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Genistein modulation of intricate signaling pathways underlying PC3 cells treatment

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To identify genes involved in the in vitro death of prostate cancer cells induced by the Genistein treatment, we analyzed gene expression profiles of the human prostate cancer cell lines PC3, androgen independent, using cDNA microarrays. Comparisson of expression patterns between Genistein untreated and treated PC-3 cells enabled us to identify several genes that were commonly up-regulated and/or down-regulated in the cell lines underlying intricate molecular pathways. Investigation of these genes should help us to decipher the molecular mechanism(s) underlying the death of prostate cancer cells treated with Genistein. In this paper, we propose a novel bioinformatics approach to predict the regulatory network of genes based on differential expressions of cDNA microarrays databases for Genistein treated human prostate cancer cells and untreated cells. The differences in regulatory networks of genes for treated and untreated prostate cancer cells reveal the information of finding possible Genistein-related genes. One exciting result of microarray technology has been the demonstration that patterns of gene expression can distinguish between Genistein treated and untreated human prostate cancer cells. Modification of existing statistical methodologies or development of new methodologies is needed for gene expression analysis. We proposed an evolutionary neural network that classifies gene expression profiles into Genistein treated and untreated prostate cancer cells. Such algorithms can play an important role in molecular profiling of underlying mechanism of Genistein treated prostate cancer cells. Neural networks can assist in the discovery of new Genistein-suppressing or -enhancing target genes, identify patterns of expression/alterations that correlate with biologically significant endpoints, and distinguish clinically meaningful outcomes (e.g., apoptosis, cell cycle arrest, signaling pathways).

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Signaling profile pathways involved in pancreatic cancer progression

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Background: The pathogenesis of pancreatic ductal adenocarcinoma (PDAC) involves multi-stage development of molecular aberrations affecting signaling pathways that regulate cancer growth and progression. This study was performed to gain a better understanding of the abnormal signaling that occurs in PDAC compared with normal duct epithelia.

Methods: Signaling profile was analyzed using xMAP array technology (Luminex 200) on samples derived from: Capan and MIA PaCa cell lines, cell cultures established from patients' tissue, tumoral and peritumoral tissue from PDAC patients and normal pancreas.

Results: Expression levels of signaling molecules were significantly increased in tumoral tissue from PDAC patients compared to normal tissue:
- cell receptors: c-KIT and EGFR were 1,5-2,0 fold increased;
- kinases: p70, p38, ERK/MAPK, JNK/SAPK were 1,7-4,0 fold increased;
- non-enzymatic signaling molecules: IRS1 was 2x increased while HSP27 over 100x;
- transcription modulators: STAT3 and CREB were around two times increased.

Similar variations were recorded in some of the peritumoral samples.

Cell cultures showed enhanced levels of expression, with a more pronunced increase in cell lines compared to primary cell cultures established from patients' tissue.

Conclusions: Our results indicate that multiple signaling proteins are over expressed and provide a higher amplification level of growth signals in PDAC. Increased levels of signaling molecules expression in peritumoral samples could be correlated with the invasivity of PDAC. Our study suggests that proteins form major cellular signaling pathways can be targets for pharmacological modulators developping thus new strategies in cancer therapy and improve the prognosis of pancreatic cancer.

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Trefoil Factor 1 (TFF1) function in cancer

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Trefoil Factor 1 (TFF1) is a small secreted protein that belongs to the Trefoil Factor Family (TFFs). It is synthesized and associated to mucin-secreting epithelial cells in the normal stomach mucosa and in damaged epithelial cells of the gastrointestinal tract. In these tissues, TFF1 plays a crucial role in mucosal defence and healing. TFF1 is essential to normal gastric mucosa differentiation and TFF1-deficient mice develop antropyloric adenomas (Lefebvre et al., Science. 1996;274:259-62). Moreover, in gastrointestinal cancer cells, TFF1 has double antiproliferative and antiapoptotic roles, which further support its function in cell differentiation (Bossenmeyer-Pourié et al., J cell Biol. 2002;157:761-70).

TFF1is frequently ectopically expressed in various human primary carcinomas as well as in their associated metastases (breast, bowel, prostate, and pancreas). In breast cancer, TFF1 overexpression is associated with a favorable prognosis (Spyratos et al., Br J Cancer. 1994;69:394-7) and is considered as a predictive factor of hormonotherapy response (Rio et al., PNAS, 1987;84:9243-7). Recent data however suggest that TFF1 could be involved in the metastatic process (Smid et al, J Clin Oncol. 2006;24:2261-7). In this context, the aim of our project is to study the role of TFF1 in breast and gastrointestinal cancer.

For this purpose, mutant and native recombinant TFF1 proteins are produced using a baculovirus/insect cell system and will be used to treat a variety of cell lines.. In parallel, breast cancer cells that show constitutive TFF1 overexpression will be silenced by a shRNA strategy.These models will be used to directly study TFF1 effect on proliferation, cell migration and apoptosis. A Transcriptome analysis of these cells will be performed to identify molecular and biological processes modulated by TFF1.

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Tumour phenotype and characteristics of metastatic brain involvement in breast cancer patient

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Background: Central nervous system metastases (CNS) occurs about 20% of patients with breast cancer. Despite of the fact that most of these patients die within a few months, substantial subgroup may survive a year or more. We performed this study to identify relations between the tumour phenotype and the incidence and characteristics of CNS dissemination, response to the local therapy and overall survival since the development of metastases in brain (OScns).

Methods: Our single institution study involved 187 breast cancer patients who developed brain metastases. Immunohistochemistry was performed on sections from primary tumors to determine following phenotypes: 1. triple-negative (negative expression of estrogen, progesterone and HER2 receptors), 2. triple-negative/basal-like (triple negative phenotype plus positivity of at least one of the basal cytokeratins and / or EGFR), 3. SHR+/HER2- (positivity at least one of the two studied steroid hormone receptors and negative expression of HER2 receptor), 4. HER2+/SHR-, 5. HER2+/SHR+, 6. SHR-/HER2? (unknown status of HER2 receptor).

Results: The incidence of monitored phenotypes was subsequent: 1. 19,4%; 2. 9,1%; 3. 21,5%; 4. 19,9%; 5. 16,7%; 6,9%, not determined 6,5%. An unambiguous dependence between the tumor's phenotype and the following attributes has been proven: a) interval between the disease diagnosis and the metastases in the CNS (TTPcns); b) interval between the first distant metastatic event and the metastases in the CNS (TTP1mts-cns); c) characteristics of CNS dissemination. The median TTPcns of monitored phenotypes was subsequent: 1. 23,6; 2. 38,0; 3. 56,4; 4. 27,7; 5. 34,6 (all in months, p=0,0111). Similarly medians of TTP1mts-cns: 1. 3,8; 2. 5,5; 3. 12; 4. 11,2; 5. 9,7 (p=0,0105). The CNS dissemination was the most

extensive in patients with HER2+ tumors in comparison with HER2- carcinomas. Surprisingly, patients with triple-negative tumors had the minimal metastatic involvement of CNS defined by size and number of lesions. Phenotype did not correlate with local response to therapy and OScns.

Conclusions: Our study has confirmed the dependence between primary tumor phenotype and the time of incidence of metastatic brain affection and character of their spread. Our results encourage the inclusion of CNS imaging examination (CT or MRI) into the regular restaging of patients with HER2 positive or triple-negative primary breast cancer, who are at high risk for early development of CNS dissemination after the first distant metastatic event have occurred. Especially, in case of triple-negative tumors, there is higher probability for early detection of limited CNS metastatic involvement. Supported by IGA Ministry of Health, CZ. Grant No.:NR/8335-3.

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Association analysis of XRCC1 and XRCC3 polymorphisms with normal tissue reactions after pelvic irradiation

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Background: The purpose of the study was to investigate the association of five polymorphisms in two DNA repair genes XRCC1 (Codon 194 Arg/Trp; Codon 280 Arg/His; Codon 399 Arg/Gln) and XRCC3 (Codon 241 Thr/Met; IVS5-14 17.893) with the development of acute side reaction after pelvic irradiation for gynecologic malignancy.

Materials and Methods: The sample included 125 women with cervical or endometrial cancer, recruited from 2005 to 2008. They received external beam radiotherapy as primary or adjuvant treatment after surgery. The acute normal tissue morbidity in the pelvic area was evaluated using the NCI CTCAE v3.0. DNA was isolated from venous blood and RFLP analysis performed for genotyping. The patients reactions were separated in two groups: "no or slight reactions" (grade 0 and 1) and "moderate and severe reactions" (grade 2 and 3). No grade 4 reactions were recorded. The side effects were subdivided into gastrointestinal and genitourinary. Moderate and severe gastrointestinal reactions were observed in 77 patients, while 48 patients had no or slight reactions. The moderate and severe genitourinary reactions were found in 48 patients and 77 patients had no or slight reactions.

Results: Significant association was found between XRCC1 Codon 280 Arg/His and moderate and severe genitourinary side effects. The genotype G/G has a protective role, while the presence of mutated allele enhance the radiosensitivity (p=0,0045). No significant difference was found for the other XRCC1 and the investigated XRCC3 gene polymorphisms.

Conclusion: The results of the present study support the contribution of XRCC1, but not XRCC3 gene for the occurrence of early genitourinary reaction after gynecologic pelvic irradiation.

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SPR label-free ranking of small molecule negative modulators of adrenomedullin

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Surface Plasmon Resonance (SPR) is one of the most informative technologies for generating binding data (kinetic, affinity, thermodynamic parameters, binding stoechiometry). Weak affinity interactions can be detected and quantified because complex formation is monitored in real-time. SPR is thus a promising tool not only for screening libraries of chemical compounds, but also for structure-activity relationship studies which require ranking of a series of related compounds for their binding properties.

Adrenomedullin (AM) is a 52 amino-acid peptidic hormone, whose dysfunction is related to several diseases, such as diabetes, hypertension, and cancer. A Surface Plasmon Resonance (SPR) biosensor (Biacore T100®, GE Healthcare Biacore) was used to screen against AM, a collection of 21 synthetic compounds generated from a previously identified AM negative modulator.

AM was immobilized on a sCM5 sensor chip surface. Compounds were injected over AM and reference surfaces at concentrations ranging between 25 and 200 µM. Binding data were obtained after reference subtraction, DMSO correction and molecular weight adjustment. Equilibrium SPR responses were low (between 1 and 14 resonance units), corresponding to binding affinities (Kd) in the 50-500 uM range.

The data generated were used to derive a three-dimensional quantitative structure-activity relationship (3D-QSAR) model which was useful to identify relevant features for an effective binding to AM. These compounds have potential interest as anti-angiogenic and anti-tumour agents.

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Genetic and epigenetic alterations in esophageal squamous cell carcinomas from Brazil

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Background: Esophageal cancer (Squamous Cell Carcinoma – ESCC) is one of the ten most common malignancies and it is the sixth cause of cancer-related death in the world. Epigenetic alterations, such as the hypermethylation of CpG islands, are important events in cancer development and are a common way of inactivating tumor suppressor genes.

Methods: In this study, we analyzed by real-time PCR the spectrum of the expression of genes involved in cell cycle and epigenetic regulation in 65 ESCC and normal adjacent mucosa from patients from Southeastern or South Brazil. We further tried to organize them in subgroups and to analyse the potential of these genes to be used as molecular markers for ESCC. The genes analysed were DNMT3B, MBD4, p14ARF, p16INK4a, HDAC1, HDAC2, p21waf/CIP1, TP53, KMT-6 and GADD45a. We also analysed the methylation status of the promoter region of p14ARF and p16INK4a.

Results: The methylation analysis revealed that p14ARF was methylated in 7.1% and p16INK4a was methylated in 35.7% (in 70% of those that presented a lower expression in the tumor when compared to the normal mucosa) of ESCC samples. We performed a cluster analysis of the data that showed that DNMT3B expression may be an important differentiator of tumors in relation to normal tissue in ESCC, and that patients from Rio de Janeiro and Porto Alegre show different profiles of gene expression.

Conclusion: Our results suggest that the low expression of p16INK4a is related to the methylation of its promoter region. Our results also suggest that a higher expression of DNMT3B in ESCC is an important event and that ESCC from patients from different regions of Brazil, and exposed to different etiological factors, may present different molecular profiles of gene expression.

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Use of a cocktail of biomarkers in serum and urine to improve detection of prostate cancer

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Prostate cancer (PCa) is one of the most common tumors in men. Early detection of PCa relies on the determination of PSA levels, digital rectal examination, and ultimately on analysis of prostate biopsy. Because PSA values over than 4 ng/mL are suggestive of PCa, patients over that threshold value have to be subjected to biopsy. A rising PSA (>0.75 ng/mL per year) is also suspicious for PCa, even if PSA is in the normal range. However, PSA can also be increased due to BPH and prostatitis and thus, a large proportion of men undergoing biopsy do not have PCa. Indeed, the PSA test has a high sensitivity (>80%), but lacks specificity (20%). This situation has prompted the search for novel non-invasive biomarkers that may predict which patients will not benefit from prostate biopsy. Because of the inherent molecular heterogeneity of PCa, measurement of a single new marker could underestimate the presence of malignant tissue. The purpose of our study was to quantify a cocktail of biomarkers in blood and urine samples with the goal of improving specificity in the diagnosis.

Urine after rectal massage, and serum samples were obtained from 113 men with ages between 50 and 78, and PSA levels from 0.4-23.3ng/mL. 15 corresponded to patients with normal prostates, 44 showed BPH, and 54 had PCa. Biomarkers analyzed in serum were the humoral response to AMACR, and MMP-2 levels (both of them by ELISA). Hypermethylation of GSTP1 and RASSF1a was evaluated in urine samples by MSP. Sensitivity and specificity were computed with Epiinfo v6.1; discriminant function analysis was performed with SPSS v15, and comparison between ROC curves areas using a Chi Square Test (computed with Stata v9 software).

Areas under the ROC curves were as follows: 0.476 for PSA; 0.532 for AMACR; and 0.706 for MMP-2. Sensitivity and specificity for methylation status was 53.3% and 47.7%, respectively. Discriminant function analysis