SAC review DNA methylation: a form of epigenetic control of gene expression

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Key content:

- Epigenetic factors such as DNA methylation play an important role in regulating gene expression.
- Aberrant DNA methylation is a feature of a number of important human diseases.
- Epigenetic changes are common in human cancer cells.

Learning objectives:

- To appreciate the role of DNA methylation as a regulator of gene expression.
- To understand the role of DNA methylation in normal gene function.
- To illustrate how DNA methylation is implicated in the regulation of genomic imprinting.
- To draw attention to how altered DNA methylation can result in human diseases such as imprinting disorders and cancer.

Ethical issues:

- What are the implications for assisted reproductive technologies?
- There are possible difficulties in interpreting the clinical significance of alterations in DNA methylation.

Keywords Beckwith–Wiedemann syndrome / cancer / genomic imprinting / Russell–Silver syndrome / tumour suppressor genes

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Introduction

The term epigenetics refers to the study of heritable changes in phenotype or expression of genes that are not due to changes in the sequence of DNA.[1] Research into epigenetics has demonstrated that epigenetic regulation of gene expression has a critical role in normal development and cell functions, including imprinting, X-inactivation and tissue-specific gene expression.[2–4] In addition, disordered epigenetic gene regulation is a feature of a number of important human diseases, including cancer.[5,6] A number of processes have been implicated in epigenetic gene regulation including DNA methylation; chromatin structure and modification; and untranslated RNAs. This review aims to introduce the reader to the concept of DNA methylation as a regulator of gene expression, with examples of its involvement in genomic imprinting and cancer.

DNA methylation: what is it?

DNA methylation refers to the addition of a methyl group ($-CH_3$) covalently to the base cytosine (C) in the dinucleotide 5′-CpG-3′ (methylated cytosine residues are sometimes referred to as the fifth nucleotide). The term CpG refers to the base cytosine (C) linked by a phosphate bond to the base guanine (G) in the DNA nucleotide sequence. Most CpG dinucleotides in the human genome are methylated. However, unmethylated CpGs are not randomly distributed, but are usually clustered together in 'CpG islands', which are in the promoter region of many genes (the region that facilitates transcription of a particular gene).[7,8] The observation that the CpG islands in the promoters of important genes that are expressed in most cells (often called 'housekeeping genes') are mainly unmethylated, and that methylation of CpG islands in cancer cells often leads to silencing of gene expression, led to the hypothesis that DNA methylation plays an important role in regulating gene expression. In general, but not always, DNA methylation is associated with loss of gene expression.[7] One theory on the evolution of DNA methylation is that it evolved as a host defence mechanism to silence foreign DNA such as viral sequences, replicated transposable elements and other repetitive sequences.[9]

How does DNA methylation affect gene expression?

For gene transcription to occur, the gene promoter should be readily accessible to transcription factors and other regulatory units (e.g. enhancers).[10] DNA methylation can directly prevent transcription factor binding and lead to changes in chromatin structure that restrict access of transcription factors to the gene promoter (**Figure 1**). For example, methylated CpGs attract methyl-CpG-binding domain proteins that recruit 'repressor complexes', resulting in histone modification. Histones are the key protein components of chromatin and they act as spools for the wrapping of DNA which can be altered. The modification of histones by the recruitment of repressor complexes leads to a more condensed chromatin structure (heterochromatin) as opposed to an open and active chromatin structure (euchromatin) required for transcription.[11–13]

Maintenance and resetting of methylation marks

The epigenetic marking of the human genome by DNA methylation is heritable (from one cell to another during cell division), stable and allows a form of epigenetic 'memory'. DNA methylation is catalysed by the DNA methyltransferases, including those which establish methylation (DNMT3a and DNMT3b) and maintain methylation (DNMT1). This allows control of expression of developmental genes at specific times of embryonic development

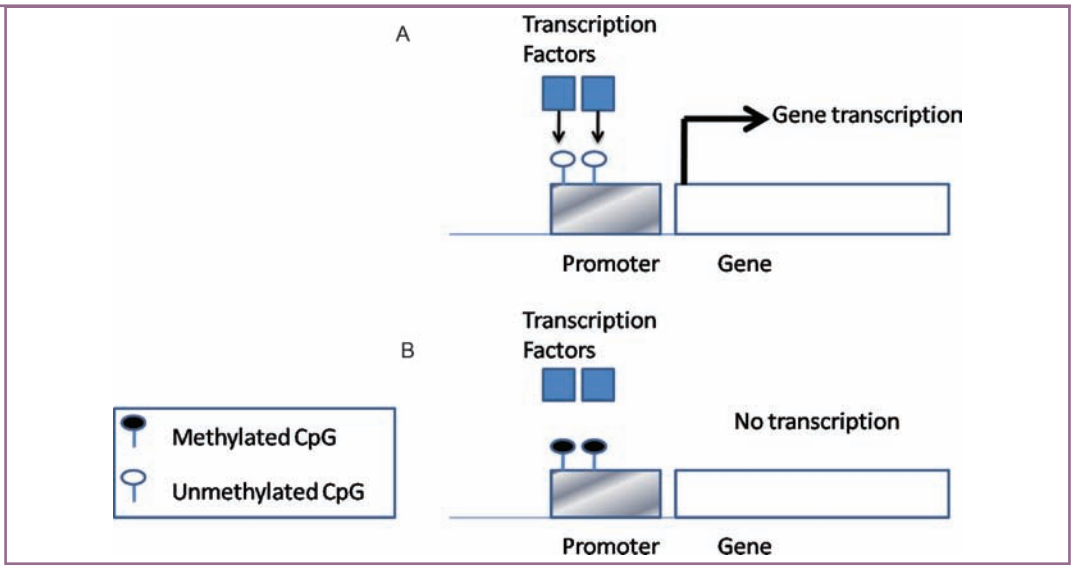

Figure 1
DNA methylation regulating gene expression. (A) The CpG island promoter is unmethylated and allows binding of transcription factors, which is required for transcription initiation. (B) The CpG island promoter methylation prevents binding of transcription factors and results in gene silencing.

in specific tissues and also ensures maintenance of the correct marking of imprinted genes.[14–16]

The role of DNA methylation in genomic imprinting and imprinting disorders

Imprinted genes are genes that are selectively expressed depending on the parental chromosome of origin. Therefore only one allele (copy) of the gene is expressed; for example, for insulin-like growth factor 2 (*IGF2*), only the copy from the father is active whereas the opposite is true for the closely linked *H19* gene (**Figure 2**). Only a minority (about 80) of human genes are imprinted but imprinted genes often play a critical role in fetal and neurodevelopment. Thus, two copies of the paternal genome (and no maternal contribution) result in a complete molar pregnancy despite the correct number of genes being present.[17]

Important information on the role of imprinted genes in human development has been derived from studies of imprinting disorders such as Beckwith–Wiedemann syndrome (BWS) and Russell–Silver, Prader–Willi and Angelman syndromes.[18–20] The majority of imprinting syndromes occur sporadically; however, familial cases of imprinting disorders such as Angelman syndrome and BWS demonstrate unusual patterns of inheritance, with parent-of-origin effects on disease phenotype. For example, in familial BWS, full-blown disease is only seen when the disease is inherited from the mother. Whilst some cases of Angelman syndrome or BWS can be caused by mutations in specific genes (*UBE3A* and *CDKN1C*, respectively), in other cases there is no genetic alteration in a specific gene but another mechanism (e.g. uniparental disomy, chromosomal deletions or duplications and epimutations affecting the methylation status of imprint control region: **Box 1**) causes abnormal expression for several imprinted genes. In this review we focus on the alteration of methylation status of the key imprinting control region resulting in a change in gene expression.[19,21]

Beckwith–Wiedemann syndrome

This syndrome is characterised by prenatal and/or postnatal overgrowth, macroglossia and anterior abdominal wall defects (which range from mild umbilical hernia to exomphalos in severe cases). Additional but variable features include facial naevus flammeus, hemihypertrophy, neonatal hypoglycaemia and childhood embryonal tumours (especially Wilms tumours).[18] Increased expression of the paternally expressed fetal growth promoter *IGF2* is implicated in some cases of BWS. The

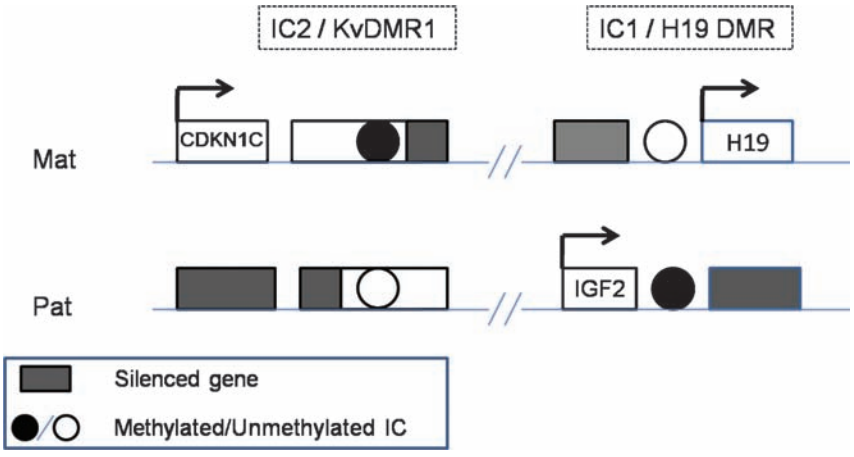

Figure 2
Genomic imprinting of chromosome 11p15.5. Diagram showing the methylation status of the two imprinting centres (IC1 and IC2) and genes that are selectively expressed from either parental allele. There is balance between the paternally expressed growth promoter *IGF2* and the maternally expressed growth suppressor *CDKN1C*. Imprinting centre 1 (IC1): the *H19* DMR is methylated on the paternal allele, allowing expression of *IGF2* from the paternal chromosome. On the maternal allele (Mat), the *H19* DMR is unmethylated, allowing expression of *H19* from the maternal chromosome. Imprinting centre 2 (IC2): KvDMR1 is methylated on the maternal allele, allowing the expression of *CDKN1C* from the maternal chromosome. On the paternal allele (Pat), KvDMR1 is unmethylated, resulting in silencing of *CDKN1C*.

Mechanism	Definition/explanation
Uniparental disomy	Both chromosomes in a cell are derived from a single parent (and there is no chromosomal material from the other parent). Uniparental disomy may affect the whole chromosome (complete) or part of a chromosome. If the chromosomal region contains an imprinted gene then uniparental disomy will be associated with alterations in imprinted gene expression.
Deletion/duplication	Deletions involving imprinted regions can affect imprinting by either: • deletion of an expressed gene on that allele (so abolishing gene expression); or • deletion of the imprinting control centre, which results in loss of regulatory control of imprinting. Duplications involving the imprinted regions can double the expression of imprinted genes expressed from that allele.
Mutation	Loss of function mutations in a gene on the expressed allele will impair gene function whereas a mutation in the silenced allele will have no apparent effect. Hence mutations in imprinted genes are associated with parent-of-origin effects on clinical phenotype.
Epimutation	An epimutation is a specific loss of methylation (hypomethylation) or gain of methylation (hypermethylation) at an imprinting control centre without any change in DNA sequence. This alters expression of imprinted genes on the same allele.

Box 1
Examples of mechanisms leading to imprinting disorders

imprinting status of *IGF2* (and *H19*) is controlled by the methylation status of an imprinting control region (IC1), which maps between *IGF2* and *H19*. On the paternal chromosome IC1 is methylated, *IGF2* is expressed and *H19* expression is silenced (the *H19* promoter is methylated). However, on the maternally inherited chromosome IC1 is unmethylated, *IGF2* is silenced and *H19* is expressed (the *H19* promoter is unmethylated) (**Figure 2**). In 5–10% of BWS cases, both maternal and paternal IC1 regions are methylated (the maternal chromosome IC1 has gained methylation) and this is associated with expression of *IGF2* and silencing of *H19* on both chromosomes (**Figure 3A**). This pattern would be predicted to double *IGF2* expression and so cause fetal overgrowth and embryonal tumour susceptibility (increased expression of *IGF2* is a feature of Wilms tumour).[21]

Russell–Silver syndrome

Another imprinting disorder has also been linked to altered *IGF2*/*H19* expression and IC1 methylation. Russell–Silver syndrome (RSS) is characterised by prenatal and postnatal growth restriction, body asymmetry, a triangular face and café-au-lait patches of the skin. Hence BWS and RSS have opposite growth phenotypes and ~40% of children with RSS have an abnormal IC1 methylation pattern which is opposite to that seen in BWS. Thus in RSS there is loss of paternal allele IC1 methylation (so that the paternal epigenotype is the same as that on the maternal allele), resulting in reduced *IGF2* expression and fetal growth retardation.[20]

The genetics of BWS and RSS are complex and other molecular mechanisms can result in a BWS or RSS phenotype. However, these disorders illustrate how alterations in DNA methylation can affect gene expression and cause specific human diseases. In most cases of BWS the cause of altered DNA methylation is unknown but there is a higher frequency of children born by assisted reproductive technologies in children with BWS who have lost genomic methylation at a second imprinting centre (IC2) between the *CDKN1C* and *IGF2* genes (**Figure 3B**).[22,23] Further studies are ongoing to determine whether the reportedly higher frequency of growth restriction and congenital abnormalities after assisted reproductive technologies may, in some cases, be related to epigenetic changes.

DNA methylation and cancer

Epigenetic changes are common in human cancer cells (**Figure 4**). Initially, most attention was paid to the DNA hypomethylation that is common in cancer cells.[24] This may promote tumourigenesis by transcriptional activation of proto-oncogenes (which promote oncogenic cell growth), loss of imprinting or genomic instability (by allowing activation of repetitive elements which are normally repressed).[25,26] Gain of CpG methylation can also be a feature of cancer cells and as methylated cytosines are highly unstable bases this will predispose to gene mutation as the methylated cytosines are often deaminated and converted to thymine. Replacement of a cytosine by a thymine can lead to inactivation of tumour suppressor genes (TSGs) (e.g. *CGA*, which encodes an arginine residue, is changed to *TGA*, which specifies a stop codon resulting in a prematurely truncated protein on translation).[27] In addition, recent research has highlighted that a major consequence of epigenetic alterations in cancer cells is acquired promoter methylation, causing silencing of expression of key TSGs.[5,6] These TSGs (e.g. *p53*, *p16*) are required for regulation of normal growth and differentiation.

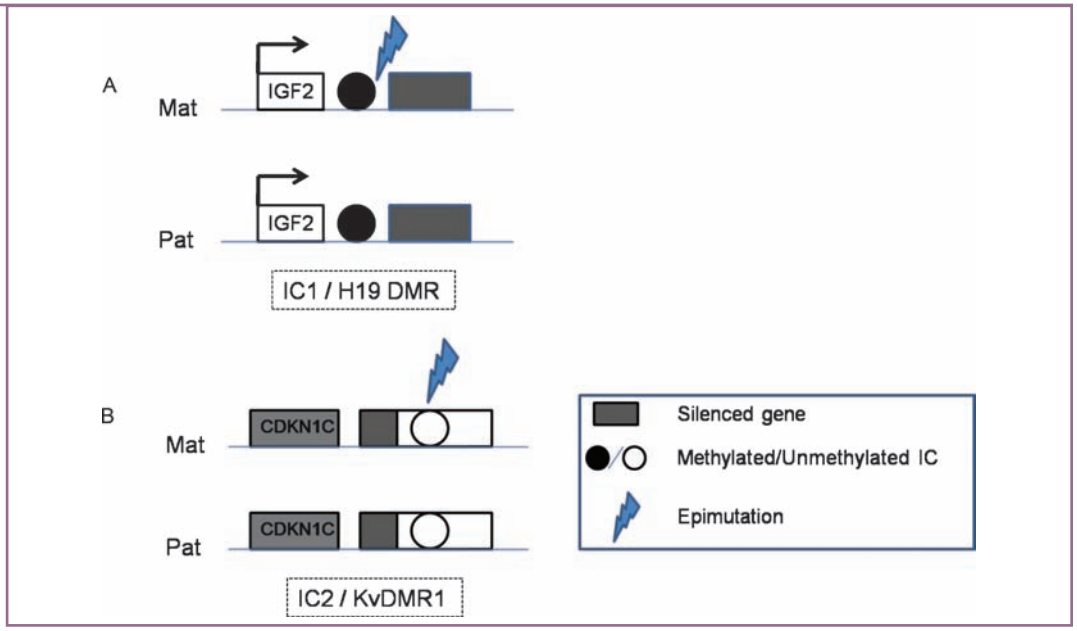

Figure 3
Example of two epigenetic errors on chromosome 11p15.5, which results in BWS. (A) Epimutation with gain of methylation at the *H19DMR* on the maternal allele (Mat), allowing biallelic expression of the growth promoter *IGF2*. This is seen in 5% of sporadic BWS cases. (B) Epimutation with loss of methylation or hypomethylation at the maternal allele KvDMR1, resulting in silencing of *CDKN1C* of both parental alleles. This is seen in 50% of sporadic BWS cases but is the predominant defect seen in BWS cases conceived by assisted reproductive technologies.

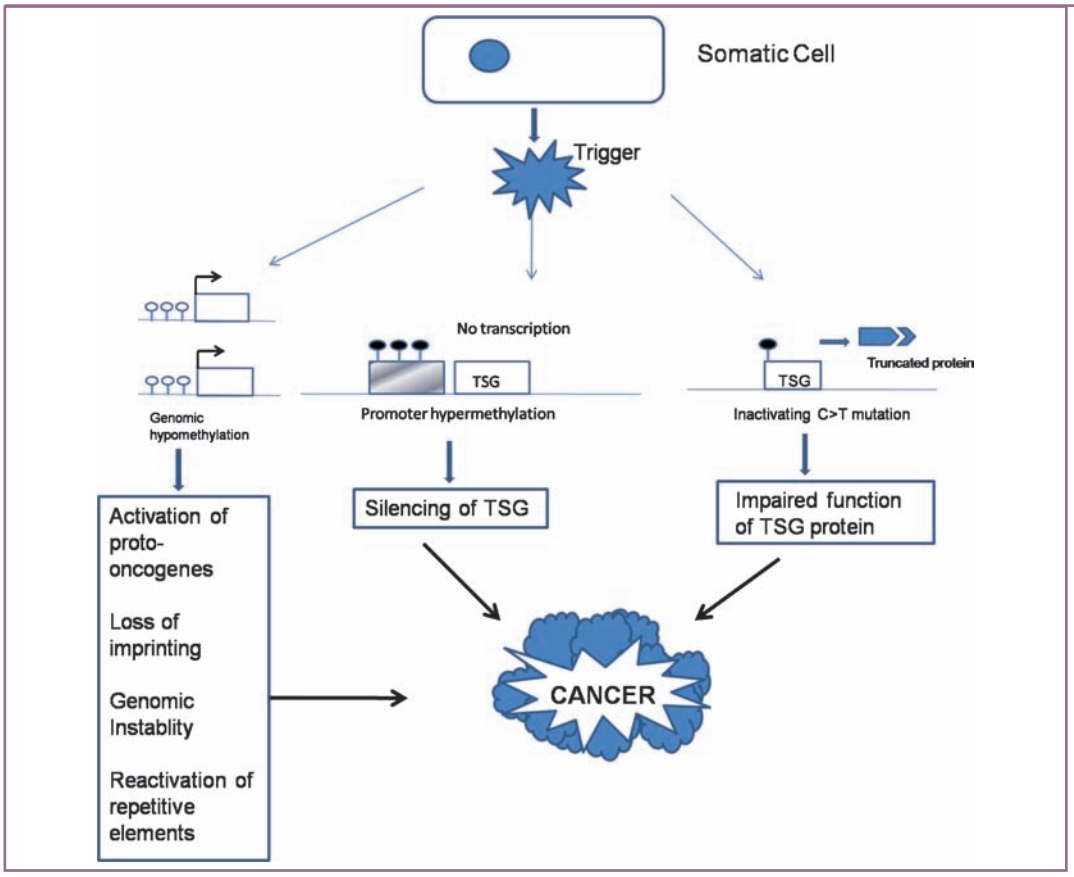

Figure 4
DNA methylation and cancer. Examples of three mechanisms leading to tumourigenesis. TSG = tumour suppresor gene

Inactivation of both alleles of TSGs is a critical event in the pathogenesis of most human cancers (which TSGs are involved depends on the specific cancer type). Initially, TSG inactivation was usually attributed to the acquisition of genetic mutations and chromosome deletions (allele loss). However, it is now clear that for some important TSGs (e.g. *RASSF1A*) promoter methylation is a more frequent cause of inactivation than mutations. Whereas genetic mutations and promoter methylation may have equivalent effects and both inactivate a TSG, epigenetic silencing by promoter methylation is a potentially reversible event. Thus, in the laboratory, treatment of cancer cells with demethylating agents can reactivate TSG expression and drugs that modify histone status are being used in clinical trials for leukaemias.[28]

In ovarian cancer cells, a number of cancer genes have been identified as having aberrant promoter hypermethylation, including *OPCML*, *BRCA1*, *p16* and *TMS1*.[29,30] Tumour suppressor gene methylation specific to cancer cells provides opportunities for novel, noninvasive early detection strategies. For example, detection of methylated TSGs in sputum may be used to detect lung cancer and in urine, bladder cancer.[31,32] Furthermore, specific patterns of TSG methylation may also be useful as prognostic indicators or predictors of treatment response. For example, aberrant hypermethylation of *MGMT* has been shown to be a biomarker for a good response to alkylating agent chemotherapy such as temozolomide.[33] In addition, the discovery of novel epigenetically inactivated TSGs can provide insights into pathways of tumourigenesis and provide a basis for further research to develop new therapeutic agents or targeted therapy such as demethylating agents.

Conclusion

This short review has covered the basic concepts of DNA methylation and its relevance to normal development and regulation of gene expression. Defects in DNA methylation can result in disorders affecting embryogenesis, genomic imprinting and cancer. Epigenetics is a rapidly developing area of human genetics. In the same way that sequencing the human genome has accelerated research into inherited diseases and cancer, it is anticipated that initiatives to define the normal human epigenome will enhance progress towards better understanding of the role of epigenetics in human disease.

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