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

similarity with TWIST1 and available structures in the PDB were performed, and the models were properly evaluated and corrected for errors using freeware programs. For molecular dynamics simulations we used GROMACS 3.3.3 package, with OPLS/AA force field and 25 ns of simulation time in solution.
Results: For the bHLH domain the protein of choice to serve as a template was NeuroD/E47 dimer (pdb code 2QL2), 2.5 Å resolution and 47% similarity with TWIST's bHLH domain. The best generated models (homodimer and heterodimer with E47) presented good atomic resolution and more than 96% of the residues at favorable regions according to Ramachandran plot. The N-terminal domain presented a high level of structural disorder (highly flexible backbone) and therefore is difficult to accurately predict its correct structure. A few mutations that affect TWIST1 in humans were also modeled and assessed: Arg119Cys, Ser144Arg and Lys145Glu. The C-terminal domain was also modeled but the template lower similarity with TWIST1 difficulted the generation of a confident model. The RMSD (root-mean-square deviation), energy fluctuation and other characteristics were assessed using molecular dynamics simulation results.
Conclusion: The models for the bHLH and C-terminal domains were obtained and corrected and have a good resolution. The Arg119Cys, Ser144Arg and Lys145Glu mutations induce a loss of DNA binding activity, but the protein is stable and forms dimers. The effect of these mutations *in vivo* will be further assessed by *in silico* docking analysis between the promoter region of specific target genes and TWIST1 dimer.

174 CD24 enhances cell migration and invasion in colorectal cancer through AKT activation

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Background: CD24 is a GPI-anchored mucin-like cell surface protein that has been found to be over-expressed in several different types of solid tumours and this has usually been linked with poor prognosis and diverse biological effects. However, the underlying molecular mechanisms of CD24-mediated effects are still unclear. We studied the function of CD24 in colorectal cancer (CRC) cell lines to scrutinize its cellular effects and the possible downstream signalling pathways through which CD24 mediates its effects.
Material and Methods: The differential expression of CD24 was assessed by q-RT-PCR and western blotting. CD24 was functionally evaluated by (1) forced expression in HCT116 using the CD24- pcDNA3.1 expression plasmid and (2) knockdown, by RNA interference, in HT29 CRC cell lines. The migratory and invasive characteristics of the cells were assessed using trans-well migration assays, matrigel invasion and wounding assays. The effects on colony formation in soft agar were assessed. To investigate downstream signaling pathways, CD24 was knocked down in GP2D cell line and the phosphorylation state of 46 kinases was assessed using the human phospho-kinase antibody array.
Results: Forced expression of CD24 resulted in increased colony formation ($p < 0.01$) compared to control cells. The migratory and invasive capacities of HCT116 cells were increased after CD24 forced expression and decreased after CD24 knockdown ($p < 0.01$). Knockdown of CD24 in DLD1 was associated with reduction of the levels of activation of different kinases predominantly phospho-AKT (S473) by 2.58 folds, phospho-FAK (Y397) by 1.88 folds, and downstream of AKT; phospho-P27 (T198) by 15.1 folds (P27 phosphorylated at T198 site is no longer inhibiting the cell cycle progression and is associated with enhanced cell motility), phospho-eNOS (S1177) by 3.56 as well as phospho-CREB (S133) showed a 2 fold decrease in activity.
Conclusions: Expression of CD24 increased the migratory and invasive capacity of the CRC cell lines, features associated with high metastatic potential. Moreover, CD24 enhanced colony formation in soft agar, a finding associated with increased tumourigenicity of cells. Furthermore, we were able to show that CD24 mediated its effects at least in part through the AKT signalling pathway, which could potentially present a therapeutic target in colorectal cancer patients.

175 Synergetic and alleviatory effects by combinational therapy of ascorbic acid and paclitaxel on sarcoma 180 implanted BALB/c mice

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Background: Paclitaxel is one of the most popular and powerful chemotherapeutic agents against a broad band of tumour type. But it leaded to severe, toxic side effects, and many patients are unable to complete the chemotherapy. Thus, in this study, we attempted to evaluate the efficacy of ascorbic acid as an adjuvant and side effect alleviator of paclitaxel in BALB/c mice implanted with or without sarcoma 180 cancer cells.
Materials and Methods: We revealed the cytotoxicity of H1299 (non small cell lung cancer cell) and H299 (human embryonic lung cell) cells using Neutral Red assay (NR assay) after treated with the gradient concentration of each or together of ascorbic acid and paclitaxel. In vivo experiments, we used eight weeks old BALB/c mice and treated with ascorbic acid and/or paclitaxel after divided into eight subgroups as with or without cancer cell induction. After anesthetized, we did hematological and biological test with whole blood cells and serum and analyzed cancer related gene expression from livers.
Results: In vitro analysis showed that the anticancer effects of combinational treatment of ascorbic acids and paclitaxel together were synergistically increased more than paclitaxel only. As a result of in vitro experiment, the cytotoxicity on lower dose of co-treatment with paclitaxel (0.35 μM) and ascorbic acid were higher than that of a paclitaxel treatment (22.4 μM) without ascorbic acid in H1299 non small cell lung cancer cells. In mouse model experiments, we observed that ascorbic acid treated mice did not show reduction of the numbers of white blood cells, red blood cells and hemoglobin compared to ascorbic acid non-treated mice after paclitaxel challenging on healthy mice. Also, we observed that ascorbic acid not only decrease side effect caused by paclitaxel but also increase anticancer effect in BALB/c mice implanted with sarcoma 180 cancer cells.
Conclusion: In conclusion, we suggested that the ascorbic acid increased the anti-cancer effects as well as reduced the toxicity of paclitaxel in vivo and in vitro trials when the combinational treatment of ascorbic acid and paclitaxel were applied.

176 Is there a role of DNA methylation in Estrogen Receptor alpha (ERa) expression in Breast Cancer (BC)?

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Background: The presence or absence **of ERα** in BC is an important prognostic indicator. Approximately 30−40% of BCs lack detectable ERα protein. Transcriptional regulation of ERa involves both genetic and epigenetic mechanisms. DNA methylation is an epigenetic modification that is associated with transcriptional silencing of ERa gene expression. The objective of the present study was to determine whether ERa expression correlates with DNA methylation status in BC.
Material and Methods: A panel of 52 patients (pts) with primary BC of known ERa status (42/52 ERa-negative; 10/52 ERa-positive) was studied. Genomic DNA was extracted from archive formalin-fixed paraffin-embedded tumour tissues. DNA methylation was determined by chemical modification of DNA and subsequent double "hot start" Methylation-Specific PCR (MSP), followed by detection on agarose gel. The methylation data were correlated with PR and HER2 status as well as with other clinicopathological characteristics in order to determine the impact of methylation in BC.
Results: Methylation of ERa gene was observed in 14/42 pts (33.3%). Correlation of these 14 cases with PR status and HER2 protein expression revealed that 14/14 pts demonstrated PR-negative status and 11/14 HER2-negative protein expression; 10/14 pts were triple-negative. The tumours were infiltrating ductal carcinomas (IDC) in 10/14 pts and of left-sided detection in 12/14 pts. Both IDC and left-sided tumours were detected in 9/14 pts. The ERa methylation status was not correlated with age, tumour size, grade and lymph node metastases. In contrast, no patients with ERa-positive BC presented methylation.
Conclusions: Our results showed that about one-third of ERa-negative tumours presented methylation. These tumours were usually characterized by simultaneously PR-negative status, HER2-negative protein expression, left-sided detection and a histological type of IDC. Whether methylation status actually acts solely or partially to silence ERa transcription is a key question. Since expression of ERα is necessary for response to endocrine therapies, inhibition of DNA methylation to restore ERα expression in ERα-negative tumours might be a therapeutic strategy in BC with ERα-negative phenotype.

177 Kinome analysis in renal cell carcinoma

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Renal cell carcinoma (RCC) accounts for 2−3% of all cancers, making it the tenth common form with an annual increase in incidence of 2%. The most common type of RCC, clear cell renal cell carcinoma, is linked to an inactivation of the VHL tumour suppressor gene in more than 60% of the patients. Loss