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