In conclusion, these results demonstrate that ZEB-1 directly inhibits SEMA3F expression in lung cancer cells. Together with its effects on E-Cadherin, these data indicate that ZEB-1 plays a critical role in the pathogenesis or progression of this disease.

**351** Poster

**Polymorphic microRNA binding sites within candidate genes are associated with the risk of colorectal cancer**

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Introduction: The individual risk to develop colorectal cancer (CRC) is hypothesized to be modulated, at least in part, by common polymorphisms within specific candidate genes. They are involved in the carcinogenic processes, through the regulation of the cell growth, differentiation, apoptosis, and the maintenance of genome stability. Recent evidences indicate that small non-coding RNA molecules, called micro-RNAs (miRNAs), bind to the 3'UTRs of mRNAs and interfere with their translation, thereby regulating cell growth, differentiation, apoptosis, and tumorigenesis. Therefore, we hypothesized that polymorphic miRNA binding sites at the 3'UTRs of cancer candidate genes could modulate the individual risk of cancer.

Materials and Methods: To confirm our hypothesis, we selected 129 genes that, according to published data and various online resources (e.g. BioCarta and KEGG pathways; http://cgap.nci.nih.gov/Pathways) are candidate genes for CRC. Fifty-one genes are involved in inflammatory processes, 37 belong to synthesis of prostaglandins and thromboxanes, 16 genes are connected with obesity and insulin resistance, and 25 genes are involved in early and late stage of this type of tumour. We identified putative microRNAs binding sites by means of specialized algorithms (PicTar, DianaMicroT, miRBase, miRanda, TargetScan, and microInspector). Then, we found 79 SNPs within the putative binding sites for their ability to affect or impair the binding with the miRNA, by assessing the variation of $\Delta G$ (Gibbs free energy) (defined as $\Delta\Delta G$) comparing the "wild-type" and their correspondent variant alleles. Considering the validation status of the SNPs and their frequencies (MAF>0.10), we found at least 15 candidate polymorphisms of biological relevance that could be investigated by performing case-control association studies on a series of samples from Czech Republic.

Results: We found statistically significant associations between risk of CRC and variant alleles of CD86 (OR=2.74 95%CI=1.24-6.04, for the variant homozygotes) and INSR genes (OR=1.94; 95%CI=1.03-3.66, for the variant homozygotes).

Conclusion: This study suggests that SNPs in miRNA binding sites may be important in the modulation of the individual risk of cancer and encouraged to undertake future works. Moreover, since the genotyping allows the screening of a relatively large number of polymorphisms in short time, the proposed study suggested also a way to restrict the number of miRNA targets to be actually experimented using time-consuming molecular biology techniques.

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**HIF-1alpha is a novel target of the SWI/SNF chromatin remodelling complex**

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Background: Hypoxia inducible factor (HIF-1) is a master regulator of the transcriptional responses to hypoxic stress. The majority of HIF-1alpha control happens at the protein level and mRNA changes in response to hypoxia are not readily observed. The SWI/SNF chromatin remodelling complex is important for activation and repression of transcription, and acts by modulating chromatin structure. Despite the importance of this complex, only a few direct targets have been identified.

Methods: Using mRNA, Western Blot and promoter analysis we have investigated how chromatin remodelling complexes regulate HIF-1alpha.

Results: We demonstrate that the HIF-1alpha is a direct target of SWI/SNF. SWI/SNF components are found associated with the HIF-1alpha promoter and their depletion results in a reduction of HIF-1alpha expression and its ability to transactivate target genes. Importantly, depletion of BAF57 (a conserved subunits of SWI/SNF) results in reduced recruitment of other SWI/SNF components as well as impaired polymerase II recruitment.

Conclusions: These results reveal a previously uncharacterized dependence of HIF-1alpha on the SWI/SNF complex, demonstrating a new level of control over the HIF-1alpha system. In addition, these studies identify BAF57, as the main targeting subunit of SWI/SNF to the HIF-1alpha promoter.

**353** Poster

**Convergent mechanisms that activate MYB transcription in colon and breast cancer which provide a therapeutic opportunity to target metastatic disease**

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Background: MYB is over-expressed in the majority of colo-rectal cancers1 (CRC) and ERalpha positive breast cancers. Activation of MYB transcription occurs at the earliest stages of adenoma formation in the colon and progressively increases in primary and finally metastatic adenocarcinoma. In CRC mutations that affect the transcriptional elongation of the gene are frequent and have been functionally validated2. Conversely in breast cancer mutations in this sequence are rare but estradiol induces ERalpha mediated induction of MYB transcription obviating the need for the mutations inherent in CRC3. Materials and Methods: Experimental mouse models have bee used to identify the interplay between c-Myb and the adenomatous polyposis coli gene in synergistically driving the c-Myc gene expression and that c-Myb over-expression increases in metastatic CRC and mammary cancer. With this in mind a DNA fusion vaccine has been devised to generate a c-Myb specific immune response to potentially treat these two common cancers. This has been achieved even though c-Myb, like many tumor antigens, is weakly immunogenic as it is a "self" antigen and thus subject to tolerance. To break tolerance, a DNA fusion vaccine was generated comprising wild-type c-Myb cDNA flanked by two potent Th epitopes derived from tetanus toxin. Vaccination was performed targeting a highly aggressive, weakly immunogenic, subcutaneous, syngeneic, colon adenocarcinoma cell line MC38 which highly expresses c-Myb. Results: Prophylactic intravenous vaccination significantly suppressed tumor growth, through the induction of c-Myb specific anti-tumor immunity for which the tetanus epitopes were essential. Vaccination generated anti-tumor immunity mediated by both CD4+ and CD8+ T cells and increased infiltration of immune effector cells at the tumor site. Importantly, no evidence of autoimmune pathology in endogenous c-Myb expressing tissues was detected 4. Conclusions: These data highlight the role of MYB in 2 common epithelial cancers and establish c-Myb as a viable antigen for immune targeting and serve to provide proof of principle for the continuing development of DNA vaccines targeting c-Myb. As c-Myb is expressed at its highest in metastatic CRC we propose that a vaccine against c-Myb may have a place in patients post-surgery and adjuvant therapy.

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**354** Poster

**Identification of proteins implicated in Kit receptor signalling**

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The receptor tyrosine kinase Kit is required for the development of germ cells, melanoblasts, interstitial cells of Cajal, erythroblasts and mast cells. Gain-of-function mutations of Kit are found in human proliferative pathologies such as mastocytosis, gastrointestinal stromal tumour (GIST), acute myeloid leukaemia (AML) of the CBF class or testicular germ cell tumours. Different kinds of mutations lead to ligand-independent activation of the receptor. The substitution of the aspartate 816 in the kinase domain occurs in 80% of the cases of mastocytosis. Substitutions or deletions in the regulatory juxtamembrane domain also induce constitutive receptor activation and subsequent cellular transformation.

Following stimulation by its ligand, Kit undergoes transphosphorylation on tyrosine residues, thus creating docking sites for signalling molecules. The JM domain of Kit contains 6 tyrosines of which Y568 and Y570 are autophosphorylation sites. We work with HMC-1 cells (a human mastocytoma cell line carrying the mutation D816V) as a model of Kit