by the ability of cells to cross porous inserts covered in matrigel. Membrane localization was determined by sucrose gradients and immunocytochemistry.
Results: Herein, we report the surprising observation that the TF protein increased by progesterone localizes to the heavy portion of the plasma membrane and does not contribute to coagulation. Instead progesterone-increased coagulative activity is localized to lipid rafts. This activity of progesterone is dependent on transcription, the progesterone receptor and is independent of caveolin-1 presence. In the presence or absence of progesterone, TF cannot be detected in lipid rafts by western blotting, but blocking antibodies against this protein eliminate coagulation. These results suggest that progesterone increases the capacity of basal levels of TF located in the lipid raft region to cleave coagulation factor X in the presence of its ligand Factor VIIa. In confirmation of this theory, the use of either 2-methoxyestradiol or inhibitors of the c-src pathway, which we have to shown to eliminate the increase in TF by progesterone, do not inhibit the capacity of progesterone to increase coagulation. Interestingly both these inhibitors eliminate the ability of progesterone to increase breast cancer cell invasion, shown previously by us to be dependent on TF.
Conclusion: We demonstrate that TF levels do not correlate to coagulative ability in breast cancer cells and show that progesterone can modulate coagulation without increasing TF levels.
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452 A novel method to enrich for glioma stem cells from glioma cell lines

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Background: Glioma stem cells (GSC) are inherently similar to stem cells except they can transform into tumours reminiscent of the pathological features of the originated tumour mass. GSCs serve as an excellent pre-clinical model to comprehend tumour re-growth and treatment resistance. Several approaches were previously described to purify GSCs, but seemingly appeared to be laborious, costly and sometimes with poor yield. Our objective was to investigate alternative strategies to cost-effectively and efficiently enrich for GSCs.
Methods-Results: We grew 3 glioma cell lines in a modified serum free media that promotes the growth of stem cells over a 10 day period and with ease of harvesting from the supernatant. The tumour spheres had cell line specific morphologies. For instance, those from U87 and DB54MG were significantly larger with tightly associated spheres, in comparison to those from U251. The tumour spheres expressed stem cell markers and in fact were 80−96% rich in CD133+ve cells. Upon growth in DMEM/10% FCS tumoursphere diffentiation occured. In addition, the tumour spheres can transform in in-vitro and with the ability to grow into tumours having similar pathological hallmarks but faster growth in comparison to xenograft tumours derived from the growth of glioma cell lines. These findings were overall similar with passages 1, 10 and 30 GSCs examined.
Conclusions: We have discovered an alternative strategy to enrich for glioma stem cells from glioma cell lines in a cost-effective, easy and efficient manner. Current efforts are undertaken to utilize our protocol to enrich for glioma stem cells from surgical tissues.

453 The effectiveness of Fas apoptosis signalling pathway determined by the combined action of functional polymorphisms at Fas, FasL and Fadd

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Background: In previous reports we described germ-line functional polymorphisms that differentiate *Fas* and *FasL* genes in two mouse strains (SEG/Pas and C57BL/6J) exhibiting extreme differences in susceptibility to gamma-radiation induced T-cell lymphomas. In this study we provide new data about the functional significance of the intra-cellular and extra-cellular polymorphisms of *Fas* and *FasL*, and report new polymorphisms in the coding sequence of *Fadd*, another key element of this pathway.
Material and Methods: Chimerical Fas and FasL proteins were constructed, combining the intra- and extra-cellular regions derived from C57BL/6J and SEG/Pas. TUNEL apoptosis assay was used to evaluate the induction of apoptosis in cells bearing wild-type/chimerical Fas and FasL molecules. Caspases cleavage assessment through Western Blot served to confirm the TUNEL assay results. C57BL/6J and SEG/Pas-*Fadd* cDNAs were genotyped and sequenced. Fadd-FLAG and Fas-HA constructs were used in co-immunoprecipitation assays.
Results: When assaying TUNEL in the chimerical systems, we found significant reductions in the levels of apoptosis they induce, as compared with those of the SEG/Pas system. This suggests evidence that the polymorphic residues we identified at the intra- and extra-cellular regions of both the Fas receptor and its ligand exhibit a different functionality. As expected, the accumulation of polymorphisms, represented in the double-chimerical systems, produces the highest differences of apoptosis. Strikingly significant seems as well the functionality of those polymorphic amino acids located on the intracellular region of Fas, through which it interacts with Fadd. These might determine different affinities of interaction between Fas and Fadd, given that none of the polymorphic residues found at *Fadd* cDNA between C57BL/6J and SEG/Pas lie in its death domain. Co-immunoprecipitation experiments show that the interaction Fas-Fadd is stronger when Fas derives from SEG/Pas, confirming the different functionality of the polymorphic residues at the intracellular region of Fas.
Conclusions: Our results support the functionality of polymorphisms located at the intra- or extra-cellular regions of Fas and FasL, but prompt us to consider that the functional consequences of any of those changes should be assessed within the general context of the system.

454 c-Met endosomal signalling and breast cancer cell migration

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Background: c-Met, the receptor of HGF (Hepatocyte Growth Factor), is a tyrosine kinase receptor overexpressed or mutated in various cancers. In breast cancer, c-Met has been associated with cancer progression and metastasis and is considered to be a marker for poor prognosis. Therefore specific targeted therapy against c-Met may provide a valuable therapy for patients.
The mechanisms of c-Met signalling that promote breast cancer progression are poorly understood. Recently, it has been shown that tyrosine kinase receptors, including c-Met, continue to signal from the endosome after internalisation. This endosomal signalling may have unique consequences on cellular outcome due to the spatial and temporal activation of downstream signalling pathways.
The objectives of this study were to investigate and compare the role of c-Met endosomal signalling in the migration of a range of human breast cancer cell lines, ranging from a pre-invasive to a highly invasive phenotype.
Material and Methods: c-Met internalisation, intracellular trafficking and downregulation were compared using FACS analysis and confocal microscopy. The relationship of c-Met trafficking to signalling, cell migration and invasion was determined using western blot analysis, transwell migration assays and 3D organotypic invasion assays.
Results: We find that the requirement of c-Met for endocytosis in the stimulation of several signalling pathways and in cell migration varies significantly between the cell lines. The more aggressive cell lines seem more reliant on c-Met endocytosis for the full activation of Gab1, ERK and AKT downstream of c-Met. Consequently, these cells require c-Met trafficking for their full migration and invasion.
Conclusions: Our results suggest that c-Met endosomal signalling might play a role in breast cancer progression.

455 Bcl-2 regulates HIF-1alpha protein stabilization in hypoxic melanoma cells via the molecular chaperone HSP90

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Background: Hypoxia-Inducible Factor 1 (HIF-1) is a transcription factor that is a critical mediator of the cellular response to hypoxia. Enhanced levels of HIF-1α, the oxygen-regulated subunit of HIF-1, is often associated with increased tumour metastasis, therapeutic resistance and poor prognosis. In this context that we previously demonstrated that the antiapoptotic protein bcl-2 cooperates with hypoxia topromote HIF-1/Vascular Endothelial Growth Factor (VEGF)-mediated tumour angiogenesis.
Material and Methods: Expression vectors encoding human bcl-2, *wild type* or hydroxylation resistant HIF-1α were used for stable and transient transfections of M14 human melanoma line. The effect of bcl-2 stable transfection will be evaluated in cells under hypoxic conditions in terms of bcl-2 and HIF-1α protein expression and localization (*western blot and confocal microscopy analyses*) HIF-1α protein stability and ubiquitination (*Western blot and immunoprecipitation analyses*) and HIF-1 transcriptional activity (*reporter assay*). The role of Heat Shock Proteins (HSPs) in the bcl-2-mediated regulation of HIF-1α expression and transcriptional activity (*Western blot analysis and reporter assay*) was evaluated by using chemical inhibitors. Immunoprecipitation experiments were also performed to investigate the possible effect of bcl-2 protein on the interaction of HIF-1α and HSPs.
Results: By using M14 human melanoma cell line and its derivative bcl-2 overexpressing clones, we demonstrated that bcl-2-induced accumulation of HIF-1α protein during hypoxia was not due to an increased gene transcription. In fact, it was related to a modulation of HIF-1α protein expression at a post-translational level, indeed its degradation rate was faster in the control lines than in bcl-2 transfectants. The bcl-2-induced HIF-1α stabilization in response