Winter Meeting of the UK Molecular Epidemiology Group

One-day Meeting for the 10th Anniversary of UK MEG
on 8th December 2006 at
The Royal Statistical Society, 12 Errol Street
London EC1Y 8LX

"Epigenomics and disease"

P R O G R A M M E

09:00 - 09:50	Registration and Coffee
09:50 - 10:00	Welcome and Introduction
	MORNING SESSION: Chair: Paolo Vineis
10:00 - 10:45	**UKEMS Keynote lecture: *'Towards a human cancer epigenome'*** Manel Esteller, Spanish National Cancer Research Center, Spain
10:45 - 11:30	**'*Epigenetics in the study of chronic diseases'*** Fei Ling Lim, Syngenta Central Toxicology Laboratory, UK
11:30 - 12:15	***'Carcinogens are not mutagens but promoters'*** James Trosko, College of Human Medicine, Michigan State University, USA
12:15 - 12.30	**UK MEG AGM**
12:30 - 13:30	LUNCH
	AFTERNOON SESSION: Chair: John Hesketh
13:30 - 14:15	***'Studies on epigenetics and lung cancer'*** Zdenko Herceg, International Agency for Research on Cancer, France
14:15 - 15:00	***'Chromatin alterations in tumorigenesis'*** Saverio Minucci, European Institute of Oncology, Italy
15:00 - 15:15	TEA
15:15 - 16:00	***'Nutrition and epigenetics - how the genome learns from experience'*** John Mathers, University of Newcastle, UK
	INTRODUCTION: Chair: Frank Martin
16:00 - 16:45	***UK MEG 10th Anniversary Award Lecture*** Christopher P Wild, University of Leeds, UK
16:45	CLOSE OF MEETING

SPEAKERS' ABSTRACTS

Towards a human cancer epigenome

Dr. Manel Esteller
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We are in an era where the potential exists for deriving comprehensive profiles of DNA alterations characterizing each form of human cancer. DNA methylation is the main epigenetic modification in humans. Tumor cells show aberrant methylation of several CpG islands, but global demethylation versus the counterpart normal cells. We have combined a candidate gene and biochemical approach to determine the overall aberrant DNA methylation in transformed cells. Our results show that CpG island promoter hypermethylation has a tumor-type specific pattern, where each gene tends to be methylated in the cancer cells driven from a particular tissue but not from others. Epigenetic silencing affects all cellular pathways: DNA repair (hMLH1, MGMT, BRCA1), cell cycle ($p16^{INK4a}$, $p14^{ARF}$, $p15^{INK4b}$, p73), apoptosis (DAPK, TMS1), hormone receptors (ER, PR, AR, RARB2, CRBP1), cell adherence (CDH1, TIMP3), detoxifiers (GSTP1) and many more (APC, LKB1, SOCS-1…). Promoter hypermethylation of particular genes have important consequences for the biology of that particular tumor. This is for example the case of the DNA repair gene MGMT which methylation-mediated silencing leads to transition mutations, but, at the same time, "marks" those neoplasms that are going to be more sensitive to the chemotherapy with alkylating drugs. Hypermethylation can be observed in hereditary tumors, where it may account for the "second hit" of the tumor suppressor gene. We have also developed massive genomic screenings to find new hypermethylated genes in cancer cell. From these assays we have identified new candidate tumor suppressor genes with important potential roles in the pathogenesis of human cancer.

Second, we have studied the global methylcytosine content of a large collection of normal tissues and sporadic and hereditary primary tumors. The picture that emerges shows that 85% of human cancer cells are hypomethylated when compared to the original normal cells. We have also found that the 5-methylcytosine DNA content and the number of CpG islands hypermethylated in a given tumor is not random, but it involves environmental factors and genetic predisposition.

Overall, our data demonstrates that human tumors suffer a profound, but specific, disturbance in their DNA methylation and chromatin patterns.

Epigenetics in the study of chronic diseases

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Epigenetics **is the term used to describe the study of heritable changes in gene function that occur in the absence of a change in DNA sequence. The principle way in which epigenetic information is stored and propagated is *via* methylation of DNA at cytosine residues, to form the modified base 5-methylcytosine, and through post-translational modification of the proteins that package genomic DNA into chromatin. Methylation of cytosines usually occurs at sites in the genome known as CpG islands, and is generally associated with the silencing of adjacent genes. Therefore, perturbation of DNA methylation status alters the spectrum of genes and proteins expressed in a cell, which in turn leads to alterations in cellular phenotype.**

Recent years have seen a surge of interest in the role of epigenetic mechanisms in normal homeostasis and disease processes, and there is now good evidence that epigenetic alterations contribute to predisposition to and development of disease. A wide range of human cancers display aberrations in DNA methylation patterns, leading to the suggestion that changes in the DNA methylation status of certain genes may contribute to the transformed phenotype. This, in turn, raises the possibility that DNA methylation changes at specific gene loci may function as diagnostic biomarkers of disease progression.

An emerging body of data suggest that epigenetic perturbations may be involved in the adverse effects associated with some xenobiotics, including certain classes of non-genotoxic chemical carcinogen. The epigenetic status of a genome is maintained between parent and daughter cells during cell division. Therefore, in theory, xenobiotics that perturb epigenetic status have the potential to induce persistent alterations in cellular phenotype that are propagated from one generation of cells to the next. It is well-established that a number of xenobiotics do indeed have the potential to produce adverse effects by inducing changes in cellular phenotype that are maintained following cell division, for example, by causing persistent changes in the molecular pathways that regulate cell growth and apoptosis. While many of these effects have been shown to be due to *mutagenic* events, there is a growing body of evidence that, in some cases, *epigenetic* alterations may play a role.

I will summarise the evidence that epigenetic changes are involved in the onset of human disease and the mechanisms of toxicity associated with some xenobiotics. I will also describe the use of DNA methylation changes as potential biomarkers of toxicity and disease progression.

References

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"Chemical carcinogens as mutagens" and "molecular epidemiology of cancer" are bankrupt paradigms: stem cells, altered cell-cell communication and epigenetic mechanisms as ignored concepts

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Mechanisms contributing to the wide variety of human diseases include [a] gene and chromosomal mutagenesis ("genotoxicity"); [b] cytotoxicity (necrosis and apoptosis) and [c] altered gene expression (epigenetic toxicology). Toxicology and carcinogenesis have been paralyzed by the prevailing paradigm that if a chemical causes birth defects, cancer, reproductive or neurological diseases, it must involve DNA damage and mutations. The genotoxicity paradigm stems from: (1) *in vitro* assays to test "genotoxicity", showing "positive" results for chemicals associated with the occurrence of various diseases; (2) with mutations in oncogenes and tumor suppressor genes in tumors; (3) with chemicals inducing oxidative stress; (4) with DNA adducts in chemically-exposed tissues; and (5) with heredity mutations associated with various diseases; Also, the lack of critical analyses of what might be the "target" cells of chemicals in exposed tissues and organs contributes to the blind acceptance of the genotoxicity paradigm. With the recent demonstration that many highly studied environmental or dietary toxins and toxicants do not induce nuclear DNA damage or mutations, even though they might induce oxidative stress, but can be teratogens, tumor promoters, immunotoxicants, reproductive-and neuro-toxicants by altering gene expression at the transcriptional, translational or posttranslational levels, indicates a strong need to assess the non-genotoxic risk of many chemicals at the epigenetic level. Implications to risk assessment are profoundly different for mutagens and epigenetic toxicants. Using the multi-stage, multi-mechanism model of carcinogenesis and ignored "hallmarks" of cancer, a new paradigm will explain the paradox of the "crises in the genotoxicity" paradigm.

Studies on epigenetics and lung cancer

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Epigenetic events affect virtually every step in tumor development and progression. Therefore, understanding epigenetic events associated with cancer onset, progression and metastasis are fundamental to improving our abilities to successfully prevent, diagnose and treat cancer. Epigenetic modifications include DNA methylation and chromatin modifications. One of the most intriguing advances is the convergence of mechanistic studies linking DNA methylation with histone modifications. Aberrant chromatin modifications including altered chromatin acetylation and methylation can cause incorrect gene activation and improper gene silencing, which can lead to cancer.

However, although the role of epigenetic events is supported by both epidemiological and experimental studies, the precise contribution of epigenetic mechanisms and cellular targets epigenetic alterations to human cancers are largely unknown. Recently, we have studied the role of epigenetic mechanisms in normal cellular processes and abnormal events that lead to oncogenic transformation and tumour development. These studies have shed new light on the epigenetic mechanisms and revealed new concepts in this emerging field. New concepts involving epigenetic events and their implication for mechanistic understanding of cancer development, risk assessment and prevention will be discussed.

Chromatin alterations in tumorigenesis

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Chromatin alterations are being increasingly found in cancer cells. We and other groups have shown that aberrant recruitment of chromatin modifying enzymes (such as histone deacetylases and methyltransferases, and DNA methyltransferases) plays a causal role in the pathogenesis of various subtypes of acute myeloid leukemias. Recently, we have started to extend these observations to other tumor types, including solid tumors. To analyze more systematically the alterations caused by the aberrant activity of histone deacetylases, we have developed a number of tools for the genome-wide analysis of chromatin accessibility and non-histone acetylation. Interestingly, histone deacetylase inhibitors are in clinical trials as antitumor agents: our findings are therefore potentially of relevance for the design of optimized anticancer treatments.

Nutrition and epigenetics – how the genome learns from experience

John Mathers
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Epigenetic marking of the genome includes DNA methylation and post-translational modification of the octet of histones around which the DNA is wrapped in the nucleus. It is now clear that these markings i) have a major influence on chromatin structure, ii) constitute a significant information store and iii) provide a sophisticated system for regulating gene expression. However, it is also apparent that, unlike the genetic code, epigenetic markings vary between cells and over time and, therefore, cell function and health.

In this presentation I will give an overview of the 4 Rs of epigenetics i.e. how the genome Receives environmental stumuli, Records and Remembers that information and Reveals effects of those stimuli through changes in gene expression with implications for cell function and health. I will illustrate some recent findings about the impact of the nutritional environment on epigenetic marking with a focus on DNA methylation. The presentation will include discussion of effects nutritional intervention at different stages of the life course and possible implications for modulation of the ageing process.

Molecular Epidemiology and Environmental Exposure Assessment: Crossing the Threshold of Hope

Christopher Paul Wild
Molecular Epidemiology Unit, Centre for Epidemiology and Biostatistics, LIGHT Laboratories, University of Leeds, UK

The majority of common complex disorders, such as cancer, vascular and neurodegenerative diseases involve both genetic and environmental risk factors. Environmental exposures are acknowledged to play an overwhelmingly important role in these diseases, which constitute the major health burden in economically developed countries. The majority of genetic variants or single nucleotide polymorphisms (SNPs) in the human genome are of low penetrance but their high prevalence means they may contribute significantly to population disease burden. Nevertheless the majority will do so only in the presence of specific environmental exposures. Despite major technical advances in assessing genetic variance, many exposure-disease associations remain ill-defined and the complex interplay with genetic susceptibility is only beginning to be addressed.

The new generation of mega-cohort studies, including the UK Biobank, provide the framework for just such investigations of genetic variation, environment, lifestyle and chronic disease. However, as measurement of the genetic portion of the gene:environment equation continues to be refined the other remains subject to a large degree of misclassification. For the most part, the accurate assessment of environmental exposures remains a largely unmet challenge in molecular epidemiology. This imbalance in measurement precision of genes and environment has consequences, most fundamentally in compromising the ability to fully derive public health benefits from expenditure on the human genome and the aforementioned cohort studies.

There is therefore a pressing need to develop methods with the same precision to measure an individual's environmental exposure as we have for the individual's genome. We need an "exposome" to match the "genome" (Wild CP, Cancer Epidemiology Biomarkers and Prevention, 14: 1847-1850, 2005). The concept of an "exposome" may be useful in drawing attention to the need for methodological developments in exposure assessment. At its most complete the "exposome" would encompass life-course exposures (including lifestyle factors), from the pre-natal period onwards. Developing reliable tools for such a complete exposure history is extremely challenging. Unlike the genome, the "exposome" is a highly variable and dynamic entity that evolves throughout life. It is not without good cause that progress has been limited in meeting this goal. However, the methodological challenge may not be more daunting than the one faced two decades ago of investigating an estimated 10 million SNPs in the human genome. In addition, as with the genome, even a partial, targeted understanding of exposure can provide significant advances.

The measurement of DNA and protein adducts and metabolites in human tissues and body fluids have contributed significantly to exposure assessment in molecular epidemiology. However, it is also pertinent to ask whether the new "omics" technologies of transcriptomics, proteomics and metabonomics can be used to characterise exposure and help unlock the problem further. Any such advances will require still greater interdisciplinary collaboration between epidemiologists, biostatisticians, experts in bioinformatics, laboratory and environmental scientists. In addition, funding agencies will need to take a medium to long-term view and encourage research that focuses on improved measures of environmental risk factors, an area that currently appears to be less of a priority for support than many others in the broad domain of medical research.

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"Epigenomics and disease"

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