the mean weight of NEIL1 males was significantly less than that of their wildtype counterparts ($p = 0.03$). In contrast, from 7 months onwards the NEIL1 knockout females weighed significantly more than the wildtypes ($p = 0.05$). Initial data from the myeloperoxidase assay suggests that whilst there is no difference in basal levels, there is significantly less neutrophil activity in the liver, heart and gut tissue of knockout animals when treated with 20 mg/kg lipopolysaccharide ($p = 0.05$).

Conclusions: The NEIL1 knockout mice show no obvious phenotypic change other than the weight differences described. No evidence of an obese phenotype was observed. The NEIL1 protein may, however, act in concert with other DNA repair proteins as a regulator of the immune system. In addition to the knockout mice, murine embryonic fibroblasts have been generated and together they should enable us to probe the biochemical and biological role of NEIL1 in genomic stability and the inflammatory process.

165 Array-based approach for early tumour detection in the biliary tract

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Background and Aim: DNA methylation has been shown to play an important role in early tumourigenesis. So far, few DNA methylation target genes have been identified in hepatopancreatobiliary (HPB) cancers. Both cholangiocarcinomas and pancreatic carcinomas have a poor prognosis due to late clinical presentation. Cholangiocarcinomas in particular may be difficult to diagnose. In this study we aimed to identify novel methylated gene targets using HPB cancer cell lines.

Methods: Gene expression profiles of cholangiocarcinoma cell lines (n = 6) were analyzed before and after treatment with a combination of 5-aza-2′deoxycytidine and trichostatin A. CpG island containing genes upregulated after drug treatment in cell lines and simultaneously downregulated in cholangiocarcinomas compared with normal tissue, were selected for further analysis. Expression profiles of primary cholangiocarcinomas were acquired from published data sets [1,2]. The methylation status of these candidates was analyzed by methylation-specific polymerase chain reaction (MSP) in HPB cancer cell lines (n = 24).

Results: Fifty-seven candidate genes displayed increased expression in cholangiocarcinoma cancer cell lines and decreased expression in primary cholangiocarcinomas. Forty-one of these targets contained a CpG island in the promoter region and were subjected to DNA promoter methylation analyses. So far, twenty-one genes are analyzed in all cell lines. Four shared no methylation, others were methylated in one or few tumour types, and six genes including *SFRP1* and *ZSCAN18* were methylated across several HPB cancer cell lines.

Conclusions: By using a genome-wide approach we have identified several epigenetically regulated genes in HPB cancer cell lines. These target genes will be submitted for methylation studies in tumour and normal tissue as well as biliary brush cytology specimens in a hunt for early biomarkers.

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166 Withdrawn

167 MicroRNA-34 family in triple-negative/basal-like breast cancer

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Background: Breast cancer is the most common malignancy of the woman after skin malignancies. “Basal-like” carcinoma is one of the most aggressive molecular subtype of breast cancer characterized by triple-negative (ER-, PR-, Her2-) and “basal-cell” phenotype and is associated with high grade, poor prognosis, and younger patient age. Adverse clinical outcome of these patients is also associated with frequent incidence of BRCA1 and p53 mutations. MicroRNAs have potential to post-transcriptionally regulate even one third of human genes, among them also significant number of important oncogenes, tumour suppressor genes and genes connected with invasion, dissemination and chemoresistance of tumours are involved. MicroRNA-34 family is under direct transcriptional control of p53 and seems to function as tumour suppressor. Mutations of p53 can induce decrease of microRNA-34 family and consequently apoptosis rate.

Material and Methods: In our study we examined expression levels of microRNA-34 family in 41 specimens of “basal-like” carcinoma by use of Real-Time PCR. Invasive breast carcinomas were immunohistochemically analysed for oestrogen receptors (ER), progesterone receptors (PR), cytokeratin 5/6 (CK5/6), epidermal growth factor receptors (EGFR), Ki67, p53 and vimentin. Tumours were considered to have basal-like phenotype if they were ER negative and HER2 negative, but positive for CK5/6 and/or EGFR and/or vimentin.

Results: Expression levels of miR-34b and miR-34c were markedly lower than those of miR-34a. We identified significantly higher levels of miR-34a in primary tumours disseminated to regional lymph nodes ($p = 0.0209$). Further, we observed increase of miR-34b levels in patients with significantly shorther overvall survival ($p = 0.05$). We did not proved association of microRNA-34 and grade, BRCA1 or clinical stage of breast cancer.

Conclusion: Our results suggest potential significance of miR-34a in invasiveness and dissemination of “basal-like” carcinoma and usage of miR-34b in diagnostic and predictive oncology of this aggressive molecular subtype of breast cancer.

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168 Grp78 activity is associated with Androgen Receptor status and upregulated in Hormone-Refractory prostate cancer

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Background: Prostate Carcinoma (PC) is the most commonly diagnosed cancer of men in the developed world and relapse of PC following androgen deprivation remains a major clinical problem. Better prognostic biomarkers may facilitate treatment stratification and improve patient outcome. The aim of our study is to investigate if Grp78 expression in prostate cancer is associated with clinic-pathologic parameters including survival and the development of castrate resistance.

Materials and Methods: Immunohistochemical analysis was performed on formalin-fixed, paraffin embedded tissue microarrays containing PC specimens. 259 primary PC samples along with 36 matched pairs of hormone naïve and castrate resistant prostate cancer (CRPC) samples were studied for Grp78 expression.

Results: Using the weighted Histoscore method graded by independent observers, upregulated Grp78 expression was found to be associated with prostate carcinogenesis. Immunohistochemical expression of Grp78 in malignant tissue (n = 164) was significantly higher than benign tissue (n = 23) ($p = 0.000$). CRPC specimens also contained a significantly greater Grp78 staining than their matched hormone naïve specimens ($p = 0.028$). A higher Grp78 stain was significantly associated with 39 samples expressing androgen receptor positivity in the nucleus ($p = 0.010$). A Kaplan-Meier Survival analysis for androgen receptor positive tumours revealed a greater median survival time of 8.011 years in samples with low Grp78 stain as compared to 4.506 years in samples with high Grp78 stain ($p = 0.049$). Grp78 did not have any correlation with the degree of metastasis in patients ($p = 0.724$). It also did not have any influence on the biochemical relapse rate ($p = 0.501$) and time to death ($p = 0.653$) in the transition of hormone-naïve to castrate-resistance.

Conclusion: Grp78 expression is significantly associated with androgen receptor status and is upregulated in CRPC. It may play a key role in prostate carcinogenesis and further investigations are warranted to validate its use as a prognostic marker.

169 Reverse phase protein arrays: a powerful tool for cancer proteomics

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Tumourigenesis implies major changes in cell signaling pathways involved in cell adhesion, proliferation and apoptosis. Activation of these pathways largely depends on post-translational modifications, such as phosphorylation, and thus cannot be analyzed at the mRNA level in transcriptome profiling. Therefore, high-throughput analysis at the protein level seems an absolute requirement to get a better insight in tumour biology. Yet, the technology required for such proteomics approaches has become available only very recently.

At the Translational Research Department of the Institut Curie (France), we developed a platform specialized in Reverse Phase Protein Arrays (RPPA). This highly quantitative technique consists of depositing in an automated manner very small amounts (1 ng) of cell- or tissue lysates onto microscope slides covered with nitrocellulose. Proteins of interest are subsequently detected using specific antibodies, directed against either modified (e.g. phosphorylated) or total protein pools. In this manner, up to 3000 samples can be analyzed simultaneously for the presence and the activation status of selected targets.

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