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

F. Entschladen[1], M.J. Voss[1], K.S. Zänker[1], B. Niggemann[1], D. Palm[1], H.O. Habashy[2], I.O. Ellis[2], D.G. Powe[2]. [1]Witten/Herdecke University, Immunology, Witten, Germany, [2]Hospitals Trust and University of Nottingham, Cellular Pathology Queen's Medical Centre, Nottingham, United Kingdom

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

L.S.M. Gulliver[1], P.R. Hurst[1]. [1]University of Otago, Anatomy and Structural Biology, Dunedin, New Zealand

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

W.E. Mesker[1], E.C. Johnstone[2], G.W. Van Pelt[1], R. Midgley[2], G.J. Liefers[1], J. Morreau[3], D.J. Kerr[2], R.A.E.M. Tollenaar[1]. [1]Leiden University Medical Center, Department of Surgery, Leiden, The Netherlands, [2]Oxford University, Department of Clinical Pharmacology, Oxford, United Kingdom, [3]Leiden University Medical Center, Department of Pathology, Leiden, The Netherlands

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

D. Hughes[1], C. O'Morain[1], N. Lee[1], L. Kelley[1], H. McMurray[2], M. Latten[2], M. Ebert[3]. [1]Trinity College Dublin/AMNCH Hospital, Department of Clinical Medicine, Dublin, Ireland, [2]Randox Laboratories, DNA Diagnostics, Crumlin, Ireland, [3]Technical University, Medicine, Munich, Germany

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.