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Roadmap for Investigating Epigenome Deregulation and Environmental Origins of Cancer

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Conflict of Interest

The authors declare that they have no competing financial interests.

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Abstract

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 stimuli and cancer risk, highlighting key developments in the field. Critical knowledge gaps and research needs are discussed and advances in epigenomics that may help in understanding the functional relevance of epigenetic alterations. Key elements required for causality inferences linking epigenetic changes to exposure and cancer are discussed and how these alterations can be incorporated in carcinogen evaluation and in understanding mechanisms underlying epigenome deregulation by the environment.

Keywords

epigenetics; environment; cancer; molecular mechanisms; research gaps; perspectives; biomarkers

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)[1] 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.[2] In recent years, accumulating evidence has highlighted the key role of epigenetics in mediating gene–environment interactions and their effect throughout the tumorigenesis process[3] (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.[4]

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,[5,6] 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[7]; however, whether these epigenetic polymorphisms may be useful as a predictor of cancer risk remains to be tested. Associations between folate status, methylation and human colon cancer have been established in the prevention of malignancy,[8] but a protective role for folate 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.[9]

Environmental contaminants (such as inorganic arsenic) were shown to be associated with methylation changes in infant cord blood,[10] suggesting the “transcription factor occupancy theory” as an underlying mechanism.[11] 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 mitochondrialrelated genes.[12] Endocrine-disrupting chemicals (EDCs) represent another example of pollutants that may deregulate the epigenome[13] and contribute to the development of specific malignancies, especially hormone-deregulated cancers, although mechanism remains largely undetermined.[14]

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.[15,16] Another example

is the epigenetic field cancerization observed in gastric cancer, where chronic inflammation induced by *Helicobacter pylori* is responsible for aberrant DNA methylation.[17] 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.[18] 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,[19] aflatoxin B1[20] and tobacco smoke.[5] 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. In males, spermatogenesis starts at puberty and continues throughout life; whereas, in females, oogenesis begins before birth and is arrested in the prophase of meiosis until puberty. Hence, in girls, oocytes remain until puberty in a haploid demethylated state, which is more susceptible to environmental stressors than the diploid methylated state of the male germline. Later during adulthood, women may exhibit other susceptible windows of exposure during the menstrual cycle, pregnancy or menopause. These timing windows of exposures must be considered when analyzing the interaction between the environment, epigenetics and cancer.

Research Gaps and Needs

Until very recently, there was a major gap in our understanding of the "normal" epigenome and its normal variability.[21] As the capacity to map the epigenome continues to increase, the catalog of epigenetic variations associated with adverse environmental exposures will undoubtedly expand.[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)

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.[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[23]) would require further evidence by Mendelian Randomization.[24,25]

(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%.[26] 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.[13] It also remains to be established to what extent the link between methylation and expression is due to loss of transcription factor binding.[27] 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.[28] However, none of the existing techniques for analysis of histone modifications is ready to use on the biospecimen types and sample size scales 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.[29–31] The fact that >50% of human cancers harbor mutations in enzymes that are involved in chromatin organization[32] 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.[33,34] 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.[34]

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 SEPT9 has been shown to be a reliable and sensitive blood-based biomarkes for colorectal cancer detection.[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 *in utero* development, within which maternal exposure factors may alter the fetal epigenome, increasing susceptibility to later-onset diseases, including cancer.[47] A recent example illustrates the complex association between *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.[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.[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."[50] Age is the strongest demographic risk factor for cancer, indicating that molecular changes upon aging trigger malignant transformation.[51] DNA methylation clock may be affected by different external and endogenous factors. Those exposures may contribute to methylation drift[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,[53] although further studies are needed to establish the synergy between exposure and age. Importantly, age-associated epigenetic silencing of 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.[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,[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 Mono graphs 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 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 are 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.

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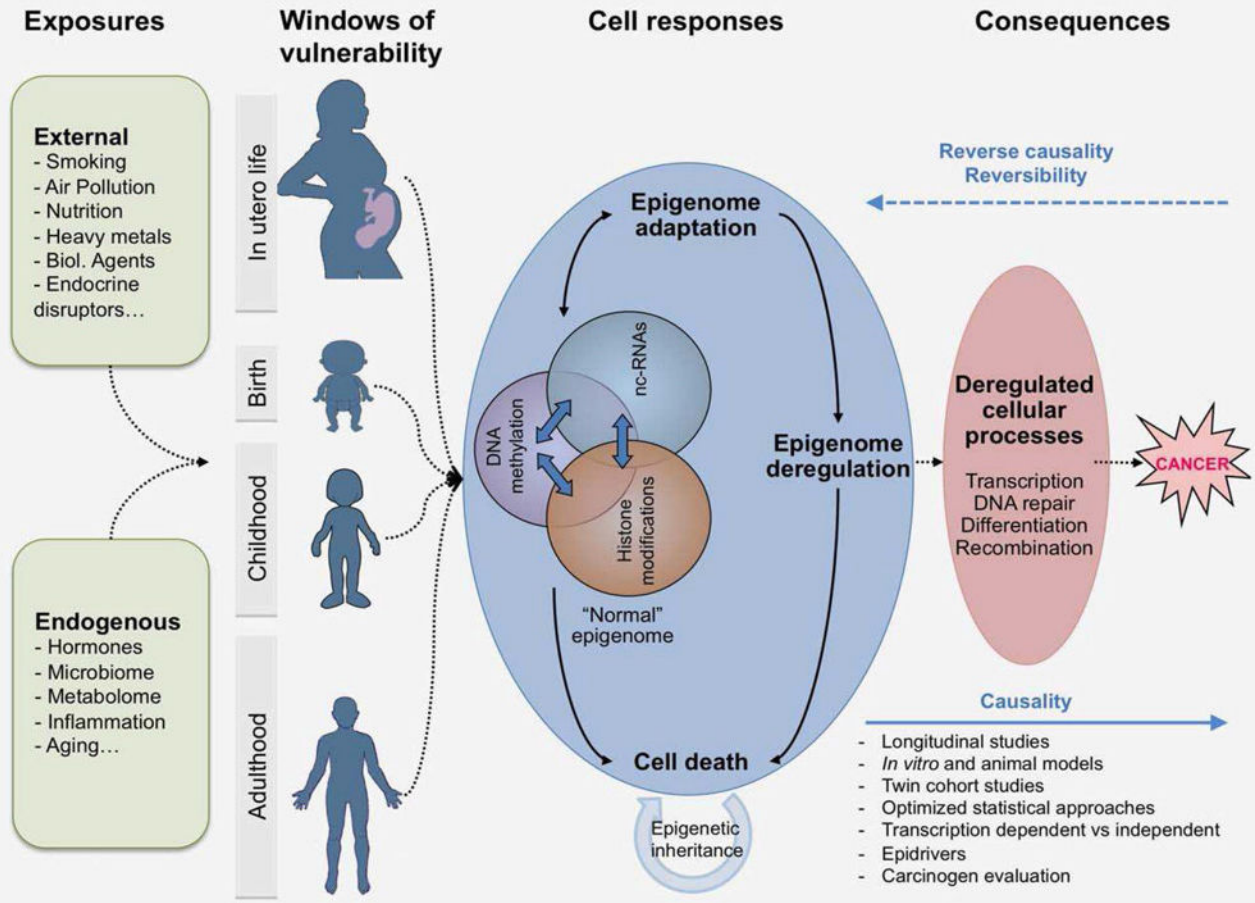

Figure 1.

Exposures arising from external sources (environmental chemicals, air pollution, infectious agents, diet, tobacco, alcohol, endocrine disruptors) and internal processes (metabolism, hormones, inflammation, gut microflora, aging) may induce stable and potentially reversible changes in the epigenome. The patterns ("signatures") and persistence of these alterations depend on multiple factors, including the type of epigenetic changes (some genomic regions remain methylated for longer periods than others), the dosage and duration of the exposure (longer and more intense exposures could minimize reversibility of DNA methylation), the tissue type and the developmental stage (*in utero* life or puberty may be particularly sensitive periods to some exposure). Thus, epigenetic mechanisms may represent "sensors" of exposure and "mediators" of the outcomes, including cancer development. Epigenome alterations should prove instrumental in discovery of new biomarkers for risk stratification and early detection and attractive targets for novel therapies and preventive strategies.

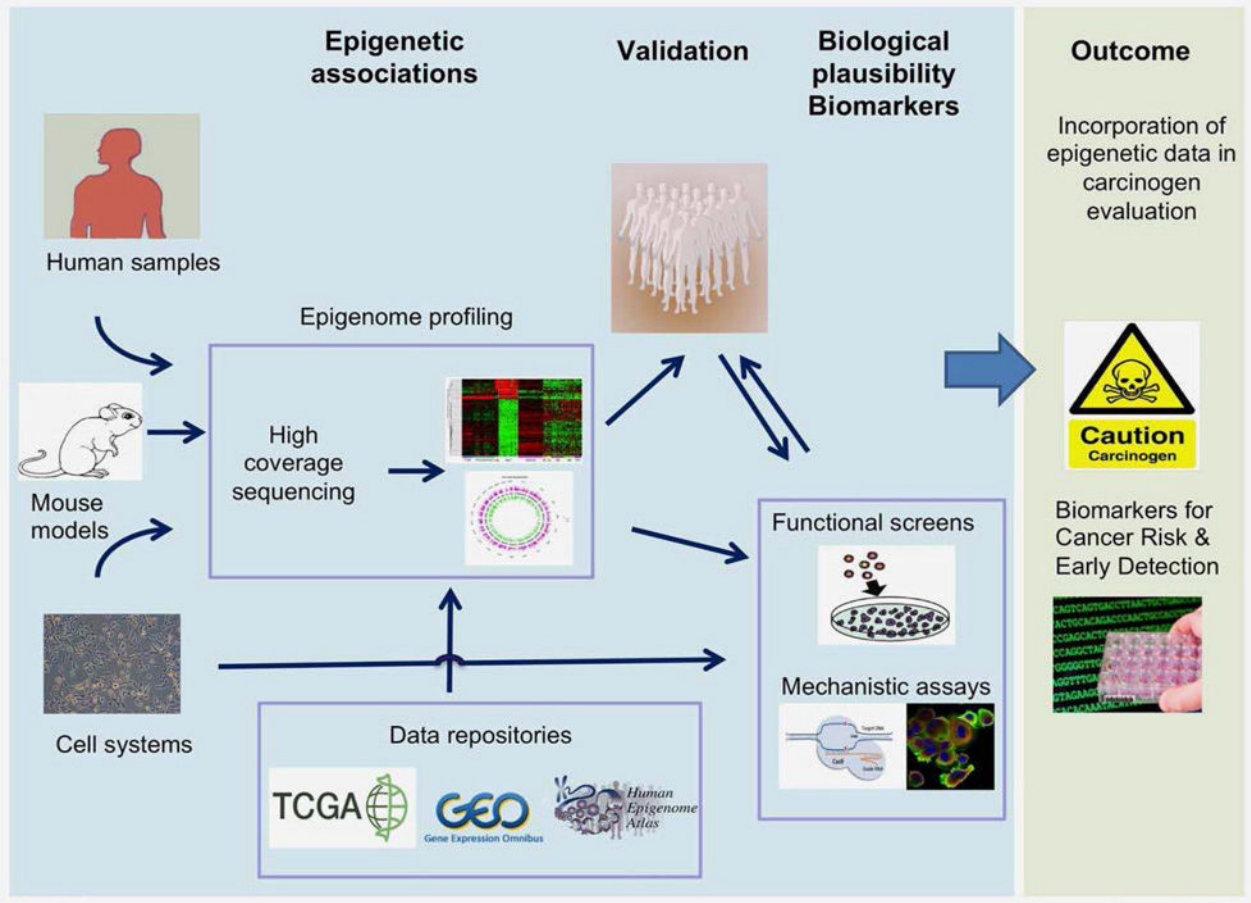

Figure 2.

An integrated approach for the production and integration of epigenetic data in carcinogen identification and evaluation. This approach implies the use of cutting edge epigenomics, population-based cohorts, and innovative bioinformatics tools for the identification, quantification, mapping of changes in the epigenome induced by known and suspected carcinogens. Human tumor samples from case– control and population-based cohorts are used in combination with *in vitro* cell systems and mouse models to perform epigenomic profiling to identify signatures, genes and pathways that are deregulated by specific risk exposure. This is followed by validation in population-based cohorts and where appropriate the data are crossed with the epigenomic databases. Identification of genes and pathways is followed by functional studies to provide biological plausibility to associations that are observed. The outcome is providing evidence base for studies directly relevant to cancer causation and prevention and identification of markers for early detection and cancer risk stratification.

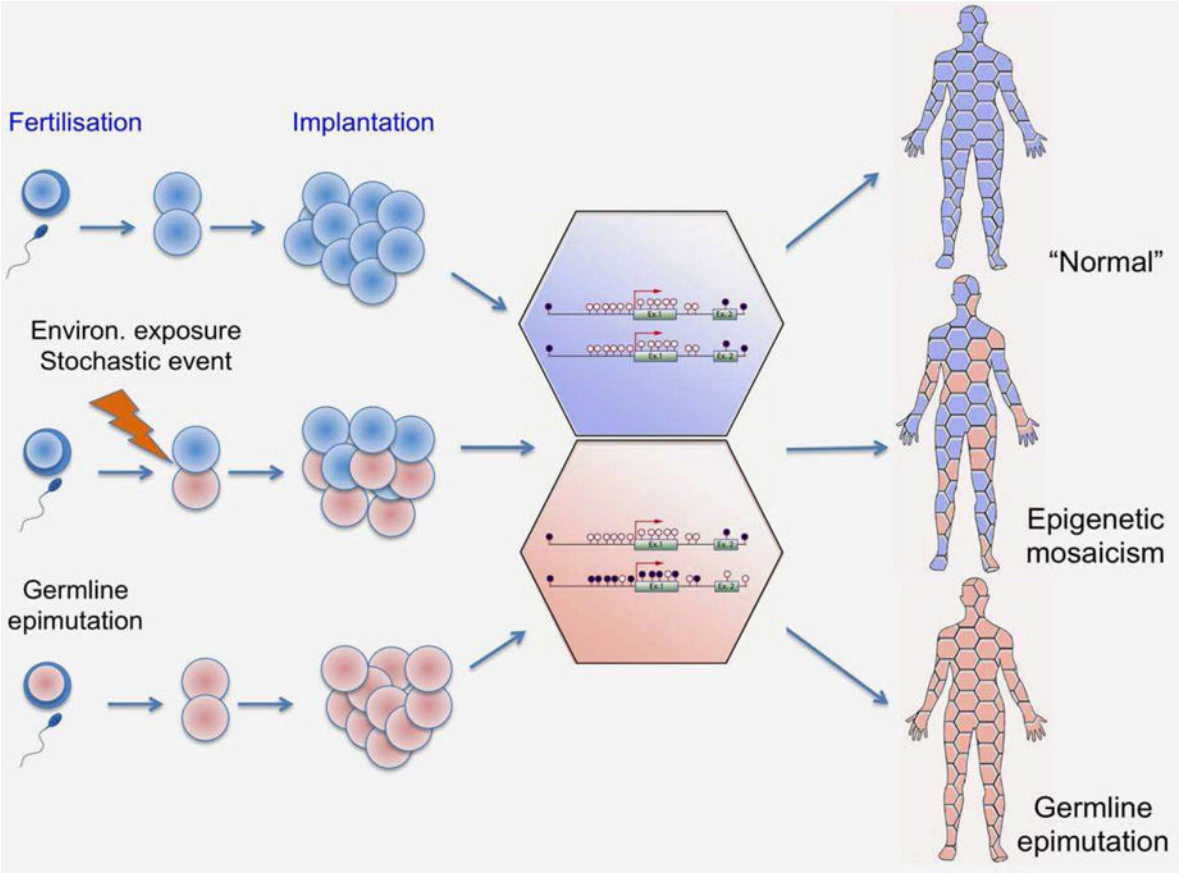

Figure 3.

Constitutional epimutations and epigenetic mosaicism as a mechanism of cancer causality and targets for biomarker discovery. Although epigenetic patterns are tissue specific, interrogating the epigenome in tissues that are not the target tissue (surrogates) may be informative of exposure history and cancer risk. Environmental exposure, stochastic event, or even germline epimutation may be propagated over life course and result in epigenetic mosaicism or germline epimutations across tissues which may constitute an increased susceptibility to cancer.