121 MicroRNA-21 is expressed in stroma of colorectal cancers and high levels identified by image analysis predict short disease-free survival in stage II colon cancer patients

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Background: Approximately 25% of all patients with stage II colorectal cancer will experience recurrent disease and subsequently die within 5 years following primary surgery. MicroRNA-21 (miR-21) is highly upregulated in several cancer types and has been associated with survival in colon cancer.
Material and Methods: In the present study we have developed a robust *in situ* hybridization assay using high-affinity locked nucleic acids (LNA) probes that specifically detects miR-21 in formalin-fixed paraffin embedded tissue samples. The expression of miR-21 was analyzed by semi-automated *in situ* hybridization in tissue samples from 130 stage II colon cancers and 67 stage II rectal cancers. The median follow-up time was 60 months and the total number of relapses was 34 and 29, respectively.
Results: The miR-21 signal was predominantly observed in fibroblast-like cells located in the stromal compartment of the tumours. The expression levels were measured using image analysis. The histological miR-21 expression estimates correlated significantly with disease-free survival ($p < 0.01$) in the colon cancer patient group. We observed no correlation with survival among the patients with rectal cancer. In multivariate analysis the miR-21 expression estimates were independent of other clinical parameters (age, gender, total leukocyte count, K-RAS and MSI).
Conclusions: We conclude that miR-21 is primarily a stromal miRNA, which when quantified by image analysis identifies a subgroup of colon cancer stage II patients with short disease-free survival.

122 Prognostic value of gene expression targeted by gain of 17q sequences in malignant peripheral nerve sheath tumours

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Background: Malignant peripheral nerve sheath tumour (MPNST) is a highly aggressive malignancy for which no consensus therapy exists besides surgery. Thus, there is clearly a need for better prognostic markers and targeted treatment strategies. We have recently confirmed in a multicentre study our initial findings that gain of parts or the whole chromosome arm 17q is a common event in MPNST [1,2] and *TOP2A* and *BIRC5* have beensuggested as gene targets for 17q gains [3,4]. Based on unpublished microarray gene expression profiling results, we here investigate the prognostic role of genes located on 17q.
Material and Methods: For 64 paraffin-embedded and 33 fresh frozen MPNSTs with up to 10 years clinical follow up we have combined *in situ* protein expression with mRNA expression of genes localized at 17q, the latter obtained from profiling analysis using the AB 1700 microarray platform. A panel of seven benign neurofibromas served as controls.
Results: From the transcriptome analyses of fresh frozen samples, we confirmed that several genes on 17q had increased expression in MPNST compared to the benign neurofibromas, including *TOP2A* and *BIRC5*. Furthermore, *TK1* was also one of the genes with highest differential expression. The mRNA expression for each of these three genes was associated with survival. The *P*-values ranged from 0.01 for *TK1* to 0.05 for *TOP2A* in univariate Cox regression analysis. The protein expression of these genes was analyzed on a tissue microarray containing the 64 paraffin-embedded samples. The TOP2A protein expression was not significantly associated with survival. The expression of BIRC5 and TK1 are currently being evaluated.
Conclusions: Gain of the terminal region of chromosome arm 17q is one of the most common genetic aberrations in MPNST, found in up to 70% of all patients [2]. We have shown that the mRNA expression of genes located on 17q are among the most differentially expressed in the transcriptome of MPNST compared to neurofibromas and that these genes carry prognostic information independent of known clinical variables.

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123 MicroRNA profile associated to clinical response in ovary cancer: biological/clinical implications

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Background: Despite important improvements over the past two decades, the overall cure rate of epithelial ovarian cancer (EOC) remains only ~30%. Although much has been learned about the proteins and pathways involved in early events of malignant transformation and drug resistance, a major challenge still remaining is the identification of markers for early diagnosis and prediction of response to chemotherapy. Recently, it has become clear that alterations in the expression of microRNAs (miRNA) contribute to the pathogenesis and progression of several human malignancies. There are accumulating evidences of the role of miRNAs in EOC pathogenesis and their dysregulated expression in EOC; however, a clear consensus on the miRNA signatures associated with prognosis or prediction of response to therapy has not yet been reached. To evaluate whether the imbalance of the miRNAs in EOC could be involved in the initial poor responsiveness and/or in the resistance acquired during treatment, we profiled a selected case materials including patients stratified for residual disease after surgery and for time to relapse after first-line therapy.
Materials and Methods: Two case materials have been used: a training set including 55 patients and a validation set including 30 patients. MiRNA profiles have been obtained on human miRNA Illumina chips (1145 miR annotated on miRBase 12.0). System biology analyses have been performed on the identified miRNA cluster for target prediction by using four different predictive algorithms and for identification of functional related networks.
Results: From the training set, applying a detection p-value <0.05 and excluding genes with data missing exceeding 50%, 744 miRNA were detected. Class comparison analysis enabled to identify 32 miRNA differentially expressed at FDR < 0.1 in responding versus non-responding patients. Ten of these miRNA were concordantly deregulated in the validation set and included a cluster of 8 miRNAs located on Xq27.3 located. Computational prediction of miRNA target genes suggested the involvement of two functional networks related to the FSH/LH hormonal pathway and cell growth and proliferation. RT-qPCR validation of relevant target genes is ongoing.
Conclusion: By the integration of functional and high-throughput methodologies applied to well-defined clinical and model systems we could contribute to the development of prognostic tools and to the identification of biology-based targeted therapeutics to better control EOC persistence or relapse.
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124 Inhibiting androgen receptor activity in prostate cancer by cofactor manipulation

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Background: Growth of prostate tumours initially depends on androgens, which act via the androgen receptor (AR) – a ligand-activated transcription factor that recruits cofactor proteins, which alter accessibility of chromatin to the transcriptional machinery. Therapies inhibiting androgen signalling via chemical castration and/or antiandrogens are initially successful but inevitably tumours progress to an advanced “androgen independent” stage. However, AR signalling remains key for their growth. It is speculated that tumours escape hormonal control via increases in coactivators or reduction in corepressor proteins. Manipulating such proteins is thus a potential therapeutic strategy. We identified prohibitin (PHB) as an androgen target protein and AR corepressor, and aimed to investigate means to and effects of altering PHB levels in prostate tumours. PHB has been identified as a target of microRNA 27a (mir27a). While introducing the whole protein as a therapy may not be feasible, altering its levels using small nucleic acids may.
Methods: Prostate cancer cells stably expressing an androgen-responsive reporter and inducible vectors to overexpress or knockdown PHB were used to assess effects on androgen signalling (real time imaging, target gene expression) and cell/tumour growth (FACs, tumour volume measurement)