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# Epigenetic modulators, modifiers and mediators in cancer aetiology and progression

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## Abstract

This year is the tenth anniversary of the publication in this journal of a model suggesting the existence of 'tumour progenitor genes'. These genes are epigenetically disrupted at the earliest stages of malignancies, even before mutations, and thus cause altered differentiation throughout tumour evolution. The past decade of discovery in cancer epigenetics has revealed a number of similarities between cancer genes and stem cell reprogramming genes, widespread mutations in epigenetic regulators, and the part played by chromatin structure in cellular plasticity in both development and cancer. In the light of these discoveries, we suggest here a framework for cancer epigenetics involving three types of genes: 'epigenetic mediators', corresponding to the tumour progenitor genes suggested earlier; 'epigenetic modifiers' of the mediators, which are frequently mutated in cancer; and 'epigenetic modulators' upstream of the modifiers, which are responsive to changes in the cellular environment and often linked to the nuclear architecture. We suggest that this classification is helpful in framing new diagnostic and therapeutic approaches to cancer.

Ten years ago, it was suggested that, in addition to oncogenes and tumour suppressor genes, epigenetic alterations disrupt the expression of hypothesized 'tumour progenitor genes' that mediate stemness at the earliest stage of carcinogenesis, even as a field effect in normal tissues[1]. Epigenetically altered tumour progenitor genes were proposed to increase the likelihood of cancer when genetic mutations occurred and these same genes were suggested to be involved throughout tumour progression, helping to explain properties such as invasion and metastasis[1]. In the 10 years since this model was proposed, several discoveries have supported the idea of tumour progenitor genes, including the identification of many of the responsible genes, the role of widespread epigenomic changes involving the nuclear architecture and chromatin compaction, and the parts played by ageing and the environment in these properties.

Nowhere else is the contribution of epigenetic changes to cancer seen more clearly than in paediatric malignancies. Systematic analyses of genetic and epigenetic alterations in a

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**Competing interests statement**

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variety of paediatric cancers have surprisingly identified tumour types with few or no mutations, suggesting that epigenetic derangements can themselves drive these cancers. The discovery of the biallelic loss of the chromatin remodeller gene *SMARCB1* (SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1; also known as *SNF5*) in highly malignant paediatric rhabdoid tumours was an early example of the disruption of epigenetic control as a driver of cancer[2]. Subsequent exome sequencing of these tumours revealed a remarkably simple genome with no other recurrent genetic mutations[3]. More recently, genome sequencing of paediatric hindbrain ependymomas revealed an absence of any recurrent somatic mutations[4]. The poor prognosis of patients with hindbrain ependymomas was instead defined by epigenetic changes, with a CpG island methylator phenotype leading to the transcriptional silencing of Polycomb repressive complex 2 (PRC2) targets. Sequencing efforts in retinoblastoma, a childhood cancer that occurs as a result of the inactivation of both copies of the tumour suppressor *RB1*, found few other genetic alterations[5]. Instead, epigenetic changes predominate, with changes in the gene expression of known oncogenes driven by alterations in histone modifications and DNA methylation. Similarly, the childhood malignant brain tumour medulloblastoma is driven by key subtype-specific somatic mutations, but has a very low mutation rate overall[6]. DNA methylation sequencing in medulloblastoma identified highly prevalent epigenetic alterations, most notably consisting of large regions of hypomethylation correlated with increased gene expression[7].

In this Review, we revisit the tumour progenitor gene model in the light of our much clearer understanding of the identity of these genes, suggesting the more appropriate term ‘epigenetic mediator’. We suggest that most driver mutations in cancer occur in ‘epigenetic modifiers’ upstream of the mediators, and we integrate the role of upstream ‘epigenetic modulators’ that sense the environment and regulate stemness epigenetically, largely through the structure of chromatin. We suggest that this framework will be useful in organizing approaches to cancer detection and treatment.

## Three types of genes in the epigenetics of cancer

There are already two non-epigenetic classification systems for cancer genes: the mutational division into dominant oncogenes and recessive tumour suppressor genes; and the selection division into gene drivers and passengers in tumour development (TABLE 1). The proposed epigenetic functional classification system divides cancer genes into epigenetic modifiers, mediators and modulators. The easiest of these to describe are the epigenetic modifiers — that is, the genes whose products modify the epigenome directly through DNA methylation, the post-translational modification of chromatin or the alteration of the structure of chromatin. These genes are frequently the target of mutations and epimutations in cancer. One of the great surprises of the past few years has been the abundance of mutations in cancer involving such genes, affecting almost all levels of the epigenetic machinery. Also within this group are the genomic sequence changes that affect the binding of chromatin regulators, such as mutations in enhancers or transcription factor binding sites. The epigenetic mediators, which we earlier called tumour progenitor genes, are often the target of epigenetic modification, although they are rarely mutated themselves; importantly, they appear to be responsible for the emergence of cancer stem cells (CSCs). The epigenetic

mediators largely overlap with the genes involved in stem cell reprogramming and their role in cancer followed directly from the discovery of their reprogramming role. Epigenetic mediators are those genes whose products are the targets of the epigenetic modifiers. For the most part, these are the genes that drive a tumour or its progenitor cells towards a more stem-like state. As the ultimate mediators of the malignant state, they are attractive targets for novel chemotherapy treatments or biological response modifiers. Last, and perhaps most arguable, are the epigenetic modulators, defined as genes lying upstream of the modifiers and mediators in signalling and metabolic pathways, and serving as the mechanism by which environmental agents, injury, inflammation and other forms of stress push tissues towards a neoplastic propensity and/or increase the likelihood that cancer will arise when a key mutation occurs by chance. We suggest that changes in the structure of chromatin are induced very early in the cancer process by epigenetic modulators and even in the non-mutated normal tissues from which tumours arise. Epigenetic modulator genes include many genes with prominent roles in conventional oncogenic signalling; these are increasingly appreciated to influence the epigenome as part of their function (TABLE 1).

## Epigenetic modifiers

A key discovery of large-scale cancer sequencing research has been the widespread occurrence of mutations in epigenetic modifiers (TABLE 2). These consist of components of nearly every level of the epigenetic machinery, including key players in DNA methylation, histone modification and chromatin organization, across a wide variety of cancer types. This has been the subject of other recent reviews[8–10], and we limit our discussion here to a number of illustrative examples.

Mutations in the DNA methylation machinery are common in haematological malignancies. DNA methyltransferase 3α (*DNMT3A*) is recurrently mutated in myeloid and lymphoid malignancies, especially in acute myeloid leukaemia (AML) and T cell lymphoma[11–13]. *DNMT3A* mutation has prognostic value and is associated with poorer outcomes in both AML and T cell lymphoblastic leukaemia[14,15]. Mouse models evaluating conditional *Dnmt3a* knockouts in haematopoietic stem cells (HSCs) revealed enhanced self-renewal and impaired differentiation of HSCs[16,17]. It has been shown that transplantation of *Dnmt3a*-null HSCs in mice predisposes for a spectrum of malignancies similar to that observed in patients with *DNMT3A* mutations, confirming that DNMT3A loss confers a pre-leukaemic phenotype in HSCs[18,19].

Frequent mutations of the methylcytosine dioxygenase enzyme TET2, a DNA methylation eraser, have likewise been observed in myelodysplastic syndrome, myeloid malignancies and T cell lymphoma[20–22] and is recognized as an unfavourable prognostic factor in AML[23]. Analyses of clonal evolution in myelodysplastic syndrome and chronic myelomonocytic leukaemia have implicated TET2 mutation as an early oncogenic event[24–26]. Mouse models of TET2 loss exhibit increased HSC self-renewal and myeloproliferation in the context of impaired erythroid differentiation, supporting the functional importance of these mutations[20,27,28].

Mutations in the chromatin remodelling machinery are widespread in solid tumours. The initial discovery of the *SMARCB1* deletion in paediatric rhabdoid tumours was followed by the identification of patients with germline *SMARCB1* mutations and the subsequent loss of the normal allele leading to the development of rhabdoid tumours, confirming a classic tumour suppressor function for this gene[29]. Cancer sequencing studies have since revealed that genes encoding components of SWI/SNF chromatin remodelling complexes are among the most common targets of mutation. Prominent examples (TABLE 2) include polybromo 1 (*PBRM1*) mutations in over 40% of clear cell renal carcinomas[30] and AT-rich interaction domain 1A (*ARID1A*) mutations in over half of ovarian clear cell carcinomas[31,32]. The identification of *ARID1A* mutations in atypical endometriotic lesions adjacent to an ovarian clear cell carcinoma suggested that *ARID1A* loss-of-function may occur early in cancer development[32].

Mutations to histone-modifying enzymes are common across a diverse range of cancer types. Mutations affecting the SET domain methyltransferase enhancer of zeste homologue 2 (EZH2), a core component of PRC2, appear to have divergent functions in different cancer types. Gain-of-function hotspot mutations and amplifications have been reported in non-Hodgkin lymphomas and a variety of solid tumours, suggesting that these tumours depend on increased H3K27 trimethylation (H3K27me3)[33,34]. This was supported by mouse studies showing that the conditional expression of activated mutant *Ezh2* induces germinal centre hyperplasia and accelerates lymphomagenesis[35]. Conversely, loss-of-function mutations of *EZH2* are frequently seen in myeloid malignancies, head and neck squamous carcinomas, and T cell leukaemia[36–40]. Further supporting a transforming influence of EZH2 loss is the finding that EZH2 disruption in mice is sufficient to induce T cell acute lymphoblastic leukaemia[41]. Interestingly, recently described Lys27Met missense mutations in histones H3.3 and H3.1 in the majority of paediatric diffuse intrinsic pontine glioma also serve to inhibit EZH2 enzymatic activity and result in a global decrease in H3K27me3 (REFS [42,43]). These observations supporting a function for EZH2 as either an oncogene or tumour suppressor in different tissue types highlights the complexity of epigenetic modifier alterations in cancer.

Epigenetic modifier mutations are also relevant to cancer progression. Translocations and mutations involving the H3K36 methyltransferases (nuclear receptor binding SET domain protein 1 (*NSD1*), *NSD2* and SET domain containing 2 (*SETD2*)) are common across haematological and solid tumours, including paediatric acute lymphoblastic leukaemia, multiple myeloma and renal cell carcinoma[44–47]. The mechanistic importance of the *SETD2* mutation to cancer progression was illustrated by a study examining intra-tumour heterogeneity in renal cell carcinoma by sequencing spatially separated samples from the same tumour. This revealed that *SETD2* underwent multiple distinct inactivating mutations in different parts of a single tumour, suggesting a selective advantage of this alteration to the progression of renal cell carcinoma[48]. Accordingly, the *SETD2* mutation is associated with poorer outcomes in renal cell carcinoma[49]. In paediatric acute lymphoblastic leukaemia, comparison of matched patient samples from diagnosis and relapse revealed an enrichment of mutations in epigenetic modifiers, including SETD2, in relapsed disease, supporting a role in cancer progression or resistance to treatment[50]. Epigenetic modifier mutations in cancer may thus be early events driving carcinogenesis (as in the inactivation of *SMARCB1*

in paediatric rhabdoid tumours or the TET methylcytosine dioxygenase 2 (*TET2*) mutation in myeloid malignancies), or late mutational changes related to progression (such as *SETD2* in renal cell carcinoma).

## Epigenetic mediators

### Role of stemness and pluripotency factors

Epigenetic modifiers often target regulatory elements that affect the levels of insulin-like growth factor 2 (*IGF2)* expression and downstream signalling in diverse tumours, such as embryonal tumours of childhood, including Wilms tumour, rhabdomyosarcoma and hepatoblastoma[51–54], as well as adult tumours such as colorectal cancer[55]. Loss of imprinting (LOI) of *IGF2* is an epigenetic change that modifies the expression of *IGF2*, leading to a doubling of dosage. LOI of *IGF2* was first identified in embryonal tumours of childhood[51–54,56]. The dosage of IGF2 is quantitatively related to the growth and number of adenoma[57] and increased levels of IGF2 are linked to both hyperproliferation in nephrogenic rests, which predisposes to Wilms tumour[58] and the increased proliferation of colon progenitor cells[59]. This information converges on the observations that the IGF2 signalling pathway is a key mediator of the self-renewal of CSCs in hepatocellular carcinoma[60]. The LOI of *IGF2* in the disorder Beckwith–Wiedemann syndrome provided the first causal argument for the role of epigenetic changes in cancer. Beckwith–Wiedemann syndrome is the canonical disorder for a causal epigenetic risk factor in malignancy, similar to tumour protein p53 (*TP53*) for conventional mutations, because the epigenetic changes in Beckwith–Wiedemann syndrome precede the development of cancer, are associated with pre-malignant growths (perilobar nephrogenic rests), the epigenetic changes are found in sporadically occurring kidney lesions in newborn infants, and the presence of LOI in Beckwith–Wiedemann syndrome is specifically associated with a substantially increased cancer risk[61].

IGF2 and IGF1 receptor (IGF1R) signalling are thus emerging as key, context-dependent regulators of stem cell self-renewal and the proliferation of early progenitor cell pools in normal tissue architectures[62–64], tumour tissues[59,60] and embryonic stem cell (ESC) cultures[65]. The properties of IGF2 in promoting stemness and tipping the balance between the stem/progenitor cell pool and differentiated progeny seems to be tightly connected with its role in cancer initiation and progression[57,59,60]. We suggest that factors contributing to a cell state change towards stem-cell-like phenotypes have central roles in cancer development and we term these factors epigenetic mediators (FIG. 1; TABLE 1). We envisage that epigenetic mediators act at all stages of cancer development by preventing differentiation and eroding barriers against dedifferentiation (FIG. 1). Epigenetic-mediator-induced alterations in the chromatin landscape of cells of origin eventually lead to increased phenotypic flexibility and heterogeneity within the epigenetically altered, precancerous progenitor cell pool; this feature is subsequently selected for and maintained in the tumour tissue during progression.

Feinberg *et al.*[1] hypothesized the existence of a group of tumour progenitor genes that counteract proper maturation programmes when ectopically expressed or overactive. Such genes, we suggest, belong to the epigenetic mediator category and include, for example,

well-known pluripotency factors such as *NANOG*[66], *OCT4* (also known as *POU5F1*)[67] and WNT signalling members[68]. Epigenetically altered genes in induced pluripotent stem cells largely overlap epigenetically altered genes in cancer[69]. Experimental evidence in mouse model systems has already established that the ectopic expression of *NANOG*[66,70] promotes hyperplastic growth. Furthermore, when challenged with an overactive WNT signalling pathway, the ectopic expression of *NANOG* in mammary epithelial cells accelerated the development of adenocarcinomas[70], demonstrating that the unscheduled expression of pluripotency genes can indeed predispose to and drive cancer development. Further highlighting the ability of mediators to reprogramme chromatin states during the initial phase of tumour development, the premature termination of *in vivo* reprogramming towards the pluripotent stem cell state led to cancer development in a mouse model system[71]. Finally, the transient, ectopic expression of OCT4 *in vivo* induces hyperplastic and dysplastic changes in mouse epithelial tissues and the intestine, with a concomitantly increased progenitor cell pool and increased β-catenin–WNT signalling pathway activity[72]. The persistent, long-term expression of OCT4, on the other hand, results in the histological features of carcinoma *in situ* and the emergence of invasive tumours in the skin. Hence, although OCT4 is not essential for somatic stem cell maintenance in the mouse model[67], somatic stem cells retain their ability to respond to pluripotency cues that can lead to impaired cellular differentiation[72]. As the cancer phenotypes in these mouse models depend on the continuous presence of reprogramming factors instead of the presence of irreversible mutations, mediators probably target the epigenome to bring about changes in cell states on the path to cancer[71,72].

To destabilize phenotypes and impair differentiation, mediators influence the epigenetic states that define differentiated cell types (FIG. 2). Cellular differentiation is accompanied by the establishment of large blocks of repressive H3K9me2 and H3K9me3 modifications, which, together with DNA methylation[73], coordinate the stable, cell-type-specific repression of developmentally regulated genes[74]. These so-called large organized chromatin K9 modifications (LOCKs) are largely absent from ESCs and cancer cell lines[74], which may underlie the phenotypic plasticity of these cell states. In line with the role of LOCKs in the maintenance of differentiated phenotypes, the generation of induced pluripotent stem cells involves the genome-wide reprogramming of DNA methylation and histone modifications[75]. Reprogramming of chromatin states are induced in part by the OCT4-mediated recruitment of H3K9me2 histone demethylase and chromatin remodelling complexes[76].

Mediator-induced epigenetic instability and phenotypic plasticity also seem to contribute to tumour evolution during the later stages of tumour development. The expression of *OCT4*, for example, plays a key part in human testicular germ cell tumour progression and malignant potential[77]. Similarly, sex-determining Y-box 2 (*SOX2*), another core pluripotency factor[78], is amplified in small-cell lung cancer and squamous cell carcinomas of the lung and oesophagus[79,80] and is linked to a poor prognosis in a range of human cancers, such as nasopharyngeal carcinoma[81], lung adenocarcinoma[82] and breast cancer[83]. Finally, *NANOG* and *OCT4* have been associated with increased metastatic potential in breast cancer[70,83] and lung adenocarcinoma[82]. The underlying mechanism may in all of these cases relate to the fact that *OCT4*, *NANOG* and *SOX2* form extensive feed-forward and feedback loops to organize a stem-cell-like transcriptional enhancer circuitry in ESCs

that not only prevents proper maturation until it is downregulated[84], but may also contribute to the heterogeneity of tumour cell states and phenotypes.

## Relevance to cancer stem cells

The presence of immature cell states with self-renewal capacity, occupying the so-called CSC states, is well established in tumours[85–87]. Although such stem-cell-like cancer cells make up only a minority of the tumour mass, they have the potential to affect tumour heterogeneity via the stochastic initiation of maturation processes[85–87] and stochastic transitions between more or less differentiated cellular phenotypes[88]. Such phenotypic flexibility of tumour cells is further illustrated by experiments showing that, irrespective of the initial differentiation status, cancer cells are able to re-establish the immature–mature tumour cell mix when cultured individually[89].

It is important to note that the cell of origin might not be synonymous with CSCs and can be represented by more or less differentiated cell types. For example, mouse model systems have established that the dedifferentiation of mature intestinal epithelial cells precedes the emergence of cancer cells with stem cell features and tumour formation in the intestine[90]. Furthermore, knocking down tumour suppressor genes in mouse post-mitotic neurons led to the generation of glioblastoma stem cells[91]. Examples where the specific targeting of somatic stem cells led to the emergence of CSC states include the observations that activation of the WNT pathway in mouse crypt stem cell populations, but not in transit-amplifying progenitor cells, induced the formation of macro-adenomas in the mouse intestine[92].

Although the identification of the cell type of origin remains largely elusive in most human cancers, there is good evidence that an initial imbalance between the somatic stem cell and differentiated cell compartments can predispose to cancer, not only in mouse model systems[59], but also in human tumours[58]. Furthermore, although the initial target in chronic myeloid leukaemia is the HSC, CSC features have been ascribed to more mature granulocyte-macrophage progenitor cells, typically with an overactive WNT signalling pathway[93]. HSCs also seem to be the cell of origin in more mature lymphoid malignancies, such as chronic lymphocytic leukaemia[94].

In a similar manner to normal stem cells, which occupy specific compartments within tissues, the so-called stem cell niches, cancer cells displaying stem-like features frequently thrive in ecological niches in which they strike a symbiotic relationship with the microenvironment to support their propagation and phenotypic plasticity. Thus an overactive *IGF2* gene in cancer-associated fibroblasts supports the propagation of lung cancer stem cells[95], whereas glioblastoma CSCs not only contribute to the endothelial lining, but also gain sustained Notch signalling induced by factors produced by the endothelial lining[96,97]. Similarly, myofibroblasts produce hepatocyte growth factor to locally support the maintenance of CSC states in the colon and their clonogenicity[98]. Strikingly, factors secreted from myofibroblasts were also reported to able to induce more differentiated tumour cells to enter into CSC states[98]. Taken together, these examples suggest that cells with stem-like features thrive due to their ability to instruct their ectopic microenvironments to render them permissive for the expansion of stem-like cells. There is thus a constant flux of information

within the expanding tumour and between the tumour and its microenvironment on the path to the increased autonomy of tumour cells[87].

The observations that epigenetic mediators contribute not only to the emergence and maintenance of CSC states, but also to tumour progression, indicates that these genes are key players from the very early stages of cancer initiation in cells of origin to metastasis formation (FIG. 2). If correct, the targeting of epigenetic mediator genes should be central in therapeutic interventions to not only reduce cancer risk, but also to antagonize the growth of the primary tumour and metastatic derivatives (see below).

## Epigenetic modulators

Given the central role of epigenetic mediators as reprogramming factors in both development and cancer, the two most important questions are: what underlies their unscheduled activation and how do they reprogramme the epigenome? We suggest introducing the term epigenetic modulators to describe the factors that influence the activity and/or localization of the epigenetic modifiers in order to destabilize differentiation-specific epigenetic states. These epigenetic modulators might also indirectly facilitate the unscheduled expression of epigenetic mediators and promote the mediator-induced reprogramming of cellular phenotypes. Epigenetic modulators thus serve to transduce signals from environmental agents, injury, inflammation, ageing and other cellular stressors towards modifiers to alter the chromatin states at tumour suppressors or oncogenes and to promote epigenetic flexibility and the acquisition of stem-like features early during cancer development. Epigenetic modulator genes are often the targets of driver mutations during the late stages of the disease (FIG. 1; TABLE 1).

### Oncogenic RAS signalling

Recent reviews have highlighted the importance of chromatin modifications in the spatiotemporal integration of diverse signals from cellular signalling and metabolic pathways[99,100]. Cancer-relevant signalling pathways thus regulate epigenetic modifiers to indirectly destabilize cellular phenotypes during tumour development (FIG. 1; TABLE 1). A notable example of epigenetic modulators is oncogenic RAS, which orchestrates global[101] and local[102–104] chromatin modifications that are essential for RAS-mediated transformation. Oncogenic KRAS-induced transformation of non-malignant cell lines thus requires the KRAS-induced downregulation of TET enzymes, leading to an increase in DNA methylation that facilitates the silencing of tumour suppressor genes[101]. KRAS-mediated silencing of a defined set of tumour suppressor genes, on the other hand, is achieved and maintained by sequence-specific transcriptional repressors that target epigenetic modifiers to regulatory elements[102–104]. Activated KRAS has thus been shown to increase the level of the ZNF304 transcription factor that binds to the SETDB1–KAP1–DNMT1 repressor complex and targets it to the promoter of tumour suppressor genes located, for example, in the *INK4A–ARF* (also known as (*CDKN2A*) locus[104]. Interestingly, silencing of the same tumour suppressor locus promotes the maintenance of pluripotency in ESCs[104] and serves as the rate-limiting factor for the generation of induced pluripotent stem cells[105]. In line with the profound effects of oncogenic KRAS on the epigenome, lentiviral delivery of mutant

KRAS into human basal cells and luminal progenitors isolated from mammary tissue induced their rapid and efficient transformation accompanied by a loss of lineage-specific gene expression. The transformed cells formed and maintained phenotypically heterogeneous, serially transplantable tumours in mice[106], indicating the successful establishment of self-renewing CSC states.

## Signalling pathways in chronic inflammation

Another prominent example of cancer-promoting pathways regulating the epigenome is represented by nuclear factor-κB (NF-κB) signalling, which, in part, mediates the effect of chronic inflammation on cancer predisposition[107–109]. Mouse models of intestinal tumorigenesis uncovered that, in the presence of an overactive WNT signalling pathway, NF-κB induced the dedifferentiation of mature cells, and promoted the acquisition of stem-like characteristics and cancer initiation[90]. Furthermore, the aberrant activation of NF-κB signalling in the mammary epithelium in doxycycline-inducible mouse models induced altered tissue architecture reminiscent of carcinoma *in situ*[109]. On the transient activation of the *Src* oncogene *in vitro*, NF-κB participated in a positive feedback loop with the inflammatory cytokine interleukin-6 and transcription factor STAT3, which mediated a stable phenotypic switch from the immortalized mammary epithelial cell state towards a stably transformed, self-renewing state[110]. Intriguingly, STAT3 (REF. [111]) is a key factor in the maintenance of *OCT4*, *NANOG* and *SOX2* expression by binding to their enhancers during early mouse development[112]. As STAT3 also promotes proliferation, survival[113] and the acquisition of stem cell features in cancer[114], one possibility is that chronic inflammation leads to unscheduled activation of epigenetic mediator genes in the cells of origin via STAT3 activation (FIG. 1; TABLE 1). Although STAT3 can interact with epigenetic modifiers, such as the p300 histone acetylytransferase (HAT), SIN3A histone deacetylase (HDAC) complexes or DNMT1 to influence gene expression, cell-type-specific transcriptional effects will probably be influenced by pre-existing chromatin marks[115]. Signalling pathways activated by chronic inflammation, such as NF-κB signalling, probably directly or indirectly modulate several layers of the epigenome[116–118], thereby modulating the effects of STAT3 activation. Using a colitis-induced mouse colon cancer model, single base methylation analyses have revealed that chronic inflammation induces the hypermethylation of several genes important in gastrointestinal homeostasis and repair, a subset of which is also hypermethylated in mouse intestinal adenomas and human colorectal cancer[116], further supporting the view that chronic inflammation is a key modulator of epigenetic lesions early during tumour development. Inflammation might contribute to the ectopic expression of epigenetic mediators in tumour-initiating cells by the activation of YAP1, a core member of the Hippo pathway[119], which is able to bind p300 (REF. [120]) and is a key regulator of intestinal epithelial regeneration in response to inflammation[119] as well as an activator of *OCT4* and *SOX2* (REF. [121]) in CSCs of non-small-cell lung cancer.

## Tumour suppressor genes as epigenetic modulators

Further examples of epigenetic modulators in cancer include the tumour suppressor protein p53 (FIG. 1; TABLE 1). Gain-of-function p53 mutations in cancer thus endow p53 with the ability to induce genes encoding the histone-modifying enzymes *MLL1*, *MLL2* (mixed-lineage leukaemia) and *MOZ*, resulting in genome-wide increases in histone H3K9

acetylation and H3K4 trimethylation[122]. Mutant p53 was likewise recently shown to enact promoter remodelling via a physical interaction with the SWI/SNF chromatin remodelling complex[123]. Similarly, the adenomatous polyposis coli (*APC*) tumour suppressor gene has been shown to control intestinal cell differentiation via the regulation of DNA methylation dynamics, as a loss of APC upregulates a DNA demethylase system and leads to the hypomethylation of key intestinal cell fate genes[124] (FIG. 1; TABLE 1). Finally, mutations in epigenetic modulators might affect DNA and histone methylation by leading to the production of oncometabolites that inhibit α-ketoglutarate-dependent epigenetic modifiers, such as histone lysine demethylases and TET hydroxylases (FIG. 1; TABLE 1). Mutations in isocitrate dehydrogenase 1 (IDH1) and IDH2 enzymes may, for example, alter the epigenome of tumour cells and block differentiation by causing the accumulation of the D-2-hydroxyglutarate oncometabilite[125]. Furthermore, mutations in fumarate hydratase (FH) and succinate dehydrogenase (SDH) might lead to the accumulation of their substrates, fumarate and succinate, which serve as competitive inhibitors of histone demethylases and TET enzymes, consequently altering DNA and histone modifications[126]. Similarly to epigenetic modifiers, modulators are thus often targeted by driver mutations in cancer to promote not only cell proliferation, but also epigenetic instability[127].

## Effects of ageing

Ageing may influence cancer risk via epigenetic change downstream of epigenetic modulators and mediators. A comparison of newborn infants and centenarians provided a strong suggestion of age-related changes in DNA methylation, subsequently borne out in multiple studies controlling for differences in cell type and exposure[128–131], also called epigenetic drift[132]. Interestingly, a recent comprehensive evaluation of age-associated DNA methylation changes in blood cells identified megabase-scale age-associated hypomethylated blocks that also showed preferential hypomethylation in age-matched cancers[131]. Analyses of chromatin modifications in ageing have also identified multiple age-associated alterations, including the loss of heterochromatin and a redistribution of activating H3K4me3 marks[133,134]. A role for epigenetic modifiers in ageing has been reinforced by studies showing that the disruption of histone-modifying enzymes affects lifespan in model systems[134]. Prominent examples include lifespan extension in *Caenorhabditis elegans* by the disruption of the H3K4 trimethylation machinery and lifespan extension in *Drosophila melanogaster* by the heterozygous mutation of PRC2 components[135,136]. A further link between ageing and chromatin alterations comes from cellular models of premature ageing disorders such as Werner syndrome and Hutchison–Gilford progeria syndrome. In an ESC model of Werner syndrome, the differentiation of ESCs to mesenchymal stem cells recapitulates cellular ageing and is marked by a global loss of H3K9me3 and changes in heterochromatin architecture[137]. Similarly, in Hutchison–Gilford progeria syndrome, skin fibroblasts show the passage-dependent loss of heterochromatin compartmentalization related to altered H3K27me3 marks[138]. Epigenetic modulator signalling upstream of age-related chromatin alterations is only beginning to be defined. In *C. elegans*, the forkhead box O (FOXO) transcription factor DAF-16 serves as an effector of an environmentally responsive insulin-like signalling pathway and regulates longevity via recruitment of the SWI/SNF chromatin remodelling complex to target genes[139]. Similarly, the energy sensor AMP-activated protein kinase mediates longevity

induced by dietary restriction in worms and flies, and impinges on chromatin regulation via the phosphorylation of HDACs and histone H2B[140,141]. Ageing is characterized by epigenetic change, and a more thorough understanding of the roles of epigenetic modifiers and modulators in this process is likely to inform our understanding of cancer aetiology and risk.

### Effects of environmental exposures

A crucial role for the dietary availability of methyl donors in cancer prevention has been demonstrated in animal models and human studies. A methyl-deficient diet is sufficient to induce liver neoplasms in rats[142,143]. Notably, the dietary deficiency of methyl donors in these animals produced global and gene-specific DNA hypomethylation[144,145]. Likewise, human studies have shown that a low dietary intake of folate or methionine increases the risk of colon adenomas[146]. Furthermore, *in utero* exposure to higher folate and similar one-carbon nutrients has been linked to a reduced risk of childhood acute lymphoblastic leukaemia, brain tumours and neuroblastoma[147]. Excessive alcohol consumption may increase cancer risk in part via folate depletion. Chronic alcohol consumption in rats results in DNA hypomethylation in the colonic epithelium[148]. In a human cohort study, low folate and a high alcohol intake were linked to the increased methylation of genes implicated in colorectal cancer[149].

Specific carcinogenic exposures have been shown to perturb the DNA methylome[150]. The aerodigestive tract epithelium of heavy smokers without evidence of cancer displays the aberrant methylation of multiple genes implicated in the pathogenesis of lung cancer[151]. Similarly, the hypermethylation of genes related to cancer progression was demonstrated in both the bronchial epithelium and peripheral lymphocytes of smokers[152]. Occupational exposure to airborne benzene in humans has been linked to the hypomethylation of repetitive elements as well as gene-specific hypermethylation, recapitulating changes also found in malignant cells[153]. Infection with *Helicobacter pylori*, an aetiological agent in gastric adenocarcinoma and lymphoma, results in increased CpG island methylation in the non-cancerous gastric mucosa[154], which is reversible on the eradication of *H. pylori* infection[155]. Asbestos, a carcinogen that is not inherently mutagenic, has been suggested to influence cancer risk via an epigenetic mechanism. Accordingly, DNA methylation profiles distinguish pleural mesothelioma from normal pleura and predict the lung burden of asbestos[156]. Although the link between epigenetic modifiers, environmental exposure and cancer risk is clearly established, much less is known about the identity of signalling pathways and epigenetic modulators that causally connect carcinogens to the writers of the epigenome.

## Deregulated 3D nuclear architecture

Alterations of the epigenome during ageing and in cancer are tightly interconnected with the 3D organization of chromatin[157] that modulates chromatin states in both development and cancer (FIG. 2). Hypomethylated blocks thus overlap with lamina-associated domains (LADs)[158], which contain repressed, gene-poor regions constitutively localizing to the nuclear periphery, and developmentally repressed genes that are recruited to the lamina in a

cell-type-specific manner[159]. In differentiated cells, a significant fraction of LADs overlaps with large domains enriched in repressive H3K9me2 and H3K9me3 histone modifications called LOCKs[74], which expand during differentiation to coordinate cell-type-specific transcriptional repression[160–162]. Interestingly, downregulation of the epigenetic mediator gene *OCT4* coincides with the formation of compact chromatin at the lamina in mice[163], suggesting that 3D chromatin compaction itself might contribute to repression[164–166] during lineage specification.

Repressive chromatin marks and peripheral localization are functionally intertwined[167] and might be particularly sensitive to ageing-related and cancer-predisposing perturbations[167–169] (FIG. 2). The recruitment of certain genomic regions to the repressive environment of the nuclear envelope is promoted by sequence-specific transcriptional repressors[170], factors that deposit and recognize repressive histone modifications (H3K9me2/me3 (REF. [170]) and H3K27me3 (REFS [170,171])), DNA methylation-binding proteins[172] and components of the nuclear envelope[167]. These factors thus act as epigenetic modulators by regulating the position of genomic regions within the 3D nucleus. In turn, the lamina modulates chromatin states by attracting repressive epigenetic modifiers, such as lysine-specific histone demethylase 1A (KDM1A; also known as LSD1)[173], histone-lysine *N*-methyltransferase EHMT2 (also known as G9A)[174], HDAC3 (REFS [175,176]) and the nuclear co-repressor (N-CoR) complex[177] that maintain a repressive environment at the nuclear periphery[167,178]. These epigenetic modifiers balance self-renewal and differentiation[179,180], affect reprogramming into the pluripotent state[181] and contribute to ageing-related chromatin changes[182,183] and cancer[183,184], suggesting that their mechanism of action ties spatiotemporal compartmentalization in the nucleus to the modulation of the epigenome and cellular states (FIG. 2).

In agreement with the role of LOCKs in the maintenance of cellular memories, tumour growth factor-β (TGFβ)-induced epithelial-to-mesenchymal transition (EMT) is preceded by the dual-specific lysine demethylase, LSD1-mediated global loss of H3K9me2 at LOCKs[185]. Chromatin changes in EMT are reminiscent of ESCs with reduced LOCKs, although the epigenetic modulators that direct LSD1 activity from H3K4me2 demethylation towards H3K9me2 demethylation within LOCKs on treatment with TGFβ and the role of this epigenetic modifier in regulating the levels of H3K9me2 and H3K9me3 in pluripotent cells have not yet been identified[74,185,186]. Importantly, these experiments might provide mechanistic support for the earlier observations that link EMT phenotypes to the acquisition of stem cell traits[74,185,186]. Cancer cells might thus gain phenotypic plasticity by acquiring EMT-related chromatin changes leading to the impaired stabilization of cellular memories (FIG. 2). Hence regions displaying a loss of H3K9me2 and H3K9me3 in various cancer cell lines overlap with hypomethylated blocks and the location of increased variability in the gene expression of cancer-relevant and developmentally regulated genes in diverse cancer types[157]. We envisage that developmental decisions are stabilized by multiple layers of epigenetic modifications, which are established and/or maintained at the lamina. The factors that regulate chromatin–lamina interactions and recruit chromatin-modifying enzymes to the nuclear periphery might thus act as epigenetic modulators by positioning the genome within the nucleus and coordinating the activity of epigenetic modifiers in space and time (FIG. 2). A failure to orchestrate such spatiotemporal crosstalk between repressive chromatin factors

will probably lead to the emergence of cells with unstable phenotypes of impaired differentiation. Some of these cells might maintain or regain self-renewal capacity due to epigenetic mediator gene products, representing transition cell fates towards CSCs (FIG. 3).

## Epigenetic stochasticity

### Large domains of epigenetic variability

We have previously suggested that a major driving force for tumour evolution is the emergence of epigenetic stochasticity, allowing rapid selection for growth-favouring tumour traits in a changing microenvironment[187–189]. Understanding the nature and genomic location of such stochastic variation, as well as the interplay between epigenetic modifiers, epigenetic modulators and epigenetic mediators that destabilize the epigenome to increase stochastic noise, is thus likely to be essential in tackling tumour evolution and resistance to treatment. Experimental evidence has confirmed that stochastic DNA methylation alterations in cancer involve large regions of the epigenome[190,191]. This stochastic epigenetic change does not occur genome-wide. Rather, genome-wide views of epigenetic variation have shown that large hypomethylated blocks, constituting up to one-third of the genome, contain the most variably methylated regions of the tumour genome[190,192]. These domains arise early during cancer development[191,193,194] and contain the most variably expressed genes regulating cancer-relevant functions[190]. Moreover, the degree of variation in methylation in early precursor lesions predicts cancer risk[193,194], suggesting a causal link between these epigenetic changes and cancer. Hypomethylated blocks in cancer largely correspond to partially methylated domains in normal cells as well as LADs and LOCKs (FIG. 2). These regions underlie much of the reported variation in methylation at CpG islands, shores and distant CpG sites, fuelling phenotypic variation in cancer[191,192]. In addition, the degree of variation in methylation[191], as well as the deviation of the variability in gene expression from the normal corresponding tissue, is a predictor of cancer progression[195]. The combination of ageing and chronic sun exposure — the two leading causes of skin cancer — induces the widespread formation of hypomethylated blocks in the epidermis at genomic regions that are hypomethylated in squamous cell carcinoma and that overlap with colon cancer-specific hypomethylated blocks[196]. These same regions are the very ones that show further alterations in methylation in squamous cell cancers arising within the same skin. Given the overlap of these regions with LADs and LOCKs, these data also indicate that the interplay between altered 3D genome organization, stochastic epigenetic change and impaired differentiation mediate the effect of environmental damage with photo-ageing[196].

### Network entropy and nuclear structure

Recent work has described cellular heterogeneity as network entropy — applied as a measure of signalling pathway promiscuity — and established that the level of network entropy provides an estimate of developmental potential[160,197]. In other words, the high entropy of a heterogeneous pluripotent stem cell population maintains a diverse range of pathways associated with more mature phenotypes in a poised state for activation. Consistent with the signalling entropy model of cellular differentiation, the variability in the expression of signalling factors and developmental regulators has been experimentally linked to the differentiation potential of ESCs[198]. In a similar manner to normal

differentiation, CSCs display a higher entropy than cancer cells, although the difference is smaller than between normal stem cells and differentiated progeny[160]. Furthermore, CSCs consistently have a lower entropy than their normal counterparts, indicating the presence of dominating oncogenic pathways. This is in agreement with models suggesting that cancers represent hybrid states between aberrantly increased as well as decreased epigenetic flexibility[188] (FIG. 3a).

Importantly, transitions between cellular states of different entropy seem to be regulated epigenetically. Using quantitative RNA fluorescence *in situ* hybridization in combination with time-lapse movies, the transient stabilization of distinct, noisy expression patterns that predict the potential for differentiation has been linked to changes in the global level of DNA methylation in ESCs[199]. These findings highlight that changes in DNA methylation might stabilize not only irreversible, but also reversible cell fate transitions, and regulate stochastic switches between states. As opposed to the short timescales of transcription bursts, the long timescales of infrequent state switching follow a stochastic bistable switch model regulated by methylation and demethylation. Interestingly, ESCs and testicular cells display a bimodal and coherent methylation pattern that becomes variable during differentiation and with age[200]. Further supporting the model in which stochastic variation in fuels aberrantly increased cellular heterogeneity, Epstein–Barr virus immortalization of human B cell cultures induces the emergence of hypomethylated blocks linked with hypervariable DNA methylation and gene expression[158]. In summary, these experiments are consistent with a model in which inherently stochastic DNA methylation variation unleashed within hypomethylated blocks continuously re-establishes tumour cell heterogeneity and thereby promotes the adaptation of the tumour tissue to changing microenvironments, facilitating the survival and growth of tumour cells outside the context of normal tissue architecture and at metastatic sites[188] (FIG. 3a,b).

## Mechanism of stochastic epigenetic variation

Recent experiments suggest that the molecular mechanisms of increased stochastic epigenetic variation might involve deregulated spatial separation between active and inactive chromatin environments and/or altered chromatin mobility between different sub-compartments of the nucleus[74,157,188,201,202]. In accordance with the reversible nature of chromatin modifications, the relocation of LADs and LOCKs away from the lamina has thus been linked to the erosion of repressive marks and an increase in transcriptional activity[203]. Importantly, the long-term stability of H3K9me2 marks in cycling cells seems to be ensured via the stochastic re-establishment of chromatin–lamina interactions in the G1 phase of the cell cycle[203]. Compromised recruitment of inactive chromatin domains to the lamina in G1 might thus lead to the heterogeneous erosion of LOCKs within a cell population, leading to stochastic reactivation of genes located within these domains. Similarly, the stochastic relocation of genes to the periphery might contribute to variegated silencing — that is, cell-to-cell variation in gene transcription depending on the subnuclear position, a phenomenon that also includes stochastic allelic exclusion that limits the production of antigen receptors to a single allele per cell[204]. Moreover, circadian chromatin transitions are also linked to the transient recruitment of clock-controlled loci to lamina[205]. 3D genome organization itself thus emerges as an epigenetic modulator that fine tunes the spatiotemporal aspects of

epigenetic modifier activities to affect phenotypic plasticity in development and cancer (FIG. 3b). We hypothesize that epigenetic mediators promote the emergence of cancer stem-like states and phenotypic flexibility in part by counteracting the formation of repressive subnuclear compartments and the spatial separation between active and inactive chromatin domains. This is likely to require crosstalk between the epigenetic mediators and epigenetic modulators that regulate the dynamics of the 3D nuclear architecture, as well as interaction with epigenetic modifiers to disrupt the multiple layers of epigenetic modifications that establish the differentiated cell state. Very little is known about how different epigenetic perturbations in cancer synergize to deregulate 3D genome organization and influence transcriptional variability. Nonetheless, an interesting opening is provided by the findings that impaired PRC2 function leads to a stochastic loss of repression and increased transcriptional variability at PRC2 target genes, which is linked to a poor prognosis[206]. H3K27me3 modifications are moreover enriched in LADs close to LAD boundaries[207] and have not only been linked to the recruitment of genomic regions to the lamina[170], but are also suggested to collaborate with H3K9me3 marks to promote HP1 binding to chromatin[208], with potential consequences on the stringency of transcriptional repression genome-wide.

## Enhancer usage

Enhancer elements integrate signals from developmental and oncogenic pathways, as well as chromatin organization, to modulate the probability and variability of transcriptional bursts at the associated transcriptional units[79,209–211]. We envisage that tumour-specific 3D chromatin organization modulates the epigenome and undermines differentiation in part by affecting the specificity and dynamics of enhancer–promoter communication. Global maps of chromatin contacts have thus uncovered long-range enhancer–promoter loops within and between chromosomes[212] that fine tune the cell-to-cell variability of gene expression, potentially providing selectable features in a cell population[213]. Conversely, the robustness of cell-type-specific gene expression is ensured by the local clustering of multiple enhancer elements *in cis* spanning tens or hundreds of kilobases[214]. These so-called super-enhancers evolved to integrate signals from multiple cell-fate-determining pathways to ensure a high probability of transcription at genes defining cellular states[209]. Factors regulating epigenetic modifiers that establish enhancer-specific chromatin states and molecular ties regulating enhancer–promoter interactions might therefore act as epigenetic modulators that influence not only the mean level of transcription, but also its variance[213], thereby affecting phenotypic variation (FIG. 3b).

Tumour cells often establish *de novo* oncogenic super-enhancers that drive proliferation[209,215] and are hypersensitive to fluctuations in the level of bromodomain containing 4 (BRD4) and the Mediator complex — an essential cofactor regulating enhancer–promoter contact[216]. Importantly, the location and activity of super-enhancers is stabilized by the cellular microenvironment of the stem cell niche[217], uncovering the surprising sensitivity of super-enhancer formation in stem/progenitor cells to environmental perturbations (FIG. 2).

Enhancer–promoter crosstalk is further constrained by the organization of the genome into topologically associated domains (TADs), which we suggest are categorized as epigenetic modulators based on their role in constituting an additional layer of regulation in setting up gene expression domains[218]. Importantly, the boundary strength of TADs is linked to the presence of architectural proteins, such as CCCTC-binding factor (CTCF)[218,219], an epigenetic modulator (TABLE 1) that binds to DNA in a methylation-sensitive manner[220]. Reprogramming of such boundaries by widespread DNA methylation alterations present in tumours might further contribute to the loss of cell-type-specific expression domains and might alter the mobility and reach of oncogenic super-enhancers. Developmentally regulated contacts between chromatin fibres thus provide a 3D framework for cell-type-specific enhancer usage that might be reprogrammed in tumours to drive variation in stochastic gene expression and diversify the array of tumour-specific cellular states to enable tumour evolution (FIGS 2,3b). Although the mechanism by which epigenetic mediators, such as OCT4, NANOG and SOX2 (TABLE 1) promote the emergence of stem-like cell states in cancer cells is not fully explored, it is likely to involve the efficient reprogramming of 3D enhancer–promoter crosstalk that maintains differentiated cell states.

## Relevance to diagnosis and treatment

Epigenetic chemoprevention to revert or prevent cancer-predisposing polyclonal epigenomic alterations in the progenitor cell compartments might be achieved by inhibiting epigenetic mediators, such as IGF2 signalling. Primary epigenetic changes are thus likely targets for early intervention to prevent tumour progression.

It will be important to consider that mutations in epigenetic modulators and modifiers can arise early in cancer, but a comparatively long time after the polyclonal epigenetic disruption of normal tissue affected by age and the environment through epigenetic modulators. For example, in renal cell carcinoma multiple distinct mutations in different parts of a single tumour converge on the same histone methylation change, suggesting that these mutations arise during progression rather than initiation[48,221]. These observations thus pinpoint epigenetic modifiers as therapeutic targets of existing tumours to prevent progression. The model also highlights the importance of overlooked approaches to epigenetic drug design and warrants new ways of thinking about assays for drugs rather than half maximal inhibitory concentration ($IC_{50}$). This is exemplified by pleiotropic, epigenome-wide changes caused by gain-of-function mutations in variant histones, such as H3.3 and H3.1 in paediatric gliomas[42,43]. The non-linear dynamics of chromatin[222,223] thus make the drug dose crucial when attacking epigenetic modifiers. An example is recent work profiling the effects of anthracycline drugs on histone eviction from chromatin[224]. The authors found that aclarubicin evicts histones from H3K27me3-marked heterochromatin and shows selective toxicity to diffuse large B cell lymphoma cells with increased levels of H3K27me3.

The prominent role of epigenetic instability in the emergence of cancer stem cells and tumour evolution provides an opportunity to reverse drug resistance and deplete CSCs by inhibiting epigenetic mediators. One remarkable opening for such a strategy is offered by the demonstration that tryptophan derivatives regulate *OCT4* transcription in stem-like cancer cells[225]. One of these compounds, 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid

methyl ester (ITE), enhanced the binding of the aryl hydrocarbon receptor to the promoter of *OCT4* to suppress its transcription. Accordingly, administration of synthetic ITE reduced the tumorigenic potential of stem-like cells in both subcutaneous and xenograft tumour models[225].

## Conclusions and future perspectives

The past decade has provided exciting new evidence demonstrating that cancer epigenomes display considerable instability, which leads to the continuous regeneration of epigenetic variation under the selection pressure of the tumour microenvironment[190,191]. One of the most surprising findings of these experiments is that certain domains of the genome seem to be particularly vulnerable to ageing- and environmental-carcinogen-induced epigenetic alterations, which can then unleash stochastic epigenetic changes within such vulnerable domains early during cancer development[190,191]. Ten years ago Feinberg *et al.*[1] argued that environmental signals and ageing could affect epigenetic modifiers and lead to the emergence of an epigenetically disrupted progenitor cell pool long before the emergence of oncogenic mutations on the path to cancer. Such epigenetic variation would then drive phenotypic variation during cancer progression and evolution[1]. Since then, experimental evidence has already accumulated to confirm this prediction, warranting the accurate assessment of the level of transcriptional variation and the contribution of deterministic versus stochastic variation within the epigenome to cancer development. Such an endeavour is likely to require the development of single-cell techniques capable of quantitatively measuring a diverse array of epigenetic modifications at high resolution.

To provide a conceptual framework for the functional characterization of the genes that rewire the epigenome during cancer development and progression (FIG. 1; TABLE 1), we have introduced here a novel classification system that differentiates between epigenetic modifiers and the epigenetic modulators that regulate modifiers, and epigenetic mediators that shape the Waddington landscape of development to shift the phenotype towards stem-like states displaying phenotypic plasticity (FIG. 3). Epigenetic modifiers and epigenetic modulators (TABLE 1) are often mutated in cancer, or transmit signals from oncogenic signalling pathways that indirectly alter local or global chromatin modifications to promote tumour development. We suggest that chromatin states at epigenetic mediator genes are vulnerable targets for cancer-predisposing environmental cues that destabilize the epigenome via signalling and metabolic pathways that impinge on epigenetic modulators. As epigenetic mediators influence phenotypic plasticity during the entire neoplastic process, from the formation of CSC states to malignant derivatives and metastases, these factors should constitute prime targets for both prevention and therapeutic interventions.

The mechanism of increased epigenetic variation in cancer appears to be functionally connected to the perturbations of the 3D organization of the genome and the architecture of the nucleus (FIGS 2,3). Factors that regulate the nuclear architecture and enhancer–promoter communication might thus modulate the epigenome by coordinating the spatiotemporal aspects of epigenetic modifier activity. Moreover, the 3D genome organization in itself seems to affect the epigenome and function as an epigenetic modulator. The physical separation between active and inactive chromatin environments and the formation of TADs

constraining enhancer–promoter contacts are thus likely to modulate the level of stochastic variation in epigenetic marks (FIG. 3b). Measuring the impact of deregulated nuclear compartmentalization on phenotypic traits that are selected for on the path to cancer requires the invention of sensitive and quantitative methods that can translate cell-to-cell variations of 3D chromatin organization to transcriptional heterogeneity in small cell populations representing transition cell fates towards CSCs.

We emphasize that the findings of the past 10 years also call for the integration of normal tissue epigenomics into precision medicine funding to promote progress in largely unexplored research areas in the context of cancer progenitors, such as RNA, tumour heterogeneity, transcriptional stochasticity, the contribution of inflammation and cell signalling, and enhancer–promoter interactions, to name but a few.

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## Glossary

| | |
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| **Field effect** | Epigenetic changes in a region of normal cells around a tumour |
| **CpG island methylator phenotype** | The classification of cancers characterized by increased methylation at CpG-rich promoter regions, best characterized in colorectal cancer and glioma and associated with distinct histological and molecular features |
| **Epimutations** | Abnormal epigenetic alterations leading to aberrant gene expression or silencing |
| **Cancer stem cells** | (CSCs). A subpopulation of cancer cells with the ability to propagate the cancer cell population |
| **Loss of imprinting** | (LOI). Loss of parent of origin-specific expression of imprinted genes in cancer |
| **Epigenetic stochasticity** | Non-deterministic changes to epigenetic marks such as DNA methylation, giving rise to epigenetic variation that underlies cellular plasticity in both normal and pathological states, and that can be localized to specific genomic regions |
| **Canalization** | The ability of an organism to produce a consistent developmental outcome despite variations in its environment |
| **Pleiotropic** | Genetic or epigenetic changes that affect multiple seemingly unrelated phenotypic traits |
| **Non-linear dynamics** | The behaviour of a system in which a small change in an input variable can induce a large change in the output. Modelling of |

| | |
|---|---|
| | chromatin structure and of the impact of chromatin states on transcription has demonstrated non-linear behaviour |
| **Waddington landscape** | A metaphor of development, in which valleys and ridges illustrate the epigenetic landscape that guides a pluripotent cell to a well-defined differentiated state, represented by a ball rolling down the landscape |

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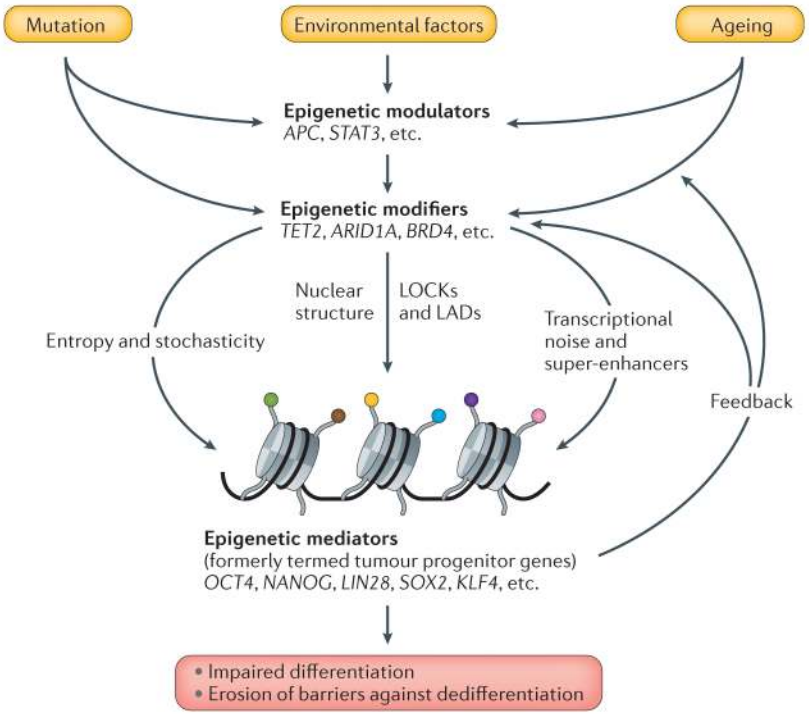


**Figure 1. Functional classification of cancer genes and their contribution to malignancy**

Ageing, inflammation and chronic exposure to carcinogens impinge on epigenetic modulators, such as adenomatous polyposis coli (*APC*) and signal transducer and activator of transcription 3 (*STAT3*), that fine tune and regulate the function of epigenetic modifiers — for example, TET methylcytosine dioxygenase 2 (*TET2*) and AT-rich interaction domain 1A (*ARID1A*) — to bring about changes in the expression of epigenetic mediators — for example, sex-determining Y-box 2 (*SOX2*) and *OCT4* — whose gene products regulate developmental potential. Chronic exposure to a fluctuating, cancer-predisposing environment and ageing promote the selection for epigenetic heterogeneity in vulnerable populations of somatic stem cells and progenitor compartments. Mutations in modulators and modifiers are often selected for during cancer development, which leads not only to increased cell proliferation, but also to the unscheduled expression of mediators that, in turn, inhibit differentiation and promote epigenetic plasticity by affecting the epigenetic modulators and modifiers in a feedback loop. The mechanism of epigenetic instability involves the erosion of barriers against dedifferentiation, such as large organized chromatin K9 modifications (LOCKs) overlapping with lamina-associated domains (LADs), and the emergence of hypomethylated blocks that contain the most variably expressed domains of the tumour genome and interfere with normal differentiation. Increased transcriptional noise at developmentally regulated genes is paralleled by the redistribution of super-enhancers from cell-fate-determining genes to oncogenes that further stabilize the cancer cell state. Stochastic changes in unstable chromatin states lead to the continuous regeneration of epigenetic heterogeneity that manifests as increased cellular entropy and provides the basis for the selection of the fittest during cancer evolution. *BRD4*, bromodomain containing 4; *KLF4*, Kruppel-like factor 4.

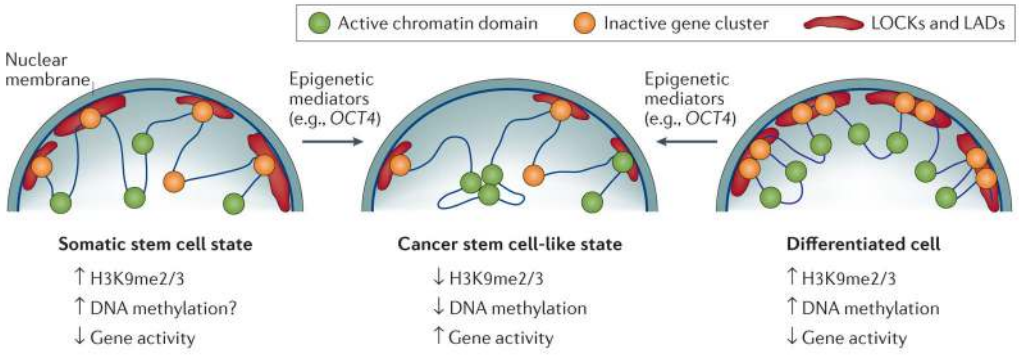


**Figure 2. Change in cell state towards cancer stem cell states induced by reprogramming of the 3D epigenome**

This hypothetical scheme explains how epigenetic mediators (for example, *OCT4*) might reprogramme the epigenome to tip over normal somatic stem cells or differentiated progenitor cells into cancer stem cell states displaying phenotypic heterogeneity. Large organized chromatin K9 modifications (LOCKs) (red cloud) overlapping with lamina-associated domains (LADs) are hypothesized to be largely absent in somatic stem cells (left panel) to ensure epigenetic flexibility associated with the multipotent state. The coordination of cell-type-specific repressed states (right panel) within the LOCKs/LADs is facilitated by epigenetic modifiers establishing multiple layers of epigenetic modifications, such as H3K9me2, H3K9me3, and DNA methylation. The localization of LOCKs/LADs to the lamina leads to the separation of active and inactive domains to reduce transcriptional noise and to provide barriers for dedifferentiation. Conversely, the unscheduled activation of epigenetic mediators leads to the erosion of LADs/LOCKs and the emergence of hypomethylated blocks during the neoplastic process. This, in turn, induces phenotypic heterogeneity by increasing the variability in expression and the probability of switches between the diverse cellular states within the tumour. A loss of LOCKs is postulated, moreover, to interfere with the constraints of enhancer–promoter communication within and between topologically associated domains (TADs), enabling the clustering of oncogenic super-enhancers and expression domains (green circles) to coordinate the expression of oncogenic pathway members (centre panel).

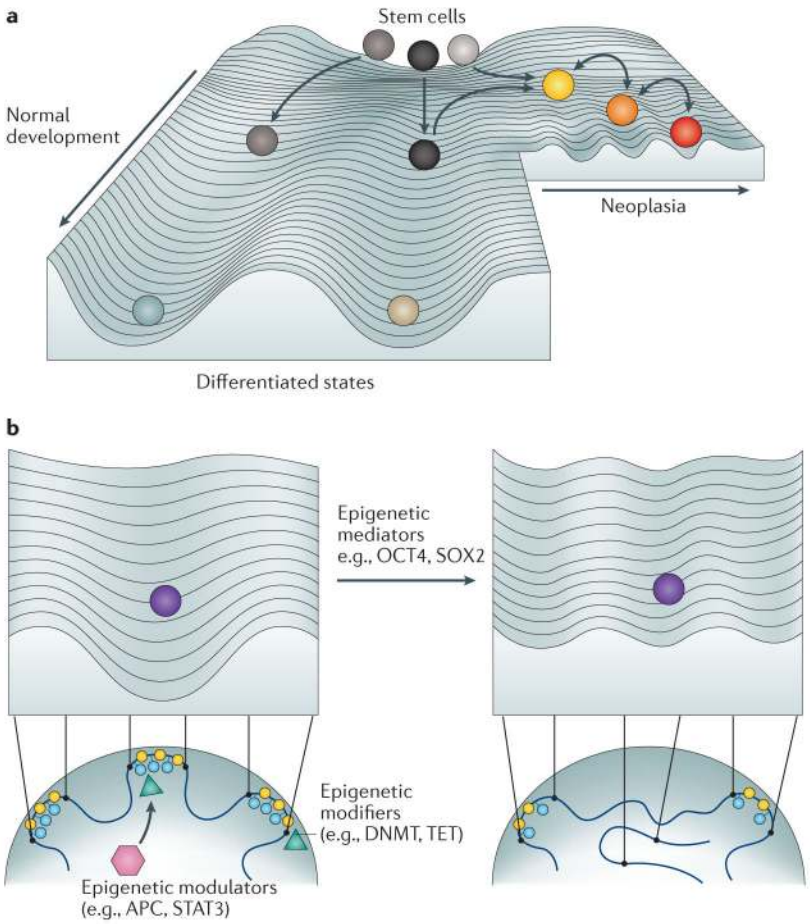


**Figure 3. Waddington landscape of phenotypic plasticity in development and cancer**

**a.** The Waddington landscape of development is adapted to compare cellular states of different entropy during normal differentiation (left side of image) and in cancer (right side of image). The developmental potential of normal somatic stem cells (grey balls) positioned on the top of the hill correlates with high entropy, which is mediated by cellular heterogeneity (different shades of grey). During differentiation, cells are guided towards well-defined cell fates (light blue and brown balls) with lower entropy, paralleled by a decrease in transcriptional noise and the stabilization of cell states (deepening of the valleys or canalization). Cancer stem cell (CSC) states (yellow ball) arise when epigenetic instability interferes with normal differentiation and leads to the erosion of barriers against dedifferentiation — for example, via the erosion of large organized chromatin K9 modifications and the emergence of hypomethylated blocks. In a similar manner to normal differentiation, CSCs with higher entropy occupy higher altitudes on the hill than cancer cells (orange and red balls), although the difference is smaller than between normal stem cells and differentiated progeny. Increased transcriptional noise (shallow valleys) and stochastic switches between diverse cell states (arrows between valleys) are regulated by the interplay between epigenetic modulators, modifiers and mediators, the deregulated epigenome and fluctuating environmental cues (for example, inflammation, repeated exposure to carcinogens, ageing or an overactive WNT pathway). Finally, cellular

heterogeneity (yellow, orange and red balls) within the tumour eventually enables selection mechanisms to drive the growth of the fittest clone. **b.** Illustration of the role of epigenetic modifiers, modulators and mediators on the Waddington landscape described in part **a**. Epigenetic modulators (pink hexagon) regulate the activity of epigenetic modifiers (green triangles) that induce the ectopic expression of epigenetic mediators. Mediators dynamically alter the contour of the landscape via feedback loops that target epigenetic modifiers such as chromatin modifications (blue circles), lamin proteins (yellow circles) and chromosomal interactions (new loop on right). The expression of epigenetic mediators thus produces a shift in the epigenetic landscape, enabling the sampling of aberrant developmental outcomes displaying increased phenotypic plasticity in neoplastic or pre-neoplastic cells. *APC*, adenomatous polyposis coli; *DNMT*, DNA methyltransferase; *SOX2*, sex-determining Y-box 2; *STAT3*, signal transducer and activator of transcription 3; TET, TET methylcytosine dioxygenase.

## Table 1

Three classification systems for cancer genes

| Class | Definition | Examples |
|---|---|---|
| *Genetic classification* | | |
| Oncogene | A gene whose activation by mutation is advantageous to the cancer cell. Acts as dominant | *MYC*, *KRAS*, *PIK3CA*, *ABL1*, *BRAF* |
| Tumour suppressor gene | A gene whose inactivation by mutation is advantageous to the cancer cell. Generally acts as recessive | *RB1*, *TP53*, *WT1*, *NF1*, *NF2*, *VHL*, *APC*, *CDKN2A* |
| *Selection classification* | | |
| Driver gene | A gene whose mutation or aberrant expression is subject to selection during tumorigenesis | *MYC*, *KRAS, PIK3CA, ABL1, RB1, TP53, WT1* |
| Passenger gene | A gene mutated in cancer that is not a driver | Estimated as 99.9% of all mutational changes in cancer |
| *Epigenetic functional classification* | | |
| Epigenetic modulator | A gene, mutated or not, that activates or represses the epigenetic machinery in cancer | *IDH1/2*, *KRAS, APC*, *TP53*, *STAT1/3*, *YAP1*, *CTCF* |
| Epigenetic modifier | A gene, mutated or not, that modifies DNA methylation or chromatin structure or its interpretation in cancer | *SMARCA4*, *PBRM1*, *ARID1A*, *ARID2*, *ARID1B*, *DNMT3A*, *TET2*, *MLL1*/2/3, *NSD1*/2, *SETD2*, *EZH2*, *BRD4* |
| Epigenetic mediator | A gene regulated by an epigenetic modifier in cancer (mutations rare or absent) that increases pluripotency or survival | *OCT4*, *NANOG, LIN28*, *SOX2*, *KLF4* |

*APC*, adenomatous polyposis coli; *ARID*, AT-rich interaction domain; *BRD4*, bromodomain containing 4; *CTCF*, CCCTC-binding factor; *CDKN2A*, cyclin-dependent kinase inhibitor 2A; *DNMT3A*, DNA methyltransferase 3α; *EZH2*, enhancer of zeste homologue 2; *IDH*, isocitrate dehydrogenase; *KLF4*, Kruppel-like factor 4; *MLL*, mixed-lineage leukaemia; *NF*, neurofibromin; *NSD*, nuclear receptor binding SET domain protein; *PBRM1*, polybromo 1; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *RB1*, retinoblastoma 1; *SETD*, SET domain containing; *SMARCA4*, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4; *SOX2*, sex-determining Y-box 2; STAT, signal transducer and activator of transcription; TET, TET methylcytosine dioxygenase; TP53, tumour protein p53; VHL, von Hippel-Lindau tumour suppressor; WT1, Wilms tumour 1; YAP1, Yes-associated protein 1.

## Table 2

Epigenetic modifier mutations in cancer

| Gene | Tumours | Refs |
|---|---|---|
| *Chromatin remodelling* | | |
| *SMARCB1* | Paediatric malignant rhabdoid tumours | 2 |
| *SMARCA4* | Lung adenocarcinoma, Burkitt lymphoma, medulloblastoma | 226_228 |
| *PBRM1* | Clear cell renal carcinoma | 30 |
| *ARID1A* | Ovarian clear cell carcinoma, hepatocellular carcinoma, colorectal cancer, lung adenocarcinoma | 227,229, 230 |
| *ARID1B*, *ARID2* | Hepatocellular carcinoma, melanoma, pancreatic cancer, breast cancer | 231_234 |
| *SMARCD1* | Breast cancer | 234 |
| *SMARCE1* | Clear cell meningioma | 235 |
| *ATRX* | Paediatric glioblastoma, pancreatic neuroendocrine tumours | 236,237 |
| *DAXX* | Paediatric glioblastoma, pancreatic neuroendocrine tumours | 236,237 |
| *CHD5* | Neuroblastoma, glioma, breast, lung, colon, ovary, prostate cancers | 238 |
| *CHD2* | Chronic lymphocytic leukaemia | 239 |
| *CHD1*, *CHD3*, *CHD4*, *CHD6*, *CHD7*, *CHD8* | Gastric, colorectal, prostate, breast, bladder, serous endometrial cancers | 240_243 |
| *DNA methylation* | | |
| *DNMT3A* | T cell lymphoma, myeloid malignancies including acute myeloid leukaemia | 11_14, 244 |
| *DNMT1* | Colorectal cancer | 245 |
| *TET2* | T cell lymphoma, myeloid malignancies including acute myeloid leukaemia | 21,22, 246 |
| *TET1*, *TET3* | Colorectal cancer, chronic lymphocytic leukaemia | 247 |
| *MBD1, MBD4* | Colorectal cancer, lung adenocarcinoma, breast cancer, melanoma | 227,230, 234,248 |
| *Histone acetylation* | | |
| *EP300* | Diffuse large B cell lymphoma, follicular lymphoma, small-cell lung cancer, transitional cell bladder cancer, serous endometrial cancer, pancreatic cancer | 33,242, 243, 249_251 |
| *CREBBP* | Diffuse large B cell lymphoma, follicular lymphoma, small-cell lung cancer, transitional cell bladder cancer, ovarian cancer, relapsed acute lymphoblastic leukaemia | 33,242, 249,250, 252,253 |
| *HDAC2* | Colorectal cancer | 254,255 |
| *HDAC4* | Breast adenocarcinoma | 256 |
| *HDAC9* | Prostate adenocarcinoma | 240 |
| *Histone methylation* | | |
| *MLL* | Myeloid and lymphoid leukaemias, majority of infant acute lymphoblastic leukaemia, solid tumours (colorectal, lung, bladder, breast) | 257_259 |
| *MLL2* | Non-Hodgkin lymphoma (90% of follicular lymphoma, one-third of diffuse large cell lymphoma) | 33,259 |
| *MLL3*, *MLL4* | Solid tumours: bladder, lung, endometrial, hepatocellular | 229,259, 260 |
| *SETD1A* | Gastric adenocarcinoma, breast cancer, chronic lymphocytic leukaemia | 234,239, 261 |
| *PRDM9* | Head and neck squamous cell carcinoma | 38 |

| Gene | Tumours | Refs |
|---|---|---|
| *EZH2* | Gain of function in non-Hodgkin lymphoma and solid tumours | 33,34 |
| | Loss-of-function in myeloid malignancies, head and neck squamous carcinoma, T cell leukaemia | 36–39, 262 |
| *NSD1* | Acute myeloid leukaemia, head and neck squamous cell carcinoma, endometrial carcinoma, melanoma, colorectal cancer, multiple myeloma | 44,263 |
| *NSD2* | Paediatric acute lymphoblastic leukaemia, colorectal cancer, melanoma | 45,263 |
| *SETD2* | Renal cell carcinoma, early T cell precursor acute lymphoblastic leukaemia, high-grade glioma | 47,262, 264 |
| *KDM5C (JARID1C)* | Renal cell carcinoma | 30,47 |
| *KDM6A (UTX)* | Multiple myeloma, oesophageal squamous cell carcinoma, renal cell carcinoma, medulloblastoma, prostate, transitional cell bladder cancer | 6,242, 265,266 |
| *KDM2B* | Diffuse large B cell lymphoma | 34 |
| *Readers* | | |
| *PHF6* | T cell acute lymphoblastic leukaemia, acute myeloid leukaemia | 267–269 |
| *PHF23* | Acute myeloid leukaemia | 270 |
| *BRD4* | NUT midline carcinoma | 271 |
| *BRD8* | Hepatocellular carcinoma | 229 |
| *ING1* | Melanoma, oesophageal squamous cell cancer, acute lymphoblastic leukaemia | 272 |
| *Histones* | | |
| *H3F3A* | Paediatric glioblastoma, diffuse intrinsic pontine glioma, giant cell tumour of bone | 237,273 |
| *H3F3B* | Chondroblastoma | 273 |
| *HIST1H3B* | Paediatric glioblastoma, diffuse intrinsic pontine glioma | 42 |
| *HIST1H1B* | Chronic lymphocytic leukaemia, follicular lymphoma, colorectal cancer | 239,256, 274 |

*ARID*, AT-rich interaction domain; *ATRX*, alpha thalassaemia/mental retardation syndrome X-linked; *CHD*, chromodomain helicase DNA binding protein; *CREBBP*, CREB binding protein; *BRD4*, bromodomain containing 4; *DAXX*, death-domain associated protein; *DNMT3A*, DNA methyltransferase 3α; *EP300*, E1A binding protein p300; *EZH2*, enhancer of zeste homologue 2; *H3F3*, H3 histone, family 3; *HDAC*, histone deacetylase; *HIST1H3B*, histone cluster 1, H3b; *ING1*, inhibitor of growth family member 1; *KDM2B*, lysine (K)-specific demethylase 2B; *KDM5C*, also known as *JARID1C; KDM6A*, also known as *UTX; MBD1*, methyl-CpG binding domain protein 1; *MBD4*, methyl-CpG binding domain 4 DNA glycosylase; *MLL*, mixed-lineage leukaemia; *NSD*, nuclear receptor binding SET domain protein; *PBRM1*, polybromo 1; *PHF*, PHD finger protein; *PRDM9*, PR domain 9; *SETD*, SET domain containing; *SMARC*, SWI/SNF related, matrix associated, actin dependent regulator of chromatin; *TET*, TET methylcytosine dioxygenase.