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