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.
This work was supported by the Fritz Bender Foundation (Munich, Germany), the German Cancer Aid (Bonn, Germany), and the Bruno and Helene Jöster Foundation (Cologne, Germany).

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.

Materials and Methods: We have tested a CRC biomarker DNA chip (*RanplexCRC®*, Randox Laboratories) which assays 28 mutations in 4 CRC-related genes (*K-ras, APC, BRAF, TP53*). These represent the most genetic markers ever to be tested simultaneously in a CRC screening study. Our test series include (1) DNA from the matched tumour and normal tissue of German CRC patients (N = 55) and (2) An Irish sample cohort (N = 250) from participants who have undergone colonoscopy following positive iFOBT results, comprising samples of stool and tissue from 100 patients with polyps and advanced adenomas, 50 with CRC, and stool from 100 individuals normal after colonoscopy. We use spin column extraction (using sample specific *Qiagen* kits) for DNA isolation.
Results: From the German CRCs, mutations were observed in 71% of the cancerous tissues (39/55) and 25% of the matched 'normal' tissue (13/55). In a majority of cases mutations were from *Kras* and *p53* (30 of the 46 mutations detected), suggesting a signature for advanced stages as the German CRCs tested were stage 2 and 3. The majority of the mutated tumours had only one mutation (25 out of 39). To test assay utility in screening for colorectal neoplasms we are currently examining in our Irish iFOBT +ve cohort how the mutation profile in the disease and normal matched tissue is reflected in DNA isolated from the stool of our patients. Mutation detection is possible in 1000-fold excess of wild-type DNA. The initial advanced adenoma results (8 mutated out of 16) indicate a maximum detection rate of 50% for these highly screen relevant growths, which if confirmed in the stool screening would be superior to FOBT. None of the matched normal adenoma tissues were mutated.
Conclusions: These data confirm the high detection rate of DNA mutations in CRCs and show that mutations associated with CRC carcinogenesis can be detected from advanced precancerous colorectal lesions using the *Ranplex* method. Genetic assays have great potential to achieve highly specific and sensitive detection of colorectal neoplasias, especially for eminently treatable early stage cancers and advanced adenomas. In addition the types of mutations detected, such as in *Kras*, will also provide important molecular prognostic information separate to detection alone.

129 Molecular diagnosis of pancreatic cancer using a combination of genetic and epigenetic markers

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Background: *KRAS* mutations have proved useful as a diagnostic marker in FNA of pancreatic masses. Methylation markers have shown promise in the early diagnosis of pancreatic carcinoma. The aim of this study was to assess the diagnostic utility of hypermethylation status of candidate genes in combination with *KRAS* mutation detection in the evaluation of pancreatic masses.
Experimental design: As a first step the methylation status of *HRH2*, *EN1*, *SPARC, CDH13, APC, RASSF1A, TMS1, CRBP1, INHBB, CPLX* and *RARB2*, was tested in paired tumoural and non tumoural tissue samples of 11 primary pancreatic adenocarcinoma by melting curve analysis (MCA) optimized to have an analytical sensitivity of 5%. Sixty-one Fine Needle Aspirates (FNA) of pancreatic masses (43 pancreatic adenocarcinomas and 18 chronic pancreatitis) were studied; their methylation status was analyzed using Melting Curve Analysis after DNA bisulfite treatment. *KRAS* mutations were detected by a Restriction Fragment Length Polymorphism/Polymerase Chain Reaction (RFLP/PCR) method.
Results: Five genes where promoter hypermethylation was detected in more than 50% of analyzed tumours were selected: *HRH2*, *EN1*, *SPARC*, *CDH13* and *APC*. The methylation panel had a sensitivity of 73% (27 of 37) and a specificity of 100% whenever two or more promoters were found hypermethylated. *KRAS* mutations showed a sensitivity of 77% (33 of 43) and a specificity of 100%. When combined, genetic and epigenetic analyses, resulted in a sensitivity of 84% maintaining the 100% specificity.
Conclusion: Both genetic and epigenetic molecular analyses offer an excellent diagnostic yield in this setting allowing cancer diagnosis in an overwhelming majority of the cases. The good clinical performance is more relevant at a time where EUS-guided FNA is increasingly used in the preoperative evaluation of small pancreatic masses.

130 GINS proteins as candidate markers for urine-based cancer screening

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Background: A urine-based screening strategy for urogenital tract malignancies would offer an affordable non-invasive alternative to current approaches. The GINS complex (a heterotetramer of Sld5, Psf1, Psf2 and Psf3) is a highly conserved DNA replication factor. GINS interacts with the MCM complex in eukaryotes and archaea. Given the value of MCM proteins as specific and sensitive markers for cancer screening, we investigated whether GINS subunits might also be of potential diagnostic value in applications where the sensitivity and/or specificity of MCM proteins is low.
Materials and Methods: We investigated the expression of GINS proteins in human cell lines by immunoblotting, and in tissue sections by immunohistochemistry. We also tested the stability of GINS proteins by incubating intact tissue culture cells or cell lysates in urine. Finally, we have set up an ongoing clinical study of Sld5 protein as a potential biomarker in urine samples from prostate and bladder cancer patients. In this study, we have screened for Sld5 and Mcm2 by multichannel immunofluorescence microscopy in the cellular fraction and measured Sld5 protein levels in the urine supernatant by ELISA.
Results: Consistent with the published findings for MCM, GINS proteins are expressed in all cycle phases of cultured proliferating cells, but are down-regulated in cells undergoing quiescence. Examination of histological sections indicates that Sld5 and Psf3 expression is restricted to the proliferative compartment in normal tissue, but spreads to the majority of cells in a wide range of dysplastic and malignant tissues, including cervix, colon and skin. Our urine spiking experiments suggest that Sld5 protein is more stable than Mcm2 in harsh extracellular environments. In the pilot clinical study of over 50 prostate and bladder cancer patients, Sld5 was readily and specifically detectable in the cellular fraction of the samples from cancer patients. We also present an evaluation of Sld5 protein levels in the supernatant portion of those same urine samples as an easy-to-screen diagnostic/prognostic marker for male urogenital cancers.
Conclusions: Owing to their stability, GINS proteins hold promise as independent or complementary markers to the MCM proteins for cancer screening in harsh extracellular environments such as urine. Work is ongoing to identify further applications of GINS in cancer screening and prognosis.

131 Relevance of copy number alterations in Ewing Sarcoma: gain of 1q defines a subset of patients with worse outcome, probably caused by DTL/CDT2 overexpression and subsequent cell cycle deregulation

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Background: Despite extensive characterization of the molecular implications of EWS-ETS, little is known about secondary genetic alterations in Ewing Sarcoma (ES), the only ones that could shape molecular subtypes of the disease with prognostic significance. Research in this field is crucial since the prognosis is poor in patients with primary disseminated disease or after relapse, with a survival rate of nearly 20%.
Material and Methods: *Patient and samples*: tumour samples from a total of 105 ES patients were included in the study along with 16 ES cell lines.
BAC-microarrays: Sanger 1Mb BAC collection was amplified and spotted in triplicate onto Codelink slides (GE). Cy5/Cy3-dCTP-labelled DNA from tumour samples and healthy donors were co-hybridized with the BAC-microarrays.
Expression microarrays and SNP-microarrays: Total RNA from *DTL*-silenced cell lines was extracted with Trizol (Invitrogen) and hybridized with the GeneChip® Human Genome U133 Plus 2.0 Array (Affymetrix) after quality assessment using an Agilent 2100 Bioanalyzer (Agilent).
Lentiviral shRNA transduction of ES cell lines for functional validations: The MISSION shRNA collection of 5 pLKO.1-shRNA constructions (SIGMA Aldrich) was selected for assays aimed at reducing *DTL* overexpression.
Results: We assessed copy number alterations (CNA) in **67 ES tumours**, finding 1q gain (31% of tumours) markedly associated with relapse and poor