deficiency of folate is connected with a variety of human diseases, such as atherosclerosis, neural tube defects, and cancer. Epidemiological studies have shown an inverse relationship between folate status and the risks of some malignancies including cancer of colon, esophagus, stomach, pancreas, lung, cervix, ovary, breast and leukemia. However, the molecular mechanism underlying of FA-induced anti-cancer activity is not fully understood. The aim of this study was to use human colon adenocarcinoma cell line COLO-205 to address this issue. Our results show that FA inhibits the growth COLO-205 through proliferation inhibition. FA-induced cell cycle arrest in COLO-205 cells occurred when the cyclin-CDK system was inhibited just as p21 and p27 protein levels increased. In vivo study demonstrates that daily i.p. injection with folic acid significantly reduced the tumor volume and prolonged the life span in the COLO-205 tumor-bearing null mice. The findings from the present *in vitro* and *in vivo* studies suggest the potential applications of FA in treating human colon cancers.

237 Investigation of the Human Epidermal Growth Factor Receptor Family as Potential Therapeutic Targets for Bladder Cancer

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Introduction: Bladder cancer is a common malignancy worldwide. Treatment typically involves surgery and chemotherapy, but outcomes remain far from optimal. Research is now focused on the identification of molecular targets which could be used for targeted therapy. The epidermal growth factor receptors 1 and 2 (EGFR and HER2) have been found to be overexpressed in some bladder cancers and have been linked to poor prognosis. These cell membrane receptors have tyrosine kinase activity and transduce signals controlling cell growth, survival and differentiation through various intracellular pathways. EGFR and HER2 are deregulated in various cancers, but their role in bladder cancer is still undefined. We aimed to investigate whether targeted drugs against EGFR and HER2 could be effective treatments for bladder cancer. These studies could provide the rationale for the use of novel targeted therapies in bladder cancer.
Material and Method: We have used six bladder cancer cell lines and three tyrosine kinase inhibitors (TKI) to inhibit EGFR and/or HER2; lapatinib, a dual inhibitor of EGFR and HER2, erlotinib, which specifically inhibits EGFR, and CP654577, a novel compound that inhibits HER2. Effect of the TKIs on cell proliferation, cell cycle and apoptosis on the bladder cancer cell lines has been analysed.
Results and Discussion: Cell proliferation of the six bladder cancer cell lines was analysed after being treated with the TKIs, obtaining variable sensitivities among the cell lines. Relative expression of EGFR and HER2 in the six bladder cancer cell lines was studied by western blot and a significant correlation between erlotinib sensitivity and EGFR expression was found. Cell cycle analysis after all three TKI treatments showed an increase in G_0/G_1 phase. A significant increase in dead cells due to apoptosis could also be seen after lapatinib and CP654577 treatment, but no apoptosis was induced after erlotinib treatment. These results suggest that inhibiting HER2 induces apoptosis on these cells, but this effect is not seen after EGFR inhibition. We are currently investigating this by analysing some apoptosis markers in these cells.
Conclusion: These results support the hypothesis that EGFR expression could be a predictive marker for sensitivity to treatments targeted at this receptor and could identify bladder cancer patients suitable for this therapy.

238 ZEB1 and Aberrant DNA Methylation Sustain Mesenchymal-stem Phenotype and the Resistance to EGFR Inhibitors in Breast Cancer

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ZEB1 is one of the drivers of EMT (Epithelial Mesenchymal Transition) that, by repressing E-cadherin and cell polarity genes, has a crucial role in tumor progression and metastasis.
In this study we investigated the role of ZEB1 in basal-like/triple negative breast cancer, a particularly aggressive subtype of breast cancer (BC) characterized by the lack of hormone receptors and HER2. Furthermore, this subtype of BC frequently expresses EGF receptor (EGFR) and is enriched with EM-transited cells showing features of cancer stem cells (CSC).
Loss of function studies in two breast cell models, MDAMB231 and MDAMB157, whose features resemble those of highly aggressive EMT transited BC, showed that actually the down-regulation of ZEB1 reduced the $CD44^+/CD24^-$ stem population, induced a luminal 'differentiation' as demonstrated by the augment of EpCAM expression, and partially reverted the EMT phenotype. Interestingly, a marked transition towards the epithelial phenotype was achieved by the simultaneous ZEB1 silencing and demethylating treatment. In fact, E-cadherin expression, a hallmark of epithelial cells, was noticeably induced in ZEB1 depleted cells by a demethylating treatment that concurrently stimulated the expression of miR200c, a regulator of several EMT-drivers. However, in these cell models we did not observe a clear contribution of miR200c in the shrinkage of stem population induced by ZEB1 downregulation.
Intriguingly, knock-down of ZEB1 promoted the activation of EGFR even in the absence of stimuli and associated with an increased sensitivity to the EGFR inhibitor 324674 in the MDAMB231 cell line.
Taken together these data suggest that the depletion of ZEB1 is not sufficient to revert the EMT phenotype in highly metastatic models. Indeed, concurrently demethylation was fundamental for a marked reversion of mesenchymal phenotype. Importantly, ZEB1 expression and EMT might affect response to EGFR-inhibitors in breast cancers prompting the needs for a better characterization of EMT features in patients receiving EGFR targeting therapies.

239 The Cancer-associated K351N Mutation Affects the Ubiquitination and the Translocation to Mitochondria of p53 Protein

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Background: Stress-induced monoubiquitination of p53 is a crucial event for the nuclear-cytoplasm-mitochondria trafficking and transcription-independent pro-apoptotic functions of p53. Although an intact ubiquitination pathway and a functional Nuclear Export Sequence (NES) are required for p53 nuclear export, the role of specific residues within this region in regulating both processes remains largely unknown.
Materials and Methods: Here we characterize the mechanisms accounting for the nuclear accumulation of a new point mutation (Lys 351 to Asn) in the NES of p53 identified in a cisplatin-resistant ovarian carcinoma cell line (A2780 CIS). In particular we evaluated the ubiquitination stauts of p53 K351N, its tanslocation to mitochondria and mitochondria-dependent apoptosis.
Results: We found that K351N substitution abrogates the monoubiquitination of p53 induced by both Mdm2 and MSL2 E3-ligases. As a consequence, cells expressing p53 K351N mutant showed defects in cisplatin-induced translocation of p53 to mitochondria, Bax oligomerization and mitochondrial membrane depolarization.
Conclusion: These data identify K351N as a critical mutation of p53 that contributes to the development and maintenance of resistance to cisplatin.

240 Hyperacetylation of Tubulin as a Chemoresistant Biomarker in Human Docetaxel-Resistance Prostate Cancer Cells

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Background: Docetaxel-based chemotherapy has generally been considered as one of the effective treatments for prostate cancer. Unfortunately, clinical treatment with docetaxel often encounters a number of undesirable side effects, including drug resistance. Therefore, it has become essential to identify molecular events that may be associated with the development of docetaxel resistance. Tubulin isoforms have been previously examined for their resistant ability to docetaxel in many cancers, but their real mechanisms remained unclear. In this study, we evaluated the feasibility of employing docetaxel as a cytotoxic agent for PC3 cells and to examine the role of acetyl-tubulin in docetaxel-resistant prostate cancer.
Materials and Methods: A series of docetaxel-resistant PC3/DX cell subclones, were established by chronically exposing PC3 to progressively increased concentrations of docetaxel. Herein, we characterized the docetaxel-mediated cytoxicity and molecular events in PC3 and PC3/DX cells by MTT assay, Western blotting, RT-PCR and real-time PCR.
Results: Our results showed that levels of acetyl-tubulin, α-tubulin, β-tubulin, γ-tubulin and βIII-tubulin were significantly higher expression in PC3/DX than in parental PC3 cells by Western blotting analysis. PC/DX with greater resistance to docetaxel had higher levels of acetyl-tubulin than in PC3 cells. The expression of acetyl-tubulin was gradually increased by docetaxel in a dose- and time-dependent manner in PC3 cells. Histone deacetylase 6, a deacetyl enzyme of tubulin, mRNA and protein levels were significantly decreased in PC3/DX than in PC3 cells. Interestingly, we also found an evident up-regulation of acetyl-tubulin protein expression after recombinant epidermal growth factor treatment in a time-dependent manner in PC3 cells.
Conclusions: Up-regulation of acetyl-tubulin may play an important chemoresistant role in docetaxel-resistant prostate cancer.