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Review

DNA hypomethylation and human diseases

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Abstract

Changes in human DNA methylation patterns are an important feature of cancer development and progression and a potential role in other conditions such as atherosclerosis and autoimmune diseases (e.g., multiple sclerosis and lupus) is being recognised. The cancer genome is frequently characterised by hypermethylation of specific genes concurrently with an overall decrease in the level of 5 methyl cytosine. This hypomethylation of the genome largely affects the intergenic and intronic regions of the DNA, particularly repeat sequences and transposable elements, and is believed to result in chromosomal instability and increased mutation events. This review examines our understanding of the patterns of cancer-associated hypomethylation, and how recent advances in understanding of chromatin biology may help elucidate the mechanisms underlying repeat sequence demethylation. It also considers how global demethylation of repeat sequences including transposable elements and the site-specific hypomethylation of certain genes might contribute to the deleterious effects that ultimately result in the initiation and progression of cancer and other diseases. The use of hypomethylation of interspersed repeat sequences and genes as potential biomarkers in the early detection of tumors and their prognostic use in monitoring disease progression are also examined.
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Keywords: DNA hypomethylation; Global demethylation; Cancer; Biomarker; Transposable element; Repeat sequence

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1. Introduction

Epigenetics describes transmission through cell division of heritable states of gene expression that do not involve sequence changes in DNA. Underlying mechanisms encompass DNA methylation patterns, histone modifications and transmitted chromatin structure that are central for the development and maintenance of normal states of differentiation and tissue-specific patterns of gene expression within different cell types. The aberrant gene expression associated with one aspect of epigenetic change, namely the alteration of DNA methylation patterns, is a key component of disease and aging and has been particularly studied in cancer.

Originally only DNA hypomethylation was linked with cancer, and was the focus of tumor research [1–3]. However, since the mid 1980s regional hypermethylation of specific cancer-associated gene promoter sites attracted greater attention and the focus changed to the role of hypermethylation in carcinogenesis [4–6]. Neoplasia is now known to be epigenetically characterised by global DNA demethylation as well as localised hypomethylation and hypermethylation of specific genes [7–11]. These processes are often observed concurrently and are frequently linked with altered chromatin structure, changes in DNA methyltransferase activity and loss of imprinting [12–18]. The resultant aberrant transcription and chromosomal instability is believed to contribute to disease onset or progression and increased tumor frequency and malignancy [12,19–26] (See Fig. 1). Some of these events have been observed in vitro and using in vivo animal models [20,27–30], but their relative importance in human disease is not understood. Recent studies suggest that some methylation patterns are discernible in risk groups and certain diseases. Indications are that the hypomethylation of specific DNA repeat elements or genes can be disease-specific [31].

Though demethylation affects a larger proportion of the genome interest in this field has only now gained momentum

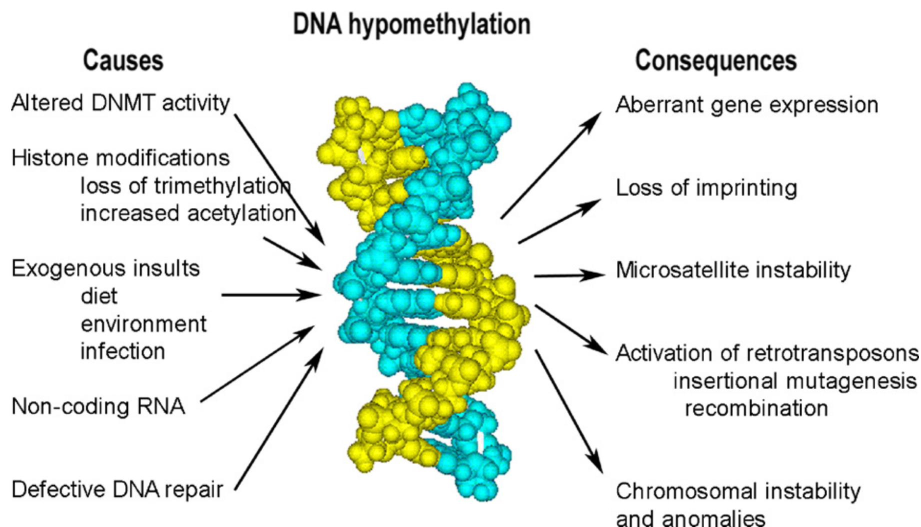

Fig. 1. DNA demethylation. The figure shows potential causes and consequences of DNA hypomethylation. Surrounding the figure of the double helix are processes linked to DNA demethylation as demonstrated by in vitro and in vivo models.

Table 1
Global demethylation and disease: studies from the last 6 years

Disease	Tissue samples	Methylation level	Comments on methylation changes	Method of determination	References
Cancer (breast, lung and colorectal)	Adjacent normal (57) Carcinoma (57)	5.50% 4.31%	Individually 70% of cancers had reduced 5 meC, 18% no change and 12% increased 5 meC relative to adjacent normal tissue. Identified a MTHFR polymorphism associated with lowered 5 meC content in normal tissue and relatively smaller subsequent hypomethylation in cancer	HPCE (high performance capillary electrophoresis) %meC of total C	Paz et al., 2002
Colorectal cancer	Colorectal carcinoma and adenomas (n=13)		Antibody staining level was reduced by 17–29% in carcinoma relative to paired normal tissue and associated with change in chromatin condensation; Southwestern blot index increased with increasing Duke stage	IHC and southwestern blot	Hernandez-Blazquez et al., 2000
	Normal colon/breast tissue (n=49) Sporadic colorectal cancer (n=26) FAP tumors (n=19) HNPCC tumors (n=13)	6.31±1.97 4.43±1.57 3.57±0.60 3.66±1.02	Global methylation 30% lower in sporadic colorectal cancers and 43% or 42% lower in hereditary FAP or HNPCC tumours respectively.	HPCE (high performance capillary electrophoresis) %meC of total C	[8]
	Normal colon mucosa (n=20) Paired normal mucosa (n=25) Colorectal cancer (n=24) Large adenoma (n=32) Small adenoma (n=21) Hyperplastic polyps (n=20)	6.5±4.9 5.2±3.8 22.2±4.4 21.5±3.4 22.8±3.8 20.3±3.6	Methyl acceptance capacity about 15% greater in cancer and polyps than in normal tissue colonic mucosa (colon mucosa about 6% lower than blood DNA)	Methyl-acceptance assay Data expressed as methyl acceptance capacity relative to peripheral blood DNA	[107]
	Normal colonic mucosa Healthy controls (n=7) Cancer patients with no LINE L1 hypomethylation (n=14) Cancer patients with LINE L1 hypomethylation 444(n=7)	 88.9±4.2% 82.0±7.7% 73.9±4.9%		Methyl acceptance assay Data expressed as total level of CpG methylation	[42]
	Normal colonic mucosa (n=76) Adenoma (n=35) Colorectal cancer (n=28)	26% increased methyl acceptance 30% increased methyl acceptance	Colonic and leukocyte hypomethylation and low folate status associated with colorectal neoplasia	Methyl acceptance assay	Pufulete et al., 2003
Gastric Cancer	Paired normal tissue (n=9) Cancer tissue (n=9)	0.82±0.07 0.64±0.12	8 of 9 tumors show hypomethylation relative to matched normal. Correlated with LINE and MAGE gene hypomethylation.	HPLC 5 meC expressed as % of total nucleotides	[103]
Breast cancer	Normal colon/breast tissue (n=49) Sporadic cancer (n=13)	6.31±1.97 2.72±0.63	Level of 5 meC is 56% lower in breast tumors; reduction methylation is less for BRCA1 (42%) and BRCA2 (30%) tumors	HPCE (high performance capillary electrophoresis) %meC of total C	[8]

	BRCA1 tumors ($n=15$) BRCA2 tumors ($n=9$) Normal Tissue Panel Cancers ($n=21$)	3.66±1.32 4.47±1.11 3.43 to 4.04%	7 of 21 cancers had 5 meC<3.43%, i.e., hypomethylated. Includes early stage/grade cancers	HPLC %meC of total C	Jackson et al. 2004
Prostate Cancer	Cancer and matched normal samples ($n=30$) Non-diseased ($n=12$)		29 of 30 patients showed decreased methylation. Average methylation scores were 99.0 for normal tissue, 100.2 for matched benign tissue and 72.5 for cancer tissue. There was no relationship to stage or Gleason grade.	Antibody staining	[198]
Wilms tumors	Normal standard (heart) Wilms tumors: No hypomethylation ($n=12$) Moderate hypomethylation ($n=10$) Strong hypomethylation ($n=9$)	3.44 3.57±0.12% 3.32±0.07% 3.09±0.11%	60% of Wilms tumors examined were found to be hypomethylated relative to least methylated normal tissue (heart). No possibility of matched normal tissue. Single gene hypermethylation studied in same samples	HPLC %meC of total C	[110]
Chronic lymphocytic leukaemia	Normal (CD19+ve B cells) CLL (peripheral blood mononuclear cells)	4.02% 3.69% (range 2.92–4.46%)	Hypomethylation level correlates with mutation level of VH genes.	HPCE, %meC of total C	Stach et al., 2003 Lyko et al., 2004
Hepatocellular carcinoma	HCC ($n=17$) Matched control tissue ($n=17$): Cirrhotic ($n=8$) Non-cirrhotic ($n=9$) Non-diseased ($n=3$)		^{3}H-methyl incorporation was higher in all 17 HCC than matched control tissue (average difference about 2 fold); correlated with histopathological grade and size of tumour. Methyl incorporation in non-diseased tissue ($n=3$) equivalent to matched control tissue. Low level LINE expression detectable in HCC, non-HCC samples as well as normal liver tissues and 3' ends were truncated	DNA-methyl acceptance assay. LINE expression determined by RT-PCR	Lin et al., 2001
Uterine leiomyoma	Leiomyoma ($n=23$) Adjacent myometrium ($n=23$)		Average 2.2 fold greater 3H-methyl incorporation in leiomyomas than matched normal tissue.	DNA-methyl acceptance assay.	[144]
Atherosclerotic vascular disease	Vascular atherosclerosis patients ($n=17$) Healthy controls, sex and age matched ($n=17$)		Average 1.5 fold greater cytosine incorporation (i.e., hypomethylation) in patients. Correlated with increased homocysteine levels.	Cytosine extension assay using HPA II RE digest	Castro et al., 2003

M.F. Paz et al., Cancer Res. 62 (2002) 4519–24; F.J. Hernandez-Blazquez et al., Gut 47 (2000) 689–93; M Pufulete et al., Gastroenterology 124 (2003) 1240–8; K. Jackson et al., Cancer Biol. Ther. 3 (2004) 1225–31; D. Stach et al., Nuc. Acid Res. 31 (2003) e2; F. Lycko et al., Electrophoresis 25 (2004) 1530–5; C.H. Lin et al., Cancer Res. 61 (2001) 4238–43; R. Castro et al., Clin. Chem. 49 (2003) 1292–6.

after a respite of almost 20 years. Feinberg and colleagues state [32], "Although individual genes vary in hypomethylation, all tumors examined so far, both benign and malignant have shown global reduction of DNA methylation. This is a striking feature of neoplasia." Yet, this area of epigenetic change remains decidedly under-investigated. The most recent reviews in DNA hypomethylation and disease, commonly referred to within this manuscript, include those by Ehrlich [12,33] and Hoffman and Schulz [31].

We will discuss the current knowledge of global demethylation of DNA in disease, predominantly in terms of the affected repeat sequences and transposable elements, by describing global hypomethylation, the different types of repeat elements within the genome and how demethylation of these elements can contribute to disease. This will be followed by analysing recent data on the underlying mechanisms that result in the unique epigenetic signatures that define different cancer types and disease progression. The understanding of these processes may improve the potential for use of DNA hypomethylation as a diagnostic or prognostic indicator. We will also highlight areas where further research is recommended and challenge current opinions in an attempt to increase interest and scientific discussion in the field of DNA hypomethylation and disease.

2. DNA hypomethylation

2.1. The CpG dinucleotide

DNA methylation principally occurs at cytosine residues located in dinucleotide CpG sites [34,35]. CpG dinucleotides are statistically under-represented in the genome but are found concentrated at the expected levels in C+G rich regions termed CpG islands that frequently coincide with promoter or gene-regulatory regions. However the bulk of lone CpG dinucleotides are found within the intergenic and intronic regions of DNA particularly within repeat sequences and transposable elements. In normal somatic cells of human tissues, between 70 and 90% of CpG dinucleotides are methylated which constitutes approximately 0.75–1% of the total number of bases in the genome [36,37].

In the healthy genome, most CpG islands are unmethylated, and associated genes expressed [34] while the lone CpGs found dispersed throughout the rest of the DNA are mostly methylated [38]. However, in specific instances gene promoter regions are methylated as part of normal developmental processes; examples include germ-line specific genes (such as MAGE genes) and certain tissue-specific genes [39]. In the case of imprinted genes and the X chromosomes in females, where only one of two copies is active, methylation of regulatory regions is involved in repression of expression of the silent loci [40]. Furthermore, it has been found that some CpG islands become methylated with age [4]. Conversely, abnormal gene-specific demethylation and global hypomethylation (involving repeat sequences throughout the genome) potentially can lead to overexpression of genes and activation of transposable elements contributing to disease.

2.2. Global hypomethylation

Measurement of the total level of 5 methyl cytosine (5 meC) in the genome by HPLC of DNA digested to mononucleotides provided the first evidence that DNA of cancer cells was hypomethylated relative to normal DNA. A number of studies using direct or indirect methods of estimating overall levels of DNA methylation have built on these initial observations (Table 1). Some key points arising are:

(i) Overall levels of hypomethylation vary widely both within and between cancer types, with a proportion of cancers not showing significant reductions in total 5 meC content. Hypomethylation relative to "normal" tissue has been observed in a range of solid tumors. A primary example is colorectal cancer where an average reduction of 10–30% total methylation levels is seen and precancerous adenomas can show a significant reduction in methylcytosine content. Among solid tumors, hypomethylation is most evident in breast cancers with reduction of up to 50% in 5 meC content. With haematological malignancies, hypomethylation has been reported for chronic lymphocytic leukaemia (CLL) while only small or no changes in overall DNA methylation was reported for CML, AML and multiple myeloma. However, in the case of haematological and some other malignancies it is difficult to define a true "normal" cell or tissue for comparison.

(ii) Global hypomethylation appears to be an early event for colon and breast cancer as well as CLL. For colon cancer hypomethylation can also be observed in the healthy tissue adjacent to these tumors suggesting a role in the initiation of disease [41,42].

(iii) For other cancers, e.g. hepatocellular carcinoma [43], the degree of hypomethylation increases with stage or histological grade. Thus the relative timing of global demethylation and its role during cancer initiation and progression may differ between cancer types.

(iv) It is evident in a number of studies, that there is a significant overlap in the levels of methylation of normal and cancerous tissues. Although DNA from an individual's cancer may be less methylated than its matched normal DNA, the normal tissue DNA for another individual may be even less methylated than that of the diseased tissue. Consequently while a loss of methylation is commonly associated with cancer development or progression, a critical level of demethylation is not associated with a particular stage of disease. However, it may not be the total methylation levels but where the hypomethylation is focussed in the genome and what genes are affected that may be of importance.

Although gene-specific demethylation occurs, much of the effects of global DNA hypomethylation are thought to occur through the activation of the normally dormant transposons and endogenous retroviruses present in the human genome. Potentially, the reactivation of the strong promoters associated

with these elements might globally alter transcription factor levels and/or modify the expression of critical growth-regulatory genes in which these elements reside [44]. It is also believed that unmethylated transposable elements permit further genomic mutations and anomalous chromosomal recombinations eventuating in the unravelling of the packed chromatin.

2.3. Repeated DNA sequences

Repeat sequences may be transposable elements found interspersed throughout the genome, or simple repeat sequences, such as DNA satellites, that are found commonly in pericentromeric or subtelomeric heterochromatin. These are normally methylated within the healthy genome. The following is a description of these various repeats in the human genome and the consequences and outcomes of hypomethylation at these sites (see Fig. 2 and Table 2).

2.3.1. Simple DNA repeats-satellite DNA

Satellite DNA comprises repeated DNA sequences arranged in tandem. The major human DNA satellites described (such as the centromeric Satα, juxtacentromeric Sat2 and Sat3 and the subtelomeric repeats) arise from the amplification of simple repeat sequences.

Current data (see Table 3) have shown hypomethylation of the Sat2 and/or Satα repeats in both the genetic ICF (*I*mmunodeficiency, *C*entromeric region instability and *F*acial anomalies) Syndrome and cancers including Wilms tumor, ovarian and breast cancer, of SatR-1 breast cancer [45–47], and extensive demethylation of the D4Z4 repeats in facioscapulohumeral muscular dystrophy and ICF syndrome [48]. The DNA repeat NBL2 was hypomethylated in neuroblastomas, hepatocellular cancers and cells from ICF patients but remained extensively methylated in ovarian and Wilms tumors [49]. These studies demonstrate an association of repeat sequence and

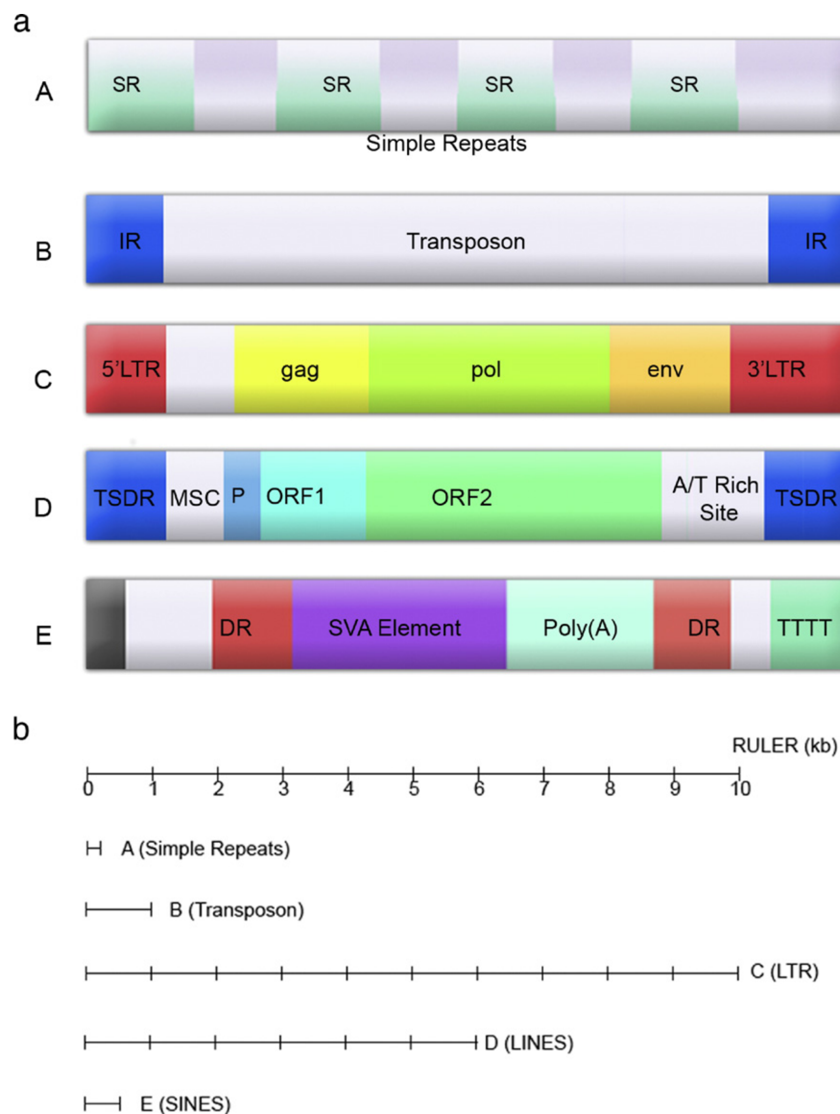

Fig. 2. Repeat elements of the human genome. (a) The following are representations of the different repeats found within the human genome. (A) Simple tandem repeat; (B) DNA transposon; (C) LTR—endogenous retrovirus; (D) Non-LTR autonomous retrotransposon: LINE; (E) Non-LTR non-autonomous retrotransposon: SINE. SR = satellite repeats, IR = inverted repeats, TSDR = target-site direct repeat, MSC = multiple stop codons, P = promoter, DR = direct repeat. (b) Underlying these figures is a comparison of relative sizes of each repeat.

Table 2
Classes of repeat sequences

	DNA transposons	Retrotransposons and endogenous retroviruses			Simple repeats
		LTR	Non-LTR		Satellite DNA
			Autonomous	Non-autonomous	
Composition [a,b]	Flanked by inverted terminal repeats ~30 bp in length; ORF = transposase moiety	Flanked by long terminal repeats; possess gag and pol genes with RT, ribonuclease and integrase activity; Nonautonomous LTR have no ORF	Strong promoter and 2 ORF with DNA binding, RT and endonuclease activity complete with polyA tail		Repeated sequence unit which are often found in tandem
Length [b,d,f,g,p–s]	~80–3,000 bp	Range = 1.5 kb–11 kb	(full length) = 4–6 kb	(full length) = 100–300 bp	Repeat unit can range from 2–70 bp
Examples [a,e,h–j]	Mariners, Tcl elements and mariner-like elements	Autonomous = HERVs; Non-autonomous = MaLR	LINEs	SINEs = Alu, SVA, MIRs; and processed pseudogenes	Satα, Sat2, (Chr 1, 16) SatR-1 (Chr 4)
Fraction of human genome [k-m]	3%	8%	21%	SINEs = 11–13%	
Number in human genome [c,k–n]	300,000	Autonomous LTR = 450,000 Nonautonomous LTR = 40,000 – 100,000	850,000	SINEs = 1,500,000	
Method of transposition [a,s]	"Local hoppers" - excised from one site to another by "cut and paste" mechanism	"Copy and paste" mechanism via an RNA intermediate, also known as target primed reverse transcription, another method is twin priming		Dependent upon LINEs for transposition	Do not transpose but rather expand
Target sites		Target site duplications (TSD)	Target cleavage sequence TTTT/A		Pericentromeric and subtelomeric heterochromatin
Age and transposition activity [d,g,i,j]	Ancient elements; Largely inactive due to mutations	Ancient and degenerate due to mutations, lone LTRs still continue to threaten genomic integrity	Many subfamilies of varying age, the oldest being degenerate. The youngest L1 can still transcribe	SVA are relatively young; Alus have many subfamilies of varying age, the oldest being degenerate.	N/A

a) H.H Jr. Kazazian, Science 303 (2004)1626–32; b) B. Charlesworth et al., Nature 371 (1994) 215–20; c) E.S. Lander et al., Nature 409 (2001) 860–921; d) M.A. Batzer et al., Nat. Rev. Genet. 3 (2002) 370–9; e) H.M. Robertson et al., Gene 205 (1997) 219–28; f) G.T. Morgan, J Mol. Biol. 254 (1995) 1–5; g) R.H. Plasterk et al., Trends Genet. 15 (1999) 326–32; h) M. Tristem, J Virol. 74 (2000) 3715–30; i) T.H. Kim et al, Mol. Cells 18 (2004) 87–93; j) B. Berkhout et al, J Virol. 73 (1999) 2365–75; k) J.D. Boeke, Nat. Genet. 16 (1997) 6–7; l) C. Esnault et al, Nat. Genet. 24 (2000) 363–73; m) W. Wei et al, Mol. Cell. Biol. 21 (2001) 1429–39; n) A.F. Smit, Nuc. Acid Res. 21 (1993) 1863–72; o) E.M. Ostertag et al, Annu. Rev. Genet. 35 (2001) 501–38; p) D.E. Promislowe et al, Proc. R. Soc. Lond. B. Biol. Sci. 266 (1999) 1555–60; q) G.D. Swergold, Mol Cell. Biol. 10 (1990) 6718–29; r) H. Hohjoh et al, EMBO J 15 (1996) 630–9; s) H. Hohjoh et al, EMBO J 16 (1997) 6034–43.

satellite DNA hypomethylation with disease and, in some cases, an apparent correlation of demethylation of a certain repeat sequence with disease group. However, the specificity of repeat sequence hypomethylation to disease type and the relationship of the demethylation of different repeated DNA sequences in a given disease(s) have only been investigated in a limited number of cases and warrants further investigation. Furthermore, investigations are required to determine the significance of satellite repeat DNA demethylation and the impact of its timing on disease progression; as with overall methylation levels some studies implicate repeat DNA hypomethylation in the initial development of cancer [47] whilst others have demonstrated an association with tumor progression and poor prognosis [46,50].

2.3.2. Transposable elements

Transposable elements are DNA sequences that have the ability to integrate into the genome at different sites. In mammals and humans they account for almost half of the DNA content [51]. There are three types of transposable elements in the human genome: DNA transposons, the retrotransposons and endogenous retroviruses.

2.3.2.1. DNA transposons. DNA transposons are probably the oldest transposable element in the human genome and are mostly degenerate due to internal deletions, end truncations or both, rendering them fixed within the genome [52]. These small elements (~1300–2400 bp in length; [53]) possess an ORF that

encodes a single transposase enzyme which is normally ineffective due to the presence of nonsense and frameshift mutations [54]. However, some remain active and a potential threat to the genome. Interestingly, DNA transposons serve as an important tool for molecular biologists in understanding mutagenesis. Nevertheless, the role of the DNA transposon in carcinogenesis and the effects and hypomethylation of transposons in disease has not yet been investigated.

2.3.2.2. Retrotransposons and endogenous retroviruses. There are two classes of retrotransposons: those flanked by long terminal repeats (LTR; largely endogenous retroviruses) and those without LTRs that generally terminate in a polyadenylated tail (non-LTR; often referred to as retrotransposons). These are able to move and integrate into other parts of the genome via a "copy and paste" mechanism involving an RNA intermediate. Although many are degenerate, they still possess strong promoters [55] that can drive transcription. Distribution of these elements within mammalian genomes is heterogeneous and non-random with densities varying across chromosomes [56,57].

2.3.2.2.1. LTR retrotransposons. Human endogenous retroviruses (HERVs) resemble simple retroviruses in structure [58], but lack a functional envelope gene. Twenty-two distinct HERV families have been identified in the human genome and are classified on the basis of their primer binding sites to host tRNAs [57]. Many of these elements are non-functional due to incomplete sequences or the presence of mutations [59]. For those that are still active, transcriptional activity is regulated, in part, by methylation [60]. Many solitary LTR remnants have also been identified in the human genome [57] and have been shown to drive gene expression [61], potentially contributing to the harmful effects associated with disease.

In a limited number of cancers, germ cell tumors and cancers of the ovary, testicles and bladder [21,31], the demethylation of HERVs has been examined. In these cases, HERV hypomethylation increases with malignancy and is often associated with transcript expression, suggesting a possible biomarker for tumor progression in these cancer groups. Expression of HERV transcripts has also been found in other diseases including multiple sclerosis, schizophrenia and cancer cell lines [62–66]. However, as the relationship to methylation levels was not investigated it is unclear whether the expression of the HERVs is a consequence of epigenetic change.

Mammalian apparent LTR-retrotransposons (MaLRs) are a superfamily of non-autonomous LTRs which comprise the most common unclassified interspersed repeats including MT, ORR-1, MstII, MER15 and MER18, all related to THE1 MaLR [67]. The ORF sequence is usually found to be excised. It is estimated that there are between 40,000 and 100,000 copies, including solitary LTRs in primate genomes. However, most of these are degenerate and have yet to be shown to cause any known human disease via insertional mutagenesis. Expression studies have detected transcripts in rheumatoid arthritis but the methylation status of critical regulatory elements were not determined [68].

2.3.2.2.2. Non-LTR retrotransposons. These elements are the most prolific retrotransposons, inhabiting almost 30% of the human genome [51]. Two types exist: autonomous (Long Interspersed Nuclear Elements—LINEs), and non-autonomous, (Short Interspersed Nuclear Elements—SINEs).

2.3.2.2.2.1. The LINE family. Full length LINEs are 6 kb in length. These elements possess strong internal promoters and encode enzymes that enable integration anywhere in the genome. The majority of LINEs are truncated at the 5′ end of the sequence and are therefore much smaller than their reported full length. A number of LINE subfamilies exist ranging in age. The oldest of these are mostly degenerate and inactive due to mutations. However the younger subfamilies (human specific L1 or L1Hs) can still transcribe when activated. The average human diploid genome has 80 to 100 active LINEs [51,69]. It is estimated that LINE insertions account for 1 in every 1200 human mutations [58] and a new LINE insertion is created in at least 1 in every 50 humans within a parental germ cell or during early embryonic development.

Evidence derived from in vitro transcription assays using site specific mutagenesis and methylation demonstrates that methylation of critical CpG dinucleotides within the LINE promoter is enough to ensure repression of transcription [55,70]. In malignant cells, hypomethylation of the 5′ end of L1Hs sequences correlates with the presence of L1Hs proteins [71]. Though in hepatocellular carcinoma, hypomethylation was not associated with increased LINE transcription [43].

The hypomethylation of LINEs has been observed in a number of cancers relative to their normal counterparts or unaffected adjacent tissues (see Table 3 of this review and [31,42,72]). LINE hypomethylation can occur early in cancer initiation, notably in cancers of the colon and prostate, where there is no significant correlation with stage. In most other cancers studied (leukemias, urothelial, ovarian and breast cancers) LINE demethylation increases with the degree of malignancy, and in some cases has been shown to correlate with clinical outcome [71]. Therefore, dependent upon cancer type, LINE hypomethylation can be useful as an early detector of cancer or a prognostic indicator. Still, what remains uncertain is whether LINE demethylation is a causative agent for cancer and what impact LINE hypomethylation has on clinical outcome.

2.3.2.2.2.2. The SINE family. This subset of non-autonomous elements relies on LINEs to enable transposition [73]. The most abundant SINE in the human is the Alu element derived from a 7SL RNA. These elements do not encode proteins but have expanded to cover 11% of the human genome with 1.1 million copies [74,75].

Normally, Alus are methylated in somatic cells. The maintenance of methylation of a number of Alu members appears to be determined by parental origin resulting in significant interindividual variability in the potential of Alus to create polymorphisms [76]. Alu-made polymorphisms can be associated with increased disease risk [77] and Alu-mediated chromosomal rearrangements, insertions/deletions and complex recombinations have accounted for over 20 different cases of human genetic disease [58]. Methylation of Alus has not been studied extensively in disease; however, in ovarian cancer and Wilms tumor, Alu demethylation follows a similar

Table 3
Demethylation of repeat sequences in disease

Juxtacentromeric Sat2 and centromeric Satα DNA	Ovarian cancer	Both satellite DNAs hypomethylated in ovarian samples, hypomethylation significantly more prevalent in advanced stage/high grade tumors, hypomethylation of Sat2 DNA in chr1 correlated with malignancy and more informative than tumor grade or stage, Sat hypomethylation correlates with global demethylation	Methylation-specific digest	[46,180]
Sat2 and Satα	Primary breast adenocarcinoma	SAT2 hypomethylation shown in ~50% breast adenocarcinomas, compared to normal, 1/19 non-malignant breast tissues with fibrocystic changes had hypomethylated Sat2, hypermethylation of E-cadherin promoter and hypomethylation in Sat2 DNA often present in the same breast cancers;	Methylation-specific digest	Narayan et al, 1998;
	Breast cancer	Sat2 demethylated in cancers compared to fibroadenomas and normal, Satα showed no change in methylation between cancer and non-cancer tissues, Sat2 demethylation correlated well with global hypomethylation even in low grade tumors, but no correlation with tumor grade, stage, axillary lymph node involvement or hormone receptor status		Jackson et al, 2004
SatR-1	Breast cancer	SATR-1 hypomethylation found in 63% of cell lines and 86% of breast tumors compared to normal; hypomethylation shown to be early event in cancer development	Methylation-specific digest/ PCR (MSAP-PCR), bisulfite sequencing, COBRA	[47]
Sat2, Sat3 and Satα	ICF Syndrome	Genetic disease characterised by impaired DNMT3B function. Strong hypomethylation of Sat2 and Sat3 sequences, more limited hypomethylation of Satα. Modest reduction in 5 meC content of genome.	Methylation specific digests Bisulfite sequencing	Miniou et al, 1997 [37] [45]
D4Z4 and NBL2 repeats	ICF Syndrome	Both these non-satellite repeats strongly hypomethylated in 4 ICF patients compared with unaffected parents	Restriction digestion/2D gel electrophoresis. Southern blotting	Kondo et al, 2000
D4Z4 repeats	Facioscapulohumeral muscular dystrophy	Severely affected patients displayed repeat sizes of 10–20 kb and pronounced D4Z4 hypomethylation whilst patients with interindividual variation in clinical severity had repeat lengths of 20–31 kb and D4Z4 hypomethylation	Methylation-specific digest	[48]
L1	Hepatocellular carcinoma	8/9 samples = LINEs hypomethylated; no obvious relationship between clinical data and the extent of L1 hypomethylation	Methylation-specific digest	[72]
	Tumors of bladder, head and neck, liver, lung, prostate, breast, oesophagus, stomach	Significant L1 hypomethylation detected in all cancers compared to normal except for kidney, thyroid lymph node and colon polyps; Note: the design of the primers enables amplification of mutated/degenerate sequences regardless of methylation status	COBRA-LINE assay	[203]
	Malignant breast cancer cell lines	L1Hs hypomethylation found on many chromosomes, 3/12 are on chromosome 15; LINEs hypomethylated in malignant germ cells are of different subset compared to those hypomethylated in non-germ cell malignancies; indicating preferential selection of LINEs that is not a random event	Methylation-specific digest	Alves et al, 1996
	Chronic myeloid leukaemia	L1 hypomethylation in 75% of blast crisis tissue compared to 38% of chronic phase tissues; hypomethylation levels correlate to L1 transcript levels and poor prognosis, cMET gene antisense transcripts, and high levels of BCR-ABL and DNMT3b4 transcripts		[71]
L1, Alu, Sat2 and Satα	Ovarian carcinoma, Wilms tumor, ICF	ICF patients had Sat2 hypomethylation whilst LINE1 and Alu remained methylated; when comparing global 5 meC data (HPLC), Alu (meth), Sat2 and LINE methylation correlated significantly; global 5 meC data showed cancer cells were generally demethylated compared to normal	MethyLight PCR assay and HPLC	[23]
L1, HERV-W	Benign and malignant ovarian tumors	Used 7 adenocarcinoma samples, 1 benign cystic mass and 2 normal ovaries; hypomethylation of L1 and HERV-W evident in malignant samples only	Methylation-specific digest	[21]
L1; HERV-E,-HC2, and THE1	Rheumatoid arthritis	RNA sequences of ORF2/L1 elements, THE1 transposon, HERV-E, HERV-HC2, isolated from different RA synovial fluid pellets	RT-PCR	[68]
L1, HERV-K	Urothelial carcinoma Renal cell carcinoma	L1 hypomethylated in 95% of urothelial carcinomas, but not renal carcinomas; hypomethylation in bladder cancers is independent of stage and increased with grade; methylation of HERV-K DNA in normal and transformed urothelial cells paralleled L1; full-length L1 transcripts detected in 2 urothelial carcinoma cell lines, HERV-K transcripts detected in teratocarcinoma cell lines	Ligation mediated PCR	[62]

trend but is not highly correlated to global hypomethylation levels [23].

SVA elements are another subset of SINEs that are heavily methylated in all adult somatic tissues [78]. These have only evolved relatively recently in the human genome as indicated by their low sequence divergence. Although there are fewer numbers of SVA elements in the genome compared to both LINEs and Alus, these have been reported to cause at least four known human diseases [79] including an X-linked blood disorder [80] and the Fukutama-type congenital muscular dystrophy [81]. The hypomethylation of these elements in disease has not been investigated as yet.

2.4. Hypomethylation of individual genes in disease

In studying gene-specific methylation changes in disease, particular emphasis has been placed on hypermethylation of critical genes and associations of silencing of their expression with disease outcomes. Yet, methylome profiling of cancer cells and studies of individual genes reveal that gene-specific hypomethylation occurs frequently in a range of cancers, e.g. cancers of the colon [82], pancreas [83,84] and the breast [85]. Demethylation of specific genes often correlates well with increased transcription levels [3,22] and Table 4.

Table 4 provides examples of single copy genes that are demethylated in disease (see also [31] for an extensive list). The ranges of genes affected by hypomethylation includes growth regulatory genes, enzymes, developmentally critical genes and tissue specific genes such as germ cell-specific tumor antigen genes, the MAGE, BAGE, LAGE and GAGE gene families. The aberrant expression of oncogenes due to cancer-linked hypomethylation such as R-Ras has also been shown [22]. For a number of genes, type or stage specific hypomethylation and expression in cancer has been shown; for example, there is a strong association of CDH3 promoter demethylation and P-Cadherin expression evident with histological grade and invasiveness in breast cancer [86], cyclin D2 activation is associated with Stage III and IV gastric cancer [87], activation of synuclein γ is associated with progression and metastatic potential in a range of solid tumors [88], and maspin expression in colorectal cancer is associated with microsatellite unstable tumors [89]. Similarly promoter hypomethylation and expression of the perforin gene is associated with active, but not inactive Lupus [90].

Another group of genes that can be disrupted by changing methylation patterns are the imprinted genes [32,91]. Loss of imprinting (LOI) is associated with cancer development [29] where the hypomethylation of imprinted genes can result in biallelic expression that can support tumor growth [12,92–95]. A well-studied example in colorectal cancer is LOI with hypomethylation of a differentially methylated region of the normally silent allele and overexpression of IGF2 [96]. Further, LOI of IGF2 has been implicated as a precursor lesion in colorectal cancer, with individuals showing systemic LOI having a higher risk of developing colorectal cancer [97]. The mechanisms involved in maintenance of imprinting (MOI) and LOI are complex and remain largely unresolved. MOI at the IGF2 locus is associated with allele-specific differential methylation of the CTCF binding site found upstream of the adjacent H19 gene. However the mechanism of LOI differs in different tumor types and can involve either biallelic CpG methylation or hypomethylation of the CTCF-binding site [96,98,99].

A number of diseases of aberrant growth develop as a consequence of hypomethylation of imprinted genes, including the Beckwith–Weidemann Syndrome (BWS), Silver–Russel Syndrome (SRS) and transient neonatal diabetes mellitus [100]. BWS is of particular interest; defined as a tumor-predisposing syndrome, it sometimes results from an extra copy of the IGF2 gene being paternally transmitted, replacing the inactive maternally inherited copy of the gene. This leads to the overexpression of IGF2 which can lead to and support tumor growth [92–94]. In children, BWS can increase susceptibility to Wilms tumors, adrenocorticol carcinoma, hepatoblastoma and neuroblastomas [101].

2.5. Relationship of repeat sequence hypomethylation and the methylation changes of single copy genes

Few studies have investigated an association of focal demethylation with global hypomethylation and fewer still have examined the link between hypomethylation of single copy genes and that of repeat sequences. The demethylation and expression of the MAGE-A1 and maspin genes have been well studied and generally correlate well with overall levels of genomic hypomethylation in a number of cell lines and primary cancers [102–105]. A similar correlation has been observed for the demethylation and expression of at least one BAGE loci with juxtacentromeric satellite DNA hypomethylation on chromosomes 9, 13, 18 and 21 [106]. Due to the close proximity of BAGE2 with the juxtacentromeric repeats on chromosome 21, it has been proposed that BAGE demethylation may be mechanistically linked to the hypomethylation of repeat sequences. Interestingly, the X chromosome on which many of the MAGE genes reside contains a high density of LINE elements that could also be subject to hypomethylation in

Notes to Table 3:

A. Narayan et al., Int. J. Cancer 77 (1998) 833–8; K. Jackson et al, Cancer Biol. Ther. 3 (2004) 1225–31; P. Miniou et al, Hum Genet. 99 (1997) 738–45; T. Kondo et al, Hum Molec Genet 9 (2000) 597–604; Alves et al, Gene 186 (1996) 39–44. M. Widschwendter et al, Cancer Res. 64 (2004) 4472–80; G. Qu et al, Mutat. Res. 423 (1999) 91–101; A. Narayan et al, Int. J. Cancer 77 (1998) 833–8; K. Jackson et al, Cancer Biol. Ther. 3 (2004) 1225–31; F.F. Costa et al, Cancer Genet. Cytogenet. 165 (2006) 135–43; P. Miniou et al, Hum Genet. 99 (1997) 738–45; C.M. Tuck-Muller et al, Cytogen Cell Genet 89 (2000) 121–8; Hassan et al, 109 (2001) 452–62; T. Kondo et al, Hum Molec Genet 9 (2000) 597–604: P.G. van Overveld et al, Ann. Neurol. 58 (2005) 569–76; D. Takai et al, Jpn. J. Clin. Oncol. 30 (2000) 306–9; K. Chalitchagorn et al, Oncogene 23 (2004) 8841–6; G. Alves et al, Gene 186 (1996) 39–44; J. Roman-Gomez et al, Oncogene 24 (2005) 7213–23; D.J. Weisenberger et al, Nuc. Acid Res. 33 (2005) 6823–36; L. Menendez et al, Mol. Cancer 3 (2004) 12; M. Neidhart et al, Arthritis Rheum. 43(2000) 2634–47; A. R. Florl et al, Br. J. Cancer 80 (1999) 1312–21.

Table 4
Examples of hypomethylated genes and their associated diseases: studies from the last 6 years

Gene	Disease	Assay method	Comments	Reference
Cytochrome P450 1B1	Prostate cancer	Methylation-specific PCR, Bisulfite-DNA sequencing, RT-PCR and immunostaining	Demethylation of promoter/enhancer region correlates to overexpression of gene; significant increases in demethylation and gene expression noted in cancer compared to surrounding muscle tissue and benign prostate tissues; no correlation of methylation levels with pathologic category	Tokizane et al, 2005
Maspin	Colorectal carcinoma	Methylation-specific PCR, microsatellite PCR, methylation-specific digest, RT-PCR, Western blot, immunostaining	Nuclear maspin expression increases with degree of dedifferentiation of cancer (high levels in microsatellite unstable tumors compared to matched benign colonic mucosa and microsatellite stable tumors ; gene promoter hypomethylation observed in all maspin expressing samples; expression frequently found at invasion front of tumor	[89]
	Thyroid cancer	Methylation-specific PCR, RT-PCR, immunostaining	Gene hypomethylation correlated well with gene expression; demethylation evident mostly in undifferentiated and poorly differentiated specimens compared to well-differentiated tumors and matched normal	Ogasawara et al, 2004
Maspin, S100P Claudin-4; lipocalin 2; S100A4; PSA; 14-3-3sigma; trefoil factor 2; mesothelin;	Primary pancreatic carcinoma	Expression microarrays, methylation-specific PCR	Promoter hypomethylation of maspin and S100P correlate with overexpression in most primary pancreatic cancers; 32 genes show overexpression in cancer, 19 of 32 were methylated in normal tissues, 7 of 19 were hypomethylated in cancer tissues (this pattern prevalent in most carcinomas examined), 1 gene overexpressed was methylated	[83,84]
Protease uPA	Breast cancer (cell lines)	Methylation-sensitive digest, MS-PCR, DNA methyltransferase activity assay DNA demethylase activity assay	Hypomethylation observed in late-stage invasive cell lines whilst MCF-7 cells were highly methylated at gene; methylation levels corresponded to maintenance DNA methyltransferase activity and inversely to demethylase activity	Guo et al, 2002
P-Cadherin	Invasive breast cancer	Methylation-specific PCR, RT-PCR, Western Blot	Demethylation of the CDH3 promoter linked to increased p-cadherin expression, overexpression of p-cadherin has shown a strong association with histological grade, p53 oncogene expression, lack of estrogen receptor and increased cell proliferation.	[86]
Synuclein γ (SNCG)	Breast and ovarian cancer (cell lines); tumors of liver, prostate, esophagus, gastric, colon, cervical, lung and breast	Bisulphite sequencing, nested methylation-specific PCR	Correlation of exon 1 hypomethylation with expression, methylation primarily occurred in exon 1 at CpG positions 2, 5, 7, 10–15 in normal breast, no such pattern observed in ovarian cells;	Gupta et al, 2003
			Gene demethylation was noted in all tumor tissues and correlated significantly with gene expression, 10–30% of normal tissues also showed some demethylation but were mostly methylated; protein expression was stage-specific (low in stage I and very high in stages II–IV) and strongly associated with distant metastasis in patients	[88]

TRAG-3	Colon, liver, lung, ovarian, pancreatic tumors (cell lines)	Bisulphite sequencing, RT-PCR	Methylation of exon 2 and promoter of TRAG-3 inversely correlates to expression; tumors overexpressed gene compared to normal cells	Yao et al, 2004
BRCA1, BRCA2	Sporadic ovarian cancer	Bisulphite-PCR, RE-digest, bisulphite sequencing	Low BRCA1 expression in tumors but high BRCA2 expression levels in tumor compared to normal; promoter hypomethylation correlated with expression levels and tumor stage	Chan et al, 2002
MEL1S	T-cell acute lymphoblastic leukemia	MS-PCR, RDA and bisulphite sequencing	Demethylation of chromosomal region contained MEL1, CACNA1H, and Nogo receptor genes, hypomethylation of MEL1S associated with increased expression in all T-ALL and a dysregulation of TGFb-mediated signaling, hypomethylation implicated in disease progression;	Yoshida et al, 2004;
HOX II			In all samples of T-ALL, promoter demethylation corresponded to HOX-11 expression	Watt et al, 2000
p53	Lung cancer	Modified methyl acceptance assay, COBRA assay	Genome-wide hypomethylation unrelated to lung cancer risk, hypomethylation of p53 in exons 5–8 (hypermutable region) associated with 2 fold increased risk of lung cancer whilst no risk associated with hypomethylation at exon 2–4	Woodson et al, 2001
MAGE 1; MAGE-A1; MAGE A3	Testicular cancer (cell lines);	Methylation-specific-PCR, bisulphite sequencing, RT-PCR and southern blot	Promoter hypomethylation in cell lines due to transient \demethylation followed by local inhibition of remethylation by the presence of transcription factors and increased promoter activity;	DeSmet et al, 2004;
	Hepatocellular cancer		Promoter hypomethylation correlated to gene expression in HCC compared to tumor free tissues and blood	Qui et al, 2006
BAGE	Breast ovarian and myeloma cell lines, tumors of sperm, testis, somatic tissue,		94% of somatic tissue is DNA methylated while 98% of tumor is demethylated but BAGE transcripts evident in 47% of tumors. Testis and sperm show demethylation in at least one BAGE loci. Hypomethylation evident at Juxtacentromeric satellite DNA	[106]
Perforin	Active SLE	Methylation-inhibitors	Gene is demethylated and overexpressed in active Lupus	[90]

T. Tokizane et al, Clin. Cancer Res. 11 (2005) 5793–801; S. Ogasawara et al, Oncogene 23 (2004) 1117–24; Y. Guo et al, J. Biol. Chem. 44 (2002) 41571–9; A. Gupta et al, Cancer Res. 63 (2003) 664–73; X. Yao et al, Cancer Genet. Cytogenet. 151 (2004) 1–13; K.Y. Chan et al, Cancer Res. 62 (2002) 4151–6; M. Yoshida et al, Blood 103 (2004) 2753–60; P.M. Watt et al, Genes Chromo. Cancer 29 (2000) 371–7; K. Woodson et al, Cancer Epidemiol. Biomarkers Prev. 10 (2001) 69–74; C. De Smet et al, Mol. Cell. Biol. 24 (2004) 4781–90; G. Qui et al, Clin. Biochem. 39 (2006) 259–66.

cancer. Thus, at least for the class of testis-expressed genes represented by the MAGE and BAGE genes, a possible connection exists between their demethylation and that of satellite and/or interspersed repeat sequences in cancer.

Studies in a number of systems including colorectal [17,82,107], urothelial [108], prostate [109], ovarian [11,46] and gastric cancers [103] as well as Wilms tumors [110] and non-Hodgkin's lymphoma [111] have examined the link between global genomic hypomethylation and hypermethylation of CpG islands. The general finding of these studies is that gene-specific hypermethylation and global hypomethylation are independent processes—that is the presence or level of global hypomethylation does not alter the likelihood of CpG island hypermethylation, and they are unlikely to be causally related. An exception is the observation in ovarian cancer where a lack of hypermethylation of two promoters (CDH13, a cadherin and RNR1, the ribosomal gene cluster) correlated with chromosome 1 Sat2 hypomethylation [11,46].

In different cancers, the relative timing of global and repeat sequence hypomethylation and hypermethylation of individual CpG islands can vary. In colorectal cancer hypomethylation is evident in early stage disease (and may be present in normal tissue adjacent to tumors) and its extent and frequency does not alter greatly with disease progression. In contrast, hypermethylation of a number of specific genes occurs early in prostate cancer, while LINE demethylation increases with tumor stage [112]. Differences in timing in different cancer types further indicate that different processes underpin common CpG island hypermethylation and demethylation of repeat sequences associated with global hypomethylation.

3. Mechanisms of hypomethylation

In order to consider possible mechanisms leading to repeat sequence hypomethylation it is important to consider what is known about the normal processes of establishment and maintenance of DNA epigenetic status.

3.1. Normal DNA methylation patterns across the genome

The mechanisms involved in the establishment and maintenance of DNA methylation patterns have been reviewed previously (see reviews [9,113,114]) and will therefore be discussed only briefly here. Tissue specific DNA methylation patterns are established early in development [115] subsequent to implantation [116]. These established patterns are then precisely transmitted via mitosis and in differentiated cells are maintained after DNA replication [117].

In mouse embryonic development waves of rapid hypomethylation are observed post-fertilisation prior to the re-establishment of methylation patterns [116]. This genome wide hypomethylation proceeds through a passive process for the maternal genome, in contrast to the paternal genome that undergoes a rapid and extensive process of active demethylation. The demethylase activity is present in the oocyte, but the molecular mechanism underlying the process is not yet established [118]. Though extensive, a level of specificity is evident in that methylation of centromeric and retrotransposon repeats and paternal imprinted genes is maintained. Active demethylation is also a feature of primordial germ cell development and is believed to be critical for re-setting the epigenetic state of the genome and erasing imprinting marks. Active demethylation of the IL2 promoter/enhancer has been shown to occur in response to T cell activation and may play a wider role in epigenetic re-programming in somatic cells [119]. Until more is understood about the molecular mechanisms involved in active demethylation, it is difficult to determine whether they are involved in the extensive hypomethylation seen in cancer.

Methylation patterns are regulated by at least 4 independent methyltransferases (DNMT1, DNMT3A, DNMT3B and DNMT3L) of partially interchangeable functions [114,120]. In mammals DNMT1 acts to conserve methylation patterns by methylating hemi-methylated sites to produce symmetrically methylated CpG dinucleotides in the double strands of DNA. In vitro studies using mammalian cells have shown the consistency of maintenance methylation from progenitor to newly synthesized strand to be as high as 97–99.9% per mitosis [121], ensuring the strict fidelity of methylation patterns following replication. DNMT3a and DNMT3b are believed to be primarily responsible for de novo methylation [122,123]; de novo methylation has been recorded at 3–5% per mitosis [20], enabling a change to occur in DNA methylation with time. DNMT3L, like its other DNMT3 members, is highly expressed during early development but does not contain any intrinsic DNA methyltransferase activity [114]. DNMT3L serves to modulate the activity of DNMT3A and DNMT3B [124,125] and positively regulates the established DNA methylation at imprinted and repeat sequence sites [126–128]. In adult cells, the role of the DNMT3 members remains unclear. The DNA methyltransferases interact with a variety of gene regulatory proteins through feedback loops that can directly and/or indirectly impact chromatin structure which may influence DNA methylation [31].

The control of DNA methylation is intrinsically linked with processes of chromatin remodelling. Chromatin states have traditionally been divided into active or euchromatic and inactive or heterochromatic. These states are characterised by a variety of modifications of histones, including acetylation, methylation, phosphorylation and ubiquitination of specific amino acids (reviewed in [129]). These modifications act as specific target sites for the binding of a variety of gene-regulatory proteins and for the establishment and maintenance of different states of gene activity. Active chromatin is characterised by an open structure, lack of DNA methylation and specific histone modifications, a key example being the acetylation of lysine 9 of histone H3 (H3Lys9). Conversely methylated DNA is associated with inactive chromatin and specific histone modifications including methylation of H3Lys9 and H4Lys20 [130]. Histone modifications are controlled by a series of enzymes including histone acetyl transferases, histone deacetylases and histone methyltransferases; direct or indirect interactions of these with the DNA methyltransferases point to the interdependence of chromatin structure and DNA methylation in determining epigenetic states.

RNA is another important component of the epigenetic regulatory system. In plant systems, the small interfering RNAs (siRNA) has been shown to directly induce transcriptional silencing via sequence specific DNA methylation and chromatin change [131] and transcription of retrotransposon sequences has been shown to be involved in heterochromatin formation and DNA methylation of centromeric repeat sequences [132]. While limited, there is growing evidence in mammalian systems for siRNA-mediated transcriptional gene silencing [133]. The existence of an endogenous system is supported by a case of α-thalassaemia characterised by an intergenic deletion [134]. The antisense transcription from an adjacent gene through the α-globin gene results in gene silencing and promoter methylation. More generally, the role of non-coding RNA in imprinting and X-inactivation is well recognised (if poorly understood) [135–137]. Recent evidence supports the idea that RNA silencing in vertebrates serves as a natural immune response to protect against pathogen (be they viral, transgene or transposon) invasion [138].

3.2. Processes leading to DNA hypomethylation

DNA hypomethylation could be triggered through disruption of control in a number of the following steps:

3.2.1. DNA methyltransferases

In animal models and in vitro experiments, the lack of DNA methyltransferase activity through knock out of an individual DNMT or a combination of DNMTs is linked to genomic hypomethylation and an associated chromosomal abnormality and instability [30,139–141]. Studies of DNMT1 knock out mice have shown that decreased methylation levels impact upon the frequency of cancer formation [142]. Knock out of DNMT1 in the min mouse model (heterozygous for mutation in the APC gene) leads to an overall decrease in incidence of intestinal cancers. Detailed analysis has indicated an increased frequency of early lesions (microadenomas), but progression of these to carcinomas is apparently impaired in the hypomethylation background. By contrast, the frequency of hepatocellular adenomas and carcinomas is significantly increased in the same mouse model. Levels of DNMT1 have been studied in a range of tumor types—where changes in expression levels correlate with cellular proliferative rate [143,144]. While there is no clear consensus, levels of DNMT1 protein or mRNA appear to be modestly elevated and/or regulation of activity disrupted in many cancers [145–148]. In colorectal cancer, neither DNMT1 nor DNMT3 expression was found to relate to changes in methylation pattern [143]; furthermore, striking heterogeneity in DNMT1 expression was observed within the colorectal carcinoma sample as well as normal colonic tissues [149]. However, there is no evidence of a link between DNMT1 levels and genome hypomethylation in any human cancers studied thus far [11,110].

In mouse germ cells, mutation of DNMT3L prevents de novo methylation of LTR and non-LTR retrotransposons without affecting the methylation of tandem repeats in the pericentric region [127]. The role of DNMT3L in regulating both dispersed repeats and satellite sequences (see below) warrants further investigation.

Studies of ICF patients have revealed that mutation of the de novo methyltransferase DNMT3B – resulting in reduced enzyme activity or the compromised stimulation of activity by DNMT3L [150] – leads to specific loss of methylation at satellite repeats (especially Sat2, Sat3 and Satα on chromosomes 1, 9 and 16) [151]. This hypomethylation is associated with chromosomal rearrangements involving the repeat sequences. This syndrome however is not linked to cancer. Since hypomethylation of satellite repeats is a common event in different cancers it was appropriate to ask whether such hypomethylation might be associated with reduced levels of DNMT3B expression. However, in a number of studies expression of DNMT3B has been found to be frequently elevated in cancer in comparison with other DNMTs [152,153]. In particular, the overexpression of the splice variant DNMT3B4 demonstrates an apparent association with repeat sequence hypomethylation. In CLL, LINE demethylation and transcription was found to correlate well with expression levels of DNMT3B4 [71]. Likewise, in hepatocellular carcinoma, DNMT3B4 overexpression has been linked to pericentromeric repeat hypomethylation [154]. However, other studies have concluded that there is not a direct causative link between DNMT3B4 and repeat sequence hypomethylation. In both Wilms tumors and ovarian cancers Ehrlich and colleagues [11,110] did not find any relationship of any isoform of DNMT3B transcripts with global hypomethylation or satellite repeat hypomethylation. Similarly, they found no association with DNMT3A expression levels.

Thus, despite the central roles of the DNA methyltransferases in establishment and maintenance of epigenetic control, evidence to date does not implicate a reduction in their expression as a significant contributing factor for cancer-associated hypomethylation. Reduction in expression, determined at the level of RNA in particular, is a crude measure of the function of the DNMTs. Interactions with many proteins, chromatin components, transcription factors, replication and repair machinery are involved in their regulated activity and it is likely that disruption at this level is responsible for the observed genome wide loss of DNA methylation. In contrast, it may be that the effects of the loss of specific DNMT activities may be evidenced in particular cancer types through specific targeted sites (repeat sequences or single copy genes) in the genome that have not yet been investigated.

3.2.2. Other proteins involved in epigenetic control

Processes of histone modification and chromatin remodelling are intricately involved with DNA methylation in establishment of normal epigenetic states and histone modifications associated with specific gene hypermethylation have been extensively studied. Recent studies demonstrate the likely importance of chromatin changes in genome-wide hypomethylation, potentially with a causative role. In addition to genome wide DNA methylation changes Fraga and coworkers [155] examined changes in histone modification in cancer cell lines and primary tumors; they found significant

loss of monoacetylation of H4Lys16 and trimethylation of H4Lys20. These histone modifications correlated well with DNA hypomethylation of Sat2, NBL2 and D4Z4 tandem repeat sequences in leukemic cells. In addition specific loss of association of the histone acetyltransferases MOF, MOZ and MORF with D4Z4, Sat2 and NBL2 repeat sequences, respectively, was observed. The specificity of association of these acetyltransferases with the different repeats is indicative of different regulatory pathways exerting epigenetic control of different repeat families.

Other histone modifications could indirectly affect methylation levels by altering chromatin structure. For example, methylation of lysine 9 on histone H3 is associated with epigenetically silenced chromatin and is essential for the establishment and maintenance of heterochromatin [156]. Mouse knock out experiments have demonstrated that loss of methylation at H3K9 results in marked genomic instability and increases cancer predisposition [157]. In human cancer, the putative oncogene GASCI (a jumonji protein found highly expressed in squamous cell carcinomas) associates with and demethylates the H3K9 [158], altering chromatin structure. Thus, disruption of chromatin structure and modifications of histone methylation and/or acetylation in cancer could have downstream effects on DNA methylation patterns.

3.2.3. Regional control of epigenetic states

In a recent genome-wide study of differential methylation levels in colon cancer cells, Weber et al. [159] identified a number of largely gene-poor, LINE rich regions on chromosomes 3, 7, 11 and 14, individually spanning up to 20 Mb that exhibited marked hypomethylation in transformed cells. Chromosomes 7 and 14 displayed a high level of overall hypomethylation and were trisomic; supporting the hypothesis that extensive demethylation contributes to chromosomal instability. Epigenetic silencing across a whole chromosomal band (4 Mb) involving histone modifications and DNA methylation has also been reported recently [160]. While these studies concentrated largely on CpG islands and gene containing regions of the DNA the observations of concordant control of epigenetic features on a regional level raise the possibility that hypomethylation of repeat sequences may proceed in a regional manner through disruption of local control. One study [106] has shown an association of gene hypomethylation with hypomethylation of nearby juxtacentromeric repeats. It will be important to determine whether the hypomethylated chromosomal regions correspond to particular repeat sequences and transposable elements; understanding of repeat sequence hypomethylation patterns on a genome-wide scale could contribute significantly to unravelling underpinning mechanisms of processes leading to hypomethylation.

3.2.4. Involvement of RNA in DNA hypomethylation in cancer

While processes of site-specific and active hypomethylation are poorly understood, there have been a number of indications that RNAs might be involved [131]. Active demethylation of specific gene sequences in a cell-free system has been shown to be dependent on the presence of RNA, with extracts obtained from different cell types promoting hypomethylation in a gene-specific manner [161]. Expression of an antisense RNA to the Sphk1 gene [162] has been shown to promote region-specific hypomethylation. The possibility that aberrant transcription of specific sequences may promote active demethylation of repeat sequences needs to be considered.

Conversely, RNA is also implicated in processes of gene silencing and promotion of DNA methylation. The role of RNA in establishing chromatin states has been recently reviewed [163] and while not established in mammalian systems, there is increasing evidence to indicate a role for antisense transcription of repeat sequences in maintaining heterochromatic states [132]. In a mouse cell system the organisation into heterochromatin of pericentric repeats has been shown to involve an RNA component binding to the heterochromatic protein HP1 [164]. High affinity binding of RNA to the de novo DNA methyltransferases DNMT3A and 3B1 as well as methyl DNA binding proteins has also been demonstrated [165]. Disruption at the RNA level of a maintenance system that likely involves other chromatin components in recruitment of DNA methyltransferases to re-enforce silencing of repeat sequences could well contribute to targeted or generalised hypomethylation.

3.2.5. Defective repair mechanisms following exogenous insult

Exogenous insults may initiate the hypomethylation process of genomic DNA via DNA damage pathways which may predispose cells to cancer development. These factors can include diet (methyl donor deficiency and metabolisers) [166], environment (UV radiation and chemicals) [167] and even bacterial infection [168]. Tumor induction through these processes has been observed, however the underlying mechanisms responsible remain speculative. What is known is that, in some cases, DNA demethylation is observed to occur following genomic damage [169]. A number of possibilities have been proposed including: i. repair of strand breaks may result in the replacement of 5 meC with cytosine resulting in local hypomethylation surrounding the site of damage; or ii. The high affinity binding of the methyltransferases to DNA lesions (such as strand breaks, gaps, abasic sites and uracil) sequesters the available methyltransferases from lesion-free DNA to promote passive replication-dependent demethylation. This may explain focal hypomethylation but not universal changes in genomic methylation levels. Furthermore, the specificity of affected repeat sequences and single-copy genes cannot be explained. Alternatively, the exogenous insults (including diet, chronic exposure to environmental conditions and so forth) may enhance the contribution and effects of other factors (that is, the proteins and enzymes mentioned above) in the initiating or spreading methylation changes.

An area of concern where environmental (cell culture) conditions may influence epigenetic programming is the use of assisted reproductive technologies (ART). Children born through ART have an increased frequency (9× more than the general public) of developing BWS that has an associated increase risk of embryonal tumors particularly Wilms tumor of

the kidney [170]. ART children also have a higher incidence of retinoblastomas (when compared to general populations) as well as an increased incidence of Angelman's syndrome associated with the hypomethylation of the imprinted SNRPN gene. Mouse studies implicate immature oocytes (i.e., the methylation patterns are not established or properly methylated) but it may also be that the change in the germ cell and embryo environment (such as artificial culture conditions) result in possible widespread changes of epigenetic patterns leading to birth defects and increased rate of prenatal tumors.

4. Consequences of DNA hypomethylation

4.1. Transcriptional consequences of transposable elements

Hypomethylation of repeated DNA sequences is expected to lead to the transcriptional activation of those repeat sequences that still contain active promoters. Repeat sequence transcription could potentially impact in a number of ways—(a) by disrupting the balance of transcription factors and other regulatory proteins through competitive binding, (b) through expression of repeat element encoded proteins—transposases, gag, pol or env genes, (c) through generation of read through transcripts into single copy sequences that encode proteins, (d) through transcriptional interference by either sense or antisense transcription in relation to neighbouring genes or (e) production of transcripts complementary to endogenous transcripts, generating double stranded RNAs. However, there is little quantitative data or direct evidence of the impact of these transcripts in the cell.

Elevated expression of endogenous retroviral sequences, especially HERV-K, has been reported in a number of cancers as well as other disease conditions (multiple sclerosis, schizophrenia and Lupus), but generally not in association with information on alterations in methylation status. In bladder cancer HERV-K sequences are commonly hypomethylated, but this is not associated with an increase HERV-K transcription [62]. Profiling of HERV transcription has demonstrated substantial tissue specificity in patterns of gene expression and expression in cancer may be cell-type dependent. In hepatocellular carcinoma there is a similar lack of increase in LINE transcripts despite reduced methylation of LINE elements [43]. A particular example of the impact of repeat sequence is the expression of LINE-1 transcripts in chronic myeloid leukaemia [71]. LINE sequences are hypomethylated in CML, particularly in blast crisis and this hypomethylation is associated with significantly elevated LINE transcription. The authors have identified a strong association between LINE expression and elevated expression of the c-MET oncogene and have raised the possibility that transcription from the antisense promoter of a LINE element within intron 2 of the c-MET gene is driving its elevated expression.

A key question that remains to be addressed is whether dysregulated expression of repeat sequences impacts on disease through generalised non-specific mechanisms or through the effects on transcription of specific individual sequences such as in the c-MET example above.

4.2. Chromosomal consequences

Evidence obtained through in vitro and in vivo models shows that the methylation status of repeated DNA sequences can significantly influence genetic stability including: the expansion of the (CGG)n repeat in Fragile X; the hyper and hypomethylation of microsatellites in certain tumors and the genetic instability that arises through altered methylation of satellite repeats and transposable elements [45–47,171–173].

4.2.1. Insertional mutagenesis

In the event of global hypomethylation transposable elements can be transcribed, potentially leading to insertional mutagenesis; mobilisation of LINE L1 elements has been demonstrated following deletion of DNMT1 [174]. Retrotransposon insertion can lead to alteration of gene expression, disruption of the coding sequences and splice sites and significant target site deletions as well as provide areas of sequence identity for both gene conversion and recombination events [44,79,175,176]. In inherited disease, 15 LINE de novo insertions and over 20 SINE-insertions have been identified that have resulted in a genetic disorder or a novel polymorphism [58,79]. Recently, in an investigation on 50 families with inherited breast cancer three families were found to have heritable Alu insertions within the BRCA1 or BRCA2 genes [177]. Interestingly, one of the insertions was created by an older AluS element (which contradicts current opinion that only young repeat sequences are able to transpose) and the insertion within the BRCA2 gene resulted in the removal of the targeted exon from its corresponding mRNA.

In mice, retrotransposition of intracisternal type A particles (IAPs, an LTR-retrotransposon) is commonly observed in the malignant transformation of haematopoietic cells, often affecting expression of cytokine genes [178]. However, despite the potential for transposition, there is no evidence thus far where global hypomethylation arising in human cancer initiation or development causes repeat element transposition. While the number of LINE and SINE elements in the genome is large, only a limited number of LINE elements, 80 to 100 per genome, are functionally competent for transposition and among these, most transpositional activity is dependent on a smaller number [69]. Additionally cell defence mechanisms sense transposition and trigger an apoptotic response [179] and thus expression of transposition-competent LINE-1 elements may be selected against.

4.2.2. Recombination

Repeated DNA sequences represent a significant threat to the structural integrity of the genome. These elements can potentially mediate recombination between non-allelic repeats causing chromosomal rearrangements or translocations. It is believed that widespread demethylation leading to an open chromatin structure at repeat sequences, can predispose chromosomes to structural and numerical aberrations. The relationship between karyotype abnormalities and hypomethylation has been analysed in a number of cancer types. The occurrence of frequent rearrangements near the chromosome 1

and 16 centromeres and the hypomethylation of Satα and Sat2 sequences in ovarian cancer are consistent with a predisposing role of hypomethylation [180]. Studies in lymphoblastoid cell lines [181] showed a clear relationship between demethylation of Sat2-rich heterochromatic regions of chromosomes 1 and 16 and rearrangements along the length of these chromosomes. In contrast, demethylation of Sat3-rich regions of chromosomes 9 and Y was not associated with chromosome instability. In Wilms tumors hypomethylation of Satα and Sat2 sequences is very common. Chromosomal rearrangements involving chromosome 1 are significantly less frequent, but are strongly associated with hypomethylation indicating that repeat sequence hypomethylation is necessary but not sufficient for chromosome rearrangement [182]. In breast cancer, copy number increases of chromosomes 1 and 16 were found associated with cancers displaying hypomethylation of Sat2 sequences, while specific chromosomal rearrangement was not [183]. Copy number increase of chromosome 1q also correlated highly with Sat2 hypomethylation in hepatocellular carcinoma [184] whilst Sat2 hypomethylation in the absence of copy number abnormalities was seen in 5 of 24 adjacent non-tumorous liver tissues, suggesting an early role in carcinogenesis.

Rearrangements are not limited to chromosomes 1 and 16—a strong correlation of loss of heterozygosity (LOH) of chromosome 9 with hypomethylation of Sat2 and Sat3 sequences was seen in urothelial carcinomas [185]. In prostate cancer hypomethylation was strongly correlated with loss of 8p or gain of 8q, while there was no association with another common chromosomal alteration, loss of 13q [186]. In colorectal cancer chromosomal instability, measured by LOH, has been linked to cancers displaying hypomethylation (of LINE sequences) and microsatellite stability [15,17]. In contrast, microsatellite unstable cancers showed more frequent specific hypermethylation and substantially lower LOH. These observations support the general conclusion that hypomethylation of simple tandem repeats predisposes such regions to illegitimate recombination events leading to genome rearrangements. The specificity of which repeats and genomic regions are involved suggests that factors in addition to DNA demethylation and chromatin de-condensation are required.

4.3. Immunological consequences

The role of DNA methylation in immune function has been extensively discussed (see Clinical Immunology 109 (2003)). The most direct link between DNA hypomethylation and immunological malfunction is exemplified by the rare recessive disorder, ICF syndrome. Here loss of activity of DNMT3B is associated with severe immunodeficiency, compromised lymphocyte function and with chromosomal rearrangements, mostly localised to the juxtacentromeric heterochromatin of chromosomes 1 and 16 [151]. It is not yet established whether hypomethylation and possible transcription from repeat sequences (e.g. Sat2, Sat3, Satα, NBL2 or D4Z4) or inappropriate hypomethylation and expression of specific single copy genes underlies the etiology of the disease.

In vivo studies have shown the potentiation of autoimmunity in mice treated with hypomethylating agents [187] that is expected to result in inappropriate gene expression. Decreased levels of 5 meC and methyltransferase activity are seen in the T cells from patients with active Lupus. This is linked to decreases in DNMT1 and DNMT3A transcript expression, possibly a result of inhibition of the ERK pathway signalling (see [188]), and demethylation [189]. The DNA hypomethylation causes overexpression of the adhesion molecule LFA-1, leading to autoreactivity and eventually autoimmunity [188]. DNA hypomethylation in patient T cells has similarly been reported in rheumatoid arthritis. Likewise, it is possible that the diseases, multiple sclerosis and schizophrenia, associated with expression of HERV and LINE transcripts (mentioned in previous sections above) may too be involve in an increased immunological response. Despite their association with DNA hypomethylation, elevated cancer risk has not been reported in either ICF or lupus patients, though their short lifespan may mask this possibility in ICF patients.

In a number of cancers, members of a class of genes normally restricted in expression to testes, the cancer/testis antigens (CTA) are found to be hypomethylated and expressed, and are immunogenic in cancer patients [190]. Almost half of the CTA families tested (14/29) have been shown to induce a cellular and/or humoral immune response in humans when expressed. Dependent upon the cancer investigated, CTA expression levels have been shown to be stage-specific. These include the XAGE 1 gene in non-small cell lung adenocarcinomas [191]; the NY-ESO 1 gene in advanced stage gastric cancer [192], advanced stage (stages III and IV) esophageal cancer [193] and hormone refractory prostate cancers where positive reactivity to the antibody correlated with poor survival in patients [194]; as well as the NY-ESO 1, MUC 1 and SSX-2 antigens in primary breast cancers [195]. Interestingly, in many cases, the expression of the CTA mRNA does not elicit an immune response and in esophageal cancer, the GAGE, MAGE, NY-ESO 1 and SSX-2 proteins did not correlate with disease progression and survival [196]. In contrast, in ovarian cancers, SCP-1 expression showed no immunoreactive antibodies in patient sera, but was found to correlate well with tumor grade and poor patient survival [197]. While the role (if any) of these antigens in cancer development is not understood, they provide unique cancer-specific targets for immunotherapeutic approaches to cancer treatment.

4.4. What is the significance of global or focal hypomethylation to clinical outcome?

A number of studies have specifically addressed whether global hypomethylation or the hypomethylation of specific repeats is associated with clinical outcome. Examples of repeat sequence hypomethylation and/or global demethylation relating to poor prognosis or clinical severity include: quantitative reduction in global levels of 5 meC in prostate cancer significantly correlated with disease recurrence after prostatectomy [198]; the hypomethylation of Sat2 DNA in chromosome 1 was associated with advanced stage ovarian cancers and

proved more informative than tumor stage or grade as a predictor of relapse or death [46]; LINE 1 hypomethylation in chronic myeloid leukemia [71] and urothelial carcinoma [108] was associated with shortened progression-free survival and the pronounced D4Z4 repeat hypomethylation (localised to chromosome 4q35) correlates with the severity of Facioscapulohumeral muscular dystrophy [48].

There is still a lack of understanding as to whether the hypomethylation of the repeat sequences causes the disease or drives the clinical outcome or is the result of natural selection in the progression of the disease. Understanding the processes and significance of methylation changes of repeat sequences and single copy genes as well as the identification of specific epigenetic signatures of certain disease classes may impact upon approaches to epigenetic therapy. Currently broad-spectrum means are being considered such as DNA demethylating agents that reactivate silenced genes and drugs targeted to chromatin-modifying enzymes. Although these are showing significant promise in hematologic malignancies [199,200] they have the potential for severe side effects, such as causing autoimmunity (Section 4.3). The presence of concurrent hyper and hypomethylation of different sequences in many cancers complicates approaches to epigenetic therapy and application of current approaches to cancers that show significant hypomethylation remains to be evaluated. Ultimately, therapy based on selective targeting to specific epigenetic control pathways may provide therapeutic selectivity with minimal side effects.

5. Hypomethylation as a diagnostic or prognostic indicator

Detection of changes in methylation patterns, as a consequence of disease or a result of treatment, is gaining relevance as an application in the clinical arena. Alteration in methylation, as a biomarker, has significant attraction as a diagnostic or prognostic indicator. Although this promise has been alluded to for many years, use of methylation (be it hypermethylation or hypomethylation) as a clinical tool remains somewhat elusive. This may be due to a number of reasons including: inadequate analytical tools, the need for specific and sensitive biomarker(s) that unambiguously and definitively characterise disease types or risk groups, modest performance of current methods to consistently and efficiently measure methylation differences (including small but significant changes), added complexity for routine use of bisulphite-based assays, acceptance of current screening applications for clinical use and the need for less invasive approaches for clinical testing.

5.1. DNA hypomethylation as a prognostic indicator

The predictive value and prognostic significance of methylation changes in disease treatment has been tested in vivo. Much of the work in this area has concentrated on the methylation changes that occur at individual gene loci which have been shown to confer a certain prognosis; such as the p15/INK4b and p16/INK4a subset of genes [201] and the CTA genes in the treatment of myeloid neoplasia. The relationship seen in certain cancers between the levels of repeat sequence hypomethylation and disease outcome as measured by progression, recurrence or death (Section 4.4) indicates that DNA hypomethylation assays could be used to provide clinically useful information. In order to translate laboratory findings to clinical application there is a need for rapid and accurate assays that can utilise limited amounts of DNA. Recently, PCR-based methods based on bisulphite treatment of DNA followed by selective amplification of unmethylated sequences derived from Alu, LINE-1 or Satα repeats [23,202,203] have been developed that allow quantification of methylation levels of these repeats. A significant challenge in the design of such assays is the sequence heterogeneity of the repeated sequences and in particular the common mutation of CpG to TpG that is not directly distinguishable from TpG sequences produced after bisulphite treatment of an unmethylated CpG site. The use of primers that allow amplification from such sequences can contribute to a high apparent background of unmethylated DNA [203]. The time appears right for evaluation of the clinical utility of such PCR-based hypomethylation assays for prediction of disease outcome.

Interest in low-dose use of demethylating agents in the treatment of haematological cancers and solid tumors with the CpG Island Methylator Phenotype (CIMP) has stimulated research in the use of methylation as a marker of efficacy [199]. PCR-based assays have been used to monitor levels of hypomethylation in patients undergoing treatment with 5-Aza-2′-deoxycytidine and appear to provide a useful predictor of clinical response [200].

5.2. Hypomethylation as a diagnostic of cancer

There are a number of tests and detection methods for identification of altered methylation of specific genes being developed for routine use as cancer diagnostics of which detection of *GSTP1* hypermethylation in prostate cancer is among the most promising [204]. Because of the high copy number of repeat sequences they offer potentially a much higher technical sensitivity for the detection of aberrantly methylated sequences using PCR-based assays such as those described above [23,202]. Key factors required for application as a clinical diagnostic are (a) the marker must be an early and common change in the disease, (b) the marker should clearly discriminate the target condition from healthy or other disease states and (c) a diagnostic assay must be able to detect the molecular change in a background of normal cells or DNA.

Genome-wide hypomethylation is a common event in many cancers and in some cases there is clear potential for the hypomethylation of specific repeats to be used for early detection of disease development (see Table 4 and [31] for extensive lists). In colorectal cancer, LINE demethylation is an early event that can be detected in both the precancerous and adenocarcinoma stages of this disease [205], whilst the hypomethylation of SatR-1 can be used to detect the early changes in breast cancer [47].

More problematic for the use of repeat sequence hypomethylation in the diagnosis of cancer is its specificity for the diseased state. The common occurrence of hypomethylation in many

tumor types also impacts on the potential specificity of any diagnostic. For a number of cancers there are potential sources of cancer cells or DNA that are local to the cancer and could be used to provide specificity, e.g. colorectal cancer (stool), breast cancer (nipple aspirates), lung (sputum), prostate (semen or urine) and bladder (urine). In the absence of disease the overall level of DNA methylation varies significantly between individuals and tissues, and there is significant overlap between DNA methylation levels seen in individual cancers and equivalent normal tissues from different individuals. Hypomethylation of specific repeats may also be seen in histologically normal tissue adjacent to cancer tissue. Similar field effects for hypermethylation of a single copy gene MGMT, that have been recorded as far away as 30 cm from the centre of the tumor [206], make it difficult to obtain a normal, unaffected adjacent tissue comparison for clinical testing. While this field effect supports the possibility that hypomethylation status could be used to identify people at risk of developing cancer, it confounds its use as a diagnostic to detect disease.

Despite these issues, there are still possible approaches to developing specificity for cancer detection.

(1) Focussing on repeats that show least hypomethylation in normal tissues can minimise the background detection of normal DNA; satellite repeats versus interspersed repeats.
(2) Identifying key changes that are unique to cancer. More detailed and extensive analysis of the methylation profiles of repeated DNA sequences may reveal subgroups of repeat families or specific subsets of CpG sites that provide higher specificity and epigenetic signatures for cancer detection. A good example is that of the NBL2 repeat sequence—DNA from Wilms tumors and ovarian carcinomas [207] characteristically displayed methylation at specific CpG sites (CpG6) and hypomethylation at others (CpG10) in comparison of normal tissues.
(3) Use of panels or patterns of markers that might include gene-specific loci and repetitive elements, for example, a panel comprising a screen for the combined demethylation of LINE, HERV-W, Satα and Sat2 repeats on chromosome 1 and 16, as well as the mixed epigenetic signature of the NBL2 repeat may prove to be a sensitive and specific detector of ovarian malignancy.

6. Conclusions

Global and focal hypomethylation is an important characteristic of neoplasia and there is increasing evidence that epigenetic changes including hypomethylation may be involved in other diseases. Use of the term “global hypomethylation” has to some extent implied that demethylation of the genome in cancer is a generalised, non-specific process and obscured the variation and complexity that has begun to be revealed over the last couple of years. Observed levels of hypomethylation vary widely both within and between different cancer types as can the timing of demethylation in relation to disease stage and grade. DNA hypomethylation can be indicative of early stage disease for some cancer types or of later stage disease poor prognosis and heightened malignancy in others. Recent data have also indicated that there can be a level of specificity in which repeat sequences become demethylated. In these respects hypomethylation is like other genome modifications – mutations, rearrangements, hypermethylation – that display different profiles in different cancer types.

It remains unclear why demethylation occurs, though recent data is starting to provide clues to the underpinning mechanisms involved in the hypomethylation of specific regions of DNA. The processes of maintaining silent heterochromatin states on repeated DNA are beginning to be understood in terms of the protein modifications and enzymes involved and the interplay of DNA methylation and histone modifications in re-enforcing metastable chromatin states. The role of specific RNA transcripts, possibly antisense, is unclear in mammalian systems but is likely to be significant. Consideration of hypomethylation within this framework can provide an understanding of the variation in demethylation of specific repeat classes in different cancers against a background of generally broad range hypomethylation of repeat sequences. There are likely to be components whose disruption induces specific demethylation of certain regions of DNA, including particular repeat sequences, genes and chromosomal regions.

The consequences of DNA hypomethylation are potentially significant but poorly understood. Of the anticipated outcomes there is only good experimental support for the link of hypomethylation with increased genome rearrangement and the CIN “chromosome instability” pathway in neoplasia. A key question is whether hypomethylation of repeat sequences contributes directly to cancer initiation or progression (through their transcripts or recombination events) or whether it is the dysregulation of specific genes (e.g. c-met) that provide growth advantage and the co-selection of hypomethylated genomes. While the significance of genomic hypomethylation in cancer and the specific case of ICF syndrome is well recognised, we need to be aware that DNA hypomethylation is not limited to these diseases. DNA hypomethylation is also observed in other diseases such as lupus, facioscapulohumeral muscular dystrophy and atherosclerosis and its role needs to be further investigated.

The link between hypomethylation of specific genes and repeat elements within the genome may serve as useful diagnostic and prognostic indicators for disease. Even though current results look promising, much is still needed to produce an effective and efficient test. More information is required with regard to what repeat elements are specific to what diseases and whether this information can be harnessed to predict disease onset or progression. With increasing interest developing in this area a useful diagnostic tool may not be too far in the future.

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