The Role of Parental and Grandparental Epigenetic Alterations in Familial Cancer Risk

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Abstract

Epigenetic alterations of the genome such as DNA promoter methylation and chromatin remodeling play an important role in tumorigenesis. These modifications take place throughout development with subsequent events occurring later in adulthood. Recent studies, however, suggest that some epigenetic alterations that influence cancer risk are inherited through the germline from parent to child and are observed in multiple generations. Epigenetic changes may be inherited as Mendelian, non-Mendelian, or environmentally induced traits. Here, we will discuss Mendelian, non-Mendelian, and environmentally induced patterns of multigenerational epigenetic alterations as well as some possible mechanisms for how these events may be occurring. [Cancer Res 2008;68(22):9116–21]

Introduction

Epigenetics is a system of gene regulation independent of DNA sequence. Aberrations in DNA methylation and histone modifications affecting the configuration of chromatin are the two most prominent epigenetic modifications in cancer. The normal developmental process of epigenetic reprogramming consists of two phases early in embryogenesis, genome-wide demethylation followed by de novo methylation (1). Perturbation of epigenetic reprogramming may lead to aberrant methylation patterning in offspring. A recent topic of discussion is whether epigenetic events that occur in a parent can be passed on to offspring to affect cancer risk. DNA mutations were thought to be the only inherited cause of cancer in individuals; however, recent findings suggest that information in the epigenetic code may be passed on to offspring and influence cancer risk.

Epigenetic Events in Cancer

Epigenetic changes including DNA methylation are frequent somatic events in tumors. Changes in the DNA methylation pattern result from simultaneous global demethylation, increased expression of DNA methyltransferases, and de novo methylation of CpG islands, which can all lead to tumors (2). Global DNA hypomethylation generally occurs in centromeric repeats and repetitive sequences and contributes to carcinogenesis by causing chromosomal instability, reactivation of transposable elements, and loss of imprinting (3). Hypermethylation leads to transcriptional repression by preventing the interaction of DNA with functional proteins (4) or recruiting proteins such as MeCP2, a methylated DNA binding protein, to further block DNA interaction with transcriptional machinery (2). In cancer patients, CpG islands in promoters and first exons of some tumor suppressor genes are hypermethylated, thereby abolishing their role in cell cycle regulation, DNA repair, angiogenesis inhibition, and metastasis suppression (3). Although typically thought to occur embryonically, epigenetic alterations leading to disease may be due to embryonic, postnatal, or adult environmental exposures (5). Several genes implicated in cancer are epigenetically altered in adult tissues. Abnormal promoter methylation has been observed in more than 37 different tumor types (6). MicroRNAs may also play a role in epigenetic regulation of tumorigenesis by establishing DNA methylation in target genes and affecting chromatin structure (7).

Environmentally Induced Epimutations

In the past, the environment was thought to contribute to carcinogenesis by introducing mutations, DNA breaks, translocations, or DNA copy number changes. However, environmental factors, diet, and lifestyle are increasingly associated with aberrant epigenetic modifications resulting in cancer. Suspected or known epigenetic carcinogens include tobacco smoke, metals, arsenic, ionizing and UV radiation, stress, alcohol, aflatoxin B1, and infectious agents such as bacteria and viruses. One mechanism by which these factors influence the epigenome is through changes in DNA methylation leading to activation of transposable element–derived promoters (8).

Transgenerational Epigenetic Events in Cancer

Unlike somatic epigenetic alterations in tumors, inherited germ-line epigenetic events in cancer are relatively understudied. Germ-line events are inherited through the transmission from parental germ cells to an embryo. This should not be confused with somatic transmission of epigenetic alterations which is when an epigenetic change is passed from parental cells to daughter cells. If an epigenetic mutation, such as promoter hypermethylation, occurs in a somatic cell, that cell can mitotically transmit the epimutation to subsequent cell divisions, which results in gene silencing. In addition, epigenetic alterations in stem cells may be transmitted to differentiated cells to promote cancer progression. For example, studies show that breast stem and progenitor cells are primary targets of estrogen imprinting, an epigenetic phenomenon that results from early exposure to estrogenic chemicals to promote adult-onset carcinogenesis (9). Epigenetic inheritance is the persistence of an epigenetic state through the germline to manifest in the next generation. Epigenetic alterations can be observed to segregate as Mendelian, non-Mendelian, or environmentally induced traits.

Mendelian Epigenetic Inheritance

CpG island methylation phenotypes in tumors are shown to positively correlate with a family history of cancer, suggesting...
familial epigenetic contributions to cancer risk (10). To date, inheritance of germline epigenetic modifications has been observed only in cases of CpG island methylation and is likely the result of incomplete erasure of epigenetic patterning in the germline or the manifestation of epigenetic-like inheritance due to cis- or trans-acting factors (1).

Studies suggest a Mendelian-like inheritance of epimutations in hereditary nonpolyposis colorectal cancer (HNPCC). Chan and colleagues described a family with allelic-specific, mosaic expression of MSH2 in multiple generations. Methylation was present in 10 of the 12 family members spanning three generations. Three siblings carrying the germline methylation developed early-onset colorectal or endometrial cancers (11). In this study, it is possible that the aberrant methylation patterns of MSH2 were erased in early development and reestablished due to cis- or trans-acting factors (Fig. 1A and B). Modifier genes functioning as cis- or trans-acting factors may affect whether an epimutation is transmitted to subsequent generations or erased in gametogenesis and reestablished in successive generations.

Another study supporting the Mendelian pattern of epigenetic allele-specific patterning showed promoter methylation and subsequent loss of expression of Death-associated protein kinase 1 (DAPK1) in heritable B-cell chronic lymphocytic leukemia (CLL; ref. 12). Raval and colleagues defined a disease haplotype, which segregated with the CLL phenotype in one large family. In affected individuals, a promoter single-nucleotide polymorphism down-regulated the expression of DAPK1 by 75% through the enhancement of HOXB7 binding. Germline transmission may be conditioned on a DNA sequence alteration; however, direct transgenerational inheritance of epigenetic information may also exist (12).

Aberrant DNA methylation patterns occur in a variety of other tumor suppressor genes to promote a cancer phenotype; however, there is no evidence to date that these abnormalities can be inherited. Studies of APC and BRCA1 failed to show aberrant promoter methylation in individuals with strong family and personal histories of cancer (13, 14), suggesting that transgenerationally inherited modifications are unlikely to be causing familial adenomatous polyposis or the hereditary breast/ovarian phenotype seen in these individuals. It remains to be seen whether germline epigenetic mutations in other cancer risk genes are heritable and if they account for a significant fraction of inherited cancer risk.

Non-Mendelian Epigenetic Inheritance

Human studies. Families with multiple individuals in multiple generations with HNPCC-like phenotypes and apparent inherited methylation of the MLH1 promoter have been described (15). In all cases, multiple tissues from multiple lineages showed an epimutation of abnormally methylated MLH1 promoter sequences, suggesting that MLH1 silencing was due to a change transmitted through the germline or one occurring early in development.

Figure 1. Mendelian inheritance. Unmethylated promoter CpG islands (small open circles) for a gene (shown in gray) allow for gene expression, whereas methylated CpG islands (small black circles) silence gene expression. A, cis-acting epigenetic factors. A cis-acting factor influences early methylation patterning of a gene implicated in cancer. The cis-acting factor segregates in a Mendelian fashion with a particular haplotype, TG. However, not everyone who has the TG haplotype also has inherited the cis-acting factor. Individuals who inherit the cis-acting factor show silencing of the gene and a predisposition to developing cancer. B, trans-acting epigenetic modifiers. A trans-acting modifier gene acts to promote epigenetic alterations of a gene implicated in cancer. Individuals must inherit both the modifier gene (black) and the haplotype of the gene implicated in cancer (TG) to develop a phenotype. Individuals who inherit the cancer-predisposing gene without the modifier gene do not show aberrant methylation patterning. Squares, males; circles, females. Black, affected; white, unaffected. Small black circles, methylated CpG islands; small white circles, unmethylated CpGs.
Interestingly, the MLH1 epimutation seems to be more readily transmitted through the maternal germline. In one family, the epimutation was transmitted from mother to son; however, promotor methylation of MLH1 was not present in spermatozoa in males carrying the silenced MLH1 allele (15). Subsequent findings suggested that MLH1 methylation was completely erased from sperm (16), suggesting that this defect is cleared during spermatogenesis. In a second family, offspring inheriting the silenced MLH1 maternal allele had reverted it back to the normal active state (15). This pattern of inheritance raises the possibility that epigenetic errors may arise more frequently during oogenesis or are more likely to be maintained during maternal transmission. In offspring that did not inherit a silenced MLH1 promoter, the normal process of resetting the epigenetic pattern during gametogenesis may have corrected the MLH1 epimutation.

Non-Mendelian inheritance patterns may also be due to preferential transmission of alleles through meiotic nondojesunction (17). Another example of preferential allelic transmission is the Delta-like 1 homologue (DLK1). DLK1 alleles exhibit a non-Mendelian inheritance pattern in obese children resulting from polar overdominance (18).

**Mammalian studies.** Transgenerational epigenetics is a type of inheritance newly identified in mouse models for testicular germ cell tumors. It may be a common mechanism of genetic susceptibility to cancer. In mice, a termination mutation in Dead-end homologue 1 (Dnd1) contributes to TCGT susceptibility. Studies of genetic modifiers of Dnd1Ter led to the identification of six genetic variants that interact to influence testicular germ cell tumor risk. Interestingly, there was a higher frequency of affected offspring in the heterozygous Dnd1/Term/+ controls than what was observed in the original crosses between Ter/− mice. When one parent carried one Ter allele and the other parent carried a different TCGT modifier, the offspring showed an increase in susceptibility to testicular germ cell tumors independent of whether the other modifiers were inherited. Therefore, the increased risk of testicular germ cell tumors was not due to direct molecular or genetic interactions, but from transgenerational epistatic interactions. It is possible that the modifiers themselves may mark the germline such that subsequent generations will have increased susceptibility, but that the offspring will need to carry other genetic susceptibility factors (such as Ter) to show a phenotype. Another possible mechanism is that environmental factors act on molecular pathways influencing genes to act as mediators and to monitor cellular conditions, which in turn induce epigenetic alterations (19).

**Nonmammalian studies.** Evidence for transgenerational epigenetic effects has been identified in nonmammalian species and plants. Mutations in the Drosophila Janus-activated kinase (JAK) gene, hop, can alter DNA methylation patterning, allowing for the inheritance of epimutations. The hopTum−4 oncomutation in JAK inhibits global heterochromatin formation and, thus, interferes with the epigenetic reprogramming process. Many of the epigenetic modifications caused by the hopTum−4 mutation persist in subsequent generations even in the absence of the mutation. This phenomenon is similar to that of a paramutation in that subsequent generations can inherit either an enhanced or a suppressed hopTum−4 phenotype even if the modifier gene mutation itself is not passed on. Inheritance of the epigenetic modification depended on which parent contained the hopTum−4 mutation, suggesting differences in epigenetic programming between maternal and paternal genomes. The hopTum−4 mutation prevents the erasure of epigenetic markers in early embryonic development, allowing the epigenetic alterations of the parental genome to be maintained in the F1 generation even when the original genetic mutation is absent (20). The mouse and fly data provide strong evidence that both epistatic and modifier genetic interactions, which influence cancer phenotypes, can occur across generations and may act via perturbations in the normal epigenetic programming process.

**Environmentally Induced Epigenetic Inheritance**

Several studies have evaluated transgenerational epigenetic inheritance induced by the environmental factors. Reprogramming events early in development are thought to erase the epigenetic modifications parental genomes acquire to protect the offspring from epigenetic mutations. However, not all epigenetic marks may be completely cleared between generations. To prove that a phenotype is truly transgenerationally inherited, an epimutation must be passed on from the initially exposed F0 generational mother and inherited by the F3 offspring (Fig. 2). This is because the F1 embryo and its F2 germline are directly exposed to the environmental factor, and one cannot distinguish between true inheritance and an exposure that causes an epigenetic alteration of both F1 and F2 genomes. If F3 individuals display the same phenotype as the F0, F1, and F2 generations, then it can be concluded that the epigenetic alteration was inherited through the germline because the F3 generation was independent of direct exposure (21).

Environmental factors may affect epigenetic patterning in third and fourth generations, increasing the likelihood that the changes are transmittable epigenetic modifications. Male F1 offspring of gestating rats exposed to vinclozolin show decreased spermatogenic capacity. This phenotype was transmitted to subsequent generations through the male germline, affecting even the F4 generation (22). The mechanism for this transgenerational phenomenon was investigated, and it was found that the endocrine disruptors reprogrammed the epigenetic state of the male germline during development and induced the presence of new imprinted-like genes/DNA sequences that are stably transmitted through the male germline (23). It is unknown why this phenotype is only transmitted through the male germline; however, gender differences in epigenetic reprogramming are likely to play a role (21).

**Diethylstilbestrol.** One example of multiple generations in families showing effects of an environmental agent are daughters of mothers who were exposed to diethylstilbestrol (DES) during the first trimester. The daughters show developmental abnormalities and an increased risk of developing a rare type of clear-cell adenocarcinoma. DES daughters also show a 2.5-fold increase in breast cancer risk after 40 years of age (24). To prove that this indeed is an inherited transgenerational effect, granddaughters and great granddaughters of the exposed mothers will need to show a DES phenotype. This analysis has not yet been completed. Mouse studies have shown that the F2 generation from a DES-exposed pregnant female had strikingly similar effects as the F1 generation, including abnormal uterine development and uterine cancer (25). The proposed mechanism of action of DES is aberrant CpG methylation of key uterine cancer genes (26). The changes in CpG methylation may be stable throughout gametogenesis, providing insight into the transgenerational effects of DES (27).
Possible Mechanisms of Transgenerational Epigenetic Inheritance

Paramutations, first described in plants, are one possible mode of inherited epigenetic modifications. A paramutation is the interaction between two homologous alleles in trans, which cause changes in gene expression that can persist in subsequent generations even when only one of the two alleles is present (Fig. 3). Several mechanisms have been suggested; however, the most striking evidence comes from plant studies of trans-acting factors such as Mediator of paramutation 1 (mop1) and Required to maintain repression 1 (rmr1). Mop1 encodes an RNA-dependent RNA polymerase and is essential for the establishment of the r1 and b1 paramutations found in maize (28). Rmr loci stabilize paramutant states. Snf2, an rmr1 protein and the catalytic subunit of the SWI/SNF chromatin remodeling complex, affects both small RNA accumulation and cytosine methylation. Rmr1 maintains heterochromatic-like chromatin structure at repetitive elements (29). RNA transmission through gametes has been suggested as a mechanism for paramutations. RNA itself can also act as an epigenetic modifier to silence homologous nuclear genes through RNA-directed DNA methylation (30).

Genomic targets that are likely susceptible to gene expression changes due to environmentally induced epigenetic alterations are promoter regions of housekeeping genes, transposable elements that lie adjacent to genes with metastable epialleles, and regulatory elements of imprinted genes (31). Metastable epialleles are genetically identical alleles, rich in CpG dinucleotide sequences, which are variably expressed because of differential DNA methylation patterning occurring during development. A\textsuperscript{Y} (Viable yellow agouti) and CAbp\textsuperscript{IAP} [Cyclin-dependent kinase-5 activator binding protein-intra-cisternal A particle (IAP)] are a few examples of genes with metastable alleles in the mouse. The A\textsuperscript{Y} allele is the result of an insertion of an IAP retrotransposon upstream of the transcription start site of the Agouti gene. Constitutive expression of Agouti causes yellow fur, obesity, and tumorigenesis. CpG methylation in the A\textsuperscript{Y} IAP correlates inversely with Agouti expression. Thus, various degrees of methylation at IAP generate a wide range of coat color. The same mechanism of action holds true for the other metastable epialleles in that IAP methylation levels determine different phenotypes (32). Interestingly, IAP is thought to be resistant to epigenetic reprogramming (33), which could explain why the methylation state of IAP in Agouti mice can be transmitted to offspring. Other retrotransposons like IAP are likely to play a role in epigenetic heritability. If retrotransposons are resistant to reprogramming, this could explain other inherited epigenetic traits in which retrotransposons are inserted in or near the affected gene. Metastable alleles have not yet been described in humans.

Gene imprinting and X-inactivation are exceptions to normal epigenetic reprogramming, in which perturbations of the normal process could result in germline transmission of epimutations. Typically, imprinting results in parental-specific allelic expression. For example, Insulin-like growth factor II (IGF2) is preferentially imprinted via promoter methylation of the maternal allele resulting in expression of only the paternal allele. In Wilm's tumors, loss of imprinting of the IGF2 gene leads to aberrant activation of the normally silenced maternal allele (34). Germline imprinted genes that do not undergo demethylation during early embryogenesis are more common than previously anticipated. Indeed, 154 additional

Figure 2. Transgenerational inheritance of environmental epigenetic alterations. In both A and B, a gestating mother is exposed to an environmental agent that induces epigenetic alteration and a disease phenotype. A, inheritance of an epimutation stops at the F2 generation. It cannot be concluded whether or not this is truly a transgenerational disease passed through the germline because the F2 germline itself was exposed to the environmental factor. B, inheritance of an epimutation persists through the F3 generation. Here the epimutation was passed through the germline because the germline of the F3 mice was not directly exposed to the environmental agent. This case would be considered to be true germline transmission of an altered epigenetic state. White, disease phenotype. Gray, wild type.
imprinted genes in human were recently identified (35). Tandem repeat sequences in or near differentially methylated regions may protect methylated imprinted alleles from passive demethylation. Genome variations may alter repeat sequences that do not undergo epigenetic reprogramming and are therefore passed on to the next generation. Some germline imprints are restricted from de novo methylation by a CCCTC-binding factor (CTCF) boundary element. The CTCF zinc finger binds to a target binding site of the imprinted gene that is recognized when it is unmethylated and acts to prevent DNA methylation. Imprinted genes may also be maintained through protective chromatin conformation (1). The process of resetting methylation is important for reestablishing sex-specific imprints and for the clearance of acquired epigenetic marks (36). X-chromosome inactivation in females, another exception to normal epigenetic programming, is the transcriptional silencing of one of two X-chromosomes. This ensures that females, with two X-chromosomes, will have gene expression levels similar to males with only one X chromosome. This process is regulated through dsRNAs. dsRNAs are believed to be inducers of DNA methylation because they appear DNA-like, and when dsRNA interacts with DNA, de novo methylation is signaled (37). Other dsRNA processes may influence transgenerational methylation patterning.

Conclusions and Thoughts for the Future
An increasing number of studies are showing transgenerational inheritance of epigenetic events through three or more generations. Some of these events seem to be associated with inherited cancer susceptibility, whereas others may be due to a transgenerational effect of a carcinogen. It remains to be seen if true germline
transmission of epimutations occurs or whether cis- or trans-acting factors that influence epigenetic patterning are mostly responsible for the observed families. Although not yet observed in humans, the transgenerational interaction effects of modifier genes or paramutations may also act to influence cancer risk through the generations. More research needs to be done to understand the role of retrotransposons and RNA elements in epistatic interactions. Whereas the molecular mechanism of transgenerational epistatic interactions remains undefined, it changes the paradigm of how we will need to think about genetic modifiers and genetic interactions for cancer susceptibility. This work implies that we may need to consider genotypes of previous generations when determining risk in an individual and not solely the genotypes within the person of study. This greatly adds to the complexity of the understanding of genetic risk and cancer susceptibility, and suggests that even if we are able to uncover all the genetic interactions present in an individual using the current state-of-the-art whole genome association scans, we may miss transgenerational effects contributed by a parental or grandparental genome. In addition to genetic studies, epidemiologic studies of environmental effects on cancer should consider how to develop models that can incorporate environmental exposures occurring in previous generations. In the search for familial cancer susceptibility epimutations, epigenomes need to be considered.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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