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CANCER (MF LEITZMANN, SECTION EDITOR)

The Link Between Early Life Nutrition and Cancer Risk

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Abstract Traditionally, cancer has been considered a disease caused by genetic alterations. However, there is growing evidence that the environment, particularly a person's early life environment, can influence cancer risk. The mechanism by which the environment has been suggested to influence cancer risk is through the altered epigenetic regulation of genes. Epigenetic processes, which include DNA methylation, induce stable changes in gene expression without altering the gene sequence. A number of environmental factors, including nutrition, have been shown to alter the epigenome, leading to long term changes in gene expression and an altered susceptibility to disease. Using evidence from epidemiological and experimental studies, this review will discuss the hypothesis that changes in diet during early development can lead to an altered susceptibility to cancer as the result of modified epigenetic regulation of genes.

Keywords Epigenetics · Nutrition · Transcription · Early life

Introduction

Traditionally, it has been widely accepted that cancer is caused by genetic alterations, such as mutations, translocations, insertions, or deletions in our DNA. However, there is now emerging evidence that some cancers, including breast cancer, may originate in early life. Epidemiological studies have shown an association between early life environment and an altered risk of breast cancer in later life. These findings have also been replicated in a variety of animal models where both undernutrition and overnutrition have been shown to influence the risk of cancer susceptibility in the offspring. The underlying mechanism by which early life environment can influence cancer risk has been suggested to involve the altered epigenetic regulation of genes. Recent studies have shown that environmental factors in early life can induce changes in the epigenome, which are then stably maintained through the lifecourse, suggesting that early life nutrition may modulate cancer risk by inducing persistent epigenetic changes that alter mammary gland development or structure, increasing later susceptibility to disease. This article will review the evidence that variations in the early life environment, particularly nutrition, can modify cancer risk through induced epigenetic changes in developing offspring.

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Early Life Nutrition and Cancer Risk

The developmental origins of health and disease (DOHaD) hypothesis is derived from epidemiological studies performed by David Barker and colleagues [1]. This hypothesis suggests that the quality of the early life environment is associated with the subsequent risk of developing chronic diseases in later life. Subsequently, this hypothesis has been supported by numerous studies in human populations that have shown low birth weight to be associated with increased risk of non-communicable diseases in later life, such as type II diabetes, obesity, and cardiovascular disease (CVD) [2], This hypothesis has also been supported by studies in animal models, whereby variations in maternal diet such as high fat or low

protein resulted in offspring that developed similar features to human cardio-metabolic disease [3].

Contrary to work on the developmental origins of metabolic diseases, the relationship between early life environment and cancer risk has not been as well characterised. Most studies have focussed on the associations between birth weight (BW) and later cancer risk [4]. However, comparisons between such studies are difficult since they differ greatly in design, for example, with respect to the number of cases analysed, whether pre- or post-menopausal women were studied together or separately, and whether other variables were included in the analysis. A meta-analysis using data from 26 studies showed that increasing birth weight led to an increasing risk of breast cancer [5] and this association was greater in pre-menopausal women. Higher birth weight has also been found to be associated with a higher mortality rate in breast cancer patients [6]. Some studies, however, have reported a U-shaped relationship between birth weight and disease risk with babies born with birth weight either below 2.5 kg or above 4 kg being associated with an increased breast cancer risk in later life compared to children born within the normal birth weight range [7–9]. However, birth weight in these studies is only thought to represent a crude indicator of the intrauterine environment, which may have been compromised through a variety of maternal, environmental, or placental factors [10].

One environmental factor that may influence future cancer risk is early life nutrition. One of the best examples of this comes from studies from the "Dutch Hunger Winter," a famine that occurred in the Netherlands in 1944. Studies have shown that breast cancer risk was higher in women who were exposed to this famine in childhood or whose mothers were exposed to the famine whilst pregnant [11, 12]. There have also been reports that breastfeeding is associated with a reduction in an infant's risk of developing breast cancer in later life [13]. Docosahexaenoic acid (DHA), the long-chain polyunsaturated fatty acid (PUFA) present in breast milk, is known to play an important role in neuronal development, but, interestingly, in animal models, diets high in n-3 fatty acids have also been shown to be protective against tumour incidence suggesting that DHA may also influence the development of the mammary gland and, subsequently, cancer risk.

Animal Models of Nutritional Programming of Cancer Risk

Studies in animal models have supported the findings of epidemiological studies that suggest that future breast cancer risk can be influenced by early life nutrition. Low birth weight offspring of rats fed a protein-restricted (PR) diet during pregnancy and lactation had a two fold increase in the incidence of mammary tumours compared to offspring from dams fed a control diet [14]. PR offspring also displayed reduced postnatal ductal branching and epithelial invasion at three weeks; this was followed by a period of rapid compensatory mammary growth and an increase in the expression of the insulin receptor, oestrogen receptor, and IGF-1 in the PR offspring compared to controls.

Overnutrition in early life has also been associated with an increased risk of mammary tumourigenesis in later life. For example, a high fat [a mixture of saturated, PUFA and mono-unsaturated fatty acids (MUFAs)] diet during pregnancy produced offspring with a higher BW. There was also an increased number of terminal end buds (TEBs) and proliferating cells within the mammary glands of the offspring. Moreover, in response to 7,12-dimethylbenz[a]anthracene (DMBA) treatment, the offspring developed mammary tumours significantly earlier than in the DMBA treated offspring from control fed dams [15]. An increase in incidence of mammary tumours was also observed in offspring from dams fed a diet high in n-6 PUFA [16, 17]. This increased susceptibility was accompanied by changes in mammary gland structure, including an increase in the number of TEB, and a reduction in alveolar bud differentiation. Feeding a diet high in n-6 PUFA during the peripubertal period in rats also led to an increase in mammary tumour incidence, compared to rats fed a high n-6 PUFA diet post puberty [18, 19], suggesting that the peripubertal along with the prenatal period may be a period of increased susceptibility where nutritional intake may impact cancer risk later in life. In contrast, feeding an n-3 PUFA diet during the peripubertal period resulted in a protective effect against mammary tumourgenesis in rats [20]. This decrease in cancer incidence was also accompanied by a reduction in mammary cell proliferation and an increase in apoptosis.

A number of animal studies have also explored the effect of micronutrient intake during early life on later cancer risk. Many epidemiological studies, although not all, have shown an inverse relationship between dietary folate intake in adulthood and cancer risk [21], although Stolzenberg-Solomon et al. (2006) reported an increased breast cancer risk with folic acid supplementation at doses ≥400 μg/d [22]. As folic acid intake has increased dramatically in many countries over the past ten years due to fortification of food with folic acid, consumption of folic acid supplements and periconceptional folic acid supplementation taken for the prevention of neural tube defects [23–25], the effect on folic acid supplementation in early life on later cancer risk has been of great interest. Sie et al. showed that folic acid supplementation for three weeks prior to mating and throughout pregnancy and lactation led to a significant reduction in the number of TEBs in the offspring compared to offspring from the dams fed the control diet [26]. Since TEBs are the structures that give rise to tumours, this reduction in TEBs would be expected to be associated with a decreased cancer risk. However, Ly et al. (2010) reported that both maternal and post-weaning folic acid supplementation

significantly increased the risk of mammary adenocarcinomas in the offspring after DMBA treatment [27•]. Maternal folic acid supplementation also significantly accelerated the rate of mammary adenocarcinoma appearance and increased the multiplicity of mammary adenocarcinomas in the offspring. This difference between the two studies in response to folic acid supplementation may reflect that in the study by Si et al., no carcinogenic agent was used, while Ly et al. studied mammary tumourigenesis in response to the carcinogen DMBA.

Early Life Nutrition, Cancer and Epigenetics

The mechanism underlying the associations seen between early life nutrition and breast cancer risk have been the subject of much debate, but there is now increasing evidence that epigenetic processes may underlie the developmental origins of cancer. Epigenetic processes are integral in determining when and where specific genes are expressed. Alterations in the epigenetic regulation of genes can lead, therefore, to profound changes in phenotype [28, 29]. The major epigenetic processes are DNA methylation, histone modification, and non coding RNAs.

DNA Methylation

Methylation at position five of cytosine in DNA within a CpG dinucleotide (the p denotes the intervening phosphate group) is a common modification in mammalian genomes transmitted through DNA replication [30]. CpG dinucleotides are not randomly distributed throughout the genome, but are clustered at the 5' ends of gene promoters in regions known as CpG islands. Hypermethylation of these CpG islands is associated with transcriptional repression, while hypomethylation of CpG islands is associated with transcriptional activation [31, 32].

DNA methylation is important for genomic imprinting [33], X chromosome inactivation [34], cell differentiation, and tissue specific gene expression [30]. Methylation of CpGs is largely established during development and early life. Upon fertilisation, the methylation marks on the maternal and paternal genomes are largely erased; this is followed by a wave of de novo DNA methylation just prior to blastocyst implantation [35], when the majority of CpGs are methylated, mainly in repressive heterochromatin regions and in repetitive sequences. Tissue specific gene methylation also occurs during this period and throughout development, which leads to cell determination and specification. De novo DNA methylation is catalyzed by DNA methyltransferases (Dnmt) 3a and 3b, and is maintained through mitosis by Dnmt1 [32, 36].

Histone Modification

DNA methylation works in concert with histone modifications to regulate gene expression. Specific covalent modifications of amino terminal tail domains of the histone proteins, around which the DNA is wrapped, lead to recruitment of effector proteins which in turn bring about specific transcriptional processes. The establishment of these marks on the histone tails is often referred to as the histone code. Histone acetylation is exclusively associated with active chromatin states and transcriptional activity, while the methylation of lysine residues can either be an active or repressive mark depending on the specific lysine involved [37]. Many families of histone-modifying enzymes have been identified; 'writers of the code' include the histone acetyl transferases and methyltransferases, while the 'erasers' include the deacetylases and demethylases [38, 39].

DNA methylation and histone modification are intricately linked. For example, methylated DNA is recognised and bound by methyl CpG binding protein-2 (MeCP2), which in turn recruits histone deacetylases (HDACs), HDACs remove acetyl groups from the histones, a signal of transcriptionally active chromatin, and histone methyl transferases (HMTs) [40], which methylates lysine 9 on H3, resulting in a closed chromatin structure and transcriptional silencing. Recent studies have also shown that Dnmt1 can itself be recruited by a number of histone-modifying enzymes such as HDAC1 and HDAC2, and the histone methyl transferases SUV39H1 and EZH2 [41, 42], suggesting that chromatin structure may also determine DNA methylation status.

Non-coding RNAs

Non-coding RNAs (ncRNAs) have also been implicated in the epigenetic regulation of gene expression. Non-coding RNAs can either act in cis or in trans. The *cis*-acting ncRNAs are the long/macro- ncRNAs (up to 100,000 nt), while the trans acting ncRNAs include the microRNAs (miRNAs). The miRNAs function by targeting the 3' untranslated region of mRNAs for degradation [43]. However, more recent studies have shown that miRNAs can also induce chromatin remodelling [44, 45], suggesting that DNA methylation, histone modification, and miRNAs may work in concert to regulate gene expression.

Epigenetic Alterations and Cancer

Over recent years it has become clear that cancer is caused both by genetic and epigenetic alterations. Cancer cells exhibit a number of characteristic epigenetic alterations. Global hypomethylation was one of the first epigenetic alterations to be

found in human cancer [46]. The loss of methylation occurs mainly in repetitive DNA sequences and within the coding regions and introns of genes [47]. Global hypomethylation increases as the cancer progresses [48]. DNA hypomethylation has been suggested to increase genomic instability, leading to the reactivation of transposable elements and the loss of imprinting [49]. However, alongside global hypomethylation, cancer cells also exhibit an increase in gene-specific hypermethylation of tumour suppressor genes [50]. Hypermethylation occurs most frequently at bivalent chromatin domain promoters [51], promoters marked by both H3K4 methylation, an active histone mark, and H3K27 methylation, a repressive histone mark. The hypermethylation of such genes has been suggested to result in the silencing of genes required for differentiation, leading to increased cell proliferation and self-renewal, and an increased cancer risk [52]. The pattern of gene specific hypermethylation appears to be the tumour type [53] and tumour stage-dependent [54]. For example, metalloproteinase inhibitor 3 (TIMP3) is frequently methylated in kidney tumours, death-associated protein kinase (DAPK) in lymphoma, while breast cancer type 1 susceptibility protein (BRCA1) is frequently methylated and silenced in breast and ovarian tumours [53]. Interestingly, mutations in BRCA1 are associated with inherited forms of breast cancer [55] suggesting that it is maybe the same pathways and genes that are disrupted, either through a genetic or epigenetic mechanism in both sporadic and inherited forms of breast cancer.

The growing realization of the importance of such epigenetic changes in cancer suggest that the level of methylation induced in early life may set the epigenetic background upon which changes induced by further environmental factors and/or aging may operate.

Early Life Nutrition and the Epigenome

There is growing evidence that the epigenome is susceptible to a number of environmental factors, specifically during certain periods of life, namely the prenatal, neonatal, and pubertal periods. One factor that has been consistently shown to modulate the epigenome is nutrition. Nutritional modulation of the epigenome has long been suggested since methyl groups for virtually all biological methylation reactions, including DNA methylation, are primarily supplied from dietary methyl donors and cofactors via 1-carbon metabolism [56]. In 1-carbon metabolism, methionine is converted to S-adenosylmethionine (SAM), the universal methyl donor. After the transfer of the methyl group to the substrate, SAM is converted to S-adenosylhomocysteine (SAH), which is then converted to homocysteine. Homocysteine is then either recycled to methionine by the enzyme betaine homocysteine methyltransferase or via a folate-dependent remethylation pathway, where 5-methyl tetrahydrofolate (THF) is reduced to 5,10-methylene THF by 5,10-methylenetetrahydrofolate reductase. The methyl group is then used by methionine synthase to convert homocysteine to methionine using vitamin B_{12} as the cofactor. The dependence on dietary sources for methyl donors and cofactors for the supply of methyl groups led to the suggestion that nutrition may affect the establishment and maintenance of DNA methylation patterns with long term consequences for health. However, it is only recently that the impact of diet in early life on the epigenome and its implications for later health has been realized.

The first demonstration that maternal diet can alter DNA methylation in offspring came from studies on Avy mice, where coat colour is determined by the methylation status of an intracisternal-A particle (IAP) retrotransposon upstream of the agouti gene. Here, they found that supplementation of the maternal diet with betaine, choline, folic acid, and vitamin B_{12} led to increased methylation of the agouti gene and shifted the distribution of coat colour of the offspring from yellow (agouti) to brown (pseudo-agouti) [57] . Subsequent studies have shown in animal models of nutritional programming, that perturbations in early life nutrition lead to the altered epigenetic regulation of key metabolic control genes within the offspring. For instance, feeding pregnant rats a PR diet induced hypomethylation of the GR and PPARα promoters in the livers of juvenile and adult offspring, which was associated with increased mRNA expression of these genes [58, 59] and alterations in the metabolic processes that they control. Plagemann *et al.* (2009) have also shown that neonatal overfeeding induced by raising rat pups in small litters induces the hypermethylation of two CpG dinucleotides within the proopiomelanocortin (POMC) promoter, a gene that plays a critical role in appetite regulation [60]. In humans, alterations in the methylation of a number of genes have also been found in individuals whose mothers were exposed to famine compared to their non-exposed siblings. Moreover, these changes were found 60 years after famine exposure suggesting that perturbations in maternal diet can induce long term epigenetic changes. Together, these findings show that early life nutrition can induce changes in the methylation of key genes involved in metabolism and appetite control, which persist long after the environmental constraint has been removed, suggesting that such changes may underpin the developmental origins of metabolic disease.

In animal models, epigenetic changes have also been shown to accompany alterations in mammary gland structure and increased mammary tumour risk in offspring from dams fed a PR or high fat diet during pregnancy. Zheng et al. reported the expression of the cell cycle inhibitors p16 and p21 were reduced in the mammary gland of the PR offspring compared to controls [61•, 62]. The decrease in p16 and p21 expression was accompanied by a decrease in histone acetylation and dimethylation of K4 on histone H3. They also examined the effect of maternal diet on p21 methylation, but

detected no change in methylation of the region examined [63•]. Feeding a high fat diet during pregnancy also induced epigenetic changes within p16 in the mammary gland, in this case, a decrease in histone H4 acetylation across the promoter region of p16 and a reduction in HDAC3 binding. But again no difference in DNA methylation was observed [62], although a MeDIP approach was used in this case to assess methylation. This approach measures methylation across a region rather than at individual CpGs, so may potentially miss changes in methylation at individual CpG sites.

Maternal, but not post-weaning, folic acid supplementation has been shown to induce a significant reduction in global DNA methylation, whereas post-weaning, but not maternal, folic acid supplementation significantly decreased DNA methyltransferase activity in non-neoplastic mammary glands of the offspring [27•], suggesting that folic acid supplementation may influence cancer risk through the altered epigenetic regulation of genes.

Identification of Epigenetic Biomarkers

If cancer risk reflects epigenetic changes induced predominantly in early life, then it should be possible to detect such epigenetic alterations prior to the onset of clinical disease to identify those individuals at increased risk of disease. However, in humans, the only readily accessible tissues for such analyses are cord blood, placenta, or buccal cells at birth, and blood or buccal cells in childhood. As DNA methylation patterns are often tissue-specific, there is a real concern that DNA methylation marks in peripheral tissues will not adequately reflect the methylation patterns in more relevant disease cell types. However, a number of studies have recently reported inter tissue methylation correlations. For example, Talens et al. reported that DNA methylation levels measured in blood were equivalent in buccal cells for half of the candidate loci examined, despite the fact that these cell types originate from different germ layers (mesoderm and ectoderm, respectively) [64]. Godfrey et al. also recently reported the methylation state of a single CpG site in the promoter region of the transcription factor *RXRA* in the umbilical cord at birth was associated with childhood adiposity in both boys and girls [65] in two independent cohorts; with *RXRA* promoter methylation explaining over a fifth of the variance in childhood fat mass. Such findings strongly support the paradigm that developmentally induced epigenetic marks make a significant contribution to later phenotype and suggests that methylation levels in cord or other readily available tissues may provide useful proxy markers of methylation in more metabolically relevant tissues. Although this may be dependent on when the environmental challenge occurred, with environmental constraints during very early development likely to affect all germ layers, while exposures in late gestation inducing only tissue specific effects.

Interestingly, Brennan et al. recently reported in prediagnostic blood samples that the methylation of an intragenic region of the ATM gene in peripheral blood DNA was associated with an increased risk of breast cancer [66••]. There was no association between ATM methylation with the time from blood collection to diagnosis, suggesting that this association may not be explained by the presence of preclinical disease, but that ATM hypermethylation represents a stable marker of predisposition in peripheral blood. Wong et al. have also reported (2011) that peripheral blood methylation of BRCA1 is associated with a 3.5-fold increased risk of early onset breast cancer with a BRCA1 mutation-associated pathology [67]. Detectable BRCA1 methylation in peripheral blood was also associated with high levels of BRCA1 promoter methylation within the tumour suggesting that constitutional BRCA1 methylation may increase susceptibility to the development of BRCA1 hypermethylated tumors. Further studies have also shown that the methylation of RASSF1A, TWIST, HIN1, and Cyclin D2 are frequently found in primary invasive breast cancers and that normal tissue adjacent to the tumour harbours the same methylation profile as the cancer [68]. Whether this reflects an inadequate surgical margin or early premalignant changes needs further investigation. Although, methylation of RASSF1A has been observed both in the tumour and in the contralateral unaffected breast (Yan et al., 2014) [69•]. However, whether alterations in the methylation of ATM, BRCA1 or RASSF1A were induced during early life when the epigenome is most susceptible to change remains to be determined. Nevertheless, these findings do suggest that epigenetic marks may provide useful predictive biomarkers of later disease risk and support the paradigm that developmentally induced epigenetic marks make a significant contribution to later cancer risk.

Conclusion

There is now a substantial body of evidence that supports the suggestion that variations in the intra-uterine environment can modify the risk of breast cancer. Although there are inherent limitations to epidemiological studies, such as the methods of data collection and variability in disease timing, histological origin, and the age and sex of patients, it is clear that higher birth weight is associated with an overall increase in risk of breast cancer. Moreover, both human and animal studies suggest one important factor in early life is nutrition, which may influence cancer risk through the altered epigenetic regulation of genes. To date, however, it is not fully understood how these changes are targeted within the epigenome, what specific nutritional factors bring about such alterations, which are the periods of susceptibility, and how stable are these

changes. Understanding these processes would allow the development of both effective predictive biomarkers of disease risk and targets for intervention strategies to reduce breast cancer risk.

Compliance with Ethics Guidelines

Conflict of Interest R. Jordan Price, Graham C. Burdge, and Karen A. Lillycrop declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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