Epigenetic gene silencing in cancer – a mechanism for early oncogenic pathway addiction?

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Abstract | Chromatin alterations have been associated with all stages of tumour formation and progression. The best characterized are epigenetically mediated transcriptional-silencing events that are associated with increases in DNA methylation — particularly at promoter regions of genes that regulate important cell functions. Recent evidence indicates that epigenetic changes might 'addict' cancer cells to altered signal-transduction pathways during the early stages of tumour development. Dependence on these pathways for cell proliferation or survival allows them to acquire genetic mutations in the same pathways, providing the cell with selective advantages that promote tumour progression. Strategies to reverse epigenetic gene silencing might therefore be useful in cancer prevention and therapy.

CpG islands
Regions of DNA that are often located proximally to the transcription start site of genes that contain a high frequency of CG dinucleotides. In most mammalian genes, these regions are normally maintained free of DNA methylation. In cancer cells, CpG islands of various tumour-suppressor genes are frequently densely methylated, which results in repression of transcription.

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doi:10.1038/nrc1799

Classically, cancer has been viewed as a set of diseases that are driven by progressive genetic abnormalities that include mutations in tumour-suppressor genes and oncogenes, and chromosomal abnormalities[1–5]. However, it is apparent that cancer is also a disease that is driven by 'epigenetic changes' — patterns of altered gene expression that are mediated by mechanisms that do not affect the primary DNA sequence[6–9]. These epigenetic alterations occur within a larger context of extensive alterations to chromatin in neoplastic cells in comparison with the normal cells from which they are derived. These involve both losses and gains of DNA methylation as well as altered patterns of histone modifications[6,8–11]. Although the molecular determinants that underlie these types of chromatin change in tumour cells are only beginning to be elucidated, the best understood component is the transcriptional repression of a growing list of tumour-suppressor and candidate tumour-suppressor genes. This suppression is associated with abnormal methylation of DNA at certain CpG islands that often lie in the promoter regions of these genes[6,8,9]. By this mechanism of 'silencing', the expression of these tumour-suppressor genes in the cancer cell can be reduced or eliminated as an alternative mechanism to genetic mutation[6,8,9].

The increasing recognition of the importance of epigenetic changes in cancer pathogenesis has led to a shift in the approaches that are used to discover genes that are affected by this process. The field has moved from studying the effects of silencing on classic tumour-suppressor genes to searching for candidate tumour-suppressor genes, on the basis of the hypermethylation of promoter regions. In fact, random searches of the cancer-cell genome are now being carried out to detect changes in methylation and chromatin status, either overall or in specific regions of the genome. The identification of genes that are specifically hypermethylated (which results in gene silencing) or hypomethylated (which results in increased transcription) might lead to the discovery of new factors that are important for tumour initiation and progression. Of particular importance is the identification of genes, the silencing of which confers a survival benefit to the cells, contributing to a neoplastic phenotype and facilitating tumour progression by allowing the accumulation of additional genetic and/or epigenetic hits. Genome methylation patterns are also being developed as biomarkers for tumour type, as markers for risk assessment, early detection and monitoring of prognosis, and as indicators of susceptibility or response to therapy.

Recent studies indicate that epigenetic alterations might initiate the expansion of pre-malignant cells during the early stages of tumorigenesis. During the earliest steps of development of principal tumour types, such as colon, lung and prostate tumours, a subset of these pre-malignant cells undergo genetic alterations that allow them to mediate tumour progression and growth. The early epigenetic changes that occur in these cells might determine the subsequent genetic changes and thereby foster progression of these clones. There has

Summary

- Epigenetic gene silencing, which is associated with aberrant methylation of promoter DNA and transcriptional repression, is an important mechanism for the loss of gene function in cancer.
- Silencing can occur during the early stages of human tumour progression — in pre-invasive lesions — and involves disruption or over-activation of key developmental pathways and cell-signalling properties.
- These early gene-silencing events might be crucial for inducing the aberrant, early, clonal expansion of cells through the above alterations in key cell pathways.
- Early gene-silencing events might 'addict' cells to certain oncogenic pathways. This 'epigenetic addiction' could predispose cells to the accumulation of genetic mutations in these same pathways, which drives tumour progression.

been increased effort to elucidate the molecular events in chromatin regulation that initiate and maintain epigenetic gene silencing in cancer cells as tumours progress. Clues are emerging as the entire field of chromatin regulation of gene expression patterns rapidly advances[12–17]. A key concept is that, in order to effectively monitor and control human neoplasia, we might need to explore the cancer-cell 'epigenome' as completely as the mutations in the cancer-cell genome.

Silenced genes

Cancer-cell genomes simultaneously show global hypomethylation and gene-promoter-specific hypermethyl-ation. The mechanisms by which hypermethylation participates in the silencing of transcription have been discussed in several recent reviews[7–9]. The ramifications of global hypomethylation for tumour progression are less well understood, but might contribute to genomic instability, structural changes in chromosomes and increases in gene expression[7]. Local changes in promoter methylation, and concomitant loss of gene expression, has been the recent focus of investigation, and the effect of these changes on tumour biology is becoming increasingly apparent. Promoter hypermethylation has become accepted as a mediator of tumorigenesis as this alteration has been observed at well-characterized tumour-suppressor genes that cause inherited forms of cancer when mutated in the germline. Half of these genes have been shown to be inactivated epigenetically, as well as genetically, in tumours of somatic-cell origin[6,8,9].

As investigators have continued to search for promoter hypermethylation in candidate tumour-suppressor genes, it has become apparent that many genes, which are located across all chromosome locations, are epigenetically silenced in cancer cells. In fact, more genes might suffer loss of function through epigenetic modification than through genetic defects. Importantly, many epigenetically silenced genes have not been found to contain any genetic mutations at all, even though they are transcriptionally repressed in many different cancer-cell types[6,8,9]. These facts underscore the potential value of screening for all epigenetic modifications, as well as genetic changes, that are associated with human tumour types. However, the ultimate value of searching for epigenetic alterations that are associated with cancer depends on demonstrating the ability of these changes to mediate tumour formation or progression. Studies of hypermethylated genes that were discovered in random screens of cancer-cell genomes have indicated that the effect of these epigenetic alterations on tumour progression is substantial.

The development of techniques that are designed to randomly screen cancer-cell genomes for epigenetic changes has recently led to the discovery of many genes that are hypermethylated in cancer cells. These techniques range from arrays that contain CpG islands to genomic screens that are based on the use of methylation-sensitive restriction enzymes to identify methylated regions of DNA in neoplastic cells, as well as gene-expression microarray analyses to identify genes that are re-expressed following pharmacological reversal of epigenetic silencing in cancer cells[18–27]. The information that comes from these approaches clearly indicates that the products of epigenetically

Table 1 | **Mutated and hypermethylated genes in colon cancer cells***

Pathway or function	Hypermethylated genes	Mutations	Biological effects
Wnt signalling	*SFRP1*, *SFRP2*, *SFRP4* and *SFRP5*	Activating mutation in *CTNNB1*	Pathway activation; stem-cell and progenitor-cell expansion; cell survival
Mismatch repair	Wild-type *MLH1* allele	Second *MLH1* allele	Defects in DNA mismatch-repair
Cell-cycle regulation	Wild-type *CDKN2A* allele	Second *CDKN2A* allele	Blocks cyclin D–RB1 pathway, which results in cell proliferation
Epithelial-cell differentiation	*GATA4*, *GATA5*, *TFF1*, *TFF2*, *TFF3* and *INHA*	*TGFBR2*	Loss of normal differentiation
p53-mediated DNA damage response	*HIC1*		Loss of apoptosis response to DNA damage
Cell invasion	*TIMP3*		Loss of inhibition of matrix metalloproteinase enzymes, which promotes cell invasion

*Partial list of genetic mutations and heritable gene-silencing events that were identified in a single culture line (HCT116) of human colon cancer cells. *CDKN2A*, the gene that encodes p16; *CTNNB1*, the gene that encodes β-catenin; *GATA*, genes that encode GATA-binding transcription factors; *HIC1*, hypermethylated in cancer 1; *INHA*, inhibin-α; MLH1, a DNA mismatch-repair protein; RB1, retinoblastoma 1; *SFRP*, secreted frizzled protein; *TFF*, trefoil factor; *TGFBR2*, gene that encodes the transforming-growth-factor-β receptor 2; TIMP3, tissue inhibitor of metalloproteinase 3.

modified genes are involved in regulating all cell functions, and that epigenetic silencing of these genes contributes to tumorigenesis[2,6,8,9].

A vivid example of how both genetic changes and epigenetic gene-silencing combine to determine the phenotype of a tumour cell can be found by examining just one extensively studied line of human colon cancer cells, HCT116. These cells contain several mutations that inactivate tumour-suppressor genes or activate oncogenes, which results in disruption of key signalling pathways and cellular functions (TABLE 1). These mutations include those in one allele of the genes that encode the DNA mismatch-repair protein, MLH1, and p16, which contribute to the mismatch-repair phenotype[28] and to disruption of the cyclin D–RB1 (retinoblastoma 1) cell-cycle-control pathway[29,30], respectively. The gene that encodes the transforming growth factor-βII receptor (TGFβ2R) is also mutated, which results in loss of control of a pathway that mediates cell differentiation[31]. Furthermore, these cells contain an activating mutation in the gene that encodes β-catenin, which results in constitutive Wnt signalling and cell proliferation[32].

However, in addition to these specific mutations, there are at least 14 epigenetically silenced genes in these cells — this is only a partial list — all of which can be reactivated by either treating the cells with DNA-demethylating agents or disrupting the genes that encode DNA methyltransferases, which catalyse DNA methylation[25,33–36]. Reactivating expression of these growth-control genes results in phenotypic changes that range from reducing proliferation to inducing senescence or apoptosis[28,29,34]. So, epigenetic alterations of these genes seem to complement mutations in determining the phenotype of these cells (TABLE 1). A particularly interesting facet of collaboration between epigenetic and genetic abnormalities occurs in *MLH1* and *CDKN2A* (the gene that encodes p16) in HCT116 cells. While one allele of each of these genes is mutated in these cells, the wild-type allele becomes silenced by hypermethylation. So, genetic and epigenetic changes can collaborate to prevent expression of a functional gene product in cancer cells.

Another epigenetic–genetic collaboration in HCT116 cells is in the Wnt pathway. Four members of the secreted frizzled-related gene family (*SFRP1*, *SFRP2*, *SFRP4* and *SFRP5*) that encode Wnt antagonists are epigenetically silenced in these cells. This contributes to the abnormal activation of Wnt signalling, even in cells that already carry activating mutations in β-catenin[34]. In addition, silencing of the genes that encode the transcription factors GATA4 and GATA5, as well as their downstream activation targets trefoil factor 1 (TFF1), TFF2, TFF3 and inhibin-α[33], could impair proper maturation of endoderm-derived epithelial cells[37–40]. Finally, *TIMP3* (tissue inhibitor of metalloproteinase 3) is silenced in HCT116 cells, and loss of function of its product might increase the invasive ability of these cells[36].

Therefore, it is clear that understanding the abnormal epigenetic profile of tumour cells, as well their genetic constitution, could enrich our understanding of how the phenotypes of different tumour types evolve. The involvement of so many genes in the progression of a single tumour type indicates that some aspects of epigenetic gene-silencing might not be completely stochastic — whole networks of genes could be simultaneously affected. It has been proposed that certain colorectal and other cancer cell types acquire a 'hypermethylator' phenotype, in which specific groups of genes are silenced through promoter hypermethylation[41]. This confers the cells with selective advantages that contribute to tumour growth and progression. The mechanistic factors that are involved in such a process remain to be elucidated, but might involve the chromatin regulation that initiates the gene silencing.

Which comes first, genetic or epigenetic changes?

Recent studies have shown that although the abnormal epigenetic silencing of genes can occur at any time during tumour progression, it occurs most frequently during the early stages of the neoplastic process, such as the pre-cancerous stages of tumour development[7,42]. Studies have shown that an epigenetic switch in gene-imprinting status might contribute to the early abnormal expansion of renal cells in patients with an inherited predisposition to Wilms tumour, and also to the early stages of colorectal and other cancers[7,43–45]. Abnormal gene silencing has also been associated with the loss of p16 expression in pre-invasive lung and breast tumours, with the loss of expression of several genes during the pre-invasive stages of colon neoplasia, and with the loss of glutathione S-transferase-π1 (GSTP1) expression in pre-invasive prostate tumours[46–55]. These early epigenetic alterations could predispose cells to the genetic abnormalities that advance the neoplastic process. For example, silencing of *CDKN2A* could allow mammary epithelial cells to escape senescence, resulting in genetic instability and other tumour-cell properties[50–52,56]. Similarly, silencing of *MGMT*, which encodes O^6-methylguanine-DNA methyltransferase, allows cells to acquire specific types of genetic mutation that arise from the inability to repair DNA guanosine adducts[28,53,57–60].

Important new roles for epigenetic abnormalities in tumour initiation and maintenance are also emerging from studies of genes that have been found through random screening of cancer-cell genomes. These studies have revealed that in some cases such epigenetic changes, and their interactions with genetic changes, could allow neoplastic cells to become addicted to various oncogenic driving pathways — a concept that was proposed by I. B. Weinstein with respect to mutations[61]. On the basis of studies of mouse tumour models and human tumours, Weinstein proposed that, through genetic changes, cancer cells become addicted to mutated oncogene products or hypersensitive to the loss of function of mutated tumour-suppressor genes. In other words, despite the multistage process of tumorigenesis, points of homeostasis are reached in which tumour cells are fully dependent on the abnormal activation or inactivation of a given cellular control pathway; this is achieved through a given gain- or loss-of-function mutation[61].

DNA-demethylating agents
5-aza-deoxycytidine and *5*-aza-cytidine are drugs that can induce DNA demethylation and are clinically effective as treatment for the pre-leukaemic syndrome myelodysplasia. They incorporate into DNA in place of cytidine, and can then bind and irreversibly inhibit DNA methyltransferases.

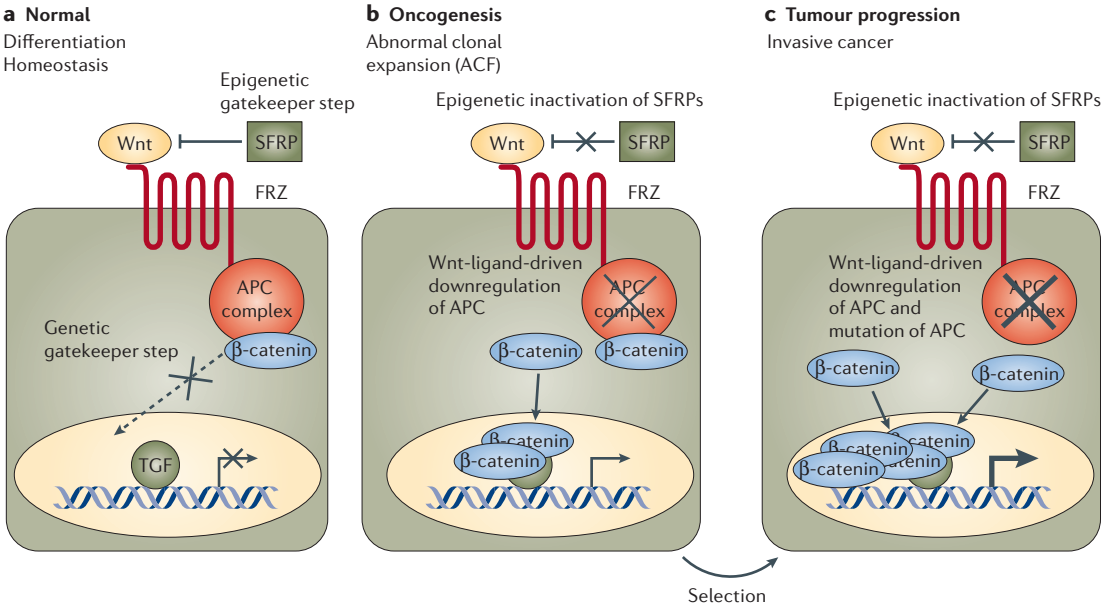

Figure 1 | **Addiction to the Wnt signalling pathway through gene-silencing events. a** | In normal colon epithelial cells, secreted frizzled-related proteins (SFRPs) function as antagonists of Wnt signalling by competing with Wnt proteins for binding to their receptor, Frizzled (FRZ). Expression of SFRPs is therefore the epigenetic gatekeeper step. When Wnt signalling is inactive, the adenomatosis polyposis coli (APC) complex phosphorylates β-catenin, leading to its degradation. This prevents the accumulation of nuclear β-catenin and therefore its ability to engage its transcription factor partners (TGF), which results in the differentiation and homeostasis of colon epithelial cells. Expression of APC is therefore a genetic gatekeeper step. **b** | When SFRP expression is lost, through epigenetic silencing of the gene that encodes it (loss of the epigenetic gatekeeper), Wnt signalling becomes activated through the receptor FRZ. This Wnt signalling potentially inactivates the APC complex (loss of the genetic gatekeeper), allowing β-catenin to accumulate in the cytoplasm and eventually in the nucleus. In the nucleus, β-catenin activates transcription of genes such as *MYC*, *cyclin D* and other genes whose products promote cell proliferation and survival rather than differentiation. This results in the expansion of colon epithelial stem and progenitor cells and formation of atypical crypt foci (ACF). **c** | Persistent activation of the Wnt pathway allows mutations to occur in other pathway components, such as those that permanently disable the APC complex and promote nuclear accumulation of β-catenin (loss of the genetic gatekeeper, as indicated by the bold cross). These cells are selected for because of their survival and proliferative advantages. This combination of epigentic and genetic events fully activates the Wnt pathway to promote tumour progression. Without the epigenetic events that silence the SFRP genes, mutations that disrupt the APC complex might not be sufficient to promote tumorigenesis or tumour progression.

So, could such pathway abnormalities also arise through epigenetic mechanisms alone? In order to undergo clonal expansion, might the pre-invasive or early-stage cancer cells become dependent on the epigenetically mediated dysfunction of a single pathway, which co-opts them from their normal differentiation and locks them into an abnormal proliferative state? This dependence could render the cells more likely to acquire subsequent mutations in the same pathway, which increases cellular reliance on the abnormalities and resultant tumour progression. In some cases, the tumour cells might actually continue to depend on both the epigenetic and genetic changes to become fully dependent on pathway abnormalities.

One example of this concept of 'epigenetic sensitization' to pathway activation has arisen from a microarray-based discovery approach for identifying hypermethylated genes that are important for colon cancer progression. This study revealed a new mechanism by which abnormalities in the Wnt pathway arise[34] (FIG. 1). Abnormal activation of the Wnt pathway results in the expansion of stem-cell and progenitor-cell populations. Mutations in *APC*, which encodes the tumour suppressor adenomatosis polyposis coli, activate the Wnt pathway and are often found in colorectal cancer cells. These mutations have been thought to be solely responsible for the initial progression of almost all cases of human colorectal cancer[62,63]. In cultured colon cancer cells, blocking activation of the Wnt pathway through restoration of APC function resulted in apoptosis. This observation was cited by Weinstein as a key example of tumour-cell addiction to a single oncogene-regulated pathway, and hypersensitivity to tumour-suppressor inactivation[61,64].

Our recent studies of early-stage, pre-invasive colon lesions that are at risk of progression to colon cancer, which are called aberrant crypt foci (ACF), indicate that epigenetic mechanisms might induce abnormal Wnt-pathway activation even before the appearance of mutations in the pathway (FIG. 1). These foci contain pre-adenomatous, pre-malignant hyperplastic cells that are derived from individual colon epithelial villi[65–72].

Atypical crypt foci
Pre-adenomatous, pre-malignant, hyperplastic cells that are derived from individual villus crypts. They are thought to constitute pre-malignant lesions with a risk of progression to colon cancer.

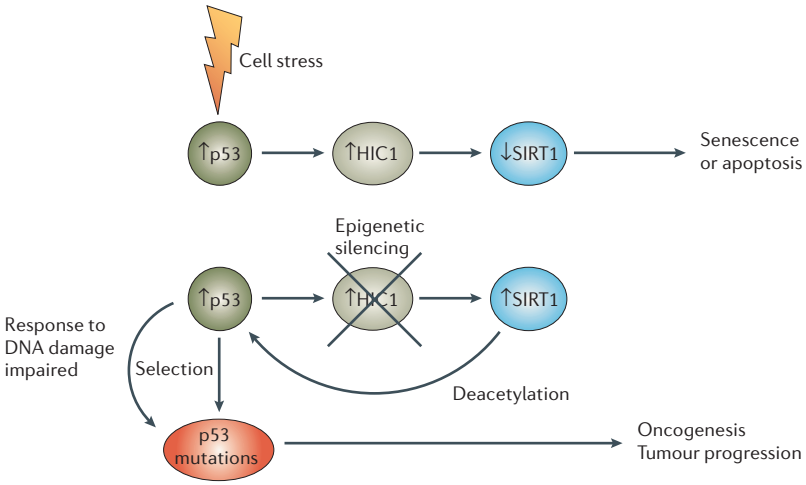

Figure 2 | **Epigenetic silencing of *HIC1* and control of DNA-damage responses.** When normal cells are faced with stressors such as DNA damage, p53 becomes activated, resulting in transcription of *HIC1* (hypermethylated in cancer 1). HIC1 represses transcription of the gene that encodes the stress-sensing protein SIRT1, allowing p53 to remain in its acetylated and active form. However, during the early stages of tumour progression, epigenetic inactivation of *HIC1* results in upregulation of SIRT1, which deacetylates p53, impairing its function and leading to a defective apoptotic response to DNA damage[83]. This allows cells to proliferate in the presence of damaged DNA and mutations to accumulate, including in p53 itself, that can disrupt cell-cycle control and promote tumour progression.

Most ACF cells do not contain mutations in genes that would abnormally activate the Wnt pathway, but they might acquire these genetic changes during tumour progression[67,68,72]. Studies of ACF cells have revealed abnormal methylation in promoter regions of members of the *SFRP* gene family. SFRP proteins have homology to the frizzled proteins, which are receptors for the Wnt secreted signalling proteins. SFRPs function at the cell membrane to antagonize Wnt-pathway activation[73–75]. Methylation of SFRP-gene promoter regions was not only present in all ACFs that were examined, but persisted for one or more of the genes in almost all primary colon cancer cells that were examined[34]. Furthermore, re-expression of SFRPs in colon cancer cells that have silenced these genes blocks Wnt signalling and results in apoptosis[34]. Importantly, these events occur in colon cancer cells that harbour the key inactivating or activating mutations in downstream factors of the Wnt pathway, and are also believed to contribute to the earliest stages of colon tumorigenesis. These Wnt-pathway genes have been called 'gatekeeper' factors. Inactivation of these factors is thought to be required for these cells to begin the process of colon cancer tumorigenesis[62,63].

So, loss of SFRP protein expression during the early stages of colon cancer development, because of epigenetic silencing, results in constitutive activation of the Wnt pathway[76]. This activation would contribute to abnormal expansion of colon epithelial stem- or progenitor-cells that rely on the Wnt pathway for proliferation, as opposed to differentiation[62,63]. These cells are then effectively addicted to the overactivity of the Wnt pathway, and can later acquire further mutations in other factors that lie downstream in this pathway, such as APC[62,63]. These additional mutations would further upregulate Wnt signalling and facilitate tumour progression (FIG. 1).

Is the loss of the epigenetic gatekeeper (the activation of SFRP expression in HCT116 cells) required for clonal expansion of cancer cells and tumour progression, as proposed in FIG. 1, or are mutations in the Wnt downstream signalling factors (such as β-catenin) sufficient to mediate this process? There are two pieces of evidence that the epigenetic change is required for clonal expansion. First, in HCT116 cells, deletions of genes that encode DNA methytransferases (DNMTs), which result in SFRP-gene promoter demethylation and re-expression, result in downregulation of Wnt signalling and induction of apoptosis, despite the fact that these cells express activated forms of β-catenin. This might be because loss of Wnt signalling decreases the amounts of β-catenin in the cell (FIG. 1), including the mutated form. Second, exogenous re-expression of SFRPs in these same cells, and in another cell line with a mutation in *APC*, similarly blocks Wnt signalling and results in induction of apoptosis. This highlights the importance of epigenetic events in driving neoplasia — reversing such events might have prevention or therapeutic potential.

Epigenetic silencing of another gene, *HIC1* (hypermethylated in cancer 1), might also be involved in pathway addiction and the early stages of tumour progression, on the basis of studies in mice and human tumour cells. *HIC1* was discovered in a random search for hypermethylated genes in a chromosome region, 17p13.3, that is frequently deleted in multiple types of human cancer[77]. The gene lies distal to, and is often deleted independently of, losses or mutations in the tumour suppressor p53. Importantly, *HIC1* is a target of active p53 (REFS 77,78) and is hypermethylated in cells from early, pre-invasive-stage breast[79] and colon[77] tumours. HIC1 is involved in a complex signalling network that actually impairs p53 tumour-suppressor activity. So, inactivation of this pathway might represent another example of oncogene addiction and tumour-suppressor hypersensitivity in cancer cells.

Homozygous disruption of *Hic1* in mice is lethal[80], but $Hic1^{+/-}$ mice survive and develop late-onset tumours, in which the wild-type allele is always retained and hypermethylated[81]. When $Hic1^{+/-}$ and $Trp53^{+/-}$ mice are crossed, there is a striking change in the timing, appearance, virulence and spectrum of tumours that arise in the $Hic1^{+/-}$ and $Trp53^{+/-}$ offspring, compared with mice that have heterozygous disruptions of either gene alone[82]. $Hic1^{+/-}$ $Trp53^{+/-}$ mice develop osteosarcomas, breast tumours and ovarian tumours — none of which develop frequently in mice of either single-mutant-background strain[82]. In the tumours that develop in $Hic1^{+/-}$ $Trp53^{+/-}$ mice, the wild-type allele of *Hic1* is almost always retained but is hypermethylated, whereas the wild-type allele of *Trp53* is almost always deleted[82]. Therefore, it is possible that loss of HIC1 in some way allows cells to acquire inactivating mutations in p53. This model is supported by the observation that crossing $Hic1^{+/-}$ mice with mice that have been engineered for disruptions in another tumour-suppressor gene,

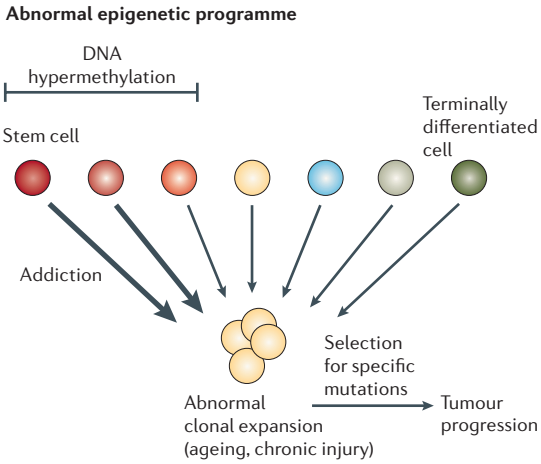

Figure 3 | **Epigenetic gene-silencing events and tumorigenesis.** Gene silencing can be a normal event in stem-cell and progenitor-cell development, as adult epithelial-cell renewal takes place. Silencing is mediated by chromatin modifications that repress transcription, but transcription can become activated as these cells mature. This homeostasis allows stem and progenitor cells to move properly along the differentiation pathway for a given epithelial cell system (moving from left to right across the figure). During chronic cell injury or inflammation, the pressure for adaptive cell renewal draws cells from the stem-cell and progenitor-cell pool for ongoing repair. This pressure might allow DNA hypermethylation to occur at promoters of genes that are normally silenced (cells under 'DNA hypermetylation' bar). This permanent silencing means that transcription cannot be easily reactivated as a cell clone expands (heavier arrows), so the stem and/or progenitor cells are channelled towards abnormal expansion at the expense of differentiation. Similar epigenetic processes might also occur in stroma, which would support abnormal epitheilial-cell expansion. This entire process leads to benign precursor tumours that are at risk of progression. This progression would be fostered by subsequent genetic or epigenetic events. The concept of 'epigenetic cellular addiction' means that the cells become dependent upon the pathways that are activated or disrupted to lead to the initial abnormal expansion. This dependency could predispose cells that have additional mutations in these pathways to be selected.

Cdkn2a, does not lead to an altered tumour spectrum in the resulting *Hic1*$^{+/-}$ *Cdkn2a*$^{+/-}$ mice[82].

Just as epigenetic events modulate the phenotypic effects of mutations that activate the Wnt pathway, the epigenetic inactivation of *HIC1* might determine the phenoptypic consequences of p53 inactivation (FIG. 2). How do we account for this interaction? One way in which HIC1 seems to function as a tumour suppressor involves its ability to directly repress transcription of the cell-stress-sensing protein SIRT1 (REF. 83) — a member of class III of the histone-deactylase family of sirtuins[84,85]. One of the deactylase targets of SIRT1 is p53 (REFS 85–87); this post-translational modification of p53 inhibits its transcriptional activity[85–87]. HIC1 and SIRT1 can also form a complex that localizes to the *SIRT1* promoter[83]. So, in the case of epigenetic inactivation of *HIC1*, increased SIRT1 levels would result in deacetylation of p53, and its response to DNA damage would be impaired[83]. In HIC1-deficient normal and neoplastic cells, this decreased p53 function would result in a reduced apoptotic response to DNA damage[83], leading to oncogenesis. In support of this model, restoration of HIC1 to deficient cells restores the apoptotic response of the cell in a p53-dependant manner[83]. So what might happen in the *Hic1*$^{+/-}$ *Trp53*$^{+/-}$ mice? During the early stages of tumour progression, loss of *Hic1* expressionthrough methylation — which appears to increase with age in mice[81–83] — results in partial loss of p53 function. Cells that acquire more powerful inactivating mutations in p53 are selected for and contribute to rapid tumour growth and progression.

Studies of the silencing of *SFRP* and *HIC1* have indicated a role for epigenetic alterations in tumorigenesis that encompasses two components. First, epigenetic silencing occurs during the early stages of tumour progression, possibly during the abnormal expansion of stem and progenitor cells. This silencing predisposes the stem cells to abnormal clonal exposition (FIG. 3). In this model, events that are known to contribute to tumour formation (for example, chronic inflammation with the concomitant generation of damaging products such as reactive oxygen species) incite vigorous cell renewal in an attempt to repair tissue. During such renewal, the occurrence of the epigenetic events can institute a heritable repression of transcription that inappropriately activates or inactivates cellular signals that foster stem-cell and/or progenitor-cell expansion. This expansion occurs at the expense of normal cell differentiation and maturation. The subsequent progression to malignancy, or the virulence of any tumours that ensue, would then depend not only on gene mutations but also on the collection of epigenetic alterations — or the 'epigenetic environment' — in which they occur. These epigenetic changes might occur continuously not only in epitheilial cells but also, as recently reported[88], in surrounding stromal cells. The importance of stromal cell abnormalities in driving tumour progression is now receiving much emphasis[89,90].

The concept that epigenetic abnormalities could be as important as genetic ones in determining the course of tumour development, and also involved in tumour-cell addiction to signalling-pathway abnormalities, is relevant to the future design of both preventive and therapeutic approaches to cancer. Whereas genetic changes can be reversed in experimental settings — a feature that is required to demonstrate the addiction determinants — this is obviously difficult to accomplish *in vivo*. However, the chromatin events that are involved in the epigenetic determinants of cancer, such as promoter methylation and histone modifications that are associated with defects in transcriptional repression, have real potential for drug targeting, as silenced genes can be induced to re-express[91,92]. Reagents that have such activities are already being tested, including DNA demethylating drugs and histone-deacetylase inhibitors[91]. The approval of the demethylating agent 5-aza-cytidine by the US Food and Drug Administration was based on studies that reported striking beneficial

Heritable repression
Epigenetic repression that is passed, through division, from cell to daughter cell.

effects of the treatment and prevention of malignant transformation in patients with the pre-leukaemic syndrome myelodysplasia[92].

Mechanisms

What are the molecular steps by which epigenetic alterations, such as DNA methylation, are initiated and maintained in pre-cancerous and cancer cells? Improving our understanding of the chromatin components of the silencing process and learning what triggers the process are important issues for cancer biology and could be essential to the reversal of gene silencing as a strategy for cancer prevention and therapy. There are several clues to the initiation of the process of abnormal promoter methylation, which results in the associated silencing of tumour-suppressor genes. These come primarily from the broad field of chromatin biology and, in particular, from studies that are beginning to combine concepts from this field with the specific issue of gene silencing in cancer. A complex series of events take place during embryonic development and during mature cell renewal, as stem and progenitor cells commit to maturation pathways that mark genes for active versus repressed transcription. The molecular basis of this marking involves activities such as chromatin remodelling, the nuclear positioning of genes and the histone modifications that distinguish active versus inactive gene states[93–99]. Chromatin-remodelling complexes, which involve proteins from the Trithorax and Polycomb families, maintain the long-term activation and repression, respectively, of gene expression states of particular loci that are necessary for the differentiation of stem and/or progenitor cells into various tissue types[96,100].

Studies of the Polycomb complexes (FIG. 4) in particular have provided important insights into the mechanisms by which genes might be abnormally silenced in cancer cells[96,99,100–102]. These protein complexes help maintain the long-term silencing of genes in *Drosophila* and other organisms that is required for the suppression of the embryonal and alternate-lineage genes that are necessary for normal development[96,99,100–102]. Polycomb constituents such as BMI1, along with complexes such as polycomb repressive complex 2 (PRC2), PRC3 and PRC4 (which contain the histone methyltransferase EZH2 and its partners), are active in stem and progenitor cells, as well as in tumour cells that have stem-cell properties[101,103–105]. However, the specific targets of these silencing complexes have been difficult to define in normal or neoplastic mammalian cells. Two important tumour-suppressor genes, *CDKN2A* and *CDKN2D*, both of which are frequently hypermethylated and silenced in cancer cells, are also known to be indirect or direct targets of BMI1 (REFS 96,102,106). However, the precise link between these interactions and the transcriptional silencing that is associated with promoter methylation is not known.

During tumour progression, levels of epigenetic promoter silencing seem to increase with respect to the individual chromatin determinants that are involved and the density of CpG-island methylation[9]. This could result in permanent gene-silencing in neoplastic cells and their progeny. This progressive silencing could be closely related to the mechanism by which transcriptionally repressive chromatin assembles at promoters, particularly with regard to the effects of methylation at key histone-amino-acid residues. Such modifications differ between genes in cancer cells that are abnormally silenced and those that are transcriptionally active. In particular, methylation of either histone H3 lysine 9 (methyl-K9-H3) or lysine 27 (methyl-K27-H3) represses transcription. Studies in *Neurospora* and *Arabidopsis*, and of mammalian X-chromosome inactivation have shown that the histone methyltransferases that catalyse these histone changes recruit DNMTs to gene promoters[107–114]. Therefore, it is possible that histone methylation occurs during the initial phases of gene silencing in cancer, and DNA methylation might then spread over the promoter with time[9]. Some experimental evidence in colon cancer cells supports this model[29].

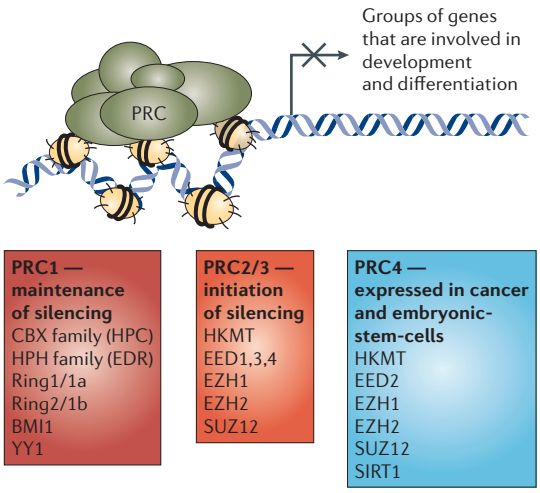

Figure 4 | **Overview of the polycomb repressive complexes (PRCs).** PRCs help organize nucleosomes (yellow ovals) and surrounding DNA (black strands) into transcriptionally repressive chromatin that mediates long-term transcriptional repression of downstream genes. There are three defined PRCs, and several protein components of these are overexpressed in cancer cells. PRC1 maintains gene silencing in human cells, and is comprised of chromobox-homologue (CBX) family, human homologue of polyhomeotic (HPH) family and ring-finger family proteins, as well as BMI1 and the transcription factor YY1. PRC2 and PRC3 initiate gene silencing, and are comprised of histone lysine methyltransferase (HKMT) proteins of the embryonic-ectoderm-development (EED) family, members of the EZH family of histone methyltransferases, and suppressor of zeste 12 (SUZ12). PRC4 exists in embryonic, stem, progenitor and cancer cells and is comprised of HKMT, EED2, EZH1 and EZH2, SUZ12, and the stress-sensing protein SIRT1. EZH–EED catalyses methylation of lysine 27 of histone H4. Normally, without DNA methylation this silencing would promote normal stem-cell function and allow gene transcription to be activated for normal cell maturation. In cancer cells, increased DNA methylation is postulated to permanently silence genes and promote cell proliferation.

An attractive model for this proposed sequential appearance of gene silencing events in cancer appears in work from F. Rauscher and colleagues[115] (for a review, see REF. 116). This study involves transient induction of silencing in human cells by binding of a specific transcriptional-repressor complex to a reporter gene. The binding induces a localized zone of histone-transcriptional-repression marks, such as methyl-K9-H3 in the promoter region. When the transcriptional repressor is removed and single-cell-derived clones are isolated, most clones revert to active transcription of the targeted gene and lose the transcriptional-repression marks. However, some clones retain the promoter-repression marks, fail to reactivate transcription of the reporter gene and, over time, stably repress transcription through promoter DNA methylation. This process might fit a model of the evolution of abnormal heritable transcriptional gene-silencing that occurs during the course of tumour progression.

We know more about the maintenance than the initiation of tumour-suppressor gene methylation (for reviews, see REFS 8,9). Multiple studies have shown that the promoters of silenced genes, when compared with actively transcribed copies of the same genes, contain localized regions of transcriptional-silencing marks that include (in addition to CpG-island methylation) the deactylation and methylation of key H3 amino acids. Examples are the deacetylation of K9 and K14, and the methylation of K9. In turn, a key activating modification, methylation of K4, is decreased[29,117–121]. In these repressive chromatin events, DNA methylation seems to be the dominant factor in gene silencing. Pharmacological induction of DNA demethylation can re-establish gene transcription and cause the appearance of transcriptional-activation marks (acetylation at K9 and K14 and methylation K4 of H3) and the loss of the silencing mark (such as methylation at amino acid K9)[117,119]. These observations are consistent with earlier studies that indicated that treatment of cancer cells with inhibitors of histone deactylases fails to transcriptionally activate tumour-suppressor genes with dense promoter-methylation until DNA-demethylating agents have first been applied[122].

Our expanding knowledge of the molecular events that mediate gene silencing in cancer cells provides a base for understanding the hierarchial chromatin events that mediate the transcriptional repression. However, much more investigation is required to completely understand all of the components. In turn, such understanding could have profound implications for attempts to reverse epigenetic gene silencing for cancer prevention and treatment.

Future directions

Epigenetic as well as genetic alterations are essential for not only the maintenance but also the initiation of many human tumour types. A complex series of chromatin-modification events arise when cells are placed under stressful conditions, such as during chronic injury and inflammation, that might 'lock in' abnormal heritable transcriptional repression of key genes, or even networks of genes, through epigenetic mechanisms. These mechanisms could addict cells to dependence on inappropriate activation or disruption of crucial cell-control pathways. This silencing can be viewed as an 'epigenetic gatekeeper' step that creates a milieu that facilitates the selection of mutations in oncogenes and tumour-suppressor genes, thereby fostering tumour progression.

Understanding the molecular events that initiate and maintain epigenetic gene silencing could lead to the development of clinical strategies for cancer prevention and therapy that reverse the silencing process. The chromatin changes that are involved, such as abnormal promoter methylation, might also be assayed in DNA from various sources as a promising molecular-marker strategy to aid cancer risk assessment, early detection and prognosis[8,9,123].

1. Hahn, W. C. *et al*. Creation of human tumour cells with defined genetic elements. *Nature* **400**, 464–468 (1999).
2. Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. *Cell* **100**, 57–70 (2000).
3. Aaltonen, L. A. *et al*. Clues to the pathogenesis of familial colorectal cancer. *Science* **260**, 812–816 (1993).
4. Kinzler, K. W. & Vogelstein, B. Lessons from hereditary colorectal cancer. *Cell* **87**, 159–170 (1996).
5. Kinzler, K. W. & Vogelstein, B. Cancer-susceptibility genes. Gatekeepers and caretakers. *Nature* **386**, 761–763 (1997).
6. Jones, P. A. & Laird, P. W. Cancer epigenetics comes of age. *Nature Genet.* **21**, 163–167 (1999).
7. Feinberg, A. P. & Tycko, B. The history of cancer epigenetics. *Nature Rev. Cancer* **4**, 143–153 (2004).
8. Herman, J. G. & Baylin, S. B. Gene silencing in cancer in association with promoter hypermethylation. *N. Engl. J. Med.* **349**, 2042–2054 (2003).
9. Jones, P. A. & Baylin, S. B. The fundamental role of epigenetic events in cancer. *Nature Rev. Genet.* **3**, 415–428 (2002).
Highlights how patterns of DNA methylation and chromatin structure are profoundly altered in neoplasia.
10. Seligson, D. B. *et al*. Global histone modification patterns predict risk of prostate cancer recurrence. *Nature* **435**, 1262–1266 (2005).
11. Fraga, M. F. *et al*. Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. *Nature Genet.* **37**, 391–400 (2005).
12. Lagger, G. *et al*. Essential function of histone deacetylase 1 in proliferation control and CDK inhibitor repression. *EMBO J.* **21**, 2672–2681 (2002).
13. Lachner, M., O'Sullivan, R. J. & Jenuwein, T. An epigenetic road map for histone lysine methylation. *J. Cell Sci.* **116**, 2117–2124 (2003).
Reviews the known complexity of histone modifications including the methylation, acetylation and phosphorylation of histone residues.
14. Strahl, B. D. & Allis, C. D. The language of covalent histone modifications. *Nature* **403**, 41–45 (2000).
15. Fischle, W., Wang, Y. & Allis, C. D. Histone and chromatin cross-talk. *Curr. Opin. Cell Biol.* **15**, 172–183 (2003).
16. Hake, S. B., Xiao, A. & Allis, C. D. Linking the epigenetic 'language' of covalent histone modifications to cancer. *Br. J. Cancer* **90**, 761–769 (2004).
17. Kuzmichev, A. & Reinberg, D. Role of histone deacetylase complexes in the regulation of chromatin metabolism. *Curr. Top. Microbiol. Immunol.* **254**, 35–58 (2001).
18. Ushijima, T. Detection and interpretation of altered methylation patterns in cancer cells. *Nature Rev. Cancer* **5**, 223–231 (2005).
19. Akama, T. O. *et al*. Restriction landmark genomic scanning (RLGS-M)-based genome-wide scanning of mouse liver tumors for alterations in DNA methylation status. *Cancer Res.* **57**, 3294–3299 (1997).
20. Costello, J. F. *et al*. Aberrant CpG-island methylation has non-random and tumour-type-specific patterns. *Nature Genet.* **24**, 132–138 (2000).
21. Yu, L. *et al*. Global assessment of promoter methylation in a mouse model of cancer identifies *ID4* as a putative tumor-suppressor gene in human leukemia. *Nature Genet.* **37**, 265–274 (2005).
22. Huang, T. H., Perry, M. R. & Laux, D. E. Methylation profiling of CpG islands in human breast cancer cells. *Hum. Mol. Genet.* **8**, 459–470 (1999).
23. Shi, H. *et al*. Triple analysis of the cancer epigenome: an integrated microarray system for assessing gene expression, DNA methylation, and histone acetylation. *Cancer Res.* **63**, 2164–2171 (2003).
24. Salem, C. E. *et al*. *PAX6* methylation and ectopic expression in human tumor cells. *Int. J. Cancer* **87**, 179–185 (2000).
25. Suzuki, H. *et al*. A genomic screen for genes upregulated by demethylation and histone deacetylase inhibition in human colorectal cancer. *Nature Genet.* **31**, 141–149 (2002).

26. Yamashita, K. *et al*. Pharmacologic unmasking of epigenetically silenced tumor suppressor genes in esophageal squamous cell carcinoma. *Cancer Cell* **2**, 485–495 (2002).
27. Toyota, M. *et al*. Identification of differentially methylated sequences in colorectal cancer by methylated CpG island amplification. *Cancer Res.* **59**, 2307–2312 (1999).
28. Herman, J. G. *et al*. Incidence and functional consequences of *hMLH1* promoter hypermethylation in colorectal carcinoma. *Proc. Natl Acad. Sci. USA* **95**, 6870–6875 (1998).
29. Bachman, K. E. *et al*. Histone modifications and silencing prior to DNA methylation of a tumor suppressor gene. *Cancer Cell* **3**, 89–95 (2003).
30. Myohanen, S. K., Baylin, S. B. & Herman, J. G. Hypermethylation can selectively silence individual *p16^{ink4A}* alleles in neoplasia. *Cancer Res.* **58**, 591–593 (1998).
31. Parsons, R. *et al*. Microsatellite instability and mutations of the transforming growth factor β type II receptor gene in colorectal cancer. *Cancer Res.* **55**, 5548–5550 (1995).
32. Morin, P. J. *et al*. Activation of β-catenin–Tcf signaling in colon cancer by mutations in β-catenin or APC. *Science* **275**, 1787–1790 (1997).
33. Akiyama, Y. *et al*. *GATA-4* and *GATA-5* transcription factor genes and potential downstream antitumor target genes are epigenetically silenced in colorectal and gastric cancer. *Mol. Cell. Biol.* **23**, 8429–8439 (2003).
34. Suzuki, H. *et al*. Epigenetic inactivation of *SFRP* genes allows constitutive WNT signaling in colorectal cancer. *Nature Genet.* **36**, 417–422 (2004).
Demonstrates that epigenetic loss of SFRP function occurs early in colorectal cancer progression, and that restoration of SFRP function in colorectal cancer cells attenuates Wnt signalling, even in the presence of downstream mutations.
35. Rhee, I. *et al*. DNMT1 and DNMT3b cooperate to silence genes in human cancer cells. *Nature* **416**, 552–556 (2002).
36. Bachman, K. E. *et al*. Methylation-associated silencing of the tissue inhibitor of metalloproteinase-3 gene suggest a suppressor role in kidney, brain, and other human cancers. *Cancer Res.* **59**, 798–802 (1999).
37. Gao, X., Sedgwick, T., Shi, Y. B. & Evans, T. Distinct functions are implicated for the GATA-4, -5, and-6 transcription factors in the regulation of intestine epithelial cell differentiation. *Mol. Cell. Biol.* **18**, 2901–2911 (1998).
38. Al-azzeh, E. *et al*. Gastroprotective peptide trefoil factor family 2 gene is activated by upstream stimulating factor but not by c-Myc in gastrointestinal cancer cells. *Gut* **51**, 685–690 (2002).
39. Bossenmeyer-Pourie, C. *et al*. The trefoil factor 1 participates in gastrointestinal cell differentiation by delaying G1–S phase transition and reducing apoptosis. *J. Cell Biol.* **157**, 761–770 (2002).
40. Wright, N. A., Hoffmann, W., Otto, W. R., Rio, M. C. & Thim, L. Rolling in the clover: trefoil factor family (TFF)-domain peptides, cell migration and cancer. *FEBS Lett.* **408**, 121–123 (1997).
41. Issa, J. P. CpG island methylator phenotype in cancer. *Nature Rev. Cancer* **4**, 988–993 (2004).
Reviews the concept of a DNA hyper-methylated phenotype.
42. Yamada, Y. *et al*. Opposing effects of DNA hypomethylation on intestinal and liver carcinogenesis. *Proc. Natl Acad. Sci. USA* **102**, 13580–13585 (2005).
43. Cui, H. *et al*. Loss of *IGF2* imprinting: a potential marker of colorectal cancer risk. *Science* **299**, 1753–1755 (2003).
44. Sakatani, T. *et al*. Loss of imprinting of *Igf2* alters intestinal maturation and tumorigenesis in mice. *Science* **307**, 1976–1978 (2005).
45. Holm, T. M. *et al*. Global loss of imprinting leads to widespread tumorigenesis in adult mice. *Cancer Cell* **8**, 275–285 (2005).
46. Belinsky, S. A. *et al*. Aberrant methylation of *p16^{INK4a}* is an early event in lung cancer and a potential biomarker for early diagnosis. *Proc. Natl Acad. Sci. USA* **95**, 11891–11896 (1998).
47. Nuovo, G. J., Plaia, T. W., Belinsky, S. A., Baylin, S. B. & Herman, J. G. *In situ* detection of the hypermethylation-induced inactivation of the *p16* gene as an early event in oncogenesis. *Proc. Natl Acad. Sci. USA* **96**, 12754–12759 (1999).
48. Swafford, D. S. *et al*. Frequent aberrant methylation of *p16^{INK4a}* in primary rat lung tumors. *Mol. Cell. Biol.* **17**, 1366–1374 (1997).
49. Lee, W. H. *et al*. Cytidine methylation of regulatory sequences near the π-class glutathione S-transferase gene accompanies human prostatic carcinogenesis. *Proc. Natl Acad. Sci. USA* **91**, 11733–11737 (1994).
50. Holst, C. R. *et al*. Methylation of *p16^{INK4a}* promoters occurs *in vivo* in histologically normal human mammary epithelia. *Cancer Res.* **63**, 1596–1601 (2003).
51. Romanov, S. R. *et al*. Normal human mammary epithelial cells spontaneously escape senescence and acquire genomic changes. *Nature* **409**, 633–637 (2001).
52. Kiyono, T. *et al*. Both Rb/p16^{INK4a} inactivation and telomerase activity are required to immortalize human epithelial cells. *Nature* **396**, 84–88 (1998).
53. Esteller, M., Hamilton, S. R., Burger, P. C., Baylin, S. B. & Herman, J. G. Inactivation of the DNA repair gene *O^6*-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. *Cancer Res.* **59**, 793–797 (1999).
54. Esteller, M. *et al*. Analysis of adenomatous polyposis coli promoter hypermethylation in human cancer. *Cancer Res.* **60**, 4366–4371 (2000).
55. Nelson, W. G., De Marzo, A. M. & Isaacs, W. B. Prostate cancer. *N. Engl. J. Med.* **349**, 366–381 (2003).
56. Wong, D. J., Foster, S. A., Galloway, D. A. & Reid, B. J. Progressive region-specific *de novo* methylation of the *p16* CpG island in primary human mammary epithelial cell strains during escape from M(0) growth arrest. *Mol. Cell. Biol.* **19**, 5642–5651 (1999).
57. Bronner, C. E. *et al*. Mutation in the DNA mismatch repair gene homologue *hMLH1* is associated with hereditary non-polyposis colon cancer. *Nature* **368**, 258–261 (1994).
58. Kane, M. F. *et al*. Methylation of the *hMLH1* promoter correlates with lack of expression of *hMLH1* in sporadic colon tumors and mismatch repair-defective human tumor cell lines. *Cancer Res.* **57**, 808–811 (1997).
59. Ahrendt, S. A. *et al*. Molecular detection of tumor cells in bronchoalveolar lavage fluid from patients with early stage lung cancer. *J. Natl Cancer Inst.* **91**, 332–339 (1999).
60. Esteller, M. *et al*. Promoter hypermethylation of the DNA repair gene O_6-methylguanine-DNA methyltransferase is associated with the presence of G:C to A:T transition mutations in *p53* in human colorectal tumorigenesis. *Cancer Res.* **61**, 4689–4692 (2001).
61. Weinstein, I. B. Cancer. Addiction to oncogenes — the Achilles heal of cancer. *Science* **297**, 63–64 (2002).
62. Gregorieff, A. & Clevers, H. Wnt signaling in the intestinal epithelium: from endoderm to cancer. *Genes Dev.* **19**, 877–890 (2005).
63. Kinzler, K. W. & Vogelstein, B. Cancer-susceptibility genes. Gatekeepers and caretakers. *Nature* **386**, 761–763 (1997).
64. Weinstein, I. B. Disorders in cell circuitry during multistage carcinogenesis: the role of homeostasis. *Carcinogenesis* **21**, 857–864 (2000).
65. Jen, J. *et al*. Molecular determinants of dysplasia in colorectal lesions. *Cancer Res.* **54**, 5523–5526 (1994).
66. DeAngelo, A. B., Geter, D. R., Rosenberg, D. W., Crary, C. K. & George, M. H. The induction of aberrant crypt foci (ACF) in the colons of rats by trihalomethanes administered in the drinking water. *Cancer Lett.* **187**, 25–31 (2002).
67. Hao, X. P., Pretlow, T. G., Rao, J. S. & Pretlow, T. P. β-Catenin expression is altered in human colonic aberrant crypt foci. *Cancer Res.* **61**, 8085–8088 (2001).
68. Mori, H., Yamada, Y., Kuno, T. & Hirose, Y. Aberrant crypt foci and β-catenin accumulated crypts; significance and roles for colorectal carcinogenesis. *Mutat. Res.* **566**, 191–208 (2004).
69. Papanikolaou, A., Wang, Q. S., Delker, D. A. & Rosenberg, D. W. Azoxymethane-induced colon tumors and aberrant crypt foci in mice of different genetic susceptibility. *Cancer Lett.* **130**, 29–34 (1998).
70. Papanikolaou, A., Wang, Q. S., Papanikolaou, D., Whiteley, H. E. & Rosenberg, D. W. Sequential and morphological analyses of aberrant crypt foci formation in mice of differing susceptibility to azoxymethane-induced colon carcinogenesis. *Carcinogenesis* **21**, 1567–1572 (2000).
71. Siu, I. M. *et al*. The identification of monoclonality in human aberrant crypt foci. *Cancer Res.* **59**, 63–66 (1999).
72. Yuan, P., Sun, M. H., Zhang, J. S., Zhu, X. Z. & Shi, D. R. *APC* and *K-ras* gene mutation in aberrant crypt foci of human colon. *World J. Gastroenterol.* **7**, 352–356 (2001).
73. Finch, P. W. *et al*. Purification and molecular cloning of a secreted, Frizzled-related antagonist of Wnt action. *Proc. Natl Acad. Sci. USA* **94**, 6770–6775 (1997).
74. Melkonyan, H. S. *et al*. SARPs: a family of secreted apoptosis-related proteins. *Proc. Natl Acad. Sci. USA* **94**, 13636–13641 (1997).
75. Rattner, A. *et al*. A family of secreted proteins contains homology to the cysteine-rich ligand-binding domain of frizzled receptors. *Proc. Natl Acad. Sci. USA* **94**, 2859–2863 (1997).
76. Taketo, M. M. Shutting down Wnt signal-activated cancer. *Nature Genet.* **36**, 320–322 (2004).
77. Wales, M. M. *et al*. p53 activates expression of *HIC-1*, a new candidate tumour suppressor gene on 17p13.3. *Nature Med.* **1**, 570–577 (1995).
78. Guerardel, C. *et al*. Identification in the human candidate tumor suppressor gene *HIC-1* of a new major alternative TATA-less promoter positively regulated by p53. *J. Biol. Chem.* **276**, 3078–3089 (2001).
79. Fujii, H. *et al*. Methylation of the *HIC-1* candidate tumor suppressor gene in human breast cancer. *Oncogene* **16**, 2159–2164 (1998).
80. Carter, M. G. *et al*. Mice deficient in the candidate tumor suppressor gene *Hic1* exhibit developmental defects of structures affected in the Miller–Dieker syndrome. *Hum. Mol. Genet.* **9**, 413–419 (2000).
81. Chen, W. Y. *et al*. Heterozygous disruption of *Hic1* predisposes mice to a gender-dependent spectrum of malignant tumors. *Nature Genet.* **33**, 197–202 (2003).
82. Chen, W. *et al*. Epigenetic and genetic loss of *Hic1* function accentuates the role of *p53* in tumorigenesis. *Cancer Cell* **6**, 387–398 (2004).
83. Chen W. *et al*. Tumor suppressor HIC1 directly regulates SIRT1 and modulates p53-dependent apoptotic DNA damage responses. *Cell* **123**, 437–448 (2005).
Reports that the silencing of HIC1 in tumorigenesis might activate a network of events that lead to partial downregulation of p53 function and then to mutations in p53.
84. Frye, R. A. Characterization of five human cDNAs with homology to the yeast *SIR2* gene: Sir2-like proteins (sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity. *Biochem. Biophys. Res. Commun.* **260**, 273–279 (1999).
85. Vaziri, H. *et al*. *hSIR2^{SIRT1}* functions as an NAD-dependent p53 deacetylase. *Cell* **107**, 149–159 (2001).
86. Langley, E. *et al*. Human SIR2 deacetylates p53 and antagonizes PML/p53-induced cellular senescence. *EMBO J.* **21**, 2383–2396 (2002).
87. Luo, J. *et al*. Negative control of p53 by Sir2α promotes cell survival under stress. *Cell* **107**, 137–148 (2001).
88. Hu, M. *et al*. Distinct epigenetic changes in the stromal cells of breast cancers. *Nature Genet.* **37**, 899–905 (2005).
89. Orimo, A. *et al*. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell* **121**, 335–348 (2005).
90. Tlsty, T. D. Stromal cells can contribute oncogenic signals. *Semin. Cancer Biol.* **11**, 97–104 (2001).
91. Egger, G., Liang, G., Aparicio, A. & Jones, P. A. Epigenetics in human disease and prospects for epigenetic therapy. *Nature* **429**, 457–463 (2004).
92. Issa, J. P., Kantarjian, H. M. & Kirkpatrick, P. Azacitidine. *Nature Rev. Drug Discov.* **4**, 275–276 (2005).
93. Peters, A. H. *et al*. Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. *Mol. Cell* **12**, 1577–1589 (2003).
94. Jenuwein, T. & Allis, C. D. Translating the histone code. *Science* **293**, 1074–1080 (2001).
95. Kouzarides, T. Histone methylation in transcriptional control. *Curr. Opin. Genet. Dev.* **12**, 198–209 (2002).
96. Lund, A. H. & van Lohuizen, M. Polycomb complexes and silencing mechanisms. *Curr. Opin. Cell Biol.* **16**, 239–246 (2004).
97. Gasser, S. M. Positions of potential: nuclear organization and gene expression. *Cell* **104**, 639–642 (2001).
98. Levine, S. S., King, I. F. & Kingston, R. E. Division of labor in polycomb group repression. *Trends Biochem. Sci.* **29**, 478–485 (2004).
99. Ringrose, L. & Paro, R. Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. *Annu. Rev. Genet.* **38**, 413–443 (2004).

100. Valk-Lingbeek, M. E., Bruggeman, S. W. & van Lohuizen, M. Stem cells and cancer; the polycomb connection. *Cell* **118**, 409–418 (2004).
101. Kuzmichev, A. *et al*. Composition and histone substrates of polycomb repressive group complexes change during cellular differentiation. *Proc. Natl Acad. Sci. USA* **102**, 1859–1864 (2005).
102. Lund, A. H. & van Lohuizen, M. Epigenetics and cancer. *Genes Dev.* **18**, 2315–2335 (2004).
103. Varambally, S. *et al*. The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature* **419**, 624–629 (2002).
104. Kleer, C. G. *et al*. EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. *Proc. Natl Acad. Sci. USA* **100**, 11606–11611 (2003).
105. Kirmizis, A., Bartley, S. M. & Farnham, P. J. Identification of the polycomb group protein SU(Z)12 as a potential molecular target for human cancer therapy. *Mol. Cancer Ther.* **2**, 113–121 (2003).
106. Lessard, J. *et al*. Functional antagonism of the Polycomb-group genes *eed* and *Bmi1* in hemopoietic cell proliferation. *Genes Dev.* **13**, 2691–2703 (1999).
107. Selker, E. U., Jensen, B. C. & Richardson, G. A. A portable signal causing faithful DNA methylation *de novo* in *Neurospora crassa*. *Science* **238**, 48–53 (1987).
108. Tamaru, H. & Selker, E. U. A histone H3 methyltransferase controls DNA methylation in *Neurospora crassa*. *Nature* **414**, 277–283 (2001).
109. Tamaru, H. & Selker, E. U. Synthesis of signals for *de novo* DNA methylation in *Neurospora crassa*. *Mol. Cell. Biol.* **23**, 2379–2394 (2003).
110. Bell, M. V. *et al*. Physical mapping across the fragile X: hypermethylation and clinical expression of the fragile X syndrome. *Cell* **64**, 861–866 (1991).
111. Jackson, J. P., Lindroth, A. M., Cao, X. & Jacobsen, S. E. Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. *Nature* **416**, 556–560 (2002).
112. Johnson, L., Cao, X. & Jacobsen, S. Interplay between two epigenetic marks. DNA methylation and histone H3 lysine 9 methylation. *Curr. Biol.* **12**, 1360–1367 (2002).
113. Cao, R. & Zhang, Y. The functions of E(Z)/EZH2-mediated methylation of lysine 27 in histone H3. *Curr. Opin. Genet. Dev.* **14**, 155–164 (2004).
114. Plath, K. *et al*. Role of histone H3 lysine 27 methylation in X inactivation. *Science* **300**, 131–135 (2003).
115. Ayyanathan, K. *et al*. Regulated recruitment of HP1 to a euchromatic gene induces mitotically heritable, epigenetic gene silencing: a mammalian cell culture model of gene variegation. *Genes Dev.* **17**, 1855–1869 (2003).
A model of the evolution of the abnormal heritable transcriptional gene silencing that occurs during tumour progression.
116. Fahrner, J. A. & Baylin, S. B. Heterochromatin: stable and unstable invasions at home and abroad. *Genes Dev.* **17**, 1805–1812 (2003).
117. Fahrner, J. A., Eguchi, S., Herman, J. G. & Baylin, S. B. Dependence of histone modifications and gene expression on DNA hypermethylation in cancer. *Cancer Res.* **62**, 7213–7218 (2002).
118. Kondo, Y., Shen, L. & Issa, J. P. Critical role of histone methylation in tumor suppressor gene silencing in colorectal cancer. *Mol. Cell. Biol.* **23**, 206–215 (2003).
119. Nguyen, C. T. *et al*. Histone H3-lysine 9 methylation is associated with aberrant gene silencing in cancer cells and is rapidly reversed by 5-aza-2′-deoxycytidine. *Cancer Res.* **62**, 6456–6461 (2002).
120. Ghoshal, K. *et al*. Inhibitors of histone deacetylase and DNA methyltransferase synergistically activate the methylated metallothionein I promoter by activating the transcription factor MTF-1 and forming an open chromatin structure. *Mol. Cell. Biol.* **22**, 8302–8319 (2002).
121. Koizume, S., Tachibana, K., Sekiya, T., Hirohashi, S. & Shiraishi, M. Heterogeneity in the modification and involvement of chromatin components of the CpG island of the silenced human *CDH1* gene in cancer cells. *Nucleic Acids Res.* **30**, 4770–4780 (2002).
122. Cameron, E. E., Bachman, K. E., Myohanen, S., Herman, J. G. & Baylin, S. B. Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. *Nature Genet.* **21**, 103–107 (1999).
123. Laird, P. W. The power and the promise of DNA methylation markers. *Nature Rev. Cancer* **3**, 253–266 (2003).

Competing interests statement

The authors declare competing financial interests; see web version for details.

Acknowledgements

The authors thank all of their colleagues whose work has contributed to the concepts in this review. Portions of the work cited has been supported by grants to S.B.B. from the National Cancer Institute and the National National Institute of Environmental Health Sciences.

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The following terms in this article are linked online to:
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
APC | *CDKN2D* | *HIC1* | *MGMT* | *p53* | *SFRP1* | *SFRP2* | *SFRP4* | *SFRP5* | *TIMP3*
National Cancer Institute: http://www.cancer.gov
breast cancer | colon cancer | lung cancer | myelodysplasia | osteosarcoma | ovarian cancer | prostate cancer | Wilms tumour
UniProtKB: http://www.expasy.org/uniprot
β-catenin | cyclin D | EZH2 | GATA4 | GATA5 | GSTP1 | inhibin-α | MLH1 | p16 | RB1 | SIRT1 | TFF1 | TFF2 | TFF3 | TGFβ2R

FURTHER INFORMATION

Chromatin structure and function site: http://www.chromatin.us/chrom.html
Gene silencing: http://www.esi-topics.com/genesil/interviews/StephenBaylin.html
***Science* epigenetics site:** http://www.sciencemag.org/feature/plus/sfg/resources/res_epigenetics.shtml
The Wellcome Trust: http://www.wellcome.ac.uk/en/genome/thegenome/hg02b002.html
Access to this interactive links box is free online.