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

mechanisms of malignant transformation and for the development of novel diagnostic and immunotherapeutic approaches in gynecologic oncology.

The aim of our study was to identify and characterize on the molecular level the ovarian cancer antigen MX35, which is recognized by monoclonal antibody MX35 (mAb) in 90% of human epithelial ovarian carcinomas. MAb MX35 was developed from mice immunized with fresh ovarian carcinoma cells and selected by extensive analysis of normal and malignant tissues and cell lines. Despite the use of humanized MX35 antibody and Fab2 fragments of mAb MX35 in several clinical trials in patients with ovarian cancer, the MX35 antigen has not been identified so far.

By screeneing an expression cDNA library from a MX35 positive ovarian cancer cell line (OVCAR-3) with MX35 mAb we identified the sodium-dependent phosphate cotransporter NaPi2b (SLC34A2, Napillb) as a likely candidate. NaPi2b is a membrane sodium-dependent phosphate cotransporter, which is involved in the regulation of inorganic phosphate metabolism and the maintenance of phosphate homeostasis. The identity of NaPi2b as MX35 antigen was further validated and confirmed based on the following experiments and results: 1) MX35 mAb specifically recognized largest extracellular loop of recombinant NaPi2b, 2) Co-typing of a panel of cancer cell lines showed a good correlation of SLC34A2 RNA expression as determined by RT-PCR and MX35 antigen cell surface expression as determined in a mixed hemadsorption assay using mAb MX35; 3) Selective down-regulation of SLC34A2 gene expression by RNA interference resulted in loss of mAb MX35 binding to MX35-expressing human cancer cells; 4) Recombinant NaPi2b proteins blocked binding of MX35 mAb to ovarian cancer tissues in immunohistochemistry.

In conclusion, we have identified sodium-dependent phosphate transporter Napi2b as a new ovarian cancer marker and a potential target for immunotherapy of cancer. Membrane transporter molecules, such as NaPi2b, represent a new family of potential cell surface targets for immunotherapy of cancer with monoclonal antibodies.

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TWIST1 overexpression is associated with nodal invasion and male gender in primary colorectal cancer

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Background: TWIST1 is a bHLH transcription factor that has been involved in tumor progression and metastasis in several cancer types, although no evidence has been provided yet on its implication in colorectal carcinogenesis.

Matherials and methods: To elucidate the involvement of TWIST1 in colorectal cancer we have examined the expression pattern of TWIST1 mRNA in 54 colorectal cancer biopsies compared to each respective adjacent normal mucosa by real-time reverse transcriptase PCR (RT-PCR) methodology.

Results: TWIST1 mRNA was found significantly overexpressed in cancer tissues compared to non tumorous colon mucosa ($p<0.0001$). Western Blot analysis was performed in some representative cases where TWIST1 mRNA levels in tumoral tissues were markedly increased. We observed that in all of these cases the protein levels were higher in tumoral tissues than in normal colon mucosa. Moreover, we have investigated the clinical relevance of TWIST1 overexpression. Receiver operating characteristic (ROC) curves analysis demonstrated that TWIST1 mRNA levels are significantly increased in patients with nodal invasion with the patient gender.

Conclusions: These findings provide the first evidence of the up-regulation of TWIST1 mRNA in colorectal cancer, suggesting its crucial role in the malignant progression of this disease.

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Investigation of melanoma progression and identification of novel prognostic markers using comprehensive tissue microarrays

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There is a clear need to improve our understanding of the molecular pathogenesis of melanoma in order to develop more effective prevention strategies, define new prognostic markers and to identify new molecular targets for therapy. The aims of this study were to firstly, develop a tissue microarray that includes all stages of melanoma development, secondly, investigate changes in the expression of key proteins during melanoma progression and thirdly, identify novel prognostic markers in primary melanomas by analysing the correlation between protein expression and patient outcome. The proteins chosen for investigation were B-catenin, bcl-2, and galectin-3 due to their central role(s) in adhesion, apoptosis and control of proliferation respectively.

A series of tissue microarrays which included 51 benign naevi, 27 dysplastic naevi, 54 in-situ melanomas, 312 primary melanomas and 64 metastatic melanomas were constructed in order to provide an efficient method of evaluating the expression of proteins by immunohistochemistry at various stages of melanoma progression. The collection of detailed clinicopathologic data for all patients with primary melanoma was undertaken in order to allow correlation of protein expression with several clinical parameters including site of melanoma and survival.

Changes in the expression of all 3 proteins during melanoma progression were seen. A significant fall in B-catenin, bcl-2 and galectin-3 expression between primary and metastatic melanomas and a rise in B-catenin and galectin-3 expression between naevi and dysplastic naevi were found. Correlation of protein expression with clinicopathologic data demonstrated that low nuclear galectin-3 expression was associated with poor survival (log-rank $p=0.0004$) and was an independent marker of poor prognosis (Hazard Ratio for death for low nuclear galectin-3 = 8, 95% CI 1.01-64, $p=0.05$).

These data reveal significant differences in expression of key proteins during melanoma progression and suggest that galectin-3 is a novel prognostic marker in primary melanoma.

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Vitamin D suppresses tumour growth and enhances cyto-toxicity of chemotherapeutic agents in cholangiocarcinoma

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Background: Cholangiocarcinoma (CCA) is a fatal cancer, poor prognosis and lacks effective therapy. Although CCA is rare worldwide, it is the most common cause of cancer death in people of the north-eastern Thailand where the incidence of CCA is highest in the world. Although surgery is potentially curative in selected patients, failure is usually occurred due to recurrence. Adjuvant or neo-adjuvant therapy by chemotherapeutic drugs has been shown to improve local control, provide palliation and prolong survival in various cancers; however, this is uncommon for CCA owing to its poor response to therapy. In the present study, we investigated the effects of a vitamin D_3 and its analog, on growth of CCA cell lines and growth of tumor in NOD-scid-Jak3 knockout mice. The possibility of using combination of vitamin D_3 or its analog and chemotherapeutic drugs to enhance the efficacy of anti-cancer drugs is demonstrated.

Materials and Methods: Vitamin D_3 or analog 0.5 and 1.0 μM were added in the culture of CCA cell lines for 24, 48 and 72 h. Viable cells were determined using MTT assay. CCA cells were inoculated subcutaneously to NOD-scid-Jak3 knockout mice. A group of 5 mice were injected intraperitoneally with vitamin D analog (10 μg/kg body weight) or buffer everyday for 20 days before being sacrificed.

Results: vitamin D_3 or analog inhibited cell proliferation in a dose and time dependent manner. Adding vitamin D_3 or analog to chemotherapeutic drugs (5-FU, mitomycin C and Pacitaxcel) significantly increased the effectiveness of anti-cancer drugs. Intraperitoneal injection of vitamin D analog significantly reduced tumor size without changing body weight and level of serum calcium comparing with those of the control group.

Conclusion: vitamin D or its analog effectively controls growth of CCA cells in vitro and in vivo. Using vitamin D or its analog as an adjuvant therapy to enhance cyto-toxicity of chemotherapeutic drugs may be an encouraged approach to increase the effectiveness of chemotherapy and improve prognosis in patients with CCA.

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