However, PM itself causes ischemia and reperfusion injury. This experimental study aimed to estimate the impact of PM in hepatic cells function, viability as well as the longest safe duration of PM in a murine model with hepatocellular carcinoma (HCC).

Material and Methods: Three groups of male Wistar rats with HCC (4 months old; N-nitrosodiethylamine 0,5 gr/L of H2O during 2 months) were subjected to a total liver ischemia period for 60 min: group A (n=12) submitted to a continuous inflow occlusion; group B (n=11) underwent to an intermittent clamping (IC) for 30 min with 5 min of reperfusion; group C (n=12) underwent an IC for 15 min with 5 min of reperfusion. The group D (n=11) was not subjected to a PM. A hepatic biopsy was done at the end of surgery. The degree of tissue injury was evaluated using: 1) Blood markers [aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), alkaline-phosphatase (AF), gamma-glutamyl-transpeptidase (GGT), total-bilirubin (TB), lactic-acid-dehydrogenase (LDH)] and hepatic extraction fraction (HEF) by radioisotopic methods three days before laparotomy (BS) and after surgery (AS); 2) hematoxylin-eosine staining; 3) apoptosis, necrosis and oxidative stress were investigated after collagenase cell isolation from hepatectomy pieces by flow-cytometry using the followed probes: propidium-iodide, annexin-V, DCFH2-DA and JC-1. Statistical analysis was carried out by variance analysis and, if applicable, post-hoc comparisons by Tukey-test (p<0.05).

Results: 1) Mortality: Group A-60%, Group B-46%, Group C-8,3%, Group D-0%.

Conclusions: We didn’t observe differences in cell viability with our model, however the PM duration bigger than 15 minutes must be avoided. We think that these results are related to tumoral cell resistance to ischemia.

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Analysis of TGFβ-Induced, as a novel gene targets in breast cancers in young women

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The aim of this study is to investigate the significance of TGFβI, identified by cDNA microarray as a promising novel marker of breast cancers in young women. Previous studies in our research group have shown that breast cancers show more aggressive features in younger than in older women (Walker et al., 1996) including a higher frequency of loss of heterozygosity (LOH) in the BRCA1, BRCA2 and p53 (Johnson et al., 2002). These findings raised the question as to whether differences are present at the molecular level and prompted us to pilot cDNA microarray to identify novel gene expression changes in sporadic breast cancers in young women.

Gene expression was investigated at the protein level using both Immunohistochemistry (IHC) and Western Blotting with clone against the pure human TGFβI protein (Proteintech Group, Inc). For IHC FFPE tissue was available from 55 breast cancer cases that were stratified by age and 12 healthy female controls. For Western Blotting, protein lysate was isolated from 6 breast cell lines (MCF-7, MDA-MB-231, HBL-100, MDA-MB-468, T47-D and ZR-75-1), tumour and normal breast cell populations (organoids) isolated by digestion of breast reduction tissue. There was stronger nuclear staining in the epithelial cells of normal breast tissue than in the cancer cases. 37 of the 55 (67.3%) breast cancer cases examined showed down-regulation of this protein. Moreover, Chi-squared analysis showed a significant difference between the grade of the tumour and the IHC protein staining distribution, and between cases aged ≤35 years and 36-49 years (p<0.05). These results were confirmed by western blotting, which showed the absence of TGFβI protein in 6 breast cancer cell lines compared to normal breast organoids. These data suggest that down-regulation of TGFβI might be an important step in the development of sporadic breast cancers in young women. Current studies are focussed on investigation of the molecular mechanisms that lead to this down-regulation.

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Characterization of zinc toxicity in PC12 cells - reactive oxygen species generation and role of voltage calcium channels

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Zinc is an essential trace element in mammalian cells and also a structural component used by many metalloenzymes and transcription factors. Recent studies indicate a possible correlation of zinc levels with the cancer risk. However, the exact role of zinc in cancer progression is unknown. The disturbance of zinc homeostasis features with a significant decrease of cellular zinc level, and excess extracellular zinc is toxic and causes death to central neurons. The mechanisms of zinc-induced cell death are still unclear. The knowledge of these mechanisms could contribute to understand the role of this metal in the different biological processes in which is implicated. In this work we used rat pheochromocytoma (PC12) cells as a model to study zinc toxicity.

In this work we characterized zinc toxicity in PC12 cells measuring cell viability by trypan blue exclusion and 3-[4,5-dimethyl thiazol-2-yl]-2,5-diphenyl-tetrazolium (MTT) reduction assays. Reactive oxygen species (ROS) generation was evaluated using flow cytometry and fluorescent labeling with 5-(y 6-)-carboxi-2´,7´-dichlorofluorescein diacetate (DCF-DA). Nuclear condensation was measured by DAPI labeling.

Zinc toxicity in PC12 cells shows a doses- and time-dependent pattern, with an EC50 value of 0.30 ± 0.05 mM and a t1/2 of 173 ± 27 min. Nuclear condensation data indicate that cell death takes place through a necrotic process, and a massive ROS generation is measured from 6 hours of zinc incubation. Differentiation of PC12 cells with neuronal growth factor (NGF) decreased two-fold EC50 value to 0.14 ± 0.02 mM. NMDA receptor blocker MK-801 (10 mM) and non-NMDA receptor blocker CNQX (10 mM) did not protect against zinc toxicity, indicating that glutamate receptors do not play a significant role. Depolarization experiments carried out with high potassium showing a synergic effect with zinc and the protection measured with nifedipine (1 mM) suggest that voltage calcium channels are implicated in zinc toxicity allowing the entry of zinc ions or alternatively through a zinc-mediated calcium entry.

In summary, zinc toxicity is mediated by a massive ROS generation and takes place mainly through a necrotic process. Voltage calcium channels have a main role in zinc toxicity, becoming an important therapeutic target. The uncovering of the molecular mechanisms underlying zinc toxicity is important to understand the different biological process where zinc plays a role.

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Specific molecular signature and developmental hypothesisfor pilocytic astrocytomas of the optic pathway

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Introduction: Pilocytic astrocytomas (PA) are common grade I gliomas that occur predominantly in childhood. They share features of both astroglial