the combination induced a good inhibition of CCF-STTG1's cell growth but was inactive on U87MG cells. Microarray gene profiling and apoptosis pathway proteins' assessments will also be presented to further characterize genes or pathways involved in the synergistic effect of Smac-083 TNFα-induced apoptosis in glioblastoma cell lines.

Reference(s)

[1] Li, L. *et al* (2004) *Science* 305(5689): 1471−4.
[2] DeAngelis L.M. (2001) *N Engl J Med* 344(2):114−23.
[3] Seneci, PF. *et al* (2009) *Bioorg Med Chem* 17(16):5834−56.

747 Human arm protein lost in epithelial cancers, on chromosome X 1 gene is transcriptionally regulated by CREB and Wnt/beta-catenin signaling

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Background: The aberrant activation of the Wnt signaling is a key process in colorectal tumourigenesis. The canonical Wnt signaling controls transcription of target genes via b-catenin and T cell factor/lymphoid enhancer factor family transcription factor complex. *Arm protein lost in epithelial cancers, on chromosome X 1 (ALEX1)* is a novel member of the Armadillo family which has two Armadillo repeats as opposed to more than six repeats in the classical Armadillo family members. The regulatory mechanism of *ALEX1* gene in normal and cancer cells remain largely unknown. Here we examined cis-regulatory elements and trans-acting factors involved in the transcriptional regulation of *ALEX1* gene.

Material and Methods: Human colon cancer cell lines (HCT116 and SW480) and pancreatic cancer cell line (PANC-1) were used in this project. The putative promoter region of the human *ALEX1* gene from −1933 to +487 was amplified by PCR and subcloned into the pCR-Blunt II-TOPO plasmid. The luciferase reporter plasmids driven by deleted mutant types of *ALEX1* promoter were generated by the same PCR-based method. The luciferase reporter assay was performed using the Dual-Glo Luciferase Assay System. PANC-1 cells were transfected with the ON-TARGETplus Non-Targeting Control siRNA or siRNAs targeting CREB using DharmaFECT1 siRNA. Chromatin immunoprecipitation was carried out with Dynabeads Protein G and polyclonal anti-CREB antibody. Indirect immunofluorescence for b-catenin was performed with Rhodamine-conjugated anti-mouse IgG antibody, whose images were obtained using a Axiovert 200M fluorescent microscope.

Results: Site-directed mutations of a cyclic AMP response element (CRE) and an E-box impaired the basal activity of human *ALEX1* promoter in colorectal and pancreatic cancer cell lines. Moreover, overexpression of CRE-binding protein (CREB) increased the *ALEX1* promoter activity in these cell lines, whereas knockdown of *CREB* expression decreased the expression level of *ALEX1* mRNA. Interestingly, luciferase reporter analysis and quantitative real-time RT-PCR demonstrated that the *ALEX1* promoter was upregulated in a CRE-dependent manner by continuous activation of Wnt/b-catenin signaling induced by a glycogen synthase kinase-3 inhibitor and overexpression of b-catenin.

Conclusions: These results indicate that the CRE and E-box sites are essential cis-regulatory elements for the *ALEX1* promoter activity, and the *ALEX1* expression is regulated by CREB and Wnt/b-catenin signaling.

748 Regulation of expression of the VE-statin/egfl7 gene in endothelial cells: a critical role for ETS and GATA factors

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The *VE-statin/egfl7* gene is specifically expressed in endothelial cells during development and in the adult [1]. We studied here the regulatory mechanisms that control this specific expression *in vitro* and *in vivo*.

A specific expression in endothelial cells is not observed with *VE-statin/egfl7*'s closest neighbor genes *notch1* and *agpat2* on chromosome 2. Further, the acetylation state of histones around the two *VE-statin/egfl7* transcription start sites shows that the chromatin is opened in endothelial cells, not in fibroblasts at the *VE-statin/egfl7* locus. Two regions are important for the endothelial-specific expression of the gene; a −8409/−7688 enhancer and the −252/+38 region located ahead of the exon-1b transcription start site. The latter contains important ETS- and GATA-binding sites which are crucial for expression of the gene in endothelial cells. Analysis of expression, chromatin immunoprecipitation and RNA interference of endogenous transcription factors showed that Erg and GATA-2 are, by far, the most highly expressed in endothelial cells and that these two factors directly control expression of *VE-statin/egfl7*.

This first detailed analysis of the mechanisms that govern the expression of the *VE-statin/egfl7* gene in endothelial cells pinpoints the specific importance of Erg and GATA-2 factors in the regulation of genes in these cells.

Reference(s)

[1] Soncin F et al EMBO J. 2003 Nov 3;22(21):5700−11.

749 C6orf69, a PKCe interacting BTB-containing protein, is a novel Cullin3 binding partner

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Protein kinase C (PKC) family members are key signaling molecules involved in many diverse cellular functions such as proliferation, differentiation, survival and death.

Among PKC isoenzymes, novel PKCε has been reported to act as an oncogene, contributing to malignancy by enhancing cell proliferation or by inhibiting cell death. PKCε seems to be involved also in mechanisms related to tumour cell invasion and metastasis, however its exact function is not known yet. By gene array approach, we identified few novel genes regulated by overexpression of constitutively active PKCε (PKCεA/E). One of the down-modulated genes, termed C6orf69, encodes a 47 kDa protein containing a single BTB/POZ domain.

C6orf69 gene is ubiquitously expressed. C6orf69 proteins were up regulated in some cancers (breast) compared to normal counterparts, suggesting a participation of C6orf69 in pathogenesis of certain tumours.

C6orf69 contains BTB domain usually involved in protein-protein interactions and implicated in the stability and dynamics of actin filaments. C6orf69 has been shown to co-immunoprecipitate with PKCε and bind to actin. Recently, BTB-domain containing proteins have been reported to regulate protein stability by recruiting proteins to the Cullin3 (Cul3) ubiquitin ligase leading to their degradation *via* ubiquitin/proteasome. Using co-immunoprecipitation method we could demonstrate that C6orf69 is interacting specifically with Cul3. We also addressed whether C6orf69 could be ubiquitylated, based on the fact that other BTB/POZ adaptor proteins have been shown to be substrates of E3 ligases. Our *in vivo* ubiquitylation assay demonstrated that C6orf69-CFP protein is efficiently polyubiquitylated and that inhibition of 26S proteasome by MG132 accumulated both unmodified C6orf69 as well polyubiquitylated C6orf69. Considering that C6orf69 binds to Cul3 and to PKCε, we are currently investigating if PKCε is ubiquitylated through the C6orf69/Cul3-based ubiquitylation system.

Analysis of the C6orf69 polypeptide showed that there are putative PKC phosphorylation sites. However, C6orf69 seems not to be a direct substrate of PKCε or other PKCs as demonstrated by *in vitro* kinase assay. Part of our current work is focused on determining which kinase (s) is responsible for C6orf69 phosphorylation, fuctional consequences of this PTM as well as determination of the precise position of the phosphorylated residues.

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750 Normal breast tissue from cancer patients is different in expression of Bmi-1 and Mel-18 compared to breast tissue from non cancer patients

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Background: Polycomb Group (PcG) proteins are epigenetic silencers involved in maintaining cellular identity, and their deregulation can result in cancer. Mel-18 and Bmi-1 are both members of PcG. Bmi-1 was initially considered an oncogene, although recent studies have suggested that Bmi-1 overexpression is associated with good outcome in breast cancer. Mel-18 is considered a tumour suppressor gene. There are different mechanisms to this tumour suppressiveness. Both Mel-18 and Bmi-1 have been studied in tumour tissue, but to our knowledge it has not been studied in normal breast epithelium. Our study compares the expression of the two genes in normal breast epithelium of cancer patients and compares this to the level of expression in breast epithelium of healthy women.

Material and Method: We studied a total of 71, 23 of which we have normal tissue from viscinity of the tumour. In addition we had 6 fibroadenomas, 2 DCIC, and 12 reduction mammoplasties. The tissue samples were stored in RNAlater, RNA was isolated and microarray performed to achieve a molecular profile. These two genes were then studied more closely first on mRNA expression level and later on protein expression level. using immunohistochemistry.

Results: Bmi-1 mRNA is significantly up regulated in normal breast tissue in breast cancer patients compared to normal breast tissue from noncancerous patients, while the mRNA expression of Mel-18 was found to be lower in normal breast from patients operated for breast cancer compared to breast tissue from mammoplasty. mRNA expression of these two genes was inversely correlated. When protein expression of these two genes was evaluated, we observed that most of the epithelial cells were positive for Bmi-1 both groups of tissue samples, although the expression intensity was stronger in normal tissue from cancer patients compared to mammoplasty tissue samples. Protein expression of Mel-18 showed stronger intensity in tissue samples from mammoplasty compared to normal breast tissue from patients operated for breast cancer.