Taken together, our results demonstrate a direct crosstalk between HIF1a and LOX in the tumour microenvironment and underline a critical role of this mutal regulation in tumour progression *in vitro* and *in vivo*.

### 532 NG2 expression identifies a tumour competent population in glioblastoma with distinct molecular signature

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**Introduction:** We previously demonstrated that NG2 expressing ($NG2^+$) cells in glioblastoma (GBM) exhibits robust proliferative and tumourigenic activity and share phenotypic and functional similarities with NG2 expressing glial progenitors (NGP). Here we conducted comparative studies to address the difference of the molecular signature of GBM-$NG2^+$ and GBM-$NG2^-$ cells.
**Methods:** GBM cell lines (GLs) were derived from clinical samples under serum-free conditions according to our Cambridge Protocol (Fael Al-Mayhani et al.; 2009). GBM-$NG2^+$ Cells were sorted using FACS. Comparative molecular studies on GBM-$NG2^+$ and GBM-$NG2^-$ cells were conducted using microarray, comparative genomic hybridization (CGH) and western blot.
**Results:** Microarray data indicated that $NG2^+$ cells over-expressed a group of genes previously recognized by Cancer Genome Atlas within the Mitosis and Cell-Cycle Module (MCM). KEGG, Transpath and Transfac studies showed over-expression of unique set of proliferative pathways and transcription factors (TFs) by GBM-$NG2^+$ cells. Gene Ontology enrichment identified more than 200 gene categories that were enriched in the GBM-$NG2^+$ cells. The top 10 categories were related to cell cycling, M phase and DNA replication. Similarly, array CGH demonstrated subtle molecular structural differences between the cytogenetic profile of GBM-$NG2^+$ and GBM-$NG2^-$ cells as unique chromosomal abnormalities were found in GBM-$NG2^+$ cells. Finally, we demonstrated that MAPK and Akt pathways were significantly over-activated in GBM-$NG2^+$ cells compared to $NG2^-$.
**Conclusion:** We previously showed the robust proliferative activity and tumourigeneity of GBM-$NG2^+$ cells. Here, we provide evidence that our previous observations are linked to the distinct molecular signature of GBM-$NG2^+$ cells. This signature includes notable structural chromosomal abnormalities, unique enrichment of TFs and MCM genes and over activation of MAPK and Akt pathways.

### 533 New cell culture models of bladder carcinoma

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**Background:** Although there are numerous bladder cancer cell lines in current use, they do not cover the whole array of disease phenotypes and participating cell types. The majority of routinely used cell lines were derived from invasive or metastatic urothelial cancers, whereas papillary urothelial carcinomas, which represent the clinically prevailing tumour type, are grossly underrepresented. Moreover, although there is ample evidence for crucial role of stromal cells in tumour development and progression, there is to our knowledge no single established cell line of bladder cancer stromal cells. We report establishment and initial characterization of a new bladder cancer cell line (BC61) and a pair of carcinoma (BC44) and carcinoma-associated fibroblast (BC44Fibr) – cell lines derived from the same tumour.
**Materials and Methods:** Cell lines were established following our published protocoll (Seifert et al., World J Urol. 2007; 25:297–302). Carcinoma associated fibroblasts were immortalized by retroviral transduction of the hTERT gene. The Fibroblast Growth Factor Receptor 3 gene was analysed by DNA sequencing, CDKN2A by PCR, protein expression by indirect immunofluorescence. Karyotyping followed a standard protocol.
**Results:** Both carcinoma cell lines retained an epithelial phenotype, as revealed by morphology, cytokeratin and E-cadherin expression. Both contain CDKN2A/p16 deletions frequent in urothelial cancers. BC61 has a pseudotriploid karyotype with changes typical of early progression stages such as loss of chromosomes 9 and 11. The cell lines displays a functional p53-response to genotoxic stress and constitutive expression of DNA damage checkpoint pathway genes. It bears an activating mutation in FGFR3 (S249C), although the receptor is expressed at a relatively low level. BC44 has an complex aneuploid karyotype and lacks p53 expression. The corresponding diploid BC44Fibr cells display typical attributes of carcinoma-associated fibroblasts like ubiquitous expression of Vimentin and Smooth Muscle $\alpha$-Actin, prevalent expression of Fibroblast Activation Protein. Focal expression of CD13 reveals their bladder stroma origin.
**Conclusion:** We believe that these new cell lines will be valuable models for numerous aspects of urothelial carcinogenesis.
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### 534 In situ immobilization in alginate foams – a novel 3D in vitro cell culture system

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The use of 3-dimensional (3D) matrices for cell culture is gaining popularity as a substitute for traditional 2D cell culture as it can approximate cell architecture and cell-cell contact found in tissues, organs and tumours. Alginate-based foams for culturing cells in 3D have been developed in which cells can be immobilized within the foam structure using an *in situ* gelation technology. The principle of the novel immobilization technique is: Cells suspended in a Na-alginate solution are applied to the alginate foam and induces *in situ* gelation as calcium ions are donated from the foam and cross-link the added alginate, effectively entrapping the cells within the pores throughout the foam.
Mouse myoblasts C2C12 (ATCC CRL-1772) were cultured in 2D and prepared as a suspension of 1.0% sodium alginate in DMEM medium. 125 µl cell suspensions were added to g-sterilized NovaMatrix-3D™ foams fitted to 24-well culture plates at cell densities of 10 000 or 25 000 cells/foam. Cell localization within the foam was visualized using a confocal microscope to identify cells fluorescently labeled cells using a carboxyfluorescein marker.
At different time points cell proliferation was measured. The foams were first de-gelled by incubating in 50 mM sodium citrate solution, then cells were counted after centrifugation and resuspension.
C2C12 myoblasts proliferated slower when immobilized within the foam compared to the standard 2D culture plate. Despite the reduced proliferation rate, the cells remained viable over extended periods of culturing (trypanblue staining).
As alginate does not provide attachment factors necessary for some cells to retain a high proliferation rate, the use of the cell attachment peptide RGD coupled to the immobilizing alginate was investigated. After two days of culture, the foam with RGD-alginate had three times as many myoblasts as the plain alginate matrix.
Use of alginate foams with concomitant *in situ* immobilization of cells results in a 3D model with the potential to approximate cell proliferation and architecture within tissues or tumours. The technology enables biomimetic approaches by varying e.g. matrix elasticity, gelling ions, attachment peptides and foam degradation. The foam may also be implanted as a xenograft, making the NovaMatrix-3D™ system truly versatile.

### 535 Tryptase up regulate VEGF and PDGF in squamous cell carcinoma cells (SCC)

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The interaction of tumour cells with their environment plays a crucial role for progression, metastasis and angiogenesis of tumours with growth factors such as VEGF, PDGF playing a crucial role. Several immunohistochemical studies indicated that mast cells, which are present in increased number in tumour-stroma, might regulate tumour progression and angiogenesis via secreted cytokines, growth factors or mediators like histamine or tryptase. In this context we were interested to study, how mast cells regulate VEGF and PDGF in cutaneous tumours.
Various squamous cell carcinoma (SCC) cell lines were cultivated for 24 h with or without conditioned medium derived from IgE-activated or non-activated primary, dermal mast cells. Protein expression of growth factors, VEGF and PDGF, was estimated by ELISA in each individual cell line in order to look for a potential modulation of these growth factors as a result of tumour-mast cell interaction. Furthermore, tumour cells were stimulated with the mast cells related mediators histamine or tryptase to examine how these mediators would influence expression of VEGF and PDGF in tumour cells.
In all cell lines examined, PDGF was released in increased amounts after co-cultivation of SCC cell lines with mast cell supernatant. Interestingly, the modulatory effect of supernatant derived from activated versus none activated mast cells on PDGF release from tumour cells differed only slightly. Stimulation of SCC cell lines with low concentrations of tryptase (0.1 µg/ml) led to a strong increase in PDGF release (in SCC-12 cells from 32 pg/ml to 150 pg/ml and in SCC-13 from 18 pg/ml to 92 pg/ml). This result suggests that mast cell derived tryptase may be a major inducer of PDGF release from SCC cells. In addition, application of the tryptase inhibitors LDTI but not of the chymase inhibitor SBTI to conditioned medium led to an attenuation of PDGF release. Unlike PDGF, SCC cells release VEGF constitutively. This continuous release was further augmented by incubation of SCC in mast cell conditioned medium. In contrast, addition of chymase or cathepsin G inhibitors SBTI elevated VEGF level. Our results indicate that mast cell-descendent mediators like tryptase and chymase have contrary effects on the release on PDGF and VEGF from SCC. Our results indicate that the two known different phenotypes of mast cells, which are defined according to their different expression of proteases ($M_{Tryptase}$ or $M_{TryptaseChymase}$), may differ with regard to their impact on the progression of cutaneous tumours.