693 Expression of Drosha, Dicer, Ago2 and TRBP, major components of the microRNA machinery, in human colon carcinomas

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Background: MicroRNAs (MiR) are small (16−29 nt), single-stranded, non-coding RNA molecules that regulate gene expression via cleavage of targeted mRNA or via translation repression. miRs are implicated in various physiological and pathological processes, including neoplasia. Production and function of miR requires a set of proteins, collectively referred as the miR machinery. Two ribonucleases, Drosha (in the nucleus) and Dicer (in the cytoplasm) process the primary transcripts (pri-miR) to generate mature miR. miR is incorporated into the RNA-induced silencing complex (RISC) that binds on target mRNA and mediates RNAi functions. Dicer, Argonaute-2, (Ago2) and TRBP are major constituents of RISC. Herein, we explored for the first time the expression and distribution of Drosha, Dicer, Ago2, TRBP in colon cancers and investigated their role in colon carcinogenesis.
Materials and Methods: Three human colon cancer cell lines (Caco-2, DLD-1, HT-29) were examined. Western blotting was performed for Dicer, Ago2, TRBP and Drosha detection. Immunoprecipitation was used for identification of possible complexes between the RISC constituents. With immunofluorescence (confocal microscopy) distribution/colocalization of the assessed molecules was investigated. The 3 cells lines together with paraffin embedded tissue from 50 patients with colon cancer of various stages and grades as well as normal colonic tissues, were evaluated via qRT-PCR for *Drosha, Dicer* and *Ago2* expression.
Results: Ago2, Dicer, TRBP and Drosha were detected in all cell lines at both protein and mRNA levels. Ago2, Dicer and TRBP displayed both cytoplasmic and nuclear localization, whereas Drosha expression was primarily nuclear. Immunoprecipitation and immunofluorescence analyses uncovered the formation of Ago2/Dicer and Ago2/TRBP complexes. *Ago2, Dicer* and *Drosha* genes were expressed in all the normal colonic epithelia and in the majority of the carcinoma cases assessed. Ago2, Dicer and Drosha mRNA levels were significantly decreased in malignant compared to normal tissues ($p < 0.05$).
Conclusions: (1) Drosha, Dicer, Ago2 and TRBP are expressed in colon cancer cells in a well-orchestrated fashion. (2) The significant downregulation of the tested genes in colon carcinomas compared to normal tissues implies that these genes may be implicated in colon carcinogenesis in humans. (3) Since the RISC complex proteins correlate with RNAi-based gene silencing it is possible that alterations of their expression levels might reflect the response of colon cancer to future RNAi-related therapies.

694 Identification of novel epigenetic biomarkers in colorectal cancer, GLDC and PPP1R14A

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Background: Colorectal cancer is one of the most common malignancies in the Western world, with an incidence of 3500 new cases per year in Norway alone. There is a need for improved early diagnostics as well as more precise cancer diagnosis to better guide the choice of treatment.
CpG island hypermethylation of tumour-suppressor genes has been established as a key molecular event in colorectal cancer. Furthermore, DNA hypermethylation occurs early during tumour development, suggesting that it could be used as a molecular marker for early detection of the disease. Determining the methylation frequencies of target genes in colorectal cancer could therefore help discover novel biomarkers with a diagnostic potential.
Materials and Methods: The objective of this study was to identify novel epigenetic biomarkers in colorectal cancer. A set of candidate genes were selected after treatment of colon cancer cell lines with AZA and TSA, and subsequent microarray gene expression analysis. Then, *in silico* analyses was performed on candidate genes to search for the presence of CpG islands in the promoter region of the gene. Ten genes were investigated *in vitro* for promoter hypermethylation by methylation-specific PCR in colon cancer cell lines ($n = 20$). Six of the ten genes were methylated in more than 14 of the cell lines and were subjected to an *in vivo* pilot methylation study of primary colorectal carcinomas ($n = 20$) and normal mucosa samples ($n = 10$). The two most promising genes, *GLDC* and *PPP1R14A*, were further investigated by quantitative real-time methylation-specific PCR in an extended series of malignant ($n = 47$) and normal ($n = 49$) colorectal tissue samples.
Results: Promoter hypermethylation of *GLDC* and *PPP1R14A* had a sensitivity of 60% and 57% in colorectal carcinomas, whereas normal mucosa samples were unmethylated for both genes, resulting in 100% specificity. Promoter methylation was independent of tumour stage, age and gender of the patients. *PPP1R14A* was significantly more methylated in tumours with microsatellite instability and thus in tumours located on the right side of the colon.
Conclusions: In the present study *GLDC* and *PPP1R14A* are identified as novel methylated gene targets in colorectal cancer.

695 Mechanisms of ER beta action in prostate cancer

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Background: Androgen-deprivation remains the predominant effective therapy for advanced prostate cancer (PCa), but invariably fails with the emergence of fatal castrate-resistant disease. To develop new therapeutic options we recently investigated the action of an oestrogen receptor beta (ERβ) specific agonist (8beta-VE2). Acting via TNFα we showed it caused apoptosis in castrate-resistant cells, in contrast to ERα, that is known to promote malignant prostate cell growth. Although the homology of their DNA-binding domains being greater than 95%, in breast cancer the two ER isoforms exhibit both shared and selective gene-targets thus divergent transcriptional effects are expected. As little is known about ERβ in PCa our aim was to identify specific gene-targets with view to understand the determinants of PCa growth regulation.
Material and Methods: Using human prostate androgen-independent DU145 cells, we first utilized quantitative RT-PCR to examine expression of TNFα and the Progesterone Receptor (PgR) over a time-course after treatment with 8beta-VE2. We next examined expression of ERα, ERβ and AR by immunoblot analysis as well as transcriptional activity on their respective luciferase-reporter constructs in cotransfected DU145 cells.
Results: Expression of TNFα was induced by 8beta-VE2 treatment of DU145 cells while PgR expression was reduced indicating that these genes are also targets of ERβ regulation in DU145 cells. No activity of neither ERα on a consensus oestrogen-responsive-element or AR activity on the probasin reporter was induced by 8beta-VE2 in contrast to positive controls with oestradiol and DHT respectively. This suggests that the growth inhibition of DU145 cells by 8beta-VE2 is unlikely due to promiscuous off-target effects on these receptors. Only transfected ERα (64 kDa) was detected in DU145 cells using an ERα specific antibody. However both endogenous and transfected ERβ (55 kDa) were detected by an ERβ specific antibody, while transfected ERα was not, confirming antibody specificity. We are currently optimizing Chromatin Immunoprecipitation (ChIP) assays with ERβ and 8beta-VE2 in these cells with view to conducting genomic sequencing and matched expression microarrays.
Conclusions: The identification of new ERβ gene-targets is essential to understand ERβ-agonist induced apoptosis that may ultimately improve therapy for advanced incurable PCa.

696 Regulation of MMP-13 (collagenase-3) by the transcription factor Pit-1

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Background: Breast cancer is the most common malignancy in women and a major cause of mortality. An important aspect of cancer biology is its ability to invade tissues and create metastases. This process involves proteolytic enzymes such as matrix metalloproteinases (MMPs), which have the ability to degrade the extracellular matrix proteins and basement membrane. Therefore, MMPs could be very interesting therapeutic targets in cancer treatment.
The Pit-1 transcription factor regulates growth hormone (GH) and prolactin (PRL) expression in both pituitary and mammary glands, two hormones that have been involved in breast cancer development. Previous results from our group seem to indicate that Pit-1 could also induce mammary tumourigenesis and metastasis directly. This study aims to evaluate the possible role of Pit-1 in the MMP-13 regulation, a protein related with breast cancer metastasis.
Material and Methods: The MCF-7 and MDA-MB-231 human breast adenocarcinoma cell lines were transfected with the Pit-1 overexpression vector (pRSV-hPit-1), and 48 hours later, MMP-13 mRNA and protein expression were evaluated by RT-PCR and Western blot. MMP-13 activation after Pit-1 was carried out by zymography. To determine the possible transcriptional regulation of MMP-13 for Pit-1 we also carried out chromatin immunoprecipitation assay (ChIP).
Results: Our data show that Pit-1 overexpression increases MMP-13 mRNA and protein expression, and induces a significant MMP-13 activation in the culture medium vs. cell extract. The ChIP assay demonstrates a direct binding of the transcription factor Pit-1 on the MMP-13 promoter.
Conclusions: Our results suggest that the transcription factor Pit-1 regulates the expression of MMP-13 at transcriptional level, by binding to the MMP-13 promoter region. The MMP-13 regulation by Pit-1 could be related with the Pit-1 induction of cell invasion and metastasis.