codon. *CpG plot* analysis identified three CpGislands in *CLSPN* promoter (Z1, Z2 andZ3). We have found that Z1 and Z2 regions were unmethylated in all the samples analysed. Data regarding the methylation status of Z3 region will also be presented. Our preliminary data suggest that the Z3 region is also unmethylated.
Conclusions: Our data suggest that *CLSPN* promoter is usually in an unmethylated state. Nevertheless, so far, these preliminary data does not exclude the possibility of this epigenetic mechanism being operant in cancer cells in which Claspin expression is lost. Further studies are needed, in as much as modulation of CpG island methylation can be used in clinical contexts as a therapeutic approach.
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No conflict of interest.

381 The ApcMin/+ mouse to identify "driver" epigenetic lesions in colorectal cancer: promoter hypermethylation of the protocadherins

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Background: There is increasing recognition that colorectal cancer could be driven by epigenetic alterations. The *de novo* methyltransferase, Dnmt3b, has been found to be overexpressed in colorectal cancer. Long range epigenetic silencing (LRES) of the protocadherins, a family of tumour suppressor genes which negatively regulate Wnt signalling, has been identified on chromosome 5q31. The subsequent hypermethylation of the protocadherins in human colorectal adenomas has been observed; however this has not been investigated in a mouse model.
Materials and Methods: DNA samples from the normal colon mucosa of ApcMin/+ mice overexpressing or not overexpressing Dnmt3b, and colorectal adenomas overexpressing or not overexpressing Dnmt3b, were subject to combined bisulfite restriction analysis (COBRA). In particular the methylation of the protocadherin promoters was investigated.
Results: The promoters of *Pcdhb12* and *Pcdhga6* showed significant hypermethylation in colorectal adenomas from Dnmt3b overexpressing mice, when compared to mice not overexpressing Dnmt3b, with an increase in the average methylation from 44% to 64% ($P < 0.02$) and 81% to 95% ($P < 0.04$) respectively. The *Pcdhgc3* promoter remained unmethylated in all mouse colon samples investigated, replicating that seen in the human colon.
Conclusions: The ApcMin/+ mouse reflects promoter hypermethylation of the beta and gamma protocadherins as seen in human colorectal cancer; this supports LRES in the mouse model. As the protocadherins show different degrees of promoter hypermethylation, this could indicate some protocadherins may be more involved than others in the regulation of the Wnt signalling pathway. Hypermethylation of the protocadherin promoters could be seen to drive the initiation and progression of colorectal tumorigenesis, through the release of inhibition of the Wnt signalling pathway and subsequent deregulation of β-catenin. The epigenetic alterations could provide biomarkers that have future implications in the diagnosis and monitoring of the response to therapy in colorectal cancer. Further to this, as methylation is a reversible modification to the genome, the ApcMin/+ mouse could provide a model for the investigation of the prevention and possible reversal of colorectal carcinogenesis.
No conflict of interest.

382 Identification of pathogenic virus sequences in pancreatic cancer

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Background: Pancreatic cancer is one of the deadliest forms of cancer, with a mortality that is nearly identical to incidence. Although individual steps of carcinogenesis have been analyzed in some detail, there is still neither a means for early diagnosis nor any real remedy for this disease. From other cancer entities, it is well known that viruses or other infectious agents are involved in or responsible for carcinogenesis. In pancreatic cancer, however, there have been no established reports yet about an identification of a virus associated with carcinogenesis. However, there are several clues that indicate that viral sequences could be involved.
Material and Method: We are performing an experimental genome-wide search for DNA-stretches that do occur in pancreatic ductal adenocarcinoma (PDAC) tissues but not in normal tissues, utilising representational difference analysis (RDA). Upon identification of sequence variations, their identity and location within the genome are analysed by next generation sequencing and confirmed by qPCR in a large cohort of samples. We are also pursuing a sequence analysis of the microRNA content of PDAC samples (miRNA-seq). Eight PDAC samples were analyzed for viral sequences using relevant databases. The two approaches (RDA and miRNA-seq) are being pursued in parallel.
Results and Discussion: Using RDA, differences in sequence composition could be isolated and amplified with very high sensitivity. Virus sequences associated with pancreatic cancer are therefore likely to be picked up by this process. The RDA procedure yielded difference products that are currently sequenced and analyzed by qPCR. The microRNA analysis revealed interesting results, too. We identified that a viral microRNA, which is an orthologue of a particular human microRNA, is expressed at significantly higher levels in PDAC samples than in normal tissue. This observation was verified by a qPCR analysis. We are currently analyzing the functional consequences of the high microRNA levels and its involvement in carcinogenesis.
Conclusion: From two different approaches, we have strong indications that the occurrence of viral sequences is associated with PDAC and could affect pathways that are relevant to carcinogenesis.
No conflict of interest.

383 Effects of different additives for detection of single nucleotide polymorphisms in promoter sequence EGFR gene in NSCLC

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Background: The aim of this study was to test the effects of several additives in various concentrations for amplification single nucleotide polymorphisms in promoter sequence of the epidermal growth factor receptor (EGFR), since the promoter sequence containing the multiple GC regions which are difficult for the amplification.
Material and Methods: PureLink™ Genomic DNA Kits (Invitrogen/LifeTechnologies, Carlsbad, CA) were used for extracion of DNA from formalin-fixedparaffin-embedded lung cancer tissue. EGFR polymorphisms −216G>T and −191C>A were genotyped using the PCR-RFLP method. Sequencing was conductedusing ABI PRISM® BigDyeTM Terminator v 3.1 Cycle Sequencing Kit in both forwardand reverse direction.
Results: Between several tested additive including; glycerol, DMSO, formamide, Tween 20, Triton X-100, PEG and BSA, only a two show effectiveness including glycerol and DMSO, and with best results at concentrations of 15% and 5% respectively. Comparison of the obtained sequence with the reference sequence of EGFR promoter region (http://www.ncbi.nlm.nih.gov; GenBank reference: M11234.1) revealed that the PCR amplification was highly specific.
Conclusions: In this study we have shown that using appropriate cosolvent it ispossible amplify promotor region of EGFR for single nucleotide polymorphisms −216G>T or −191C>A.
No conflict of interest.

384 The role of ODZ4 in epithelial cancers

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Introduction: The teneurins are a family of transmembrane glycoproteins that play fundamental roles in embryogenesis and central nervous system development in a wide range of animal species. Vertebrates express four distinct teneurins encoded by the pair-rule genes *TENM-1* to *TENM-4* (or *ODZ1* to *ODZ4*). Little is known about the function of teneurins in adult organisms.
It has recently been shown that *ODZ4* is an upstream effector of FAK signaling, and several studies also suggest that it may be implicated in tumorigenesis in various ways. It is highly overexpressed in malignant brain tumours compared to normal brain tissue, and forms a fusion gene in the breast cancer cell line MDA-MB-175, where it secretes a chimeric protein whose function may be important in the tumorigenic process. *ODZ4* is an insertion site of MMTV-induced tumorigenesis along with such well-known cancer genes such as *FGF* and *Wnt*; it is thus very possible that it is an oncogene.
Materials and Method: We are investigating rearrangements of *ODZ4* in breast and oesophageal adenocarcinomas (OACs). By paired-end sequencing of OACs from the current ICGC study, fusions in *ODZ4* were predicted, and then validated by PCR and Sanger sequencing. By RNA-Seq, we characterized the fusion gene in MDA-MB-175 and predicted additional ones in it and MDA-MB-134. By qRT-PCR we established the expression levels of *ODZ4* in these two breast cancer cell lines and a panel of 31 other cell lines. Finally, shRNA experiments were used to silence *ODZ4* in MDA-MB-134.