**Epigenetic Epidemiology for Cancer Risk**

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**ABSTRACT**

Epigenetic epidemiology is the study of epigenetic traits in populations and their influence upon disease risk. The epigenome is modifiable through environmental exposures, such as air pollution, tobacco smoke and diet, and epigenetic dysregulation of tumor-suppressor genes such as mutL homolog 1 (*MLH1)* and breast cancer 1 (*BRCA1)* have been identified in healthy individuals that are associated with increased cancer risk. Through the study of these aberrant epigenetic marks and the effect of environmental exposures, epigenetic epidemiology can bring significant insight into the mechanisms of carcinogenesis and the risk of disease. In this chapter, the design and selection of appropriate tissues for studies will be addressed. Evidence for epigenetic changes in response to environmental exposures and for the presence of ‘epimutations’ in oncogenes and tumor-suppressor genes will be reviewed. In addition, novel approaches to integrated analysis of the epigenome, such as the ‘epigenetic clock’ model of aging, will be discussed and the future directions of the field will be commented upon.

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**1. INTRODUCTION**

Epigenetic epidemiology concerns the study of epigenetic traits in populations and how they may influence disease risk. Epigenetic marks, such as DNA methylation and histone modifications, are modifiable through environmental exposures and may lead to aberrant expression of genes that drive carcinogenesis. By gaining a greater understanding of environmental influences upon the epigenome and the associations between aberrant epigenetic marks and the initiation of disease, this field of study can aid in primary prevention by identifying risk factors and effective methods of intervention, and can serve to identify biomarkers of early disease that can improve treatment outcomes.

Disruption of the epigenome is an early event in carcinogenesis that can be observed prior to the detection of tumors (1). Furthermore, many carcinogenic compounds have been shown to impact upon global or gene-specific DNA methylation patterns in healthy tissues. Therefore, epigenetic epidemiology is particularly well suited to the study of cancer risk. Therapies that target regulation of epigenetic marks, such as the DNA methyltransferase inhibitor, azacitidine (2), have wide-ranging effects and are therefore not suitable in prevention, but there is evidence for dietary intervention being able to counteract the harmful effects of some environmental exposures upon the epigenome.

In this chapter, the appropriate study design and the suitability of blood for epigenetic analysis will be discussed. Data from studies of the influence of environmental and early-life exposures upon the epigenome and evidence for the epigenetic dysregulation of oncogenes and tumor-suppressor genes in early carcinogenesis will be presented. The predominant focus of the chapter will be on changes in DNA methylation, due to their more extensive study and their ready utility as biomarkers that can be applied to the clinic.

**2. STUDY DESIGN**

Epigenetic epidemiology studies aiming to identify biomarkers of cancer risk or early changes associated with disease development must carefully consider the study design. Prospective studies remain the gold standard for the identification of aberrant epigenetic traits present prior to diagnosis that may confer disease risk. The epigenome is substantially reshaped in cancer, commonly characterized by global hypomethylation and promoter hypermethylation, which inhibits the identification of events associated with carcinogenesis within tumor samples. Indeed, a common theme in the study of cancer biology is the need to distinguish between ‘drivers’ and ‘passengers’; that is, genetic or epigenetic changes that have a functional role in driving the initiation or progression of the tumor, rather than being a result of the disease. Retrospective studies cannot readily establish causality, and therefore prospective studies are highly preferable for this purpose. However, such studies require substantially more expense and effort. Cohorts must be large enough to obtain a sufficient number of cancer cases to provide the statistical power to identify aberrant epigenetic traits that may be small in effect size. Such studies also require extended follow-up to fully identify disease incidence, and therefore require more time to perform than retrospective case-control studies.

The effects of environmental exposures can frequently be observed near instantaneously, and functional mechanisms can be studied through *in vitro* systems. However, their associations with disease incidence are more complex to truly establish. Prospective studies can again provide insight into how exposures can influence the epigenome and modify cancer risk, but consideration must be paid to the relative timing of exposures, such as the stage of development or lifecycle. As will be described later, diet and carcinogenic compounds have been shown to have differential effects upon the fetus according to the trimester of exposure (3). Furthermore, the effects of such exposures may be observed at different timepoints, as exemplified by hypomethylation of the nucleosome-binding protein 1 gene (*Nsbp1)* being immediately detectable following pre-natal exposure of rats to bisphenol A while hypomethylation of the phosphodiesterase type 4 variant 4 gene (*Pde4d4)* is not seen until the rats reach sexual maturation (4). Therefore, identifying environmental agents that influence cancer risk requires careful consideration of how and when they might act.

Longitudinal studies may be required to establish the stability of epigenetic traits. The epigenome readily responds to environmental influence, and when exposures are removed the epigenetic marks may revert to their previous state, unlike genetic aberrations. This is demonstrated by methylation of the aryl hydrocarbon receptor repressor gene (*AHRR)*, a biomarker of exposure to tobacco smoke, reverting to levels seen in non-smokers following the cessation of smoking (5). The plasticity of the epigenome makes it an excellent candidate for the study of cancer risk, but this property also serves to complicate the relationship between exposures, epigenetic marks and disease risk.

**3. TISSUE-SPECIFICITY OF EPIGENETIC MARKS**

An important consideration when describing advancements in this field is the suitability of the analyzed tissue. Many epidemiological studies utilize blood samples due to their ready availability, but early epigenetic changes in carcinogenesis are frequently confined to the tissue of tumor origin and therefore cannot be observed in blood. Furthermore, DNA methylation patterns are tissue-specific (6) and so results cannot necessarily be extrapolated from one tissue to another. However, this does not preclude the use of surrogate tissues, such as blood.

Recent advances in the field have sought to address the heterogeneity of cell types in the blood, using epigenetic profiling to estimate their proportions and to adjust measured methylation values accordingly (7). Indeed, for epigenetic studies blood should not be perceived as a single tissue, but rather a heterogeneous mix of cells, each with their own epigenetic profile. However, this diversity can be utilized in the study of environmental exposures and carcinogenesis. Tumors generate a distinct immune response, with an enrichment of regulatory T cells in the tumor microenvironment. Koestler and colleagues (8) demonstrated that 50 leukocyte differentially methylated regions (DMRs) can be used to distinguish between blood samples from solid tumor patients (head and neck squamous cell carcinoma, ovarian cancer, and bladder cancer) and healthy controls. The authors identified eight DMRs that could distinguish between cases and controls for each of the three solid tumors, and these were enriched for genes implicated in immune function. This work demonstrates the possible utility of analyzing epigenetic profiles in blood for the study of non-hematological malignancies. Work conducted within the longitudinal Normative Aging Study identified hypermethylation of the interferon-gamma (*IFN)* and intercellular adhesion molecule-1 (*ICAM-1*) genes as significantly associated with a 43 - 49% increase in the risk of prostate cancer, with *ICAM-1* hypermethylation detectable more than ten years prior to diagnosis (9). Similar to eight DMRs identified by Koestler and colleagues, epigenetic silencing of the interferon gamma (*IFNG)* and (*ICAM-1)* genes is associated with immunosuppression and a weakening of the immune response against the tumor (10). Studies conducted using blood samples can therefore bring insight into the biology of carcinogenesis outside the cell of origin.

**4. ENVIRONMENTAL EXPOSURES**

The epigenome is modifiable through environmental influence, including exposures to air pollutants and diet. By studying the effect of these agents upon the epigenome, it is possible to gain insight into the mechanisms of carcinogenesis and potentially identify markers of disease risk. However, as will be demonstrated, consideration must be paid not only to the type of exposure, but also to the timing of exposure and tissue-specific effects.

**4a. TOBACCO SMOKE**

Microarray-based studies into the effect of tobacco smoke exposure upon DNA methylation in blood have shown remarkable consistency in identifying three CpG sites as hypomethylated among smokers. These map to the aryl hydrocarbon receptor repressor (*AHRR*) gene body (Illumina Infinium methylation microarray probe ID: cg05575921), the coagulation factor II (thrombin) receptor-like 3 (*F2RL3*) gene body (cg03636183), and to 6p21.33 (cg06126421). *AHRR* encodes the aryl hydrocarbon receptor repressor that is implicated in the metabolism of carcinogenic products in tobacco smoke, and which also regulates cellular proliferation and differentiation *(11)*. *F2RL3* encodes protease-activated receptor 4, and has roles in blood clotting and inflammation. Interestingly, methylation at the sites mapping to *AHRR* and *F2RL3* do not correlate with that of neighbouring CpG sites (12), suggesting a very localized effect. It has been demonstrated that methylation at cg05575921 is associated with expression of the *AHRR* gene (5) and this loci maps to an RNA polymerase binding site, thereby offering a clear biological rationale for this observation.

The most extensive analysis of these genes in relation to smoking is that of Fasanelli *et al (13)*. Utilizing data from four cohorts, hypomethylation of the two CpG sites mapping to the *AHRR* and *F2RL3* genes were reported to be associated with increased risk of lung cancer. As all four cohorts came from prospective studies, this work provides strong evidence for the use of these genes as biomarkers of disease risk. Studies elsewhere have demonstrated that methylation of *AHRR*, *F2RL3* and *6p21.33* are independently able to predict lung cancer incidence but, as might be expected of loci that are independently associated with tobacco smoke exposure, do not offer synergy when their analysis is integrated (12,14). The degree of hypomethylation of these genes is associated with the level of risk, as work within the Copenhagen City Heart Study reported that individuals with strongest hypomethylation of *AHRR* have a hazard ratio of 4.87 for lung cancer incidence (15).

It is not clear whether these genes are directly implicated in neoplastic transformation or whether their methylation may simply serve as a surrogate marker of smoking history. Methylation of *F2RL3* displays a dose response with pack-years of smoking, and former smokers display intermediate levels of methylation according to time since cessation (16). Similarly, *AHRR* methylation is correlated with pack-years of smoking, and among former-smokers methylation levels regress over time to that observed in non-smokers (5,17). While this may suggest that these genes serve merely as biomarkers of exposure, Zhang and colleagues (18) reported that *F2RL3* methylation remained a predictor of cancer risk following adjustment for pack-years of smoking. However, the hazard ratio decreased substantially in comparison to the unadjusted model. Furthermore, *AHRR* has been described as a tumor suppressor gene and Zudaire *et al (11)* have provided *in vitro* evidence that silencing of *AHRR* in a lung cancer cell line promotes anchorage-free growth and resistance to apoptosis.

DNA methylation within the *AHRR* and *F2RL3* genes and at 6p21.33 therefore appear to serve as strong markers of tobacco smoke exposure and lung cancer incidence, and their utility have been replicated across many studies and prospective cohorts. However, further work is required to determine whether these sites exclusively serve as biomarkers of recent smoking history or whether they may be more directly implicated in carcinogenesis.

**4b. AIR POLLUTION**

Air pollution was recently classified as carcinogenic by the World Health Organization. A meta-analysis of 17 prospective cohorts through the European Study of Cohorts for Air Pollution Effects reported a 22% increase in risk of lung cancer incidence per 10 g/m PM10 exposure, and an 18% increase in risk per 5 g/m PM2.5 exposure (19). Exposure to high levels of particulate matter (PM, defined by particle size in micrometers (m)), referred to as lung particle overload, is associated with an inability of the lungs to clear the particles, leading to neutrophilic inflammation and the generation of reactive oxygen species (ROS) that induce DNA damage (20). There is increasing evidence for a further epigenetic effect of air pollution exposures, demonstrated by ROS-induced protein kinase B activation leading to increased expression of DNA methyltransferase 3 beta (*Dnmt3b*) in mice and subsequent hypermethylation of the tumor protein p53 gene (*Tp53) (21)*. Furthermore, the use of the DNA methyltransferase inhibitor azacitidine has been shown to reduce the development of lung neoplasms by 30% in mice (2), suggesting carcinogenesis is in part through the accumulation of epigenetic defects in the cell.

Mice exposed to high levels of ambient PM2.5 were reported to show hypermethylation of the p16 INK4a tumor-suppressor gene (*CDKN2A*) in lung tissue (22). This gene is commonly hypermethylated in lung tumors and cell lines, thereby providing a mechanistic link between air pollution exposure and carcinogenesis. This is supported by evidence from a cross-sectional study that reported hypermethylation of the *CDKN2A* and adenomatous polyposis coli (*APC*) gene promoters in peripheral blood leukocytes from steel workers exposed to PM (23). However, the authors also reported decreased promoter methylation of two other tumor-suppressor genes, *TP53* and Ras association domain family 1 isoform A (*RASSF1A*), and therefore the inference is not immediate clear. Furthermore, the analysis of DNA methylation in leukocytes cannot necessarily be extrapolated to the lung due to the tissue-specific effects of many carcinogens.

Benzene exposure is associated with increased risk of acute myelogenous leukemia, and it has been demonstrated to induce significant hypomethylation of LINE-1 and *Alu* elements in workers exposed to low levels of benzene through traffic (24). Repetitive elements such as long interspersed nuclear element-1 (LINE-1) and *Alu* are often utilized as surrogate markers of global methylation levels due to their abundance throughout the genome (25), and the authors commented that the effects of this carcinogen are consistent with the global hypomethylation observed in malignancies that leads to chromosomal instability. However, care must be taken with extrapolation of pyrosequencing-based analysis of repetitive elements. LINE-1 and *Alu* elements incorporate many subfamilies that have diverged over the evolution of the genome, and Byun *et al (26)* reported that these subfamilies display differential susceptibility to the effects of environmental pollutants such as benzene, with evolutionary older LINE-1 subfamilies and younger *Alu* subfamilies displaying the greatest hypomethylation. The widely used pyrosequencing assays interrogate only the L1HS (young) and *Alu*Sx (intermediate age) subfamilies and therefore may not truly represent the effect of particulate matter on the epigenome. Nonetheless, the observation of a clear epigenetic effect of benzene exposure has provided insight into its association with the development of leukemia.

**4c. ARSENIC**

Arsenic exposure can deplete s-adenosyl methionine levels (due to its use in the methylation and detoxification of arsenic) and inhibit the expression of DNA methyltransferase 1 (*DNMT1*), thereby leading to global hypomethylation (27). Rea *et al (28)* demonstrated that arsenic slightly decreased expression of the CTCF transcription factor in cell lines and led to reduced CTCF occupancy at DNA methyltransferase gene promoters, and thereby reduced the expression of these genes. The subsequent deregulation of DNA methylation maintenance led to differential methylation of genes involved in processes such as cell adhesion and epithelial-mesenchymal transition (EMT). Arsenic-induced epigenetic changes have been identified in association with the development of skin lesions that can give rise to skin cancers (29), with marked changes of >10% observed in the blood of individuals identified as having newly developed skin lesions. Further evidence for epigenetic dysregulation being implicated in exposure-associated carcinogenesis was brought by Yang *et al (30)*, who reported that arsenic-associated and non-arsenic-associated urothelial carcinomas display differential methylation patterns, with nine genes significantly hypermethylated among exposed individuals. However, the form of arsenic can be influential. It has been reported that dimethylated arsenics have less of an effect on the epigenome in comparison to inorganic and monomethylated arsenic (31), and therefore care needs to be taken in the study of arsenic exposure in relation to cancer risk.

Exposure to arsenic during early pregnancy has been shown to have wide-ranging site-specific effects on the epigenome of newborns (32). While global DNA methylation levels remained similar, approximately three-quarters of differentially methylated sites were hypomethylated with exposure. The authors reported enrichment for differential methylation of cancer-associated genes in boys, supporting evidence from elsewhere of greater cancer risk in arsenic-exposed males in comparison to females, and that associations were much stronger following exposure earlier in development.

Interestingly, there is evidence for an interaction between arsenic exposure and dietary folate upon cancer risk. Folate has a critical role in one-carbon metabolism and the maintenance of global DNA methylation patterns through the regeneration of s-adenosyl methionine, the sole source of methyl groups for DNA methyltransferases. Furthermore, folate enhances the detoxification of inorganic arsenic through its methylation. The offspring of pregnant mice exposed to arsenic and fed a normal diet did not display significant changes in their global DNA methylation patterns in the fetal liver, but the offspring of mothers who were exposed and fed a high-folate diet showed differential methylation of nearly 3,000 genes (33). These were enriched for imprinted genes such as growth factor receptor-bound protein 10 (*Grb10*) and mesoderm specific transcript (*Mest*), and for cancer-associated pathways such as Wnt signaling. This underlines the complexity in establishing the effect of environmental exposures upon cancer risk. While *in vitro* systems and animal models enable greater control to study single factors in isolation, an individual’s cancer risk is the sum of many exposures and their genetic and epigenetic predisposition to their influence. Comprehensive cohort studies with extensive characterization of diet and environmental exposures, and especially prospective studies, offer the most incisive approach to further our knowledge of cancer risk factors.

**4d. BISPHENOL A**

Bisphenol A (BPA) is a compound used to strengthen plastics but which is known to be an endocrine disruptor through its interaction with the estrogen receptor. It has been shown that pre-natal BPA exposures have wide-ranging effects upon the epigenome in rat mammary glands, with no specific tendency for either hyper- or hypomethylation (34). Importantly, though, loci displaying differential methylation between the exposed and non-exposed groups were not consistent across post-natal days 4, 21 and 50. Indeed, only 38 of the 675 sites that were hypomethylated at day 4 were maintained at day 21, and only 2 of those by day 50. This suggests that the observed changes in the epigenome represent temporal effects of BPA on the mammary gland, rather than early epigenetic activation of oncogenes in tumor development. This hypothesis is supported by the observation of the most substantial differences in the epigenomes of exposed and unexposed rats coming at day 21, which coincides with the secretion of estrogen by the ovaries. BPA exposure may therefore enhance the response to estrogen in the rat mammary gland, driving the growth of neoplasias.

In contrast, the risk of prostate cancer following BPA exposure may indeed be through the epigenetic activation of oncogenes. Neonatal exposure has been reported to induce hypomethylation of the phosphodiesterase type 4 variant 4 (*Pde4d4*) gene, which normally becomes increasingly methylated with age, leading to its increased expression (35). This hypomethylation and increased expression were maintained in the adult rats, thereby suggesting that pre-natal BPA exposure could have long-lasting effects. PDE4D4 plays a role in the degradation of cyclic AMP and thereby serves to promote cellular proliferation and differentiation, and its persistent expression may therefore help to drive the growth of malignancies. This evidence in an animal model is supported by observations of *PDE4D* being overexpressed in human prostate cancers, and having been demonstrated to drive the growth and migration of malignant cells (36). While *Pde4d4* hypomethylation was not observable until after sexual maturation of the rats, hypomethylation of nucleosome binding protein-1 (*Nsbp1*) was immediately observable following BPA exposure and was similarly maintained over time (4). NSBP1 binds to nucleosomes to modify chromatin architecture and promote gene expression, and it has been shown to promote the growth of prostate cancer cell lines (37). Pre-natal exposure to BPA may therefore confer increased risk of prostate cancer via the epigenetic dysregulation of *PDE4D* and *NSBP1*, which may serve to drive the growth of malignancies.

**4e. DIET**

The agouti mouse model has demonstrated the importance of maternal nutrition in shaping the epigenome of offspring. The abundance of methyl donors in the maternal diet, such as folic acid and S-adenosyl methionine, determines the methylation of the *Agouti* gene, which in turn renders the mouse’s coat to be yellow when hypomethylated and dark when methylated (38). The yellow-coloured mice have a predisposition to obesity and the development of cancer. Interestingly, a maternal diet rich in methyl donors has been shown to overcome the negative effects of *in utero* BPA exposure. BPA was demonstrated to lead to global hypomethylation of the offspring epigenome, but supplementation of the maternal diet with folic acid, betaine, vitamin B 12, choline or genistein was found to negate these effects of BPA (39). Indeed, the ability of diet to offset the effects of other environmental exposures, such as that of air pollution, is proving to be an area of increasing research interest as it represents a means of intervention to reduce disease risk.

Studies of the Dutch Hunger Winter of 1944-45 have provided particular insight into the effect of diet and malnutrition upon the epigenome and cancer risk. Women aged between 2 and 33 at the time of the Dutch Hunger Winter of 1944-45 have a 48% increase in risk of developing breast cancer in comparison to women not exposed to the famine (40), but the incidences of other cancers were not affected (41). However, it is not only such extreme circumstances that influence cancer risk. A recent study found that adolescents consuming a diet lacking in vegetables and high in sugar, red and processed meats and margarine had a 35% increase in risk of developing breast cancer prior to the menopause, which was hypothesized to be the product of chronic inflammation at a time when the mammary gland is developing (42). There is evidence for high fat diets inducing aberrant expression of DNA methyltransferase 3 alpha (*DNMT3A) (43)*, although no evidence of an effect upon global DNA methylation patterns was presented.

**5. EARLY LIFE EXPOSURES**

There is now extensive evidence for pre-natal exposures influencing the epigenome beyond birth, which has led to substantial interest in the potential health implications. The Developmental Origins of Health and Disease (DOHaD) hypothesis originated with the observation by David Barker that fetal growth and development were associated with coronary heart disease later in life (44), and there is increasing evidence for cancer risk being modified by *in utero* exposures, such as through maternal diet and environmental pollutants. Indeed, a meta-analysis of 57 studies revealed that increased birth weight and length are associated with breast cancer risk later in life (relative risks of 1.15 and 1.28 respectively) (45).

Individuals who were conceived during the Dutch Hunger Winter of 1944-45 displayed hypomethylation of *IGF2* differentially methylated region 0 (DMR0), a gene implicated in fetal growth and a widely studied oncogene, when analyzed more than 60 years later, while their siblings did not (46). This observation demonstrates both the effect of the pre-natal environment and the persistence of subsequently acquired epigenetic traits. The timing of exposures also appears to be critical, as Tobi and colleagues reported differential methylation of genomic loci related to the regulation of growth and metabolism in individuals exposed to the famine particularly during early gestation (3). This observation may be due to the differentially methylated genes being expressed in the early stages of fetal development, and therefore most susceptible to environmental influence during this window. Other studies have conversely shown increased effects of environmental exposures with sequential trimesters (47), which may suggest consequences are dependent upon both the type and timing of exposure.

Maternal smoking is associated with differential methylation of the *AHRR* and cytochrome P450 family 1 member A1 (*CYP1A1*) genes in the cord blood of newborns (48). However, the long-term consequences for the child are not clear. As previously described, *AHRR* methylation is a biomarker for recent exposure to tobacco smoke (5), and it could therefore be expected that these measurements in cord blood will represent an acute response to the maternal environment that will diminish if and when the selection pressure, exposure to tobacco smoke, is removed. There is crucial evidence provided by the Avon Longitudinal Study of Parents and Children (ALSPAC) that *AHRR* methylation does indeed normalize to levels seen in the children of non-smokers by age 7. However, differential methylation of *CYP1A1*, myosin 1G (*MYO1G*) and contactin associated protein-like 2 (*CNTNAP2*) persists at ages 7 and 17 in individuals with prenatal exposure to tobacco smoke (49). These findings have been supported by work conducted as part of the European Childhood Obesity Project (CHOP), which has similarly observed persistent hypermethylation of *MYO1G* and hypomethylation *CNTNAP2* at age 5 (50).

DNA methylation at the *AHRR* locus in children exposed to pre-natal and early life tobacco smoke has been shown to be associated with genomic deletions in acute lymphoblastic leukemia (51). However, the strength of *AHRR* methylation as a biomarker for tobacco smoke exposure, in addition to the rarity of childhood leukemias, may suggest it has little clinical utility in predicting disease risk.

While it is clear that *in utero* exposures can influence the epigenome, much further study is required to establish the long-term consequences. Longitudinal studies are required to establish the stability of the epigenetic changes in response to the environment, and the implications for human health.

**6. TRANSGENERATIONAL INHERITANCE OF RISK**

Transgenerational inheritance of epigenetic traits concerns the transmission of acquired DNA methylation marks on to future generations. DNA methylation marks are normally erased in the zygote following fertilization, with the exception of imprinted loci. However, there is increasing evidence of incomplete removal of DNA methylation from the genome, and subsequently the potential for passing of altered DNA methylation traits on to offspring. True transgenerational inheritance requires the trait to be observed in the F2 generation and beyond, due to the potential for it being acquired by a fetus (F1) or the germline of the fetus (F2) (*Figure 1*). Subsequently there is scant evidence of true transgenerational inheritance in humans due to the inherent difficulty in its study. There is, however, evidence from animal models to support this ‘epigenetic Lamarckism’. Mice who were induced to fear the odor from acetophenone were observed to have hypomethylation of the olfactory receptor 151 (*Olfr151*) gene, and the F1 and F2 offspring of these mice inherited both the behavioural sensitivity to this odor and hypomethylation of this gene (52). It has also been shown in mice that pre-natal exposure to the endocrine disruptor vinclozin disrupts pathways related to the Blimp1 transcription factor via differential expression of the microRNAs miR-23b and miR-21, which was maintained in subsequent generations and resulted in their decreased fertility, although no changes in DNA methylation were observed (53).

**\*\*\*INSERT FIGURE 1\*\*\***

The concept of transgenerational inheritance of epigenetic traits remains highly controversial. There is currently no convincing evidence to suggest its presence in humans, but work in animal models has been sufficiently promising to suggest it should be an avenue of further study. This is inhibited by the need to observe the epigenetic trait in multiple generations and the inter-individual variation in epigenetic and genetic profiles, which has to date restricted investigation to animal models. Furthermore, caution must be taken to distinguish between acute responses to *in utero* exposures or shared environments and true inheritance of epigenetic traits. Should evidence of transgenerational inheritance in humans be submitted, there would be clear implications for the study of inherited cancer risk.

**7. EPIGENETIC DYSREGULATION OF ONCOGENES AND TUMOR-SUPPRESSOR GENES**

While genetic predisposition to cancer has been demonstrated, such as germline mutations in the *BRCA1* and *APC* genes, fewer ‘epimutations’ conferring increased cancer risk have been identified. For some of these genes suggested to be targets of epimutations, there is conflicting evidence and a lack of reproducibility.

**7a. LYNCH SYNDROME**

Lynch syndrome, previously referred to as hereditary non-polyposis colorectal cancer (HPNCC), is a disorder of mismatch repair genes mutL homolog 1 (*MLH1*), mutS homolog 2 (*MSH2*), mutS homolog 6 (*MSH*6*)* or PMS1 homolog 2 (*PMS2*), leading to microsatellite instability and subsequently to frameshift mutations. It is estimated that Lynch syndrome accounts for 3% of colorectal cancer cases, and estimates of lifetime colorectal cancer risk range from 30 to 74%, with the risk higher for men (54). Lynch syndrome patients are also at increased risk of other cancers, such as that of the breast and prostate (54). The disorder most commonly arises through genetic mutations in the aforementioned genes, but can occur through monoallelic *MLH1* promoter hypermethylation. This epigenetic route to microsatellite instability is estimated to occur in approximately 16% of Lynch syndrome patients (55). Microsatellite stability is not confined to individuals with Lynch syndrome as approximately 20% of sporadic colorectal tumors display hypermethylation of *MLH1 (55)*, and it is detectable in a similar proportion of adenomas as tumors (56).

Silencing of *MLH1* is a clear epigenetic marker of cancer risk, but screening strategies for this defect are problematic. Individuals with familial history of the disease are likely to have genetic, rather than epigenetic, defects in mismatch repair genes, although there is evidence for the heritability of a germline epimutation in *MLH1 (57)*. While *MLH1* promoter hypermethylation also appears to be an early event in sporadic colorectal carcinogenesis, screening the general population is unlikely to prove cost-effective.

**7b. Insulin-like growth factor 2 (*IGF2*)**

The *IGF2* gene is important in growth and development. It is imprinted, with expression from the paternal allele, and loss of imprinting (LOI) of this gene is associated with the Beckwith-Wiedemann and Silver-Russell syndromes (58). It has also been demonstrated to be an oncogene. Changes in DNA methylation at DMR0 and DMR2 have been reported in colorectal, breast and liver cancers, and are frequently associated with aggressive disease (58-60). However, while some studies have reported loss of allele-specific expression associated with epigenetic changes (61), others have reported that monoallelic expression of the gene is retained (58). Models of genomic imprinting often hold true in animal models, but the relationship between allele-specific methylation and expression is less clear in humans. Subsequently, studies into LOI in cancer and disease risk need to investigate both epigenetic regulation and allele-specific expression of candidate genes.

It has been proposed that loss of *IGF2* imprinting in blood can serve as a risk biomarker for colorectal cancer. Cui and colleagues (62) analyzed methylation of *IGF2* DMR0 in peripheral blood samples and reported odds ratios for hypomethylation of 21.7 in patients with colorectal cancer and 3.5 for patients with adenomas. Epigenetic dysregulation of this region was also associated with familial history of the disease. These findings would suggest that a simple blood-based assay could identify patients at increased risk of colorectal cancer for colonoscopy-based screening, and thereby have substantial clinical utility. Other studies have failed to replicate these findings, with no hypomethylation of *IGF2* observed in blood samples from patients with colorectal (63) or breast tumors (58), and no evidence for a correlation in the methylation of imprinted genes measured in blood and solid tumors (64). The most powerful evaluation of *IGF2* methylation as a biomarker for cancer risk is perhaps that of Kaaks *et al (65)*. The authors utilized samples from the Northern Sweden Health and Disease prospective study, and they reported no difference in *IGF2* methylation between cases and controls. Therefore, the use of blood as a surrogate tissue for *IGF2* screening remains controversial.

**7c. Breast cancer 1 (*BRCA1*)**

The *BRCA1* gene encodes a protein involved in homologous recombination to repair double-strand DNA breaks. Defects in this gene have been well characterized in conferring familial risk of breast and ovarian cancers, primarily through inherited genetic mutations (66). *BRCA1* inactivation can also occur through promoter hypermethylation, which has been reported to be present in 17 - 60% of sporadic breast tumors (67). Although the epigenetic profile of breast tumors largely do not correlate with that of peripheral blood leukocytes (64), *BRCA1* promoter hypermethylation is detectable in the blood of more than 20% of all breast cancer patients, and in more than two-thirds of patients with tumoral hypermethylation (68). This observation of hypermethylation across multiple tissues may suggest that it originates early in development and therefore may be detectable prior to disease diagnosis as a marker of susceptibility. However, there is currently little evidence to support its utility in predicting cancer risk. A case-control study of more than 1800 participants identified no association between *BRCA1* methylation in blood and breast cancer risk (69). Furthermore, while *BRCA1* methylation has been reported as more common among cancer patients than healthy controls in seven studies, this difference was only statistically significant in four of them (70).

**7d. Septin 9 (*SEPT9*)**

The *SEPT9* promoter is hypermethylated in colorectal cancer, with measured methylation levels increasing from non-advanced adenomas to colorectal tumors (71), and early studies of *SEPT9* methylation in peripheral blood or plasma samples have generated great interest in its utility in the clinic. A case-control study by Grützmann and colleagues identified *SEPT9* promoter hypermethylation as being detectable in plasma samples from patients with colorectal cancer (72), with the sensitivity and specificity of the assay reported to be 72% and 90% respectively. Due to the initial promise of the results, Epigenomics AG developed a *SEPT9*-based assay for the detection of colorectal cancer which is available to clinics in Germany and Switzerland. However, a recent study in a prospective cohort of 53 cases and 1,457 controls suggested that this biomarker may be of more limited use (73). The sensitivity of the assay to detect advanced adenomas and Stage I tumors were reported as 11.2% and 35.0% respectively, and therefore it does not appear to hold promise in detection of early disease.

**8. INTEGRATED MODELS**

Early studies into cancer epigenetics took the candidate gene approach, in part due to the inability or cost of performing epigenome-wide analysis of DNA methylation. The advent of cost-effective means of doing this, such as the Illumina Infinium DNA methylation microarray platform, revolutionized the field. While initial studies primarily focussed on identifying differentially methylated genes, recent advances have demonstrated the power of integrated approaches that utilize DNA methylation across many genomic loci to understand the effect of environmental exposures and predict cancer risk.

**8a. GLOBAL METHYLATION LEVELS**

The cancer epigenome is frequently characterized by gene promoter hypermethylation and global hypomethylation, and changes in the epigenome are believed to be an early event in carcinogenesis (74). Therefore, simple analysis of global DNA methylation levels may have utility in predicting cancer incidence. The relative infrequency of gene-specific epimutations, such as those in *IGF2* or *BRCA1*, may limit their application to population-based screening for cancer risk, and broader analysis of the epigenome may offer superior sensitivity.

A recent study utilizing prospective peripheral blood samples was able to detect global hypomethylation many years prior to diagnosis of mature B-cell neoplasms such as multiple myeloma and follicular lymphoma (75). More specific analysis revealed that hypermethylation of promoter regions and hypomethylation of non-promoter regions were each independently associated with a greater than 1.5-fold increase in risk. More than half of the 436 cases were diagnosed with neoplasms more than 10 years after sampling, and the authors reported no effect of the time between sampling and diagnosis upon the detection of global hypomethylation. These points would imply that simple analysis of global DNA methylation could be of use in the clinical setting in identifying patients at risk of developing hematological malignancies.

Similarly, it has been reported that global DNA methylation levels measured in blood, estimated by analysis of *LINE-1* methylation, were associated with a 1.6-fold increased risk of head and neck cancers (76). However, some of the other findings from the study highlight inherent difficulties in this approach. Dietary folate levels, methylenetetrahydrofolate reductase gene (*MTHFR*) SNP variants and pack-years of smoking, which are known risk factors for head and neck cancers, were independently associated with measured methylation levels. Further work is required to elucidate the relationship between global DNA methylation levels and other risk factors in the development of head and neck cancers, and therefore to establish their potential utility in identifying cancer risk.

**8b. DNA METHYLATION VARIABILITY**

Models integrating methylation at many loci have demonstrated significant promise in estimating disease risk. Teschendorff and colleagues developed the algorithm Epigenetic Variable Outliers for Risk prediction Analysis (EVORA) utilizing data from the Illumina Infinium DNA methylation microarray platform to examine loci with differential variability in methylation between tissues (1). In a prospective cohort, the authors were able to distinguish between cytologically-normal cervical cells from individuals who developed cervical neoplasia within three years and those from age-matched controls who remained cancer-free, based upon the number of variable or outlier methylation measurements at 140 ‘risk’ CpG sites. Increased stochastic variation in methylation therefore appears to be an early event in cancer development, and its presence in cytologically-normal cells suggests that its examination could have significant clinical utility. Indeed, the authors had previously reported that epigenetic aberrations in Polycomb group target genes could be detected three years prior to morphological changes (77). The power of this approach was also demonstrated by the absence of significantly differentially methylated sites identified by conventional analysis between the cases and controls. Indeed, variability in methylation may predict the propensity for a tissue to develop genetic and epigenetic aberrations that propagate carcinogenesis. However, as the authors described, this model relies upon the study of the cell of origin for the cancer, and therefore cannot be utilized with peripheral blood samples for the study of solid tumors, for example.

Further work by Teschendorff and Widschwendter led to the development of a ‘mitotic clock’ (78). This model focusses upon promoters within Polycomb group target genes that are unmethylated in fetal tissues and show increasing methylation with age, and incorporates estimated rates of stem cell division in normal tissues, which is in itself associated with cancer risk (79). The authors were able to demonstrate increased ‘ticking’ of the mitotic clock in pre-cancerous lesions and in a variety of cancers. Furthermore, and importantly for analysis of disease risk, they were also able to demonstrate increased ticking following exposure of epithelial cells to carcinogenic compounds. This model is highly promising, but it has yet to be applied to prospective studies and so its potential utility in identifying cancer risk has not been established.

**8c. AGING AND THE ‘EPIGENETIC CLOCK’**

Age is the most significant risk factor for a variety of different cancers. There has been a great deal of interest in understanding the molecular mechanisms that underpin the aging process, which could also provide insights into cancer risk. This research has focussed on topics such as the acquisition of DNA damage and telomere length, but more recently there has been increased interest in studying the epigenome for this purpose.

There have been attempts to develop epigenetic models of aging, with the most notable being that published by Steve Horvath in 2013 (80). This model utilizes 353 CpG sites that are interrogated by Illumina’s Infinium microarray platform (HumanMethylation27 and HumanMethylation450 beadchips) and was demonstrated to accurately estimate chronological age to within three years across a variety of healthy tissues. These sites are enriched at genes implicated in carcinogenesis, with 57 of the sites having been reported as differentially methylated between colorectal tumors and surrounding normal tissue (81). Since the publication of the model, age acceleration, defined as the difference between chronological age and methylation age, has been reported in the liver of obese individuals (82), in the blood of HIV-infected patients (83) and patients with Parkinson’s disease (84), and has also been shown to be associated with all-cause mortality in an elderly population (85). However, the relationship between this ‘epigenetic clock’ and cancer is more complex. Age acceleration is routinely observed in a variety of cancer types and is inversely associated with the number of somatic mutations, and especially with *TP53* mutations, but there appears to be no clear association with tumor grade (80). It is of course to be expected that a model of aging predicated on marginal changes in DNA methylation over many years will become less insightful when applied to malignant cells that display widespread disruption of the epigenome.

**\*\*\*INSERT TABLE 1\*\*\***

The epigenetic clock could provide significant insight into cancer risk. Promisingly, there is evidence to suggest that age acceleration is observable before diagnosis, as Levine *et al* reported that age acceleration is significantly associated with future lung cancer incidence among current and former smokers (86). Further evidence of this was provided by Zheng *et al (87)*, who reported a significant association between age acceleration and cancer incidence in a prospective cohort. The observation that this effect was particularly pronounced in individuals diagnosed with cancer within five years of blood draw may suggest that age acceleration is an acute event, rather than a more nuanced one over time. The epigenetic clock may also have prognostic significance, as it has been demonstrated to be significantly associated with risk of cancer mortality in an elderly cohort (88).

There is further evidence for the utility of the epigenetic clock in establishing cancer risk in that lifestyle factors associated with disease risk appear to influence the rate of epigenetic aging. Diet, alcohol consumption and BMI are all cancer risk factors and significantly associated with age acceleration (89). Models such as the epigenetic clock could represent a summation of many factors influencing cancer risk and therefore bring unique insight. However, care must be taken in considering the tissue of study. For example, the rate of age acceleration measured in a peripheral blood sample may not truly represent the effect of diet upon colonic tissue, or smoking upon the lungs.

While the epigenetic clock model has been proposed as a biomarker for early carcinogenic processes (87), age acceleration does not display specificity for cancer-risk as it is also associated with cardiovascular disease incidence and all-cause mortality. Nonetheless, elucidation of how the genes integrated into the clock influence biological aging and disease risk can bring significant insight into the biological mechanisms associated with conferring cancer risk.

**9. MITOCHONDRIAL EPIGENETICS**

The field of mitochondrial epigenetics is still in its infancy, but there is evidence to suggest that it may prove a fruitful area of research, in particular the observations of differential methylation in cancer and in response to environmental exposures.

The displacement loop (D-loop) promoter sequence of the mitochondrial genome has an important role in the regulation of mitochondrial gene expression, and it has been shown to be hypomethylated in colorectal tumors and that this is associated with an increase in mitochondrial DNA copy number, with lower levels of methylation observed in more advanced tumors (90,91). However, these studies reported methylation of this region in the surrounding normal tissue when most investigations into the mitochondrial epigenome have reported very low levels of methylation, to the point where some critics have suggested it may be a technical artifact from incomplete bisulfite-conversion of the DNA. Regardless, to date there has been no evidence offered of D-loop promoter hypomethylation prior to diagnosis, and therefore it is not possible to determine whether it is associated with cancer risk or is rather a product of the cancer. While the genes encoded in the mitochondrial genome are associated with the production of ATP, and are therefore not putative oncogenes or tumor suppressor genes, there is *in vitro* evidence that depletion of the mitochondrial genome in a breast epithelial cell line can induce transformation of the cells (92). Furthermore, there is evidence of age-related changes in the mitochondrial epigenome, with the mitochondrially encoded 12S RNA gene (*MT-RNR1*) increasingly hypomethylated with age (93). Therefore, aberrant epigenetic regulation of the mitochondrial genome could be implicated in cancer risk. One mechanism for such an association could be through increased production of ROS as part of the oxidative phosphorylation process, which can lead to the development of mutations in the mitochondrial and nuclear genomes alike. Further work is required to understand the relationship between the mitochondrial epigenome and ROS production, which could bring insight into cancer risk.

It has been shown that mitochondrial DNA methylation is modifiable through environmental exposures. Exposure to the flame retardant BDE-47 is associated with increased risk of breast cancer (94) and has also been demonstrated to impact upon the mitochondrial epigenome through decreased methylation of the cytochrome c oxidase subunit II gene (*MT-CO2*) in the frontal lobe of exposed rats (95). Chromium, which is known to be genotoxic and carcinogenic, has been demonstrated to be associated with hypomethylation of the mitochondrially encoded TRNA phenylalanine (*MT-TF*) and *MT-RNR1* genes, encoding tRNA phenylalanine and 12s RNA respectively (96). Exposure to PM2.5 airborne particles has also been shown to be associated with increased methylation of the D-loop region and *MT-RNR1* in placental tissue (97). While these observations are interesting and open up novel avenues of research, it is not yet clear whether changes in the mitochondrial epigenome may be directly associated with increased cancer risk or whether they are by-products of exposure to carcinogenic compounds.

However, it should be noted that there are several challenges inherent in this field. In addition to the tissue-specificity of epigenetic marks observed in nuclear DNA, each cell contains varying numbers of mitochondria (approximately 2 – 2,000, according to cell type) with their own copies of the mitochondrial genome. It has not yet been established whether the mitochondrial epigenome differs between mitochondria within a single cell, nor how these epigenetic profiles vary between cells and cell types. Furthermore, sequences from the mitochondrial genome are present within the nuclear genome, referred to as nuclear mitochondrial DNA sequences (‘numts’). These are believed to have originated through retrotransposition from the mitochondrial genome (98), and they have the ability to confound bisulfite-sequencing-based approaches to the study of mitochondrial DNA methylation. Care must be taken to design mitochondrial DNA-specific PCR primers when using a candidate gene approach, or to ensure isolation of pure mitochondrial DNA samples (without nuclear DNA contamination) for whole-epigenome sequencing.

**10. FUTURE DIRECTIONS**

Much progress has been made into understanding epigenetic changes associated with carcinogenesis, and this promises to continue with advances in our understanding of the epigenome and with the development of new technologies. Epigenome-wide analysis of DNA methylation, either through microarrays or sequencing, is becoming increasingly affordable and is now being applied to large-scale population studies, facilitating the identification of novel epigenetic risk markers. Nonetheless, there are a number of challenges faced within the field.

Approaches taken to the analysis of the epigenome are becoming increasingly holistic and complex, and to great effect. The ‘epigenetic clock’ model of aging has shown remarkable accuracy in predicting age and the risk of disease (87), while measuring stochastic variation in DNA methylation at specific risk loci can provide insight into the risk of malignant transformation that genetic or morphological analysis cannot (1). While these approaches have brought insight into the development of cancer, there remain substantial challenges in the application of such models to the clinical setting.

Further work is required into the long-term stability of biomarkers of environmental exposures, and how they can best be utilized to predict cancer risk. This is exemplified by *AHRR* methylation serving as an excellent biomarker of cumulative tobacco smoke exposure among current smokers, but regressing to normal levels following the cessation of smoking and subsequently unable to represent lifetime exposure (5). Furthermore, many pollutants display differential effects according to the stage of development or the life cycle, and therefore biomarkers of cumulative exposure may not adequately predict disease risk.

It has been clearly demonstrated that pre-natal exposures can increase cancer risk through epigenetic mechanisms. However, the transgenerational inheritance of aberrant epigenetic traits remains a highly debated topic. Currently there is no evidence for this phenomena in humans, but it remains a very intriguing field of study. Its investigation is of course hampered by the relatively long human lifespan, but epigenetic analysis of trans-generational cohorts may be able to provide some insight.

Novel avenues of research may be able to bring insight into the mediation of cancer risk following environmental exposures. Extracellular vesicles generated from the plasma membrane facilitate communication between cells and tissues, and their release can be induced by environmental stresses. They have subsequently been hypothesized to play a role in the mediation of the effects of environmental exposures. While this field is in its infancy, increased presence of microRNAs implicated in the regulation of inflammatory pathways have been observed in extracellular vesicles following PM exposure (99,100). This novel and challenging field of study could provide important insight into the response of the body to harmful exposures.

The identification of epigenetic biomarkers of disease risk can also inform the identification of protective factors. One of the most intriguing areas of research is in diet modulating the effect of environmental exposures to reduce cancer risk. As previously described, supplementation of the diet with methyl donors such as folic acid can overcome the adverse effects of BPA exposure (39), but can also exacerbate the effects of arsenic exposure (33). Further work is required to investigate the ability of diet to offset the negative effects of exposures associated with cancer risk.

**11. CONCLUSIONS**

The study of epigenetics is providing substantial insight into cancer risk. It has identified the epigenetic dysregulation of oncogenes and tumor-suppressor genes that are directly implicated in carcinogenesis, and also biomarkers of exposures that can describe the influence of an individual’s environment over many years. Furthermore, integrated analysis of epigenome-wide variation in DNA methylation is not only identifying potential biomarkers for assessing disease risk, but also providing insight into mechanisms of carcinogenesis.

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**REFERENCES**

1. Teschendorff AE, Jones A, Fiegl H, Sargent A, Zhuang JJ, Kitchener HC, Widschwendter M. Epigenetic variability in cells of normal cytology is associated with the risk of future morphological transformation. Genome Med 2012;4(3):24.

2. Belinsky SA, Klinge DM, Stidley CA, Issa JP, Herman JG, March TH, Baylin SB. Inhibition of DNA methylation and histone deacetylation prevents murine lung cancer. Cancer Res 2003, Nov 1;63(21):7089-93.

3. Tobi EW, Goeman JJ, Monajemi R, Gu H, Putter H, Zhang Y, et al. DNA methylation signatures link prenatal famine exposure to growth and metabolism. Nat Commun 2014, Nov 26;5:5592.

4. Tang WY, Morey LM, Cheung YY, Birch L, Prins GS, Ho SM. Neonatal exposure to estradiol/bisphenol A alters promoter methylation and expression of *Nsbp1* and *Hpcal1* genes and transcriptional programs of *Dnmt3a/b* and *Mbd2/4* in the rat prostate gland throughout life. Endocrinology 2012, Jan;153(1):42-55.

5. Shenker NS, Ueland PM, Polidoro S, van Veldhoven K, Ricceri F, Brown R, et al. DNA methylation as a long-term biomarker of exposure to tobacco smoke. Epidemiology 2013, Sep;24(5):712-6.

6. Byun HM, Siegmund KD, Pan F, Weisenberger DJ, Kanel G, Laird PW, Yang AS. Epigenetic profiling of somatic tissues from human autopsy specimens identifies tissue- and individual-specific DNA methylation patterns. Hum Mol Genet 2009, Dec 15;18(24):4808-17.

7. Houseman A, Accomando P, Koestler C, Christensen C, Marsit J, Nelson H, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. BMC Bioinformatics 2012, Jul;13:86.

8. Koestler DC, Marsit CJ, Christensen BC, Accomando W, Langevin SM, Houseman EA, et al. Peripheral blood immune cell methylation profiles are associated with nonhematopoietic cancers. Cancer Epidemiol Biomarkers Prev 2012, Aug;21(8):1293-302.

9. Joyce BT, Gao T, Liu L, Zheng Y, Liu S, Zhang W, et al. Longitudinal study of DNA methylation of inflammatory genes and cancer risk. Cancer Epidemiol Biomarkers Prev 2015, Oct;24(10):1531-8.

10. Janson PC, Marits P, Thörn M, Ohlsson R, Winqvist O. CpG methylation of the *IFNG* gene as a mechanism to induce immunosuppression [correction of immunosupression] in tumor-infiltrating lymphocytes. J Immunol 2008, Aug 15;181(4):2878-86.

11. Zudaire E, Cuesta N, Murty V, Woodson K, Adams L, Gonzalez N, et al. The aryl hydrocarbon receptor repressor is a putative tumor suppressor gene in multiple human cancers. J Clin Invest 2008, Feb;118(2):640-50.

12. Baglietto L, Ponzi E, Haycock P, Hodge A, Bianca Assumma M, Jung CH, et al. DNA methylation changes measured in pre-diagnostic peripheral blood samples are associated with smoking and lung cancer risk. Int J Cancer 2017, Jan 1;140(1):50-61.

13. Fasanelli F, Baglietto L, Ponzi E, Guida F, Campanella G, Johansson M, et al. Hypomethylation of smoking-related genes is associated with future lung cancer in four prospective cohorts. Nat Commun 2015, Dec 15;6:10192.

14. Zhang Y, Elgizouli M, Schöttker B, Holleczek B, Nieters A, Brenner H. Smoking-associated DNA methylation markers predict lung cancer incidence. Clin Epigenetics 2016;8:127.

15. Bojesen SE, Timpson N, Relton C, Davey Smith G, Nordestgaard BG. *AHRR* (cg05575921) hypomethylation marks smoking behaviour, morbidity and mortality. Thorax 2017, Jan 18.

16. Zhang Y, Yang R, Burwinkel B, Breitling LP, Brenner H. *F2RL3* methylation as a biomarker of current and lifetime smoking exposures. Environ Health Perspect 2014, Feb;122(2):131-7.

17. Shenker NS, Polidoro S, van Veldhoven K, Sacerdote C, Ricceri F, Birrell MA, et al. Epigenome-wide association study in the European Prospective Investigation into Cancer and Nutrition (EPIC-Turin) identifies novel genetic loci associated with smoking. Hum Mol Genet 2013, Mar 1;22(5):843-51.

18. Zhang Y, Schöttker B, Ordóñez-Mena J, Holleczek B, Yang R, Burwinkel B, et al. *F2RL3* methylation, lung cancer incidence and mortality. Int J Cancer 2015, Oct 1;137(7):1739-48.

19. Raaschou-Nielsen O, Andersen ZJ, Beelen R, Samoli E, Stafoggia M, Weinmayr G, et al. Air pollution and lung cancer incidence in 17 european cohorts: Prospective analyses from the European Study of Cohorts for Air Pollution Effects (ESCAPE). Lancet Oncol 2013, Aug;14(9):813-22.

20. Lim WY, Seow A. Biomass fuels and lung cancer. Respirology 2012, Jan;17(1):20-31.

21. Zhou W, Tian D, He J, Wang Y, Zhang L, Cui L, et al. Repeated PM2.5 exposure inhibits BEAS-2B cell P53 expression through ROS-Akt-DNMT3B pathway-mediated promoter hypermethylation. Oncotarget 2016, Apr 12;7(15):20691-703.

22. Soberanes S, Gonzalez A, Urich D, Chiarella SE, Radigan KA, Osornio-Vargas A, et al. Particulate matter air pollution induces hypermethylation of the p16 promoter via a mitochondrial ROS-JNK-DNMT1 pathway. Sci Rep 2012;2:275.

23. Hou L, Zhang X, Tarantini L, Nordio F, Bonzini M, Angelici L, et al. Ambient PM exposure and DNA methylation in tumor suppressor genes: A cross-sectional study. Part Fibre Toxicol 2011, Aug 30;8:25.

24. Bollati V, Baccarelli A, Hou L, Bonzini M, Fustinoni S, Cavallo D, et al. Changes in DNA methylation patterns in subjects exposed to low-dose benzene. Cancer Res 2007, Feb 1;67(3):876-80.

25. Barrow TM, Michels KB. Epigenetic epidemiology of cancer. Biochem Biophys Res Commun 2014, Dec 5;455(1-2):70-83.

26. Byun HM, Motta V, Panni T, Bertazzi PA, Apostoli P, Hou L, Baccarelli AA. Evolutionary age of repetitive element subfamilies and sensitivity of DNA methylation to airborne pollutants. Part Fibre Toxicol 2013;10:28.

27. Reichard JF, Schnekenburger M, Puga A. Long term low-dose arsenic exposure induces loss of DNA methylation. Biochem Biophys Res Commun 2007, Jan 5;352(1):188-92.

28. Rea M, Eckstein M, Eleazer R, Smith C, Fondufe-Mittendorf YN. Genome-wide DNA methylation reprogramming in response to inorganic arsenic links inhibition of CTCF binding, DNMT expression and cellular transformation. Sci Rep 2017, Feb 2;7:41474.

29. Seow WJ, Kile ML, Baccarelli AA, Pan WC, Byun HM, Mostofa G, et al. Epigenome-wide DNA methylation changes with development of arsenic-induced skin lesions in Bangladesh: A case-control follow-up study. Environ Mol Mutagen 2014, Jul;55(6):449-56.

30. Yang TY, Hsu LI, Chiu AW, Pu YS, Wang SH, Liao YT, et al. Comparison of genome-wide DNA methylation in urothelial carcinomas of patients with and without arsenic exposure. Environ Res 2014, Jan;128:57-63.

31. Rager JE, Tilley SK, Tulenko SE, Smeester L, Ray PD, Yosim A, et al. Identification of novel gene targets and putative regulators of arsenic-associated DNA methylation in human urothelial cells and bladder cancer. Chem Res Toxicol 2015, Jun 15;28(6):1144-55.

32. Broberg K, Ahmed S, Engström K, Hossain MB, Jurkovic Mlakar S, Bottai M, et al. Arsenic exposure in early pregnancy alters genome-wide DNA methylation in cord blood, particularly in boys. J Dev Orig Health Dis 2014, Aug;5(4):288-98.

33. Tsang V, Fry RC, Niculescu MD, Rager JE, Saunders J, Paul DS, et al. The epigenetic effects of a high prenatal folate intake in male mouse fetuses exposed in utero to arsenic. Toxicol Appl Pharmacol 2012, Nov 1;264(3):439-50.

34. Dhimolea E, Wadia PR, Murray TJ, Settles ML, Treitman JD, Sonnenschein C, et al. Prenatal exposure to BPA alters the epigenome of the rat mammary gland and increases the propensity to neoplastic development. PLoS One 2014;9(7):e99800.

35. Ho SM, Tang WY, Belmonte de Frausto J, Prins GS. Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. Cancer Res 2006, Jun 1;66(11):5624-32.

36. Rahrmann EP, Collier LS, Knutson TP, Doyal ME, Kuslak SL, Green LE, et al. Identification of PDE4D as a proliferation promoting factor in prostate cancer using a sleeping beauty transposon-based somatic mutagenesis screen. Cancer Res 2009, May 15;69(10):4388-97.

37. Jiang N, Zhou LQ, Zhang XY. Downregulation of the nucleosome-binding protein 1 (*NSBP1*) gene can inhibit the in vitro and in vivo proliferation of prostate cancer cells. Asian J Androl 2010, Sep;12(5):709-17.

38. Dolinoy DC, Weidman JR, Waterland RA, Jirtle RL. Maternal genistein alters coat color and protects avy mouse offspring from obesity by modifying the fetal epigenome. Environ Health Perspect 2006, Apr;114(4):567-72.

39. Dolinoy DC, Huang D, Jirtle RL. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. Proc Natl Acad Sci U S A 2007, Aug 7;104(32):13056-61.

40. Elias SG, Peeters PH, Grobbee DE, van Noord PA. Breast cancer risk after caloric restriction during the 1944-1945 Dutch Famine. J Natl Cancer Inst 2004, Apr 7;96(7):539-46.

41. Elias SG, Peeters PH, Grobbee DE, van Noord PA. The 1944-1945 Dutch Famine and subsequent overall cancer incidence. Cancer Epidemiol Biomarkers Prev 2005, Aug;14(8):1981-5.

42. Harris HR, Willett WC, Vaidya RL, Michels KB. An adolescent and early adulthood dietary pattern associated with inflammation and the incidence of breast cancer. Cancer Res 2017, Mar 1;77(5):1179-87.

43. Govindarajah V, Leung YK, Ying J, Gear R, Bornschein RL, Medvedovic M, Ho SM. *In utero* exposure of rats to high-fat diets perturbs gene expression profiles and cancer susceptibility of prepubertal mammary glands. J Nutr Biochem 2016, Mar;29:73-82.

44. Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet 1986, May 10;1(8489):1077-81.

45. Xue F, Michels KB. Intrauterine factors and risk of breast cancer: A systematic review and meta-analysis of current evidence. Lancet Oncol 2007, Dec;8(12):1088-100.

46. Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci U S A 2008, Nov 4;105(44):17046-9.

47. Grevendonk L, Janssen BG, Vanpoucke C, Lefebvre W, Hoxha M, Bollati V, Nawrot TS. Mitochondrial oxidative DNA damage and exposure to particulate air pollution in mother-newborn pairs. Environ Health 2016, Jan 20;15:10.

48. Joubert BR, Håberg SE, Nilsen RM, Wang X, Vollset SE, Murphy SK, et al. 450K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. Environ Health Perspect 2012, Oct;120(10):1425-31.

49. Richmond RC, Simpkin AJ, Woodward G, Gaunt TR, Lyttleton O, McArdle WL, et al. Prenatal exposure to maternal smoking and offspring DNA methylation across the lifecourse: Findings from the Avon Longitudinal Study of Parents and Children (ALSPAC). Hum Mol Genet 2015, Apr 15;24(8):2201-17.

50. Rzehak P, Saffery R, Reischl E, Covic M, Wahl S, Grote V, et al. Maternal smoking during pregnancy and dna-methylation in children at age 5.5 years: Epigenome-wide-analysis in the European Childhood Obesity Project (CHOP)-study. PLoS One 2016;11(5):e0155554.

51. de Smith AJ, Kaur M, Gonseth S, Endicott AA, Selvin S, Zhang L, et al. Correlates of prenatal and early-life tobacco smoke exposure and frequency of common gene deletions in childhood acute lymphoblastic leukemia. Cancer Res 2017, Feb 15.

52. Dias BG, Ressler KJ. Parental olfactory experience influences behavior and neural structure in subsequent generations. Nat Neurosci 2014, Jan;17(1):89-96.

53. Brieño-Enríquez MA, García-López J, Cárdenas DB, Guibert S, Cleroux E, Děd L, et al. Exposure to endocrine disruptor induces transgenerational epigenetic deregulation of microRNAs in primordial germ cells. PLoS One 2015;10(4):e0124296.

54. Giardiello FM, Allen JI, Axilbund JE, Boland CR, Burke CA, Burt RW, et al. Guidelines on genetic evaluation and management of Lynch syndrome: A consensus statement by the US Multi-society Task Force on colorectal cancer. Gastroenterology 2014, Aug;147(2):502-26.

55. Li X, Yao X, Wang Y, Hu F, Wang F, Jiang L, et al. *MLH1* promoter methylation frequency in colorectal cancer patients and related clinicopathological and molecular features. PLoS One 2013;8(3):e59064.

56. Bai AH, Tong JH, To KF, Chan MW, Man EP, Lo KW, et al. Promoter hypermethylation of tumor-related genes in the progression of colorectal neoplasia. Int J Cancer 2004, Dec 10;112(5):846-53.

57. Hitchins MP, Wong JJ, Suthers G, Suter CM, Martin DI, Hawkins NJ, Ward RL. Inheritance of a cancer-associated *MLH1* germ-line epimutation. N Engl J Med 2007, Feb 15;356(7):697-705.

58. Barrow TM, Barault L, Ellsworth RE, Harris HR, Binder AM, Valente AL, et al. Aberrant methylation of imprinted genes is associated with negative hormone receptor status in invasive breast cancer. Int J Cancer 2015, Aug 1;137(3):537-47.

59. Cui H, Onyango P, Brandenburg S, Wu Y, Hsieh CL, Feinberg AP. Loss of imprinting in colorectal cancer linked to hypomethylation of *H19* and *IGF2*. Cancer Res 2002, Nov 15;62(22):6442-6.

60. Martinez-Quetglas I, Pinyol R, Dauch D, Torrecilla S, Tovar V, Moeini A, et al. IGF2 is up-regulated by epigenetic mechanisms in hepatocellular carcinomas and is an actionable oncogene product in experimental models. Gastroenterology 2016, Dec;151(6):1192-205.

61. Murphy SK, Huang Z, Wen Y, Spillman MA, Whitaker RS, Simel LR, et al. Frequent *IGF2/H19* domain epigenetic alterations and elevated *IGF2* expression in epithelial ovarian cancer. Mol Cancer Res 2006, Apr;4(4):283-92.

62. Cui H, Cruz-Correa M, Giardiello FM, Hutcheon DF, Kafonek DR, Brandenburg S, et al. Loss of *IGF2* imprinting: A potential marker of colorectal cancer risk. Science 2003, Mar 14;299(5613):1753-5.

63. Ito Y, Koessler T, Ibrahim AE, Rai S, Vowler SL, Abu-Amero S, et al. Somatically acquired hypomethylation of *IGF2* in breast and colorectal cancer. Hum Mol Genet 2008, Sep 1;17(17):2633-43.

64. Barault L, Ellsworth RE, Harris HR, Valente AL, Shriver CD, Michels KB. Leukocyte DNA as surrogate for the evaluation of imprinted loci methylation in mammary tissue DNA. PLoS One 2013;8(2):e55896.

65. Kaaks R, Stattin P, Villar S, Poetsch AR, Dossus L, Nieters A, et al. Insulin-like growth factor-II methylation status in lymphocyte DNA and colon cancer risk in the Northern Sweden Health and Disease cohort. Cancer Res 2009, Jul 1;69(13):5400-5.

66. Smith SA, Ponder BA. Predisposing genes in breast and ovarian cancer: An overview. Tumori 1993, Oct 31;79(5):291-6.

67. Wu L, Wang F, Xu R, Zhang S, Peng X, Feng Y, et al. Promoter methylation of *BRCA1* in the prognosis of breast cancer: A meta-analysis. Breast Cancer Res Treat 2013, Dec;142(3):619-27.

68. Iwamoto T, Yamamoto N, Taguchi T, Tamaki Y, Noguchi S. *BRCA1* promoter methylation in peripheral blood cells is associated with increased risk of breast cancer with *BRCA1* promoter methylation. Breast Cancer Res Treat 2011, Aug;129(1):69-77.

69. Bosviel R, Garcia S, Lavediaux G, Michard E, Dravers M, Kwiatkowski F, et al. *BRCA1* promoter methylation in peripheral blood DNA was identified in sporadic breast cancer and controls. Cancer Epidemiol 2012, Jun;36(3):e177-82.

70. Tang Q, Cheng J, Cao X, Surowy H, Burwinkel B. Blood-based DNA methylation as biomarker for breast cancer: A systematic review. Clin Epigenetics 2016;8:115.

71. Semaan A, van Ellen A, Meller S, Bergheim D, Branchi V, Lingohr P, et al. *SEPT9* and *SHOX2* DNA methylation status and its utility in the diagnosis of colonic adenomas and colorectal adenocarcinomas. Clin Epigenetics 2016;8:100.

72. Grützmann R, Molnar B, Pilarsky C, Habermann JK, Schlag PM, Saeger HD, et al. Sensitive detection of colorectal cancer in peripheral blood by Septin 9 DNA methylation assay. PLoS One 2008;3(11):e3759.

73. Church TR, Wandell M, Lofton-Day C, Mongin SJ, Burger M, Payne SR, et al. Prospective evaluation of methylated *SEPT9* in plasma for detection of asymptomatic colorectal cancer. Gut 2014, Feb;63(2):317-25.

74. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990, Jun 1;61(5):759-67.

75. Wong Doo N, Makalic E, Joo JE, Vajdic CM, Schmidt DF, Wong EM, et al. Global measures of peripheral blood-derived DNA methylation as a risk factor in the development of mature B-cell neoplasms. Epigenomics 2016, Jan;8(1):55-66.

76. Hsiung DT, Marsit CJ, Houseman EA, Eddy K, Furniss CS, McClean MD, Kelsey KT. Global DNA methylation level in whole blood as a biomarker in head and neck squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev 2007, Jan;16(1):108-14.

77. Zhuang J, Jones A, Lee SH, Ng E, Fiegl H, Zikan M, et al. The dynamics and prognostic potential of DNA methylation changes at stem cell gene loci in women's cancer. PLoS Genet 2012, Feb;8(2):e1002517.

78. Yang Z, Wong A, Kuh D, Paul DS, Rakyan VK, Leslie RD, et al. Correlation of an epigenetic mitotic clock with cancer risk. Genome Biol 2016, Oct 3;17(1):205.

79. Tomasetti C, Vogelstein B. Cancer etiology. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. Science 2015, Jan 2;347(6217):78-81.

80. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol 2013;14(10):R115.

81. Galamb O, Kalmár A, Barták BK, Patai ÁV, Leiszter K, Péterfia B, et al. Aging related methylation influences the gene expression of key control genes in colorectal cancer and adenoma. World J Gastroenterol 2016, Dec 21;22(47):10325-40.

82. Horvath S, Erhart W, Brosch M, Ammerpohl O, von Schönfels W, Ahrens M, et al. Obesity accelerates epigenetic aging of human liver. Proc Natl Acad Sci U S A 2014, Oct 28;111(43):15538-43.

83. Horvath S, Levine AJ. HIV-1 infection accelerates age according to the epigenetic clock. J Infect Dis 2015, Nov 15;212(10):1563-73.

84. Horvath S, Ritz BR. Increased epigenetic age and granulocyte counts in the blood of Parkinson's disease patients. Aging (Albany NY) 2015, Dec;7(12):1130-42.

85. Marioni RE, Shah S, McRae AF, Chen BH, Colicino E, Harris SE, et al. DNA methylation age of blood predicts all-cause mortality in later life. Genome Biol 2015, Jan 30;16:25.

86. Levine ME, Hosgood HD, Chen B, Absher D, Assimes T, Horvath S. DNA methylation age of blood predicts future onset of lung cancer in the Women's Health Initiative. Aging (Albany NY) 2015, Sep;7(9):690-700.

87. Zheng Y, Joyce BT, Colicino E, Liu L, Zhang W, Dai Q, et al. Blood epigenetic age may predict cancer incidence and mortality. EBioMedicine 2016, Mar;5:68-73.

88. Perna L, Zhang Y, Mons U, Holleczek B, Saum KU, Brenner H. Epigenetic age acceleration predicts cancer, cardiovascular, and all-cause mortality in a German case cohort. Clin Epigenetics 2016;8:64.

89. Quach A, Levine ME, Tanaka T, Lu AT, Chen BH, Ferrucci L, et al. Epigenetic clock analysis of diet, exercise, education, and lifestyle factors. Aging (Albany NY) 2017, Feb 14;9(2):419-46.

90. Feng S, Xiong L, Ji Z, Cheng W, Yang H. Correlation between increased ND2 expression and demethylated displacement loop of mtdna in colorectal cancer. Mol Med Rep 2012, Jul;6(1):125-30.

91. Gao J, Wen S, Zhou H, Feng S. De-methylation of displacement loop of mitochondrial DNA is associated with increased mitochondrial copy number and nicotinamide adenine dinucleotide subunit 2 expression in colorectal cancer. Mol Med Rep 2015, Nov;12(5):7033-8.

92. Kulawiec M, Safina A, Desouki MM, Still I, Matsui S, Bakin A, Singh KK. Tumorigenic transformation of human breast epithelial cells induced by mitochondrial DNA depletion. Cancer Biol Ther 2008, Nov;7(11):1732-43.

93. Mawlood SK, Dennany L, Watson N, Dempster J, Pickard BS. Quantification of global mitochondrial DNA methylation levels and inverse correlation with age at two CpG sites. Aging (Albany NY) 2016, Apr;8(4):636-41.

94. Holmes AK, Koller KR, Kieszak SM, Sjodin A, Calafat AM, Sacco FD, et al. Case-control study of breast cancer and exposure to synthetic environmental chemicals among Alaska Native women. Int J Circumpolar Health 2014;73:25760.

95. Byun HM, Benachour N, Zalko D, Frisardi MC, Colicino E, Takser L, Baccarelli AA. Epigenetic effects of low perinatal doses of flame retardant BDE-47 on mitochondrial and nuclear genes in rat offspring. Toxicology 2015, Feb 3;328:152-9.

96. Yang L, Xia B, Yang X, Ding H, Wu D, Zhang H, et al. Mitochondrial DNA hypomethylation in chrome plating workers. Toxicol Lett 2016, Jan 22;243:1-6.

97. Janssen BG, Byun HM, Gyselaers W, Lefebvre W, Baccarelli AA, Nawrot TS. Placental mitochondrial methylation and exposure to airborne particulate matter in the early life environment: An ENVIRONAGE birth cohort study. Epigenetics 2015;10(6):536-44.

98. Tsuji J, Frith MC, Tomii K, Horton P. Mammalian NUMT insertion is non-random. Nucleic Acids Res 2012, Oct;40(18):9073-88.

99. Bollati V, Angelici L, Rizzo G, Pergoli L, Rota F, Hoxha M, et al. Microvesicle-associated microRNA expression is altered upon particulate matter exposure in healthy workers and in A549 cells. J Appl Toxicol 2015, Jan;35(1):59-67.

100. Pavanello S, Bonzini M, Angelici L, Motta V, Pergoli L, Hoxha M, et al. Extracellular vesicle-driven information mediates the long-term effects of particulate matter exposure on coagulation and inflammation pathways. Toxicol Lett 2016, Sep 30;259:143-50.

**ABBREVIATIONS**

Aryl hydrocarbon receptor repressor gene (*AHRR*)

Avon Longitudinal Study of Parents and Children (ALSPAC)

Adenomatous polyposis coli gene (*APC*)

Bisphenol A (BPA)

Breast cancer 1 gene (*BRCA1*)

p16 INK4a gene (*CDKN2A*)

European Childhood Obesity Project (CHOP)

Contactin associated protein-like 2 gene (*CNTNAP2*)

Cytochrome P450 family 1 member A1 gene (*CYP1A1*)

Displacement loop (D-loop)

Differentially methylated region (DMR)

DNA methyltransferase 3 alpha gene (*DNMT3A*)

DNA methyltransferase 3 beta gene (*Dnmt3b*)

Developmental Origins of Health and Disease (DOHaD)

Epithelial-mesenchymal transition (EMT)

Epigenetic Variable Outliers for Risk prediction Analysis (EVORA)

Coagulation factor II (thrombin) receptor-like 3 gene (*F2RL3*)

Growth factor receptor-bound protein 10 gene (*Grb10*)

Hereditary non-polyposis colorectal cancer (HPNCC)

Intercellular adhesion molecule-1 gene (*ICAM-1*)

Insulin-like growth factor gene (*IGF2*)

Interferon-gamma gene (*IFN*)

Long interspersed nuclear element-1 (LINE-1)

Loss of imprinting (LOI)

Mesoderm specific transcript gene (*Mest*)

MutL homolog 1 gene (*MLH1*)

MutS homolog 2 gene (*MSH2*)

MutS homolog 6 gene (*MSH6*)

Cytochrome c oxidase subunit II gene (*MT-CO2*)

Methylenetetrahydrofolate reductase gene (*MTHFR*)

Mitochondrially encoded 12S RNA gene (*MT-RNR1*)

Mitochondrially encoded TRNA phenylalanine gene (*MT-TF*)

Myosin 1G gene (*MYO1G*)

Nucleosome-binding protein 1 gene (*Nsbp1*)

Nuclear mitochondrial DNA sequences (numts)

Olfactory receptor 151 gene (*Olfr151*)

Phosphodiesterase type 4 variant 4 gene (*Pde4d4*)

Particulate matter (PM)

PMS1 homolog 2 gene (*PMS2*)

Ras association domain family 1 isoform A gene (*RASSF1A*)

Reactive oxygen species (ROS)

Septin 9 gene (*SEPT9*)

**FIGURE LEGENDS**

Table 1: Application of the ‘epigenetic clock’ model of aging to the study of disease. Summary of the major findings of age acceleration (epigenetic age > chronological age) in relation to human disease.

Figure 1: The influence of *in utero* exposures on three generations. Environmental exposures during pregnancy can induce epigenetic changes in the mother (F0), the fetus (F1), and the gametes of the fetus (F2). Studies of transgenerational inheritance of epigenetic traits therefore need to demonstrate effects beyond the F3 generation.

Table 1: Application of the ‘epigenetic clock’ model of aging to the study of disease.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Year** | **Disease** | **Tissue** | **Findings** | **Reference** |
| 2014 | Obesity | Liver, blood, adipose, muscle | Age acceleration confined to the liver (2.2 years / 10 BMI) | *82* |
| 2015 | HIV | Brain, blood | Mean increase in age of 7.4 years in the brain, 5.2 years in blood | *83* |
| 2015 | Parkinson’s | Blood | Significant age acceleration after adjustment for cell type composition | *84* |
| 2015 | Lung cancer | Blood | 1 year increase in age associated with 50% increase in cancer risk | *86* |
| 2015 | All-cause mortality | Blood | 5 year increase in age associated with 21% increase in mortality | *85* |
| 2016 | All cancer | Blood | 5 year increase in age associated with 22% increase in cancer mortality | *88* |
| 2017 | Lifestyle | Blood | Decreased aging with poultry consumption, increased aging with BMI | *89* |
| 2016 | All cancer | Blood | 1 year increase in age associated with 6% increase in risk of cancer incidence and 17% cancer mortality | *87* |