709 SOX2 expression is repressed by the BMP pathway and H.pylori in AGS gastric cell line

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Background: Infection with Helicobacter pylori (Hp) may trigger the development of intestinal metaplasia (IM), a preneoplastic lesion of the stomach characterized by a phenotypic switch from a gastric to an intestinal phenotype. Ectopic expression of CDX2 *per se* was sufficient to induce IM both in the stomach and other tissues. CDX2 is not expressed in the normal gastric mucosa therefore mechanisms of CDX2 repression may be lost in the progression to IM. One candidate for CDX2 repression is the transcription factor SOX2, since CDX2 and SOX2 expression in the gastrointestinal tract are mutually exclusive, with SOX2 being expressed in the esophagus and stomach and CDX2 in the intestine. Independent studies have shown that CDX2 is positively regulated by Hp and Bone Morphogenetic Proteins (BMPs) in the gastric context. Our aim was to evaluate if Hp and BMP/SMAD were negatively regulating SOX2 expression.
Material and Methods: (I) *BMP treatments:*AGS gastric carcinoma cells were treated with BMP-2 or BMP-4 or vehicle for 24 h. CDX2 and SOX2 expression were assessed by Real-Time PCR. For Western Blotting analysis, BMP-treated AGS cells were submitted to SDS-PAGE and incubated with polyclonal anti-SOX2 antibody. (II) *SMAD4 RNAi:* SMAD4 Knock-down cells were established using a retroviral short hairpin RNA expression system. CDX2 and SOX2 expression of SMAD4 RNAi AGS cells was assessed by Real-Time PCR. (III) *SMAD6/7 transfection*: AGS cells were transfected with SMAD6 or SMAD7 expression vectors using lipofectamine 2000. Cells were collected 48h after transfection for SOX2 and CDX2 expression assessement by Real-Time PCR. (IV) *Hp co-cultures:*AGS cells were co-cultured with Hp cagPAI negative and cagPAI positive strains and collected 8 hours post-culture. SOX2 expression was assessed by Real-Time PCR.
Results: In AGS cells, BMP treatment led to a decrease in the SOX2 levels, both at the RNA and protein levels. This tendency was reverted upon inhibition of the BMP canonical effector SMAD4. Transfection with BMP/SMAD inhibitors SMAD6 and SMAD7 increased SOX2 RNA levels. Finally, Hp infection decreased SOX2 RNA levels, independently of the Hp cagPAI status.
Conclusion: BMPs and Hp downregulate SOX2 expression by facilitating loss of gastric differentiation and gain of intestinal differentiation in gastric cells.

710 Sarcosine induces up-regulation of HER2/neu in androgen dependent prostate cancer cells

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Background: The oncoprotein HER2/neu is associated with invasiveness and proliferation of cancer cells. Additionally, there is increasing evidence that HER2/neu is also involved in prostate cancer progression. Sarcosine was recently reported as a novel biomarker for aggressive cancer and exogenous sarcosine induces an invasive phenotype in benign prostate cells [1]. Thus far, the pathways downstream HER2/neu and sarcosine are both elusive. The purpose of this study was to assess a possible relation between sarcosine and the expression of HER2/neu in prostate cancer cells. Insight into the progression of prostate cancer might pave the way for development of novel therapies.
Materials and Methods: Prostate cancer cells (LNCaP, PC-3 and DU145) were obtained from ATTC. Cells were exposed to 25–100 μM sarcosine for 24, 48 or 72 h. Relative amounts of HER2/neu and AR transcripts were determined using real-time quantitative reverse transcription PCR. Expression of HER2/neu and the androgen receptor (AR) was confirmed by Western blot. HER2/neu molecules displayed on the cell surface were probed using HER2/neu specific Affiprobes [2] and imaged by confocal laser scanning microscope.
Results: Exposure of LNCaP cells to 50 μM sarcosine for 24 h resulted in a 58% increase of the HER2/neu mRNA level ($P < 0.001$), indicating that addition of sarcosine influenced HER2/neu expression on the level of transcription. Up-regulation of HER/neu protein was confirmed by Western blot. However, no change in HER2/neu expression was found for the other cell lines studied. Control experiments with the sarcosine analogue alanine showed no significant effect on the HER2/neu mRNA level. The phosphorylated form of the HER2/neu protein, HER2/neu-P was also detected but sarcosine treatment did not confer any increase in activation of HER2. Imaging by confocal microscopy showed an increase of HER2/neu on the surface of LNCaP cells after 48 and 72 h of sarcosine treatment, with a more pronounced effect at the later time point.
Conclusions: This is the first report that exogenous sarcosine significantly increases HER2/neu expression in prostate cancer cells. Sarcosine seems to be involved in the regulation of HER2/neu on the level of transcription. Thus, sarcosine may induce progression and aggressivity of prostate cancer by increased HER2/neu expression.
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711 New phenol-substituted thiazolidinediones: an improved antiproliferative PPARgamma-independent effect on breast cancer cell lines

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Background: PPARs are ligand-activated transcription factors that belong to the nuclear receptor superfamily. Thiazolidinediones (TZD) are synthetic PPARγ agonists, which inhibit proliferation and migration of breast cancer cell lines. However, together wither other groups, we have shown that several anticancer effects of TZD do not require PPARγ activation. Despite hopeful results in preclinical studies, TZD display a low therapeutic index for breast cancer treatment. More potent and more selective compounds need to be developed. Therefore we synthesized new TZD, without PPAR? agonist activity and used biotinylation to improve the delivery of the molecules to cancer cells.
Material and Methods: The new compounds derived from an inactive analogue of Troglitazone (TGZ) named Δ2-TGZ. Biotin was added to the OH function of the phenol group (bΔ2-TGZ). The effects were studied in the hormone-dependent breast cancer cell line MCF-7 and in the hormone-independent cell line MDA-MB-231. Proliferation was studied using the "cell titer glo" test. Proteins were analyzed by western blotting and immuno-localisation.
Results: Our study shows that bΔ2-TGZ inhibited proliferation of both MCF-7 and MDA-MB-231 cells more drastically than TGZ or Δ2-TGZ. These data are correlated to cyclin D1 level since inhibition of proliferation is associated to cyclin D1 proteasomal degradation. MDA-MB-231 cells are more sensitive than MCF-7 cells. These effects are related to ER stress which is not triggered to the same extent by the different compounds as shown by DDIT3/CHOP expression.
In competition experiments, the presence of free biotin in the culture medium did not decrease the antiproliferative action of bΔ2-TGZ. Besides, other compounds that had no biotin but that were substituted at the same position of the phenolic group of the chromane moiety of Δ2-TGZ decreased cell viability similarly to bΔ2-TGZ. The relationship with ER stress is currently studied. These results suggest an important role of the OH substitution on the phenol group rather than a direct involvement of biotin.
Conclusions: Taken together, we developed new TZD compounds displaying a strong anticancer activity on both hormone-dependent and hormone-independent breast cancer cell lines. Their mechanism of action is not fully elucidated, but clearly the PPARγ-independent action is partially mediated by ER stress. These compounds could be important tools for developing new strategies for breast cancer treatment.

712 ICI 182,780 induces p-cadherin overexpression in breast cancer cells through chromatin remodelling at the promoter level: role of C/EBPbeta in CDH3 gene activation

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Background: *CDH3*/P-cadherin is a classical cadherin which overexpression has been associated with proliferative lesions of high histological grade, decreased cell polarity and poor patient breast cancer survival. *In vitro* studies showed that it can be up-regulated by ICI 182,780, suggesting that the lack of ERα signalling is responsible for the aberrant P-cadherin overexpression and for its role in inducing breast cancer cell invasion and migration. However, the mechanism by which ER-signalling inhibition leads to P-cadherin expression is still unknown. The aim of this study was to explore the molecular mechanism linking the ERα-signalling and P-cadherin-regulated expression in breast cancer cell lines.

Results: This study showed that ICI is able to increase *CDH3* promoter activity, inducing high levels of the active chromatin mark H3 lysine 4 dimethylated (H3K4me2). We also observed for the first time, that the transcription factor C/EBPβ is induced by ICI, being able to up-regulate *CDH3* promoter activity in breast cancer cells. Moreover, we showed that the expression of P-cadherin and C/EBPβ are highly associated in human breast carcinomas and linked with a worse prognosis of breast cancer patients.
Conclusions: This study demonstrates the existence of an epigenetic regulation by which ICI up-regulates P-cadherin expression in MCF-7/AZ breast cancer cells through chromatin remodelling at *CDH3* promoter, bringing forward the growing evidence that ERα signalling-abrogation by anti-oestrogens is able to induce the expression of ERα-repressed genes which, in the appropriate cell biology context, may contribute to a breast cancer cell invasion phenotype.

713 miR-210 is associated to endothelial cell fusion

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Background: Endothelial cells (EC) play a major role maintaining the homeostasis and adequate function(s) of different organs. Given the homeostatic functions of EC, recent studies have attempted at originating EC from undifferentiated endothelial progenitors (EPC); the regenerative potential of such an EC source is attractive and theoretically unlimited. Very little is known about EPC differentiation at molecular level. We have recently characterized cord blood-derived progenitor cells generating coding-gene expression profiles during EPC differentiation into mature EC using microarrays. As miRNA may regulate up to 30% of the protein-coding genes in the human genome and are involved in several biological processes we aimed to identify miRNAs highly expressed in EPC and investigate its role in the endothelial cell biology.
Material and Methods: To identify miRNAs highly expressed in EPC, we performed a screening for miRNA sequences using microarrays enriched for intronic noncoding RNAs of EPC and at different time points of differentiation. We have focused this study in one miRNA and to investigate its role in EC biology we have modulated miRNA levels transfecting endothelial cells with anti-miR or pre-miR molecules to decrease or increase miRNA expression, respectively and phenotypes the obtained were characterised.
Results: From miRNAs identified, the expression of hsa-miR-210 was highly up-regulated in undifferentiated EPC and decreases with differentiation into mature EC. We investigated the role of miR-210 in EC biology and observed that reduction of the miR-210 on mature EC lead to appearance of giant multinucleated (3n-12n) cells, which represented 2−3% of the total endothelial population. These aberrant EC had a disarranged actin cytoskeleton and a high lipidic content. We demonstrate that multinucleated EC arise from cell fusion events and that inhibition of cell fusion by calpeptin reduced the number of multinucleated EC, even when miR-210 levels are decreased. miR-210 target genes that could be associated to endothelial cell fusion, specifically syntaxin-11 were also investigated. Due to importance of this miRNA in EC function we are currently assessing the level of expression of this miRNA in different human cancers.
Conclusions: Taken together, our data suggests a crucial function for miR-210 in regulating EC homeostasis, its reduction being strongly associated with EC function.

714 Investigating the role of viral infections in the etiology of common Acute Lymphoblastic Leukemia through an epigenomic approach

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Background: It is believed that the onset of common Acute Lymphoblastic Leukemia (c-ALL) in children involves two stages, or mutational "hits": the first hit would promote mutations or epigenetic alterations that create a pre-leukemic clone and the second one, would precipitate ALL through a proliferative expansion of this clone. Epidemiologic evidence strongly supports a role for infections in the leukemogenesis process and may impact this secondary clone expansion. We investigated the role of a specific bone marrow tropic infection in c-ALL etiology.
Material and Methods: We analyzed the expression profiling of 11 genes related to JAK/STAT pathway, by Real Time PCR, to evaluate the role of aberrant immune response as a trigger of c-ALL. We also verified ALL methylation profile, using beadarrays (Illumina, Inc.), to identify possible alterations that could distinguish ALL subgroups and could be associated with viral infections. 1,505 CpG loci related to oncogenic process were studied. These molecular results categories were correlated with serology, IgM and IgG anti-parvovirus B19 (PVB19) levels, measured by ELISA. We started validation of beadarrays results through analysis of *DAPK* gene methylation by Methylation Specific PCR (MSP).
Results: Samples of 121 childhood ALL, classified as pro-B, common and pre-B were used. *MX1* and *SP110* genes presented higher expression levels (2.5 and 2 times, respectively) in c-ALL when compared to other subtypes ($p = 0.03$ and 0.05, respectively). On the other hand, *LY6E* showed lower expression (2 times) in c-ALL. There is an association between anti-PVB19 IgM levels and higher expression of genes ($p < 0.01$). Leukemia immunophenotypic subtypes (pro-B ALL, c-ALL and pre-B ALL) could be distinguished based on methylation profiling of some genes such as *DAPK* and *IFNG*. An association between PVB19 infection and hypermethylation of some genes, for instance, *DAPK*, *PTGS2* and *NRAS* was observed. *DAPK* gene altered methylation in PVB19 positive samples was confirmed by MSP ($P < 0.05$).
Conclusion: A cell's response to viral infection leads to expression of IFN stimulated-genes and also activation of methylation processes in efforts to control viral gene expression. It is proposed that leukemias related to infections will harbor altered DNA methylation patterns compared to leukemias that arise from other etiologies. Our expression profiling results suggest that immune response is related to c-ALL. Also, altered DNA methylation patterns and genes in c-ALL may be a signature of infection etiologies. DNA methylation profiles are also associated with leukemia immunopathologic subgroups and age at onset.

715 Effect of tumour necrosis factor (TNF) alpha on HER2/neu expression in ovarian cancer cells

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Background: Host protection against incipient tumour progression involves the production of tumour necrosis factor a (TNFa) by immune cells in the stroma as part of the antitumour innate immune response. Resistance to this immune response may result in tumour progression instead of regression. Human epidermal growth factor receptor 2 (HER2/neu or HER2), a tyrosine kinase receptor, is a potent oncoprotein in different cancers including breast, ovarian, lung and bladder cancer, and is associated with cancer progression and poor prognosis. The aim of this study was to investigate the effect of TNFa on HER2/neu expression in ovarian cancer cells.
Materials and Methods: Ovarian cancer cells (SKOV-3) obtained from ATTC were used for the experiments. SKOV-3 cells were exposed to 10 nM TNFa for 24 h. Relative amounts of HER2/neu transcripts were determined using QPCR, normalizing for the β-actin reference gene. Expression of proteins was analyzed by Western blot. HER2/neu receptors on the cell surface were probed using the HER2/neu-specific fluorescent Affiprobe z(HER2)-red [1] and imaged by a Leica TCS SP5 confocal laser scanning microscope. Experiments were repeated at least three times.
Results: A 24-h exposure to 10 nM TNFa resulted in a significant ($p < 0.005$), 20% decrease in the relative mRNA levels of HER2/neu in SKOV-3 cells. Western blot confirmed the decrease in HER2/neu expression after 24 h of TNFa treatment, but no alteration of the activated form, HER2/neu-P, was observed. Interestingly, imaging using confocal microscopy showed that cells treated with TNFa displayed a strong increase in formation of HER2/neu-containing microvesicles compared to control cells.
Conclusions: This is the first report indicating that TNFa significantly down-regulates HER2/neu expression in ovarian cancer cells. Moreover, the strongly induced shedding of HER2/neu-containing vesicles possibly reveals a role for TNFa in the recently reported intercellular transfer of oncogenes via microvesicles referred to as 'oncosomes' [2]. The effect of cytokines on HER2/neu expression and microvesicle formation remains to be elucidated.
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