Here, we will present the RPPA technique and provide examples of applications in the field of translational cancer research, based on our in-house projects and collaborations. We aim to put forward the possibilities of the technique and of our platform, which is now open for external collaborations.

170 A chemical genetics screen identifies novel steroid inhibitor drugs that inhibit the growth of glioma cell lines

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Background: Gliomas are among the top 5 causes of cancer related deaths, representing about ~60% of the cases in adults and ~30% in children. Despite current treatments (surgery, radiation, and chemo-therapy), the overall survival is still poor. Current promises exist with patients treated with adjuvant temozolomide, however only 10−15% typically have a positive response with combined surgery and radiation therapies leading to prolonged survival of up to 2 years. Since a wide range of steroid receptors are expressed in gliomas, our objective was to investigate whether novel classes of steroid inhibitor drugs can be used efficiently to inhibit glioma growth. To achieve this, we studied the effect of these drugs on the growth of glioma cell lines.
Methods-Results: We screened using a candidate chemical structure approach, a library of 400 steroid inhibitor drugs on 5 human glioma cell lines, and a normal human astrocyte cell line. We discovered 4 potent new drugs of the Androsterone family that can induce significant death of glioma cell lines ($n = 5/5$) within a 24 hour period in contrary to normal human astrocytes. These drugs induced significant apoptosis resulting in an overall decreased viability and proliferation of the cells in a dose dependent manner (5 μM and 10 μM). Furthermore, significant inhibition of transformation was noted.
Conclusions: We have discovered a novel chemically distinct class of drugs that can significantly inhibit the growth of glioma cell lines. Current efforts are undertaken to study more of the mechanistic function of these drugs.

171 Impact of TACSTD1 germline deletions as Lynch syndrome causing mutations in Spanish hereditary non-polyposis colorectal cancer – suspected patients

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Lynch syndrome (LS) is an autosomal dominant inherited cancer syndrome characterized by the occurrence of early-onset cancers of colorectum, endometrium and other tumours. The ethiology of LS is related to the DNA mismatch repair (MMR) inactivation caused by germline mutation of a MMR gene (*MLH1*, *MSH2*, *MSH6* or *PMS2*) followed by somatic inactivation of the second allele. Standard genetic testing for LS frequently delivers unsatisfactory non-informative results with a lack of pathogenic mutation in the MMR genes. Very recently, it has been shown that germline deletions involving the last exons of a non-MMR gene as *TACSTD1* may produce the silencing of its neighbouring gene *MSH2* by its promoter hypermethylation.
The aim of this study was to evaluate the prevalence of *TACSTD1* deletions as LS causing effect in Spanish population, and the clinical implications in a Genetic Counselling Unit.
A total of 501 index subjects from LS suspected families (Bethesda guidelines) from the Genetic Counselling in Cancer Units at the Comunidad Valenciana (Spain) were included. Standard procedures were approached for the analysis of MMR proteins expression (immunohistochemistry), MSI (with five mononucleotide markers), *BRAF* mutation (direct sequencing) and *MLH1* methylation (MS-MLPA); as well as *MLH1*, *MSH2* and *MSH6* germline mutation analysis (direct sequencing and MLPA).
Subjects with no mutation at the MMR genes, loss of expression (LOE) of *MSH2,* and MSI were analyzed for large deletions on *TACSTD1* locus by MLPA. Detected deletion was confirmed and mapped by long range PCR experiments from genomic DNA.
The number of cases with LOE of *MSH2* and MSI was 25. From those, we found 15 mutated subjects at *MSH2* ($n = 10$) or *MSH6* genes ($n = 5$). The remaining 10 cases with non-detected mutation were selected to *TACSTD1* deletion analysis. One case was found to harbour a large deletion in that locus (1/10). This deletion expand for 8.6 Kb including *TACSTD1* exons 8 and 9. A second affected member of this family carried the same deletion. In both cases the tumours showed *MSH2* promoter hypermethylation. The family fulfilled the Amsterdam I criteria.
The *TACSTD1* deletion analysis, and the subsequent *MSH2* methylation testing in the tumour, is a fast and low-cost procedure that may help in the identification of LS causing mutations, and should be incorporated in the LS genetic analysis strategy in clinical setting. We propose a decision-tree flow diagram to help with this analysis.

172 Trefoil factor 3: a potential diagnostic and prognostic marker whose expression contributes to malignant feature in endometrial carcinoma cells

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Background: Endometrial carcinoma (EC) is the most common gynecologic malignancy in Western world. To date, no good marker for EC screening or disease monitoring is available. Trefoil Factor 3 (TFF3) is a secreted glycoprotein that we recently found elevated in the serum of patients harbouring poorly-differentiated (G3) endometrial carcinoma (EC) compared to healthy patients (NE).
Materials and Methods: We extended TFF3 serum determination by an in-house sandwich ELISA to 32 well-differentiated (G1), 70 moderately-differentiated (G2) and 58 G3 ECs, other than 43 NEs. Moreover, to determine TFF3 clinicopathological significance in EC, its serum levels were correlated with clinical characteristics including tumour grade, histology, FIGO stage, myometrial and cervical invasion, adnexal and lymph node metastasis and peritoneal cytology. Finally, we permanently transfected an estabilished cell line derived from a G3 EC (J cells) with either TFF3 expression vector or blank vector, testing them in assays of cell proliferation and response to chemotherapeutic agents (carboplatin and taxol).
Results: TFF3 serum levels were elevated (>690 ng/ml, cut-off chosen at 90% specificity on healthy patients) in 25% G1 ECs, in 46% G2 ECs and in 50% G3 ECs. Median preoperative TFF3 value was 586 ng/ml (range, 265–2523) for G1 ECs, 677 ng/ml (range, 191–6520) for G2 ECs and 721 ng/ml (range, 197–3345) for G3 ECs, compared with 495 ng/ml (range, 254–912) for NEs. Differences in TFF3 serum levels were significant in NEs vs G2 ECs and NEs vs G3 ECs (all $p < 0.01$). Interestingly, elevated TFF3 serum levels were significantly associated with high tumour grade (G2+G3 vs G1, $p = 0.04$), advanced FIGO stage ($\geqslant$IIB vs <IIB, $p = 0.02$) and deeper myometrial invasion (M2 vs M1, $p = 0.007$) in EC patients. Moreover, we were able to permanently transfect J cells with TFF3 gene, whose expression was successfully demonstrated both at mRNA and protein level. TFF3-expressing J cells (clone 5D7) showed a significantly prolonged doubling time (27.6±1.1 hours) compared to cells transfected with blank vector (mock E9) (21.5±0.05 hours). Treatment with carboplatin and taxol caused a moderate increase of cell death in mock E9, while no difference in cell death between treated cells and controls was found in clone 5D7 cells.
Conclusions: In conclusion, our results confirm on a large cohort of EC serum samples that TFF3 preoperative levels are frequently elevated in G2 and G3 EC patients compared with normal controls. Furthermore, our data show for the first time that high TFF3 serum levels correlate with a more aggressive EC malignant phenotype, aiding to identify high-risk patients who could benefit from individualized treatments. TFF3 ectopic expression in an endometrial carcinoma cell line resulted in reduced proliferation rate which contributes to resistance to chemotherapy-induced cell death.

173 Structure and molecular dynamics of metastasis biomarker TWIST1

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Background: Approximately 90% of cancer-related death is due to tumour metastasis complications and treatment. TWIST1, a bHLH transcription factor, is reported to regulate cancer metastasis by inducing Epithelial-Mesenchymal Transition (EMT) program. For EMT to occur epithelial cells undergo a transitory transformation into mesenchymal cells by changing the gene expression program, and epithelial markers such as E-cadherin and α-catenin are suppressed while mesenchymal markers, as N-cadherin and vimentin are activated, changing the cell phenotype. In breast cancer (BC) metastasis TWIST1 seems to be the key protein responsible for changing the tumour phenotype to an aggressive and metastatic carcinoma. The crystallographic structure of TWIST1 protein is not available yet, which hampers the study of its characteristics, function and, most importantly, the possibility of rational drug design to block metastasis.
Objective: Our aim is to resolve by computational modeling the TWIST1 dimer structure and three described mutations, and to study their behavior using molecular dynamics simulations.
Material and Methods: Comparative modeling (MODELLER program) with atomic coordinate information from homologous proteins that share sequence