517 Genomic Instability is a Hallmark Feature of Serous Epithelial Ovarian Cancer and May Contribute to MicroRNA Dysregulation

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Introduction: Genomic instability is a hallmark of ovarian cancer. MicroRNAs (miRNAs) are small RNA molecules that negatively regulate gene expression, and are often dysregulated in cancer. The aim of this study is to determine whether copy number variations (CNV) lead to altered miRNA expression and downstream effects on target gene expression in serous epithelial ovarian cancer (SEOC).
Materials and Methods: CN, mRNA and miRNA expression profiling was performed on 4 SEOC (OV167, OV202, OVCAR-3, PE01) and 1 normal (HOSE) cell lines using Affymetrix Cytogenetic 2.7M Arrays, Affymetrix GeneChip® Gene 1.0 ST arrays and Exiqon MiRCURY™ LNA arrays respectively. Chromosomal positioning of miRNAs was performed using Bowtie v 0.12.7 [1].
Results and Discussion: We determined that 60% (361/605) of miRNAs assessed were in regions of CNV in at least 2 cancer cell lines. Of these miRNAs, 32% (115/361) had changes in expression levels that correlated with CNV. One of these, miR-23a, is predicted to target *AXL*, an oncogenic receptor tyrosine kinase upstream of the PI3K/mTOR signalling pathway. AXL is over-expressed in ovarian cancer, and an inverse relationship was identified between the expression of *AXL* and miR-23a in 2 of the 4 cell lines. Axl has been previously reported to be over-expressed in 73% of ovarian tumour samples [2]. Transient transfection of miRNA mimics (Ambion) into ovarian cancer cell lines confirmed AXL down regulation after miR-23a addition.
Conclusion: We demonstrate that approximately one third of miRNAs that are located in regions of CNV have altered expression which correlates with the chromosomal change. We conclude that genomic instability may contribute to miRNA dysregulation in ovarian cancer.

Reference(s)

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518 Genetic and Epigenetic Alterations of PTPRD in Hepatocellular Carcinoma

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Background: Hepatocellular carcinoma (HCC) is one of the most deadly malignancies worldwide. Therefore detailed knowledge of the genetic and epigenetic aberrations in hepatocarcinogenetic process is vital. The use of DNA arrays has increased ability to detect deletion and amplifications and possible genes located in these regions that might be involved in HCC development. Our SNP microarray analysis of 14 HCC cell lines revealed some chromosomal aberrations. One of the most remarkable aberrations was a homozygous deletion located at 9p23 harboring *PTPRD* gene. *PTPRD* (Protein Tyrosine Phosphatase, Receptor Type, D) is frequently inactivated through deletions, mutations or epigenetic mechanisms and already regarded as a tumor suppressor gene. In this study, we investigated genetic and epigenetic alterations of *PTPRD* gene in human HCCs.
Material and Methods: A panel of 14 HCC cell lines were systematically screened for genome-wide chromosomal aberrations using 10K SNP microarray platform. Mutation analysis of *PTPRD* gene were performed by direct sequencing in HCC cell lines and archival HCC samples. *PTPRD* expression was investigated in HCC cell lines and in commercially available HCC samples by using multiplex semiquantitative RT-PCR, quantitative RT-PCR and immunohistochemistry. Combined bisulfite restriction analysis (COBRA) was used to analyse *PTPRD* promoter methylation status. *PTPRD* mRNA expression was rescued with DNA methyl transferase inhibitor (5-AzaC) and/or histone deacetylase inhibitor (TSA) treatments.
Results: Our SNP microarray study revealed a homozygous deletion site (~1Mb) at 5′ UTR region of *PTPRD* in one HCC cell line (Mahlavu). Also, according to our genomic PCR analysis, eight coding exons of *PTPRD* were deleted only in Snu475 cell line which is in concordance with Sanger SNP array data.
We analysed three exons of *PTPRD* for mutations; exon 19, 20 and 28, which have been shown to be commonly mutated in various cancers. We found only an SNP (rs10977171, Q447E) at exon 20 in one liver sample and in Snu182 cell line.
We found that *PTPRD* mRNA expression was very low or absent in six out of 14 HCC cell lines and significantly reduced in 19 out of 23 (82.6%) primary HCCs compare to normal liver tissues (*P*-value = 0.013). According to our preliminary immunohistochemical results, level of *PTPRD* protein expression was also lower in tumor part of the HCC tissue compare to adjacent normal tissue.
Our COBRA assay results showed tumor specific promoter hypermethylation in six out of 27 (22%) normal-tumor paired HCC samples. We restored the *PTPRD* mRNA expression in Hep3B and PLC cell lines by 5-AzaC and/or TSA treatments.
Conclusions: *PTPRD* is partially deleted and epigenetically downregulated in human HCCs and suggested to be involved in hepatocarcinogenesis.

519 MLPA Analysis of 1p/19q Deletion in Patients With Astrocytoma and Oligodendrogliomas

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Background: A genetic hallmark of oligodendroglial tumours is the combined chromosomal deletion of the short arm of chromosome 1 (1p) and the long arm of chromosome 19 (19q). Combined losses of 1p/19q are also found in approximately 50% of oligoastrocytomas. These aberrations are associated with favourable prognosis, improved overall survival and chemosensitivity not only in oligodendrogliomas, but also in astrocytomas even though they are rarer in astrocytic tumours. Mutations in *IDH1* and *IDH2* genes (coding for isocitrate dehydrogenase enzymes) in astrocytic tumours are commonly found together with loss of 1p and 19q.
Methods: In this study, 12 glial tumours (6 astrocytomas with *IDH1* mutation and 6 oligodendrogliomas) were analyzed for 1p/19q loss by MLPA technique. MLPA analysis of large genomic deletions within chromosome 1p and 19q was performed using SALSA MLPA kit P088 which is specific for these regions. The data were interpreted using an Excel-based program which facilitates calculation of the results.
Results: MLPA analysis showed loss of 1p and/or 19q in 9 out of selected 12 glial tumours (75%). Only 3 gliomas (2 astrocytomas and 1 oligodendroglioma) showed no chromosomal aberrations in 1p/19q region. Codeletion of 1p and 19q was found in 6 patients (50%). The most frequent chromosomal deletion observed in our study was the partial loss of 1p chromosomal arm − 7 tumours. We also found chromosomal loss of 1q in one patient with glioblastoma. Because of the small groups of patients, no statistically significant conclusions about survival benefit could be made.
Conclusion: Chromosomal deletions of 1p and 19q are likely to be a frequent aberration in Bulgarian patients with oligodendrogliomas and astrocytomas with *IDH1* mutation. Further studies are required to evaluate the clinical importance of 1p/19q loss.

520 Promoter Hypermethylation of HIST1H4K and RASSF2 in Urine From Prostate Cancer Patients

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Introduction: Prostate cancer (PC) is the most commonly diagnosed cancer and is the second leading cause of cancer death in men. Current diagnosis of PC relies on PSA screening and/or digital rectal examination. Due to low specificity of the PSA testing there are high number of unnecessary biopsies and many undiagnosed cases. PC is a polygenic disease accompanied by many epigenetic changes. Hypermethylated DNA can be detected in body fluids from PC patients and thus it may serve as useful noninvasive biomarker.
Materials and Methods: Using MethyLight technology we analyzed promoter hypermethylation of *RASSF2* and *HIST1H4K* genes in urine from 57 patients with PC and 72 controls, 43 of which were young asymptomatic men and 29 were patients with benign prostatic hyperplasia (BPH). Our goal was to determine the role of these genes as new non invasive diagnostic and prognostic biomakers in Bulgarian PC patients.
Results and Discussion: *RASSF2* did not show any methylation either in the patients or in the two control groups, which is in contrast with the findings of previous studies. This result might be due to differences in the investigated populations or the used methods.