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Validation of potential molecular markers of papillary thyroid carcinoma by quantitative real-time PCR

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Introduction: In our previous DNA microarray profiling studies we specified a multigene classifier of papillary thyroid carcinoma, which included some novel transcriptps, not analyzed until now in the context of PTC biology. The aim of this study was to validate them by quantitative real-time PCR on an independent set of PTC samples. We included such new genes as EVA1, CDH3, KCJN2, LRP4, Q-PCT GALE (up-regulated) and HGD, TACSTD2 (down-regulated) and compared them to the well known PTC markers, which were confirmed by our microarray analysis (DPP4, FN1, KRT19, MET, RXRG, ADORA1, up-regulated, and TFF3, down-regulated).
Material and Methods: Total RNA was isolated from 31 paired normal and PTC samples by Chomczynski-Sacchi method. 0.5 mg of RNA was used in the reverse transcription reaction. cDNA was further used in Q-PCR reaction. The expression was measured by Universal Probe Library LNA probes (Roche) and was normalized by the index obtained from 3 reference genes (UBE2D2, HADHA, EIF3S10) by GeNorm software.
Results. All analyzed genes except TACSTD2 were highly significantly differentiating tumor and normal samples. When the diagnostic efficiency of these genes was assessed by ROC (relative operating characteristic) analysis, we found out that for overexpressed markers, the area under ROC curve (AUC) was higher than 0.9 for all of them and for down-regulated ones, this criterion was met for TFF3 and HGD. We trained the multigene classifier on the obtained data and currently we are performing its validation on independent set of samples.
Conclusions: Our data supply a next step of validation of PTC markers, before the required verification on routine diagnostic material obtained by fine needle biopsy of benign and malignant thyroid nodules
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The association between the MTHFR 677C>T polymorphism and breast cancer risk in premenopausal women

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Background: Methylenetetrahydrofolate reductase (MTHFR) is involved in folate metabolism and plays a role in DNA biosynthesis, methylation, and repair in actively dividing cells. A common 677C>T polymorphism in the gene for MTHFR, leading to a thermolabile enzyme with decreased activity, has been associated with reduced plasma folate levels and elevated homocysteine levels. The aim of our case-control study was to analyze to role of the MTHFR 677C>T for breast cancer risk in premenopausal women.
Materials and Methods: MTHFR genotypes were determined in 105 premenopausal breast cancer patients and 105 sex- and age-matched premenopausal healthy control subjects using a 5′nuclease assay (TaqmanTM). Statistic analysis was done using SPSS 11.0 for Windows. Numeric values were analyzed by Student's t-test, proportions of groups were compared by χ^2-test. Odds ratio (OR) was calculated to estimate the risk for breast cancer. Threshold for significance was $p < 0.05$.
Results: At the time of breast cancer diagnosis, mean patient age was 40 ± 7 years. Controls were age-matched to the case subjects (±1 year), the mean age was 40 ± 7 years. Although not statistically significant, we observed a higher MTHFR 677T allele frequency in breast cancer cases (61.9%) than in controls (51.5%, $p = 0.082$).
Conclusions: The present data support the hypothesis that the MTHFR 677 C>T polymorphism might influence the risk for breast cancer in premenopausal women. However, well powered, large prospective population-based studies will be necessary before a final conclusion can be drawn.

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Apoptosis in Bladder and prostate cancer: The role of FAS and FASL polymorphisms

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Background: The FAS/FASL system is one of the major pathways in apoptosis and is important to regulate cell proliferation and tumor cell growth. Functional promoter polymorphisms of FAS and FASL genes alter the transcriptional activity. The role of FAS polymorphisms in prostate and bladder cancer has not been studied.
Material and Methods: DNA extracted from peripheral blood from 1230 individuals: 657 prostate cancer patient, 140 bladder cancer patients treated with BCG (Bacille Calmette-Guérin) therapy and from 433 healthy controls was analyzed by Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) for FAS −670 A/G and FASL −844 T/C polymorphisms.
Results: We found that the AG and GG genotypes of FAS −670 A/G represent significantly protection (OR = 0.30; confidence interval (CI): 0.20−0.44 and OR = 0.22; CI: 0.12−0.74, respectively) for prostate cancer extra-capsular invasion. Taken together these data, a significantly 72% protection was found for G allele carriers (OR = 0.28; CI: 0.19−0.41). It was also verified that there were no statistically significant association between G allele carriers and bladder cancer susceptibility or response to BCG treatment. No association was found between BCG treatment responder and non responder bladder cancer patients for FASL polymorphism.
Conclusions: It is well known that allele G reduces the transcriptional activity of FAS gene. Fas exist as membrane bound and soluble forms and with opposite roles. They derive from the same gene by alternative splicing. Membrane Fas receptor trigger apoptosis and in other hand soluble Fas (sFas), which lacks transmembrane domain, binds to Fas ligand antagonizing Fas-Fas ligand apoptotic pathway. It is reported that serum sFas is elevated in prostate cancer and associated with advanced disease. Our results suggest that G allele may reduce sFas levels preventing the apoptotic inhibition caused by the soluble form. In other hand, we could also conclude that FAS polymorphism does not relate with bladder cancer risk and that FAS and FASL polymorphisms appear not have influence in the BCG treatment response.

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The Glu228Ala polymorphism in the ligand binding domain of death receptor 4 is associated with prostate cancer metastases

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Background: Death receptor 4, encoded by the TNFRSF10A gene, is an important mediator of apoptosis and its dysfunction may be related to cancer development and distant tumor spread. A single nucleotide polymorphism in TNFRSF10A (Glu228Ala, rs20576) within a conserved region of the extracellular cysteine-rich domain of death receptor 4 has been associated with an increased risk for a variety of tumor entities. Aim of the present study was to evaluate the role of the TNFRSF10A polymorphism in metastatic progression of prostate cancer.
Materials and Methods: TNFRSF10A genotypes were determined by a 5′-nuclease assay (TaqMan) in 702 participants from the Austrian PROCAGENE (Prostate Cancer Genetics) study. Finally, 95 samples were reanalyzed and results were identical for all samples. Statistical analysis was done using SPSS 11.0 for Windows.
Results: Within a median follow-up time of 10 months (range 0−60 months), 24 (3.4%) patients developed metastases. In a Cox regression model including age at diagnosis and risk group as potential confounders, carriage of an 228Ala allele was associated with a relative risk of 2.47 (95% CI 1.10−5.54; $p = 0.028$) for metastases. TNFRSF10A genotypes were not associated with risk group or age at diagnosis.
Conclusion: The TNFRSF10A Glu228Ala polymorphism may be a novel independent risk factor for prostate cancer metastases. Recent studies have indicated that stimulation of apoptosis by agonistic TRAIL or TRAIL receptor antibodies may be useful in anticancer therapy, especially in combination with ionizing radiation or other DNA-damaging agents. In this context it might be interesting to explore the potential pharmacogenetic role of the DR4 228Ala variant for TRAIL receptor targeting therapies.