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 Based on unpublished microarray gene expression profiling results, we here investigate the prognostic role of genes located on 17q.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)

in culture and in xenograft models. PHB regulation was investigated using reporter genes coupled to upstream and downstream regulatory sequences and microRNA mimic and antisense oligonucleotides, assaying PHB levels, AR activity and cell growth.
Results: PHB overexpression inhibited AR activity, target gene expression and androgen-dependent growth of cells, inducing rapid G0/G1accumulation. Conversely, reduction of PHB increased AR activity, PSA expression, androgen-mediated growth and S-phase entry. *In vivo*, overexpression led to tumour growth arrest, whereas knockdown resulted in accelerated tumour growth, even in castrated mice, and an increase in tumour spread.
We demonstrated that mir27a is upregulated by androgens in prostate cancer cells, with a concomitant decrease in PHB expression and upregulation of androgen target genes. We are able to abrogate this effect using an anti-sense oligonucleotide to mir27a.
Conclusions: AR promotes "androgen-independent" prostate tumour growth by down-regulating its own repressors. In the case of PHB this is via microRNA(s) in a positive feedback loop, which could be interrupted by small molecule therapy.

125 Beta-blockers inhibit tumour cell migration: a potential use as anti-metastatic drugs

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Over ninety percent of all deaths from cancer are not due to the primary tumour, but a consequence of metastasis development. It is thus a pressing need in tumour biology to investigate the molecular mechanisms of metastasis formation and to deduce anti-metastatic strategies from this knowledge. Cell migration is a prerequisite for metastasis development, and we have shown by means of our specifically developed three-dimensional, collagen-based *in vitro* cell migration assay that the stress-related neurotransmitter norepinephrine is a potent stimulus of cell migration from tumour cell lines of breast, colon, and prostate tissue origin. This effect of norepinephrine was inhibited by the clinically established beta-blocker Propranolol. The *in vivo* relevance of these results for metastasis formation was confirmed in athymic BALB/c nude mice: Tumour cells were injected into the thigh muscles and grew in to a local tumour. After five weeks the mice developed lumbar lymph node metastases, which were more than two-fold larger when the mice were exposed to norepinephrine (applied by osmotic mini-pumps). Additional treatment with Propranolol reduced metastasis formation under control levels. In order to evaluate a potential clinical benefit of beta-blockers to inhibit metastasis formation, we retrospectively analyzed the course of the disease in 466 breast cancer patients. Forty-three of these patients received beta-blockers due to hypertension. This group showed a significantly longer cancer specific survival as well as significantly reduced metastasis formation. Patients treated with other anti-hypertensive drugs did not show any difference in comparison to non-hypertensive patients. In summary, beta-blockers have a strong impact on breast cancer metastasis formation, and might constitute a first class of anti-metastatic agents, which can be rapidly transferred to clinical use.
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126 Estrogen receptor protein: high resolution immunofluorescent profiling and quantitation in older mouse OSE

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Background: Downregulation of estrogen receptor (ER)-β occurs in many epithelial cancers including ovarian, a cancer of mainly older women. Changes to ER expression are measured chiefly at mRNA level yet levels of ER mRNA do not always correlate with immunoreactive protein. Conventional immunohistochemistry does not sharply define locus or shared loci of ER subtype and permits only qualitative analysis of ER *in vivo*. This study used immunofluorescence and confocal microscopy to optimally define ERα and ERβ loci and quantify ERβ protein in ovarian surface epithelium (OSE) after treatment with estradiol valerate ®(EV).
Material and Methods: Older Swiss Webster mice (7−10 month) were injected with EV 10 μg/g body weight while diestrous controls received oil. Mice were killed 48 hours later. Ovaries were taken for ovarian estradiol RIA and immunohistochemical analysis of ER using polyclonal antibody directed against ERα, and monoclonal antibody directed against ERβ-1. Definitive localization used triple label immunofluorescence – Alexa Fluor fluorochromes 488 (ERα), 555 (ERβ) and the nuclear marker TO-PRO-3, visualized using a Zeiss Upright Confocal microscope and LSM software. Quantitative analysis of ERβ was from 5 ovary sections per animal (N = 5/group) using captured images from 3 randomly selected OSE areas per section. Fluorescence intensity emitted from 50 μm lengths of OSE was measured with Image J software and scores averaged. Optimized LSM settings from control ovary scans provided baseline scan settings for positive and negative immunofluorescence controls and treated ovaries.
Results: ERα was the dominant ER in OSE and had a uniform, mainly nuclear distribution. Cytoplasmic expression of ERα varied with cell shape. ERβ formed clusters in nuclei and cytoplasm. Cluster size also varied with OSE cell shape with large nuclear clusters detected in columnar OSE. Co-localization of ER was cytoplasmic and frequent in cuboidal OSE. Ovarian estradiol was elevated in treated animals ($p < 0.01$), causing an 11-fold reduction in ERβ expression ($p < 0.0001$).
Conclusion: Triple label immuofluorescence and confocal microscopy provides sharp definition of ER locus in OSE and allows for the study of variable expression patterns of ER protein subtype. Furthermore, immunofluorescent profiling is an unbiased method to quantify ER protein expression *in vivo*.

127 Stroma production within the primary tumour correlates with poor survival for stage I-II colon cancer patients

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Background: Recent models on metastatic invasion focus on the tumour-"host" interface, in particular the role of the stromal tissue. The biological meaning of the stromal compartments are thought to be part of the process of wound healing, but there is also strong emphasis that CAF's (cancer-associated fibroblasts) are important promotors for tumour growth and progression. Assuming these models are correct we anticipated that changes in the proportion of stroma in the primary tumour could reflect progression. We therefore investigated if the amount of intra-tumour stroma could be applied as a candidate marker to identify patients for adjuvant therapy.
Methods: We have investigated the proportion of intra-tumour stroma, on heamatoxylin-eosin (H&E) stained histological sections and distinguished between patients with a high amount of stroma (stroma-high) and patients with less stroma (stroma-low).
We have analyzed 135 stage I-II colon cancer patients for the proportion of tumour related stroma and for TGFβ-R2, SMAD4 and β-catenin, markers involved in pathways related to stromal production and epithelial-to-mesenchymal transition (EMT).
Treatment with a COX-2 inhibitor might improve patient outcome in those patients with high percentage of stroma (with or without chemotherapy). A series of 596 patients treated either with placebo or COX-2 inhibitor was analyzed (VICTOR-trial).
Results: Of 136 analyzed patients 35 (25.7%) patients were stroma-high and 101 (74.3%) stroma-low. Significant differences in survival were observed between the two groups, with stroma-high patients showing poor survival (OS $p < 0.0001$, HZ 2.59; DFS $p = 0.0002$, HZ 2.31).
A high-risk group was identified with stroma-high and SMAD4 loss (OS $p = 0.008$, HZ 7.98, CI 4.12–15.44, DFS $p = 0.005$, HZ 6.57, CI 3.43–12.56); 12 of 14 (85.7%) patients died within 3 years. In a logistic-regression analysis a high proportion of stroma and SMAD4 loss were strongly related (HZ 5.42, CI 2.13–13.82, $p < 0.001$).
Results of the COX-2 inhibitor are currently under evaluation but will be presented at the conference.
Conclusions: Conventional haematoxylin-eosin stained tumour slides contain more prognostic information than previously fathomed. This can be unleashed by assessing the tumour-stroma ratio. The combination of analyzing the tumour-stroma ratio and staining for SMAD4 results in an independent parameter for confident prediction of clinical outcome. It should be considered to implement this parameter in standard pathological reports in addition to the TNM classification.

128 Use of a biochip assaying 28 mutations in the KRAS, BRAF, TP53, and APC genes for detection of colorectal neoplasia

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Background: Ireland has one of the highest worldwide colorectal cancer (CRC) mortality rates. We recently used the immunochemical faecal occult blood test (iFOBT) to perform the first CRC pilot screening program in Ireland. However, studies testing genetic variants associated with CRC development show a definite potential to better target colonoscopy resources by developing a non-invasive, affordable, user-friendly alternative to improve on the low sensitivity of FOBT for premalignant growths.