its substrate GSK3β and either restores or increases the levels of expression of E-Cadherin. Moreover, the treatment with the MEK inhibitor U0126 induces similar effects to those observed after elimination of B-RAF expression, revealing that these effects are mediated by the canonical MEK/ERK-MAPK pathway. Furthermore, cell migration and invasion decrease when B-RAF expression and MEK activity are inhibited in these cells.
All these data show that ^{V600E}B-RAF plays a key role in EMT of thyroid cancer cells by increasing ILK expression and the concomitant decrease of E-Cadherin levels. These and further investigations may contribute to explain the role of ^{V600E}B-RAF in thyroid cancer progression and will help to understand the biological processes involved.

420 Impact of altered expression of E2Fs gene family on tumourigenic and phenotypic process in human colon cancer

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Background: In order to identify molecular genomic markers predictive of initiation and/or progression of human colon cancer (CC), a genome-wide analysis highlighted a micro-deletion at the 1p36.11−12 region in 23% (n = 115) and 47% (n = 59) of adenomas and carcinomas, respectively. Also, RT-QPCR evidenced a E2F2 transcript decrease in human colon tumours. Within the micro-deleted region, a potential target gene, E2F2 is described as either oncogenic or tumour suppressor, depending on the tissue or cell type. Thus, the aim of this study was to specify the functions of some of the E2F genes in CC, and the impact of the E2F2 deletion in the human colon carcinogenesis process.
Material and Methods: Two activators, E2F1 and E2F2, and one repressor, E2F4 were over-expressed in the human CC cell line TC7 (a well-differenciated subclone of Caco-2 cells) via stable transfection with plasmids containing the cDNA of each gene or the cDNA coding for the green fluorescent protein as a control. Survival (clonogenic assay) and migration properties (wound healing assay) of the cells were then assessed. In parallel, these cells were subjected to RNA interference experiments using siRNA directed against E2F1, -2 and -4. Transfection efficiency was validated by RT-QPCR and Western blot and phenotypic studies evaluated by immunocytochemistry.
Results: The number of colony-forming cells and the cell migration process were decreased in cells overexpressing E2F1 or E2F2 and to a lesser extent in those overexpressing E2F4.
E2F1, -2 and -4 silencing induced gene-specific morphological modifications, notably by modulating the expression of markers involved in differentiation, cytoskeleton organisation and cell-cell junctions. In addition, alteration of E2Fs expression in tumour colon cells induced compensation/regulation mechanisms between E2Fs themselves and with known associated proteins such as the pocket protein pRB and their dimerization partner DP. Finally, the tumourigenic potentiel of E2Fs overexpression has been evaluated by subcutaneous injections of the transfected cells into nude mice.
Conclusions: These data suggest that in colon TC7 cells, altered expression of E2F2, as well as of two other E2Fs members, brings the cells to change their migratory, survival and phenotypic properties asssociated with disseminating process. These results will be confronted with the on-going *in vivo* xenograft model experiments to strengthen the hypothesis that E2F2 expression deregulation could play a key role in human colon tumour initiation/progression.

421 The OPCML tumour suppressor functions as a repressor-adaptor, negatively regulating receptor tyrosine kinases in ovarian cancer

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Background: We previously identified opioid binding protein cell adhesion molecule (OPCML) as epigenetically inactivated in 83% of ovarian cancers demonstrating it was a functional tumour suppressor *in vitro* and *in vivo*[1]. OPCML belongs to the "IgLON" family of glycosylphosphatidylinositol (GPI)-anchored cell adhesion molecules with 3 extracellular I-type immunoglobulin domains[2–4]. The GPI anchor may sequester IgLONs to cholesterol-rich "rafts" that could confer specificity of signaling capability and choice of interacting partners[5]. We hypothesized that OPCML might interact with trans-membrane receptor tyrosine kinases (RTKs) and abrogate growth factor mediated signalling.
Materials: The ovarian cancer line SKOV3 shows very low expression of OPCML and is heavily hypermethylated. Stable transfected OPCML lines (BKS2.1 & SKOBS3.5) as well as vector-only control (SKOBS-V1.2) were used to identify differences in RTK expression and phospho-signaling upon growth factor stimulation. RNAi strategies in the normal ovarian surface epithelial line OSE-C2[6] were generated to look at the activation RTK signaling upon depletion of physiological OPCML. Interaction with RTKs in co-IP experiments used the pAb (R&D Systems) in SKOV3 cell lines. Refinement of the OPCML-RTK interactions was carried out using nGST-OPCML fusion proteins with either cell lysates or *in vitro* with expressed RTK fragments.
Results: We show that OPCML interacts with and downregulates HER2 and FGFR1, leading to inhibition of those signaling pathways in ovarian cancer cells. siRNA knockdown of OPCML in OSE-C2 strongly upregulated HER2 with concomitant increase in EGFR signaling. Interrogating multiple RTKs upon constitutive OPCML expression in SKOV3 cells or knockdown in OSE-C2 has highlighted FGFR1 as another target for interaction and down-regulation. The *in vitro* translated HER2 ECD interacted with GST-OPCML protein, but not a truncated fusion protein with the 3rd juxtamembrane Ig domain (Ig-III) deleted suggesting Ig-III is crucial for interaction with HER2. Exploring whether OPCML interacted with FGFR1 & 2 suggested both nGST-OPCML and the truncated version interacts with FGFR1, therefore Ig-III is not essential for FGFR1 binding to OPCML.
Conclusions: The OPCML tumour suppressor functions by negatively regulating HER2 and FGFR1, abrogating their pro-oncogenic functions. This has general implications for understanding the relationship of IgLONs to the RTK pathways, and their role in cancer biology.

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422 LPA receptor 4 mediates autotaxin-induced invadopodia production and invasion

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The ability of cancer cells to invade and metastasize is the major cause of death in cancer patients. Recent studies indicate that tumour cell invasion and metastasis may be initiated by the formation of the actin-rich cell protrusions, invadopodia. Autotaxin (ATX) is a secreted lysophospholipase whose expression level within tumours correlates strongly with their aggressiveness and invasiveness. ATX produces LPA, a phospholipid with known tumour promoting functions that acts through the G-protein coupled receptors, LPA_{1-6}, although only LPA_{1-3} have been linked to tumour progression. The purpose of this study was to assess the implication of ATX and LPA signaling in tumour cell invasion and invadopodia production.
We used the invasive fibrosarcoma cell line, HT1080, in fluorescent matrix degradation assays to study the influence of ATX on invadopodia production. Our results using HT1080 cells stably transfected with ATX or shRNA targeting ATX indicate that ATX is implicated in the production of invadopodia resulting in an increase in both their formation and function. By adding LPC or LPA, the substrate and product of ATX, to our assays we demonstrated that invadopodia production is dependent on the production of LPA from LPC. Among the LPA receptors LPA_4 has the highest expression in HT1080 cells. Using LPA_4 shRNA as well as agonists and inhibitors of the cAMP pathway we provide evidence that LPA_4 signaling through the cAMP-EPAC-Rap1 axis regulates invadopodia formation downstream of ATX. Furthermore, inhibition of Rac1, a known effector of Rap1 and invadopodia formation, abolished EPAC-induced invadopodia production, suggesting downstream participation of Rac1. Finally LPA_4 knockdown was further correlated with a decrease in cell invasion and in vivo metastasis in a 3D assay and a lung metastasis assay, respectively.
Our results suggest that ATX through LPA_4 is a strong inducer of invadopodia formation that correlates with the ability of the cells to invade and metastasize. This study also revealed an unexpected signaling pathway for cell invasion involving LPA_4-driven cAMP production and subsequent activation of the EPAC-Rap1-Rac1 axis.