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