Published in final edited form as:

Nat Rev Cancer. ; 12(7): . doi:10.1038/nrc3220.

Developmental reprogramming of cancer susceptibility

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Abstract

Gene–environment interactions have been traditionally understood to promote the acquisition of mutations that drive multistage carcinogenesis, and, in the case of inherited defects in tumour suppressor genes, additional mutations are required for cancer development. However, the developmental origins of health and disease (DOHAD) hypothesis provides an alternative model whereby environmental exposures during development increase susceptibility to cancer in adulthood, not by inducing genetic mutations, but by reprogramming the epigenome. We hypothesize that this epigenetic reprogramming functions as a new type of gene–environment interaction by which environmental exposures target the epigenome to increase cancer susceptibility.

Cancer is now understood to have both a genetic and an environmental component. Studies of hereditary cancer syndromes, and targeted and genome-wide mutation analyses, have provided ample evidence for the participation of genetic alterations in carcinogenesis. Evidence for an environmental component in cancer aetiology has been gathered from studies that examine cancer incidence in twins and in families, as well as from population-based studies of environmental and occupational exposures. As highlighted in the recent President's Cancer Panel report¹, these studies have identified many human environmental carcinogens, including asbestos (mesothelioma), arsenic (skin cancer) and tobacco (lung, bladder and kidney cancer).

For the vast majority of cancers, both genes and the environment have prominent roles. For example, in breast cancer, inherited genetic factors, such as alterations in *BRCA1* and *BRCA2* (which account for 5–10% of breast cancers)², and environmental factors, such as exogenous hormone exposure (for example, hormone replacement therapy), contribute to the risk of developing several forms of this disease. Exposure to tobacco carcinogens can induce mutations in genes such as *TP53* (which encodes p53) in lung cancer; and defects in several genes, such as adenomatous polyposis coli (*APC*), *KRAS* and *TP53*, contribute to multistage carcinogenesis of the colon, as does a diet high in fat and red meat. It is thought that approximately 40% of the world's cancer burden is caused by avoidable environmental

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Competing interests statement

The authors declare no competing financial interests.

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exposures to carcinogens, including ultraviolet (UV) radiation, ionizing radiation, viruses, radon gas, alcohol and obesity³.

Gene–environment interactions have been traditionally understood to describe mechanisms whereby individual genetic risk factors modify the effects of environmental exposures to increase or to decrease cancer risk, with gene–environment interactions thought to be a major determinant of individual risk for this disease⁴. Typically, gene–environment interaction refers to an increased (or a decreased) sensitivity to an environmental carcinogen owing to a specific germline alteration that is carried by exposed individuals, often as a single nucleotide polymorphism (SNP). This is best illustrated in colon cancer, in which hereditary defects in DNA mismatch repair genes such as *MSH2* decrease repair efficiency, which enhances the efficacy of genotoxic carcinogens, induces microsatellite instability and predisposes to hereditary non-polyposis colorectal cancer (HNPCC). Similarly, SNPs in genes that encode metabolic enzymes, such as *N*-acetyltransferase 1 (*NAT1*) and *NAT2*, which are involved in the activation (for example, polycyclic aromatic hydrocarbons (PAHs) in the colon) or the detoxication (such as arylamines in the bladder) of carcinogens can increase or decrease the potency of these carcinogens. Even in familial cancer syndromes, such as hereditary breast and ovarian cancer caused by germline mutations in *BRCA1* or *BRCA2*, the predisposing genetic alteration is highly, although not fully, penetrant (that is, not all BRCA-mutation carriers develop breast or ovarian cancer)^{5,6}. Although an inherited defect in a tumour suppressor gene remains the strongest known risk factor for cancer, the penetrance of tumour suppressor gene defects, such as BRCA mutations, remains variable among families and across generations⁵. In such settings, environmental exposures are thought to be an important determinant of penetrance by inducing ‘second hits’ in the normal allele of the tumour suppressor gene (which induces the loss of gene function) or additional mutations in other genes that are required for multistage carcinogenesis. Thus, for gene–environment interactions, in the setting of both highly prevalent, low penetrant genetic alterations (such as SNPs), and low prevalence, high penetrance genetic alterations (such as tumour suppressor gene defects), our understanding of how environmental factors contribute to cancer development has focused on how these exposures promote the acquisition of additional genetic alterations that participate in multistage carcinogenesis.

The DOHAD hypothesis

Although the aforementioned studies have been highly informative and have generated many mechanistic insights into multistage carcinogenesis, they have so far provided little insight into how the epigenome might participate in gene–environment interactions. Recently, however, clear evidence has emerged that the exposure of developing tissues or organs to an adverse stimulus or insult during crucial periods of development can increase the risk of many diseases, including cancer, later in life^{7,8}.

During development, organogenesis and tissue differentiation occur through a continuous series of tightly regulated and precisely timed molecular, biochemical and cellular events. It is now being appreciated that adverse environmental exposures during this period can have life-long consequences for exposed individuals. For example, congenital limb malformations can be caused by exposure of the developing foetus to thalidomide; folate deficiency *in utero* is associated with spina bifida; and maternal alcohol consumption can cause a host of neurological deficits that are associated with foetal alcohol syndrome⁹. Although the effect of these environmental exposures manifests at birth as congenital abnormalities, it is also becoming clear that environmental exposures can have adverse effects at the cellular and molecular levels that may be undetectable at birth, but that may nonetheless have a profound effect on the health of an individual throughout their life.

The developmental origins of health and disease (DOHAD) hypothesis posits that increased risk of disease in adulthood can result from an adverse developmental environment that reprogrammes cellular and tissue responses to normal physiological signals in a manner that increases disease susceptibility¹⁰. The link between the early environment and adult disease was first noted by Ravelli and colleagues¹¹, who reported increased rates of obesity in individuals that were exposed to famine *in utero*. As reported in this and subsequent studies, maternal starvation during the Dutch 'hunger winter' of the Second World War correlated with an increased risk in exposed offspring of cardiovascular disease and metabolic diseases such as obesity, metabolic syndrome and diabetes in adulthood^{12–14}. In the 1980s, using birth weight as a surrogate for poor *in utero* nutrition, Barker reported a similar link between birth weight and increased coronary heart disease in adulthood¹⁵. This initial report and later confirmatory studies¹⁰ found that infants with the lowest birth weights had the highest rates of coronary heart disease, hypertension and stroke as adults. We have since learned that birth weight per se is not responsible for increased disease risk, but rather it is a surrogate for a poor *in utero* nutritional environment^{16,17}. For example, infants whose mothers were exposed to famine only during the first trimester were not born smaller or lighter than non-exposed infants, but became more obese, had higher incidences of coronary heart disease and had poorer health as adults^{12,13,18}. In the context of the DOHAD hypothesis, the mechanism responsible for the increased risk of disease in adulthood is not thought to be mutation of the genome, but reprogramming of the epigenome.

Epigenetics is used to describe heritable changes in gene expression that are not caused by alterations in the nucleotide sequence of the genome. From its earliest stages, development is directed by epigenetic programmes that are 'installed' on the genome by epigenetic 'writers', such as histone methyltransferases (HMTs) and DNA methyltransferases (DNMTs), which add methyl groups to lysine and arginine residues on histone tails and to CpG sites in DNA, respectively^{19,20}. In the case of histone modifications, the epigenetic programmes that are installed by these writers form a 'histone code' that is interpreted by 'readers' (which are effector molecules that recognize methylated arginine and lysine residues of histones) and that is modified by 'erasers' (histone demethylases). Histones H3 and H4 are the primary targets for methylation, and the methyl marks are by convention denoted by specific lysine or arginine residues that are monomethylated, dimethylated or trimethylated. Histone methyl marks both activate (for example, H3K4 dimethylation) and repress (for example, H3K27 trimethylation) gene expression. Gene-specific patterns of histone modifications can generate binding sites for histone code readers, such as proteins containing plant homeodomain (PHD) domains, as well as other epigenetic writers, such as DNMTs^{21–25}.

It has now begun to be appreciated that developmental programming of the epigenome exhibits a high degree of plasticity, and that it is modifiable by both extrinsic factors, such as environmental chemicals, and intrinsic factors, such as maternal influences²⁶. During the early stages of embryogenesis, DNA methylation is dynamic, with methylation patterns erased and re-established on both the maternal and paternal germline, albeit with different kinetics²⁷. Prenatal exposure to the famine of the Dutch hunger winter and season of conception in areas of rural Gambia that experience dramatic seasonal fluctuations in nutritional status have both been associated with epigenetic changes at specific gene loci in affected individuals^{28,29}. Histone methylation is also dynamic. In the case of the collinear HOX genes — for example, where gene expression is under both temporal and spatial control — deposition of both repressive and activating methyl marks by Polycomb and trithorax HMT complexes, respectively, during development induces dynamic changes in chromatin modifications that repress or activate the expression of specific HOX genes to regulate patterning in the developing embryo³⁰. This process of developmental programming of the epigenome continues through organogenesis, which, in some tissues

and organs, such as the breast (humans and rodents), reproductive tract and kidney (rodents), may extend into the perinatal, early childhood and peripubertal periods.

It is thought that this plasticity affords opportunities for modifying epigenetic programming in response to environmental cues and thus can assist the developing organism in preparing for the adult environment. For example, in the case of maternal starvation, developmental programming *in utero* could function to regulate physiological ‘set points’ in such a manner as to prepare the offspring for an environment in which nutrients are in short supply, thus providing a survival advantage. However, in settings in which the adult environment is inconsistent with the fetal environment — for example, nutrient deficit *in utero* and nutrient abundance in adulthood — it is hypothesized that this disconnection can lead to metabolic diseases in adulthood, such as obesity and type II diabetes. This has given rise to what was coined by Hales and Barker as the “thrifty phenotype hypothesis” (REF. 31) (FIG. 1).

In addition, the effect of such epigenetic reprogramming in early life may remain dormant until engaged in response to later-life adult events³². An example is altered DNA methylation that is observed in the uterus in response to *in utero* exposure to the xenoestrogen diethylstilbestrol (DES). The adult uterus of an animal exposed to this xenoestrogen *in utero* exhibits alterations in the methylation of the lactotransferrin (*Ltf*) gene after puberty³³. If ovariectomized, the aberrant DNA methylation that is observed in intact animals is not observed in the castrated female uterus. Similarly, early life exposure to DES or to the phytoestrogen genistein can reprogramme high-mobility group nucleosome-binding domain 5 (*Hmgn5*; also known as *Nsfp1*) gene methylation, causing the promoter of this gene to become hypomethylated in the adult uterus, which increases gene expression³². However, if genistein-exposed animals are ovariectomized, aberrant hypomethylation of the *Hmgn5* promoter does not occur. Therefore, although some developmental reprogramming can be observed immediately after exposure (discussed below), both aberrant epigenetic programming and altered disease susceptibility may manifest only later in life, long after the environmental exposure occurred. The mechanisms that are responsible for such dormancy are far from clear. However, one possibility is that the initial reprogramming of the developing epigenome targets histone methylation that serves to direct later life changes in DNA methylation at CpG sites — for example, in response to hormone exposure during puberty. In such a situation, altered patterns of DNA methylation (directed by the reprogrammed aberrant histone methylation) would not manifest until after puberty when changes in CpG methylation would normally occur (FIG. 2).

Importantly, the installation of epigenetic programmes that direct cell-specific differentiation during development is unidirectional, with development always moving inextricably forwards. If an adverse environmental exposure is encountered during this crucial ‘install phase’, faulty epigenetic programming may be imparted on the genome of the developing organism, or the epigenome may be reprogrammed in a manner that alters the response to normal physiological signals in adulthood, thus promoting the development of disease later in life. The potential exists for even a brief exposure to an environmental agent to disrupt epigenetic programming during development, and reprogramme the epigenome for life. An example is the reprogramming of the fetal epigenome that is observed in response to maternal dietary intake of methyl donors. During *in utero* development, the formation of *S*-adenosylmethionine — the methyl donor for both histone and DNA methylation — is dependent on methyl groups obtained from the maternal diet. Studies from the Jirtle laboratory and others using mutant agouti mice have convincingly demonstrated that changing the *in utero* environment by increasing or decreasing maternal dietary intake of the methyl donors methionine and choline, or folate (which participates in one-carbon metabolism that generates *S*-adenosylmethionine), increases or decreases DNA methylation,

respectively, in the genome of the offspring. This consequently alters patterns of DNA methylation, coat colour and susceptibility to obesity³⁴. Although this effect has been best studied at the metastable agouti viable yellow (*Avy*) locus in mice, methylation at both metastable alleles and imprinting of homologous loci in humans can also be modulated in response to dietary folate levels^{29,35,36}.

Mechanisms of epigenetic reprogramming

If developmental exposures are reprogramming the epigenome to increase susceptibility to diseases such as cancer, the effects of this reprogramming should be apparent in the 'normal' tissue prior to the development of disease. Ample evidence exists that developmental reprogramming induces epigenetic changes that can be detected in at-risk tissues prior to the development of tumours (TABLE 1). In the reproductive tract, examples of genes in which developmental reprogramming induces persistent alterations in DNA methylation include *FOS*, *LTF*, homeobox A10 (*HOXA10*), *HMG5* and phosphodiesterase 4D variant 4 (*PDE4D4*)^{32,33,37–40}. Early studies demonstrated that neonatal DES exposure induced aberrant methylation of specific CpG sites in *Ltf*³³ and *Fos*³⁸ in the mouse uterus. More recently, developmental exposure to several endocrine-disrupting compounds (EDCs), including xenoestrogens, was found to modulate the expression and promoter methylation of HOX genes, which have key roles in uterine development. Exposure to DES *in utero* results in increased promoter methylation and decreased *HOXA10* expression³⁹, whereas bisphenol A (BPA) exposure results in decreased methylation of *HOXA10*, increased oestrogen receptor (ER) binding by *HOXA10* and increased *HOXA10* expression in the adult uterus⁴¹.

Similarly, neonatal exposure of mice to DES or genistein also induces persistent hypomethylation of the *Hmgn5* promoter and aberrant overexpression of this gene in the uterus throughout life, and exposure is associated with an increased risk of developing uterine tumours in adult female mice³². Additional studies have demonstrated that exposure of the developing uterus to environmental oestrogens reprogrammed many oestrogen-responsive genes, including S100 calcium-binding protein G (*S100G*; also known as *CALB3*), glutamate receptor ionotropic AMPA2 (*GRIA2*), growth differentiation factor 10 (*GDF10*) and matrix metalloproteinase 3 (*MMP3*), causing them to become hyper-responsive to oestrogen^{42,43}. As described above, increased gene expression as a result of this epigenetic reprogramming in early life may be triggered by later-life events such as the presence of ovarian steroid hormones during puberty. In these settings, ovariectomy before puberty completely eliminates the effect of epigenetic reprogramming on gene expression and uterine tumour development, pointing to the inter-dependency between early life reprogramming and later-life events. In the prostate, *PDE4D4*, the gene product of which regulates intracellular levels of cyclic AMP (cAMP), is developmentally reprogrammed in response to perinatal xenoestrogen exposure⁴⁰. In the normal adult prostate, *PDE4D4* is gradually hypermethylated with age. However, this gene undergoes persistent hypomethylation in the adult prostate of animals that are neonatally exposed to oestradiol or to BPA. This epigenetic reprogramming results in the overexpression of *PDE4D4* and is correlated with increased susceptibility to developing precancerous lesions in the prostate⁴⁰. Although these epigenetic changes correlate with altered gene expression and increased susceptibility to cancer in the developmentally reprogrammed tissues, whether they are biomarkers of developmental reprogramming (as is the case for *LTF*) or contribute to increased cancer susceptibility (as might be the case for *PDE4D4*) remains to be fully explored.

Reprogramming cancer susceptibility

Importantly, evidence is now emerging that, in addition to cardiovascular and metabolic diseases, developmental reprogramming can also influence the risk of developing cancer. To date, hormone-dependent cancers of the male and female reproductive tracts and breast cancer have provided the strongest evidence of developmental reprogramming of cancer susceptibility by early life exposures, providing support for the DOHAD hypothesis in cancer.

Effects of the maternal environment on cancer risk

Human epidemiological studies show a positive association between birth weight and adult breast cancer risk. Increased birth weight (a surrogate for over-nutrition *in utero*) positively correlates with increased risk for breast cancer, especially in premenopausal women. By contrast, low birth weight and maternal pre-eclampsia (hypertension, fluid retention, increased weight gain and protein in urine) are associated with decreased breast cancer risk^{44–46}. High circulating hormone levels at puberty (ascertained by large pelvic intercrystal diameter) have been linked to increased breast cancer in daughters⁴⁷, as has prostate cancer risk in sons born before 40 weeks gestation⁴⁸. In the male reproductive tract, prostate cancer has been linked to high birth weight, which increases the risk of more aggressive disease^{49–51}. In addition, *in utero* exposure to high levels of endogenous pregnancy hormones, including testosterone, oestrogen and insulin-like growth factor 1 (IGF1) — which contribute to high birth weight, jaundice and later increased adult height — are linked to an increased risk of prostate cancer and/or more aggressive disease. Recently, maternal steroid hormones, specifically testosterone, were found to be elevated in the cord blood of African Americans, and this correlated with increased prostate cancer risk in male offspring, whereas no such correlation was observed in European Americans⁵². Differences in maternal levels of IGF1 and IGF2 might also contribute to the ethnic disparity in prostate cancer prevalence between African Americans and European Americans.

Recently, the maternal environment has been linked to the development of both lung and breast cancer. In the case of lung cancer, evidence has emerged that both the size of the fetus (high ponderal index equals birth weight divided by length³) and the placental size (surface area) correlate with increased risk for this disease^{53,54}. As a result of these and other investigations, the possible role of ‘placental programming’ in determining individual disease risk in adulthood is rapidly emerging as one of the central themes in DOHAD research.

Early life environmental exposures and reproductive tract cancer

The xenoestrogen DES, a synthetic stilbene oestrogen, was administered to pregnant women from the 1940s to the 1970s to prevent complications of pregnancy. In the early 1970s, the daughters of women who took DES during the first trimester (so-called ‘DES daughters’) were frequently diagnosed with congenital reproductive tract abnormalities (specifically, a ‘T-shaped’ uterus), dysplasia and cervical intraepithelial neoplasia, and with a significantly increased rate of an otherwise rare type of vaginal clear cell adenocarcinoma⁵⁵. Continued follow-up of DES daughters has now revealed that they also have an increased relative risk for developing breast cancer (~twofold to threefold that of unexposed women) and uterine leiomyoma, although not all data on this point are concordant^{56–59}. DES sons may also be at a higher risk of developing prostate cancer; however, the final verdict on this point requires additional investigation⁶⁰.

Animal studies that simulate the human DES experience show that exposure of the developing reproductive tract to DES and other xenoestrogens imparts a permanent

oestrogen imprint that alters reproductive tract morphology, induces persistent expression of oestrogen-responsive genes and induces a high incidence of uterine adenocarcinoma^{38,61,62}. Uterine leiomyoma, sometimes referred to as fibroids, arise from the smooth muscle layer of the uterus and are the most common benign tumour in women⁶³. In rats carrying a genetic defect in the tuberous sclerosis 2 (*Tsc2*) tumour suppressor gene, exposure to DES during uterine development causes the tumour suppressor defect to become fully penetrant, increasing tumour incidence from 65% to 100%⁴² (discussed below). This increased penetrance is associated with the reprogramming of oestrogen-responsive genes, which become hyper-responsive to hormone and promote the development of hormone-dependent uterine leiomyomas⁴³. DES exposure during this crucial perinatal period of development also modulates IGF signalling in the adult endometrium, decreasing negative feedback to insulin receptor substrate 1 (IRS1) and increasing the incidence of endometrial hyperplasia in rats that are genetically predisposed to develop these preneoplastic lesions^{64,65}.

Inappropriate exposure to environmental oestrogens, such as DES, the plasticizer BPA or the soy phytoestrogen genistein, during mammary gland development alters the susceptibility of the mammary gland to chemical carcinogenesis in adulthood⁶⁶. The mammary gland begins developing *in utero* and is not fully mature until after pregnancy and lactation⁶⁷. Importantly, the effect of xenoestrogen exposure during mammary gland development differs throughout life⁶⁸. Xenoestrogen exposure *in utero* can increase the risk for mammary tumorigenesis, in part by altering mammary gland architecture and increasing the number of target cells for transformation in terminal end buds (TEBs) of the mammary gland⁸². Conversely, xenoestrogen exposure during the postnatal period can decrease susceptibility to mammary gland tumorigenesis by inducing a programme of differentiation in mammary epithelial cells that mimics the protective effects of pregnancy. In addition to xenoestrogen exposure, there is a suggestion that prenatal famine may increase breast cancer incidence in exposed individuals⁶⁹.

Testicular cancer, one of the features of testicular dysgenesis syndrome (TDS), which includes poor semen quality, undescended testis and hypospadias, has also been linked to early life environmental exposures. Experimental studies from animal models and human epidemiology support a causal association between TDS and exposure to compounds that disrupt the endocrine system, such as DES, and anti-androgens, such as vinclozolin, during male reproductive tract development⁷⁰. In humans, the majority of testicular cancers are derived from germ cells, and epidemiology data suggest *in utero* DES exposure may be a risk factor⁷¹. Testicular cancer is preceded by carcinoma *in situ*, and recent studies suggest that these lesions have a gene expression pattern that is similar to gonocytes (spermatogonia)⁷² and exhibit DNA hypomethylation and altered methylation of histones H3K9, H3K27 and H3K4, which is analogous to the epigenetic pattern that is seen in fetal germ cells⁷³. These data have led to the suggestion that germ cells in the developing testis are the target for the developmental reprogramming that is associated with TDS.

The maternal environment might also play a part in susceptibility to testicular cancer. Low birth weight is a risk factor for testicular germ-cell cancer (which comprises more than 95% of testicular cancer)⁷⁴, and a recent meta-analysis of 18 epidemiological studies indicated that low birth weight is also a risk factor for testicular cancer⁷⁵. Although germ-cell testicular cancer is very rare in experimental animal models, by contrast, interstitial cancers, benign tumours and adenocarcinoma of the rete testis are frequently observed in rodent testes that have been prenatally exposed to DES^{76,77}. In the prostate, early life exposure of rodents to xenoestrogens such as methoxychlor, BPA or genistein leads to prostate hypertrophy and increased inflammation with age in the prostate of adult animals⁷⁸ and can also predispose to prostate carcinogenesis⁴⁰.

A new gene–environment interaction?

Studies in Eker rats with a defect in *Tsc2* first pointed to the fact that exposure to environmental oestrogens during development could cooperate with a tumour suppressor gene defect to increase the penetrance of the defective tumour suppressor gene⁴². In this model, brief neonatal exposure to environmental oestrogens, such as DES, during uterine development significantly increased tumour incidence (that is, penetrance), as well as tumour multiplicity and size. Neonatal xenoestrogen exposure also resulted in the reprogramming of oestrogen-responsive gene expression, which manifested as an increased expression of oestrogen-responsive genes in the adult myometrium at 5 months of age, many months prior to tumour development. This suggests that the combined increase in oestrogen responsiveness (reprogramming) and the defect in *Tsc2* promoted the development of hormone-dependent leiomyoma, effectively increasing tumour suppressor gene penetrance^{42,43}. Importantly, in the absence of the tumour suppressor gene defect, environmental oestrogen exposure alone failed to induce tumours⁴², even though gene expression was reprogrammed (K. L. Greathouse and C.L.W., unpublished observations). Conversely, ovariectomy almost completely ablated tumour development in genetically predisposed animals, indicating that, in the absence of ovarian hormones, the tumour suppressor gene defect was not sufficient to induce tumorigenesis⁴³. Thus, developmental reprogramming can cooperate with a tumour suppressor gene defect to increase its penetrance, thereby functioning as a new type of gene–environment interaction.

Recently, a direct mechanism by which environmental oestrogens can disrupt the epigenetic machinery of a cell during developmental reprogramming was identified⁷⁹ (FIG. 3). Environmental oestrogens can bind to membrane-associated ER to activate rapid, non-genomic (or more appropriately pre-genomic) ER signalling. Among the targets for this non-genomic signalling are epigenomic writers such as the HMT enhancer of zeste homologue 2 (EZH2), which is a member of the Polycomb repressive complex⁸⁰. This pre-genomic signalling activates PI3K signalling and the kinase AKT. Phosphorylation of serine 21 of EZH2 by AKT inactivates EZH2 and reduces the levels of the repressive trimethylation of H3K27 in the developing uterus. This rapid non-genomic ER signalling provides a mechanism by which environmental oestrogens can inappropriately activate kinases such as AKT (and probably others such as ERK in the MAPK pathway) to modulate the activity of HMTs and to disrupt the epigenetic machinery during developmental reprogramming. Although EZH2 is the only epigenetic target modulated by non-genomic signalling in response to environmental agents that has been identified to date, it is likely that other readers, writers and erasers of the epigenome can also be affected, as many of them contain consensus motifs for phosphorylation by AKT, ERK and other kinases (M. T. Bedford and C.L.W., unpublished observations).

As a result of this developmental reprogramming, oestrogen-responsive genes in the uterus become hyper-sensitive to hormone, which promotes tumour development in adult animals that are neonatally exposed to environmental oestrogens (FIG. 4). In Eker rats that are neonatally exposed to DES, more than 50% of the oestrogen-responsive genes that have been examined in the adult uterus displayed an inappropriate, exaggerated response to steroid hormones: reprogrammed genes were overexpressed during the oestrus cycle when oestrogen levels were high, and remained elevated even during periods of the oestrous cycle when hormones were at their lowest. As a result, the uterus displayed an ‘oestrogenized phenotype’ that cooperated with the tumour suppressor gene defect to increase tumour suppressor gene penetrance. Together, these data lead us to propose that developmental reprogramming is a type of gene–environment interaction that can cooperate with a genetic predisposition, not by inducing mutations, but by reprogramming the epigenome to modulate gene expression in order to promote tumour development.

The timing of exposure during development also seems to be an important determinant for developmental reprogramming by environmental agents. For some tissues, such as the breast, which continues to develop well into adulthood, this window of susceptibility might be quite large, stretching from *in utero* to the first full-term pregnancy. In the uterus, development is completed in the first trimester in humans, and in the first few weeks of neonatal life in rodents. Although it is not known when the window of susceptibility to developmental reprogramming begins, we have found in the rat that the window of susceptibility in the uterus closes around post-natal day 17 (REF. 81). Rats exposed to xenoestrogens prior to day 17 exhibit developmental reprogramming of oestrogen-responsive genes and increased tumour suppressor gene penetrance, whereas neonates exposed after this time do not become developmentally reprogrammed nor do they exhibit any increase in tumour suppressor gene penetrance.

It remains an open question as to what defines the window of susceptibility for developmental reprogramming, which will probably exhibit both species specificity and tissue specificity. In rodents, the female reproductive tract develops in an oestrogen-naïve environment; high levels of steroid hormone-binding proteins such as α -fetoprotein (AFP) protect the developing uterus from maternal hormones of pregnancy, and from oestrogen produced by organs such as the adrenal gland during neonatal life. Xenoestrogens are not recognized by these steroid hormone-binding proteins, and thus evade this defence system. The timing for window 'closure' coincides with the period in rodents when the liver stops producing AFP and steroid hormone-binding proteins are cleared from the neonate; after this time, cells and tissues are exposed to low levels of circulating endogenous oestrogens. It is tempting to speculate that this window of susceptibility for developmental reprogramming is defined as the period when the uterus would normally be developing in an oestrogen-naïve environment, and that after day 17, the uterus is ready to experience oestrogens, and, therefore, neither endogenous oestrogen nor xenoestrogen exposure disrupts epigenetic programming.

Conclusions and perspectives

There is an emerging consensus that development is a time of increased susceptibility to the effects of environmental agents, and that reprogramming of the epigenome by environmental exposures early in life can determine the risk of many adult diseases, including cancer, decades before disease onset. For metabolic diseases, these exposures primarily occur at the maternal–fetal interface; for example, maternal starvation or obesity. But for cancer, exposures to extrinsic environmental agents, such as endocrine-disrupting compounds, also seem to have prominent roles. The contribution of developmental reprogramming to the risk of cancers that are not hormonally regulated is currently mostly unknown, although lung cancer is emerging as one such candidate^{53,54}. It is also an open question as to whether other types of environmental exposures — for example, exposure to classic genotoxic carcinogens such as ionizing radiation or nitrosoamines — can contribute to cancer risk not just via mutations, but via developmental reprogramming of the epigenome.

In genetically susceptible individuals, developmental reprogramming represents a new type of gene–environment interaction that can promote tumour development by reprogramming gene expression in a way that cooperates with a genetic predisposition to promote tumorigenesis. Although existing data provide proof-of-concept that reprogramming of the epigenome can interact with a tumour suppressor gene defect to increase risk, it is likely that alterations in the epigenome can cooperate with other less penetrant, more prevalent genetic defects such as SNPs to similarly increase cancer risk. Importantly, the effect of developmental reprogramming on cancer susceptibility may not be evident for many years

or even decades after the initial environmental exposure. As a result, our ability to recognize (and to measure) specific environmental exposures that contribute to adult cancer risk is severely limited by studies that focus only on adult exposures. The identification of the epigenetic alterations that are induced by developmental reprogramming that cooperate with inherited genetic defects will open new avenues for developing biomarkers to identify individuals who are at an increased risk of developing cancer as a result of early life environmental exposures. In this regard, one of the next challenges in this field lies in the area of exposure assessment. We must develop the capability to measure environmental exposures throughout life in order to identify environmental agents that can reprogramme the epigenome and adversely affect cancer risk during developmental windows of susceptibility.

Finally, additional insights into how developmental reprogramming increases cancer risk holds promise for interventions to reverse the effect of this new type of gene–environment interaction. In contrast to germline alterations in tumour suppressor genes and cancer-associated SNPs, which are generally irreversible, epigenetic alterations that are induced by developmental reprogramming are potentially reversible with epigenetic therapies. By increasing our knowledge regarding how epigenetic reprogramming increases cancer risk, we may not only be able to better identify who has an increased risk of developing cancer from early life environmental exposures, but may also be able to develop interventions that can reverse the epigenetic effects of developmental reprogramming to decrease cancer risk associated with this type of gene–environment interaction.

Acknowledgments

C.L.W. is supported by grants from the US National Institutes of Health (ES018789, ES008263 and ES020055) and the Cancer Prevention and Research Institute of Texas (CPRIT RP120855). S.-M.H. is supported by grants from the NIH (P30ES006096, U01ES019480, R01ES015584, RC2ES018758 and RC2ES018789) and a VA award (I01BX000675).

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Figure 1. The thrifty phenotype hypothesis

Plasticity of the epigenome during development affords an opportunity for the developing organism to ‘pre-adapt’ to the future adult environment, which provides a survival advantage. However, in settings in which the fetal environment does not match the adult environment — for example, fetal development in a nutrient-poor environment (such as maternal starvation) coupled with a nutrient-rich adult environment — the resulting ‘catch-up’ growth and disconnection between fetal programming and the adult environment can predispose to adult metabolic disease, including obesity and type II diabetes.

Figure 2. Developmental reprogramming dormancy

Programming of the epigenome primarily occurs during development, although some epigenetic programmes are executed in response to later-life cues such as puberty. As a result, epigenetic reprogramming by environmental oestrogens may remain dormant until after these cues later in life, such as until after puberty. In the reproductive tract of intact animals that are perinatally exposed to environmental oestrogens, lactotransferrin (*Ltf*) and high-mobility group nucleosome-binding domain 5 (*Hmgn5*) become hypomethylated, which increases their expression in adult animals. However, in the absence of hormone (that is, castration), in adult animals that are neonatally exposed to environmental oestrogens, these target genes remain hypermethylated, which decreases their expression.

Figure 3. Model of the engagement of the epigenetic machinery by environmental oestrogens via non-genomic ER signalling

Environmental oestrogens can activate non-genomic oestrogen receptor (ER) signalling pathways in cells during developmental reprogramming. These signalling cascades target epigenetic 'readers', 'writers' and 'erasers' for phosphorylation (P) (and perhaps other post-translational modifications), modulating their activity and disrupting epigenetic programming. In the case of the histone methyltransferase (HMT) enhancer of zeste homologue 2 (EZH2), phosphorylation by AKT inhibits its ability to methylate histone H3, which reduces the levels of repressive tri-methylation of H3K27 in the developing uterus. This developmental reprogramming results in elevated expression of oestrogen-responsive genes in the adult uterus, promoting tumorigenesis. Although we know little about how post-translational modifications might affect the activity of other readers, writers and erasers, it is possible that non-genomic signalling could also increase their activity. Depending on whether the effect is on a repressive or an activating methyl mark, the resulting reprogramming could decrease or increase gene expression in the adult tissue

Figure 4. Developmental reprogramming of the penetrance of defects in tumour suppressor genes

Developmental exposure to xenoestrogens disrupts the epigenetic machinery in the cell. **a** | In animals carrying a defect in the tuberous sclerosis 2 (*Tsc2*) tumour suppressor gene, this 'oestrogenized' phenotype in the adult uterus promotes tumour development in genetically susceptible animals, which increases the penetrance of the *Tsc2* defect from 65% to 100%. **b** | This reprogramming may act via alterations in both histone and DNA methylation. For example, diethylstilbestrol (DES) exposure inhibits enhancer of zeste homologue 2 (EZH2) and decreases the levels of repressive trimethylation of H3K27. As a result, hormone-responsive genes in the uterus become hyper-responsive to hormone, exhibiting significantly

elevated expression to even low levels of steroid hormones, which again increases the penetrance of tumour susceptibility.

Table 1

Developmentally reprogrammed genes

Gene	Tissue or organ	Exposure	Reprogrammed phenotype	Refs
<i>HOXA10</i>	Uterus	BPA	Hypomethylation and constitutive expression	39,41
		DES	Hypermethylation and reduced expression	
<i>PDE4D4</i>	Prostate	BPA	Elevated expression	40
<i>LTF</i>	Uterus	DES	Elevated expression	33,37
<i>FOS</i>	Uterus	DES	Elevated expression	37,38
<i>HMGN5</i>	Uterus	DES or genistein	Elevated expression	32
<i>S100G</i>	Uterus	DES	Hyper-responsiveness to oestrogen	42,43
<i>GRIA2</i>	Uterus	DES	Hyper-responsiveness to oestrogen	42,43
<i>GDF10</i>	Uterus	DES	Hyper-responsiveness to oestrogen	42,43
<i>MMP3</i>	Uterus	DES	Hyper-responsiveness to oestrogen	42,43

BPA, bisphenol A; DES, diethylstilbestrol; *GDF10*, growth differentiation factor 10; *GRIA2*, glutamate receptor ionotropic AMPA2; *HMGN5*, high-mobility group nucleosome-binding domain 5; *HOXA10*, homeobox A10; *LTF*, lactotransferrin; *MMP3*, matrix metalloproteinase 3; *PDE4D4*, phosphodiesterase 4D variant 4; *S100G*, S100 calcium-binding protein G.