Roadmap for investigating epigenome deregulation and environmental origins of cancer

Zdenko Herceg, International Agency for Research on Cancer
Akram Ghantous, International Agency for Research on Cancer
Christopher P. Wild, International Agency for Research on Cancer
Athena Sklias, International Agency for Research on Cancer
Lavinia Casati, University of Milan
Susan J. Duthie, Robert Gordon University
Rebecca Fry, University of North Carolina
Jean-Pierre Issa, Fels Institute for Cancer Research & Molecular Biology
Richard Kellermayer, Baylor College of Medicine
Igor Koturbash, University of Arkansas

Only first 10 authors above; see publication for full author list.

Journal Title: International Journal of Cancer
Volume: Volume 142, Number 5
Publisher: Wiley: 12 months | 2018-03-01, Pages 874-882
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1002/ijc.31014
Permanent URL: https://pid.emory.edu/ark:/25593/t0sbk

Final published version: http://dx.doi.org/10.1002/ijc.31014

Copyright information:
© 2017 International Agency for Research on Cancer (IARC/WHO); licensed by UICC.

This is an Open Access work distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License (http://creativecommons.org/licenses/by-nc/3.0/).
Supporting your research with our capabilities

BD Accuri™ C6 Plus Personal Flow Cytometer
BD FACSCelesta™ Cell Analyzer
BD LSRFortessa™ X-20 Cell Analyzer
BD FACSMelody™ Cell Sorter
One of the largest portfolios of reagents

Learn more>
Roadmap for Investigating Epigenome Deregulation and Environmental Origins of Cancer

Zdenko Herceg1, Akram Ghantous1*, Christopher P. Wild1, Athena Sklias1, Lavinia Casati2, Susan J. Duthie3, Rebecca Fry4, Jean-Pierre Issa5, Richard Kellermayer6, Igor Koturbash7, Yukata Kondo8, Johanna Lepeule9, Sheila C. S. Lima10, Carmen J Marsit11, Vardhman Rakhyan12, Richard Saffery13, Jack A. Taylor14, Andrew E. Teschendorff15,16, Toshikazu Ushijima17, Paolo Vineis18, Cheryl Lyn Walker19, Robert A. Waterland20, Joe Wiemels21, Srikant Ambatipudi1, Davide Degli Esposti1 and Hector Hernandez-Vargas1

1 International Agency for Research on Cancer (IARC), 150 Cours Albert-Thomson, Lyon 69008, France
2 Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy
3 School of Pharmacy and Life Sciences, The Robert Gordon University, Aberdeen, United Kingdom
4 Gillings School of Global Public Health, UNC, Chapel Hill, NC
5 Fels Institute for Cancer Research & Molecular Biology, Philadelphia, PA
6 Texas Children’s Hospital, Baylor College of Medicine, TX
7 University of Arkansas for Medical Sciences, AR
8 Aichi Cancer Center Research Institute, Nagoya, Japan
9 INSEMr, Albert Bonniot Institute, Grenoble, France
10 National Cancer Institute (INCa), Rio de Janeiro, Brazil
11 Emory University, Atlanta, GA
12 Centre for Genomics and Child Health, Blizard Institute, London, United Kingdom
13 Murdoch Childrens Research Institute, Melbourne, Australia
14 National Institute of Health, NC
15 Statistical Cancer Genomics, UCL Cancer Institute & Dept. of Woman’s Cancer, University College London, United Kingdom
16 CAS-MPG Partner Institute for Computational Biology, Shanghai Institute for Biological Sciences, Shanghai 200033, China
17 National Cancer Center Research Institute, Tokyo, Japan
18 MRC/PHE Centre for Environment and Health, School of Public Health, Imperial College London, London, UK
19 Departments of Molecular & Cellular Biology and Medicine, Baylor College of Medicine, Houston, TX
20 Baylor College of Medicine, USDA/ARS Children’s Nutrition Research Center, Houston, TX
21 UCSF School of Medicine, Epidemiology & Biostatistics, San Francisco, CA

The interaction between the (epi)genetic makeup of an individual and his/her environmental exposure record (exposome) is accepted as a determinant factor for a significant proportion of human malignancies. Recent evidence has highlighted the key role of epigenetic mechanisms in mediating gene–environment interactions and translating exposures into tumorigenesis. There is also growing evidence that epigenetic changes may be risk factor-specific (“fingerprints”) that should prove instrumental in the discovery of new biomarkers in cancer. Here, we review the state of the science of epigenetics associated with environmental

**Key words:** epigenetics, environment, cancer, molecular mechanisms, research gaps, perspectives, biomarkers

**Conflict of Interest:** The authors declare that they have no competing financial interests.

*Z.H. and A.G. contributed equally to this work

**Grant sponsor:** Institut National du Cancer (INCa, France); **Grant sponsor:** European Commission (EC) Seventh Framework Programme (FP7) Translational Cancer Research (TRANSCAN) Framework; **Grant sponsor:** Fondation ARC pour la Recherche sur le Cancer (France); **Grant sponsor:** Plan Cancer-Eva-Inserm; **Grant sponsor:** International Agency for Research on Cancer; **Grant sponsor:** Marie Curie Actions – People – Co-funding of regional, national and international programmes (COFUND); **Grant number:** EC FP7; **Grant sponsor:** Fonds National de la Recherche, Luxembourg; **Grant number:** 10100060

**DOI:** 10.1002/ijc.31014

This is an open access article distributed under the terms of the Creative Commons Attribution IGO License IARC’s preferred IGO license is the non-commercial: https://creativecommons.org/licenses/by-nc/3.0/igo/legalcode which permits non-commercial unrestricted use, distribution and reproduction in any medium, provided that the original work is properly cited. In any reproduction of this article there should not be any suggestion that IARC/WHO or the article endorse any specific organization or products. The use of the IARC/WHO logo is not permitted. This notice should be preserved along with the article’s URL.

**History:** Received 1 June 2017; Accepted 3 Aug 2017; Online 24 Aug 2017

**Correspondence to:** Zdenko Herceg, PhD, Epigenetics Group, International Agency for Research on Cancer (IARC), 150 Cours Albert

Thomas, F-69008 Lyon, France, Tel.: +33-4-72-73-8398, Fax: +33-4-72-73-8322, E-mail: herceg@iarc.fr

Int. J. Cancer: 142, 874–882 (2018) © 2017 International Agency for Research on Cancer (IARC/WHO); licensed by UICC
Epidemiological studies have uncovered robust and consistent associations between environmental factors and cancer risk. However, these associations provide little information on the mechanism by which a given exposure leads to cancer. The interaction between the (epi)genetic makeup of an individual and his/her environmental exposure record (exposome) may determine a large fraction of human malignancies.

Epigenetic disruption is a near-universal feature of human malignancy and a key driver of many cancers. In recent years, accumulating evidence has highlighted the key role of epigenetics in mediating gene–environment interactions and their effect throughout the tumorigenesis process (Fig. 1). This progress has been catalyzed by advances in the epigenomic field, including the emergence of powerful technologies and state-of-the-art in vitro and in silico computational approaches. Well-established risk factors of cancer, such as age, inflammation, diet, and smoking have been studied in the context of epigenome deregulation, along with some less widely studied exposures and lifestyle factors such as air and water pollution, fungal toxins and endocrine disruptors (Fig. 1). Notably, numerous international cohorts have been established enabling an investigation of life course exposures on epigenetic profiles in the context of large-scale epidemiological studies.

Here, critical knowledge gaps and research needs are discussed and advances in epigenomics that may help an understanding of the functional relevance of epigenetic alterations induced by environmental exposures. All co-authors of this work have met during the first Environmental and Epigenetics Origin of Cancer meeting, held at IARC, Lyon, in June 2016 and have extensively interacted during and after the meeting to concretize in this article the valuable conclusions, arguments and highlights in the field. Accordingly, this manuscript is not intended to be a meeting report as it does not merely summarize different scientific opinions nor does it represent a review of the literature. Instead, it is intended to bring forward critical questions that need to be answered, approaches and study designs that could help answering them, methodology developments that could be implemented, important findings attained so far as examples, future utilities of the field and the direction(s) toward which all these developments could steer the field.

**Risk Factors Associated With Epigenome Deregulation and Cancer**

The mechanisms by which environmental factors can have long-lasting effects on cancer outcome remain poorly understood (Fig. 1). For example, tobacco smoke has well-established effects on blood DNA methylation of newborns, children and adults, though it remains unclear how these effects contribute to tumorigenesis. In addition, nutrition was shown to affect metastable epialleles (MEs), exhibiting systemic (not cell type-specific) interindividual variation in DNA methylation; however, whether these epigenetic polymorphisms may be useful as a predictor of cancer risk remains to be tested. Associations between folic acid status, methylation and human colon cancer have been established in the prevention of malignancy, but a protective role for folic acid against carcinogenesis has recently been questioned, with increasing evidence that excessive intake of synthetic folic acid may actually increase the risk of certain human malignancies.

Environmental contaminants (such as inorganic arsenic) were shown to be associated with methylation changes in infant cord blood, suggesting the “transcription factor occupancy theory” as an underlying mechanism. Air pollution represents another epigenome disruptor; a recent meta-analysis showed that nitrous dioxide exposure during pregnancy is associated with cord blood differential DNA methylation in mitochondrial-related genes. Endocrine-disrupting chemicals (EDCs) represent another example of pollutants that may deregulate the epigenome and contribute to the development of specific malignancies, especially hormone-deregulated cancers, although mechanism remains largely undetermined.

Infection agents and chronic inflammation are also known to affect epigenetic states. For example, the maternal microbiome and the postnatal gut microbiome seem to play a role in modulating intestinal mucosal epigenetic patterning and consequent susceptibility to inflammatory bowel disease (IBD) and young-onset colorectal cancer. Another example is the epigenetic field cancerization observed in gastric cancer, where chronic inflammation induced by Helicobacter pylori is responsible for aberrant DNA methylation. In addition, oncogenic viruses such as Hepatitis B virus and Epstein–Barr virus are known to hijack the host epigenetic machinery to promote its replication and to cloak itself from the host surveillance system, but potentially leaving a recognizable epigenetic signature. The fact that infection-related cancers are often characterized by DNA methylation changes extending to noncancer adjacent tissues suggests that these alterations may be the result of a complex process involving chronic inflammation, immune response and changes in cell distribution in addition to possible direct effects of infectious agents or their mediators (e.g., viral proteins).

**Exposure Timing and Epigenome Deregulation**

In addition to the type of environmental exposure, timing also plays an important role in influencing disease risk. Embryonic life and fetal life comprise sensitive periods in the human life cycle due to the capacity for changes in cell fate during...
Embryonic development, with potentially lifelong health outcomes. Epigenetic mechanisms represent likely “mediators” of these outcomes because they are implicated in (i) pathways driving embryogenesis, including tissue differentiation, (ii) mitotically heritable mechanisms with long lasting effects, and (iii) environmentally sensitive and potentially reversible molecular drivers of disease. There is increasing evidence showing how in utero exposure leaves epigenetic marks in the fetus, and these include food contaminants such as arsenic and heavy metals, aflatoxin B1, and tobacco smoke. The influence of many of these environmental contaminants on childhood cancer has yet to be evaluated. These findings do suggest, however, that critical time points for intervention and prevention strategies may occur early in life.

In addition to the embryonic period, environmental and epigenetic influences may alter other developmental stages, such as childhood and puberty, especially in females.

**Research Gaps and Needs**

Until very recently, there was a major gap in our understanding of the “normal” epigenome and its normal variability. As the capacity to map the epigenome continues to increase, the catalog of epigenetic variations associated with adverse environmental...
exposures will undoubtedly expand.\textsuperscript{22} The specific research questions highlighted below warrant particular attention in that they remain equivocal or have not been fully addressed.

**Strengthening causal inference**

To better infer causality of epigenetic associations linking environmental exposure and cancer, several critical scientific approaches are needed (Figs. 1 and 2):

1. Establishing mechanistic causes through the use of cellular and animal models, which allow the systematic manipulation of variables (Fig. 2). Based on mouse models, an important question of epigenetic cause versus consequence is being addressed across several windows of mouse development, showing that developmental reprogramming of H3K4me3 is acutely induced by EDCs, persists across the life-course, increases responsiveness to hormones without being dependent on abnormal transcription and promotes the development of hormone-dependent tumors.\textsuperscript{13}

2. Coupling epigenetic mechanisms to other molecular players (including cross-omics). For example, epigenetic marks can be functionally annotated to gene expression data and can be associated with causality through genetic variant randomization. Epigenetic variants that are causal to cancer would likely demonstrate functional consequences on gene activity or cellular function. Optimized statistical approaches are equally important and this is demonstrated through the example on the aryl-hydrocarbon receptor repressor (AHRR) methylation, which is to date the most consistent epigenetic signature of tobacco smoking. Although cigarette smoke is the strongest exposure factor causing lung cancer, the role of AHRR methylation in the causal pathway from smoking to lung cancer (as estimated by mediation analysis\textsuperscript{23}) would require further evidence by Mendelian Randomization.\textsuperscript{24,25}
Mini Review

(3) Integrating epigenetics within well-designed epidemiological studies, particularly prospective cohort designs (Fig. 1). Cohort studies enable the identification of “driver” epigenetic alterations that occur prior to disease onset, and hence, avoid confounding by “passenger” events that are induced by the disease (reverse causality). Moreover, longitudinal cohorts that start in early life can contribute to our understanding of how the epigenome changes over critical periods throughout life, while cohort studies based on twin pairs can help disentangle the causal contribution of genetics relative to epigenetics in mediating the response to environmental cues and risk to cancer (Fig. 1). Evidence from the Peri/Postnatal Epigenetic Twins Study showed the role of both environment and genetic variation in determining neonatal epigenetic profile, with the heritability of DNA methylation profiles estimated at 15–20%. The environmental exposures per se also should be better estimated, especially given that long-term exposures cannot be measured with the same degree of accuracy as in short-term experimental studies. Another criterion in well-designed studies is their ability to reproduce observed associations in multiple cohorts and large sample sizes. The Pregnancy and Childhood Epigenetics Consortium (PACE) and Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortia provide interesting examples of the largest studies to date analyzing the effects of environmental exposure on epigenetic alterations in birth and adult cohorts, respectively.

(1) Epigenetic mechanisms in relevance to biochemical precursors of DNA methylation. Although folate, a methyl donor, has a strong impact on DNA methylation and cancer, the directionality of those effects remains questionable. For example, high folate levels may lead to both high and low DNA methylation and to both increased and decreased risk of cancer. These seemingly contradicting findings become more biologically plausible upon dissecting the effect of folate exposure by dosage, timing, target genes and cancer types. Mouse studies directly testing the effect of folic acid intake at various stages of the life course and on various tissues may be particularly important for fine tuning the intricate associations between folate exposure, epigenetics and cancer. Additionally, the influence of folate species on methylation and cancer risk remains to be established.

(2) Epigenetic mechanisms in relevance to transcriptional machinery. Although DNA hypermethylation in the promoters of many genes is generally associated with transcriptional silencing, the importance of the link between epigenetics and transcription remains an open question. CpG methylation that is not associated with RNA expression may have little functional relevance, but this is questioned by the evidence showing how developmental reprogramming involving the remodeling of chromatin marks may lead to increased responsiveness to hormones without necessarily altered transcription. It also remains to be established to what extent the link between methylation and expression is due to loss of transcription factor binding. Moreover, much remains to be learned about the functional regulation of ultralow methylation regions (ULMRs), which are methylated at 1–20% and rarely studied using traditional methodologies (J.P. Issa, unpublished data).

(3) Epigenetic mechanisms in relevance to chromatin landscape. While DNA methylation is known to be transmitted with high fidelity across cell divisions, the chromatin landscape is less characterized, and it is still unclear how a defined chromatin domain is reproduced following cell replication. Recently, the Polycomb Repressive Complex 2 has been implicated in the inheritance of histone modifications across cell divisions. However, none of the existing techniques for analysis of histone modifications is ready to use on the biospecimen types and sample sizes that are utilized in population-based studies. The advent of new technologies in chromatin biology holds promise for future studies aiming to investigate the role of chromatin in mediating effects of environmental exposures on cancer.

(4) Epigenetic mechanisms in relevance to epidrivers. The role of epidrivers (the genes involved in epigenetic regulation exhibiting recurrent disruption in cancer through mutational or nonmutational mechanisms) in carcinogenesis requires particular attention. The fact that >50% of human cancers harbor mutations in enzymes that are involved in chromatin organization argues that epidrivers may represent an early and central event in tumorigenesis. To confirm this, mechanistic studies of epidrivers altered by specific carcinogenic agents should be considered using in vitro human and mouse models and state-of-the-art approaches (epigenome-wide shRNA or CRISPR library screens, epigenome editing, and functional genomics). Characterization of epidriver events is expected to advance the knowledge of mechanisms of carcinogenesis and underpin studies of cancer etiology, therapy and prevention.

Analytical considerations

In population-based epigenome-wide studies, the generation, analysis and interpretation of data are not straightforward. Several studies demonstrated the robustness of wet lab and bioinformatics pipelines and capacity to perform epigenome analysis in high throughput and genome-wide settings; however, there is a lack of consensus on the pertinent optimal analytical approaches. While recent studies of epigenome-wide changes associated with some known risk factors used a GWAS-like strategy that treats individual CpG sites independently, there is wide recognition that more advanced approaches (including CpG regional clusters) may be more informative.

Epigenetic reversibility, effect size and rate of change. Several studies highlight the reversibility or lifetime persistence of some specific epigenetic changes associated with environmental exposures (Fig. 1). This seems to depend on multiple factors, including the type of epigenetic signatures (some CpGs remain methylated for longer periods than others, given the same exposure), the level and duration of the exposure, the tissue type, and the developmental stage (in utero life or puberty are sensitive periods to exposure and can be prone to epigenetic alterations with long-term effects). More studies and cohorts with repeated time points are needed to enhance
the resolution of the epigenetic snapshots taken at different developmental stages of life (Fig. 1). Such study designs can also enable the assessment of the “rate” of change (and not only the effect size) of DNA methylation in response to exposure over time. These studies may also need to consider if the reversal of the change leads to reversal of risk, as specific epigenetic events during a critical developmental period could initiate a program which later in life could be important, regardless of the continued presence or absence of that initiator (a “hit and run” effect).

**Cell type heterogeneity.** This remains a major concern in epigenetic studies, the deconvolution of which becomes more intricate in tumor tissues, which exhibit both clonal and genetic intratumor heterogeneity.\(^{35}\) For example, head and neck squamous cell carcinomas exhibit extensive heterogeneity in etiological, environmental, cellular and molecular features, hampering accurate prognosis, treatment planning and identification of causative genes that may serve as molecular drug targets.\(^{36–38}\) Recent advances in bioinformatics have helped correct for possible changes in cell subpopulations using DNA methylome-based prediction algorithms that rely on reference tissues (initially using peripheral blood\(^{39}\) and recently cord blood\(^{40}\)) and reference-free methods (a recent but rapidly developing field.\(^{41,42}\) Emerging methods for single-cell epigenomics should also provide exciting tools for resolving the issues related to the variability of the epigenome among different cells and cell clones in complex tissues.\(^{43,44}\)

**Target tissue.** Epigenetic changes are abundant and directly measureable in tumor biopsies, especially when compared with adjacent tissue. However, aberrant DNA methylation has also been observed in surrogate tissues such as peripheral blood of cancer patients. Epigenetic alterations can arise in early stages of embryonic development, when epigenetic patterns undergo large-scale reprogramming (Fig. 3), and, hence, may be propagated in most, if not all, tissues, thereby generating identical constitutional epimutations throughout the body\(^7\) or creating a mosaic pattern of the epigenome in a given organ.\(^{45}\) In this scenario, the timing of the epigenetic event and proliferation history of the affected cells will determine the proportion and distribution of the cells harboring...
epimutations across different tissues (Fig. 3). These considerations provide the basis for developing epigenetic biomarkers in blood, which can serve as a surrogate for diagnostics and risk stratification of cancer in other tissues. For example, methylation of \textit{SEPT9} has been shown to be a reliable and sensitive blood-based biomarker for colorectal cancer detection.\textsuperscript{46}

Besides blood and urine samples, additional body fluids and different types of biospecimens collected through noninvasive or minimally invasive techniques, such as buccal swabs, breast lavage and cervical smears, may provide attractive targets for the discovery of biomarkers of exposure or early detection of cancer.

**Early-life exposures**

As described earlier, “windows of vulnerability” exist during \textit{in utero} development, within which maternal exposure factors may alter the fetal epigenome, increasing susceptibility to later-onset diseases, including cancer.\textsuperscript{47} A recent example illustrates the complex association between \textit{in utero} exposure to tobacco smoke and childhood cancer. A study of neonatal blood spots showed that DNA methylation at birth was altered in association with early pregnancy maternal folate status.\textsuperscript{48} DNA methylation marks of smoking demonstrated a difference between cases and controls (J. Wiemels, unpublished data), consistent with the interaction between maternal smoking in cancer risk in the offspring.\textsuperscript{49} International collaboration on such a rare disease (to assimilate large samples and replicate findings in multiple cohorts) may help decipher this complex exposure-to-phenotype pattern.

**Epigenetic clock and cancer risk**

One of the best-characterized DNA methylation signatures in population-based studies is chronological age. Age-associated epigenetic changes have been identified and provide the basis for an “epigenetic clock.”\textsuperscript{50} Age is the strongest demographic risk factor for cancer, indicating that molecular changes upon aging trigger malignant transformation.\textsuperscript{51} DNA methylation clock may be affected by different external and endogenous factors. Those exposures may contribute to methylation drift\textsuperscript{52} and “accelerated” aging, emphasizing that the often ignored rate of change in methylation can be important even though the magnitude of methylation differences might be minimal. As DNA methylation landscape is altered as a function of age (independently of exposures), there is a need to explore synergistic epigenetic effects between age and environmental exposures. For instance, DNA methylation profiling in a large prospective cohort revealed an association between the epigenetic age acceleration and breast cancer risk,\textsuperscript{53} although further studies are needed to establish the synergy between exposure and age. Importantly, age-associated epigenetic silencing of \textit{HAND2} seems to be an early event in endometrial carcinogenesis, leading to gradual inactivation of the progesterone tumor suppressor pathway and sensitizing endometrial epithelial cells to oncogenic estrogen.\textsuperscript{54} Therefore, this may serve as a paradigm for aging-associated epigenetic changes sensitizing (priming) the cells for subsequent exposure to oncogenic stimuli. Further studies are needed to test the presence of synergistic age-exposure mediating effects on the DNA methylome and cancer risk.

**Toward incorporating epigenetic data into carcinogen identification and evaluation**

Recent advances in epigenetics represent an exciting opportunity toward the incorporation of epigenetic mechanisms into carcinogen evaluation and safety assessment (Fig. 2). In spite of recent data on epigenetic mechanisms as biological mediators of certain exposures (such as EDCs discussed above), evidence for a causal role of epigenetic changes in carcinogenesis is limited. Although the incorporation of epigenetic mechanisms into carcinogen evaluation is at an early stage,\textsuperscript{21,55} important data have been generated, and valuable scientific resources could be applied in the main international programs of carcinogen evaluation (such as the IARC Monographs Programs and National Toxicology Programs in the US). There will be value in designing integrated approaches aiming to interrogate all layers of the epigenome in response to carcinogen exposure in populations followed by validation in population-based studies and functional characterization in \textit{in vitro} model systems (Fig. 2). There is an urgent need to develop epigenetic assays that incorporate scientifically sound experimental designs with particular consideration for dose and route of exposure. Identifying a set of priority carcinogens to be studied in detail will be an important start. We propose that particular attention should be paid to potential “epigenetic carcinogens” (such as those classified by IARC as probably carcinogenic or possibly carcinogenic to humans [Groups 2 A and 2B] that seem to act through nonmutational mechanisms), as opposed to established mutagens.

**Conclusions**

Remarkable progress in the field of epigenetics provides a better understanding of the etiology of human cancers and suggests a potential causal role for epigenetic disruptions linking environmental exposure to tumorigenesis. The emergence of powerful sequencing technologies has enabled the analysis of the epigenome with high resolution in both genome-wide and high-throughput settings, thus dramatically accelerating investigations in cancer biology and molecular epidemiology. Major international efforts have brought about critical advances, with the establishment of reference epigenomes for many normal cell types and cancer-specific epigenomes for several tumor types. Recent studies contributed to the identification of epigenetic events deregulated by specific environmental and lifestyle stressors, supporting the hypothesis that the epigenome may function as an interface between environmental factors and the genome. Importantly, many studies provided evidence that environmental exposures can induce specific changes in the epigenome. Such epigenetic “fingerprints” will
prove instrumental in carcinogen evaluation and identification and in the discovery of new biomarkers for risk stratification and novel interventions for prevention.

Acknowledgements

This article was instigated by the "Epigenetics and Environmental Origins of Cancer" (EEOC) meeting which was held with 140 international participants at the International Agency for Research on Cancer (IARC, Lyon, France) on the 11th and 12th of June, 2016. Presentations from leading scientists in the field were grouped thematically around the following topics: Environmental Agents and Lifestyles, Nutrition and Metabolism, Endocrine Disruptors, Carcinogen Evaluation, Early Life Exposures, and Infections and the Microbiome. The meeting highlighted important research advances, needs and gaps in the field, including critical assessments and interdisciplinary approaches. The EEOC was organized by the Epigenetics Group, IARC, Lyon, and supported by grants from the Institut National du Cancer (INCa, France), the European Commission (EC) Seventh Framework Programme (FP7) Translational Cancer Research (TRANSCAN) Framework, the Fondation ARC pour la Recherche sur le Cancer (France), and Plan Cancer-Eva-Inserm research grant to Z.H. S.A. was supported by the postdoctoral fellowship from the International Agency for Research on Cancer, partially supported by the EC FP7 Marie Curie Actions – People – Co-funding of regional, national and international programmes (COFUND). A.S. is supported by the PhD fellowship from the Fonds National de la Recherche, Luxembourg (AFR Code: 1000060). Special thanks to Ms Elizabeth Page for her help in organizing the conference, to Epigenie, represented by Dr Angelika Merkel, for covering the meeting and to the members of the Section of Mechanisms of Carcinogenesis at IARC: Dr Szilvia Ecsedi, Dr Nora Fernandez Jimenez, Ms Hana Huskova, Ms Diana Maria Narvaez Noguera, Dr Vibha Patil, Dr Fadlu Rahman Talukdar, Dr Hae DON Woo and Ms Maria Zhivagui for their help during the conference.

References

1. Wild CP. The exposome: from concept to utility. Int J Epidemiol 2012;41:24–32.
2. Jones AP. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet 2012;13:484–92.
3. Feil R, Fraga MF. Epigenetics and the environment: emerging patterns and implications. Nat Rev Genet 2011;11:397–109.
4. Ng JW, Barrett LM, Wong A, et al. The role of DNA methylation in cancer. Cancer Epidemiol Biomarkers Prev 2015;24:1348–52.
5. Ambatipudi S, Cuenin C, Hernandez-Vargas H, et al. Tobacco smoking-associated genome-wide DNA methylation changes in the EPIC study. Epigenomics 2016.
6. Silver JM, Kessler JR, Henning JB, et al. Independent genome-wide screens identify the tumor suppressor TTNRA2 as a human epistle responsive to periconceptional environment. Genome Biol 2015;16.
7. Duthe JS, Folate and cancer: how DNA damage, repair and methylation impact on colon carcinogenesis. J Inherit Metab Dis 2011;34:101–9.
8. Cole BF, Baron JA, Sandler RS, et al. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. JAMA 2007;297:2351–9.
9. Laine EJ, Bailey AK, Rubino-Andrade M, et al. Maternal arsenic exposure, arsenic methylation efficiency, and birth outcomes in the Biomarkers of Exposure to Arsenic (BEAR) pregnancy cohort in Mexico. Environ Health Perspect 2015;123:186–92.
10. Martin EM, Fry RC. A cross-study analysis of prenatal exposures to environmental contaminants and the epigenome: support for stress-responsive transcription factor occupancy as a mediator of gene-specific CpG methylation patterns. Environ Epigenet 2016;2.
11. Gravina O, Xu CJ, Breton CV, et al. Epigenome-wide meta-analysis of methylation in children related to prenatal NO2 air pollution exposure. Environ Health Perspect 2016.
12. Wang Q, Trevino SL, Lee Yean Wong R, et al. Reprogramming of the epigenome by MLIL links early-life environmental exposures to prostate cancer risk. Mol Endocrinol (Baltimore, Md) 2016; me20151310-me.
13. Gruzieva O, Xu CJ, Breton CV, et al. Epigenome-wide meta-analysis of methylation in children related to prenatal NO2 air pollution exposure. Environ Health Perspect 2016.
14. Fleisch FA, Wright OR, Baccarelli AA. Environmental epigenetics: a role in endocrine disease? J Mol Endocrinol 2012;49:R61–R7.
15. Shah R, Cope JL, Naga-Szakal D, et al. Composition and function of the pediatric colonic mucosal microbiome in untreated patients with ulcerative colitis. Gut Microbes 2016; 1–13.
16. Tahara T, Yamanoto E, Madireddi P, et al. Colorectal carcinomas with CpG island methylator phenotype 1 frequently contain mutations in chromatin regulators. Gastroenterology 2014;146:530–46.
17. Hattori N, Ushijima T. Epigenetic impact of infection on carcinogenic mechanisms and applications. Genome Med 2016;8:10.
18. Herceg Z, Palwai A. Epigenetic mechanisms in hepatocellular carcinoma: how environmental factors influence the epigenome. Mutat Res 2011; 727:55–61.
19. Green BB, Karagas MR, Punshon T, et al. Epigenome-wide assessment of DNA methylation in the placenta and arsenic exposure in the New Hampshire Birth Cohort Study (USA). Environ Health Perspect 2016;124:1253–60.
20. Hernandez-Vargas H, Castelino J, Silver JM, et al. Exposure to aflatoxin B1 in utero is associated with DNA methylation in white blood cells of infants in The Gambia. Int J Epidemiol 2015.
21. Herceg Z, Lambert M-P, van Veldhoven K, et al. Towards incorporating epigenetic mechanisms into carcinogen identification and evaluation. Carcinogenesis 2013;34:1955–67.
22. Busche S, Shao X, Caron M, et al. Population-wide genome bisulitide sequencing across two tissues highlights the environment as the principal source of human methylyation variation. Genome Biol 2015;16:290.
23. Pasanelli F, Baglioni L, Ponzi E, et al. Hypomethylation of smoking-related genes is associated with future lung cancer in four prospective cohorts. Nat Commun 2015:6.
24. Richmond RC, Hemeni G, Tilling K, et al. Challenges and novel approaches for investigating molecular mediation. Hum Mol Genet 2016;25:R149–R56.
25. Latvala A, Olliikainen M. Mendelian randomization in (epi)genetic epidemiology: an effective tool to be handled with care. Genome Biol 2016; 17:58.
26. Gordon L, Joo JE, Powell JE, et al. Neonatal DNA methylation profile in human twins is specified by a complex interplay between intrauterine environmental and genetic factors, subject to tissue-specific influence. Genome Res 2012;22:1395–406.
27. Ziller MJ, Go H, Muller F, et al. Charting a dynamic DNA methylation landscape of the human genome. Nature 2013;500:477–81.
28. Plass C, Pfister SM, Lindroth AM, et al. Mutations in the epigenome and their connections to global chromatin patterns in cancer. Nat Rev Genet 2013;14:765–80.
29. Vogelstein B, Papadopoulos N, Velculescu VE, et al. Cancer genome landscapes. Science 2013; 339:1546–58.
30. Gonzalez-Perez A, Jene-Sanz A, Lopez-Bigas N. The mutational landscape of chromatin regulatory factors across 4,623 tumor samples. Genome Biol 2013;14:kr106.
31. Jones PA, Issa JP, Baylin S. Targeting the epigenome for therapy. Nat Rev Genet 2016;17: 630–41.
32. Breton CV, Marist J, Faustman E, et al. Small-magnitude effect sizes in epigenetic end points are important in Children's Environmental Health Studies: the Children's Environmental Health and Disease Prevention Research Center's epigenetics working group. Environ Health Perspect 2017;125:511–26.
33. Mill J, Heijmans BT. From promises to practical strategies in epigenomic epidemiology. Nat Rev Genet 2013;14:585–94.
34. Gerlinger M, Rowan AJ, Horswell S, et al. Intra-tumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 2012;366:883–92.
35. Rothenberg MS, Ellisien WL. The molecular pathogenesis of head and neck squamous cell carcinoma. J Clin Invest 2012;122:1951–7.
36. Lima SC, Hernandez-Vargas H, Simao T, et al. Identification of a DNA methylyome signature of esophageal squamous cell carcinoma and potential epigenetic biomarkers. Epigenetics 2011;6: 1217–27.
37. Leemans RC, Braakhuys MBJ, Brakenhoff HR. The molecular biology of head and neck cancer. Nat Rev Cancer 2011;11:9–22.
38. Houseman AE, Acardiino PW, Koestler CD, et al. DNA methylation arrays as surrogate
measures of cell mixture distribution. *BMC Bioinformatics* 2012;13.

40. Bakulski KM, Feinberg JI, Andrews SV, et al. DNA methylation of cord blood cell types: applications for mixed cell birth studies. *Epigenetics* 2016;11:354–62.

41. Houseman EA, Molitor J, Marsit CJ. Reference-free cell mixture adjustments in analysis of DNA methylation data. *Bioinformatics* 2014;30:1431–9.

42. Houseman EA, Kile ML, Christiani DC, et al. Reference-free deconvolution of DNA methylation data and mediation by cell composition effects. *BMC Bioinformatics* 2016;17:259.

43. Clark SJ, Lee HJ, Smallwood SA, et al. Single-cell epigenomics: powerful new methods for understanding gene regulation and cell identity. *Genome Biol* 2016;17:72.

44. Herceg Z, Hernandez-Vargas H. New concepts of old epigenetic phenomena and their implications for selecting specific cell populations for epigenomic research. *Epigenomics* 2011;12:383–6.

45. Hitchins MP. Constitutional epimutation as a mechanism for cancer causality and heritability? *Nat Rev Cancer* 2015;15:625–34.

46. Warren JD, Xiong W, Bunker AM, et al. Septin 9 methylated DNA is a sensitive and specific blood test for colorectal cancer. *BMC Med* 2011;9:133.

47. Ghantous A, Hernandez-Vargas H, Byrnes G, et al. Characterising the epigenome as a key component of the fetal exposome in evaluating in utero exposures and childhood cancer risk. *Mutations* 2015;30:733–42.

48. Gomjeth S, Roy R, Houseman EA, et al. Periconceptional folic acid consumption is associated with neonatal DNA methylation modifications in neural crest regulatory and cancer development genes. *Epigenetics* 2015;10:1166–76.

49. Stjernfeldt M, Berglund K, Lindsten J, et al. Maternal smoking during pregnancy and risk of childhood cancer. *Lancet* 1986;1:1350–2.

50. Horvath S. DNA methylation age of human tissues and cell types. *Genome Biol* 2013;14:R115.

51. Lin Q, Wagner W. Epigenetic aging signatures are coherently modified in cancer. *PLoS Genet* 2015;11.

52. Issa JP. Aging and epigenetic drift: a vicious cycle. *J Clin Invest* 2014;124:24–9.

53. Ambatipudi S, Horvath S, Perrier F, et al. DNA methylation analysis identifies accelerated epigenetic aging associated with postmenopausal breast cancer susceptibility. *Eur J Cancer* 2017;75:299–307.

54. Jones A, Teschendorff AE, Li Q, et al. Role of DNA methylation and epigenetic silencing of HAND2 in endometrial cancer development. *PLoS Med* 2013;10:e1001551.

55. Smith TM, Guyton ZK, Gibbons FC, et al. Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. *Environ Health Perspect* 2016;124:713–21.