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 transwell 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