Materials and Methods: Expression of HIF-1alpha was assayed by Werstern Blotting and qPCR. Transfection of siRNA against HIF-1alpha was done to demonstrate its transcriptional activity. A pull down assay using as bait the oxygen binding domain of HIF-1 was used to assay PHD activity.

Results: Here, we demonstrate that exposure of T84 colon cancer cells to C1845 bacteria induces the expression of HIF-1, a key transcription factor involved in VEGF expression. In contrast to hypoxia which inhibits the activity of prolyl hydroxylases (PHD) and as a consequence stabilizes HIF-1alpha protein, C1845 bacteria do not inhibit PHD activity but rather induce translational mechanisms. C1845 stimulation of HIF-1alpha required the binding of F1845 adhesin to the apical DAF/CD55 receptor. HIF-1alpha expression was inhibited by treating the cells with inhibitors of Src like tyrosine kinase, MAP kinase and phosphatidylinsositol 3-kinase signaling pathways. These inhibitors also blocked the C1845-induced phosphorylation of the translational regulatory protein p70 S6 kinase thus providing a mechanism for the modulation of HIF-1alpha protein synthesis. In addition to VEGF, C1845 bacteria induce the expression of BNIP3, a major regulator of autophagy. Autophagy is a process by which cytoplasmic organelles can be catabolized to provide macromolecules for energy generation under conditions of nutrient starvation.

Conclusion: Thus we propose that C1845-induced HIF1alpha expression could promote the survival of human colon cancer cell.

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A crosstalk between HIF-1a and LOX in the tumor microenvironment

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The Lysyl oxidase gene family (LOs) comprises five members acting as extracellular modulating enzymes. Lysyl oxidase (LOX), the first member of the family, catalyzes the cross-linking of collagen and elastin and its expression correlates with metastatic potential in tumor cell lines. Importantly, recent data revealed an overexpression of LOX under hypoxic conditions. This up-regulation is under the control of the Hypoxia-Inducible Factor-1a (HIF-1a), a key transcription factor involved in cellular adaptation to changes in O2 level. In addition to LOX, our results suggest that other LOs isoforms are regulated by hypoxia in several tumorigenic cell lines, confirming the tight control of LOs by the cancer micro-environment. Reciprocally, we pointed out that LOX can also act on the HIF-1a pathway. We showed this new link using human colorectal carcinoma cell lines in which the expression of LOX is modulated under both normoxic and hypoxic conditions. Indeed, LOX is able to regulate the expression of HIF-1α protein, as well as the downstream effector Carbonic Anhydrase IX. Taken together these results underline an inter-relation and a positive feedback loop between two main actors of tumoral progression: HIF-1a and LOX.

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Role of lymph vessels in progression of breast cancer; morphological characteristics and prognostic implication

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Background: In spite of the growing evidence about the important role of lymphatics in progression of breast cancer, this issue is still a matter of controversy. Aims of the following study were (a) to investigate lymphatic characteristics (lymph vessel density (LVD) and lymphovascular invasion (LVI)) in breast cancer and their role as prognostic factors (b) to study the role of vascular endothelial growth factors (VEGF)-A, VEGF-C and VEGF-D in regulation of lymphangiogenesis (C) to distinguish between LVI and blood vascular invasion (BVI) to find which type of vessels play the major role in metastasis.

Materials and methods: Paraffin embedded sections of 177 invasive breast cancer, with 10 years follow up, were stained immunohistochemically with the lymphatic markers, podoplanin and D-40 to assess LVD and LVI, with CD34 and CD31 to identify BVI and with VEGF-A, VEGF-C and VEGF-D. LVD, LVI and expression of growth factors were correlated together and with survival. Ethical approval was obtained for the study from Nottingham Local Research Ethics Committee.

Results: in breast cancer the majority of lymphatics are located in the peripheral and the peritumoural areas. Tumours with higher LVD are significantly associated with the presence of LN metastasis (P<0.001) and shorter overall survival (OS) (P=0.04). High expression of VEGF-A and -C but not of VEGF-D were associated with high LVD (P= 0.047, <0.001 and 0.187 respectively) and with poorer survival. Vascular invasion was detected in 56/177 specimens (31.6%); 54 (96.4%) were LVI and 2 (3.5%) were BVI. The presence of LVI was significantly associated with the presence of LN metastasis, development of distant metastasis, regional recurrence and worse disease free interval (DFI) and OS. In multivariate analysis LVI but not LVD was an independent poor prognostic factor.

Conclusion: lymphatics in breast cancer play an essential role in disease progression by being the major routs of dissemination. VEGF-A and VEGF-C are important factors for lymphangiogenesis.

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Non small cell lung cancer xenografts as preclinical models for epidermal growth factor receptor (EGFR) - targeted therapies

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Background: The EGFR plays a crucial role in human cancer. It is involved in tumor development and progression, cell proliferation and regulation of apoptotic cell death. In lung cancer the EGFR is frequently overexpressed in 50-80% of the patients. With the tyrosine kinase inhibitors (TKI) Gefitinib and Erlotinib as well as with the monoclonal antibody Cetuximab targeted drugs are available for the treatment of patients with lung cancer. The evaluation of clinical trials using Erlotinib and Gefitinib revealed that only a small group (adenocarcinomas, women, never-smokers and people with asian origin) did benefit from the treatment with TKIs. In addition, the role of mutations in the exon 18- 21 of the EGFR gene was widely investigated and debated.

Method: Up to now, in our group 101 tumors had been transplanted from which 25 transplantable models were generated.

Results: It could be demonstrated that the murine passages coincide with the original tumor regarding histology, the expression of the surface proteins E-Cadherin, EpCAM, the cell proliferation marker Ki-67 and in gene profiling. The analysis of the EGFR gene revealed no mutations relating to a better response to TKIs. With the exception of five models all express a wild type EGFR. Five K-ras mutations were found in the xenografts and 11 different mutations could be located in the p53 gene. Furthermore, the sensitivity of the xenografts was tested against five clinically used cytotoxic agents (Etoposid, Carboplatin, Gemcitabine, Paclitaxel and Navelbine) and two EGFR inhibitors (Erlotinib and Cetuximab). It could be shown that there exist strong differences in responses among the xenografts.

Conclusion: In summary, we have available a panel of well characterized NSCLC xenografts correlating with the clinical situation and being able to identify biomarkers and their regulation after therapeutic interventions both at genetic and at protein level.

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Microarray analysis and functional studies in a novel human colon cancer model of EMT: TAF12 regulates E-cadherin and Fra-1 regulates Vimentin expression

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Background: The process of epithelial mesenchymal transition, is a fundamental process of embryogenesis and cancer invasion/metastasis. TFIID is composed of the TATA box-binding protein (TBP) and its associated factors (TAFs). Interestingly, the TFIID activity can be regulated by cellular signals to specifically alter transcription of particular subsets of genes.

Materials and methods. In order to examine the distinctive functions in cancer development in the colon, we introduced constitutively active mutant Ras genes into an intermediate stage colon adenoma cell line (Caco-2).

Results. We found that Ha-RasV12 was very efficient in transforming these cells, which developed a mesenchymal morphology. We conducted microarray analysis in an attempt to reveal the genes whose aberrant expression is a direct result of overexpression of either Ki-RasV12 or Ha-RasV12 (1) and then arrays of more than 25,000 genes (2). We present that vimentin, a key molecule of epithelial mesenchymal transition, was

differentially regulated between Ha-RAS and Ki-RAS leading to Ha-RAS specific induction of migrative phenotype. We demonstrate that the AP-1 sites in vimentin promoter are involved in the regulation of vimentin and FRA-1 binds to vimentin promoter in vivo, regulates its expression as well as migration and invasion properties (3).We identified TAF12 levels as being up-regulated in cell lines bearing natural RAS mutations or stably overexpressing a mutated RAS isoform and was dependent on the MEK pathway. We further identified a functional ETS binding site on the TAF12 promoter. Reduction of TAF12 levels by siRNA treatment enhanced E-cadherin mRNA and protein levels and reduced migration and adhesion properties of RAS transformed cells with Epithelial to Mesenchymal Transition (4).

Conclusions. Overall, our study has identified a signature of metastatic gene expression in colon and reveals new mechanisms of regulation of the two major EMT related genes, that of vimentin and e-cadherin by Fra-1 and TAF12 transcription factors respectively

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Role of COX-2 and Ras activation in pancreatic adenocarcinogenesis

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Since the majority of pancreatic adenocarcinomas are highly aggressive and therapeutically non–accessible, basic research on pancreatic carcinogenesis is crucial. Cyclooxygenase-2 (COX-2), a key enzyme of prostaglandin (PG) biosynthesis, is over-expressed in 75 % of carcinomas including those of the pancreas. In our group, the pathologic and molecular changes of transgenic mouse pancreas with keratin 5 promoter-driven over-expression and activity of COX-2 were characterized. Transgenic pancreata developed cystic intra-ductal papillary mucinous neoplasms resembling human lesions in this organ. Multiple inflammatory clusters comprised of B- and T-cells as well as macrophages, were found to be spread throughout the pancreata. Mutational activation of the K-Ras gene, predominantly in codon 12, is known to be an initiating event in human pancreatic adenocarcinogenesis. Pyrosequencing of DNA from transgenic pancreatic cysts for mutations in cancer-relevant codons 12, 13, and 61 of this gene revealed wild-type sequences. Nevertheless, activation of Ras (measured as increased levels of GTP-Ras) and Ras-downstream effector kinases such as Mitogen-Activated Protein Kinase (MAPK) and AKT was enhanced. Celebrex treatment of transgenics suppressed the accumulation of PG, the activation of Ras, MAPK, AKT, the pathologic changes, including the inflammatory phenotype. Analysis of PGE2 receptors EP1-4 in pancreata of transgenic mice showed an over-expression of EP-1 and EP-4 as compared to wild type organs, while EP-2 and EP-3 expression was not modulated. By indirect multi-colour immunofluoresence stainings all receptors were located in the keratin 19-positive pancreatic ducts, in macrophages, and with the exception of EP-2, in CD31-decorated blood vessels. EP-1 was only observed in CD45/B220- and CD4-positive lymphocytes. In ongoing studies the role of individual EP receptors in pancreatic carcinoma cells with respect to proliferation, migration, and Ras signaling is studied. In conclusion, there is strong evidence for a causal relationship between aberrant COX-2 expression, COX-2-mediated PG signaling via Ras, and the development of the pre-invasive lesions including the inflammatory phenotype.

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Antitumoural effect of cannabinoids in an animal model of breast cancer

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We have previously shown that Δ^9-tetrahydrocannabinol (THC), the most important cannabinoid in terms of potency and abundance, reduces human breast cancer cell proliferation in vitro by blocking the progression through the cell cycle and by inducing apoptosis. Here we show that cannabinoids have antitumoural properties in an animal model of breast cancer: the MMTV-neu mouse. These transgenic mice carry an inactivated neu oncogene under the transcriptional control of the mouse mammary tumour virus promoter/enhancer and, as a consequence, they develop mammary tumours with a latency period of approximately 7 months. Mice were palpated twice weekly for the detection of mammary gland nodules. At the time of appearance of the first tumour, cannabinoid peritumoural treatment was started and maintained for three months (twice per week). Tumour volume was measured during this period. Our results show that both THC and JWH-133, a selective ligand for the CB_2 -non psychotropic-cannabinoid receptor, drastically reduce tumour growth and the number of tumours per animal. The presence of cannabinoid receptors in these tumours was confirmed by confocal microscopy and real-time quantitative PCR. In order to elucidate the mechanism of cannabinoid antitumoural action in this model, we performed different experiments in (i) tumour samples, (ii) cells isolated from tumours, and (iii) an established human breast cancer cell line that naturally overexpresses neu (SkBr3). Preliminary data indicate that the mechanism underlying cannabinoid effect include inhibition of proliferation, metastasis and angiogenesis, together with a modulation of tumour immune infiltration.

In summary, our results show for the first time that cannabinoids have an antitumoural effect in a genetic model of cancer, and confirm the potential of these compounds as anticancer therapeutic tools.

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Mechanisms of apoptosis and cell cycle arrest in sub-cutaneous breast tumours treated sequentially with doxorubicin followed by zoledronic acid

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Background: Late stage breast cancer involving metastasis to the bone is often treated with a chemotherapeutic agent in combination with the anti-resorptive drug zoledronic acid (Zol). We have previously reported that administration of doxorubicin (dox) 24h prior to zoledronic acid inhibits intra-osseous breast tumour growth, inhibits tumour cell proliferation and increases apoptosis in vivo. This is the first report of the potential molecular mechanisms by which doxorubicin and zoledronic acid exert their synergistic anti-tumour effects.

Materials and methods: MDA-MB-436-GFP cells were inoculated into the right flank of female MF1 nude mice (n=3/array). Mice were treated 1x per week for 6 weeks with saline, 2mg/kg dox, 100μg/kg zol, dox and zol simultaneously, dox followed 24h later by zol, or zol followed 24h later by dox. Animals were sacrificed 24h following final treatment. Biotin labelled RNA from each group was hybridised to a GEArray cell cycle pathway specific microarray. Genes that showed a 2 fold or greater change in expression were considered relevant, and changes were confirmed by qPCR, and Western blot.

Results: Molecular analysis of subcutaneous MDA-G8 tumours showed no effect on tumour cell cycle or apoptosis following administration of 100ug/kg zol. 2mg/kg dox caused a cell cycle block at G1-S with a down regulation of cyclin E/CDK2; whereas apoptosis-related genes were unaffected. However, when dox was administered 24h prior to zol cell cycle progression was further suppressed, was accompanied by a down regulation of cyclins E1, B, D1 and D3 as well as their related cyclin dependent kinases CDK2, CDC2, CDK4 and CDK7 compared with dox alone. Tumours treated sequentially with dox then zol also showed an induction in the apoptotic pathway, with an up regulation in Bax, a down regulation in Bcl2 and an increase in caspase 3 cleavage.

Conclusions: This is the first report showing that sequential treatment of sub-cutaneous breast tumours in vivo with doxorubicin followed by zoledronic acid induces changes in a number of specific genes associated with regulation of the cell cycle and apoptosis.

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ERK1/2 as a modulator of the cross-talk between VEGFR-2 and S1P-receptor signalling pathways in follicular thyroid ML-1 cells

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The sphingosine 1-phosphate receptors (S1P1-3, 5) and the vascular endothelial growth factor receptor 2 (VEGFR-2) interact in the follicular thyroid carcinoma cell line ML-1. In addition to secreting substantial amounts of VEGF-A and –C, ML-1 cells also express receptors for VEGF (VEGFR-2), opening the possibility for autocrine signalling with VEGF. We have previously shown a complex interplay, in part dependent on Akt,