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Medicine in focus

DNA methylation, epimutations and cancer predisposition

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ABSTRACT

Hereditary cancer syndromes caused by germline mutations give rise to distinct spectra of cancers with characteristic clinico-pathological features. Many of these hereditary cancer genes are silenced by methylation in a similar spectrum of sporadic cancers. It is likely that the initiating event in some of those cases of sporadic cancer is the somatic epigenetic inactivation (epimutation) of the same hereditary cancer gene. Recently, it has been shown that epimutations of certain hereditary cancer genes can be constitutional i.e. present throughout the soma. These epimutations may be inherited or arise very early in the germline. The heritability of these epimutations is very low as in most cases they are erased by passage through the germline. In other cases, predisposition to epimutations rather than the epimutations themselves can be inherited. These cases are characterised by Mendelian inheritance and are likely to be associated with sequence variants. Other sequence variants and environmental influences may also affect methylation propensity at a global level.

1. Introduction

This review will assess the role of epimutations, in particular epimutations of hereditary cancer genes, in the development of cancer. It will consider the evidence that there are cases of cancer in which epimutations not only directly predispose to the cancer, but are also widespread through adjacent tissues and unrelated tissues indicating a soma-wide event.

Epigenetic silencing of tumour suppressor genes associated with promoter methylation in tandem with an overall global reduction in DNA methylation is considered to be a hallmark of cancer cells (Esteller, 2008). As promoter methylation can lead to silencing that is mitotically transmissible, the term "epimutation", was introduced for any heritable change such as methylation that did not affect the actual sequence of the DNA (Holliday, 1987).

It is important to clarify the terms "somatic", "constitutional" and "germline" used to describe epimutations in this review. We will endeavour to use the terms as tightly as possible within this review while acknowledging that they may be more loosely used in the literature.

By "somatic", we refer to any epimutations that are observed in the tumour. The somatic epimutation may also be present as a precursor lesion in the apparently non-cancerous tissue from which the tumour arises. The presence of methylation in adjacent normal tissues is often referred to as a field effect and indicates that the apparently normal tissue is clonally related to the malignant cells.

By "germline", we refer to an epimutation that is found in all cells of the body and for which there is conclusive evidence of transmission of an actual epigenetic mark from the previous generation. As germline epimutation is present in every cell in the body, the risk of developing cancer will be similar to that of an individual that carries a germline mutation. However, it is still controversial whether germline epimutations occur in humans (Chong et al., 2007; Horsthemke, 2007; Leung et al., 2007; Suter and Martin, 2007).

By "constitutional", we refer to an epimutation that is found in all tissues of the body. There may be no, or equivocal, evidence of transmission from the previous generation. The epimutations may have occurred very early in development. In some cases, constitutional epimutations may be mosaic, i.e. they are present in all tissues but not all cells in those tissues have the epimutation.

Germline epimutations are constitutional but not all constitutional mutations are germline. Germline and constitutional epimutations have the common property that the same allele is methylated in all tissues of the individual. Somatic epimutations may arise more than once and thereby different alleles may be affected. Any one of these types of epimutations may be the

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first step in tumour development and thus directly predispose to cancer.

2. Hereditary cancer genes and cancer predisposition

Studies of familial cancer have identified a group of genes whose mutational inactivation results in predisposition to a characteristic spectrum of cancers. The tumour suppressor gene, *RB* which is mutated in retinoblastoma, was the first hereditary cancer gene to be identified (Friend et al., 1986). Subsequently, other tumour suppressor genes operating through a diverse range of mechanisms were identified by their role in other familial cancers e.g. *APC* mutations were identified as underlying familial adenomatous polyposis coli (Nishisho et al., 1991) and *CDKN2A* (*p16*) mutations were identified in familial melanoma (Hussussian et al., 1994). DNA repair genes (also referred to as caretaker or stability genes) such as the *BRCA1*, *MLH1*, and *MSH2* genes are also often involved in predisposition to familial cancer.

Inactivating mutations in hereditary cancer genes are recessive and the carrier usually has no phenotypic abnormalities apart from the cancer predisposition. The incidence of cancer however displays an often highly penetrant dominant pattern of inheritance as there is a high likelihood that a mutation, loss of heterozygosity or a silencing event will inactivate the remaining normal allele in a susceptible population of cells.

3. Methylation of hereditary cancer genes in sporadic tumours

It is reasonable to suppose that cancers with the same underlying driving genetic lesion will resemble each other whether they are sporadic or familial. Thus sporadic retinoblastoma also involves mutational inactivation of the retinoblastoma gene, *RB*. Similarly, many cases of sporadic colorectal cancer involve mutational inactivation of the *APC* gene. However, many other cases of sporadic cancers that resemble hereditary cancers but have no mutations in the corresponding hereditary cancer gene have been identified.

Reports of the methylation of *RB* were the first demonstrations that a gene involved in hereditary cancer predisposition could be epigenetically inactivated in the corresponding sporadic cancers (Greger et al., 1989; Sakai et al., 1991). Subsequently, many other hereditary cancer genes were shown to undergo methylation in the corresponding sporadic cancers including the *VHL* (Herman et al., 1994), *MLH1* (Kane et al., 1997), *APC* (Hiltunen et al., 1997) and *BRCA1* (Dobrovic and Simpfendorfer, 1997) genes.

4. *MLH1* and *MSH2* epimutations in colorectal carcinoma

Mutations in *MLH1* and *MSH2* are a frequent cause of hereditary non-polyposis colorectal cancer (HNPCC). The mutations also predispose to a characteristic spectrum of extra-colonic cancers including endometrial, gastric and ovarian cancer. Both genes code for components of the mismatch repair apparatus and their inactivation give rise to microsatellite instability in the tumours, (reviewed in de la Chapelle, 2004).

It has been shown that the *MLH1* gene can be methylated in sporadic colorectal carcinoma (Kane et al., 1997) giving rise to the same mismatch repair deficiency phenotype and similar clinicopathological features as hereditary tumours. *MLH1* methylation is a frequent occurrence in sporadic colorectal cancers (Kane et al., 1997), and it is strongly associated with cancers displaying the CpG island methylator phenotype (CIMP). CIMP positive cancers show frequent methylation of a characteristic set of CpG islands (Weisenberger et al., 2006). These cancers mainly arise in the ascending colon and are particularly frequent in elderly women.

Gazzoli et al. (2002) were the first to show that the *MLH1* gene can be methylated in the peripheral blood as well as in the tumours of colorectal carcinoma patients. They studied 14 apparent cases of HNPCC with microsatellite instability, but for which no mutation in a mismatch repair gene had been identified. In a female diagnosed at 25 years of age, high levels of methylation (approximately 50%) of *MLH1* were found in DNA from normal blood. The observation that one allele was methylated in a clearly uninvolved tissue resulting from a different embryonic germ layer indicated that the methylation was constitutional and possibly germline. Samples from the parents were not available, so it could not be concluded whether or not the epimutation was inherited or had arisen *de novo* in early embryogenesis.

The very early age of onset of this patient's tumour is noteworthy. Most reports of constitutive methylation of hereditary cancer genes describe patients who develop early onset tumours and frequently develop multiple tumours. This is reminiscent of the properties of inherited mutations in cancer predisposition genes though intriguingly, the ages of onset seem even earlier and the cases with multiple tumours even more frequent.

A subsequent study reported further colorectal cancer patients in which one allele of the *MLH1* gene appeared to be constitutionally methylated (Suter et al., 2004). The methylated *MLH1* allele was present in cell types derived from all three embryonic germ layers of the two colorectal cancer patients (aged 43 and 46 when first diagnosed) who subsequently developed further tumours. Bisulfite sequencing of a fragment containing a heterozygous polymorphism (−93G/A) within the 5′-untranslated region of *MHL1* confirmed that only one allele was methylated. The unmethylated allele was lost in all four informative tumours and all the tumours showed complete loss of *MLH1* as judged by immunohistochemistry. It could not be determined if the epimutations were maternally or paternally transmitted or had arisen *de novo* in early embryogenesis as no tissues from any of the parents were available. Tissues from four out of five of the two patients' children were available, but no *MHL1* methylation was detected.

Further studies of *MLH1* methylation in normal tissues from patients with colorectal cancer have confirmed that epimutations are rarely, if ever inherited, and occur in a limited and non-Mendelian fashion (Hitchins et al., 2005, 2007; Valle et al., 2007; Morak et al., 2008). Complete erasure of epimutations during spermatogenesis has been observed (Hitchins and Ward, 2007), and so far evidence is only present for maternally derived inheritance (Hitchins et al., 2007; Morak et al., 2008), suggesting that epimutations are less likely to be erased during oogenesis.

If epimutations are substantially or totally erased in the germline, families with strong histories of cancer are unlikely to have underlying epimutations. Consistent with this prediction, only one case of constitutive *MLH1* methylation was found in a cohort of 160 probands from suspected HNPCC families without germline mutations in the mismatch repair genes (Hitchins et al., 2005). The unaffected parents and siblings of this 39-year-old male did not show any *MLH1* methylation. This indicates that to identify constitutional epimutations of tumour suppressor genes in cancer patients, tumours with characteristic clinico-pathological features will be a better guideline than family history.

Heritable transmission of propensity to *MSH2* methylation in a family with HNPCC has been reported (Chan et al., 2006). Interestingly, the *MSH2* gene is not otherwise known to be methylated in cancer. In contrast to the apparent allelic methylation observed for the *MLH1* gene in most of the *MLH1* constitutional methylation patients, the observed *MSH2* methylation was not at the 50% level expected for allelic methylation. In fact, the degree of methyla-