1-IS
Successes and failures of molecular cancer epidemiology

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Molecular epidemiology was introduced in the study of cancer in the early 1980s, with the expectation that it would help overcome some of the limitations of epidemiology and facilitate cancer prevention. The expectation was that biomarkers would improve exposure assessment, document early changes preceding disease, and identify subgroups in the population with greater susceptibility to cancer, thereby increasing the ability of epidemiological studies to identify causes and elucidate mechanisms in carcinogenesis. An early example of a successful application of molecular epidemiology in cancer research includes the measurement of chemical-specific DNA adducts formed by the interface between environmental exposures, like polycyclic aromatic hydrocarbons (PAHs) and DNA, a toxicological target. Using this type of biomarker as a biologic dosimeter, researchers could identify individuals who, when exposed to specific carcinogens, are likely to be at increased risk for developing health conditions, including cancer. Another success, also involving a genotoxic endpoint, has been the demonstration that increased chromosomal aberrations or specific pre-leukemic changes are associated with risk of cancer. In addition, molecular epidemiology has successfully identified certain subgroups, such as the fetus and young child, or those with specific genetic polymorphisms or nutritional deficits as being more susceptible. However, the failure to measure the full spectrum of pre-clinical alterations resulting from carcinogen exposure has precluded clear gains in terms of cancer prevention. Recently, new epigenetic and "-omic" biomarkers have become available to address these gaps, thanks to the development of high-throughput technologies based on advances in molecular biology. While these new biomarkers hold promise to revolutionize the field of molecular epidemiology, most have not yet been adequately validated and their role in the causal paradigm is not clear. To achieve their potential, there is a need for systematic validation of these newer biomarkers using principles and criteria established over the past several decades in the epidemiology and molecular epidemiology of cancer. They can then be used in combination with the earlier validated biomarkers of exposure, risk and susceptibility to identify "at risk" individuals, increase our understanding of mechanistic carcinogenic pathways, and mount more effective interventions to prevent cancer occurrence.

2-IS
Novel viral markers

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Infection with distinct types of Human papillomaviruses (HPV), Herpes viruses, Hepatitis viruses, very recently identified Merkel polyoma virus but also bacteria, e.g. Helicobacter pylori, are essential factors in the pathogenesis with specific human tumors. We have developed high-throughput multiplex technologies for the simultaneous, quantitative detection of (i) antibodies to up to 100 different viral or bacterial proteins, or (ii) the genomes of broad varieties of viruses and bacteria.

Multiplex serology uses full-length proteins bacterially expressed as Glutathione S-Transferase (GST) fusion proteins as antigens, affinity-purified in a single step in situ on different sets of glutathione-coated fluorescence-labeled polystyrene beads (Luminex®). Multiplex genotyping uses multiplex PCR to amplify the target sequences followed by hybridization of the PCR products to specific oligonucleotide probes covalently bound to Luminex beads. Antibody bound to antigens or hybridized PCR products on beads are stained with fluorescent secondary reagents and quantified specifically for each antigen or probe in a modified 2-colour FACS machine.

Examples for application of these technologies to large cross-sectional case-control and infection prevalence studies will be described and discussed.

3-IS
Application of epigenetics to cancer epidemiology

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It is now recognized that non-genetic heredity is important for understanding the causes of complex diseases such as cancer. Recent discoveries provided strong evidence arguing that epigenetic mechanisms play key roles in virtually all stages of cancer development and progression. A number of critical processes found in cancer cells, such as silencing of tumour suppressor genes, activation of oncogenes, aberrant cell cycle, and defects in DNA repair, can be a consequence of not only genetic but also epigenetic changes. The term "epigenetic" refers all heritable changes in gene expression and associated phenotypic traits that are not coded in the DNA sequence itself. Epigenetic inheritance include DNA methylation, histone modifications, and microRNAs, all of which are essential mechanisms that allow the stable propagation of gene activity states from one generation of cells to the next. Despite the fact that epigenetic changes induced by environmental, dietary and lifestyle factors are likely to be important mechanisms of cancer development, epigenetic epidemiology is in its infancy, and little is known of the precise contribution of epigenetic changes to cancer burden. A list of genes and gene networks that are targets of epigenetic alterations are likely to grow with the development of powerful screening approaches. Almost spectacular technological advances in epigenetics and epigenomics now allow powerful screening of large series of samples of different cancer types. These approaches are beginning to reveal a number of genes (tumor suppressors and other cancer-associated genes) susceptible to inactivation through epigenetic mechanism. Epigenetic profiling using both genome-wide and candidate-gene approaches in different tumor types will help in elucidation the mechanism underlying tumourigenesis. Technological advances in epigenetics and epigenomics as well as ongoing studies aiming to identify specific epigenetic targets, environmental factors, and the critical windows of vulnerability to environmentally induced epigenetic alterations will be discussed.

4-IS
Expression microarrays and cancer epidemiology; lessons from breast cancer

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Expression profiling in relation to breast cancer (BC) have been performed on healthy breast tissue and various tumor tissues from benign to malignant in order to identify profiles related to BC risk, aggressiveness, metastatic potential and therapy response. At least five distinct molecular subgroups have been identified associated with different clinical outcome (Sørlie et al PNAS 2001, 2003). There is growing evidence that analyzing blood-derived gene expression profiles may lead to a diagnostic test for BC. A gene signature of 82% accuracy, 87% sensitivity and 76% specificity has been identified (Sharma et al BCR 2005, Aarøe et al 97thAACR and 19thEACR, 2006, Børresen-Dale AL et al 97thAACR 2007). In a current study to investigate the efficacies of a blood based test in another ethnic population, 720 subjects with or without BC from diverse areas of India are recruited. The results of interim analyses of approximately 350 cases indicate that the previously identified 96 gene set efficiently discriminates BC and non-BC samples, providing evidence for a gene expression signature as a potential additional tool in BC diagnostic work-up.

To improve the reliability and accuracy of the various expression profiles, the role an individual's genotype (SNPs, CNVs) and exposure (eg hormones) has on these profiles is of importance to identify. Recently, GWAS analysis of BC has revealed SNPs in 5 novel genes associated to susceptibility. With the notion that the probability of developing a given subclass of breast cancer is genetically determined, we might expect to find that the newly discovered susceptibility genes are differentially expressed in the various tumor subclasses, and that their transcription is regulated in cis by SNPs within them. With this in mind, we retrieved the mRNA expression data of these 5 genes from 112 breast tumors representing all 5 subclasses, and significantly different mRNA levels between the subtypes were found for all the 5 genes by ANOVA analysis (Nordgard et al 2007 BCR, Kristensen and Borresen-Dale 2008 Mol. Onc.). This illustrates the necessity to conduct stratified SNP-disease association studies. Stratification of patients by their molecular subtypes may give much more power to the classical case control studies, and genes of no or borderline overall significance may be highly penetrant for certain subclasses, and therefore identifiable.

5-IS
Integrated gene expression analysis in PBL and in the derived Lymphoblastoid Cell Lines to define individual radiotoxic risk profiles

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Microarrays are a powerful tool for the simultaneous analysis of expression of thousands of genes to outline similarities and differences among samples. This technology is extensively used to explore biological causes