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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Reference(s)

[1] Lyakhov I, Zielinski R, Kuban M, Kramer-Marek G, Fisher R, Chertov O, Bindu L, Capala J. HER2- and EGFR-specific Affiprobes: novel recombinant optical probes for cell imaging. ChemBioChem 2009; 10:1−7.
[2] Al-Nedawi K, Meehan B, Micallef J, Lhotak V, May L, Guha A, Rak J. Intercellular tranfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. Nature Cell Biol 2008; 10:619–624.