



**First European Cancer and Environment Research Institute Workshop**

**ENVIRONMENTAL CARCINOGENESIS: A FOCUS ON  
EPIGENETICS**

-

**October the 26<sup>th</sup> 2012,  
Académie Royale de Médecine de Belgique,  
*Belgium Royal Academy of Medicine,*  
Salle Albert I,  
Brussels, Belgium**

Under the patronage of Michael Skinner (US) and Luc Montagnier (Nobel Prize, France)



On 26 October 2012, the European Cancer and Environment Research Institute (ECERI) held its first International Workshop in the 'Albert the 1st' Room of the Royal Academy of Medicine of Belgium, entitled "Environmental carcinogenesis: a focus on epigenetics".

The newly created ECERI (headquartered in Brussels, chaired by Prof. D. Belpomme, oncologist in Paris) is an association of several European research centers aimed at advancing research environment links to cancer and the resulting need for primary prevention methods. Professors Janos Fruhling and Luc Montagnier co-chair the Scientific Council of ECERI, which includes internationally recognized scientific personalities. Although ECERI is focused primarily on cancer, it does not ignore other chronic diseases such as obesity, type 2 diabetes, autism and Alzheimer's disease (the latter has recently shown a link with the environment--pointed out by Prof. Belpomme in his introductory lecture at the Workshop).

Prof. Belpomme was one of the first to promote the concept of environmental carcinogenesis. In the vast majority of countries, the incidence of cancer has increased in recent decades. The traditional cancer risk factors of aging populations and/or lifestyle, such as smoking, alcoholism, dietary imbalances, sedentary lifestyle, etc.. do not, alone account for that increase. So physical, chemical and biological degradation of our environment - in particular the presence of many carcinogenic, mutagenic and / or toxic for reproduction pollutants must be considered. Professor Belpomme along with Professor Montagnier had already delivered such a message in 2005, at the Belgium Royal Academy of Medicine.

Since then progress in molecular biology--particularly epigenetics--enable us to understand the role of the environment in carcinogenesis (and other diseases).

## **Epigenome changes induced by environmental epimutagens and cancer**

**Zdenko HERCEG, Ph.D.**

*Head, Section of Mechanisms of Carcinogenesis,*

*Head, Epigenetics Group, International Agency for Research on Cancer (IARC), 150 Cours Albert Thomas, F-69008, Lyon, France.*

Recent advances in epigenetics and epigenomics has a tremendous impact on our thinking and understanding of biological phenomena and importance of environmental stressors in complex diseases, notably cancer. Environmental and lifestyle factors are thought to be implicated in the development of a wide range of human cancers by eliciting epigenetic changes, however the underlying mechanisms remain poorly understood. The epigenome can be viewed as an interface between genome and environmental influence, therefore aberrant epigenetic events associated with environmental exposures (epimutagens) and factors in cell microenvironment are likely to play an important role in the onset and progression of different human malignancies. At the cellular level, aberrant epigenetic events influence critical cellular events (such as gene expression, carcinogen detoxification, DNA repair, and cell cycle), which are further modulated by risk factor exposures. However, there is little understanding about whether epigenetic changes in surrogate tissues can be used as biomarkers for exposure assessment, early detection and an intermediate biomarker for different health outcomes. Remarkable advances in epigenomics and the advent of powerful technologies for analyzing epigenetic patterns in both cancer tissues and normal cells indicate that the next few years will be fundamental for the identification of critical cancer-associated and exposure-associated epigenetic changes and for their evaluation as biomarkers. Here I will discuss recent progress in our understanding of epigenetic mechanisms through which environmental stressors and endogenous factors may promote tumour development and progression as well as its implications for biomarker discovery, risk assessment and cancer control.



## What is environmental carcinogenesis

**Dominique BÉLPOMME, M.D. MSC**

*Paris Descartes University, ARTAC Research Center, ECERI*

The growing incidence of a variety of cancers since the Second World War confronts scientists with the question of their genesis. In economically developed countries, expansion and ageing of the population and new/improved diagnostic and screening tests to detect previously hidden developing tumors/cancers do not fully account for the observed growing incidence of cancer. Our hypothesis is that in addition to oncogenic viruses and radiation, exposure to synthetic chemicals plays a more important role in cancer genesis than it is usually agreed: (1) Over the last 2-3 decades, alcohol consumption and tobacco smoking in men have significantly decreased. (2) Obesity is increasing in many countries and adipose tissue has been shown to be enhanced by environmental chemicals and to be a reservoir for carcinogenic lipophilic environmental pollutants. (3) Our environment has changed over the same time scale as the recent rise in cancer incidence (including the accumulation of many new carcinogenic chemicals in the biosphere). (4) Endogenous carcinogenesis susceptibility factors, such as individual's genetic polymorphism, cannot so change over one generation and some may favour the role of exogenous carcinogens/procarcinogens, via gene-environment interaction. (5) Population aging does not explain rising incidence as most time series adjust for population aging and the increase is seen across all age categories, including children. (6) The foetus is specifically vulnerable to exogenous carcinogenic agents. Chemical exposure during prenatal and early postnatal life could alter gene expression by genetic and epigenetic changes, causing biochemical functional changes in specific organs and tissues that may cause cancer occurrence during adult life, and increase cancer susceptibility in successive generations. This seems particularly the case for the increases in breast and prostate cancers, which have been shown in rodent models to result from the perinatal administration of endocrine disruptors. A fetal exposure during a critical window period may explain why current epidemiological studies fail to find a link between cancer and environment in adults. Moreover carcinogenesis is being revealed to be a far more complex process than initially thought: It should be considered not only direct mutagenesis via the classical somatic mutation theory; but also epigenomic processes leading to metabolic dysfunction and indirect mutagenesis through environment-related epigenomic alterations. We therefore propose that the involuntary exposure to many carcinogens in the environment may contribute to the recent rising trend in cancer incidence; that the risk attributable to environmental carcinogens may be far higher than it is usually proposed; and that cancer should be considered to be a model of multifactorial environmental disease.

## Methylation analysis of colorectal cancer genes

**Lucia MIGLIORE, Ph.D.**

*Department of Human and Environmental Sciences, Medical Genetics Laboratories, University of Pisa, Pisa, Italy.*

*ITT, Istituto Toscano Tumori, Florence; Italy.*

Since epigenetic modifications are reversible, methylation studies are extremely promising to better characterize colorectal cancer (CRC) and to identify new tools for diagnosis and prognosis. We performed methylation analysis in promoters of APC, CDKN2A, hMLH1, MGMT and RASSF1A genes in almost 80 CRC and healthy adjacent tissue specimens and we analyzed the correlation among the methylation status of these genes and the clinical-pathological features of the patients; we tested also a possible association among MTHFR C677T, DNMT3B C-149T polymorphisms and the methylation levels of APC, CDKN2A, hMLH1 and MGMT gene promoters. Methylation analyses were performed by means of methylation sensitive-high resolution melting (MS-HRM) and pyrosequencing, followed by comparison of the results. We observed specular profiles of methylation and a high correlation between the MS-HRM and pyrosequencing techniques and elaborated a novel algorithm to make MS-HRM a quantitative assay. We also noticed a higher gene promoter methylation in CRC tissue with respect to the healthy adjacent tissue. No statistically significant association between stage (TNM), gender, sex, tumor size, location with regard to the methylation profile of each of the analyzed genes was found. However, a positive association between age and both hMLH1 and MGMT methylation levels and a significant interaction between MTHFR 677C>T, DNMT3B -149C>T polymorphisms and gene promoter methylation were found.



## **Epigenetic Regulation as the molecular link between gut microflora and colon cancer**

**Vincent CASTRONOVO, M.D., Ph.D.**

*Department of Biology, Faculty of Medicine, Director, Metastasis Research Laboratory, Head of GIGA-Cancer, University of Liege, Belgium.*

Colorectal cancer (CRC) is the third most frequently diagnosed cancer in men and women and the second highest cause of cancer deaths in Western societies. Because of the steadily increased incidence of CRC, it becomes urgent to develop effective primary prevention strategies. Both genetic and environmental factors are associated with the risk of CRC development. Altered nutrition, especially in the Western countries, is considered to account for around 70% of death by CRC. A clear link has been established between consumption of foods rich in fibers and lower incidence of CRC. Recently, it has been demonstrated that the fructo-oligosaccharose and inulin, present in the protective fibers, stimulate the activity of eubiotic bacteria, which produce n-butyrate, as one of the metabolic byproducts. This short chain fatty acid induced the programmed cell death of premalignant and malignant colonic cells by inhibiting histone deacetylases, a family of enzymes involved in the epigenetic regulation of the transcription of several genes. In the light of these observations, it appears clear that strategies enabling the stimulation of high level of n-butyrate by the colonic bacteria could be of high value for the primary prevention of CRC.

## Transplacental and transgenerational epigenetic origins of childhood cancer

**Ernesto BURGIO, M.D.**

*ISDE Scientific Committee.*

Cancer's continuous increase in incidence, observed throughout the 20th century in all industrialized countries, is classically explained clinically by improvement of diagnostic capacities and biologically by the progressive accumulation of oxidative stochastic genetic damage. Yet this rising incidence (blighting individuals of all ages, the young included) has been too often underestimated. Some recent reports of a significant increase in childhood cancer in Europe and especially in Italy have caused concern, forcing us to fundamentally revise the dominant model of carcinogenesis. Noteworthy is that at least the first stages of the malignant process of cancer in infants are present at time of birth. The importance of genetic changes in utero has been long suspected for many years, on the basis of studies of twins with leukemia. In utero genesis is also suggested by blood samples at birth (Guthrie cards) of infants who subsequently develop leukemias that show translocations and gene sequences corresponding to the fusion genes which are later found in leukemic blasts. Today pro-leukemic translocations and clones in foetuses (the common leukemic fusion genes, TEL-AML1 t (12; 21) (p12; q22) or AML1-ETO t (8; 21) (q22q22)) have been found in the cord blood with a frequency 100 times greater than the corresponding frequency of leukemia. The classical interpretation is that translocations do not necessarily determine the onset of leukemia, which requires additional genetic events in the postnatal period. A possibly more realistic interpretation is that if less than 1% of children who have "been associated with" a translocation have developed leukemia; it may be that the translocation leads to an active potentially positive adaptive genomic change which could yield cells to be responsive to toxic exposures in the utero. Researchers who adhere to the paradigm of stochastic mutations and more generally to a linear and gene-centric model of DNA, have obviously some difficulty to accept this new hypothesis. However if we accept the concept that the epigenome is a unitary, dynamic, responsive and complex molecular network; it is possible to that epigenetic (e.g. global DNA hypomethylation, hyper-methylation of promoter sequences of tumor suppressor genes), genetic (genomic instability, mobilization of transposable sequences), and chromosomal (translocations) environmental alterations are steps of a



failed or distorted evolutionary/adaptive and essentially defensive process leading to cancer progression.

## **Epigenetics and Epigenomics in Health and Diseases**

### **François FUKS**

*Laboratory of Cancer Epigenetics Faculty of Medicine, Free University of Brussels (U.L.B.), 808 route de Lennik, 1070 Brussels, Belgium.*

DNA methylation plays an important role in cancer and is associated with gene silencing of tumour suppressor genes. The methylation of CpG sites is established by the DNA methyl transferases - the DNMTs. One main interest in our lab is to better decipher the mechanisms by which these enzymes function and participate to cancerogenesis.

In recent years, we have contributed to show that DNA methylation and the modifications of histones work hand-in-hand as parts of an epigenetic program that integrates gene-silencing networks within the cell. In particular, a close connection was found between DNMTs and several histone modification enzymes.

Another more recent interest of our lab is to better understand the involvement of the DNA methylome in cancers. Indeed, the cancer epigenetic field has evolved from a gene-by-gene approach to more global epigenomic strategies, with far-reaching fundamental and clinical implications. Using genome-wide approaches, we have recently performed DNA methylation profiling in human breast tumor tissues. Results will be presented that highlight precise epigenetic portraits in breast cancers, uncovering a key contribution of the DNA methylome to the complexity of the disease.

### **Selected publications**

- Brenner et al. EMBO J. 2005 24:336-46.
- Viré et al. Nature 2006 439:871-4.
- Brenner & Fuks Dev. Cell 2007 12:843-4
- Villa et al. Cancer Cell 2007 11:513-25
- Feldman et al. Nature SMB. 2008 15:1176-83
- Fuks F. Nature. 2010 Jul 22;466(7305):417.
- Ndlovu et al. Trends BiochemSci. 2011 Jul; 36(7):381-7.
- Denis et al. EMBO Rep. 2011 Jul 1;12(7):647-56.
- Dedeurwaerder et al. EMBO Molecular Medicine. 2011 Dec;3(12):726-41.
- Volkmar et al. EMBO J. 2012 31:1405-26.

# Low level chronic environment exposures such as metals and cancer: role of epigenetics in complex diseases

**Nick OSBORNE, Ph.D.**

*European Centre for Environment and Human Health, Truro, Cornwall, United Kingdom.*

In recent years there has been an exponential increase in the demand and production of tungsten as well as its use in diverse applications. This has seen it recently placed on the EPA USA chemicals of concern list as it is now understood to be more bioavailable in soil than previously understood with risk of it entering aquifers.

## Methods

We investigated the association between quartiles of urinary tungsten concentration and cancer in 6 waves of the National Health and Nutrition Examination Survey (NHANES). Tungsten was measured by inductively coupled plasma dynamic reaction cell mass spectrometry and from the same sample, urinary concentration of creatinine was quantified. We utilised logistic regression models adjusted for a range of confounders including demographic and clinical factors, smoking, and other urinary metals. Further we stratified our data to investigate associations in a subset of data for younger individuals (18-50 years, n=5760).

## Results

Our results indicated that individuals in the highest quartile of tungsten were at an increased risk of developing cancer (OR: 1.5, 95%CI 1.0-2.1). The OR remained similar when the common cancers (breast, bowel, lung and prostate) were removed (OR: 1.6, 95%CI 1.1-2.5). Significance was lost when other heavy metals were included in the model. In a younger cohort (18-50 years) the association between urinary tungsten and cancer remained (OR: 2.1, 95%CI 1.1-4.0) even after adjusting for other heavy metals.

## Discussion

We have demonstrated that higher urinary tungsten concentrations appear to increase the risk of cancer but caution should be taken in interpreting results. We hypothesize that the pathological pathway of tungsten may involve an inflammatory response. The potential role of epigenetics will be discussed.



## **Obesity, Diabetes and Cancer: is mitochondrial dysfunction a common key target ?**

**Philippe IRIGARAY, Ph.D.**

*ARTAC Research Center, ECERI Scientific Coordinator*

Obesity, type 2 diabetes and cancer are recent epidemic plagues that concern mostly but not exclusively the developed world. Among classical lifestyle-related risk factors are a combination of excess dietary calories and a lack of physical activity. In addition to genetic predisposition, environmental risk factors, including endocrine disruptors has been proposed as possible underlying mechanisms to explain these incidence increases.

Accumulating evidence suggest indeed that exposure to common man-made environmental CMR chemicals increase the individual's risk of cancer but also of obesity and type 2 diabetes.

In humans, persistent organic pollutants (POPs) are stored primarily in adipose tissue. And it was recently observed that POPs levels in adipose tissue and serum correlate with biological markers of obesity-related dysfunctions. I have previously discovered a novel molecular mechanism of toxicity for one environmental pollutant benzo[a]pyrene (B[a]P) and introduced the concept that chronic exposure to B[a]P and possibly to other polycyclic aromatic hydrocarbons (PAH) could be not only carcinogenic but also obesogen. Moreover at the foetal stage endocrine disruptors can not only induce hormone-dependent adulthood cancers, such as testis, breast and prostate cancer but also increase the number of pre-adipocytes, thus furthering obesity.

The obesity-related diabetes and insulin resistance (IR), increasing in incidence, may also be increased by chemicals - e.g. arsenic and polychlorinated biphenyls (PCBs).

IR can be a common key mechanism of diseases such as obesity, diabetes and cancer. Our hypothesis is that in addition to specific factors, mitochondrial dysfunction might be a key common target accounting for these disorders. IR may originate in the womb as the result of a mitochondrial dysfunction. Moreover mitochondria not only have the essential role in powering cells, by generating ATP, but are also the major source of intracellular reactive oxidative species (ROS), which can be genotoxic and epigenotoxic and so far have a regulatory role in cell death and cell proliferation. Dysregulation of mitochondrial metabolism has been frequently observed in metabolic diseases and in human tumors, as have mutations in mitochondrial DNA, a finding that strongly reinforce our hypothesis.

## From complex disease genetics to complex disease epigenetics

**J Andrew POSPISILIK, Ph.D.**

*Max Planck Institute for Immunobiology and Epigenetics, Freiburg Area, Germany.*

The prevalence of diabetes and obesity has been estimated to exceed 1 billion by the year 2030. Since energy homeostasis is intimately tied to cell lineage decisions, to pluripotency, and to most cellular functions, it is therefore perhaps no surprise that these metabolic disorders are critical risk factors for heart disease, cancer and stroke. Thus, obesity and diabetes currently represent one of the world's chief economic and health care challenges. To date, little is known about the contribution of epigenetic regulation towards the etiologies of these diseases. Our aim is to unravel some of the epigenetic regulatory systems that contribute to the susceptibility and development of complex disease. Our approach is simple, combine the power of mouse and fly genetics with high-level phenotyping, and ultimately apply molecular biology tools to dissect mechanism. At present this strategy is taking us into Polycomb, Trithorax and ncRNA regulation of insulin secreting beta-cells, into germline transmission of paternal feeding state information, into stochastic and epigenetic regulation of adipose tissue development and function, and across organ systems, bridging lipid sensing, microbiota, intracellular metabolism and immune activation. What is clear at present is that these processes embody the product of epigenetic control; the fundamental outstanding question we are interested in is how these influence the susceptibility and development of human disease.



# **Epigenetics of the impact of early traumatic stress on behavior across generations in mammals**

**Isabelle MANSUY**

*Brain Research Institute, University/ETH Zürich, Winterthurerstrasse 190, Zürich, Switzerland.*

Complex behaviors and brain functions in mammals are strongly influenced by environmental factors. When favorable and positive, these factors facilitate the development and expression of adapted and normal behaviors, but when adverse and stressful, they can lead to brain pathologies and psychiatric conditions such as major depression, personality and conduct disorders, and antisocial behaviors. Traumatic events experienced chronically early in life are particularly strong risk factors for such conditions, and can not only affect the individuals directly exposed to trauma, but also following generations. The mechanisms underlying the etiology and inheritance of the effects of early traumatic stress are known to involve epigenetic processes, but remain poorly understood. This talk will discuss initial evidence for the contribution of epigenetic mechanisms to the inherited impact of stress on behavior based on an experimental model of early traumatic stress in mice. This model shows that chronic and unpredictable maternal separation causes depressive-like behaviors, social withdrawal, lack of behavioral control and cognitive defects in adult mice, and that these symptoms are transmitted to the offspring across several generations. It shows that these alterations are associated with persistent changes in DNA methylation in the promoter-associated CpG island of several genes, and in the expression of small non-coding RNAs in the brain and germ cells. These findings suggest that negative environmental conditions early in life broadly and persistently alter epigenetic processes in mammals.

## CONCLUSION

This Workshop--at the Royal Academy of Medicine of Belgium, October 2012--aimed at understanding the underlying biological mechanisms that promote and cause environmental carcinogenesis.

As introduced by Zdenko Herceg from IARC, recent advances in epigenetics and epigenomics have a tremendous impact on our understanding of the genesis of complex diseases such as cancer, to which environmental factors contribute, or cause outright (indeed it is important to clearly distinguish lifestyle risk factors from exogenous disease agents). In this new approach, the epigenome is considered to be an interface between the genome and the environment, allowing environmental factors to alter the control of the genome, causing disease occurrence. Hence the concept of epimutagens and epimutations. Examples involving alterations of DNA methylation of cancer genes and silencing of tumor suppressor genes associated with DNA methylation and histone modifications were provided.

Moreover, it is now accepted that the initiation of chronic diseases such a cancer happens *in utero* or peri-natally. In fact, epigenetic dysregulation in foetus can be imprinted onto the genome and thus be passed on generations.

However disease genesis is probably even more multi-factorial than this, even more than is currently conceived. For example, environmental factors modify microbiota such as the gut microflora and cause mitochondria dysfunction, leading cells to produce an excess of free radicals, the ultimate cause of secondary epigenetic alterations.

Finally, a very important animal experiment revealed that due to epigenetic mechanisms, the behavioral impact of psychological stress could be imprinted and be passed on generations.

This international workshop was organised and supported by ECERI. ECERI is an independent network of European and international scientific experts and researchers specialized in the fields of carcinogenesis, epigenetics, gene-environment interactions and environmental diseases.

ECERI's objectives are :

- boosting European research in the field of environmental carcinogenesis,
- spreading its research to all European countries by developing international scientific collaborations,
- creating pan-Europe data bases,
- providing scientific expertise to the European institutions,
- making health recommendations to the public.

This workshop is the first step towards future scientific developments currently involving more than fifteen European research institutions.

