DNA Methylation Changes in Cancer

John P. Thomson and Richard R. Meehan

Abstract Although cancer is a genetic disease, broad changes in epigenomic profiles are a key observation in many distinct cancer types that can be diagnostic, reflect altered signalling/gene regulatory networks and may directly contribute to the disease state. In this short review we will focus on how DNA modification changes have contributed to our understanding of cancer progression and the hypothesis that cancer cells have an epigenome reflecting altered dependencies compared to the tissue of origin.

Keywords DNA methylation reprogramming • 5-hydroxymethylcytosine landscapes • Cancer diagnostics • Tet-1/2/3 enzymes

1 Introduction

The concept of 'epigenetics' was originated by Conrad Waddington to resolve a potential paradox of cellular differentiation; how can embryological cells with similar genetic material differentiate into multiple and distinct cell types [1, 2]. Importantly, whatever the epigenetic mechanism is, it had to incorporate the idea of inheritance of altered gene expression states during subsequent divisions of committed cells, even after the signals that initiated epigenetic changes may have long ceased [2–5]. This concept runs in parallel with the idea that signalling pathways and gene regulatory networks organise the development of an organism from a fertilized egg through embryogenesis and adulthood, a fundamentally genetic basis of development and disease [6–8]. Waddington's illustrative 'epigenetic landscapes' are bedded on the action of genes, which influence the epigenetic states adopted by differentiating cells [1, 9]. In Waddington's landscape, as the cell progresses down the valleys, its genetic information becomes modified (but not lost) which restricts its developmental potential. Subsequent molecular analysis identified chromatin-centred mechanisms which can promote the selective gene silencing and activation

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profiles that are characteristic of cell types [10]. However, the ability to transdifferentiate cells with core transcription factors offers strong evidence for gene regulatory networks (GRN) as the dominant mode of development specification and it is within this context that epigenetic mechanisms operate [11]. One question that then arises, can embryo development or cancer transformation occur without active epigenetic pathways? Understanding the potential roles of epigenetic processes in cancer is predicated on comprehending their role in development, how and why they are altered during cellular transformation and what are the functional consequences of these alterations [12, 13]. It is becoming increasingly clear that disruption of the "epigenome" as a result of alterations in epigenetic regulators is a fundamental mechanism in cancer, which has implications for both molecular diagnostics and small molecule cancer therapies.

2 DNA Methylation Machinery

A core feature of 'epigenetics' is that developmental potential is linked with changes in gene activity independently of genetic alterations. This concept has driven the identification of DNA and chromatin modifying activities which participate in regulating gene expression profiles in development and disease states [9, 10]. However, the targeting of many of these activities depends on a classical transcriptional mechanism, where DNA-binding proteins recruit chromatin/DNA-modifying activities in concert with the transcriptional machinery [7].

In mammals, DNA methylation is the best studied epigenetic mark in development and disease contexts, especially in cancer studies [14]. This is partly due the relatively simple models that correlate changes in chromatin function with altered DNA methylation profiles, efficient data generation and sophisticated bioinformatics analysis [15–20]. It is well established that CpG Island (CGI) methylation can provide strong and heritable repression of transcription, and that ectopic de novo methylation of CGI's associated with tumour suppressor genes can potentially contribute to establishing the cancer state [21–23]. However, there is still strong debate as to whether observed promoter methylation alterations are a cause or consequence of gene inactivation in cancer, as much of the analysis relies on correlative evidence [21, 22]?

The maintenance methyltransferase Dnmt1 and its cofactors have been classically considered responsible for the perpetuation of DNA methylation during cell divisions, whereas de novo DNA methylation is initially established in development by a combination of Dnmt3A and Dnmt3B acting in concert with the cofactor Dnmt3L [24–27]. Dnmt3L itself is essential during germ cell development to ensure that endogenous retrotransposons are inactivated [28]. Somatic patterns of DNA methylation participate at multiple levels (locus specific, genome stability and indirectly) in most epigenetic mechanisms, including X-chromosome inactivation, ensuring genomic imprinting, retrotransposon silencing and gene repression [16, 29–32]. 5-methyl cytosine (5mC) is enzymatically generated on mammalian DNA

by the addition of a methyl group to the carbon-5 position of the pyrimidine ring of cytosine, mostly in the context of the dinucleotide CpG [33]. Both DNA strands are symmetrically methylated at CpGs and during replication hemi-methylated DNA is potent substrate for the maintenance DNA methyltransferase, Dnmt1; perpetuating the parental pattern [33]. Genome-wide profiling demonstrate that methylated CpG (MeCpGs) are pervasive throughout mammalian genomes, with the exception of discrete non-methylated CGI which feature as regulatory landmarks, as they are mostly associated with gene promoters [34–38]. Changes in DNA methylation profiles and content are indicative of an altered cellular state, as first exemplified in early cancer studies [39–42]. This has been replicated many times culminating in nucleotide resolution modification maps of cancer cell lines and tumours, for example colon cancer, which exhibit characteristic alterations [19, 43]. DNA methylation data from many cancer genome consortia are being continuously incorporated with comprehensive resources of somatic mutations in human cancer to improve definitions of disease types at presentation, remission and reoccurrence [44]. DNA methylation profiling has also been used extensively in reprogramming and disease studies to chart changes in cell state, which can also be linked to physiological processes, such as ageing and metabolism [45–51].

The attraction of DNA methylation as an epigenetic mark was the observation that symmetrically methylated DNA is relatively stable in the originating cell and the patterns can be propagated through cell division by the DNA methylation machinery, which integrates with DNA replication pathways [23, 52]. In general, the occurrence of DNA methylation at regulatory regions such as enhancers or active CGI promoters is associated with induced transcriptional repression, which may be mediated by direct inhibition of Transcription Factor (TF) binding or by attracting chromatin silencing activities [13, 16]. Differentially methylated promoters associated with gene inactivation in different tissues types have been identified, but these may correspond to a remarkably small number of genes that are normally expressed in the germline [29, 30]. Most silent non-methylated CGI genes are associated with a histone repressive modification profile that is dependent on Polycomb Repressive Complex's 1 and 2, which are responsible for adding a ubiquityl moiety to histone H2A at Lys119 (H2AK119ub1; PRC1)) and the addition of one to three methyl groups to histone H3 at Lys27, leading to H3K27me1, H3K27me2 and H3K27me3 (PRC2) respectively [53, 54]. It is the H3K27me3 mark that is resolutely associated with gene repression.

DNA methylation undergoes extensive reprogramming during early embryo development and in primordial germ cell (PGC) progression (PGCs), which have been linked with signal induced pathways that shift DNA methylation profiles in mouse ES cells [32, 50, 55–58]. Similar developmental changes have been observed in other somatic cell contexts [59, 60]. Until recently it was unclear what the molecular mechanisms were that underpinned 'DNA demethylation' pathways, whose disruption could account for the altered patterns of hyper- and hypo-DNA methylation observed in many cancers [61–63].

3 DNA De-methylation

Two basic mechanisms leading to DNA demethylation can be considered; (A) a passive mechanism in which re-methylation of hemi-methylated substrates during DNA replication is prevented, thus leading to progressive loss of 5mC in concert with cellular proliferation and (B) active processes that remove the modification or modified bases from DNA [64–69]. 5mC marks can be converted back to an unmodified state via methylcytosine dioxygenase enzymes known as the Ten-Eleven-Translocases (TETs 1, 2 & 3) that can generate intermediates in a potential DNA demethylation pathway; 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) [70–72] (Fig. 1). 5hmC has gathered much interest in recent years as its stable relative abundance predicts that it may have biological functions in addition to its role as a DNA demethylation intermediate [72–75]. The presence of oxidation derivatives may also lead to passive demethylation because

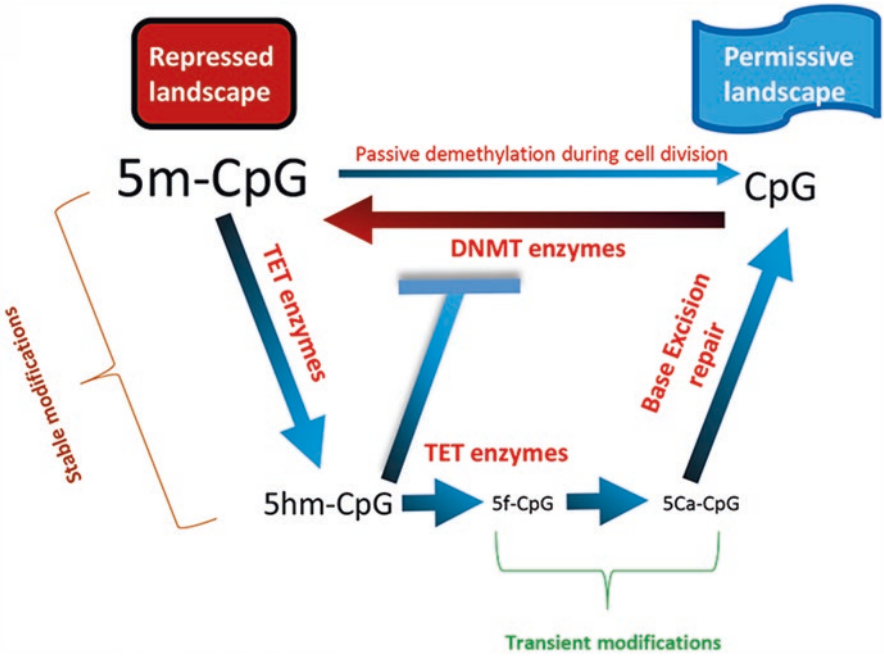

Fig. 1 Overview of the DNA methylation cycle. DNA modification occurs largely at CpG (cytosine-phosphodiester-guanine) dinucleotides in the mammalian genome. The bulk of CpGs are modified by methylation (5m–CpG). Recent reports reveal that these methylated cytosines can be converted to 5-hydroxymethylcytosine (5hm-CpG) through the actions of the TET enzymes. 5hmC is relatively stable but can be further converted into 5-formylcytosine (5f–CpG) and 5-carboxylcytosine (5Ca–CpG) which are rapidly removed by base excision repair, resulting in an unmodified CpG dinucleotide. Relative amounts of each modification are suggested through the font size of the CpG text. The presence of 5hmC in the genome can dampen the activity of DNA methyltransferases (DNMTs) leading to passive hypomethylation via DNA replication

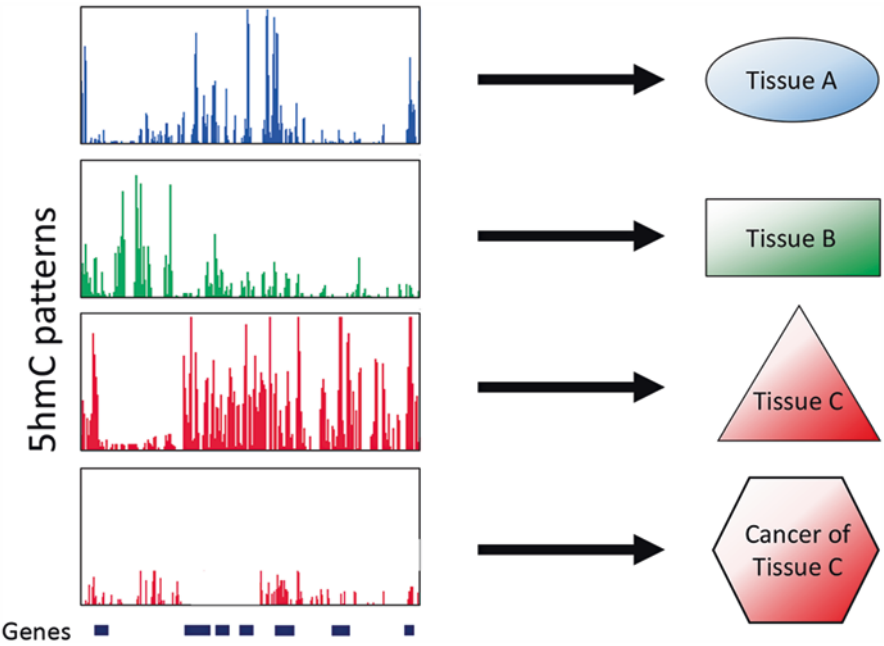

Fig. 2 DNA modification patterns act as identifiers of cell state. Both 5mC and 5hmC patterns are unique to given cell types and are strongly altered in cancer. Understanding how these epigenetic changes relate to transcriptional outcomes for a given cell is important for understanding their significance in cancer

they are not properly recognized by the methylation maintenance machinery, for example DNMT1 is not active on hemi-hydroxymethylated DNA [76]. Another possibility is that 5hmC, 5caC, or 5fC trigger erasure by DNA glycosylases such as thymine DNA glycosylase (TDG), followed by base excision repair [77–79]. However oocyte-specific *Tdg* conditional knockout gives rise to normal offspring which do not exhibit altered levels of zygotic 5hmC [80]. This result may indicate the existence of as-yet-unknown demethylation mechanisms downstream of 5mC oxidation [81].

TET enzyme conversion of 5-methyl modified cytosine bases to 5-hydroxymethyl marked bases by oxidation occurs in an iron and α-ketoglutarate (αKG) dependent manner [70, 71]. Changes in TET activity is linked with altered 5mC patterns in many cancers [82–87]. Although less abundant in absolute terms than 5mC (between 0.1% and 0.7% of all cytosines) the levels of 5-hydroxy-marked cytosines are far greater than the downstream DNA demethylation modifications; 5fC and 5caC; the more abundant 5hmC may also have a functional role throughout the genome [69, 74].

The patterns of the modifications vary greatly between tissue and cell types – to the extent that 5hmC profiling can be used as an exquisite identifier of cell state or tissue type [49, 59, 88, 89] (Fig. 2). A consensus view is such that 5hmC modified

CpGs are generally depleted over the majority of promoter elements but are enriched over the bodies of transcriptionally active genes and enhancer elements as well as a small number of transcriptional starts sites associated with silenced genes [90, 91]. This contrasts with 5mC profiles which are present genome wide and enriched at satellite and repeat DNA sequences [90]. Proximal enrichment of 5hmC at enhancers upstream of annotated transcriptional start sites (TSS) suggests a role for these regions in the regulation of gene expression [92]. Histone modification profiles around genes strongly overlaps with peaks of 5hmC in normal tissues, for example active enhancer marks, H3K4me1/H3K27ac, are associated with 5hmC at regions flanking transcription start sites (TSS) [59, 93]. The fact that 5hmC profiles are related to the transcriptional landscape means that it is a far more dynamic modification than 5mC – which is typically thought of as a stable lock on inactive chromatin states.

4 DNA Modification Perturbations in Cancer

Disruption of epigenetic landscapes, including 5hmC and 5mC patterns, is a hallmark of cancer [93–97] (Fig. 3). Although the underlying mechanisms of cancer-specific methylation changes are still largely unclear, it is apparent that they can occur early in both cancer initiation and progression [98]. Focal hypermethylation of specific regions of the genome was first reported in 1986 and inactivation of the RB1 gene in retinoblastoma cells by de novo methylation of its CGI was reported in 1989 [99, 100]. Subsequently causation, mechanism, scope, and the potential for experimental artefacts were addressed in multiple studies investigating the relationship between alterations in genomic methylation patterns and carcinogenesis [12, 14, 101]. Accumulative evidence suggests that DNA methylation patterns are often drastically different in cancer compared to those found in the normal healthy tissue, which can create altered epigenetic dependencies [23]. Three major epigenetic alterations are frequently observed: (A) global DNA hypo-methylation in cancer across large domains and affecting repetitive DNA sequences, (B) global hypo-hydroxymethylation across the majority of the genome including over promoters and gene bodies, and (C) discrete gene-specific hypermethylation of CGIs, CGI shores and enhancer elements affecting hundreds of loci [82, 101–104]. Given the dynamic interplay that 5mC and 5hmC exhibit, the observed changes in each modification throughout cancer are dynamically linked [87, 105, 106].

5 Discrete Hyper-Methylation Events in Cancer

Recent evidence suggests that disruption of the normal DNA methylation/demethylation cycle during carcinogenesis may be one mechanism that is responsible for aberrant CGI hyper-methylation events [87, 93, 97, 99, 102, 103] (Figs. 1 and 2).

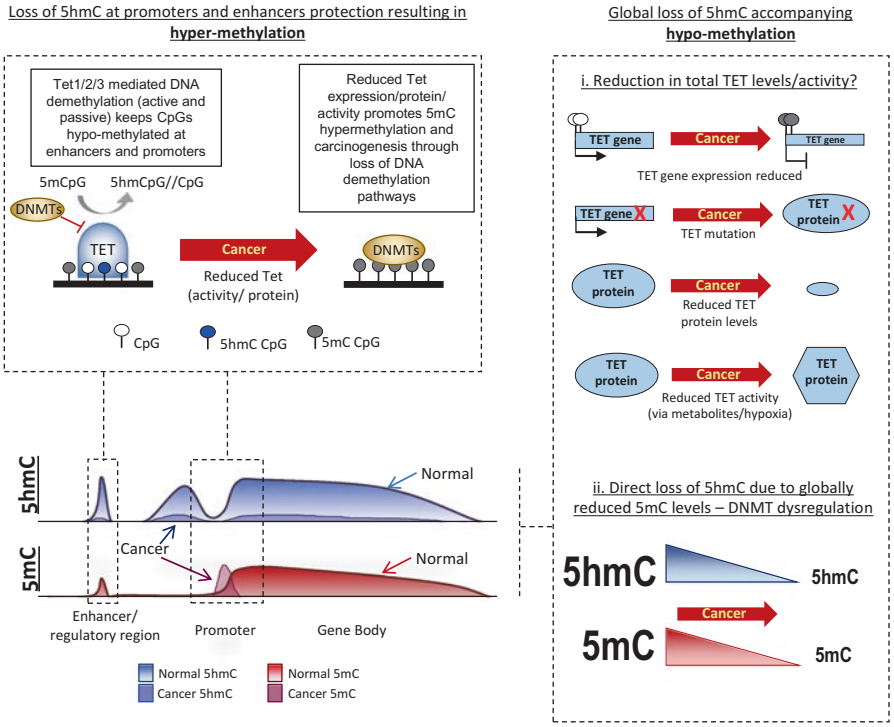

Fig. 3 Schematic for 5hmC and 5mC patterns across the genome in normal and cancer cells. Coloured plots show typical 5hmC and 5mC patterns across the genome. Typically 5hmC is found enriched over enhancers, promoter proximal and genic regions, whilst 5mC is found at enhancer, genic and repetitive elements (not shown). In cancer 5hmC is lost from promoter and enhancer regions contributing to aberrant hypermethylation events. In contrast, genic 5hmC loss accompanies loss of 5mC. *Dashed boxes* indicate possible mechanisms for these two observations

Mutations in the *TET1* gene are associated with hematopoietic malignancy where loss of 5hmC and/or gain of 5mC on promoters in *tet1*$^{-/-}$ cells may result in down-regulation of expression and derailment of the differentiation process [107]. Of interest is the observation that oncogenic KRAS can inhibit TET1 expression via the ERK-signalling pathway; restoration of TET1 expression by ERK pathway inhibition or ectopic TET1 reintroduction in KRAS-transformed cells reactivates a select number of target genes [85]. This indicates a dichotomy between signalling induced regulation of methylation of a discrete number of CGI target genes and generalised tissue determined CGI methylator profiles that are variable within tumour types [3, 97]. In the latter case, *de novo* methylation occurs predominantly at already silenced genes (passenger genes) and therefore does not affect their expression status while in the former silencing by DNA methylation of select target genes is dependent on an active signalling pathway and therefore is not strictly epigenetic in character [85, 96]. Oncogenic RAS or BRAF is required for both initiation of the pathway and maintenance of repression via the activation of pathway

intermediates which can direct methylation at select target CGI genes [108–110]. This consideration leads to two questions; how do tumour suppressor genes become methylated and how is DNA methylation of tumour suppressor genes inherited through multiple generations [3]? One idea would be that DNA methylation at TSGs is not epigenetically inherited, but is maintained by an instructive transcriptional mechanism that can potentially repress multiple genes [3]. In contrast, we have recently shown that the aberrant CGI hyper-methylation in several mouse models of liver cancer occurs at sites marked by a unique chromatin state in the healthy liver [87]. The promoter proximal sites destined to become hyper-methylated in liver cancer were found to be rich in 5hmC and associated with "bivalently" marked histone tail modifications (H3K27me3 and H3K4me3), which are typically associated with a transcriptionally poised but not active expression states. We observe loss of 5hmC occurs at these sites prior to accumulation of 5mC and this is related to a reduction in the levels of the TET1 enzyme, which has previously been shown to bind preferentially to CGIs. Loss or reduced binding of TET1 from these CGIs would ultimately result in a loss of active, 'protective' DNA demethylation and acquisition of 5mC (Fig. 3). The activity of TET enzymes can also be reduced by tumour hypoxia in human and mouse cells, which occurs independently of hypoxia-associated alterations in TET expression and depends directly on oxygen shortage [86]. This can result in increased hypermethylation at gene promoters in vitro, patients exhibit markedly more methylated at selected promoters in hypoxic tumour tissue, independently of proliferation, stromal cell infiltration and tumour characteristics. Increased hypoxia in mouse breast tumours also increases hypermethylation, while restoration of tumour oxygenation abrogates this effect.

Hyper-methylation at CGIs is often invoked as a mechanism of transcriptional inactivation of tumour suppressor genes that directly drives the carcinogenic process, however many of the genes associated with hyperethylated CGIs in cancer are already silent in the host tissue to begin with [12, 87, 96, 97, 111–113]. Recent data suggests that changes in 5mC profiles over enhancer elements may instead be related to the phenotypic and transcriptomic changes observed during cancer progression [93]. Enhancers are consistently the most differentially methylated regions during the progression from normal tissue to primary tumours and subsequently to metastases, compared to other genomic features. Changes in the 5mC levels at these loci have been linked to cancer type as well as the overall patient outcome [93, 103].

The anti-cancer effects of DNA methyltransferase inhibitors has been linked with upregulation of immune signalling in cancer through the viral defence pathway, independently of CpG island methylator profiles [114, 115]. In these examples, upregulation of intergenic hypomethylated endogenous retrovirus (ERV) genes accompanies, and may drive, the response. This anti-viral response may underlie some of the anti-tumour activity of these drugs, e.g. 5-Azacytidine (AZA), as transfection of dsRNA derived from AZA-treated cells, but not control cells, induced an antiviral response in recipient cells. Interferon pathway genes were also upregulated by AZA, and this was correlated with increased expression of endogenous retroviral transcripts rather than de-repression of interferon pathway transcription factors [114, 115] (Fig. 4).

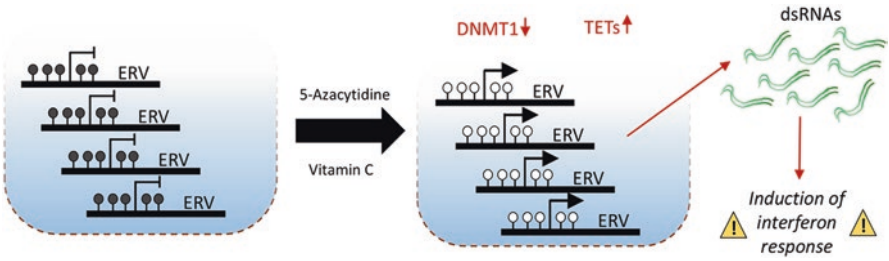

Fig. 4 Schematic of the molecular mechanisms of combined 5-Azacytidine and Vitamin C treatment in generating an anti-tumour response. Loss of 5mC at multiple endogenous retrovirus (ERV) genes occurs through inhibition of DNMT1 by 5-Azacytidine and stimulation of active DNA demethylation through elevated TET enzyme activity following vitamin C treatment. This results in the induction of double stranded RNAs (dsRNAs) which are recognised by the cell and stimulate an interferon response, enhanced immune signalling resulting in reduced cell proliferation and ultimately apoptosis of tumour cells

6 Changes to the 5hmC Landscape in Cancer

Studies using immunohistochemistry, immuno-dot blot and mass spectroscopy, consistently report a strong global loss of 5hmC in cancer cell lines and tumours [87, 88, 104, 116–120] (Fig. 3). In concert with these reduced global levels of 5hmC, genome-wide patterns of 5hmC are also markedly altered between tumour samples and normal surrounding tissue [87, 93, 104, 120, 121]. In melanoma, there is both loss and gain of genic 5hmC at a large number of gene bodies: although the changes in 5mC are far more subtle than for 5hmC. These genes tended to be associated with melanoma related pathways, Wnt signalling components and not surprisingly, general cancer progression. In lung and liver cancers the specific relationships between 5hmC and sets of chromatin marks present in normal tissue is largely absent in tumours, which may drive or reflect altered regulation of gene expression [93]. In both mouse liver cancer and in human cancer cell lines, 5hmC is lost from a series of promoter regions, resulting in aberrant hyper-methylation event at such sites and reinforcing the reciprocity between these two marks in the regulation of DNA modification landscapes [86, 87, 122]. As well as genic and promoter regions, 5hmC is typically strongly enriched over promoter–proximal enhancer elements. In mouse ES cells, loss of TET enzymes results in hypermethylation at such enhancer elements and delays nearby gene induction during differentiation [123]. Similar results have also been observed in acute myeloid leukemia (AML) where loss of the TET2 enzyme was linked to hypermethylation of ~25% of active enhancer elements [124]. These results indicate that the TET enzymes are fundamentally required to maintain normal epigenetic and transcriptomic landscapes in a given cell at least in part through the protection of key regulatory loci such as enhancers and promoter elements. This dysregulation can, in turn, provide the cell with a growth advantage through increases stem cell-like proliferation and silencing of tumour suppressor genes (Fig. 3). Studies comparing 5hmC changes between tumour types and subtypes are essential to shed light on the molecular events associated with cancer progression, and to the identification of biomarkers for clinical use.

7 The Role of the TET Enzymes in Cancer

The TET methyl cystosine dioxygenase enzymes (TET1, 2 and 3) – as well as several of their cofactors, are often mutated, transcriptionally downregulated or reduced at the protein level [86, 87, 125]. There is substantial amount of overlap in 5hmC deposition by the three members as Tet-1, -2 or -3 null mice are viable and that loss of 5hmC is not absolute in *Tet1* null mouse livers [87, 126]. Short hairpin RNA (shRNA) reduction of each of the TET enzymes in human embryonic carcinoma cells has shown that loss of TET1 resulted in the greatest elevation of 5mC at promoter elements as well as widespread reduction of 5hmC, while depletion of TET2 and TET3 reduces 5hmC at a subset of TET1 targets suggesting functional co-dependence [122]. All TET mediated 5hmC can prevent hypermethylation throughout the genome, particularly at CGI shores where loss of all three TETs was related to hypermethylation events [93, 104, 121]. Loss of 5hmC at enhancers in Tet2$^{-/-}$ mouse ES cells resulted in their hypermethylation and impacted on gene expression during early stages of ES cell differentiation [123].

Analysis of large numbers of human cancer studies (such as those recorded in the Catalogue of Somatic Mutations in Cancer – "COSMIC" – database) reveals a differing number of mutations across the three TET enzymes. TET2 is the most frequently mutated of the three however such mutations are more or less exclusively found in haematopoietic and lymphoid cancers (14.18% COSMIC datasets). TET1 and TET3 are by comparison only found mutated in a rare number of cases (both typically <0.5% of human cancers; TET3 mutated ~5% of skin cancers and 3% of colorectal cancers) [44]. Although specific mutations within the TET1 gene have not been directly associated with cancer progression, reduced transcriptional and/or protein levels of TET1 has been reported in colon, gastric, lung and liver cancers whilst TET2 transcription/protein levels are more typically reduced in leukaemia and melanoma [83, 105, 116, 120, 127–129]. TET1 downregulation has also been shown to promote malignancy in breast cancer and to act as a tumour suppressor that can inhibit colon cancer growth by de-repressing inhibitors of the WNT pathway [83]. In addition, reduced levels of TET1 has also been shown to result in elevated rates of metastasis in gastric cancer through the miss-regulation of downstream pathways required for tumour migration [128]. Interestingly TET1 is itself found both methylated and transcriptionally repressed in a series of cell lines and primary tumours of multiple carcinomas and lymphoma although, whether or not the methylation is itself causative or reflective of TET transcriptional inactivation is still to be fully elucidated [85, 127].

The activity of TET enzymes can be inhibited or stimulated by several cofactors, metabolites, and post-translational modifications. This is most evident in cancers harbouring gain-of-function mutations in the genes *IDH1* and *IDH2* – the Krebs cycle enzymes isocitrate dehydrogenase 1 and 2 – which results in the aberrant conversion of αKG into 2-hydroxyglutarate (2HG), a potent inhibitor of TET activity [105]. Mutations in two other Krebs cycle proteins; Fumarate hydratase (FH) and succinate dehydrogenase (SDH) are relatively common in a subset of human

cancers including Gastrointestinal stromal tumours (3–8% of SDH cases), Renal cell carcinomas (1–4% of SDH and 71–93% of FH cases) and Paraganglioma (12–15% of SDH cases) [130–132]. Mutations in FH and SDH lead to an accumulation of fumarate and succinate which can inhibit multiple αKG-dependent dioxygenases, including the TET family of enzymes. Loss of TET activity in tumour hypoxia was found to result in a loss of 5hmC and gain of 5mC over gene promoters and enhancer elements, once again reinforcing the "protective" role that these enzymes play at these loci in the normal cell [86].

In contrast, it has been shown that increasing the levels of ascorbic acid (vitamin C) stimulates TET protein enzymatic activity in both cultured cells as well as mouse tissues [49, 133]. The addition of vitamin C to low doses with AZA results in a synergistic inhibition of cancer-cell proliferation and increased apoptosis. These effects are associated with enhanced immune signals including increased expression of bidirectionally transcribed ERVs, increased cytosolic dsRNA, and activation of an IFN-inducing cellular response [134]. Many patients with hematological neoplasia are markedly vitamin C deficient, treatment of patients with hematological and other cancers with vitamin C may improve responses to epigenetic therapy with DNA methyltransferase inhibitors [134]. Treatment with DNA methylation inhibitors to activate a growth-inhibiting immune response may also be an effective therapeutic approach for colon cancers [135]. Taken together, these results highlight the complex relationship between 5hmC disruption and cancer progression that is not only reliant directly on the transcriptional state of the TET enzymes but also the overall environment in the cancer cell [136].

8 Indirect Impact of DNA Methylation Reprogramming on Cancer Epigenomes

Two major differences can be observed between normal mammalian DNA methylation landscapes and those found in cancer cell lines and tumours, (i) as discussed above, many CGIs become aberrantly hypermethylated in cancerous cells whereas (ii) hypo-DNA methylation occurs at other genomic regions [137]. Genes that are subject to CGI promoter hypermethylation are frequently marked by PRC2-deposited H3K27me3 in early development [138, 139]. The persistence of H3K27me3 at these regions in normal cells and in development is dependent on the global 5mC content [50, 140–142]. Induced hypomethylation results in loss of H3K27me3 from previously unmethylated CGIs, which in a somatic cell context can lead to gene activation [142]. Importantly, DNA hypomethylation results in the accumulation of the PRC2 complex components and H3K27me3 to genomic locations that were previously DNA methylated, suggesting that dense DNA methylation prevents PRC2 binding to chromatin. In addition, TET1 is required for a significant proportion of PRC targeting in mouse ES cells, connecting this putative demethylation pathway to PRC recruitment [87, 143]. In this context it is formally

possible that delocalisation of PRC complexes in tandem with loss of demethylation activities makes CGI genes formerly marked by H3K27me3 susceptible to *de novo* methylation during the epigenomic reprogramming phase of carcinogenesis [18]. This brings the relationship between DNA methylation and the Polycomb system to the forefront of cancer epigenomics, and also has implications for genome regulation [144]. An important inference is that reprogramming of DNA methylation patterns in cancer could trigger mis-regulation of transcriptional programs through subsequent redistribution of the repressive activity of PRCs, that in addition also feeds back on ectopic targeting of de novo methylation to CGIs previously marked by H3K27me3 [18]. These targets include a large number of genes with key functions in cell lineage decisions and the regulation of the cell cycle, which have a major impact on the development and progression of cancer [54]. Mutations resulting in histone variants in cancers that are resistant to modification can impact on diverse aspects of chromatin biology including DNA methylation and gene expression [20]. The functional interplay between DNA methylation and PRC pathways are also likely to be important in other biological systems, especially ageing [46, 145]. In cancer, these mechanisms promote a transcriptome that facilitates cancer formation, plasticity, and progression; analysis of multiple cancer DNA methylomes implies that altered TF binding occurs contributing to altered enhancer activities, which impacts on the transforming processes during carcinogenesis [87, 113, 146]. The checkpoints that enable correct preservation of transcriptional circuits and metabolic programs are often absent in tumorigenic lesions, thus imparting cancer cells with the ability to generate novel transcriptional, metabolic and epigenetic dependencies [147].

9 Future Perspectives

Future studies should concentrate on dissecting the cause-consequence relationships involved in cancer transformation, the role of epigenetic plasticity in driving tumour progression and identity, the epigenetics of cellular heterogeneity and exploring potential points of combinatorial therapeutic intervention. This will involve patient/tumour stratification by genetic, metabolic and epigenetic profiling, which in themselves may provide new markers for early diagnosis (Fig. 5).

The ability to identify DNA based markers for liquid biopsies of circulating tumour cells, may be effective in identifying origin of the tumour, especially during metastasis [148]. Regulation of the inhibitory immune receptor programmed cell death-1 (PD-1) is governed by cis-DNA elements, TFs, and epigenetic modifications [149, 150]. Of note is a report that PD-1 promoter methylation is an independent prognostic biomarker for biochemical recurrence-free survival in prostate cancer patients, which may linked with immune surveillance [151, 152]. Finally the availability of new gene-editing tools may enable exquisite manipulation of cancer epigenetic profiles, including Tet gene function, that promote cell death rather that cell proliferation [13, 153].

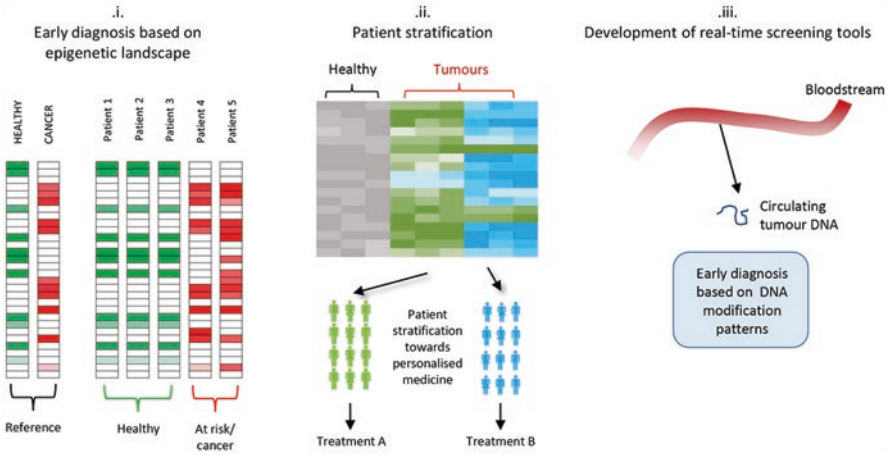

Fig. 5 Utility of DNA modification based assays towards clinical application. Analysis of genome wide DNA modification landscapes holds potential towards the development of novel early stage diagnostic screens (**i**), stratification of tumour subclasses towards more efficient personalised medicine regimes (**ii**) and the development of new real-time screening tools such as epigenetic based liquid biopsy screens (**iii**)

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