breast cancer cell migration. Furthermore, we have evidence that an inhibitory antibody to JAM-A reduces breast cancer cell migration *in vitro*. Therefore, it is tempting to speculate that, akin to Herceptin targeting the HER2 receptor, targeting JAM-A may represent a new therapeutic modality for future breast cancer treatment.

405 p38a is required for cancer-specific metabolism and survival

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Background: Increased glycolysis, even in the presence of high oxygen tension, is a common feature of tumour cells, a process known as 'Warburg effect'. A key mechanism sustaining aerobic glycolysis is the stabilization and activation of the transcription factor HIF1α. The p38 pathway is often activated by stress-associated stimuli and cytokines leading to diverse and sometimes opposite cell type-specific responses, such as cell survival and apoptosis. Earlier reports indicate that p38α is responsible for the stabilization of HIF1α in hypoxic MEFs and in a pancreatic cancer cell line. Our previous studies indicate that p38α inhibition induces cell cycle arrest, autophagy and cell death in cancer cells, suggesting that this kinase might be involved in the regulation of cancer-specific energy balance.
Material and Methods: Colorectal, ovarian and prostate cancer cells were treated with p38α inhibitors and characterized by cellular and molecular approaches to evaluate survival, death, autophagy, and protein and gene expression.
Results: Colorectal cancer cells treated with p38α inhibitors showed reduced levels of ATP, together with reduced glucose uptake and lactate extrusion. These results correlated well with the reduction of HIF1α protein levels and the down regulation of a specific subset of HIF1α target genes, which encode for glycolytic rate-limiting enzymes, thus affecting the most important steps of this metabolic pathway. The use of inhibitors of prolyl hydroxylases (i.e. DFO), whose enzymatic activity triggers HIF1α proteasomal degradation, led to the recovery of HIF1α protein levels and activation of its transcriptional program, indicating that p38α is required for HIF1α protein stability. These results were reproduced in both ovarian and prostate cancer cells. The p38α-dependent energetic imbalance triggered the activation of FoxO3A transcriptional program, which promotes energy retrieval for survival; however, prolonged activation of p38α led to autophagic cell death.
Conclusions: We showed that p38α is required for HIF1α stability, probably through a mechanism involving prolyl hydroxylases. Thus, p38α blockade inhibits cancer-specific aerobic glycolysis by switching off the expression of glycolytic rate limiting enzymes. The energetic balance is first maintained by the activation of energy producing pathways; however, prolonged inhibition of p38α eventually leads to autophagic cell death.
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406 The BH4 domain is required for proangiogenic function of bcl-2 protein

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Background: We previously demonstrated that the antiapoptotic protein bcl-2, in addition to act as an antiapoptotic protein, cooperates with hypoxia to promote Vascular Endothelial Growth Factor (VEGF)-mediated tumour angiogenesis, through a mechanism involving the transcription factor Hypoxia Inducible Factor 1 (HIF-1). In the present work we analyzed in which domain(s) map the proangiogenic function of bcl-2.
Materials and Methods: Human melanoma cell line M14 was used for stable and transient trasfections of expression vectors encoding *wild type* or mutated forms bcl-2 protein. Cells expressing different forms of bcl-2 was characterized for secreted VEGF protein level (*ELISA assay*), HIF-1 protein expression (*Western blot*) and transcriptional activity (*Reporter assay*). Conditioned media from cells expressing *type* or mutated forms of bcl-2 were tested for their angiogenic activity using *in vivo* (*Matrigel plugs assay*) and *in vitro* (*endothelial cells proliferation and morphogenesis*) assays.
Results: We showed that removal of or mutations at BH4 domain abrogate bcl-2 ability to induce VEGF secretion, HIF-1α protein expression and HIF-1 trascriptional activity under hypoxic conditions. Conditioned medium from cells expressing bcl-2 deleted of the BH4 domain under hypoxia markedly reduced *in vitro* angiogenesis-related endothelial cell functions and *in vivo* neovascularization when compared to the effect induced by conditioned medium from cells overexpressing *wild type* bcl-2. By contrast BH1 and BH2 domains are not required for the activation of proangiogenic signaling by bcl-2. We also found that the exposure to a cell-permeable form of BH4 domain of bcl-2 is sufficient to induce HIF-1/VEGF protein expression in melanoma cells under hypoxia. Finally, transient overexpression of wild type or deleted forms of bcl-2 extends this observation to other melanoma cell lines and tumour cell lines with different origin.
Conclusions: These results lead to elucidation of the importance of HIF-1 in bcl-2-mediated angiogenic response under hypoxia and show a regulation of angiogenesis by bcl-2 through a mechanism that requires its BH4 domain.

407 Direct protein and peptide imaging in breast tumour by mass spectrometry

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Background: Matrix-assisted laser desorption/ionization (MALDI)-imaging mass spectrometry (IMS) has been used for detection and verification of peptides or other polymers of biological interest. Direct application of MALDI-time of flight (TOF)-MS on tissue sections makes it possible to obtain specific information on local molecular composition, relative abundance, and spatial distribution for image profiling. The identification directly from tissue sections is important for the diagnosis of tumours, and is the main aim for developing the MALDI-IMS technology.
Material and Methods: MALDI measurements and image analyses were performed using a linear Autoflex instrument equipped with a Smartbeam laser and FlexImaging 2.1 and ClinProTools 2.1 software packages (Bruker Daltonics).
Results: For successful tissue MALDI-IMS results, preparations of the tissue samples are crucial. On testing the condition of tissue preparation embedded in optical cutting temperature polymer (OCT), which are detected in the *m*/*z* 921.7–2,022.4 range, OCT polymer signals suppress the peptide signals and interrupt peptide imaging because of contaminant noisy peaks. With Tissue MALDI-IMS, we obtained protein peaks ranged in 3−17 KDa from sinapinic acid matrix, peptide peaks ranged in 0.8−2.9 KDa from DHB matrix. Peptide images enable to be better as detected more MS peaks than protein peaks. 25 peptide images were obtained in OCT removed tissue and 18 peptide images in OCT embedded tissue, whereas only 10 protein images were produced in the same sample. Therefore, embedding of tissue should be avoided contamination with OCT by using the blade of the cryostat to remove OCT, and embedding in only one side bottom of sample. Peptide imaging is preferable in effectiveness and more information than protein imaging. On PCA analysis to evaluate the quality of tissue spatial features based on MALDI-IMS data, signals with three different regions were extracted and peptide-specific ions was identified. Ions at 2,032 *m*/*z* were most abundant in interface zone (IZ), whereas those at 911.8 *m*/*z* were most abundant in normal zone, and those at 1,542 *m*/*z* were found specifically in tumour burden. We will perform peptide sequencing directly from the tissue section using MS/MS and identify a significant increase peptide of 2,302 *m*/*z* peak in IZ.
Conclusions: We compared peptide with protein imaging patterns by using MALDI-IMS technology in crude samples of fresh-frozen tissue samples based on tumour, normal and IZ. The best imaging results were generated with peptide imaging in OCT removed. Given update of current theoretical concept, molecular margin could be practically developed.

408 The metabolic microenvironment finely regulates invadopodia ECM proteolysis through an ezrin-PKA-RhoA-NHE1 signaling axis

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Background: Degradation of the extracellular matrix (ECM) is one of the critical steps of tumour cell invasion and invadopodia are thought to mediate invasion through focal proteolysis of the ECM. Invasion of the surrounding tissue needs a continuous communication between the tumour cells and the surrounding ECM and, in this context, the tumour microenvironment assumes a fundamental importance since alterations in the ECM, in cytokines and in growth factors can influence cellular behavior. Invadopodia form as a consequence of the tumour cell interaction with the ECM via activation of ECM receptors. However, tumour associated stromal components find themselves in a markedly different environment when in a tumour. In particular the tumour environment is hypoxic and has a low extracellular pH/nutrients. Indeed, it is increasingly clearer that the selective events underlying metastatic progression often involve interactions with elements of both the tumour-specific stromal and metabolic microenvironments. Therefore, a fundamental question is how the invasive mechanism(s) are, in turn, regulated by the other components of the tumour metabolic microenvironment such as low serum and/or hypoxia.
Materials and Methods: Experiments were conducted in the metastatic breast cancer cell line, MDA-MB-231, seeded onto Matrigel containing the quenched fluorescent substrate, DQ-Green-BSA, such that proteolysis produces fluorescence in a dark background. Proteolysis was evaluated microscopically in 3D co-localization analysis with cortactin and/or actin to localize invadopodia.
Results: Here, we observe significant differences in invadopodial-driven focal ECM digestion and cell shape/size when cells on Matrigel are subjected

to hypoxia or serum deprivation. Co-IP and ezrin phosphorylation negative mutants demonstrate that ezrin plays a a fundamental role together PKA and phospho-RhoA in driving these alterations.
Conclusions: Altogether, these data lead to the recognition of a synergistic, positive-feedback interaction between the tumour cell and both the metabolic and stromal microenvironments in tumours that can lead to transient changes in the biochemistry and physiology of the tumour cells and elicit further changes in these microenvironments that support invasion. An important aspect for further research will be to determine the signaling systems that integrate the interplay of these various tumour microenvironmental compartments in driving invadopodial proteolysis.

409 Role of reactive oxygen species and autophagy in the generation of neoplastic cells from senescent keratinocytes

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Senescence is a non-proliferative state that occurs in response to telomere shortening or reactive oxygen species (ROS) accumulation. Using normal human keratinocytes, we recently reported that some rare senescent cells can spontaneously reactivate a mitotic process that generate so-called emergent cells which are transformed and able to form skin hyperplasias in nude mice. Several data suggest that the oxidative DNA damage occurring in senescent cells would be the mutagenic motor of this emergence [Cancer Res, 2009. 69, 7917−25]. In parallel, we have shown that most of the senescent cells end-up in programmed cell death through over-activation of (macro)autophagy [Am J Pathol, 2009. 174, 423−35]. We investigated here the relationships between oxidative stress, emergence and autophagy.
Young keratinocytes treated with H_2O_2 underwent premature senescence followed by massive autophagic cell death. Conversely, a catalase treatment, that degrades H_2O_2, delayed senescence and decreased autophagic cell death, hence evidencing the role of oxidative stress in inducing autophagic senescent-cell death. Inhibiting the initiation of the autophagic process with 1 mM 3-methyladenine increased the emergence frequency, suggesting that emergence requires an escape from autophagic cell death. However, a higher drug concentration (5 mM) almost completely abolishes the emergence process, indicating that a minimal level of housekeeping autophagy remains necessary to senescent cells for reinitiating their mitotic program. To determine the more prone to emerge senescent cell subpopulation, we sorted senescent cells according to their Lysotracker® staining as an indicator of their autophagic activity, or to their H_2-DCFDA staining as an indicator of their ROS level, and then monitored for emergence. The results indicated that the more prone to emerge are the senescent cells displaying a moderate autophagic activity, and a moderate level of ROS.
Taken together, these results indicate that the outcome of a senescent cell is dictated by its ROS level. A high ROS level induces a high and lethal autophagic activity. At a lower ROS level, the cell induces a housekeeping autophagic activity that clears up the oxidized components and avoids cell death, and by the way becomes permissive for neoplastic evolution consecutively to the putative oxidative alteration of some oncogenes, tumour suppressor genes or other crucial cell regulators.

410 HER2 as a relevant molecule in tumour initiating cells

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Background: Recent studies on breast cancer cell lines over expressing HER2 have suggested that tumour initiating cells (TICs) cultured as spheres have greater levels of HER2 as compared to the parental counterpart and the therapeutic activity of Trastuzumab seems to be related to its ability to target not only the bulk tumour but also the tumour initiating cells in HER2 amplified tumours.
We investigated whether HER2 is expressed at higher levels in TICs derived from other carcinoma than breast expressing low HER2 levels in comparison with parental cell lines and this peculiar expression can drive TICs more sensible to anti-HER2 therapies.
Materials and Methods: Human cancer cell lines obtained from prostatic (DU-145), vulvar (A-431), head and neck (Cal-27), and pancreatic (PACA44, GER) tumours characterized by low levels of HER2 were used in our experiments in vitro and in vivo. Sphere forming assays were performed and the activity of Aldheyde Dehydrogenase (ALDH) enzyme, the expression levels and the percentage of CD133, CD44v6, ALDH and HER2 positive cells were evaluated using flow cytometry in spheres and in the parental cell lines. Cells were also treated with Trastuzumab and Lapatinib and sphere forming efficiency (SFE) was evaluated. Experiments *in vivo* were performed on nude mice. Animals were injected subcutaneously with tumour fragments and treated with Trastuzumab or saline. At the end of schedule of treatment, tumours were excised and desegregated to obtain a cellular suspension; tumour sphere assays and serial transplantability of cells were assessed.
Results: Spheres were enriched in cells positive for ALDH, CD133 and CD44v6 in comparison with the parental counterpart (1.2 to 8-fold increase), showed higher HER2 levels and higher percentages of CD133/HER2, CD44v6/HER2 and ALDH/HER2 double-positive cells as compared to the parental cell lines (2 to 3.4-fold increase and 1.5 to 7-fold increase, respectively). The SFE of cells treated *in vitro* with Trastuzumab or Lapatinib was significantly lower than in untreated cells ($p = 0.0043$).
Cells isolated from Trastuzumab-treated xenograft tumours showed a decrease up to 4-fold of SFE and the loss of serial trasplantability in comparison with cells from saline-treated xenograft tumours.
Conclusion: Our results provide evidence that HER2 is expressed at higher levels in TICs of solid tumours than in the correspondent parental cell lines suggesting the use of anti-HER2 therapies for the destabilization of tumour stem cell niche.

411 Epithelial plasticity during Epithelial-Mesenchymal Transition (EMT) is associated with alterations of histone H3 modifications

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Background: During cancer progression epigenetic events, like alteration of histone modification markers, co-exist with genetic events and affect cell properties line cell migration and invasion. We analysed the role of global histone modifications, how this modifications may be affected by pathways activated by oncogenes and their association with epithelial plasticity.
Materials and Methods: We generated oncogene-transformed colon cell lines by RASV12, BRAFV600E oncoproteins. Notably, the phenotype of the H-RASV12 oncoprotein-transformed cells (Caco-H) is associated with Epithelial-Mesenchymal Transition (EMT) characteristics [3]. We have shown that E-cadherin is regulated by TAF12 transcription factors [4].
Results: A global histone modification analysis revealed a general de-regulation of histone modification markers, in particular H3K27me3 by H-RAS. Variations of methyl- and acetyl-transferase enzymes as EZH2, JMJD3, PCAF GNC5 and HDACs are associated with appearance of aggressive tumour properties. ChIP analysis has been used to follow histone markers on the promoter of two selected genes Cyclin D1 and the EMT marker gene E-cadherin. Interestingly, Cyclin D1 and E-cadherin genes demonstrate inverse histone repression patterns on their promoter, associated to their inverse expression levels. Furthermore, we verified the dependence of histone modification marker by MER-ERK signalling pathways [5].
Discussion: We show that (a) Cyclin D1 and E-cadherin promoters are regulated by histone modifications in a RAS-dependent manner. (b) EMT associated E-cadherin expression correlates with existence of H3 histone methylation markers on the promoter (c) global histone modification changes and/or their histone modifiers can be proven reliable tumour markers.

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412 An acetylation/phosphorylation signalling network governs turn-over and activity of the splicing factor SC35 in response to cisplatin

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Background: SC35 belongs to the family of serine/arginine-rich (SR) proteins that are crucial regulators of pre-mRNA splicing. It is well established that SR