480 Implications of cellular ERalpha/ERbeta ratio in uterine smooth-muscle cells carcinogenesis

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Background: Uterine LM are very common benign tumours of uterine muscle cells. The types in terms of histological criteria consist of ordinary leiomyoma (LM), bizarre or atypical leiomyoma (AL), and cellular leiomyoma (CL). In contrast, malignant tumours or leiomyosarcomas are rare but with a high risk of local recurrence and metastasis. Although the etiology of uterine LM is unknown, their development is considered to be estrogen dependent. It was also shown that the ratio of estrogen receptor a (ERa) to estrogen receptor b (ERb) expression, rather than the individual expression levels, possibly determines the growth potential of LM. The factors affecting transformation of uterine smooth-muscle cells to LMS is unknown although the estrogen may have effect on this process.
The estrogen receptors ratio is a determinant factor in several estrogen-dependent cancers such as in breast, colon, prostate and more recently, endometrium. There are no previous data about ERb in malignant smooth-muscle uterine neoplasms. Our aim was to determine the cellular ratio of ERa to ERb expression in three types of uterine smooth muscle tumours, LM, AL, LMS, and compare it with healthy myometrium. This was contrasted with the expression of the proliferation factor, Ki67.
Methods: We used a significant number of formalin-fixed and paraffin-embedded tumoural and healthy uterine smooth muscle tissue samples, from Hospital Universitario de Canarias and Hospital La Colina, to perform immunohistochemical detection.
Results: In healthy myometrium and LM, ERa is predominantly expressed but LM show and increase of ERb-ir. Interestingly in AL the ratio ERa/ERb is reverse and greater expression of ERb compared to ERa is observed. Even more, all leiomyosarcomas studied were ERb-ir, while only a low percentage of them were ERa-ir This inversion of the ratio ERa/ERb observed during tumour progression was statistically significant.
Conclusions: These results are correlated with Ki67 expression, also suggesting that the ERb/ERa ratio is a useful index of progression in myometrial tumours and that ERb has an important role in uterine smooth-muscle malignancy.

481 Dissection of the NG2 involvement in extravasation phenomena simulated in vitro

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Background: The metastatic cascade entails an initial step in which tumour cells intravasate lymphatic or haematic circuits and develop the ability to interact with the endothelium of distant sites, followed by a subsequent interaction with the underlying ECM to pursue their tissue infiltration. To explore more in detail some of these metastatic passages and address the role played by membrane-bound proteoglycans (PGs), we have devised an experimental paradigm that allows us to investigate how these PGs) may directly or indirectly affect cell-cell and cell-ECM interactions in settings that mimic *in vitro* the rheological conditions encountered by tumour cells in peripheral blood.
Materials and Methods: A perfusion system that mimics the microcirculation was devised and exploited to explore the interactions of sarcoma cells harbouring diverse constitutive expression of NG2 or manipulated levels of the PG through RNAi, with activated and non-activated endothelial cells (HUVEC) or with native ECM isolated according to a specifically devised protocol from vascular smooth muscle cells. To investigate the extravasation capabilities of the same cells, we have combined our protocol for deriving native, cell-free ECM with transmigration assays involving porous membranes.
Results and Conclusions: In perfusion experiments cells enriched by immunosorting for high surface levels of NG2, bound significantly more avidly to activated HUVEC and, most strikingly, clustered in the proximity of the endothelium. Chondroitin sulphate chains of NG2, and possibly other unidentified cell surface PGs, seemed to play a key role in these cell-cell interactions. Antagonists of known cell-cell adhesion molecules and signal transduction probes are currently adopted to dissect the involved molecular mechanisms. In similar experiments with cell-free native ECM, we observed a differential capability of tumour cells to interact with it and even in this case the presence of NG2 modulated their adhesion to the underlying matrix.
Preliminary transmigration data show that expression of NG2 also modulate the capability of cells to extravasate in presence of native ECM, especially in the initial phases of the process. The findings highlight a crucial role of sarcoma NG2 in mediating the tumour cell-endothelium binding and the cells interaction with vascular matrix suggesting a fundamental role of the PG in the control of intra- and extravasation events.

482 Characterising the role of the metastasis associated cell surface glycoprotein CDCP1 in cancer cell lines – possible roles in cell adhesion and survival

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Background: CUB domains are immunoglobulin-like folds often involved in protein-protein interactions. Expression of CUB Domain Containing Protein 1 (CDCP1) in cancer is associated with an increased frequency of metastasis. Our investigations have focussed on CDCP1's role in colon cancer cell line adhesion and survival.
Methods: The CDCP1 negative Colo320 cell line was stably transfected with an expression vector encoding a CDCP1-FLAG fusion protein, yielding the Colo320-CDCP1 cell line. Apoptosis assays, cell cycle analysis, cell-cell aggregation assays and CDCP1 cell surface expression assays were performed by flow cytometry. Antibody-mediated CDCP1 endocytosis was assessed by immunocytochemistry. Cell adhesion assays were conducted in 24 well plates +/− a coating of 10 μg/ml Matrigel.
Results: In fixed A549 cells, CDCP1 was enriched along points of cell-cell contact. The rate of CDCP1 antibody-mediated endocytosis was reduced at points of cell-cell contact in both SW480 and A549 cell lines. Calcium supplementation of growth media preferentially increased aggregation of Colo320-CDCP1 cells and reduced binding to tissue culture plates. Matrigel reduced the binding of the Colo320-CDCP1 cell line to tissue culture plates. Reduction of CDCP1 protein by RNA interference (RNAi) in SW480 cells decreased cell binding to Matrigel coated tissue culture plates.
CDCP1 RNAi reduced the growth rate of SW480 cells forced into suspension, whereas it had negligible effects on adherent SW480 cells. SW480 cells forced into suspension for 20 hours displayed a lower level of sub-G1 propidium iodide (PI) staining (an indicator of apoptosis) than cell lines known to undergo anoikis (cell death induced by loss of adherence to substrate) such as HaCaT and 3T3. However the apoptotic frequency (assayed by sub-G1 PI stain) of SW480 cells forced into suspension for 20h following 48h CDCP1 RNAi was not altered.
Conclusions: CDCP1 modulates the adhesive properties of colon cancer cell lines. Some CUB domains have been demonstrated to bind Ca^{2+}. Given that calcium affected the adhesive properties of Colo320-CDCP1 cells it is possible that one or more of CDCP1's CUB domains bind Ca^{2+}. It remains to be investigated whether CDCP1 regulates cell adhesion directly or through association with other Ca^{2+} binding proteins such as Cadherins. Further work is required to determine the role of CDCP1 in the prevention of anoikis in SW480 cells.

483 Dual role of the extracellular matrix glycoprotein EMILIN2 in the tumour microenvironment

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Background: Elastin Microfibril Interface Located ProteINs (EMILINs) are a family of extracellular matrix (ECM) glycoproteins characterized by the presence of an N-terminal cystein rich EMI-domain and a coiled-coil region. We have recently demonstrated that EMILIN2 is a pro-apoptotic molecule that significantly reduces the viability of different tumour cell lines resulting not toxic for normal cells. The peculiar mechanism of action involves a direct binding of EMILIN2 to TRAIL receptors and the activation of the extrinsic apoptotic pathway. The binding to death receptors triggers receptor clustering, colocalization with lipid rafts, DISC assembly and caspase activation. The aim of this study was to identify the EMILIN2 pro-apoptotic region and to verify its antitumourigenic potential *in vivo*.
Material and Methods: Cell viabilty and apoptosis were evaluated by MTT and TUNEL assays, respectively. The interaction between EMILIN2 and its deletion mutant with TRAIL receptors was analyzed by GST pulldown using *in vitro* transcribed and translated proteins. Tumourigenicity in the presence of EMILIN2 or the deletion mutants was analyzed by soft agar colony and clonogenic assays. For *in vivo* experiments fibrosarcoma or malignant glioblastoma cells were subcutaneously injected in nude mice and pharmacologically treated every other day. Tumours apoptosis was quantified by TUNEL assay and analysis of caspases-8 and -3 activity while tumour vasculature was analyzed by immunofluorescence.