Epigenetic modification and cancer: mark or stamp?

William D Foulkes

Program in Cancer Genetics, Departments of Oncology and Human Genetics, 546 Pine Avenue West, McGill University, Montreal, Quebec, Canada H2W 1S6

(Correspondence should be addressed to W D Foulkes; Email: william.foulkes@mcgill.ca)

Abstract

Hypotheses are built upon data, but data require hypotheses before they can be understood. The development of the ‘two-hit’ hypothesis of carcinogenesis was a key event in cancer genetics because it provided a testable model of how tumours develop. In this commentary on ‘Promoter hypermethylation patterns in Fallopian tube epithelium of BRCA1 and BRCA2 germline mutation carriers’ by Bijron et al. published in the February 2012 issue of Endocrine-Related Cancer, the need for new grammar and some new hypotheses in epigenetics is discussed. Meanwhile, data suggesting an important role of epigenetic modification in the cause, progression and treatment of cancer continues to accumulate.

Introduction

In hereditary tumours, the first hit occurs in the germ line, whereas in non-hereditary tumours, the first hit occurs in the cell from which the tumour arises. The second hits are always somatic, and can inactivate the second allele in various different ways. The development of the ‘two-hit’ hypothesis of carcinogenesis was a key event in cancer genetics because it provided a testable model of how tumours develop (Knudson 1971, 1978). Although there have been extensions and revisions to the basic model (Tomlinson et al. 2001), the essential elements of the basic hypothesis remain intact, 40 years on. In the original ‘test case’ of RB-1 mutations in retinoblastoma, these events were physical alterations in the structure of the chromosome or gene (Cavenee et al. 1983), and the perception was such that physical changes put a ‘stamp’ on the tumour that could be detected by examination of genomic DNA.

At the same time as the classic paper of Cavenee et al. (1983) on the mechanisms of loss of heterozygosity in retinoblastoma was being published, another theme was being explored. Feinberg & Vogelstein (1983) studied hypomethylation in colon cancer and found that there was significant epigenetic modification of DNA in fresh colorectal tumours. While this first study focused on hypomethylation, rather than hypermethylation (the latter is generally thought to lead to gene silencing (Baylin et al. 2000), this work expanded the class of alterations that lead to loss of normally functioning protein to include reversible ‘marks’, such as those made by methylation of DNA. There have been thousands of studies since that time, studying various aspects of epigenetic modification of cancer. If we fast-forward to 2011, Manel Esteller has provided an excellent survey of progress (Esteller 2011a). so, what has been learnt?

CpG hypermethylation

Most studies have focused on the epigenetic marks left on cancers by methylation of cytosines within CpG islands. Now it seems that so-called ‘CpG’ shores can also be hypermethylated: almost every type of common cancer has been subjected to methylation analysis of promoters of candidate tumour suppressor genes, with widely (and wildly) varying results. Histone marks have also been studied to a lesser degree, and these methylation and histone marks have been used to diagnose cancer and predict its outcome. Some alkylating agents such as temozolomide cause DNA damage that is repaired by methyltransferases – when these enzymes are absent in cancer cells, drug-induced DNA damage is irreparable and the cell dies. The ‘hypermethylome’ is emerging (Esteller 2007). This vast body of knowledge is expanding daily but lacks a
clear expiatory narrative. There is, however, one clear story that emerges – that of genomic imprinting.

Genomic imprinting

The seeds of this work that were sown in experiments in the 1950s by Janice Spofford have been largely forgotten. Dr Spofford showed that the extent of pigmentation in white-variegated eyes in Drosophila was enhanced if the rearrangement responsible for this so-called ‘position-effect variegation’ was paternal, rather than maternal in origin (Spofford 1959, 1961). This was one of the first experimental examples of what animal breeders had known for years – the phenotype of the offspring of the mating of two closely related animals (e.g. donkey and horse, which have 62 and 64 chromosomes respectively) can differ depending on which is the male parent and which is the female parent of such a cross. This effect of marking the DNA for silencing – imprinting – is obliterated when the genes are passed onto the next generation (perhaps it is not surprising that mules and nearly all hinnies are infertile). Spofford’s work was confirmed in the late 1980s when it was shown in Prader–Willi syndrome, maternal uniparental disomy at chromosome 15q11–13 was found to underlie some cases of the disorder (Nicholls et al. 1989). In contrast, in Beckwith–Wiedemann syndrome, both copies of chromosome region 11p15.5 are derived from the father – paternal uniparental disomy (Henry et al. 1991). It turned out that this was being driven by IGF2, as this is located within the imprinted region and, during embryonic development, is only expressed from the paternal allele. More recently, Rahman and colleagues showed that abnormalities at 11p15, including H19 differentially methylated region (DMR) epimutations, uniparental disomy and H19 DMR imprinting centre mutations were present in lymphocyte DNA from a small but significant fraction of children with Wilms’ tumour – thus implicating inherited mutations that affect imprinting in the aetiology of a relatively common childhood tumour (Scott et al. 2008). Mutations that affect methylation of nearby genes have also been seen in hereditary forms of colorectal cancer (Ligtenberg et al. 2009). Thus, the classical mutation-based two-hit model and the vaguer, plastic biology of epigenetics can both be seen to play a part in inherited cancers, but the hard-wired genetic effects are clearly paramount for most highly penetrant cancer susceptibility syndromes. The classic example of an inherited cancer predisposition where imprinting plays a major role is paraganglioma, caused by germ line SDHD mutations. In this condition, there is a fascinating interplay between the inherited mutation in genomic DNA and two different epigenetic factors – one being imprinting on chromosome 11q23, such that the disease only develops if the mutation is inherited from the father – there has yet to be a well-documented case of maternally transmitted paraganglioma in an SDHD mutation carrier. The second factor is altitude (or more exactly oxygen tension). As SDHD mutations might be less penetrant for carotid body tumours at higher oxygen tensions, mutations in populations living at low altitudes might have a higher fitness and might become quite prevalent. Indeed, there are multiple different founder mutations in the Netherlands, lending credence to this hypothesis (Baysal 2004).

For non-inherited cancers, the first inkling of link between loss of heterozygosity and imprinting came from work showing preferential loss of a particular parental allele of a tumour suppressor gene in sporadic childhood tumours – the first hit is imprinting of one parental allele (inactivating one copy of the tumour suppressor gene) and the second hit is loss of the other allele (usually the maternal one). Loss of imprinting is the mirror image of loss of heterozygosity – it results in gene activation; and it is quite commonly seen in apparently sporadic Wilms tumour (Feinberg 1993). Murine experiments suggest that loss of imprinting can by itself predispose cells to develop tumours (Holm et al. 2005).

Hypermethylating the tube

Turning to the common cancers of adulthood, such as breast and ovarian cancer, hypermethylation of the promoter of tumour suppressor genes, such as BRCA1, has been found in tumours that phenocopy breast cancers arising as a result of inherited mutations in this gene (Esteller et al. 2000). These results have not always been confirmed by others (Turner et al. 2007), but some have extended this work to include even germ line methylation of BRCA1 (Wong et al. 2011). One question that arises from studies such as these is that if methylation affects a broad swathe of DNA in a tumour, why would it tend to be accompanied by loss of heterozygosity of one of the two alleles?

In a recent issue of Endocrine-Related Cancer, Bijron et al. (2012) have taken a different approach. They have looked at patterns of promoter hypermethylation in fallopian tube epithelium of women carrying BRCA1 and BRCA2 germ line mutations. Bijron et al studied 14 such women (from a much larger set of BRCA1/BRCA2 carriers) whose tubal epithelium showed TP53 over-expression, and compared their findings with those obtained from normal areas of tubal epithelium in 13 controls and eight non-BRCA1/2 tubal
cancers. The key new finding of their study was that \textit{BRCA1} and \textit{BRCA2} mutation carriers display increased methylation of tumour suppressor genes in their TP53-positive non-malignant fallopian tube epithelium, compared with tubal epithelium in untested women who were (reasonably) presumed to be non-carriers. Despite this difference, within the \textit{BRCA1/2} carrier group, the levels of methylation did not depend on the level of TP53 staining – a somewhat surprising finding, given that these ‘TP53 signature’ cells are thought to be precursors of the invasive cancers (\textit{Folkins et al. 2008}). But the levels of methylation in TP53-overexpressing cells arising in \textit{BRCA1/2} carriers were closer to those seen in tubal cancer than those seen in the normal control tubal epithelium.

The authors used a cumulative methylation index (CMI) – a measure they have used before (\textit{Suijkerbuijk et al. 2008, Bol et al. 2010}) – to calculate these differences. They chose a cut-off of 15% to denote positivity for hypermethylation – that is, 15% of possible promoters of a gene under study were hypermethylated. To look for methylation, they used an ‘off-the-shelf’ methylation-specific multiplex ligated probe amplification kit that covers 70 tumour suppressor genes, and was selected, at least in part, to cover genes that are believed to be important in adnexal cancers. The average number of methylated genes was greater in TP53-positive epithelium in \textit{BRCA1/2} carriers than in normal non-\textit{BRCA1/2} epithelium, but \textit{BRCA1/2} non-TP53 signature cells did not show an increase in methylation compared with these controls (and as stated earlier, there was no difference in the average number of methylated genes between the TP53-positive and TP53-negative regions of the tube in \textit{BRCA1/2} carriers). Interestingly, the same group studied methylation levels in sporadic and hereditary breast cancer and found the CMI to be higher in normal breast epithelium from \textit{BRCA1/2} mutation carriers compared with non-mutation carriers (\textit{Suijkerbuijk et al. 2008}), but they did not find differences in CMI between sporadic and hereditary ovarian cancer (\textit{Bol et al. 2010}) – a confusing set of results. So what does it all mean? Some questions come to mind. For example, are these genes merely markers of some other more fundamental problem in the fallopian tubes of \textit{BRCA1/2} carriers? \textit{RASSF1} is hypermethylated in many cancers (including hereditary breast and ovarian cancers), but its role in carcinogenesis is uncertain (\textit{Gordon & Baksh 2011}). Does hypermethylation actually turn off the expression of the targeted gene? The authors note these caveats. We are left with a lot of data but not much in the way of hypotheses.

Non-coding RNAs

If it is more data you want then a vast new terrain has begun to be explored – that of non-coding RNAs. As well as the well-known microRNAs (of which there are nearly 1000 known species), non-coding RNAs include PIWI-interacting RNAs (piRNAs), transcribed ultraconserved regions (T-UCRs), small nucleolar RNAs (snoRNAs) and large intergenic non-coding RNAs (lincRNAs) (\textit{Esteller 2011b}). This new world will add further complexity to the epigenetic universe, because we know that in the case of microRNAs, one microRNA can control 30 or more genes, and each gene can be controlled by many microRNAs. Another twist to the story is added by the fact that the genes that encode the microRNA processing machinery are themselves subject to mutations that alter their functions – this could disrupt either the general production of microRNAs or could bias the microRNA profile towards or away from one particular species. For example, somatic genomic mutations in several pathway members such as XPO-5 and TRBP (encoded by \textit{TARBP2}), and both somatic and germ line mutations in \textit{Dicer1} can alter microRNA profiles and functions (\textit{Bahubeshi et al. 2011, Heravi-Moussavi et al. 2011}).

Do marks become stamps?

Despite these tremendous advances, the field of epigenetics has been held back due to lack of a single over-arching hypothesis or canonical set of experiments that can be called upon to explain the observed phenomena. Perhaps this is because these complex epigenetic marks (by their very nature) can be applied and removed. Moreover, portraits of the epigenetic modification of a tumour are likely to be unstable and if it is unstable, it is unpredictable. Without predictive ability, data lose their power. Nevertheless, there is nothing to say that an epigenetic mark cannot become a permanent stamp. Considering the established effect of early-life experience on health outcomes in adulthood, which can operate via DNA methylation (\textit{Szyl et al. 2008, McGowan et al. 2009}), it is reasonable to presume that some epigenetic effects can be as permanent as genetic ones. Then the only difference between the two is that one involves a physical change to DNA sequence whereas the other does not – perhaps that is the simplest definition of epigenetics (\textit{Esteller 2011a}). The original definitions of epigenetics do not help as they are so broad, but restricting epigenetics to inherited examples such as Beckwith–Wiedemann is also limiting because there is clearly more to
epigenetics than genomic imprinting. So we need a new grammar and some new hypotheses in epigenetics. Until that time, we will have to continue to accumulate data. But already from the vast amounts of data that have been produced so far, it is very clear that epigenetic reprogramming of gene expression is one of the major mechanisms driving cancers to their ends. Perhaps methylation of key genes in susceptible precursor cells, as suggested by the work of Bijron et al, is an important step on the road to a cancer itself.

Declaration of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

References


Feinberg AP & Vogelstein B 1983 Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature* 301 89–92. (doi:10.1038/301089a0)


Tomlinson IP, Roylance R & Houlston RS 2001 Two hits revisited again. *Journal of Medical Genetics* **38** 81–85. (doi:10.1136/jmg.38.2.81)


Received in final form 13 January 2012
Accepted 30 January 2012
Made available online as an Accepted Preprint 30 January 2012