The epigenetic progenitor origin of human cancer

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Abstract | Cancer is widely perceived as a heterogeneous group of disorders with markedly different biological properties, which are caused by a series of clonally selected genetic changes in key tumour-suppressor genes and oncogenes. However, recent data suggest that cancer has a fundamentally common basis that is grounded in a polyclonal epigenetic disruption of stem/progenitor cells, mediated by 'tumour-progenitor genes'. Furthermore, tumour cell heterogeneity is due in part to epigenetic variation in progenitor cells, and epigenetic plasticity together with genetic lesions drives tumour progression. This crucial early role for epigenetic alterations in cancer is in addition to epigenetic alterations that can substitute for genetic variation later in tumour progression. Therefore, non-neoplastic but epigenetically disrupted stem/progenitor cells might be a crucial target for cancer risk assessment and chemoprevention.

Cancer has been defined in many ways. Starting from Hippocrates' observation of angiogenesis, the word cancer itself refers to the thick blood vessels that feed the tumours and that resemble the claws of a crab. Since the time of Laennec, pathologists have viewed cancer as acquiring properties of cells at different developmental stages, but appearing inappropriately in the tumours[1]. In the past century the genetic model of cancer has predominated, beginning with Boveri who first suggested a role for abnormal chromosomes in cancer[2]. In the modern era the diverse molecular changes that occur among cancer types have led to the idea that cancer encompasses many diseases. Solid tumours are thought to progress from benign, relatively well-differentiated and non-invasive tumours, probably involving one or a small number of mutations, to '*in situ*' cancers with genetic instability but still relatively low invasive and metastatic potential, to invasive and metastatic tumours with increasing numbers of genetic changes, which presumably allow selection for diverse metastatic environments throughout the body. By contrast, leukaemias arise from specific chromosomal rearrangements without a benign precursor stage, but with increasing numbers of mutations late in the course of the disease. Despite these differences, there nevertheless seems to be something central to what cancer ultimately is, something that should be reflected in a common mechanism that involves the inappropriate timing of normal cellular functions.

Great advances have been made in basic research on cancer and in identifying the genetic changes that underlie tumour cell biology. Nevertheless, the main approaches to treating common adult solid tumours still involve non-specific cytotoxic drugs and radiation. This fact does not diminish the outstanding accomplishments in identifying genetic changes such as *HER2/NEU* amplification in breast cancer, *ERBB2* mutations in lung cancer, *EGFR* (epidermal growth factor) mutations in lung cancer, and the *BCR–ABL* (breakpoint cluster region–Abelson murine leukaemia viral (*v-abl*) oncogene homologue) rearrangement in chronic myelocytic leukaemia (CML), and their attendant specific therapies[3].

Similarly, great advances have been made in characterizing epigenetic alterations in cancer. These include global alterations, such as hypomethylation of DNA and hypoacetylation of chromatin, as well as gene-specific hypomethylation and hypermethylation[4]. Global DNA hypomethylation leads to chromosomal instability and increased tumour frequency, which has been shown in *in vitro* and *in vivo* mouse models[5–8], as well as gene-specific oncogene activation, such as *R-ras* in gastric cancer, and cyclin D2 and maspin in pancreatic cancer[9–13]. In addition, the silencing of tumour-suppressor genes is associated with promoter DNA hypermethylation and chromatin hypoacetylation, which affect diverse genes such as retinoblastoma 1 (*RB1*), *p16* (also known as cyclin-dependent kinase inhibitor 2A (*CDKN2A*)), von Hippel–Lindau tumour suppressor (*VHL*) and MutL protein homologue 1 (*MLH1*); this topic has been well reviewed

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Table 1 | **Hypomethylation and hypermethylation in cancer**

	Hypomethylation	Hypermethylation
Frequency	Ubiquitous even in the earliest benign tumours	Some early hypermethylation, with increasing frequency with tumour progression
Targets	Repetitive sequences, coding regions, promoters	Promoters
Primary/secondary change	Primary?	Can be secondary to gene silencing, chromatin changes
Possible effects in humans	Chromosomal instability, loss of imprinting, oncogene activation	Maintenance of tumour-suppressor-gene silencing
Effects in animal models	Lymphoma, increased intestinal tumour initiation, liver cancer	Increased intestinal tumour progression
Variation in the age of onset	Yes	Yes
Therapy	Inhibitor side effect?	Inhibitor therapy

elsewhere[4,14–16]. Methylation changes occur early and ubiquitously in cancer and their roles are summarized in TABLE 1.

The early onset of epigenetic changes and the growing view that stem cells are the target cells for cancer, together with the idea that epigenetic changes probably distinguish stem cells from somatic cells, make it likely that epigenetic disruption of stem cells is a common unifying theme in cancer aetiology. Our goal in this article is to point out that epigenetic alterations in stem cells might precede the differences that so clearly distinguish tumour types. These differences between tumour types might largely be due to the genetic gatekeeper alterations that arise on the background of epigenetically altered stem cells. These epigenetic changes could be in addition to the important role they have as surrogates for the genetic gatekeeper alterations, such as tumour-suppressor-gene inactivation and oncogene activation. Epigenetic changes in pluripotent precursor cells might explain many of the heterogeneous properties that are commonly associated with tumour cell-growth, invasion, metastasis and resistance to therapy. We propose that a more intensive focus on the role of epigenetic changes in progenitor cells might offer significant advances in cancer prevention, diagnosis and treatment. Furthermore, an epigenetic progenitor model might provide a simple explanation for both the age dependency of disease incidence and the environmental sensitivity of cancer risk, an idea that could be valid for common human diseases in general[17].

The clonal genetic model of cancer

For the past 30 years cancer has been thought to arise from a single cell. According to this view, a series of genetic alterations is responsible for continued clonal selection and tumour cell heterogeneity (FIG. 1). This process gives rise to tumour proliferation, invasion, metastasis and drug resistance[18]. The clonal genetic model has been supported by the discovery of dominantly acting oncogenes and recessively acting tumour-suppressor genes. The large number of such genes that have been discovered so far has led to the view that cancer is a heterogeneous group of diseases with diverse aetiology and pathogenesis[19]. For example, two of the best-understood malignancies, CML and colorectal cancer, seem to arise and progress by completely distinct mechanisms. CML is defined by the Philadelphia chromosome translocation, which results in a fusion between the *BCR* gene on chromosome 9 and the *ABL* gene on chromosome 22 (REF. 20). Patients with CML do not have any other genetic alterations until late in the course of the disease. By contrast, colorectal cancer involves a series of alterations in oncogenes and tumour-suppressor genes, including adenomatosis polyposis coli (*APC*), Kirsten rat sarcoma-2 viral (*v-Ki-ras2*) oncogene homologue (*KRAS*) and tumour protein 53 (*TP53*), that roughly follow the progression from small benign adenomas to advanced metastatic tumours, with the stages of disease, from early to late, directly related to the acquisition of these sequential genetic changes[21].

The clonal genetic model of cancer has been particularly successful in predicting so-called gatekeeper mutations, that is, those that seem to be necessary for the earliest stages of tumour growth, such as those in *APC* in colorectal cancer[22] and *VHL* in renal-cell carcinoma[23]. Accumulation of genetic changes during tumour progression has been well documented for many tumours. Importantly, the clonal genetic model has inspired specific drug development, such as imatinib for CML — a product of rational drug design for compounds that inhibit the *BCR–ABL*-encoded tyrosine kinase[24].

Despite its strengths, this classical view has significant limitations. First, progression-related genetic changes are inconsistent. In colorectal cancer, which might be the best-defined example of genetic changes in tumour progression, no known mutations are necessary and sufficient for specific stages of tumour progression other than the gatekeeper mutation itself. It has been suggested that a series of sequential genetic changes accounts for each pathological stage of colorectal cancer progression, although the specific order of genetic changes after the gatekeeper mutation might not be important in tumour progression[21]. Despite determined efforts by many laboratories, so far no recurrent mutations have been identified that are responsible for invasion or metastasis of that tumour. By contrast, many studies have linked specific changes in gene expression to these properties, but with no underlying mutation to account for the expression changes. Overexpression of the *MET* gene induces *in vivo* breast cancer metastasis in mice[25], and *in vivo*

Adenoma
A benign epithelial tumour.

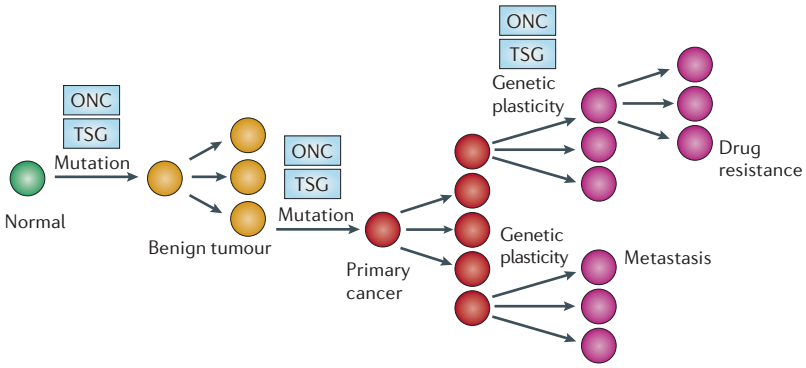

Figure 1 | **The clonal genetic model of cancer.** The classical view of cancer is that it arises through a series of mutations, including dominantly acting oncogenes (ONC) and recessively acting tumour-suppressor genes (TSG). Each mutation leads to the selective overgrowth of a monoclonal population of tumour cells, and each significant tumour property (invasiveness, metastasis and drug resistance) is accounted for by such a mutation. Epigenetic changes are viewed in this model as surrogate alterations for mutations.

ribozyme-mediated reduction of its expression reduces prostate cancer metastasis in mice[26]. Overexpression of the basic helix–loop–helix (bHLH) transcription factor Twist also promotes breast cancer invasion in mice by promoting an epithelial–mesenchymal transition[27]. Similarly, the intercellular adhesion protein intercellular adhesion molecule 1 (ICAM1) is epigenetically silenced in human cancer, and overexpression of ICAM1 in mice inhibits liver metastasis of colorectal cancer[28].

Additionally, it is difficult to reconcile tumour kinetics with the idea of multiple clonally selected mutations. On the one hand, most common adult tumours require many years of exposure (from an epidemiological perspective, which includes both age and environmental insult) simply to arise in their earliest recognizable form. Although the multiple-mutation model might explain tumour progression, there is no evidence for anything other than a critical gatekeeper mutation at the earliest stage, so why the latency? On the other hand, the multiple-mutation model could take too long to account for tumour progression. If it takes decades for the tumour to arise, then it should take decades to progress as well, given that most solid tumours do not divide significantly more rapidly than normal cells. To circumvent this problem, genetic instability has been suggested, as discussed later. Although gene-mutation rates are increased in some tumours as a result of microsatellite instability, the rate of point mutation does not seem to be increased, at least in the case of colorectal cancer[29].

Epigenetic surrogates for genetic changes

Genetic mechanisms are not the only path to gene disruption in cancer. Pathological epigenetic changes — non-sequence-based alterations that are inherited through cell division — are increasingly being considered as alternatives to mutations and chromosomal alterations in disrupting gene function[30]. These include global DNA hypomethylation, hypermethylation and hypomethylation of specific genes, chromatin alterations and loss of imprinting. All of these can lead to aberrant activation of growth-promoting genes and aberrant silencing of tumour-suppressor genes[4].

Altered DNA methylation. Most CG dinucleotides are methylated on cytosine residues in vertebrate genomes[31]. CG methylation is heritable, because after DNA replication the DNA methyltransferase 1, DNMT1, methylates unmethylated CG on the base-paired strand. CG dinucleotides within promoters tend to be protected from methylation[32]. Although individual genes vary in hypomethylation, all tumours examined so far, both benign and malignant, have shown global reduction of DNA methylation[33–35]. This is a striking feature of neoplasia.

In addition to global hypomethylation, promoters of individual genes show increased DNA methylation levels. This applies to many tumour-suppressor genes, including *RB1* in retinoblastoma[14,36], *p16* in melanoma[37], *VHL* in renal-cell carcinoma[38], and *APC* and Wnt-signalling genes in colorectal cancer[39,40]. Hypermethylation of tumour-suppressor genes can be tumour-type specific[41]. An increasing number of genes are found to be normally methylated at promoters but hypomethylated and activated in the corresponding tumours. These include *R-RAS* in gastric cancer[9], melanoma antigen family A, 1 (*MAGE1*) in melanoma[42], maspin in gastric cancer[11], *S100A4* in colon cancer[43], and various genes in pancreatic cancer[13]. An intriguing recent example of hypomethylation promoting tumour cell-growth is the paired box 2 (*PAX2*) gene in endometrial cancer, which is responsible for the responsiveness of the cancer to oestrogens and promotes tumour proliferation[44]. Studies of model organisms support a role of both hypomethylation and hypermethylation in cancer. For example, DNA methyltransferase hypomorphs that are crossed with *Tp53* knockout mice show an increased frequency of lymphoma[7], and when crossed with an *Apc* mutation show reduced numbers of macroadenomas but increased numbers of microadenomas[45]. This implicates both hypomethylation and hypermethylation in tumour formation. Therefore, both hypomethylation and hypermethylation are important in cancer. Hypomethylation generally arises earlier and is strongly linked to chromosomal instability and loss of imprinting[6–8,46,47], whereas hypermethylation is associated with promoters and can arise secondary to gene silencing[48–50], but might be a target for epigenetic therapy[30].

Chromatin alterations. Our genetic material is complexed with proteins in the form of histones in a one-to-one weight ratio[51]. Core histones H2A, H2B, H3 and H4 form nucleosome particles that package 147 bp of DNA, and the linker-histone H1 packages more DNA between core particles, forming chromatin[52]. It is chromatin, and not just DNA, that is the substrate for all processes that affect genes and chromosomes. In recent years, it has become increasingly evident that chromatin, like DNA methylation, can impart memory to genetic activity[53].

There are dozens of post-translational histone modifications[54]. The best studied are those that occur on unstructured histone tails, which protrude from the nucleosome core particle and interact with DNA, other

Microsatellite
A class of repetitive DNA that is made up of repeats that are 2–8 nucleotides in length. They can be highly polymorphic and are frequently used as molecular markers in population genetics studies.

Hypomorph
A mutant allele that has reduced function, or an organism that carries such a mutation.

Lymphoma
A tumour of the lymphoid system.

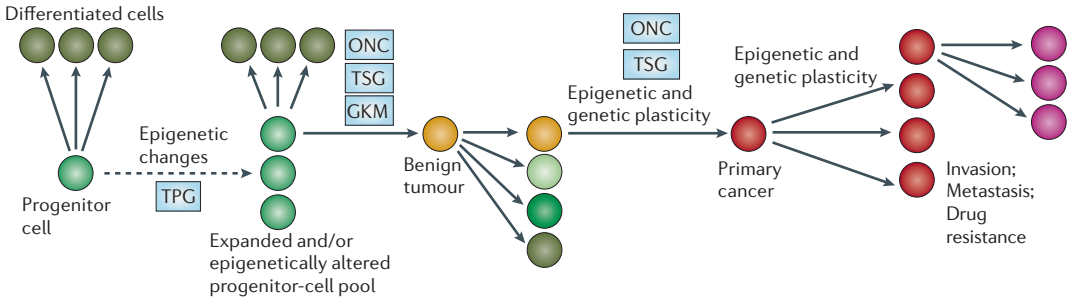

Figure 2 | **The epigenetic progenitor model of cancer.** According to this model, cancer arises in three steps. First is an epigenetic alteration of stem/progenitor cells within a given tissue, which is mediated by aberrant regulation of tumour-progenitor genes (TPG). This alteration can be due to events within the stem cells themselves, the influence of the stromal compartment, or environmental damage or injury. Second is a gatekeeper mutation (GKM) (tumour-suppressor gene (TSG) in solid tumours, and rearrangement of oncogene (ONC) in leukaemia and lymphoma). Although these GKMs are themselves monoclonal, the expanded or altered progenitor compartment increases the risk of cancer when such a mutation occurs and the frequency of subsequent primary tumours (shown as separately arising tumours). Third is genetic and epigenetic instability, which leads to increased tumour evolution. Note that many of the properties of advanced tumours (invasion, metastasis and drug resistance) are inherent properties of the progenitor cells that give rise to the primary tumour and do not require other mutations (highlighting the importance of epigenetic factors in tumour progression).

nucleosomes and many chromatin-associated proteins. Some tail modifications, such as acetylation of lysine residues, alter charge, and so change the bulk electrostatic properties of nucleosomes and can potentially alter their interactions with other nuclear components[55]. Others, especially methylation, provide specific binding platforms for chromatin-associated proteins such as the α, β and γ-isoforms of heterochromatin-associated protein 1 (HP1) (REF. 56). Studies in many model systems have shown that particular histone modifications are enriched at sites of active chromatin (histone H3 and H4 hyperacetylation, lysine 4 at H3 (H3-K4) dimethylation and trimethylation, and H3-K79 methylation) and others are enriched at sites of silent chromatin (H3-K9 and H3-K27 methylation)[57]. These and other histone modifications survive mitosis and have been implicated in chromatin memory.

Overproduction of key histone methyltransferases that catalyse the methylation of either H3-K4 or H3-K27 residues are frequent events in neoplasia[58,59]. Global reductions in monoacetylated H4-K16 and trimethylated H4-K20 are general features of cancer cells[60]. Histones are also distinguished by variants[61]. Variant forms of histone H2A are involved in DNA repair, X-chromosome inactivation and other basic cellular processes. Histone H3 has two universal variants, one at centromeres (centromere protein A (CENPA) in humans) and one at actively transcribed genes (H3.3) in dividing cells. CENPA becomes overproduced in colorectal cancer; a probable factor leading to aneuploidy[62]. The possible role of other variants in cancer progression remains to be explored.

Loss of imprinting. Genomic imprinting is parent-of-origin-specific gene silencing. It results from a germline mark that causes reduced or absent expression of a specific allele of a gene in somatic cells of the offspring. Imprinting is a feature of all mammals, affecting genes that regulate cell growth, behaviour, signalling, cell cycle and transport; moreover, imprinting is necessary for normal development. Imprinting is important in neoplasia because both gynogenotes (embryos derived only from the maternal genetic complement) and androgenotes (embryos derived only from the paternal genetic complement) form tumours — ovarian teratomas and hydatidiform moles/choriocarcinomas, respectively[63]. Loss of imprinting (LOI) refers to activation of the normally silenced allele, or silencing of the normally active allele, of an imprinted gene. LOI of the insulin-like growth factor 2 gene (*IGF2*) accounts for half of Wilms tumours in children[64]. LOI of *IGF2* is also a common epigenetic variant in adults and is associated with a fivefold increased frequency of colorectal neoplasia[46,65]. LOI of *IGF2* might cause cancer by increasing the progenitor cell population in the kidney in Wilms tumour[64], and in the gastrointestinal tract in colorectal cancer[47]. Other genes that show LOI in cancer include *PEG1/MEST* (paternally expressed gene 1/mesoderm-specific transcript homologue) in lung cancer[66], $p57^{KIP2}$ (also known as *CDKN1C*) in pancreatic cancer[67], *DIRAS3* (GTP-binding RAS-like 3) in breast cancer[68], and *TP73* in gastric cancer[69].

The epigenetic progenitor model of cancer

Given the above examples, cancer seems to have both a genetic and epigenetic basis. Clonal genetic changes are common in tumours and clearly account for defining genetic changes in both leukaemia (chromosomal rearrangements) and solid tumours (gatekeeper mutations). Epigenetic alterations are ubiquitous and serve as surrogate alterations for genetic change (oncogene activation, tumour-suppressor-gene silencing), by mimicking the effect of genetic change. For example, gene silencing and associated hypermethylation can involve gatekeeper genes such as SFRP (secreted frizzled-related protein) genes in colorectal cancer[40] and *VHL* in renal-cell cancer[38], or *BCL2* (B-cell CLL/lymphoma 2) activation and hypomethylation in B-cell lymphocytic leukaemia[70].

Genomic imprinting
The parent-of-origin-specific silencing of a specific allele of a gene; loss of imprinting of *IGF2* increases cancer risk and shifts the balance of normal intestinal epithelium to a less differentiated state.

Epigenetic changes also contribute to chromosomal loss and rearrangement during tumour progression[7,71]. However, we believe that not enough attention has been given to the further role of progenitor cells before the apparent initiation of genetic change. The stem cell is increasingly recognized as the target of initiating events (reviewed in REF. 72), and epigenetic alterations probably provide stem cell identity (as suggested by nuclear-cloning experiments, for example). The fact that epigenetic changes are found so early in tumorigenesis, and even in normal tissues before tumours arise, indicates to us that early epigenetic changes in stem cells might provide a unifying view of cancer aetiology. We suggest that epigenetic disruption of progenitor cells is a key determinant not only of cancer risk, but of tumour progression and heterogeneity late in the course of the tumours that arise from these cells. Epigenetic changes can provide mechanistic unity to understanding cancer, they can occur earlier and set the stage for genetic alterations, and have been linked to the pluripotent precursor cells from which cancers arise. Importantly, early epigenetic changes could explain many of the heterogeneous properties that are commonly associated with tumour cell-growth, invasion, metastasis and resistance to therapy. To integrate the idea of these early epigenetic events, we propose that cancer arises in three steps: an epigenetic disruption of progenitor cells, an initiating mutation, and genetic and epigenetic plasticity (FIG. 2).

The first step — epigenetic disruption of progenitor cells. The first step involves an epigenetic disruption of progenitor cells in a given organ or system, which leads to a polyclonal precursor population of neoplasia-ready cells. Until recently, comparatively little attention has focused on the apparently normal tissue of patients who develop cancer, with some notable exceptions[46,73], but these cells represent a main target of environmental, genetic and age-dependent exposure that largely accounts for the long latency of cancer. Epigenetic disruption might perturb the normal balance between undifferentiated progenitor cells and differentiated committed cells within a given anatomical compartment, either in number or in their capacity for aberrant differentiation, which provides a common mechanism for unifying neoplasia.

Five lines of evidence suggest the existence of an epigenetically disrupted progenitor-cell population from which tumours arise (BOX 1). First, classical *in vitro* studies reveal stable but reversible tumour-related growth properties — for example, in differentiating leukaemic cells[74,75] — which implicates an epigenetic mechanism[76]. Second, all tumours show global changes in DNA methylation[33,34], and DNA methylation is clonally inherited through cell division[77]. Because the conventional genetic changes in cancer are also clonal, global hypomethylation would have to occur universally, at the same moment as the mutational changes, which seems unlikely. This suggests that global DNA hypomethylation (and global reductions of specific histone modifications) precedes genetic changes in cancer. Similarly, hypermethylation of tumour-suppressor genes has been observed in the normal tissue of patients in which the same gene is hypermethylated in the tumour tissue[73,78,79].

Third, recent cloning experiments show that a mouse melanoma nucleus can give rise to an entire mouse[80]. The fact that almost all the phenotypic properties of cancer were reversed indicates that the information causing the tumour was largely epigenetic, and that some cells within the tumour were pluripotent. That the tumours

Box 1 | **Evidence in support of an epigenetic progenitor model**

The epigenetic progenitor model states that cancer has a fundamentally common basis that is grounded in a polyclonal epigenetic disruption of stem/progenitor cells, mediated by tumour-progenitor genes. A second step involves monoclonal genetic mutation of gatekeeper genes (or characteristic chromosomal rearrangements in leukaemia or lymphoma), followed by a third step that involves acquisition of genetic and epigenetic plasticity.

The epigenetic progenitor model includes a key step before commonly recognized neoplasia, which can help to explain the late onset of most adult cancers, recurrent disease, environmental effects, tumour heterogeneity and the genetics of cancer risk.

Evidence for the epigenetic progenitor model

- *In vitro* studies of tumour cells demonstrate reversibility of phenotype in both leukaemia and solid tumour development[74–76].
- Global epigenetic changes precede the initial mutations in cancer; the changes involve widespread DNA hypomethylation in all tumours examined[33,34], and promoter hypermethylation in many cases[73,78,79]. These changes must precede the earliest genetic alterations as the epigenetic alterations are always found, even in benign neoplasms.
- Cloned mouse melanoma nuclei can differentiate into normal mice, which indicates that most of the properties of tumour cells can be reprogrammed to normal development — that is, they are epigenetically controlled[80].
- Serial grafting of tumour tissue. Daughter cells retain a diverse range of primary tumour markers, which indicates that a subpopulation of tumour cells possesses a self-renewal mechanism that is similar or identical to stem cells[72,81,82]. Imatinib resistance might be largely due to *BCR–ABL* (breakpoint cluster region–Abelson murine leukaemia viral (*v-abl*) oncogene homologue) mutations in chronic myelocytic leukaemia, but an important contribution seems to be the clonal expansion of progenitor cells[85].
- Loss of imprinting of *IGF2* (insulin-like growth factor 2) is common in the normal colonic epithelium of patients that are at risk of colorectal cancer, and mouse studies show that this epigenetic change shifts the balance of the intestinal epithelium towards an expanded progenitor-cell population[46,47,65]. Altered methylation is also found in the stroma of cancer patients[88,146].

Polyclonal
Arising from multiple cells.

are not entirely epigenetic in origin is revealed by the fact that cloned mice show an increased incidence of melanoma[80].

Fourth, neoplastic clones can be maintained solely by a small population of cells with stem cell properties[72,81,82]. This can be demonstrated by serial grafting of selected tumour-cell populations: for example, only CD113-positive cancer cells (CD113 is a cell-surface marker for self-renewing brain stem cells and early progenitor cells) were able to produce tumours that could be serially grafted into non-SCID mice[82]. In addition, all grades of astrocytoma show stem cell characteristics, whereas early pre-symptomatic lesions reside within a region of the brain, the subventricular zone of the lateral ventricle, that contains neurogenic stem cells[83]. Recent kinetic studies of *BCR–ABL* transcript levels in patients with CML that are on imatinib indicate that the drug eradicates differentiated leukaemic cells but not the underlying stem cell population that gives rise to the tumour[84]. Although most imatinib resistance might be due to mainly *BCR–ABL* mutations, an important contribution seems to come from the the clonal expansion of progenitor cells[85]. A recent study of hyman embryonic stem cells directly showed that promoter hypermethylation can arise in some cases after continued passage *in vitro*[86]. Although most of these data could be explained by genetic or epigenetic changes, they also strongly support the idea that progenitor cells are the actual target of epigenetic change in cancer.

Fifth, recent data demonstrate LOI of *IGF2* throughout the apparently normal colonic epithelium of patients who have LOI-associated colorectal cancer[46,87]. LOI is associated with increased risk of intestinal cancers in both humans and mice[46,47,65]. A specific change in the epithelium is seen in mice that are engineered to have biallelic expression of *Igf2* — a shift in the proportion of progenitor to differentiated cells throughout the epithelium; a similar abnormality was observed in humans with LOI of *IGF2* (REF. 47). Epigenetic alterations in normal stroma also commonly occur and confer tumorigenicity on prostatic epithelial cells[88], although a specific link with progenitor cells has not yet been established. A recent report that altered methylation of the stroma of patients with breast cancer[146] supports the idea that the tumour microenvironment can affect the epigenetic state of progenitor cells.

To account for epigenetic abnormalities in preneoplastic tissues, we propose that 'tumour-progenitor genes' promote epigenetic disruption of stem/progenitor cells. The normal function of tumour-progenitor genes might be to regulate 'stemness' itself, affecting pluripotency and unlimited self-renewal. They might also normally regulate injury response — for example, through the sonic hedgehog (SHH) signalling pathway[89]. When considering the roles of potential inducers of epigenetic lesions in stem cells, it is important to recognize that epigenetic marks that control a transcription unit might constitute parts of an epigenetic network, with positive and negative feedback, similar to the networks that mediate signal transduction[90]. Although such a network should normally be sufficiently robust to maintain cellular memory in a fluctuating microenvironment, it must also be plastic enough to allow for the laying down of new and stable cellular memories during maturation. As a consequence, the stem/progenitor cell might survive and propagate epigenetic lesions either because of a self-renewal imbalance, or because dysregulated functions are manifested only in derived cells. Indeed, although embryonic stem cells that have a hypomethylated genome survive and are apparently normal, they are unable to support normal post-implantation development[91]. We propose therefore that epigenetic lesions might be frequent in a subpopulation of stem cells, owing to, for example, chronic inflammation, injury and nutrition. According to our model, cancer stem cells will arise if tumour-progenitor genes are targeted in this process.

What are tumour-progenitor genes and how do they work? We define them as genes that mediate epigenetic expansion of progenitor cells, and increase their cancer proneness, perhaps by increasing their stemness — that is, their capacity for self-renewal and pluripotency — over their tendency towards limited replicative potential and differentiation. One example discussed above is *IGF2*, for which LOI promotes an increase in the progenitor-cell compartment, increasing the probability of neoplasia. A good candidate for a tumour-progenitor gene would be one that acts directly on DNA, with both genetic and epigenetic consequences. This class might include the APOBEC (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide) family of genes that encode DNA cytosine deaminases[92,93]. Many B-cell lymphomas are evidently caused by mutations that originated with activation-induced deaminase (AID)-dependent deamination events in tumour-suppressor genes[94–96]. Methylated cytosines are especially interesting in this context, because AID prefers to bind to methylated CGs that are preceded by AG *in vitro*, and the same sequence bias is seen for *APC* mutations in colorectal tumours[97]. APOBEC-related proteins might also be responsible for global demethylation in cancer: 5-methylcytosine is deaminated to thymine, and the mismatched TG base pair that results is usually repaired back to CG by the mismatch-repair pathway[93]. Deamination and repair would therefore gradually lead to genome-wide erasure of DNA methylation in lineages that have elevated or misregulated APOBEC family members. So, enzymatic DNA deamination in tumour-progenitor cells would lead to both gatekeeper mutations and global DNA demethylation, both of which are general characteristics of cancer.

Another example of candidate tumour-progenitor genes are those that seem to be necessary for pluripotency in the early embryo, such as the POU transcription factor OCT4 (REF. 98), the forkhead transcription factor FOXD3 (REF. 99), and the homeodomain protein Nanog[100]. Overexpression or inappropriate expression of these genes could tilt the balance towards stem cell renewal and proliferation over normal differentiation within a given tissue. Indeed, such stem cell genes are overexpressed in diverse tumours[101,102]. Genes that directly affect chromatin might also serve as tumour-progenitor genes. For example, the polycomb group protein EZH2 (enhancer of zeste homologue 2) has been

SCID
Severe combined immunodeficiency disorder. Mice that have this disorder are used as hosts for tumour xenografts.

Astrocytoma
An early stage brain tumour.

Stem/progenitor cells
Stem cells are pluripotent cells that have an unlimited capacity for self-renewal, but limited replication frequency, that live within a tissue-specific compartment or niche. Tissue-specific progenitor cells are derived from stem cells and have a limited capacity for self-renewal.

shown to be overexpressed in prostate cancer[103], and it has recently been shown that AKT phosphorylates EZH2, which could lead to aberrant epigenetic gene activation or silencing[104].

The class of tumour-progenitor genes might also include those that are aberrantly silenced, allowing cancer stem cells to escape the confines of the niche. Stem cells normally occupy niches where they can reside for an indefinite period of time and produce progeny cells while self-renewing. Many recently characterized niches have common features, including cell–cell communication, which are organized by E-cadherin, β-catenin and integrins that anchor the stem cell to the niche cells and gap-junction proteins (connexins)[105]. These properties position the stem cell for signals from the niche microenvironment, such as bone morphogenetic proteins (BMPs), Wnt, SHH and IGF1 or IGF2, which affect conserved, intracellular signalling pathways with differing degrees of crosstalk[106–109]. The mechanistic basis for the Wnt-signalling pathway that underlies the maintenance of the self-renewal properties of niche stem cells might be analogous to the function of the *Drosophila melanogaster* orthologue of the tumour suppressor APC. This protein, which is part of the Wnt pathway, controls asymmetrical cell division — a hallmark of stem cells — of male germline stem cells by regulating the orientation of the mitotic spindle perpendicular to the niche[110]. By analogy, the human APC function might ensure that one daughter cell remains in the niche with stem cell properties, whereas the other daughter cell is displaced away from the niche to initiate differentiation. In addition to *IGF2*, which is discussed above, genes that control adherens, gap junctions or intrinsic cell-signalling systems that are sensitive to the niche microenvironment are subject to epigenetic silencing; these include E-cadherin[105], β-catenin[111], connexins[112] and APC[39]. Disruption of stem cell signals from the niche microenvironment might itself be driven by stromal alterations and/or environmental damage or injury.

The second step — initiating mutation. The second step in cancer involves an initiating mutation within the subpopulation of epigenetically disrupted progenitor cells at the earliest stages of what we currently recognize to be a neoplasm. This event has until now been considered to be the first step; it has received great attention and is reviewed elsewhere[113–115]. Initiating mutations are specific for tumour type; for example, mutations in genes that encode APC or β-catenin are specific to colorectal cancer, or the *BCR–ABL* rearrangement in CML[113,115]. As noted earlier, epigenetic alterations can substitute for mutation-induced oncogene activation or tumour-suppressor-gene silencing.

The third step — genetic and epigenetic plasticity. The third step in cancer involves genetic and epigenetic plasticity — that is, an enhanced ability to stably evolve its phenotype, with both a genetic and epigenetic basis. It is well recognized that epigenetic states can be continuously modified to become heterogeneous at all stages of the neoplastic process[116]. In many cases, the underlying basis for increases in genetic plasticity is well understood[115,117]. For example, telomeres erode because polymerases cannot extend 5′ ends, and telomere erosion results in chromosomal shortening beyond which their ends can no longer be capped with telomere-binding proteins. The uncapped ends begin to fuse, and the resulting dicentric chromosomes break at anaphase. This process in turn creates new uncapped ends, and this bridge-breakage–fusion cycle begins anew, stabilizing only because telomerase is induced and new telomeres are synthesized *de novo*[118].

In other cases, the molecular basis of genetic plasticity is less well understood. For example, DNA palindromes have recently been found to form at high levels in cancer cells[119]. Like telomere erosion, DNA palindrome formation can lead to genetic instability by initiating bridge-breakage–fusion cycles. However, it is not known how or exactly when palindromes form, although they appear early in cancer progression.

Epigenetic changes might also drive phenotypic plasticity. In 1984 Schmid and colleagues showed that DNA hypomethylating agent 5-azacytidine causes decondensation of centromeric heterochromatin and widespread chromosomal rearrangement[5]. This could arise as a result of a new centromere forming at sites of decondensation, followed by bridge-breakage and fusion when two centromeres on one chromosome are pulled to opposite poles at anaphase. Consistent with this observation, the DNMT1 hypomorphic mouse shows a marked increase in chromosomal instability[5]. Other evidence comes from the fact that CENPA and CENPH (centromere protein H), proteins that specify the fundamental active chromatin component of centromeres, are overexpressed in cancer, and their overexpression results in aneuploidy[62,120]. Therefore, epigenetic formation of new centromeres might underlie much of the genomic plasticity that is seen in cancer.

Epigenetic instability can also promote cancer through pleiotropic alterations in the expression of genes that modify chromatin. For example, the levels of expression of *HP1α* regulate invasiveness of cancer cells[121]. Other examples include *EZH2*, a homologue of *D. melanogaster* Enhancer-of-zeste that encodes a histone methyltransferase that is specific for H3-K27 (REF. 122). Expression of *EZH2* and consequent secondary widespread gene silencing, which is presumably associated with methylated H3-K27, is seen in metastatic prostate cancers, whereas *in vitro* silencing of *EZH2* inhibits tumour cell-growth. Myeloid/lymphoid or mixed-lineage leukaemia (*MLL1*), a methyltransferase that is specific for histone H3-K4, is the human homologue of *D. melanogaster trithorax*, which is required for heritable gene activation during development, and methylated H3-K4 is a consistent marker for transcriptionally active chromatin[58,59]. Chromosomal rearrangements that activate *MLL1* expression are the most common rearrangements in human cancers, which indicates that disruption of chromatin memory is a frequent event during cancer progression. These associations of cancer with overproduction of either a silencing or an activating

Palindrome
A DNA sequence that is followed by its inverted repeat.

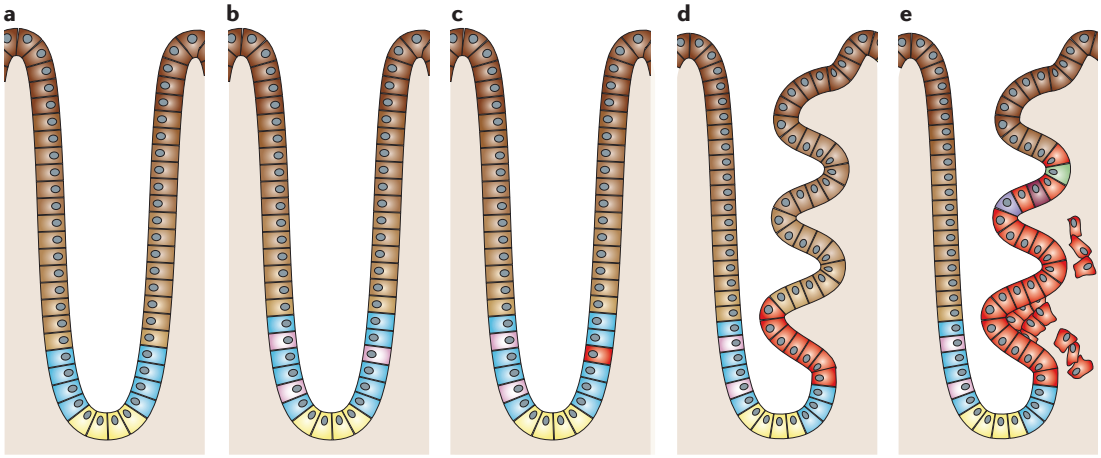

Figure 3 | **The epigenetic progenitor model in the context of a stem cell niche.** Normal colonic epithelium (first panel) includes a proliferative zone that contains stem cells (blue), which give rise to differentiated cells further up the crypt (shades of brown represent differentiation stages) (**a**). The epigenetic progenitor model suggests that the stem cell compartment is altered epigenetically (**b**), which can involve an expansion of the progenitor compartment or other epigenetic changes in gene expression (pink), followed by genetic mutation (**c**, red). Subsequent evolution of the tumour involves genetic and epigenetic plasticity; the latter allows expression of phenotypic features (invasion, metastasis and drug resistance, the last of which is denoted by altered colour) that are inherent properties of the stem cell progenitor (**d** and **e**).

histone modification implicate both gene activation and gene repression in the epigenetic basis of cancer.

Unlike genetic changes, such as mutations and chromosomal rearrangements, epigenetic changes are reversible. These reversible changes can initiate irreversible genetic changes. For example, reduced levels of the protein chaperone, heat shock protein 90 (HSP90), can lead to phenotypic changes in *D. melanogaster*[123,124]. These changes are typically thought to be genetic, in that they can be explained by uncovering pre-existing genetic variation. Surprisingly, the same treatments that reduce HSP90 also uncover epigenetic variation: reductions in HSP90 cause morphological defects in flies from which genetic variation has been eliminated by extensive backcrossing[125]. Similar heritable morphological defects are uncovered by mutations in genes that are thought to act by modifying chromatin, such as Trithorax group genes[125]. This implies that permanent phenotypic changes can have an epigenetic basis. Given the conservation of *Hsp90* and histone-modifier genes in animals, it is likely that similar processes underlie phenotypic plasticity and tumour heterogeneity. By uncovering variants that might be deleterious to the whole organism and suppressed epigenetically in familial transmission, cancer cells might gain a selective growth advantage. On this background of cancer-associated epigenetic instability, the effects of mutations in oncogenes and tumour-suppressor genes might be exacerbated. Therefore, the risk of developing malignancy would be much higher for a given mutational event if it occurred on the background of epigenetic disruption.

Implications of the epigenetic progenitor model

The epigenetic model of cancer has important advantages for understanding cancer biology, the role of the environment, tumour heterogeneity, cancer epidemiology and, importantly, therapeutic intervention and chemoprevention.

Cancer biology. The proposed existence of epigenetically disrupted progenitors of cancer implies that the earliest stages in neoplastic progression occur even before what a pathologist would recognize as a benign pre-neoplastic lesion. Such alterations are inherently polyclonal. This is in contrast with the widely accepted model of cancer as a monoclonal disorder that arises from an initiating mutation — a model which was proposed and accepted when little was known about epigenetic phenomena in cancer. This classic idea of tumour promotion in fact referred to a loss of differentiation induced by chemical agents, and was assumed to occur after the initiating mutation[126]. As recently noted by Klein when describing the expansion of intestinal progenitor cells by LOI, promotion could be epigenetic and could precede mutation[127]. The epigenetic progenitor model in the context of a stem cell niche is shown in FIG. 3.

Role of the environment. The existence of epigenetic precursor lesions could explain several important properties of cancer. It could explain the relationship between environmental exposure or injury and cancer. Tumours, even benign ones, generally take many years to develop. Chronic injury is a major cause of cancer even though it is not inherently mutagenic (for example, asbestos exposure). Environmental insults might affect the expression of tumour-progenitor genes, leading to both genetic and epigenetic alterations. Direct evidence supports an important role for dietary regulation of DNA methylation. For example, liver regeneration after tissue injury leads to widespread hypomethylation[128–130] and hypermethylation of individual genes; both of these epigenetic changes occur in cancer (see above). In human studies, deficiency in folate, a precursor for methionine

Chaperone
A protein that assists in protein folding.

Monoclonal
Arising from a single cell.

biosynthesis, as well as methionine deficiency, increases the risk of colorectal neoplasia[131].

Environmental agents might lead to cancer by misregulating protein chaperones and epigenetically disrupting key signalling pathways that are required for anchoring stem cells. For example, environmental stress titrates out HSP90, which is required to fold the SMYD3 (SET and MYND domain containing 3) histone H3-K4 methyltransferase[132]. Ruden *et al.*[133] have proposed that this event would reduce expression of Wnt-target genes that are needed for attachment of stem cells to niche cells. In the colon, such a reduction in Wnt signalling would be a predisposing factor for APC-mediated escape of stem cells from their niches. The environmental dependence of cancer fits an epigenetic model generally for human disease — the environment might influence disease onset not simply through mutational mechanisms but in epigenetically modifying genes that are targets for either germline or acquired mutation; that is, by allowing genetic variants to be expressed[17].

Tumour heterogeneity. One of the most vexing problems in cancer research and therapy is the acquisition of traits within a tumour that were not there previously, such as metastatic capability and drug resistance. It is generally assumed that these properties are entirely due to the acquisition of new genotypes within the tumour through clonal evolution and selection for these advanced tumour phenotypes. Although this is likely to be a frequent underlying mechanism, tumour heterogeneity and progression could be explained independently of genetic clonal evolution. If, as we propose, tumours arise from a polyclonal population of epigenetically altered progenitor cells, there are two other ways in which tumour progression might occur. First, the epigenetically defined tumour-progenitor cells that have acquired a gatekeeper genetic mutation, or initiated progenitor cells, are likely to be quite different epigenetically from the bulk of the tumour itself. These cells probably resemble early progenitor cells, are relatively slow to divide and lack many differentiated features that characterize the tumour we see. They also differentiate spontaneously into what we recognize as the bulk of the tumour mass, which we characterize biochemically or at the level of gene expression. However, these initiated progenitor cells themselves probably lack many of these differentiated properties and can differentiate in other directions and/or eventually expand in a less differentiated state. In other words, the properties of tumour-cell progression are present all along in the initiated progenitor cell and in the tumour-progenitor cells that precede it. A mechanism for this latency of tumour heterogeneity could be differentiation-dependent manifestation of underlying epigenetic marks (FIG. 3). Importantly, the epigenetic progenitor model can explain the common occurrence of late metastatic disease that has markedly different properties from the primary tumour, years after resection of a small primary malignancy. The metastastic property does not require subsequent mutation and clonal selection within a large tumour mass. Rather it is an inherent property of the progenitor cell from which the tumour arises even in early stage disease, which requires common epigenetic (rather than rare mutational) change.

Second, epigenetic changes might occur in progenitor cells but remain silent without a stress response, as in the case of the action of HSP90 that buffers against phenotypic change or related proteins in *D. melanogaster*[123]. Therefore, stress that is caused by the tumour microenvironment itself (for example, hypoxia) or induced by therapy (for example, cytotoxic drugs or radiation) might unmask the underlying epigenetic heterogeneity within the progenitor cell. In the last case, although the primary tumour is cured, advanced disease might be induced at the same time.

Many experiments support this model for tissue heterogeneity. For example, Wilms tumour involves epigenetic heterogeneity in a subpopulation of mesenchymal cells preceding tumour development[134]. Explanted haematopoietic and solid tumour cells not only acquire other phenotypes *in vitro* or *in vivo*, but these phenotypes can be modified depending on the cellular and tissue matrix milieu into which they are introduced[135–137], indicating that the heterogeneity is an intrinsic and plastic property of the primary tumour.

The model might also explain why tumours often recur *in situ* many years after the primary lesion is resected, and often with a much less differentiated phenotype. This is true for both solid tumours and for leukaemias, again indicating that our model tends to unite cancers of diverse types. If the tumour-progenitor cell itself has the capacity for pluripotent differentiation — that is, these properties do not require continued clonal evolution from the primary malignancy — then it might be necessary to identify and treat the tumour-progenitor cell population that remains after a primary tumour is resected, either by conventional or epigenetic therapy. This might be why, for example, limited surgery and local radiation therapy are as effective as radical mastectomy in the treatment of breast cancer[138].

Late onset of adult cancers. The epigenetic progenitor model could help to explain the late onset of most adult cancers. Patients with gatekeeper mutations in the *APC* gene do not develop adenomas until late in childhood, and even with hundreds of tumours, the overwhelming majority of colon epithelial cells are not neoplastic. By contrast, patients with germline mutations in the *RB1* and Wilms tumour 1 (*WT1*) genes develop retinoblastoma and Wilms tumour, respectively, almost from birth and even before. Such early onset of these tumours could be explained by the fact that loss of the corresponding gene function is directly involved in an expansion of a progenitor-cell population — retinoblasts in patients with mutations in *RB1* and nephroblasts in patients with mutations in *WT1* — so these genes function as both tumour-progenitor and gatekeeper genes. The late onset of cancer might be in part due to the time-dependent erosion of epigenetic marks in progenitor cells[17].

Epigenetic therapy. The heterogeneity and irreversibility that are implicit in the clonal genetic model for cancer provide little hope for much improved

therapies. But if cancer in fact begins with pre-neoplastic epigenetic changes, then we are confronting a disease that might be detectable at an early stage, treatable with generic agents and reversible. Indeed, there are already promising indications that epigenetic therapies work. Agents that modify the epigenome globally, such as 5-aza-2′-deoxycytidine that inhibits DNA methylation, and SAHA (suberoylanilide hydroxamic acid) and others that inhibit histone deacetylases, are available. Although some of these agents, such as 5-aza-2′-deoxycytidine[139], are in clinical use already one must be cautious about their effects, as non-specific epigenetic modification can lead to the activation and silencing of many genes, and it is not yet clear whether regression has an epigenetic basis. These concerns reflect the early stage of this field and emphasize the importance of understanding the fundamental biological mechanisms of epigenetic regulation, much as we have come to understand the mechanism by which conventional cytotoxic agents inhibit replication or induce apoptosis. Ultimately, it might be possible to tailor epigenetic therapy to the crucial epigenetic modification or gene target — for example, by using engineered targeting transcription factors[140] or microRNA[141].

Cancer risk. The epigenetic progenitor model fits well with what we know about the human genetics of cancer risk. Although mutations in gatekeeper and tumour-suppressor genes are a model for cancer biology, and are ubiquitously involved in the tumours themselves, they are quite rare in normal cells at the population level. All germline mutations in colorectal cancer-related genes together account for only 3% of cases[142]. Mutations in *BRCA1* are also rare, accounting for approximately 3% of cases[143]. This is a surprise as a substantial fraction of the population develops these tumours at a relatively young age if there is a strong family history, and genetic predisposition is thought to account for about one-third of cancer. By contrast, LOI, an epigenetic change, is found in 5–10% of the population, with an adjusted odds ratio of approximately five for colorectal neoplasia in three studies (REFS 46,65; M. Cruz-Correa, personal communication). Little is known about epigenetic predispositions to cancer, but a recent twin study indicates that, similar to cancer risk, global epigenetic changes show striking increase with age[60].

Conclusions

It is now widely accepted that cancer is in part an epigenetic disease, although epigenetic alterations are still viewed largely as a surrogate of genetic alterations. Studies of DNA methylation in tumour tissues have revealed at least as many epigenetic as genetic alterations for a given gene, but this is likely to be just the tip of the iceberg. We propose an epigenetic progenitor model in which cancer involves epigenetic disruption of progenitor cells, an initiating mutation, and genetic and epigenetic plasticity. The initial epigenetic disruption might perturb the normal balance between undifferentiated progenitor cells and differentiated committed cells within a given anatomical compartment, either in number or in their capacity for aberrant differentiation, thereby providing a common mechanism that unifies neoplasia.

Early epigenetic events might also be crucial for understanding tumour heterogeneity and progression, which might as much reflect the epigenetic state of cancer precursor populations as clonal genetic evolution during tumour progression. Although clonal genetic evolution is important, pre-existing polyclonal epigenetic disruption within progenitor populations provides a unifying explanation for the pluripotency of tumour cells and for their capacity for unlimited self-renewal. Careful epigenetic analysis at the earliest stages of recognizable cancer, and the tissue from which the tumour arises, might be the most important target for finally cracking this clinically vexing problem in cancer biology.

It is imperative to understand the nature of global and gene-specific epigenetic variation in normal cells of those with cancer or at risk of developing cancer. The global changes to the epigenome, including hypomethylation, hypermethylation and chromatin alterations, will be made clearer by a systematic examination of the epigenome in cancer, at the molecular level. DNA-resequencing efforts that are directed at tumours will miss these important epigenetic changes. Continued epigenetic plasticity must be viewed as an equal partner with genetic plasticity in the late stages of tumour evolution. Therefore, large-scale tumour analysis must examine the relationship between genetic instability and the genes that regulate chromatin.

Tumour-progenitor genes might be identified by the candidate-gene approaches that have been described above in mouse models, to determine whether their overexpression or aberrant expression promotes tumorigenesis. More direct evidence might come from combined genetic epidemiological and mouse models, such as those that implicate alterations in imprinted genes. A comprehensive understanding of tumour-progenitor genes will come from a better understanding of the normal stem cell compartment, particularly the genes whose expression is crucial for maintenance and expansion of that compartment during development, and will therefore benefit from epigenetic studies of stem cell biology. There are important differences in the approach to identifying the epigenetic versus genetic basis of cancer — with a greater emphasis on normal tissues of patients with cancer or those who are at risk of cancer — and methylation and chromatin analysis rather than DNA sequencing.

Finally, tumour-progenitor genes should present attractive drug targets for therapeutic intervention. Such drugs might prevent the onset of cancer, analogously to the way in which the use of drugs to lower cholesterol prevents heart disease. Drugs that are targeted at tumour-progenitor genes might be useful in preventing relapse after a primary treatment results in remission. For example, imatinib therapy becomes ineffective when drug-resistant *ABL* mutations appear. By that time, the tumour-progenitor gene that is responsible for the pre-neoplastic alteration might be identified and targeted, making possible long-term remission. It is encouraging that *Apobec1* knockout mice are phenotypically normal and the symptoms of patients

that have a defective or null AID protein seem to be limited to acquired immunity, reflecting its role in generating antibody diversity[144]. Therefore, despite the widespread expression of these potential tumour-progenitor genes, specific inhibitors of this class of enzymes might be well-tolerated by cancer patients. Another possibility are specific inhibitors of IGF2 signalling in patients who are at risk of colorectal cancer[145]. The key step in identifying such agents will be to focus on epigenetic events in apparently normal tissue that arise long before the neoplasms we currently recognize, and we hope that this article helps in this effort.

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Competing interests statement
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