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Cancer as a dysregulated epigenome allowing cellular growth advantage at the expense of the host

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Abstract

Although at the genetic level cancer is caused by diverse mutations, epigenetic modifications are characteristic of all cancers, from apparently normal precursor tissue to advanced metastatic disease, and these epigenetic modifications drive tumour cell heterogeneity. We propose a unifying model of cancer in which epigenetic dysregulation allows rapid selection for tumour cell survival at the expense of the host. Mechanisms involve both genetic mutations and epigenetic modifications that disrupt the function of genes that regulate the epigenome itself. Several exciting recent discoveries also point to a genome-scale disruption of the epigenome that involves large blocks of DNA hypomethylation, mutations of epigenetic modifier genes and alterations of heterochromatin in cancer (including large organized chromatin lysine modifications (LOCKs) and lamin-associated domains (LADs)), all of which increase epigenetic and gene expression plasticity. Our model suggests a new approach to cancer diagnosis and therapy that focuses on epigenetic dysregulation and has great potential for risk detection and chemoprevention.

Even before the discovery of epigenetic modifications in cancer, classical tumour biology suggested that generalized disruption of gene expression might underlie the key properties of unregulated tumour growth, invasion and metastasis. Perhaps the earliest person to recognize the importance of gene expression in cancer was Sidney Weinhouse, who described a generalized disruption of the biochemistry of cancer cells that was focused on isozymes that were primarily related to metabolism[1]. However, since the discovery of oncogene mutation in human tumours[2], the principal focus of cancer genetics has been on mutations. We argue in this Opinion article that, although key mutational changes are

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necessary for the initiation of what we currently recognize as neoplastic growth and are likely to be required for escape from a cellular niche, epigenetic modifications also have a crucial role: these modifications allow rapid cellular selection in a changing environment, thus leading to a growth advantage for the tumour cells at the expense of the host. This view does not contradict and indeed collaborates with the genetic model, but it puts epigenetics at the very heart of cancer biology, from normal precursor cells at the sites where cancer arises, and through all stages of tumour progression, to advanced metastatic disease.

The first experiments on DNA methylation in human cancer, which compared samples of human colorectal cancer with matched normal mucosa isolated from the same patients, showed widespread hypomethylation involving approximately one-third of single-copy genes[3]. In response to the discovery of tumour suppressor genes[4], later studies focused on identifying silenced genes as surrogates for mutation, beginning with the observation of promoter hypermethylation of *RB1* by Horsthemke and colleagues[5,6]. During the 2000s, the maturation of microarrays and the advent of next-generation sequencing technologies in combination with the rise of data-driven discovery in biology have led to important new insights. These include the discovery of genome-wide loss of epigenetic stability, which is common across disparate tumour types. This seems to be the underlying mechanism for both the hypomethylation and the hypermethylation of individual genes, which was the historical focus of this field[7]. In addition, recently discovered mutations in the epigenetic apparatus probably contribute to epigenetic disruption in cancer. We review these recent discoveries and point to the possibility that cancer is a state in which the epigenome is allowed to have greater plasticity than it is supposed to have in normal somatic tissues. This increased epigenetic plasticity is a normal component of development or postnatal responses to injury, but its constitutive activation in cancer causes epigenetic heterogeneity that leads to most of the classical cancer hallmarks. We discuss below how this perspective provides new research avenues for diagnostics and treatment.

Large epigenetic structures

Just as the field of cancer epigenetics was presaged by early studies of abnormal gene expression, the role of large epigenetic structures in cancer was indicated by the earliest studies of cancer epigenetics by Theodor Boveri, who described abnormal chromatin in cancer cells in photomicrographs in 1929 (REF. 8). Alterations in nuclear shape are often used for diagnosis and are potentially symptomatic of the disorganization of this carefully regulated state[9]. In addition, nuclear lamina proteins (which serve to retain nuclear organization) show altered gene expression in cancer[10]. We describe below advances from whole-genome analyses that begin to provide molecular detail to these altered structures in cancer.

Chromatin LOCKs and LADs

Euchromatin refers to genes that are more open to transcription owing to post-translational modifications of histones and lower nucleosome density, whereas heterochromatin is the opposite: genes that are less open to transcription owing to greater nucleosome density and certain post-translational histone modifications. Typically, facultative heterochromatin — that is, a region that can switch between transcriptionally repressive states and activated

states — is examined at a local gene level. However, in addition to small-scale changes, the genome is partitioned into large euchromatic and heterochromatic domains, which have been given different names for mostly overlapping structures by laboratories that have approached this organization using varying methods. We recently reported large organized chromatin lysine modifications (LOCKs), which are defined by genomic domains enriched for heterochromatin post-translational modifications, such as histone H3 lysine 9 dimethylation (H3K9me2)[11]. LOCKs expand during differentiation and are lost in cancer[11] (FIG. 1a,b). Heterochromatic regions can also be defined by their organization and position within the nucleus: DNA sequences associated with proteins in the nuclear lamina are known as lamina-associated domains (LADs)[12]. Heterochromatic regions defined by histone modifications (LOCKs) and those defined by nuclear location (LADs) have been shown to have 80% overlap in different samples[11,13,14], but a causal relationship, as in LADs controlling chromatin or chromatin informing nuclear location, has not yet been proved.

LOCKs and LADs change during development, generally increasing in size. Genes in LADs are typically transcriptionally repressed[15], but by artificially reorganizing the nucleus to move genes to the nuclear periphery, transcription profiles and histone modifications of chromatin containing these genes are drastically altered[15]. Genes encoding proteins that are involved in organizing the nuclear membrane also have altered expression in many different cancer types[16]. Different laboratories have observed dynamic changes in chromatin state by examining different histone sites — for example, H3K9me2, H3K9 trimethylation (H3K9me3) or H3K27me3 — but still note that the prevalence of heterochromatic regions is associated with the differentiation state of the cell[17,18]. LOCKs are also altered in cells undergoing epithelial-mesenchymal transition (EMT), an important behaviour in cancer progression: during EMT, chromatin is reprogrammed in bulk, which results in a dramatic loss of H3K9me2 and an increase of H3K4me3 and H3K36me3 (REF. 19). Chromatin immunoprecipitation followed by microarray (ChIP-chip) experiments carried out on mouse chromosomes 4–14 showed loss of H3K9me2 in 96% of LOCKs but not in non-LOCK regions[19].

The study of LOCKs and LADs in cancer is very new, and even chromatin modifications that are known targets of mutations, such as H3K27me3, have not yet been analysed systematically at a genome-scale sequencing level in cancer. A great deal of detail and mechanism needs to be fleshed out. For example, LOCKs and LADs may themselves be nuanced with regard to combinations of chromatin marks that define physiologically distinct domains[20]. To date, other than pilot studies, there has been no systematic analysis of primary human cancers and matched normal tissues with respect to LOCKs and LADs. More detailed study has been carried out on blocks of DNA methylation (discussed below), but these need to be related to LOCKs and LADs to form a complete picture of large-scale epigenetic alterations in cancer. Euchromatin islands provide a clue to a possible connection between LOCKs and LADs; these islands are small regions within the larger LOCKs and LADs that have reduced amounts of heterochromatin and are enriched for DNase hypersensitive sites and differentially methylated regions in cancer[21].

Hypomethylated blocks

We recently made a surprising discovery by using whole-genome bisulphite sequencing of human colorectal cancer samples, and this finding helps to explain the earliest observation in cancer epigenetics: the widespread hypomethylation of genes in cancer[3]. By comparing three samples of colorectal cancer to matched normal mucosa from the same patients, we identified long blocks of hypomethylated DNA in cancer with a median size of 28 kb and a maximum size of 10Mb (a range of 5kb–10Mb)[7] (FIG. 1a,b). In blocks, normal samples exhibited methylation levels of ~80%, and the cancer samples ranged from 40% to 60%. One-third of transcriptional start sites are contained within the large hypomethylated blocks. Furthermore, these hypomethylated blocks mostly corresponded to LOCKs and LADs, uncovering a surprising relationship between large nuclear domains of both DNA and chromatin that are disrupted in cancer[7]. These findings were subsequently confirmed by others[22].

There is a point of confusion in the literature that we wish to clarify: in older literature based on Southern hybridization of long interspersed nuclear element (LINE) or *Alu* sequences, it seems that the DNA hypomethylation in cancer is due to repetitive sequences and not to single-copy genes[23]. However, modern whole-genome bisulphite sequencing methods have demonstrated that repetitive sequences, although somewhat enriched in hypomethylated blocks, are in fact no more hypomethylated than non-repetitive sequences in the blocks[7].

What is the potential role of hypomethylated blocks in cancer? An intriguing suggestion comes from an analysis of gene expression. Although the overall level of gene expression in hypomethylated blocks remains low in cancer, the hypomethylated blocks contain the most variably expressed genes in tumours compared with normal controls[7]. Furthermore, the DNA methylation levels in these regions were not only reduced, they were also extremely variable in the quantitative levels of DNA methylation[7]. Thus, although mean changes in gene expression and DNA methylation in cancer are important, their heterogeneity may be equally or even more important in tumour heterogeneity and cancer progression and may underlie tumour cell heterogeneity.

Furthermore, similar structures — known as partially methylated domains (PMDs) — have been found to be relevant in differentiation and reprogramming. PMDs are large regions that are differentially methylated between embryonic stem cells and fibroblasts[24], as well as between induced pluripotent stem (iPS) cells and fibroblasts[25]. These areas generally overlap with the blocks found in cancer[7] and are hypomethylated in more differentiated cells. This reinforces the idea that there is a strong link between the epigenetic loci dysregulated in cancer and the loci that show controlled alteration in differentiation.

In addition, the hypomethylated blocks may contribute to mutation. Hypomethylated loci in cancer often coordinate with DNA-break hotspots, and may therefore contribute to copy number changes[26]. As these primary observations are so new, it is likely that additional mechanisms linking these considerable regional DNA methylation changes to cancer will be uncovered over time.

5-hydroxymethylcytosine is a proposed intermediate in the demethylation of cytosine, but it is indistinguishable from 5-methylcytosine by bisulphite- or restriction enzyme-based techniques[27]. Affinity-based methods (for example, 5-hydroxymethylcytosine DNA immunoprecipitation (hMeDIP) and methyl-CpG binding domain (MBD)-binding assays[28]) and chemical methods (for example, liquid chromatography-mass spectrometry (LC-MS)[29]) can distinguish hydroxymethyl from methyl modifications. Although the absolute level of hydroxymethylation in most normal tissue is low, recent work has shown a relative reduction in the levels of hydroxymethylation in melanoma[30], liver cancer[29] and colorectal cancer[31]. New single-base-resolution methods that are sensitive to hydroxymethylation are emerging to aid in distinguishing differences in hydroxymethylation from total methylation in cancer[32,33].

Small epigenetic structures

The role of DNA methylation in smaller regions of DNA, such as CpG islands (CGIs), is part of the classical cancer epigenetics literature, but here too our perspective has been greatly changed by the advent of newer genomic technologies. For example, the existence of CpG island shores (CGI shores) and of asymmetric division of nucleosomes during DNA replication were unknown until recently.

CGIs and shores

In 1982, Wolf and Migeon[34] discovered highly CpG-enriched sequences that, when methylated on the inactive X chromosome, are associated with silencing of housekeeping genes. Bird and colleagues[35] later identified what they termed islands of CpG-rich sequences enriched at genes throughout the genome.

The observation of the hypomethylation of genes in human colorectal cancer[3] was extended shortly thereafter to a larger series of tumours, including pre-malignant adenomas, with hypomethylation as an apparently ubiquitous feature of cancer[36] (FIG. 1a,b). The overall global reduction of 5-methylcytosine in tumours was confirmed by quantitative high-performance liquid chromatography (HPLC)[37]. Many laboratories identified genes activated by hypomethylation, including oncogenes, such as *HRAS*[38], and the families of genes expressed normally in testis and aberrantly activated in tumours, such as the melanoma-associated antigen (MAGE) family in melanoma[39]. Additional high-throughput array-based methods have identified hundreds of genes that are epigenetically activated in various cancers, including lung, gastric, colon, pancreatic, liver and cervical cancers[40–48].

Arguing that epigenetic gene silencing might involve tumour suppressor genes, in 1991 Horsthemke[5] and Dryja[6] independently identified hypermethylation of a CGI upstream of the *RB1* tumour suppressor gene[5,6]. Many tumour suppressor genes have since been associated with hypermethylated CGIs[49] (FIG. 1a,b). However, there are several conundrums in this work. One issue is that much of this research was dedicated to the analysis of stable tumour cell lines and immortalized cell lines, which show marked hypermethylation of CGIs in general[50]. Furthermore, as Bestor and others[51–53] have repeatedly pointed out, most hypermethylated tumour suppressor gene-associated CGIs are not in the promoters of these genes, and thus the hypermethylation of these sequences is

likely to be consequential rather than causal[51–53]. It has been proposed that *a priori* methylation is a mechanism of tumour suppressor gene silencing that can cause cancer predisposition (rather than being a late event in tumorigenesis) in a similar manner to the cancer predisposition that is caused by germline mutations; however, the data supporting this have been relatively sparse[51]. The most exciting example is *MLH1* methylation transmitted as a germline trait, but this report was repudiated by most of its authors owing to contamination of the germ cells with stroma[54,55].

Indeed, we believe the mechanism of tumour suppressor gene silencing to be primarily driven by chromatin modification and not by DNA methylation. Vogelstein and colleagues[56] showed in 2003 that tumour suppressor gene silencing seems to be driven by histone modifications before DNA methylation changes[56]. Recently, Sproul and colleagues[57] directly showed that DNA hypermethylation of tumour suppressor genes in breast cancer occurs at sites that are already repressed in normal cells of the same lineage[57]. The same group extended these convincing results regarding the lack of a role for DNA hypermethylation in cancer development to 1,154 human cancer samples from seven different tissue types[58]. Similar findings at individual loci have also been shown by others[52,53].

Loss of CGI boundary stability in cancer

With the advent of whole-genome epigenetic analysis, it was possible to broaden the focus of cancer epigenetics to consider regions outside the relatively limited CGIs. We designed a microarray using an algorithm that is agnostic to genes and CGIs[59], and found that most methylation differences between tissues (tissue-specific differentially methylated regions (tDMRs)) occurred outside CGIs, often within 2kb of CGI boundaries in regions that are now commonly referred to as CGI shores[60]. A retrospective analysis of previous work agrees with this result; tDMR locations are more common in low-CpG-density promoters, although the connection to CGI shores was not identified[24].

Furthermore, compared with the fibroblasts of origin, 70% of altered methylation regions (reprogramming-specific differentially methylated regions (rDMRs)) in iPS cells are located in CGI shores. This suggests that shores are important for differentiation and reprogramming[61]. Indeed, colon cancer can in most cases be distinguished from normal colon tissue using these rDMRs, suggesting that carcinogenesis may involve a partial reprogramming of the epigenome towards a more stem cell-like state[61].

What is the function of CGI shores? One possibility is that they are sites of alternative transcription and enhancer binding regions; this is in contrast to CGIs, which Wolf and Migeon[34] and others demonstrated are strongly protected from DNA methylation to maintain housekeeping gene function[34]. Indeed, hypomethylated CGI shores were shown to activate alternative transcriptional start sites proximate to cancer-specific differentially methylated regions (cDMRs), as shown by 5′ rapid amplification of cDNA ends (RACE) experiments[60].

Another clue to the function of CGI shores comes from whole-genome bisulphite sequencing. Cancers lose the sharply demarcated boundary between high and low

methylation that is defined by CGIs; that is, at the CGI shores[7]. Thus, when the boundary between high and low methylation shifts inwards towards the CGI, the CGI shore becomes hypermethylated. When the boundary shifts outwards, the CGI shore becomes hypomethylated (FIG. 1c). The erosion of these sharply defined boundaries results in altered gene expression[7].

Furthermore, a striking hypervariability in DNA methylation is found at these CGI shores or boundaries in cancer samples, similar to the hypervariability of DNA methylation described above for the large blocks[7] (FIG. 1c). This same property of hypervariable DNA methylation at the CGI shores or boundaries and blocks is a general property of cancer, affecting at least breast, colorectal, kidney, lung and thyroid tumours[7]. Tissue heterogeneity does not explain this hypervariability because normal tissue displays even greater heterogeneity than cancer samples[7]. In fact, an increase in methylation hypervariability in phenotypically normal tissue is predictive of future cancer development[62]. A similar hypervariability is found in gene expression in cancer[63], and the most hypervariable of these genes are found within the large blocks[7]. We emphasize that mean changes in gene expression are important but that variance may be equally important in tumour progression.

Small chromatin domains

Individual mucleosomes, as opposed to the large domains described above, are also likely to be involved in affecting tumour progression. The organization of chromatin into euchromatic and heterochromatic structures is controlled by nucleosome positioning, which functions together with post-translational modifications of histone tails (for example, in enhancers)[64,65]. Physical access to DNA is restricted by nucleosome positioning and packing — chromatin remodelling complexes act to alter this in cancer[66]. For example, transcriptional activity is associated with nucleosome depletion[67], with transcription-activating histone modifications such as acetylated H3K14 (REF. 68) and with the presence of specific histone variants such as H3.3 (REF. 69) and H2A.Z[70]. It is important to consider the complex combinatorial nature of the histone code; different histone modifications often act together, meaning that each modification must be considered in context with the other modifications that are present on the nucleosome[71]. Some regions are even bivalent, with nucleosomes having both H3K4me3 (a euchromatic, transcriptionally active modification) and H3K27me3 (a heterochromatic, repressive modification), implying a metastable pluripotent state[72]. Although previous work has demonstrated that bivalent modifications do not occur on the same histone tail[73], more recent work has shown that these opposing modifications localize to a single nucleosome, with repressive and activating marks on different H3 proteins within the same nucleosome[74]. These same regions are associated with hypermethylated CGIs in cancer[75,76] (FIG. 1a,b) and reprogramming[61], and in fact a relationship has been shown between DNA methylation and nucleosome positioning[77] and histone modification[18].

We and others[78,79] have identified one mechanism for heterochromatin-induced silencing of tumour suppressor genes. Antisense expression of cyclin-dependent kinase inhibitor 2B (*Cdkn2b*, which encodes p15) generates heterochromatin formation at the sense promoter, leading to gene silencing, and this mechanism seems to be important in leukaemia[78,79].

Hypermethylation ensues on cell differentiation, and the expression of antisense RNA has been shown to act as a mediator of chromatin remodelling and heterochromatin formation (FIG. 1a,b). Intriguingly, CGI hypermethylation does occur but only arises after heterochromatin formation[79]. *Cis*-acting non-coding RNAs at promoters and enhancers can regulate chromatin at gene promoters[80]. The observation of chromatin modifications preceding DNA methylation during differentiation resonates with the observations that CGI hypermethylation arises secondarily to chromatin modifications in tumour suppressor gene silencing[56], and that the hypermethylated CGIs in cancer are located at genes that have already been silenced in normal tissue[57,58], presumably through chromatin.

Mutations and the epigenome

The recent discovery of several mutated epigenetic modifiers in human cancer provides a potential mechanism by which DNA mutation might lead to epigenetic alterations. Given the apparently universal presence of DNA methylation and chromatin alterations in human cancer, we summarize below the frequency of mutations of epigenetic modifying genes, going gene by gene and tumour by tumour, beginning with the Catalogue of Somatic Mutations in Cancer (COSMIC) database[81] and then reviewing the original citations. The classes of genes include histone variants (direct substitution of a mutant histone isoform); DNA methyltransferases; histone acetyltransferases; histone deacetylases; histone methyltransferases; histone demethylases; and chromatin remodelling factors, which can induce changes in euchromatin and heterochromatin (TABLE 1). Mutations in chromatin readers are also occasionally involved in cancer but apparently not as drivers of cancer progression[82].

Our analysis of the mutation frequency of epigenome modifiers in cancer reveals a surprising pattern. Although there is clearly a relationship between mutations and epigenetic modification in cancer, most of the epigenetic-associated mutations in solid tumours identified to date involve either rare aggressive variants of adult tumours or paediatric cancers. For example, the common form of pancreatic adenocarcinoma shows an 8% mutation frequency in the histone acetyltransferase p300 (*EP300*)[83], but the rarer pancreas neuroendocrine cancer has a 44% frequency of the histone methyltransferase multiple endocrine neoplasia I (*MEN1*)[84] (TABLE 1). Similarly, childhood glioblastoma, an extremely rare brain cancer, shows frequent (35.6%) mutations in histone H3 family 3A (*H3F3A*), but adult glioblastomas show a drastically lower frequency of *H3F3A* mutations (3.4%)[85] (TABLE 1).

By contrast, haematological cancers frequently involve chromosomal rearrangements of epigenetic modifiers, and this has been known for many years; for example, in mixed-lineage leukaemias[86]. Acute myeloid leukaemia involves mutations in several genes encoding proteins that modify DNA methylation, including DNA methyltransferase 3A (DNMT3A), isocitrate dehydrogenase 1 (IDH1) and IDH2. These mutations lead to either decreases in DNA methylation (IDH1 and IDH2 mutations) or increases in DNA methylation (DNMT3 A mutations)[87–89]. Chronic myelomonocytic leukaemia involves mutations in tet methylcytosine dioxygenase 2 (TET2), which is involved in DNA demethylation[90]. Lymphomas involve frequent inactivating mutations in the histone

acetyltransferases *EP300* and CREB binding protein (*CBP*; also known as *CREBBP*), leading to increased heterochromatin and resulting in gene silencing[91,92]. The histone methyltransferase mixed-lineage leukaemia (MLL), which undergoes translocations as a defining characteristic of mixed-lineage leukaemia, functions through DOTl-like (DOT1L), an H3K79me2 methyltransferase, which leads to specific gene activation[93].

Epigenetic mutations also frequently occur in cancers that relapse or that are otherwise resistant to therapy, such as mutations of *CBP* in relapsed ALL[94]. A detailed analysis of these mutations can be found in TABLE 1. Chromatin remodelling proteins — for example, AT-rich interactive domain-containing protein 1A (ARID 1A) — are perhaps the most frequently mutated class of epigenetic modifying proteins in common solid tumours, and their consequent inactivation leads to increased levels of euchromatin and gene activation (TABLE 1). A dramatic example of epigenetic gene mutation coupled with aggressiveness in cancer is the recent finding that the histone H3 variant H3.3 is itself mutated in paediatric glioblastoma, thus preventing H3.3K27 modifications[85,95]. Such a mutation would be expected to substantially affect chromatin structure, causing aberrant gene expression and potentially allowing for the acquisition of aggressive properties; it should be noted that adult glioblastomas do not frequently contain this mutation. A similar argument can be made for ovarian clear cell carcinoma — mutations in the chromatin remodeller ARID1A were found to be common in this aggressive subtype in two different studies (57% and 46% frequency)[96,97] but were not present in high-grade serous ovarian carcinoma[97].

A very important possibility in considering the relationship between DNA mutations and epigenetic modification in cancer is that altered DNA methylation or chromatin modifications may change the mutation rate itself. For example, guanine quadruplexes (G4s) increase the risk of DNA breakage and activation of the homologous recombination DNA repair pathway; these breaks are inhibited by DNA methylation. Hypomethylated loci in cancer often coordinate with DNA break hotspots, and these are enriched in G4s[26]. Methylation-mediated mutation through spontaneous deamination may also give rise to mutation; 18.2% of inherited gene mutations occur as C–G>T–A mutations in CpG dinucleotides[98]; C–G>T–A mutations also make up the bulk of substitutions in many cancers[99], even specifically at CpG dinucleotides in some cases[83]. More substantially, chromatin state has been shown to correlate extremely well with somatic mutation rate: H3K9me3 levels alone are predictive of >40% of somatic mutation loci in human cancer samples[100]. Common DNA fragile sites, which are implicated in copy number variation in cancer, also have decreased stability in regions of heterochromatin[101]. The organization of chromatin and genetic architecture of the nucleus have a direct effect on the rate and effectiveness of copy number alterations and rearrangements in cancer[102]. These data represent clear correlations between areas of epigenetic dysregulation and mutation, suggesting a collaborative effort between epimutation and genetic mutation in cancer development.

Abnormal expression of epigenetic modifier genes

It is also important to note that many alterations in the expression of epigenetic modifiers have been reported, and some of these seem to have an important role in tumour progression

(and have been reviewed extensively elsewhere[64,65,103]). Perhaps the most notable example is overexpression of enhancer of zeste homologue 2 (EZH2), which results in increased H3K27me3 levels and thus silences tumour suppressor gene expression and promotes metastasis[104]. Therefore, these alterations in the epigenetic machinery are more complex to understand, and they should be viewed not as equivalent to the mutations summarized in TABLE 1 but rather as members of a positive feedback loop that leads to epigenetic dysregulation. An example of a positive epigenetic feedback loop in malignant transformation has recently been demonstrated directly in the nuclear factor-κB (NF-κB) pathway[105]. Another example is the class of reprogramming factors that lead to the generation of iPS cells[106]. We have also observed a significant overlap of rDMRs and cDMRs[61]. Furthermore, many of the reprogramming genes are overexpressed in cancer[107]. A non-exhaustive list of examples of alterations in gene expression of epigenetic modifiers in cancer is provided in TABLE 2.

One lesson from examining the expression of epigenetic modifiers in cancer is that the balance of euchromatic and heterochromatic histone modifications is crucial — a modification too far in either direction towards euchromatin or heterochromatin leads to dysregulation of gene expression and is advantageous for tumour growth (see REF. 108 for an example). This imbalance could be a target for therapy: for example, histone modifications could be brought back into balance through small-molecule inhibitors of histone deacetylases, histone acetyltransferases or histone methyltransferases[108].

Cancer as epigenetic dysregulation

In summary, most cancers may share several common epigenetic modifications: large-scale alterations in chromatin involving LOCKs or LADs and hypomethylated blocks, and loss of methylated boundary stability at CGIs leading to hypermethylated CGIs, hypomethylated CGI shores and aberrant gene expression. This would lead to a drift towards a hybrid stem-somatic cell state, with increased methylation of Polycomb target regions and loss of methylation at pluripotency loci. A simple unifying explanation of these results is that cancer is caused by epigenetic dysregulation, which could account for the high degree of phenotypic variability that is observed among individual cancers and that leads to selection for cancer cell survival independent of the host.

To visualize this process, consider the classic ‘epigenetic landscape’ described by Waddington[109], by which the normal epigenetic signature of the cell is represented by a ball trapped in a valley, the walls of which represent a restoring force constraining the ball in its normal state (FIG. 2a). Although this signature differs among tissues, it is highly regulated, invariant among individuals and ultimately defined genetically, according to the classic view[109]. In order to be dynamic and flexible, the epigenetic signature must allow for variation, provided in the form of intrinsic noise: that is, a biochemical characteristic of the system that leads to random departure from a set point[110]. For example, methylation inheritance shows an error rate that is estimated to be 4% for a given CpG motif per cell division in a cell population[111]. This is also consistent with the idea that epigenetic variability can lead to phenotypic selection on a much shorter timescale than can mutation[112]. Furthermore, variation in the epigenome may be controlled by factors in the

genetic code, providing a potential mechanism for control of the level of this variation (which is represented by the slope of the valley walls)[113].

In cancer, an initial dysregulation of the epigenome would flatten this valley, breaking the delicate balance of regulation that maintained the stable epigenetic signature in the face of noise (FIG. 2b). This could be the result of repeated restructuring of the epigenetic landscape through inflammatory insult, as in the case of Barrett's oesophagus[114], or it could be through an initial mutation in one of the genes directing the fragile balance of the epigenome (almost any of the mutations presented in TABLE 1). A splicing variation in an epigenetic modifier gene could also cause dysregulation. For example, an isoform of DNMT3B commonly found in cancer, DNMT3B7, results in increased variation in the methylation signature[115]. Even the classical 'gatekeeper' mutations may have a role in unleashing epigenetic variation, given that cancer-associated mutants of adenomatous polyposis coli (APC), BRCA and p53 interact with epigenome-controlling proteins. We propose that after regulation of the epigenetic signature is relaxed, stochastic variation becomes the driving force in patterning the epigenome, allowing DNA methylation or chromatin structure to gradually diffuse away from its initial state. Natural selection within the host then allows each cancer to move differently, also leading to substantial epigenetic variation between a given cancer type across individuals or between metastases of a given cancer.

This epigenetic signature is constantly buffeted by stochastic variation, as if the ball is rocking randomly from side to side. The restoring force — that is, the network of genes that maintain epigenetic homeostasis — prevents the epigenetic signature from wandering too far from its equilibrium point in normal tissue. We can model this variation using an Ornstein-Uhlenbeck process (FIG. 2c); such processes are already used in biology for modelling selection pressure versus random genetic drift[116]. On the basis of this model, our argument that cancer involves a loss of regulation of the epigenome means that the restoring force is reduced or lost altogether (the positive feedback referred to above).

We generated simulated methylation levels using this process (FIG. 2c). Before carcinogenesis, methylation levels have relatively low variability, oscillating stochastically around their equilibrium point. When the simulation reaches the carcinogenesis point, we reduced the restoring force in the process, flattening the Waddington valley (FIG. 2b), and allowed the simulation to continue. The simulated methylation levels subsequently exhibited a random walk away from their previously well-ordered profile. It is important to note that the distance from the original equilibrium point increases over time, but not directionally: instead it increases by a diffusive spreading of the epigenetic signature (FIG. 2c).

We then applied our model to existing DNA methylation data from our previous work[7], selecting a CpG from within a hypomethylated block as an example. The density plot of methylation values for normal, adenoma and cancer tissue at this CpG demonstrate the tight distribution in normal tissue, with a progressive relaxation from normal to adenoma to carcinoma (FIG. 2d). The simulated values match the actual data extremely well (FIG. 2d,e). This model is intended to suggest the underlying behaviour that may explain the increased distance from the normal profile we observe in tumour cells over time. We have not studied

this exhaustively, and only one CpG is used as an example of the type of stochastic process that might apply. There are two interesting implications of this suggested model. First, the increased variation over time shown in data and matched by the model supports the idea that disruption of epigenetic regulation occurs at the earliest stages of cancer. Second, it shows that the idea of looking for a defined epigenetic signature for cancer is flawed. Rather, we should be looking for an anti-profile: that is, stochastic departure from a normal epigenetic signature.

Epigenetics and cancer hallmarks

Cancer is usually viewed as a complex group of multiple disorders that are mostly driven by somatic mutation and that involve the accretion of ten proposed properties: enhanced proliferation, growth suppressor evasion, anti-apoptosis, replicative immortality, angiogenesis, inflammation, altered metabolism, genomic instability and metastasis signalling[117]. We suggest that epigenetic disruption lies at the heart of all of these processes and that mutations enable and collaborate in these disruptions. The hallmark properties of cancer arise not only by mutation but also generally through stochastic epigenetic variation (as described above) and by natural selection of phenotypes that are advantageous to cancer cell survival and growth at the expense of the host. This is described graphically in FIG. 3, in which the epigenome sits at the intersection of the environment, genetic mutation and tumour cell growth.

Epigenetic damage arises from carcinogens or diet (for example, methionine), as well as injury and inflammation (for example, altered LOCKs and LADs in EMT, as described above). Errors in the maintenance of the epigenome over time — that is, simply ageing — may also have a role in accumulating stochastic epigenetic damage. The mechanism for maintaining epigenetic integrity is damaged in either of two ways: through mutation in the genes that encode these mechanisms (TABLE 1) or through epigenetic modifications themselves in such genes (TABLE 2) resulting in positive feedback. Examples of epigenetic modifications that lead to positive feedback include epigenetic silencing of *EZH2* and loss of imprinting (LOI) in colorectal cancer. This damaged epigenetic maintenance machinery leads to stochastic drift from a normal epigenetic set point (FIG. 2) and selection for cancer hallmarks that give the cell a growth advantage at the expense of the host.

Some epigenetic modifications directly affect the hallmark properties of cancer; for example, shifts in the methylation boundaries at CGIs and CGI shores result in enhanced proliferation and metabolic change, and hypomethylated blocks lead to increased invasive potential[61]. In addition, shifting DNA methylation at CGI boundaries leads to hypomethylated shores and the activation of cell cycle genes that are overexpressed in cancer[7], another cancer hallmark. In this case loss of DNA methylation, which seems to be a ground state for embryonic stem cells[24], seems to promote gene expression, normally in development and abnormally in cancer. Similarly, LOI can directly change the balance between apoptosis and proliferation[118,119] (FIG. 3).

Epigenetic dysregulation occurs very early in cancer. For example, DNA methylation is altered in the normal tissue of cancer patients, and these changes increase with age,

suggesting a mechanism by which cancer frequency increases in an age-dependent manner[120–122] (FIG. 3). A second example is LOI of insulin-like growth factor 2 (*IGF2*) in colorectal cancer, in which the normally silent allele becomes activated, leading to a double dose of this mitogen. In colorectal cancer, LOI of *IGF2* is found in both the tumours and the normal tissue of affected patients[123], and induced LOI of *Igf2* in mice doubles the frequency of tumour development[124]. A third example is that Barrett's oesophagus shows epigenetic modifications long before progression to overt malignancy[114]. Epigenetic modifications, particularly increased heterogeneous outlier variability in DNA methylation, are also predictive of malignant change in cervical cancer[125].

Where do mutations fit into this process? We have previously suggested that evolution may favour mechanisms through genetic selection that allow for epigenetic heterogeneity by providing a selective advantage in a changing environment[113]. We suggest a similar mechanism might occur in cancer: that is, selection for epigenetic plasticity itself. More generally, we suggest that the initial dysregulation of the epigenome collaborates with crucial mutations to provide the phenotypic variation that allows the selection of the hallmark properties of cancer. There is interplay between epigenetic dysregulation, which provides the phenotypic heterogeneity that generates the hallmark properties of cancer and that potentially alters the mutation rate, and genetic mutations, which directly alter genes and pathways to confer hallmark properties of cancer, as well as enabling and assisting in epigenetic dysregulation (FIG. 3). Furthermore, the epigenetic changes themselves may contribute to increased mutation frequency.

We note that these mutations in epigenetic controlling genes generally occur in two types of tumours: haematopoietic malignancies and rare solid tumours (childhood solid tumours or aggressive subtypes of common adult solid tumours). The implication of this observation, which seems fairly well supported by a great deal of sequencing data, is that primary epigenetic modifications are a more prominent mechanism for cancer progression in common solid tumours than are mutations in epigenetic modifiers. The converse of this argument is that mutations in epigenetic modifiers have extremely strong effects on cellular behaviour, which is consistent with the profound aggressiveness of such tumours and their relative rarity. Haematopoietic malignancies are consistent with this view, as they commonly exhibit mutations in epigenetic modifier genes but arise almost completely progressed (that is, widely disseminated) compared with solid tumours. Even lymphomas, which can be relatively indolent for long periods, are more aggressive when associated with mutations in epigenetic modifiers.

Note that we do not exclude a primary role for mutations in conferring the hallmark properties of cancer, and in fact we believe that they do. We simply suggest that the epigenome is not relegated to merely a surrogate for mutation but rather that it has important non-local ramifications. Limiting our understanding of epigenetics in the context of cancer to the gene-centric view may neglect valuable insights into how cancer causes a general disruption of genetic regulation through the epigenome. With that in mind, even genes that are not normally thought to have a primary epigenetic role may have a strong interaction with the epigenome. For example, BRCA2, which at one time was thought to be a histone acetyltransferase[126], is actually a binding partner of the histone acetyltransferase PCAF (also

known as KAT2B)[127]. SMAD4 is mutated in pancreas adenocarcinoma and has been shown to be an important driver of this cancer type, but it also interacts with chromatin remodellers[83]. p53 directly interacts with DNMT1, which act together to control the expression of anti-apoptotic genes[128]. The epigenome can also be indirectly affected through pathways that are commonly mutated in cancer that can affect the expression level of epigenetic controlling proteins. Examples include the regulation of histone deacteylase 2 (HDAC2)[129] and DNMT1 (REF. 130) by mutations of the WNT-β-catenin pathway, such as APC mutations in colorectal cancer. In fact, the protein stability of DNMT1, rather than its expression, may be altered by the PI3K-AKT pathway so that levels of the DNMT1 protein (and not mRNA) are changed without new expression[131].

Implications for diagnosis and therapy

Viewing epigenetic dysregulation itself as a common driver of cancer progression has important implications for cancer diagnosis and therapy. For diagnosis it suggests a promising approach for identifying patients very early in the course of disease, and for therapy it suggests novel targets that could be the focus of therapeutic intervention early or even before the development of overt cancer.

In epigenetic detection, a great deal of effort has been invested in identifying CGI hypermethylation, with limited success[49]. We suggest that this is because an implication of the recent whole-genome epigenetic work shows that departure from a normal profile is more pathognomonic than a specific cancer epigenetic signature. For example, septin 9 (*SEPT9*) was reported to be a sensitive and specific serum-based marker for colorectal cancer[132]. However, a case-control study carried out independently of the commercial developer showed 90% sensitivity and 88% specificity for colorectal cancer but only 71% sensitivity for early cancers and 12% sensitivity for adenomas[133], and therefore this marker is not useful for early screening. Similarly, methylation of bone morphogenetic protein 3 (*BMP3*), eyes absent homologue 2 (*EYA2*), ALX homeobox 4 (*ALX4*) or vimentin (*VIM*) was found in 66%, 66%, 68% and 72%, respectively, of primary colorectal cancers but in 7%, 5%, 11% and 11%, respectively, of normal mucosa[134]. One notable exception to the disappointing history of epigenetic detection of cancer is glutathione *S*-transferase pi 1 (*GSTP1*) in prostate cancer[135], and this is probably because this particular gene has a crucial role in the early stage of the disease rather than being an indicator of epigenetic dysregulation per se.

If a detection scheme was developed on the basis of anti-profiling instead, a much higher sensitivity and predictive value might be achieved. Consistent with this idea, Teschendorff and colleagues[125,136] recently showed that the DNA methylation variability was more predictive of cancer progression than mean changes in DNA methylation in both cervical[125] and breast[136] cancers. This test can differentiate between normal and neoplastic cervical tissue with 95% sensitivity and 78% specificity[125]. In fact, this test is predictive using tissue taken before the development of cervical neoplasia; neoplasia development within 3 years of sample collection is predictable with a 71% sensitivity and 50% specificity[125]. DNA methylation instability at specific loci is predictive of survival in endometrial, ovarian, cervical and breast cancers, emphasizing the usefulness of epigenetic instability in

chemoresistance[136]. Such an approach could even be used in screening patients for early cancer: epigenetic anti-profiling of tumour cells using a sophisticated digital PCR-based approach has already shown considerable utility for the early diagnosis of colorectal cancer in stool[137].

The methylation signature at specific CGIs can also suggest specific forms of tailored therapy, as in O^6-methylguanine DNA alkyltransferase (*MGMT*) methylation, which can indicate the therapeutic choice for glioma[138]. However, other hypermethylated CGI markers have not shown a high degree of sensitivity or specificity for cancer or for key phenotypes, such as drug resistance, although such efforts are still ongoing[139]. In these cases DNA methylation is being used as a surrogate measure of gene expression and/or gene regulation.

In the case of haematological malignancies, in which mutations in epigenetic regulators are most frequent (TABLE 1), therapy targeted towards these pathways is already quite promising, including EZH2 inhibitors for lymphoma[140,141], inhibitors of mutant IDH1 for acute myeloid leukaemia and myelodysplasia[142], and HDAC inhibitors for cutaneous T cell lymphoma[108]. Additionally, a clever approach has been taken to inhibiting DOT1L, which is not mutated itself but which is a common target of *MLL* translocations[143].

In our view, the most exciting potential application of this model of epigenetic dysregulation as a common driver throughout cancer progression is the potential for targeted chemoprevention. Currently, chemoprevention involves either nutritional recommendations or the use of non-prescription agents such as cyclooxygenase 2 (COX2; also known as PTGS2) inhibitors targeted towards individuals with a high risk of developing colorectal cancer, such as those with a strong family history of colorectal cancer[144,145]. The approach to chemoprevention is far more limited than in cardiology, in which chemoprevention with prescription medication (statins) is extremely effective at preventing heart disease[146]; this was a controversial approach when statins were first introduced for widespread use in the population. But what if we could identify the epigenetic disruption in patients before neoplastic growth even begins (as has been shown for cervical cancer[125])? Then we might treat such patients with specific inhibitors even before they develop cancer. Similarly, LOI of *IGF2* is associated with an increased frequency of colorectal cancer[123] and may be associated with gastric cancer risk[147]. LOI of *IGF2* also substantially increases the frequency of colon preneoplastic aberrant crypt foci in mice treated with the carcinogen azoxymethane, and inhibition of signalling at the IGF2 receptor reduces the incidence of neoplasms to a level even lower than that found in mice with normal *Igf2* imprinting[148]. Thus, we might be able to identify disruption of the epigenome or even the risk of such disruption through epigenetic or genetic testing before cancer arises, and then treat patients preventively to reduce cancer incidence. This seems to us to be a potentially far more effective mechanism for reducing cancer mortality than the treatment of late-stage disease, and it would argue strongly for an epigenome-centred approach.

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Glossary

Bivalent modifications	Nucleosomes containing both euchromatic histone H3 lysine 4 trimethylation (H3K4me3) and heterochromatic H3K27me3 post-translational modifications.
Cancer hallmarks	Ten biological properties of cancer that are said to define the disease we argue that they arise by natural selection for cellular survival at the expense of the host in the setting of epigenetic dysregulation and random variation.
Cancer-specific differentially methylated regions	(cDMRs). Differentially methylated regions that distinguish cancer cells from normal cells.
Chemoprevention	Administration of pharmacological compounds to reduce cancer incidence without certain knowledge of its effect on a given patient.
CpG islands	(CGIs). Areas of high CpG dinucleotide density in the genome, typically defined as a region at least 200 bp long with > 50% GC dinucleotides and an observed-to-expected CpG ratio of > 0.6.
CpG island shores	(CGI shores). The region 2 kb on either side of a CpG island, and the location of most cancer-specific, tissue-specific and reprogramming-specific differentially methylated regions.
Epigenetic dysregulation	The loss of normal control of DNA methylation or chromatin as a result of injury, epigenetic change or mutation, leading to phenotypic drift.
Epigenetic variability	Increased inter-sample variation in the methylation or chromatin state. This was recently identified as a common property of cancer, allowing for more accurate detection between samples.
Euchromatin	Areas of the genome that are more open to transcription owing to post-translational modifications of histones and with less nucleosome density.
Heterochromatin	Areas of the genome that are less open to transcription owing to post-translational modifications of histones and with greater nucleosome density. Facultative heterochromatin can change between the two states. Large organized chromatin lysine modifications and lamina-associated domains describe heterochromatin over relatively large regions and are associated with the nuclear membrane.

Hypomethylated blocks	Large (mean 144 kb) regions that are broadly hypomethylated in cancer and that mostly overlap with large organized chromatin lysine modifications and lamina-associated domains.
Lamina-associated domains	(LADs). Genomic regions located in the nuclear periphery that are associated with lamina (an inner nuclear membrane-associated protein) and usually have low expression levels.
Large organized chromatin lysine modifications	(LOCKs). Large heterochromatic regions characterized by low gene expression that are altered between somatic and stem cells; they are typically lost in cancer cells.
Loss of imprinting	(LOI). Loss of parent of origin-specific expression in cancer of imprinted genes, first observed for insulin-like growth factor 2 (*IGF2*) in Wilms' tumour and colorectal cancer.
Ornstein–Uhlenbeck process	An overdamped Brownian harmonic oscillator — that is, stochastic variation from a normal state with no persistence of the rate of change — opposed by a stronger restoring force towards the equilibrium point. We are using this to model stochastic change in DNA methylation.
Reprogramming-specific differentially methylated regions	(rDMRs). Differentially methylated regions that distinguish reprogrammed stem cells from somatic cells.
Tissue-specific differentially methylated regions	(tDMRs). Differentially methylated regions that distinguish normal tissues from each other.

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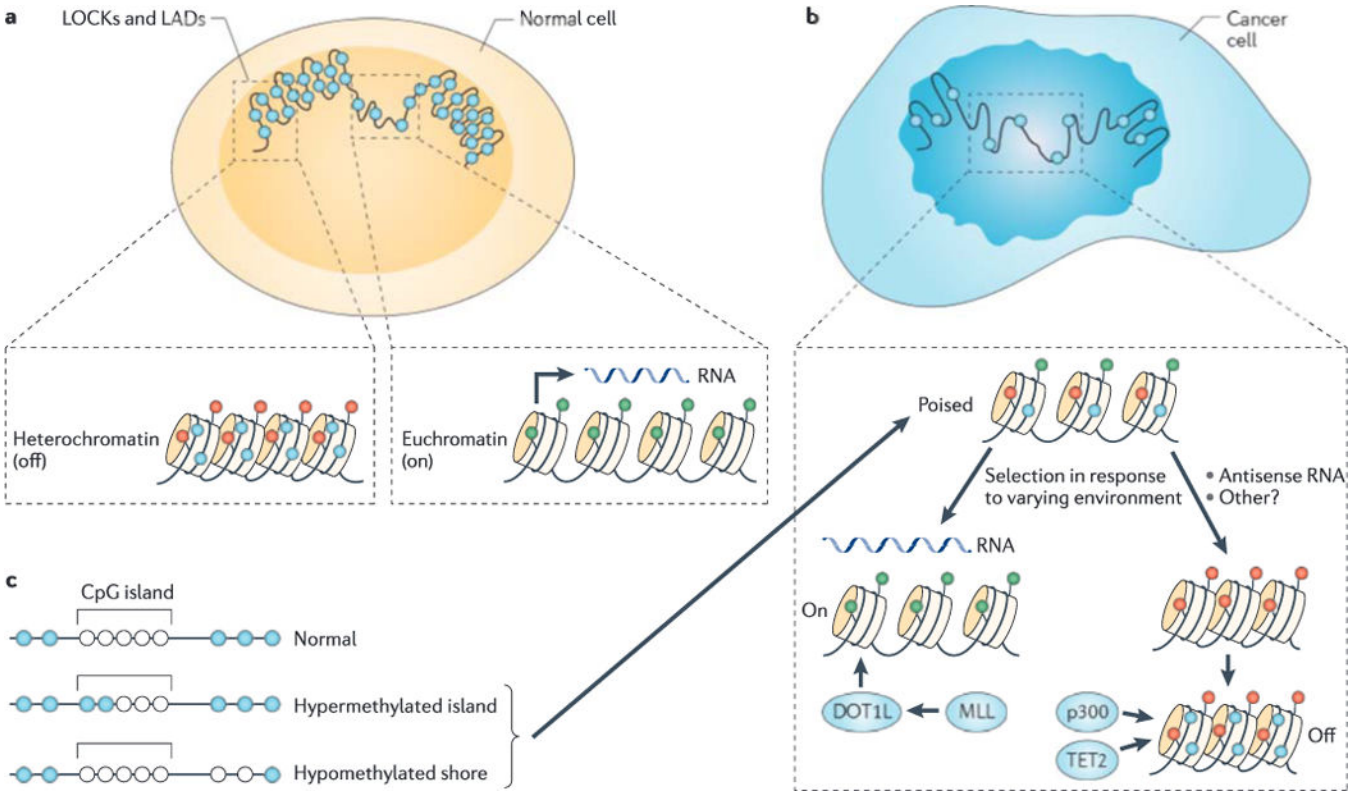

Figure 1. Alterations in the cancer epigenome that can cause epigenetic dysregulation

a | Large organized chromatin lysine modifications (LOCKs) and lamin-associated domains (LADs) (shown here in large scale) are associated with the nuclear membrane and are generally heterochromatic, with a high level of DNA methylation. Transcriptionally active genes have less compact nucleosomes than silent genes, and active and silent genes are distinguished by differing post-translational modifications of histones (green represents on and red represents off), as well as increased DNA methylation (shown in blue) of silent genes, **b** | In cancer there is a reduction of LOCKs, as well as general disorganization of the nuclear membrane and hypomethylation of large blocks of DNA corresponding approximately to the LOCKs and LADs. Chromatin is in a more stem cell-like state with the ability to differentiate into euchromatin and hypomethylated genes, or into heterochromatin and hypermethylated genes. Our argument is that epigenetic dysregulation allows for selection in response to the cellular environment for cellular growth advantage at the expense of the host. Mechanisms include mutations in epigenetic regulatory genes (for example, DOT1-like (*DOT1L*), mixed lineage leukaemia (*MLL*), *EP300* (which encodes p300) and tet methylcytosine dioxygenase 2 *(TET2*)) and primary epigenetic modifications with positive feedback. **c** | Loss of boundary stability of methylation at CpG islands includes the encroaching of boundaries, leading to CpG island hypermethylation, and the shifting out of boundaries, leading to hypomethylated CpG shores. Both mean shifts in methylation and hypervariability allow for selection.

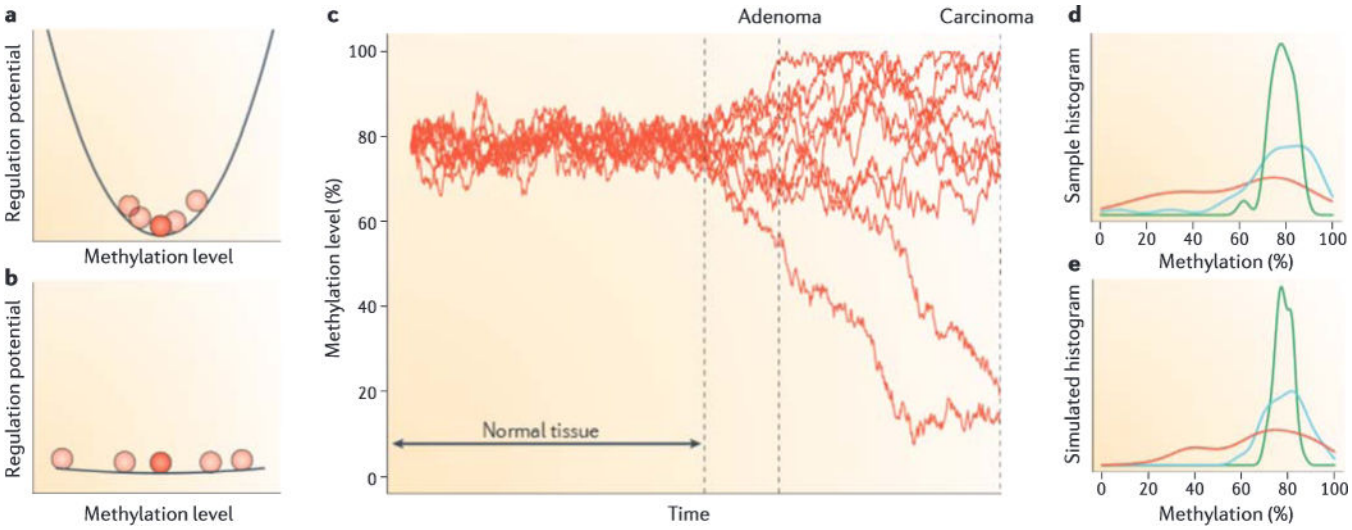

Figure 2. Modelling epigenetic dysregulation using an Ornstein-Uhlenbeck process

We used an Ornstein-Uhlenbeck process to model stochastic change in DNA methylation opposed by regulatory proteins. **a** | In normal tissue, the methylation level can be represented as a ball at the bottom of a valley—stochastic noise allows it to vary slightly, but regulatory forces represented by the walls of the valley keep the levels clustered around a single point, **b** | An early carcinogenic event flattens the landscape, leading to more variable methylation levels, **c** | Using the Euler-Murayama method, we can model this behaviour as = $\theta(\mu—M)dt + \sigma\, dW$, with M being the methylation value, μ the equilibrium point, θ the restoring force, σ the noise level (4%) and dW a Wiener process increment. Shown are ten example traces of simulated methylation levels. Regulatory forces (θ) are set high in the normal tissue and low after a carcinogenic event. As time progresses, samples of the simulation are taken, representing different stages of cancer progression: that is, normal, adenoma and carcinoma. **d** | Density plots of methylation data of a single CpG site from REF. 7 (Gene Expression Omnibus number: GSE29381) showing methylation histograms for normal (green; N = 29), adenoma (blue; N = 31) and carcinoma (red; N = 10) colon samples. The plots were generated by Gaussian kernel smoothed density function in *R*.**e** | Density plots of simulations for the same CpG showing the combined results from 100 simulations using the same sampling methods as in part **d**. The model provides an excellent fit to the data.

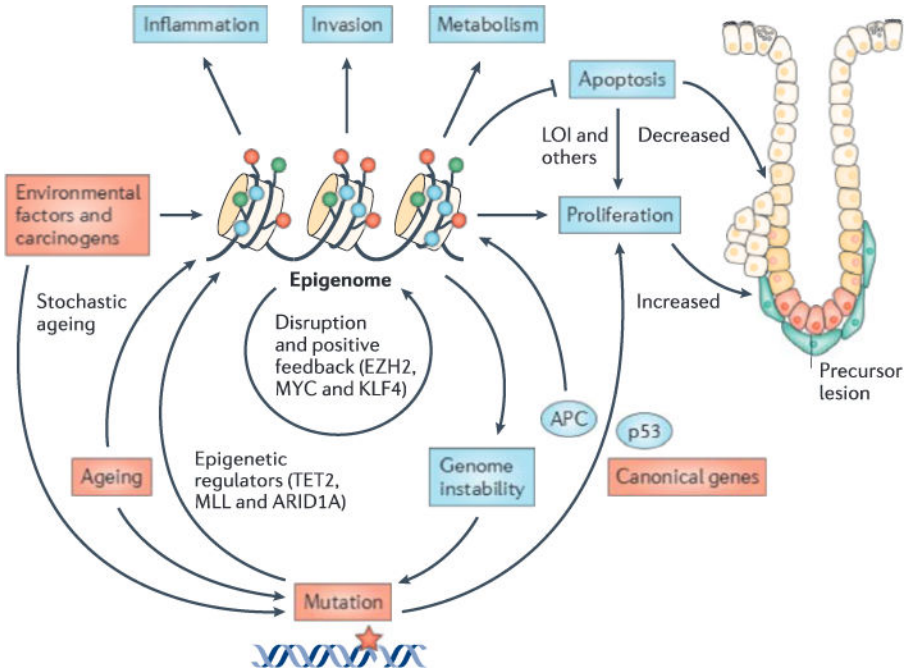

Figure 3. Collaboration of epigenetic modification and mutation in the hallmarks of cancer

The epigenome sits at the intersection of the environment, genetic mutation and tumour cell growth. Environmental factors, such as carcinogens or diet, as well as injury and inflammation, cause epigenetic reprogramming. The epigenome also accumulates damage stochastically and through ageing. The machinery for maintaining epigenetic integrity can be stably disrupted in either of two ways: by mutation or by epigenetic change itself with positive feedback. Examples of mutation include epigenetic regulator mutations (TABLE 1), whereas examples of epigenetic change include loss of imprinting (LOI) of insulin-like growth factor 2 (*IGF2*) in colorectal carcinogenesis, enhancer of zeste homologue 2 (*EZH2*) silencing in prostate cancer (TABLE 2) and overexpression of reprogramming factors. The disruption of epigenetic integrity maintenance leads to the loss of epigenetic regulation and stochastic drift from a normal set point, followed by selection for cellular growth at the expense of other cells (FIGS 1,2). Some epigenetic modifications, such as shifting methylation boundaries at CpG islands and shores, lead to metabolic change and enhanced proliferation. Others, such as hypomethylated blocks, lead to increased invasion. Still others, such as LOI, directly change the balance between apoptosis and proliferation. Canonical mutations, such as in adenomatous polyposis coli (*APC*) and *TP53* (which encodes p53), directly affect cancer hallmarks but can also cause epigenetic dysregulation. Similarly, epigenetic disruption, such as regional hypomethylation or CpG hypermethylation, can lead to increased chromosomal rearrangements and mutations, respectively. Instability of CpG island methylation boundaries also contributes to epigenetic dysregulation, allowing for selection in response to the cellular environment for cellular growth advantage at the expense of the host. ARID1A, AT-rich interactive domain-containing protein 1A; KLF4, Krüppel-like factor 4; MLL, mixed lineage leukaemia; TET2, tet methylcytosine dioxygenase 2.

Table 1

Epigenome-modifying gene mutations in human cancer

Gene	Cancer	Frequency or stage of cancer	Frequency of mutation (*N*)	Effect	Refs
Histone variants					
HIST1H1B	Colorectal cancer	Common	4% (24)		149
HIST1H1C	Non-Hodgkin's lymphoma	Common	7% (127)		150
H3F3A	Paediatric glioblastoma	Rare aggressive paediatric, high grade	36% (90)	Prevents PTMs on H3K27 or H3K36	85
	Diffuse intrinsic pontine glioma	Rare aggressive paediatric	60% (50)	Prevents PTMs on H3K27	95
HIST1H3B	Diffuse intrinsic pontine glioma	Rare aggressive paediatric	18% (50)	Prevents PTMs on H3K27	95
DNA methyltransferases					
DNMT1	Colorectal cancer		2% (29)	Mutation	151
DNMT3A	AML	Stage M4	13.6% (66)		87
		Stage M5	20.5% (112)		87
	AML	Common	22.1% (281)		88
DNA demethylases					
TET2	BCR-ABL-negative myeloproliferative neoplasms	Rare form	13% (239)		152
	CMML	Common form	50% (88)		90
	MDS	Rare	26% (102)		153
IDH1	Anaplastic astrocytoma	Rare	73% (52)		154
	Diffuse astrocytoma	Rare	90% (30)		154
	AML	Common	6.2% (385)		89
IDH2	AML	Common	8.6% (385)		89
Historie acetyltransferases					
EP300 (which encodes p300)	Pancreas adenocarcinoma	Common form	8% (24)	Mutation	83
	DLBCL	Common form	10% (134)	Mutation	91
	Follicular lymphoma	Uncommon form	8.7% (46)	Mutation	91
	Head and neck squamous cell cancer	Common	11% (74)	Mutation	155
	Transitional cell carcinoma (bladder)	Common	13% (97)	Mutation	156

Gene	Cancer	Frequency or stage of cancer	Frequency of mutation (*N*)	Effect	Refs
CREBBP (which encodes CBP)	Ovary	Common	3% (75)	Inactivated	157
	Breast adenocarcinoma	Common	8% (183)	Gain of copy	158
	Lung cancer	Common	5.3% (95)	Mutation	159
	DLBCL	Common form	22.4% (134)	Mutation	91
	DLBCL	Common form	18% (111)	Mutation	92
	Follicular lymphoma	Uncommon form	32.6% (46)	Mutation	91
	Relapsed ALL		18.3% (71)	Mutation	94
	Transitional cell carcinoma (bladder)	Common	13% (97)	Mutation	156
ELP4	Breast adenocarcinoma	Common	4% (183)	Gain of copy	158
Historie deacetylases					
HDAC4	Breast adenocarcinoma	Common	4% (24)	Mutation	149
HDAC9	Prostate adenocarcinoma	Common	42.9% (7)	Mutation	160
Historie methyltransferases					
SETD2	Renal clear cell carcinoma	Common	3% (407)	Mutation	161
MEN1	Pancreas neuroendocrine cancer	Rare	44% (68)	Mutation	84
	Parathyroid cancer	Rare	35% (185)	Mutation	162
MIL	Squamous cell lung cancer	Rare	3% (63)	Mutation	158
	Transitional cell carcinoma (bladder)	Common	7% (97)	Mutation	156
	Mixed lineage leukaemia	Common	100% (definition)	Fusion	86
MLL2	Renal clear cell carcinoma	Common	4% (407)	Mutation	161
	Childhood medulloblastoma	Rare	8.7% (92)	Mutation	163
	Childhood medulloblastoma	Rare	13.6% (88)	Mutation	164
	DLBCL	Common form	32% (37)	Mutation	150
	DLBCL	Common form	22.8% (92)	Mutation	92
	Follicular lymphoma	Uncommon form	89% (35)	Mutation	150
	Head and neck squamous cell cancer	Common	11% (74)	Mutation	155
MLL3	Childhood medulloblastoma	Rare	3.4% (88)	Mutation	164
	Transitional cell carcinoma (bladder)	Common	5% (97)	Mutation	156

Gene	Cancer	Frequency or stage of cancer	Frequency of mutation (*N*)	Effect	Refs
	Colorectal cancer	Common	20.8% (24)		149
EZH2	Non-Hodgkin's lymphoma	Common	7.8% (681)	Mutations	165
	DLBCL	Common form	5.6% (107)	Mutation	92
	MDS and MPNs	Rare	12% (219)	Mutations	166
	Myelofibrosis	Rare	13% (30)	Mutations	166
	Follicular lymphoma	Uncommon form	12% (221)	Mutations	167
Histone demethylases					
KDM5C (also known as *JARID1C*)	Renal clear cell carcinoma	Common	3% (407)	Mutation	161
KDM6A (also known as *UTX*)	Transitional cell carcinoma (bladder)	Common	20% (97)	Mutation	156
	Childhood medulloblastoma	Rare	3.2% (92)	Mutation	163
KDM2B	DLBCL	Uncommon form	7.4% (54)	Mutation	92
Chromatin remodelling factors					
ARID1A	Pancreas adenocarcinoma	Common	8% (24)	Mutation	83
	Ovarian clear cell carcinoma	Rare	57% (42)	Mutation	96
	Ovarian clear cell carcinoma	Rare	46% (119)	Mutation	97
	Endometrial cancer	Common	30% (33)	Mutation	97
	Transitional cell carcinoma (bladder)	Common	13% (97)	Mutation	156
	Hepatocellular carcinoma	Common	16.8% (125)	Mutation	168
	Colorectal adenocarcinoma	Hypermutated	37% (30)	Mutation	169
		Non-hypermutated	5% (165)		
ARID1B	Breast adenocarcinoma	Common	5% (100)	Mutation	170
ARID2	Hepatocellular carcinoma	Common	5.6% (125)	Mutation	168
	Melanoma	Common	9% (121)	Nonsense mutation	171
CHD1	Prostate adenocarcinoma	Common	42.9% (7)	Mutation	160
CHD5	Prostate adenocarcinoma	Common	42.9% (7)	Mutation	160
PBRM1	Clear cell renal carcinoma	Common	41% (227)	Mutation	172
ATRX	Pancreas neuroendocrine cancer	Rare	25% (68)	Mutation	84

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Gene	Cancer	Frequency or stage of cancer	Frequency of mutation (*N*)	Effect	Refs
DAXX	Pancreas neuroendocrine cancer	Rare	17.6% (68)	Mutation	84
SMARCD1	Breast adenocarcinoma	Common	4% (100)	Mutation	170
SMARCB1 (also known as *SNF5* and *INI1*)	Malignant rhabdoid cancer	Rare	100% (29)	Loss of copy or mutation	173
SMARCA4	Childhood medulloblastoma	Rare	4.3% (92)	Mutation	163

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; *ARID*, AT-rich interactive domain; *ATRX*, α-thalassaemia/mental retardation syndrome X-linked; *CHD*, chromodomain helicase DNA binding protein; CMML, chronic myelomonocytic leukaemia; *CREBBP*, CREB binding protein; *DAXX*, death-domain associated protein; DLBCL diffuse large B cell lymphoma; *DNMT*, DNA methyltransferase; *ELP4*, elongator acetyltransferase complex subunit 4; EZH2, enhancer of zest homologue 2; *H3F3A*, histone H3 family 3A; *HDAC*, histone deacetylase; *HIST1H1B*, histone cluster 1, H1b; *HIST1H1C*, histone cluster 1, H1c; *HIST1H3B*, histone cluster 1, H3b; *IDH*, isocitrate dehydrogenase; *KDM*, lysine-specific demethylase; MDS, myelodysplastic syndrome; *MEN1*, multiple endocrine neoplasia I; *MLL*, mixed lineage leukaemia; MPNs, myeloproliferative neoplasms; *PBRM1*, polybromo 1; PTMs, post-translational modifications; *SETD2*, SET domain containing 2; *TET2*, tet methylcytosine dioxygenase 2.

Table 2

Altered expression of some epigenetic modifying genes in cancer

Gene	Change	Cancer	Refs
IGF2	Increased	LOI in colorectal, gastric and breast cancers	123,174,175
Class 1 HDACs	Increased	Gastrointestinal, prostate, breast and cervical cancers	176–180
EZH2	Increased	Prostate cancer	104
EZH2	Increased	Breast cancer	181
HDACs	Increased	Several	182
HATs	Decreased	Several	182
HDACs	Increased	Colon cancer	183,184
HDAC6	Increased	Breast cancer	185
SIRT1	Increased	Prostate cancer	186
SIRT3	Increased	Breast cancer	187
KDM5C	Increased	Breast cancer	188
SMYD3	Increased	Liver, colon and breast cancers	189
EHMT1	Decreased	Medulloblastoma	190
DNMT1	Increased	Pancreas, liver, bladder and breast cancers	191–194
DNMT3B	Increased	Breast cancer	195
AID	Increased	Leukaemia	196

AID, activation-induced cytidine deaminase; *DNMT*, DNA methyltransferase; *EHMT1*, euchromatic histone-lysine *N*-methyltransferase 1; *EZH2*, enhancer of zeste homologue 2; HAT, histone acetyltransferase; HDAC, histone deacetylase; *IGF2*, insulin-like growth factor 2; *KDM5C*, lysine-specific demethylase 5C (also known as *JARID1B*); LOI, loss of imprinting; *SIRT*, sirtuin; *SMYD3*, SET and MYND domain-containing 3.