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

using a combination of the biomarkers resulted in a ROC curve area of 0.806, which was significantly higher (p=0.0033) than that of PSA (0.58), or any of the variables alone. Our results show that the use of a combination of biomarkers in serum and urine improves the diagnosis of prostate cancer, which could avoid a significant number of unnecessary prostate biopsies.

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Proliferative/Angiogenic genetic profile is associated with progression-free-interval in androgen blockade treated prostate cancer patients

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Background: Androgen blockade therapy (ABT) is frequently used in prostate cancer (PC) advanced stages, albeit most men will eventually fail this therapy and die from recurrent hormone-resistant prostate cancer (HRPC). The epidermal growth factor (EGF), the transforming growth factor beta 1 (TGFβ1) and the vascular endothelial growth factor (VEGF) are key molecules in prostate cancer (PC) cell proliferation and tumoral angiogenesis. The combined effect of functional genetic variants in these genes (EGF +61G>A; TGFB1 +869T>C; VEGF +405G>C) in PC outcome are still uncovered. Their role in PC and HRPC oncobiology increases the rationale for selecting these molecular markers for studying PC prognosis and pharmacogenomics. We hypothesize that PC tumor microenvironment might be modulated through combined effect of EGF, TGFB1 and VEGF functional polymorphisms.

Methods: We conducted a case-control study in histopathologically confirmed PC patients (n=178) and healthy individuals without evidence of neoplastic disease (n=171). EGF +61G>A and VEGF +405G>C genotyping was performed through PCR-RFLP and the TGFB1 +869T>C polymorphism was analysed through allelic discrimination Real-Time PCR. Genotypes from the three polymorphisms were combined into 2 categories according to functional phenotype: low and intermediate/high risk profile (proliferative/angiogenic profile according to gene expression levels).

Results: Genotype frequencies are similar between patients and controls, according to the proliferative/angiogenic profile (P=0.173). The progression free interval (PFI) was significantly shorter in intermediate/high carriers, comparatively with low proliferative/angiogenic genetic profile carriers (36.3±6.5 and 56.4±6.5 months, respectively, P=0.007). Multivariate Cox-regression analysis showed that the proliferative/angiogenic genetic profile is an independent and significant variable for an earlier development of hormone-resistance, in the course of androgen-blockade therapy, even after adjustment for age, Gleason grade and clinical stage (HR=10.3, 95%CI=1.2-90.6, P=0.036).

Conclusion: Combined analysis of target genes from synergistic pathways may reveal interesting functional outcomes and help to define PC susceptibility and pharmacogenomic profile. Results from the present study show an independent effect of the proliferative/angiogenic genetic profile in the response to androgen blockade therapy. The genes studied may be included in further PC pharmacogenomic profiling.

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Relevance of autoantibody profiles in the early detection of cancer

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Circulating autoantibodies against tumour-derived proteins have been observed in the most if not all cancer patients hence they may serve as non-invasive biomarkers for the screening, diagnosis, prognosis or monitoring of cancer. We recently commenced a study aiming to identify a comprehensive set of antigens eliciting B cell responses in patients with melanoma, prostate and gastric cancer and to establish the relevance of autoantibodies for the early detection of cancer and prediction of response to immunotherapy. Nine T7 phage displayed cDNA expression libraries were constructed from testis, melanoma and gastric cancer tissues and prostate cancer cell lines, and the serum-reactive phage clones were selected via biopanning followed by the immunoscreening of the enriched libraries with sera from 76 cancer patients. This resulted in the identification of 1049 different serum-reactive phage clones. However, only ~10% of them represented known genes translated in their natural reading frame and included known TAAs such as CTAG1B, GAGE and Annexin XI-A, and several novel antigens. The remaining clones contained DNA fragments in non-natural reading frames that most likely represent mimotopes, nevertheless, they may turn out to be valid biomarkers. So far a panel of 750 serum-reactive phage clones was assembled and exploited for the production of phage-displayed antigen microarray that was applied to analyse the autoantibody profiles in the sera from 123 melanoma patients (not included in the screening set), 33 patients with systemic autoimmune disorders and 80 healthy controls. A cut-off value for defining melanoma specific antigens was set as >4SDs above the mean value for the healthy control sera. This revealed 194 antigens that reacted with serum from at least one melanoma patient and not with control sera with CTAG1B/CTAG2 being the most frequently recognised (p=0.0007) followed by two out-of-frame peptides (p=0.006). Based on this set of antigens we could classify the sera as “melanoma” or “normal” with 78% sensitivity and 100% specificity. Moreover, the sensitivity for the detection of stage I melanoma was 77% and 73, 77 and 82% for the stage II, III and IV, respectively that demonstrates the relevance of autoantibody profiling in the early detection of cancer.

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Diagnostic role of new circulating markers in bone metastases from breast cancer

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More than 50% of breast cancer patients who relapse with distant metastases present bone lesions, which are responsible for high morbidity. A diagnostic, non-invasive test to detect metastases is needed in order to provide patients with specific, effective treatments. The study was carried out on an overall 54 individuals: 18 healthy donors (median age 43 years [23-76]) and 36 breast cancer patients, 18 of whom were disease-free (median age 49 years [32-77]) and 18 at first diagnosis of bone metastases (median age 63 years [36-86]). OPG and RANKL transcripts were determined using quantitative PCR analysis. The diagnostic accuracy of each marker and their ratio were calculated using receiver operating characteristic (ROC) curves. OPG and RANK-L values were not significantly correlated in healthy donors, disease-free patients, or bone metastasis patients. Median values were independent of age in all the subgroups and, in patients with bone metastases, were not correlated with the number of bone lesions or the presence of visceral metastases. Although the median OPG value was lower in patients with lytic lesions than in those with osteoblastic/mixed lesions (0.3 vs. 1.5), the difference did not reach statistically significance. Moreover, whilst there was no statistically significant difference in median OPG or RANK-L/OPG values between healthy donors and the entire patient group, within the latter subgroup, median OPG was threefold lower (p <0.003) and the RANK-L/OPG ratio about threefold higher (p <0.003) in patients with bone metastases with respect to those who were disease-free. However, median RANK-L values were not statistically different in these two subgroups. The area under the curve (AUC) in disease-free patients was 0.88 for OPG and 0.83 for RANK-L/OPG, with 78% sensitivity and 89% specificity for OPG. The ratio between the two markers reached 44% sensitivity and 89% specificity. A parallel analysis showed about 100% specificity for CEA and CA153, but much lower sensitivity (57% and 50%, respectively) than that observed for RANK-L/OPG. Our preliminary results show that markers of bone damage, in particular OPG, could play a potentially important role in the diagnosis of bone metastases. Confirmation of these data is now required in a larger case series.

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The sodium-dependent phosphate transporter NaPi2b is a new target antigen in ovarian carcinoma and is recognized by the anti-cancer antibody MX35

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Epithelial ovarian cancer is the most common gynecologic cancer that is usually far advanced before it is diagnosed and thus patients have a poor prognosis and survival rate. Identification and characterization of novel ovarian cancer markers is important for understanding the molecular