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

control (3% corn oil diet), high corn oil (20%) or high olive oil (17% olive oil + 3% corn oil) diets from weaning or after carcinogenic induction. Firstly, we analysed the clinical and histopathological features of the mammary tumours. Then, we analysed the expression and activity of the antioxidant enzymes superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx), as well as the non enzymatic antioxidant capacity by means of reduced (GSH) and oxidized (GSSG) glutathione levels and the GSSG/GSH ratio. Levels of F2-isoprostane (F2-IsoP) were measured as markers of lipid peroxidation. To evaluate oxidative DNA damage we determined 8-oxo-2′-deoxyguanosine (8-oxo-dG) levels and DNA breaks levels by the comet assay. Results showed that the HCO diet increased oxidative stress in the mammary gland related with its clear promoting effect on the carcinogenesis. The HOO diet conferred to the adenocarcinomas a clinical behaviour and histopathological pattern compatible with a lower degree of malignancy in comparison with the HCO diet. However, these effects were also associated to an increase in some oxidative stress parameters in the mammary gland, although the different 8-oxo-dG and GSH levels suggest a differential influence compared to the HCO diet. Oxidative stress was higher in mammary adenocarcinomas compared to adjacent normal tissue irrespective of the diet and the timing of administration, as evidenced by the lower antioxidant capacity of tumours. This effect was more important in the high fat diet groups. Nevertheless, high fat diets did not clearly modify the oxidative status of mammary adenocarcinomas which would be more dependent on the neoplastic transformation. In conclusion, changes in the oxidative stress levels of the mammary gland may be a mechanism by which high fat diets influence the carcinogenesis but in tumours this status would be more independent on the dietetic factors such as dietary lipids.

879 Increased MGMT expression by dietary constituents may be involved in their chemopreventive effects

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Alkylating agents present in the diet or endogenously produced may be mutagenic and carcinogenic. There have been several reports, including our own unpublished observations, of dietary chemoprevention conferred against alkylating agents. O^6-Methylguanine (O^6MeG) is an important promutagenic DNA adduct produced by SN-1 methylating agents. If these methyl adducts are not repaired by the cell, specifically by methylguanine methyltransferase (MGMT), they are converted in O^6MeG:T mismatch, which can be repaired by the mismatch repair system (MMR). O^6MeG still remains in one of the template strands and this will cause the repair process to be repeated, creating a "futile repair loop". This loop will eventually result in toxic double-strand breaks and will induce apoptosis. When both of these systems fail to repair, O^6MeG results in point mutations that can possibly initiate the carcinogenic process. In the present study the possible role of dietary constituents in the induction of expression and activity of the enzyme MGMT in colon cell has been investigated. A modified version of the comet assay has been developed to assess MGMT repair activity.

UA, a dietary triterpenoid, increased MGMT expression in Caco-2 after 6 hour incubation. In the attempt to see if this increase corresponds to an increase of O^6MeG repair, two colon cell lines (Caco-2, MMR efficient, and HCT116, MMR defective) were treated with the MGMT inhibitor BG (2hrs) followed by MNU exposure, and the DNA damage was evaluated 1, 24, 48 and 72 hrs later, by the comet assay. In Caco-2, after 1 hr MNU induced DNA damage detected by comet assay. This damage decreased in a time-dependent manner, in which this decrease was most marked in cells without BG treatment. After 72 hrs, the cells treated with BG present significantly more damage than cells without BG. This difference corresponds to O^6MeG that were converted in strand breaks by the MMR system. To prove this idea, the same treatments were performed in HCT116 cells and we found that there were no differences between cells with and without BG, due to a deficient MMR system.

In conclusion, we showed that it is possible to quantify O^6MeG by the comet assay in MMR proficient cells. As we found that UA increased MGMT expression, we can now use the comet assay to investigate if this increase corresponds to an increase in O^6MeG repair by MGMT. Evaluation of the effects of UA on damage induced by therapeutic alkylating agents should also be done.

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880 Diets high in corn oil or extra-virgin olive oil provided from weaning differentially modify sexual maturation and susceptibility to mammary carcinogenesis in rats

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Diverse evidence has shown the importance of early life events, including nutrition, in the modification of breast cancer risk. We investigated the effects of high-fat diets on sexual maturation, mammary gland development and its susceptibility to malignant transformation. Female Sprague-Dawley rats were fed a low fat (LF), a high corn oil (HCO) or a high extra-virgin olive oil (HOO) diet from weaning, gavaged with 7,12-dimethylbenz[a]anthracene (DMBA), and euthanized at 24, 36, 51, 100 and 246 days. We studied several parameters of growth and sexual maturation: body weight and mass index, vaginal opening, serum hormone levels, histological evaluation of the ovaries activity and morphological evaluation of mammary gland differentiation. Moreover, the expression of kisspeptin in hypothalamus as well as β-casein and estrogen and progesterone receptors in mammary glands was assessed by real time PCR. The clinical manifestation of carcinogenesis was studied by the tumour incidence (percentage of affected rats) and tumour yield (total number of tumours per group). The results showed that the body weight and body mass index increased in the HCO group in relation to the LF group. Vaginal opening was advanced in both high-fat groups, especially in the HCO group. Such group had also an increased body weight around puberty, higher number of corpora lutea at post-puberty, and tended to have higher mRNA levels of kisspeptin in hypothalamus. Both high-fat diets induced subtle modifications in the mammary gland morphology. No changes were found on serum hormone levels or the mRNA expression of β-casein and hormone receptors of the mammary gland among groups. The HCO diet had a clear stimulating effect of the carcinogenesis, inducing the highest tumour incidence and yield, whereas the HOO diet seem to have a weak enhancing effect, since only increased the tumour yield in comparison with the LF diet. Our data suggest differential effects depending on the oil that is consumed, showing that administration from pre-puberty with the HCO diet strongly advanced maturation and increased breast cancer risk, while the animals fed the HOO diet were more similar to the controls.

881 Effect of Pteridium aquilinum in gastric epithelial cells: potential synergistic effect with Helicobacter pylori infection

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Helicobacter pylori (Hp) is an important etiological factor in the development of gastric cancer. The molecular mechanisms of *Hp*-induced gastric carcinogenesis and the interplay with other environmental factors, such as toxins present in the human diet, are not fully understood. *Pteridium aquilinum* – bracken fern – is the only higher plant known to naturally cause cancer in animals. The carcinogenic toxin of bracken, ptaquiloside, was identified not only in the plant, but also in cow's milk and water. Moreover, epidemiological studies have consistently shown an association between bracken exposure and gastric cancer. The aim of the present work is to evaluate the involvement of *Pteridium aquilinum* in the gastric carcinogenesis process and its potential synergistic effect with *Hp* infection.

Bracken fern extracts damaging effects were investigated on *Hp* culture *in vitro*. Alterations associated with extract exposure were also analyzed on gastric epithelium either in the presence or absence of *Hp*. In addition, *Hp*-infected or not infected C57Bl/6 mice were also treated with *Pteridium aquilinum* in order to identify histological and molecular alterations in the gastric mucosa.

An inhibitory effect of bracken fern extracts was observed on *Hp* growth and survival *in vitro*. In gastric epithelial cells culture the bracken fern extract (20−40 mg extract/ml) decreased the number of viable cells in proliferation. Both γH2AX immunofluorescence and western blot analysis showed induction of DNA double strand breaks in cells exposed to extracts. *In vivo*, KI-67 immunostaining also indicated an increased proliferative index on gastric mucosa cells of mice exposed to bracken fern independently of the infection status. We further evaluated glycosylation alterations on the mice gastric mucosa upon *Pteridium aquilinum* exposure, and an increased expression of sialylated structures was observed.

Our *in vitro* results show the DNA damaging effects of *Ptequiridium aquilinum* extracts on gastric epithelial cells. In addition, results from the mouse model suggest that *Ptequiridium aquilinum* extract in association with *Hp* infection might have an important role in the human gastric carcinogenesis process.