of the known inhibitor cerulenin caused 40% inhibition. PA treatment led to increased expression of phosphorylated-eIF2α after treatment for 6 hours with 2.5 and 5.0 μg/mL. Constitutive activation of STAT3 in U266 multiple myeloma cells was not affected by PA and activation of ERK1/2 in RPMI-8226 multiple myeloma cells was only partially inhibited.
Conclusion: The results suggest that the anti-proliferative and pro-apoptotic effects of PA are not primarily mediated through inhibition of signalling from growth factor receptors but may be the consequence of ER-stress possibly related to disturbed lipid metabolism.

871 Deficiency of the WWOX Fragile Gene Impairs DNA Damage Response

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Common chromosomal fragile sites are preferential targets of replication stress in preneoplastic lesions, resulting in deletions involving archetypal fragile genes encoded at these conserved chromosome regions, such as *FHIT* and *WWOX*. The *WWOX* (WW domain-containing oxidoreductase) gene encompasses the second most active chromosomal fragile site, FRA16D; a region involved in loss of heterozygosity and homozygous deletions in cancers and cancer-derived cell lines, in chromosome translocations in multiple myeloma, and its promoter region is frequently hypermethylated in cancers. Indeed, Wwox expression is reduced or absent in most common human cancers and its restoration in Wwox-negative cells suppresses tumourigenicity both *in vitro* and *in vivo*. Targeted ablation of the *Wwox* gene in mice demonstrated *bona fide* tumour suppressor function. Recently, it has been suggested that damage to fragile sites, with lost function of genes located at these sites, is coincident with activation of DNA damage response (DDR) checkpoint proteins suggesting that fragile sites might function as DNA damage warning sensors. Nevertheless, role of the *WWOX* fragile gene and the mechanism it might play in DDR are largely elusive. Here, we demonstrate that *Wwox*-deficient murine fibroblasts (MEF) display increased number of total chromosomal breakage as compared to wild type counterparts following treatment with aphidicolin, a mild DNA replication inhibitor. Overexpression of Wwox in *Wwox*-deficient MEF rescued this phenotype. Moreover, our findings show that this genomic instability in murine fibroblast is associated with delayed gH2AX foci formation. Furthermore, manipulation of Wwox expression in human cancer cell lines is associated with altered DDR checkpoint activation and DNA repair. Our data suggest that loss of the *WWOX* fragile gene product impairs DDR thus contributing to genomic instability. These findings present a fresh prospective on the role of Wwox as a tumour suppressor, which is inactivated early in pre-neoplastic cells, and how its loss may provide a selective advantage for clonal expansion of neoplastic cells.

872 Promoter hypermethylation in Bulgarian patients with glial and laryngeal cancer

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Background: Promoter hypermethylation is one of the major mechanisms in the transcriptional inactivation of certain carcinoma-associated genes. O6-methylguanine-DNA methyltransferase (MGMT) repairs the cytotoxic and mutagenic O6-alkylguanine produced by alkylating agents such as chemotherapeutic agents and mutagens. *hMGMT* expression is inversely linked to hypermethylation of the CpG island in the promoter region. Methylation in the promoter region of the DNA mismatch repair gene *hMLH1* is responsible for its inactivation and is associated with increased mutations in simple repeats in genomic DNA and microsatellite instability. The methylation analysis of these DNA repair genes may provide important information about laryngeal and glial carcinogenesis.
Materials and Methods: Genomic DNA was extracted from 50 tumour tissue samples (30 glial and 20 primary laryngeal tumours) and bisulfite conversion was performed. All samples were analyzed for promoter hypermethylation of *MGMT* gene by using a methylation-specific polymerase chain reaction (MSP) assay. The other DNA repair gene *hMLH1* was analyzed by MSP in 20 primary laryngeal carcinomas.
Results: MSP analysis demonstrated hypermethylation of *hMGMT* gene in 9 patients (30%) with glioma and 6 patients (30%) with laryngeal cancer. Promoter hypermethylation of *hMLH1* was observed in 11 (55%) of the cases with laryngeal cancer, whereas promoter hypermethylation of both *hMLH1* and *hMGMT* was found only in 3 cases (15%). The epigenetic inactivation of *hMLH1* and *hMGMT* in Bulgarian patients was detected in similar frequencies to relative studies of both cancers.
Conclusions: Our results indicate that methylation modifications in *hMLH1* and *hMGMT* genes are implicated in a significant proportion of cases with glial and laryngeal cancer.

873 Association study of polymorphic variants in chromosome locus 8q24 linked with prostate cancer in Bulgarian patients

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Background: In developed countries, prostate cancer (PC) is the most common noncutaneous malignancy in men. The molecular pathology of PC is not clear yet. Twin studies and epidemiologic observations have suggested a substantial genetic contribution to the disease risk. Linkage, admixture mapping and genome-wide studies have identified variants with moderate effects on PC risk at multiple loci in 8q24. Three distinct regions within this hot spot locus in the genome have been associated with PC risk. The locus itself is a 1.2-Mb region devoided of genes, delimited by the genes *FAM84B* and *MYC*. It is not yet known how 8q24 variants influence PC development.
Material and Methods: We have performed a case control study of the polymorphic variants rs1447295, rs16901979, and rs10505477 on locus 8q24 for association with PC. One hundred and ten PC samples and 195 controls were genotyped by using TaqMan® method.
Results: The three polymorphic variants did not show association with increased PC risk after comparison of all samples and controls. Significant association was found for rs6983267 and rs10505477 when we compared genotype and allele frequencies of patients with Gleason score above seven with the controls samples. The A/A genotype of rs10505477 (OR = 3.29, 95% CI=1.38–7.83, p = 0.007) and G/G genotype of rs6983267 (OR = 3.04, 95% CI=1.28–7.24, p = 0.011) showed association with PC in patients with Gleason score above 7. The results for the A allele (OR = 2.06, 95% CI=1.10–3.89, p = 0.016) and the G allele (OR = 1.94, 95% CI=1.03–3.65, p = 0.027) of the same variants are analogous and show statistical significance.
Conclusions: Although rs1447295 is not associated with the total PC risk or with grade and stage of the carcinoma, rs6983267 and rs10505477 demonstrated association with PC in Bulgarian patients with high Gleason score. These two polymorphisms lead to three fold increased risk for development of aggressive form of the disease.

874 Large genomic aberrations in MSH2 and MLH1 genes in Bulgarian colorectal cancer patients

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Background: Hereditary nonpolyposis colorectal cancer is caused by inactivating mutations in the genes of the DNA mismatch repair (MMR) system. Previous studies have shown that large-fragment aberrations in MMR genes are responsible for a considerable proportion of hereditary colorectal cancer (CRC) in different populations.
Material and Methods: In the present study we performed ligation-dependent probe amplification analysis (MLPA) of large genomic *MLH1*/*MSH2* alterations in 38 Bulgarian patients with CRC, in which neither epigenetic changes nor mutations were found by traditional screening methods.
Results: The frequency of the large genomic *MLH1*/*MSH2* alterations was 13.2%, which was in consistency with previous studies in other populations. One deletion was found in *MLH1* (2.6%): **del MLH1 ex 7** in a patient from family with Lynch syndrome. The observed genomic alterations in *MSH2* were four (10.5%). Two patients from HNPCC families possessed **dup MSH2 ex 9** and **del MSH2 ex 4**, respectively. The **del MSH2 ex 1** and **del MSH2 ex 3** were found in two patients with sporadic CRC and early onset, correspondingly. All cases with deletions/duplications correlated with high microsatellite instability.
Discussion: Our results indicate that genomic large-fragment deletions and duplications in *MLH1* and *MSH2* genes play a role in the pathogenesis of Bulgarian patients with both familial and sporadic CRC, as reported in other populations.

Conclusions: In conclusion, combination of MLPA with the conventional methods for mutation screening will assist the discovery of all spectra of mutations in patients with CRC in the Bulgarian population.

875 Methylation and mRNA expression profile provide supplementary information about the molecular characteristics of breast cancer tumours with clinical implications

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Background: DNA methylation plays an important part in development of breast cancer. The mechanisms by which DNA methylation can influence cancer development include hypermethylation of CpG islands in tumour suppressor genes resulting in gene inactivation, and global genomic hypomethylation causing chromosome instability, aneuploidy, and up-regulated gene expression.
Breast cancer is a heterogeneous disease that can be divided in subtypes (luminal A, luminal B, normal-like, ErbB2 positive and basal-like) based on mRNA expression with significantly different prognosis and survival. Distinct global DNA methylation profiles have been reported in breast tumours, but the influence of the tumour methylome on the development of the mRNA expression subgroups of breast cancer has not yet been defined.
Material: DNA material from 80 tumours with existing information from whole genome expression analysis was available for methylation analysis. The samples were collected at hospitals in Oslo/Akershus and all patients have given informed consent and the projects are approved by the local ethical committee.
Results and Conclusions: Three major clusters were identified based on methylation profiling of the 80 breast tumours, and these 80 tumours were also classified as belonging to one of the five mRNA expression subgroups. Cluster 1 (N = 23) contained 65.3% luminal A, 21.7% luminal B and 13% normal-like tumours. Cluster 2 (N = 28) contained 39.3% ErbB2 positive, 25% basal-like, 17.9% luminal B, 10.7% normal-like and 7.1% luminal A tumours. Cluster 3 (N = 24) contained 66.7% luminal A, 16.7% basal-like, 8.3% ErbB2 positive, 4.2% luminal B and 4.2% normal-like tumours. A strong concordance between the methylation and expression based classification was observed. Interestingly, luminal A were split between cluster 1 and 3, basal-like tumours were split between cluster 2 and 3, and both luminal B and normal-like were split between cluster 1 and 2. This distribution suggests that despite the strong concordance to the mRNA expression clusters, additional information was provided by the clustering by methylation.
The three major methylation clusters of patients were studied for differences in survival, and significant differences were found with Cluster 2 showing shortest survival times. We will also present analyses comparing survival between patients within the same mRNA expression subgroup but in different methylation clusters to determine the clinical implication of methylation profile combined with expression profile.

876 Role of classical Protein kinase C (PKC) in gastric cancer

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Background: PKCs represent a family of serine/threonine kinases consisting of a least 10 isoforms, divided into three subclasses based on their Ca2+ and DAG dependency. The classical PKC (cPKC) isoforms alpha, beta-I, beta-II and gamma are calcium and diacylglycerol (DAG) dependent whereas novel PKCs (delta, epsilon, eta and theta) are calcium independent. In contrast the atypical PKC zeta and lambda/iota do neither need calcium ions nor DAG for their activation. All PKCs are thought to be involved in cell growth and differentiation. In particular studies focusing on expression profiles of PKCs during tumour formation and progression imply a functional link. For example have we been able to show that PKCalpha and -beta are differently regulated in the APCMin mouse model which represents a well established model for gastrointestinal cancer. Based on these data we further have identified PKCalpha as to act like a tumoursuppressor in this context. Given the fact that PKCs are activated by PMA/TPA therefore are defined as tumour promotors, this was a very surprising observation.
Material and Methods: This project make use of mouse intestinal epithelial cell lines we have established from various genetic backgrounds. Using standard western blot protocols first we analyse the abundance and activation status of different signal cascades upon epidermal growth factor (EGF) stimulation.
Results: Earlier studies by us (Oster and Leitges, 2006) have indentified an alteration of the EGF receptor signalling in the APCMin model due to PKCalpha deficiency. To understand the underlying mechanism we analyse EGF signalling in mouse intestinal epithelial cell lines. Thus far it became obvious that PKCalpha deficiency causes a prolonged Erk1/2 and Akt activity whereas JNK activation was only detectable in the alpha deficiency.
Conclusion: In sum we have shown that EGFR signalling capacity is functionally linked to PKCalpha activity.

Reference(s)

Henrik Oster and Michael Leitges (2006): Protein kinase C alpha but not PKC zeta suppresses intestinal tumour formation in APCmin mice. Cancer Res 66: 6955–6963.

877 Measuring the level of genomic distortion in breast tumours with increasing histological grade – a progression model

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Background: Recently we developed an algorithm for identification of two different types of genomic distortions observed in breast tumours:
- gains/losses of whole chromosome arms
- complex aberrations

Presence of complex aberrations, previously described as genomic firestorms, has been associated with more aggressive disease and poor survival. In this study we explore whether the level of these two distinct aberration patterns changes with progression of the disease, by comparing normal tissue, ductal carcinoma in situ (DCIS) and invasive ductal carcinomas (IDCs) of various histological grades.
Material and Methods: In total, 444 breast tissue samples have been analyzed using 244K Agilent Human Genome CGH Microarrays. The samples comprise 20 normal tissue samples of mammographically dense breast, 30 pure DCIS' and 394 IDCs. Data segmentation was performed using piecewise constant fitting (PCF) and the above mentioned algorithm was used to measure arm-wise aberration patterns.
Tissue samples were available through collaboration with F. Wärnberg, B. Naume, R. Kåresen and J. Overgaard.
Results: We identified whole arm aberrations (WAAs) on all chromosomes, with the most frequent alterations (>20%) being gain of 1q, 8q, 16p and 20q and loss of 16q, genomic patterns that are well known in breast tumours. We observed no WAAs in the samples obtained from normal dense breast tissue and the overall frequency was lower in DCIS' compared to all IDCs. We observed a significantly increased frequency of 9p loss and a decreased frequency of loss of 16q and 22 with increasing histological grade. Gain of 1q was significantly more frequent in lower grade tumours compared to those of higher grade.
Complex arm aberrations (CAAs) were identified on all chromosome arms, and were most frequent (>20%) on 8p, 11q, 14 and 17q. The frequency of CAAs seems to rise with increasing histological grade for a large proportion of the chromosome arms and the difference was significant on 2q, 3p, 7q, 10pq, 17q, 18q and 20q. Interestingly, of the arms most frequently affected by CAAs, only 17q was significantly different when stratified by grade, indicating that CAAs on the remaining three chromosome arms are crucial events at early stages of breast cancer development. Furthermore, the frequency of CAAs on 16q was significantly higher among the DCIS' (~33%) compared to the invasive carcinomas (~8%).
Conclusions: We have developed a method for identification of two different mechanisms of genomic distortion in breast tumours. By using this tool we observed an escalating level of genomic complexity with increasing tumour grade in certain genomic regions. An improved algorithm for identification of CAAs is currently being developed to be able to extract the specific genomic regions spanned by complex rearrangements facilitating identification of hotspots for such aberrations. This will enable a more gene driven approach and might potentially help us understanding key mechanisms of breast cancer progression.

878 Effects of high corn oil and high virgin olive oil diets on the oxidative stress in an experimental mammary cancer model

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Breast cancer is a significant cause of mortality in women worldwide and nutrition may be involved in its aetiology. We have previously demonstrated the differential modulatory effects of high corn oil (HCO) and high virgin olive oil (HOO) diets, stimulatory and protective respectively, on experimental mammary cancer. The purpose of this study was to determine the role of oxidative stress on the dietary lipid effects in the mammary gland and adenocarcinomas in the rat DMBA-induced cancer model. Animals were fed