Human Molecular Genetics, 2007, Vol. 16, Review Issue 1 ***R50–R59***
doi:10.1093/hmg/ddm018

Epigenetic gene silencing in cancer: the DNA hypermethylome

Manel Esteller*

Cancer Epigenetics Laboratory, Spanish National Cancer Centre (CNIO), Melchor Fernandez Almagro 3, 28029 Madrid, Spain

Received January 9, 2007; Revised and Accepted January 19, 2007

Epigenetic gene inactivation in transformed cells involves many 'belts of silencing'. One of the best-known lesions of the malignant cell is the transcriptional repression of tumor-suppressor genes by promoter CpG island hypermethylation. We are in the process of completing the molecular dissection of the entire epigenetic machinery involved in methylation-associated silencing, such as DNA methyltransferases, methyl-CpG binding domain proteins, histone deacetylases, histone methyltransferases, histone demethylases and Polycomb proteins. The first indications are also starting to emerge about how the combination of cellular selection and targeted pathways leads to abnormal DNA methylation. One thing is certain already, promoter CpG island hypermethylation of tumor-suppressor genes is a common hallmark of all human cancers. It affects all cellular pathways with a tumor-type specific profile, and in addition to classical tumor-suppressor and DNA repair genes, it includes genes involved in premature aging and microRNAs with growth inhibitory functions. The importance of hypermethylation events is already in evidence at the bedside of cancer patients in the form of cancer detection markers and chemotherapy predictors, and in the approval of epigenetic drugs for the treatment of hematological malignancies. In the very near future, the synergy of candidate gene approaches and large-scale epigenomic technologies, such as methyl-DIP, will yield the complete DNA hypermethylome of cancer cells.

INTRODUCTION TO EPIGENETIC GENE SILENCING

From the initial observation of the presence of DNA methylation differences in the vicinity of beta-globin genes (1,2), and the characterization of the first tumor-suppressor genes undergoing CpG island-methylation-associated silencing (3–7) to the present-day human epigenome projects (8,9), the clinical approval of epigenetic therapies (10), and the hypermethylation-associated down regulation of microRNAs (miRNAs) (11,12), epigenetic gene silencing has been the protagonist in the biomedical arena, and its representation in the scientific literature continues to increase (http://www.esi-topics.com/genesil2006). The scenario is further enriched by the discovery that transcriptional repression mediated by DNA methylation occurs in the chromatin-'receptive' context of histone modification and chromatin-remodeling factors (13,14), and that these histone methylation and acetylation markers are also disrupted in human cancer (15,16), leading to further aberrations in gene silencing. We should also consider the spectrum of interindividual differences in CpG island DNA methylation patterns (17,18). The aspects of epigenetic gene silencing are therefore myriad, but in this review I shall focus on promoter CpG island hypermethylation, clarifying some of the unsolved issues and emphasizing the latest relevant 'hot' research in the area.

Let us start at the beginning by returning to the essentials of CpG islands. The frequency of the CpG dinucleotide in the human genome is lower than expected (19). The proposed reason for this lack of CpG in our genome is spontaneous deamination in the germline during evolution. However, approximately half of the human gene-promoter regions contain CpG-rich regions with lengths of 0.5 to several Kb, known as 'CpG islands' (19). Although the majority of these are associated with 'house-keeping' genes, half of the 'tissue-specific' genes also contain a promoter CpG island (19). The questions of which and how DNA methylation changes in tissue-specific genes occur in cancer remain largely unanswered. The Maspin is still the main representative gene in this class (20), but larger epigenomic studies have begun to

*To whom correspondence should be addressed at: Tel: +34 912246940; Fax: +34 912246923; Email: mesteller@cnio.es.

address this issue (18,21). Due to the complexity of the problem and the small amount of information available, I shall not discuss this in the current review.

It should also be noted that although the most significant proportion of CpG islands is located in the 5′-unstranslated region and the first exon of the genes, certain CpG islands can occasionally be found within the body of the gene, or even in the 3′-region. CpG islands in these atypical locations are more prone to methylation (22), and the RNA transcript can cross over them without any evident impediment (23). Exceptionally, certain small genes can be considered in their totality as a whole CpG island. Typical CpG islands are entirely unmethylated at all stages of development and allow the expression of a particular gene if the appropriate transcription factors are present and the chromatin structure is accessible to them. In the transformed cell, certain CpG islands of tumor-suppressor genes will become hypermethylated, as I will discuss below.

MOLECULAR MACHINERY FOR EPIGENETIC GENE SILENCING

The enzymes directly responsible for CpG island hypermethylation of tumor-suppressor genes are the DNA methyltransferases (DNMTs). DNMT1 exhibits a 5- to 30-fold preference for hemimethylated substrates. This property led to the identification of DNMT1 as the enzyme responsible for maintaining the methylation patterns following DNA replication. In fact, the somatic genetic knockout of DNMT1 in human cells shows an aberrant nuclear structure and disorganizes the distribution of the heterochromatin protein 1 (24), demonstrating that the two processes are closely linked. DNMT3a and DNMT3b were soon after identified from searching EST databases and were proposed as being the enzymes responsible for *de novo* methylation (25). Although DNMTs were originally classified as maintenance or *de novo* DNMTs, several strands of evidence indicate that all three DNMTs not only cooperate, but also may possess both *de novo* and maintenance functions *in vivo* (26–29). The knockout cell lines for DNMT1, DNMT3b and both enzymes demonstrated that while no effective CpG island demethylation and restoration of gene expression were observed in the single knockouts, the double knockout of DNMT1 and DNMT3b showed complete hypomethylation at the studied CpG islands and corresponding gene activation (27,29). The double-knockout cell line has also been shown to be a useful tool for identifying new hypermethylated genes in human cancer (29) thanks to the use of global genomic methylation strategies, such as AIMS and CpG island arrays (30). Taken together, these results strongly suggest that both enzymes, DNMT1 and DNMT3b, participate in, and are necessary for, effective CpG island hypermethylation.

The DNA methylation code has to be read by the cell. The information stored by hypermethylated CpG islands is in part interpreted by methyl-CpG binding proteins (MBDs). MBDs are important 'translators' between DNA methylation and histone-modifier genes that establish a transcriptionally inactive chromatin environment. This family of proteins consists of five well-characterized members (MeCP2, MBD1, MBD2, MBD3 and MBD4) (31). MBD proteins are associated with hypermethylated CpG island promoters of tumor-suppressor genes and their transcriptional silencing (31), showing remarkable specificity *in vitro* (32) and *in vivo* (33–35). In fact, most hypermethylated promoters are occupied by MBD proteins, whereas unmethylated promoters generally lack MBDs, with the exception of MBD1 (35). Treatment of cancer cells with a demethylating agent causes CpG island hypomethylation, MBD release and gene re-expression, reinforcing the notion that association of MBDs with methylated promoters is methylation-dependent. Whereas several promoters are highly specific in recruiting a particular set of MBDs, other promoters seem to be less exclusive. Thus, it may be concluded that the specific profile of MBD occupancy for the hypermethylated CpG islands of tumor-suppressor genes is gene- and tumor-type-specific (35).

The finding that MeCP2 represses transcription of methylated DNA through the recruitment of a histone deacetylase (HDAC)-containing complex (36,37) established for the first time a mechanistic connection between DNA methylation and transcriptional repression by the modification of chromatin. Most histone modifications occur in their protruding N-terminal tails. A specificity in the pattern of modifications under particular conditions led to the proposal of the histone code hypothesis, according to which, histone modifications act sequentially or in combination to form a code that may be read by nuclear factors (38,39). Several modifications are compatible with gene silencing. In general, histone deacetylation leads to gene silencing, while histone acetylation leads to gene activation. Thus, hypermethylated CpG islands of silenced tumor-suppressor genes are known to display a histone code or modification maps characterized overall by histone hypoacetylation and histone methylation (30,33,40). Additional connections have been found: DNMTs are able also to recruit HDACs (41,42), while on the other hand, both DNMTs and MBDs recruit histone methyltransferases (HMTs) that modify lysine 9 of histone H3 (43,44). In the hypermethylated CpG islands of tumor-suppressor genes, all this active recruitment of multiple repressors leads to a characteristic histone modification pattern featuring deacetylation of histones H3 and H4, methylation of lysine 9 of histone H3, and demethylation of lysine 4 of histone H3 (33,40,45–47). Finally, Polycomb-group genes are epigenetic silencers that are part of multitask protein complexes, including HDACs and HMTs activity (48). Regarding DNA methylation, Polycomb and MBD proteins collaborate in long-term gene-silencing events such as X-chromosome inactivation and imprinting (49). Most importantly, the Polycomb protein EZH2 associates with DNMTs, and is required to establish DNA methylation in a subset of target genes (50–52). Thus, Polycomb proteins may serve as recruitment platforms for DNMTs involved in the hypermethylation of tumor-suppressor genes, highlighting another connection between various epigenetic repression systems.

Thus, the promoter hypermethylated CpG islands of tumor-suppressor genes seem to be a popular and busy location, where at a deep layer of densely methylated CpGs, certain proteins, such as MBDs, are more permanent residents and other proteins, such as DNMTs, HDACs, HMTs and

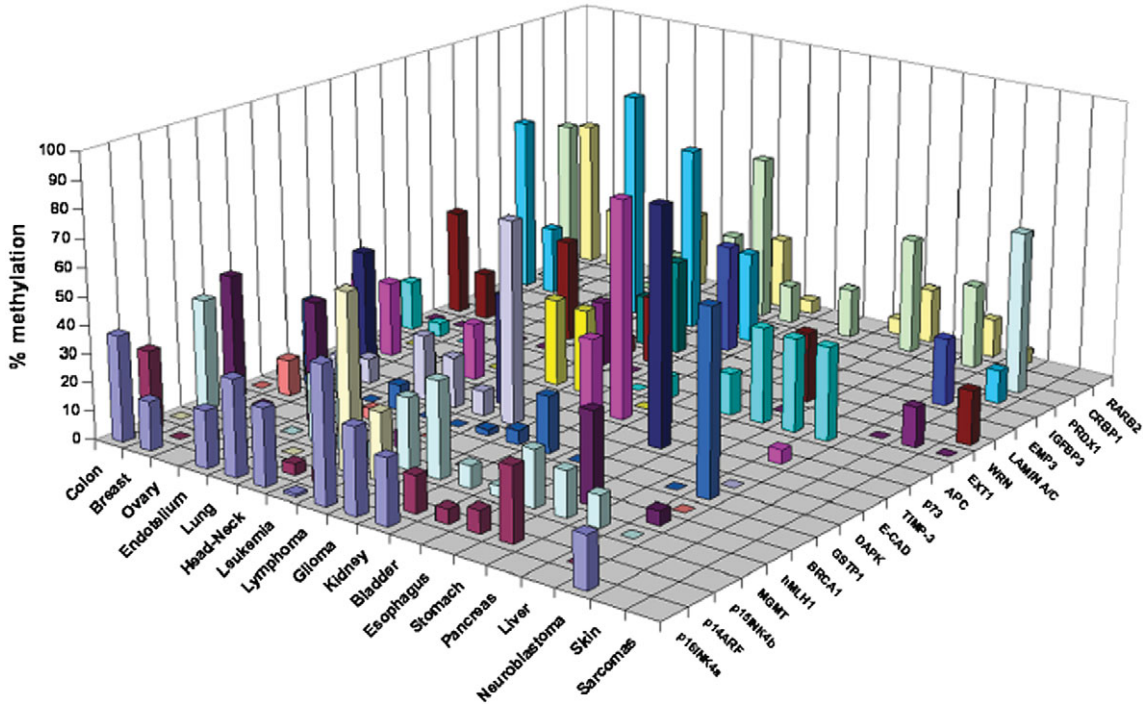

Figure 1. A CpG island hypermethylation profile of human cancer. *Y*-axis, frequency of hypermethylation for each gene in each primary.

Polycomb, shuffle to maintain a chromatin conformation compatible with stable epigenetic transcriptional silencing.

THE HYPERMETHYLOME OF A CANCER CELL

The earliest studies reported local areas of DNA hypermethylation in tumors that were absent from their normal counterparts (53). The subsequent detailed characterization of *bona fide* tumor-suppressor genes, with their respective familial syndromes, such as p16INK4a, hMLH1 and BRCA1, in which transcriptional silencing was associated with the hypermethylation of their respective 5′-CpG islands (54) gave rise to this new epigenetic world. Several concepts are worth reviewing.

First, the profiles of CpG island hypermethylation of tumor-suppressor genes vary according to the tumor type (55,56). This carefully respected pattern of epigenetic inactivation is not only a property of the sporadic tumors, but also neoplasms appearing in inherited cancer syndromes display CpG island hypermethylation specific to the tumor type (57), and even occasionally acts as a second hit (57). The tumor-type-specific CpG island hypermethylation profile is even maintained in long-established human cancer cell lines (58,59). Drawing together all these data, several reports have characterized the spectrum of gene hypermethylation for particular tumor types. We can call these 'early DNA hypermethylomes'. Some tumor types have more methylation of the known CpG islands than others: for example, the most hypermethylated tumor types are those of the gastrointestinal tract, while significantly less hypermethylation has been reported in other types such as ovarian tumors and sarcomas (30). One explanation is that the hypermethylated genes for this type have not yet been found, but another attractive reason may be that the more hypermethylated types are those that are more exposed to external carcinogen agents. Another conclusion is that there is a clear gradient in the distribution of tumors with different degrees of CpG island methylation (55,56). Figure 1 shows a profile of CpG island hypermethylation in human primary tumors.

Secondly, we know that cancer is a disease involving multiple pathways and genetic lesions, all of which are necessary for a tumor to become fully established. The story is the same for epigenetic lesions. The presence of CpG island-promoter hypermethylation affects genes involved in cell cycle, DNA repair, carcinogen metabolism, cell adherence, apoptosis, etc. Table 1 lists a selection of hypermethylated genes among different pathways in human cancer. It is also important to mention that we need to identify the role of any newly methylated genes in the biology of the tumor. We can examine a number of aspects: (a) whether the reintroduction of the gene in a deficient cancer cell line reduces colony formation, or inhibits xenograft growth in nude mice; (b) whether the hypermethylation of that gene is associated with a particular molecular or clinical phenotype, as is the case with the methylguanine DNMT (MGMT) methylation associated with the appearance of transition mutations and chemosensitivity to alkylating agents (60); (c) whether methylation-mediated silencing has the same effects as a frameshift mutation, as is the case for BRCA1 (61); and (d) whether mutations for that gene have not been described, generating a knockout mouse, as has been accomplished for HIC-1 (62). We can also rescue the functionality of the hypermethylated gene by using a DNA demethylating agent, as it has been shown for $p14^{ARF}$, hMLH1, DAPK, EXT1 and WRN (54,63,64). These two types of functional assays that restore gene function, by

Table 1. Cellular pathway disrupted by promoter CpG island hypermethylation of tumor suppressor genes

Pathways	Representative hypermethylated genes
DNA repair	hMLH1, MGMT, WRN, BRCA1
Hormone response	Estrogen, progesterone, androgen, prolactin and thyroid-stimulating hormone receptors
Vitamin response	RARB2, CRBP1,
Ras signaling	RASSFIA, NOREIA
Cell cycle	p16INK4a, p15INK4b, Rb
P53 network	p14ARF, p73, HIC-1
Cell adherence and invasion	E-cadherin, H-cadherin, FAT cadherin, EXT-1, SLIT2, EMP3
Apoptosis	TMS1, DAPK1, WIF-1, SFRP1
Wnt signaling	APC, DKK-1, IGFBP-3
Tyrosine kinase cascades	SOCS-1, SOCS-3, SYK
Transcription factors	GATA-4, GATA-5, ID4
Homeobox genes	PAX6, HOXA9
Other pathways	GSTP1, LKB1/STK11, THBS-14, COX-2, SRBC, RIZ1, TPEF/HPP1, SLC5A8, Lamin A/C
microRNAs	miR-127 (targeting BCL6), miR-124a (targeting CDK6)

chemical demethylation or exogenous overexpression, are very useful tools for highlighting the relevance of genes that undergo CpG island hypermethylation in human cancer.

Thirdly, we still do not clearly understand why certain CpG islands are hypermethylated in cancer cells while others remain methylation-free. We can hypothesize, as has been done in the case of genetic mutations, that a particular gene is preferentially methylated with respect to others in certain tumor types because its inactivation confers a selective clonal advantage, or because other chromatin players, such as Polycomb proteins, pinpoint 'methylable' islands (50–52). Both these points are briefly discussed below. It is possible that CpG islands undergoing DNA hypermethylation have a particular nucleotide distribution. Indeed, genome-wide analysis of DNA methylation using the methyl-DIP approach in colon and prostate cancer cells suggests that there may be common sequence motifs in promoters that undergo CpG island hypermethylation (65). Other characteristic sequence motifs in hypermethylated genes have been found in cells overexpressing DNMT1 (66). A personal observation of the genomic structure of numerous genes that have CpG island hypermethylation is that they usually have two different promoters, both with CpG islands, although with different CpG density: a strong one, from which the main transcript originates, and a weak one, from which a minor transcript is started. This is the case for BRCA1, APC, RASSF1A, ER, ENDBR and RARB2. Another interesting finding is that many of the hypermethylated CpG islands in human cancer have bidirectional promoters, as is commonly seen in methylated imprinted genes, e.g. WNT9A/BC040563 (67), MAPK10/PTPN13 (68), SURF-1/SURF-2 (29) and WRN/PURG (64). Certain chromosomal regions may be more prone to hypermethylation events than others. A chromosomal mapping of hypermethylated tumor suppressor genes is shown in Figure 2. For example, many hypermethylated promoter CpG islands have been identified in 3p. However, are these genome areas really prone to disregulated methylation or do they merely accommodate a high density of genes with tumor-suppressor-gene functions? Can the aberrant methylation spread from surrounding highly methylated sequences, such as Alu regions (69)? Most intriguing of all, a recent study has shown that epigenetic silencing by DNA hypermethylation can span large (1 Mb) regions of the chromosome (70). This is an epigenetic lesion that resembles the loss of heterozygosity often observed in human tumors. Clearly, many possible explanations, most of which probably contribute to varying degrees to the ability to discriminate between a 'methylable' cancer and an 'unmethylable' CpG island.

Fourth, epigenetic gene silencing could play a role in cancer stem cells. These are cells that have the same DNA sequence, but a different gene activity: the post-translational histone modifications and DNA methylation could explain the different programming. The use of epigenomic technologies for different acetylation and methylation modifications of histones (71,72) and methylated DNA (51,52) in embryonic stem cells provides a chromatin signature of pluripotent cell lines. The resulting epigenetic profile is distinct from that of embryonic carcinoma cells (51,52,72). These findings and others have led to the proposal of an epigenetic progenitor origin of human cancer (73). In this scenario, a polyclonal epigenetic disruption of stem/progenitor cells would be an early event in the non-malignant tissue, and later other genetic and epigenetic lesions would drive to full-blown tumor progression (73). The epigenetic variation in the progenitor cells would be a good explanation for the well-known plasticity and heterogeneity of tumor cells. It can also explain the existence of histologically normal human mammary epithelia with the potential to acquire malignant epigenetic phenotypes (74).

Finally, there is a big question of how many genes are hypermethylated in a given cancer cell. An inspection of the literature might give one the impression that almost every gene can be hypermethylated in a tumor, but this is definitely not the case. There is a clear under-reporting of good candidate CpG islands that, on careful experimental analysis, have been found to be unmethylated in all primary tumors and cancer cell lines examined. The reports of absence of hypermethylation of BRCA2, hMSH2, hMSH3, hMSH6, PTEN, p19INK4d, CHK1, CHK2, MTAP, NKX3.1 and other genes are a welcome addition to our knowledge. Although the number of hypermethylated genes in cancer can be estimated by candidate gene approaches, the epigenomic technologies have provided us with probably the most accurate data. Chromatin immunoprecipitation using antibodies against MBDs associated with microarray hybridization (ChIP-on-CHIP) (33), DNA immunoprecipitated with an antibody against 5-methylcytosine hybridized to genomic microarray platforms (methyl-DIP) (65,75), and the comparison of mRNA levels from cancer cell lines before and after treatment with a demethylating drug (47,76,77), among other techniques such as AIMS (29) and RLGS (55), suggest a range of 100–400 promoter hypermethylated CpG islands in a given tumor (30). We may expect that, in time, we will have a more accurate estimate.

EPIGENETIC AND GENETIC LESIONS EXCHANGE PUNCHES IN THE CANCER BOXING RING

One of the most compelling examples of the role of epigenetic gene silencing in the development of human cancer is the

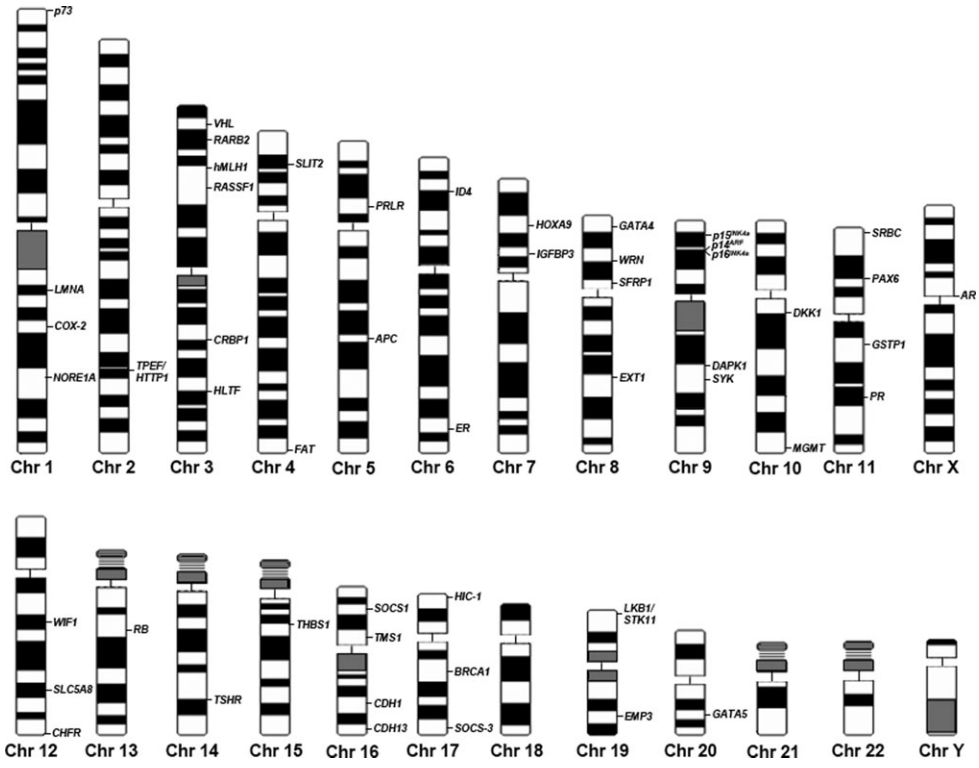

Figure 2. A CpG island hypermethylation map of human cancer. Hypermethylated genes are shown in their respective cromosomal loci.

inactivation of DNA repair genes by promoter CpG island hypermethylation. Thus, an epigenetic lesion leads to a genetic lesion, a hypermethylation event that changes the entire molecular environment of the cell. For reasons of brevity, I will mention only the three cases of hMLH1, MGMT and WRN.

Microsatellite-unstable (MSI) tumors are characterized by the presence of aberrant insertions or deletions of mono- or dinucleotide repeats induced by defects in the DNA mismatch repair (MMR) pathway. MSI is a typical feature of tumors from patients with hereditary non-polyposis colorectal carcinoma (HNPCC), attributed to germline mutations in the DNA MMR genes, mainly hMLH1, and hMSH2. However, MMR mutations are extremely rare in sporadic MSI+ tumors; the main cause of MSI in sporadic cases of colorectal, endometrial and gastric cancers is the transcriptional inactivation of hMLH1 by promoter hypermethylation (78). Thus, we see how a DNA mutator pathway in human cancer is activated by a hypermethylation event.

Another illustrative example is that of the DNA repair gene O6-MGMT (60). The nucleotide base guanine can undergo a chemical modification, the addition of a methyl- or alkyl-group. The abnormally generated O6-methylguanine is read as an adenine by the DNA polymerases and thus may generate G → A mutations, which are a common hallmark of many human tumors. Our cells are protected against this mutation by MGMT (60), which removes the promutagenic O6-methylguanine. However, we have shown that the DNA repair gene MGMT is transcriptionally silenced by promoter hypermethylation in primary human tumors (79). These tumors may accumulate a considerable number of G → A transitions, some of which affect key genes in similarly to the way in which the loss of hMLH1 MMR by methylation targets other genes. This information has led to the discovery that the hypermethylation-associated inactivation of MGMT gives rise to the appearance of G → A transition mutations in the K-ras oncogene and the universal tumor suppressor p53 in many tumor types, including colon, lung, brain and stomach (60). Thus, this is another DNA mutator pathway set in train by another, different, hypermethylation event.

Finally, Werner syndrome (WS) is our third example. The WRN protein has helicase and exonuclease activity (80), and, most importantly, cultures of WS cells show increased chromosomal instability, with abundant deletions, reciprocal translocations and inversions (80). We have shown that human cancer cells with an unmethylated WRN promoter experience minimal chromosomal breakage upon exposure to DNA-damaging agents. In contrast, both cells from WS patients and cancer cells with WRN-aberrant methylation are extremely sensitive to these drugs, displaying a high frequency of chromosomal breakages, sometimes even in the characteristic form of quatriradial chromosomes (64). In WRN-methylated cells, resistance to chromosomal breakage is acquired upon transfection with the WRN gene (64). It would be extremely interesting to determine whether the same type of chromosomal fragility occurs in primary tumors with WRN hypermethylation. In the meantime, however, we can at least consider it a third example of a genomic mutator pathway triggered by another, different, hypermethylation event.

It is more difficult to argue for the reciprocal process whereby genetic lesions lead to epigenetic gene silencing. It has been proposed that fusion proteins, such as the

promyelocytic leukemia/retinoic acid receptor alpha (PML/RARalpha), which is expressed in some leukemias, contribute to aberrant CpG-island methylation by recruiting DNMTs and HDACs to specific target genes (81), although this does not seem to be a general mechanism in this type of cancer (82). Oncogenes may be good candidates for hypermethylating tumor-suppressor genes. Again, we can consider three examples: Myc, which associates with DNMTs *in vivo* in osteosarcoma cells (83); BRAF mutations, which are more common in tumors with a putative predisposition to hypermethylating a subset of CpG islands (84); EZH2, which has already been associated with CpG island hypermethylation (49,50), and may 'pre-mark' a subgroup of CpG islands that will later undergo hypermethylation in cancer cells (51,52).

We can also think about natural selection instead of molecular targeting. Certain CpG islands become hypermethylated while others do not because the former confer a selective advantage to the survival of that particular cancer cell. For example, BRCA1 undergoes promoter hypermethylation only in breast and ovarian tumors (53,54,80) because only in these tumor types does the lack of this transcript have important cellular con sequences. A similar explanation can be advanced for hMLH1: epigenetic silencing occurs only in colon, gastric and endometrial tumors because it is advantageous in the biology of these tumors but not others. This Darwinian concept is supported by the classical genetic studies of familial tumors: carriers of BRCA1 germline mutations predominantly develop breast and ovarian tumors, whereas carriers of hMLH1 germline mutations mostly develop colorectal, gastric and endometrial tumors. There is a perfect correspondence between the genetic and epigenetic realms. Thus, selection for hypermethylation and for targeting as a result of genetic defects are probably not exclusive events, and both are likely to occur during the generation and maintenance of hypermethylated CpG islands of tumor-suppressor genes.

AGING AND CANCER: THE CASE OF THE EPIGENETICALLY SILENCED PROGEROID GENES

Aging is the main risk factor associated with cancer development (85). Thus, it makes sense that genes involved in 'preventing' the aging process are inactivated in cancer cells. The first evidence of a gene that is directly involved in aging, exhibits tumor-suppressor-like activity, and is frequently repressed in cancer by promoter hypermethylation, was recently observed for the lamin A/C (86). Lamins can be subdivided into two subfamilies: A-type, whose members are expressed in most differentiated somatic cells; B-type, whose members are expressed in nearly all cells and are essential for cell viability (87,88). A-type lamins are intermediate filaments that, in conjunction with B-type lamins, form the nuclear lamina located on the inner side of the nuclear membrane (87,88). It has recently been shown that nuclear lamins are highly dynamic, which suggests a role for them in the non-random positioning of subchromosome domains in the overall chromatin structure, and possibly in the regulation of gene expression (87,88). The lamin A/C gene encodes the lamins A and C, which are two isoforms that arise as a result of alternative RNA splicing. Mutations in the lamin A/C gene have been shown to cause Hutchinson–Gilford progeria and atypical WS (87,88).

The knowledge that atypical WS commonly features mutations of the lamin A/C gene (87,88), and that its expression is reduced in hematological malignancies (89), prompted the search for epigenetic alterations of its promoter in human cancer. We demonstrated for the first time promoter CpG island hypermethylation at the lamin A/C gene that is associated with loss of RNA and protein expression in leukemia and lymphoma tumors (86). The epigenetic loss of lamin A/C expression in malignant B- and T-lymphocytes is thought to be directly involved in the abrogation of the differentiation of hematological cells (89) and is a predictor of poor outcome of these patients (86).

However, the most striking case of epigenetic gene silencing of a progeroid gene is provided by the Werner gene (WRN), as we have briefly discussed above. WS patients undergo many features indicative of accelerated aging, including cataracts, type 2 diabetes, osteoporosis, arteriosclerosis and, most interestingly, cancer (90,91). Deaths of WS patients have two major causes: atherosclerosis (44% of patients) and neoplasia (39.5%). The tumor type of neoplasms appearing in WS patients is different from that observed in people who do not have the syndrome: the ratio of mesenchymal:epithelial cancers is 1:1 (85–87), compared with 1:10 in the normally aging population. Thus, it seems that the accelerated aging process in WS patients contributes to the higher incidence of tumors, but the specific loss of the WRN gene confers a particular tumor-type-prone phenotype. Almost all the mutations identified in WS patients are nonsense or insertion/deletion/substitution mutations that result in the truncation of protein translation before the nuclear localization signal (91). The WRN protein is a member of the RecQ helicases that includes proteins that are defective in human genomic-instability diseases such as Rothmund Thompson (RecQ 4) and Bloom (BLM) syndromes (92). WRN possesses DNA-dependent ATPase and $3' \rightarrow 5'$ exonuclease and helicase activities (91). WRN has a role in several pathways, such as DNA replication, DNA repair, p53-mediated pathways and telomere metabolism. It has been suggested that RecQ family proteins act as tumor suppressors (92), and we demonstrated for the first time that the WRN gene undergoes epigenetic inactivation by CpG island promoter hypermethylation in various tumor types of both mesenchymal and epithelial origin, including those commonly observed in WRN patients (64). The re-introduction of the WRN gene in deficient cells has tumor suppressor-like features and the use of a DNA demethylating in cancer cells hypermethylated at the WRN promoter agent restores the exonuclease activity of the WRN protein (64).

Thus, Lamin AC and WRN are the two founding fathers of a new class of tumor suppressor genes undergoing methylation-associated silencing in human cancer, the aging genes.

EPIGENETIC SILENCING OF MICRORNAS IN CANCER

We are beginning to understand a great deal about epigenetic silencing of tumor-suppressor genes in human cancer by CpG island promoter hypermethylation. However, one important

piece of the puzzle is still missing: the putative role of epigenetic disruption in producing the aberrant patterns of expression of miRNAs in cancer cells. miRNA expression patterns can be developmentally regulated, tissue-specific or steadily expressed in the whole organism and are thought to play important roles in cell proliferation, apoptosis and differentiation (93,94). In diseases, recent studies have shown that miRNA expression profiles differ between normal tissues and the derived tumors, and between different tumor types (95,96). Interestingly, downregulation of subsets of miRNAs has been found in many of these studies, suggesting that some of these miRNAs may act as tumor-suppressor genes (95,96). The downregulation of many tumor-suppressor genes of importance in human cancer has been closely linked to the presence of CpG island promoter hypermethylation, as described above, and this has prompted us and other researchers to wonder whether the same mechanism plays a role in the described loss of miRNA expression in tumors.

The role as tumor suppressors of miRNAs has been investigated in more detail for particular cases. For example, the downregulated let-7 and miR-15/miR-16, and miR-127 are known to target the oncogenic factors RAS and BCL-2, respectively (97,98). This may be explained by the failure of these miRNAs during post-transcriptional regulation in cancer cells (99), but additional mechanisms such as CpG island hypermethylation could also be invoked. Two critical sets of data have recently appeared that are relevant to this matter. First, it has been observed that ~5% of human miRNAs are upregulated by treatment of bladder cancer cells with DNA demethylating agent and HDAC inhibitor (11). In particular, miR-127, which is embedded in a CpG island, was strongly induced by a decrease in DNA methylation levels around the promoter region of the miR-127 gene, and the proto-oncogene BCL6, a potential target of miR-127, was translationally downregulated after treatment (11). Second, using a genetic approach that takes advantage of the genomic disruption of DNMT1 and DNMT3b in cancer cells, we have demonstrated that CpG island hypermethylation is a mechanism that can account for the downregulation of miRNAs in human cancer (12). Most importantly, the epigenetic silencing of miR-124a, one of the DNA methylation-associated silenced miRNAs isolated using this approach, leads to the activation of cyclin D kinase 6 (CDK6), a *bona fide* oncogenic factor, and the phosphorylation of the retinoblastoma (Rb) tumor-suppressor gene (12).

We should be aware that these two independent sets of results (11,12) potentially open up an avenue of anticancer therapy based on the epigenetic regulation of miRNAs. If the already known connection between aberrant expression of miRNAs and the development of cancer suggests that miRNAs are potential therapeutic targets, these recent findings suggest that the functional restoration of tumor-suppressor activities of epigenetically silenced miRNAs could be beneficial to anticancer strategies. Until now, therapy with epigenetic drugs, such as DNA demethylating agents and HDAC inhibitors, has been based on classical protein-coding tumor-suppressor genes. In the near future, with the identification of many more hypermethylation-silenced miRNA genes with tumor-suppressor function in human cancer, the epigenetic silencing of these miRNAs will become an excellent target for the epigenetic drugs that are currently available and for those that will be developed. Our expectations are high.

Conflict of Interest statement. None declared.

REFERENCES

1. McGhee, J.D. and Ginder, G.D. (1979) Specific DNA methylation sites in the vicinity of the chicken beta-globin genes. *Nature*, **280**, 419–420.
2. Van der Ploeg, L.H. and Flavell, R.A. (1980) DNA methylation in the human gamma delta beta-globin locus in erythroid and nonerythroid tissues. *Cell*, **4**, 947–958.
3. Greger, V., Passarge, E., Hopping, W., Messmer, E. and Horsthemke, B. (1989) Epigenetic changes may contribute to the formation and spontaneous regression of retinoblastoma. *Hum. Genet.*, **83**, 155–158.
4. Herman, J.G., Latif, F., Weng, Y., Lerman, M.I., Zbar, B., Liu, S., Samid, D., Duan, D.S., Gnarra, J.R., Linehan, W.M. *et al*. (1994) Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. *Proc. Natl. Acad. Sci. USA*, **91**, 9700–9704.
5. Merlo, A., Herman, J.G., Mao, L., Lee, D.J., Gabrielson, E., Burger, P.C., Baylin, S.B. and Sidransky, D. (1995) 5' CpG island methylation is associated with transcriptional silencing of the tumour suppressor p16/CDKN2/MTS1 in human cancers. *Nat. Med.*, **1**, 686–692.
6. Herman, J.G., Merlo, A., Mao, L., Lapidus, R.G., Issa, J.P., Davidson, N.E., Sidransky, D. and Baylin, S.B. (1995) Inactivation of the CDKN2/p16/MTS1 gene is frequently associated with aberrant DNA methylation in all common human cancers. *Cancer Res.*, **55**, 4525–4530.
7. Gonzalez-Zulueta, M., Bender, C.M., Yang, A.S., Nguyen, T., Beart, R.W., Van Tornout, J.M. and Jones, P.A. (1995) Methylation of the 5' CpG island of the p16/CDKN2 tumor suppressor gene in normal and transformed human tissues correlates with gene silencing. *Cancer Res.*, **55**, 4531–4535.
8. Jones, P.A. and Martienssen, R. (2005) A blueprint for a Human Epigenome Project: the AACR Human Epigenome Workshop. *Cancer Res.*, **65**, 11241–11246.
9. Esteller, M. (2006) The necessity of a human epigenome project. *Carcinogenesis*, **27**, 1121–1125.
10. Mack, G.S. (2006) Epigenetic cancer therapy makes headway. *J. Natl. Cancer Inst.*, **98**, 1443–1444.
11. Saito, Y., Liang, G., Egger, G., Friedman, J.M., Chuang, J.C., Coetzee, G.A. and Jones, P.A. (2006) Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. *Cancer Cell*, **9**, 435–443.
12. Lujambio, A., Ropero, S., Ballestar, E., Fraga, M.F., Cerrato, C., Setien, F., Casado, S., Suarez-Gauthier, A., Sanchez-Cespedes, M., Gitt, A. *et al*. (2007) Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. *Cancer Res.*, **67**, 1424–1429.
13. Fraga, M.F. and Esteller, M. (2005) Towards the human cancer epigenome: a first draft of histone modifications. *Cell Cycle*, **4**, 1377–1381.
14. Kubicek, S., Schotta, G., Lachner, M., Sengupta, R., Kohlmaier, A., Perez-Burgos, L., Linderson, Y., Martens, J.H., O'Sullivan, R.J., Fodor, B.D., Yonezawa, M., Peters, A.H. and Jenuwein, T. (2006) The role of histone modifications in epigenetic transitions during normal and perturbed development. *Ernst. Schering Res. Found. Workshop*, **57**, 1–27.
15. Fraga, M.F., Ballestar, E., Villar-Garea, A., Boix-Chornet, M., Espada, J., Schotta, G., Bonaldi, T., Haydon, C., Ropero, S., Petrie, K., Iyer, N.G., Perez-Rosado, A., Calvo, E., Lopez, J.A., Cano, A., Calasanz, M.J., Colomer, D., Piris, M.A., Ahn, N., Imhof, A., Caldas, C., Jenuwein, T. and Esteller, M. (2005) Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. *Nat. Genet.*, **37**, 391–400.
16. Seligson, D.B., Horvath, S., Shi, T., Yu, H., Tze, S., Grunstein, M. and Kurdistani, S.K. (2005) Global histone modification patterns predict risk of prostate cancer recurrence. *Nature*, **435**, 1262–1266.
17. Fraga, M.F., Ballestar, E., Paz, M.F., Ropero, S., Setien, F., Ballestar, M.L., Heine-Suner, D., Cigudosa, J.C., Urioste, M., Benitez, J., Boix-Chornet, M., Sanchez-Aguilera, A., Ling, C., Carlsson, E., Poulsen, P., Vaag, A., Stephan, Z., Spector, T.D., Wu, Y.Z., Plass, C. and Esteller, M. (2005) Epigenetic differences arise during the lifetime of monozygotic twins. *Proc. Natl. Acad. Sci. USA*, **102**, 10604–10609.

18. Eckhardt, F., Lewin, J., Cortese, R., Rakyan, V.K., Attwood, J., Burger, M., Burton, J., Cox, T.V., Davies, R., Down, T.A., Haefliger, C., Horton, R., Howe, K., Jackson, D.K., Kunde, J., Koenig, C., Liddle, J., Niblett, D., Otto, T., Pettett, R., Seemann, S., Thompson, C., West, T., Rogers, J., Olek, A., Berlin, K. and Beck, S. (2006) DNA methylation profiling of human chromosomes 6, 20 and 22. *Nat. Genet.*, **38**, 1378–1385.
19. Bird, A.P. (1986) CpG-rich islands and the function of DNA methylation. *Nature*, **321**, 209–213.
20. Futscher, B.W., Oshiro, M.M., Wozniak, R.J., Holtan, N., Hanigan, C.L., Duan, H. and Domann, F.E. (2002) Role for DNA methylation in the control of cell type specific maspin expression. *Nat. Genet.*, **31**, 175–179.
21. Khulan, B., Thompson, R.F., Ye, K., Fazzari, M.J., Suzuki, M., Stasiek, E., Figueroa, M.E., Glass, J.L., Chen, Q., Montagna, C., Hatchwell, E., Selzer, R.R., Richmond, T.A., Green, R.D., Melnick, A. and Greally, J.M. (2006) Comparative isoschizomer profiling of cytosine methylation: the HELP assay. *Genome Res.*, **16**, 1046–1055.
22. Nguyen, C., Liang, G., Nguyen, T.T., Tsao-Wei, D., Groshen, S., Lubbert, M., Zhou, J.H., Benedict, W.F. and Jones, P.A. (2001) Susceptibility of nonpromoter CpG islands to *de novo* methylation in normal and neoplastic cells. *J. Natl. Cancer Inst.*, **93**, 1465–1472.
23. Jones, P.A. (1999) The DNA methylation paradox. *Trends Genet.*, **15**, 34–37.
24. Espada, J., Ballestar, E., Fraga, M.F., Villar-Garea, A., Juarranz, A., Stockert, J.C., Robertson, K.D., Fuks, F. and Esteller, M. (2004) Human DNA methyltransferase 1 is required for maintenance of the histone H3 modification pattern. *J. Biol. Chem.*, **279**, 37175–37184.
25. Okano, M., Bell, D.W., Haber, D.A. and Li, E. (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for *de novo* methylation and mammalian development. *Cell*, **99**, 247–257.
26. Rhee, I., Jair, K.W., Yen, R.W., Lengauer, C., Herman, J.G., Kinzler, K.W., Vogelstein, B., Baylin, S.B. and Schuebel, K.E. (2000) CpG methylation is maintained in human cancer cells lacking DNMT1. *Nature*, **404**, 1003–1007.
27. Rhee, I., Bachman, K.E., Park, B.H., Jair, K.W., Yen, R.W., Schuebel, K.E., Cui, H., Feinberg, A.P., Lengauer, C., Kinzler, K.W., Baylin, S.B., Vogelstein, B. (2002) DNMT1 and DNMT3b cooperate to silence genes in human cancer cells. *Nature*, **416**, 552–556.
28. Kim, G.D., Ni, J., Kelesoglu, N., Roberts, R.J. and Pradhan, S. (2002) Co-operation and communication between the human maintenance and *de novo* DNA (cytosine-5) methyltransferases. *EMBO J.*, **21**, 4183–4195.
29. Paz, M.F., Wei, S., Cigudosa, J.C., Rodriguez-Perales, S., Peinado, M.A., Huang, T.H. and Esteller, M. (2003) Genetic unmasking of epigenetically silenced tumor suppressor genes in colon cancer cells deficient in DNA methyltransferases. *Hum. Mol. Genet.*, **12**, 2209–2219.
30. Esteller, M. (2007) Cancer epigenomics: DNA methylomes and histone modification maps. *Nat. Rev. Genet.*, **8**, doi:10.1038/nrg2005.
31. Ballestar, E. and Esteller, M. (2005) Methyl-CpG-binding proteins in cancer: blaming the DNA methylation messenger. *Biochem. Cell. Biol.*, **83**, 374–384.
32. Fraga, M.F., Ballestar, E., Montoya, G., Taysavang, P., Wade, P.A. and Esteller, M. (2003) The affinity of different MBD proteins for a specific methylated locus depends on their intrinsic binding properties. *Nucleic Acids Res.*, **31**, 765–774.
33. Ballestar, E., Paz, M.F., Valle, L., Wei, S., Fraga, M.F., Espada, J., Cigudosa, J.C., Huang, T.H. and Esteller, M. (2003) Methyl-CpG binding proteins identify novel sites of epigenetic inactivation in human cancer. *EMBO J.*, **22**, 6335–4635.
34. Klose, R.J., Sarraf, S.A., Schmiedeberg, L., McDermott, S.M., Stancheva, I. and Bird, A.P. (2005) DNA binding selectivity of MeCP2 due to a requirement for A/T sequences adjacent to methyl-CpG. *Mol. Cell.*, **19**, 667–678.
35. Lopez-Serra, L., Ballestar, E., Fraga, M.F., Alaminos, M., Setien, F. and Esteller, M. (2006) A profile of MBD protein occupancy of hypermethylated promoter CpG islands of tumor suppressor genes in human cancer. *Cancer Res.*, **66**, 8342–8346.
36. Jones, P.L., Veenstra, G.J., Wade, P.A., Vermaak, D., Kass, S.U., Landsberger, N., Strouboulis, J. and Wolffe, A.P. (1998) Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. *Nat. Genet.*, **19**, 187–191.
37. Nan, X., Ng, H.H., Johnson, C.A., Laherty, C.D., Turner, B.M., Eisenman, R.N., Bird, A. (1998) Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature*, **393**, 386–389.
38. Jenuwein, T. and Allis, C.D. (2001) Translating the histone code. *Science*, **293**, 1074–1080.
39. Turner, B.M. (2002) Cellular memory and the histone code. *Cell*, **111**, 285–291.
40. Fahrner, J.A. and Baylin, S.B. (2003) Heterochromatin: stable and unstable invasions at home and abroad. *Genes Dev.*, **17**, 1805–1812.
41. Robertson, K.D., Ait-Si-Ali, S., Yokochi, T., Wade, P.A., Jones, P.L. and Wolffe, A.P. (2000) DNMT1 forms a complex with Rb, E2F1 and HDAC1 and represses transcription from E2F-responsive promoters. *Nat. Genet.*, **25**, 338–342.
42. Fuks, F., Burgers, W.A., Godin, N., Kasai, M. and Kouzarides, T. (2001) Dnmt3a binds deacetylases and is recruited by a sequence-specific repressor to silence transcription. *EMBO J.*, **20**, 2536–2544.
43. Fuks, F., Hurd, P.J., Deplus, R. and Kouzarides, T. (2003) The DNA methyltransferases associate with HP1 and the SUV39H1 histone methyltransferase. *Nucleic Acids Res.*, **31**, 2305–2312.
44. Fuks, F., Hurd, P.J., Wolf, D., Nan, X., Bird, A.P. and Kouzarides, T. (2003) The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation. *J. Biol. Chem.*, **278**, 4035–4040.
45. Stimson, K.M. and Vertino, P.M. (2002) Methylation-mediated silencing of TMS1/ASC is accompanied by histone hypoacetylation and CpG island-localized changes in chromatin architecture. *J. Biol. Chem.*, **277**, 4951–4958.
46. Stirzaker, C., Song, J.Z., Davidson, B. and Clark, S.J. (2004) Transcriptional gene silencing promotes DNA hypermethylation through a sequential change in chromatin modifications in cancer cells. *Cancer Res.*, **64**, 3871–3877.
47. Fraga, M.F., Herranz, M., Espada, J., Ballestar, E., Paz, M.F., Ropero, S., Erkek, E., Bozdogan, O., Peinado, H., Niveleau, A. *et al*. (2004) A mouse skin multistage carcinogenesis model reflects the aberrant DNA methylation patterns of human tumors. *Cancer Res.*, **64**, 5527–5534.
48. Sparmann, A. and van Lohuizen, M. (2006) Polycomb silencers control cell fate, development and cancer. *Nat. Rev. Cancer*, **6**, 846–856.
49. Matarazzo, M.R., De Bonis, M.L., Strazzullo, M., Cerase, A., Ferraro, M., Vastarelli, P., Ballestar, E., Esteller, M., Kudo, S. and D'Esposito, M. (2007) Multiple binding of methyl-CpG and polycomb proteins in long-term gene silencing events. *J. Cell Physiol.*, **210**, 711–719.
50. Vire, E., Brenner, C., Deplus, R., Blanchon, L., Fraga, M., Didelot, C., Morey, L., Van Eynde, A., Bernard, D., Vanderwinden, J.M., Bollen, M., Esteller, M., Di Croce, L., de Launoit, Y. and Fuks, F. (2006) The Polycomb group protein EZH2 directly controls DNA methylation. *Nature*, **439**, 871–874.
51. Schlesinger, Y., Straussman, R., Keshet, I., Farkash, S., Hecht, M., Zimmerman, J., Eden, E., Yakhini, Z., Ben-Shushan, E., Reubinoff, B.E., Bergman, Y., Simon, I. and Cedar, H. (2006) Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for *de novo* methylation in cancer. *Nat. Genet.*, doi:10.1038/ng1950.
52. Widschwendter, M., Fiegl, H., Egle, D., Mueller-Holzner, E., Spizzo, G., Marth, C., Weisenberger, D.J., Campan, M., Young, J., Jacobs, I. and Laird, P.W. (2006) Epigenetic stem cell signature in cancer. *Nat. Genet.*, doi:10.1038/ng1941.
53. de Bustros, A., Nelkin, B.D., Silverman, A., Ehrlich, G., Poiesz, B. and Baylin, S.B. (1988) The short arm of chromosome 11 is a 'hot spot' for hypermethylation in human neoplasia. *Proc. Natl. Acad. Sci. USA*, **85**, 5693–5697.
54. Esteller, M. (2005) Aberrant DNA methylation as a cancer-inducing mechanism. *Annu. Rev. Pharmacol. Toxicol.*, **45**, 629–656.
55. Costello, J.F., Fruhwald, M.C., Smiraglia, D.J., Rush, L.J., Robertson, G.P., Gao, X., Wright, F.A., Feramisco, J.D., Peltomaki, P., Lang, J.C., Schuller, D.E., Yu, L., Bloomfield, C.D., Caligiuri, M.A., Yates, A., Nishikawa, R., Su Huang, H., Petrelli, N.J., Zhang, X., O'Dorisio, M.S., Held, W.A., Cavenee, W.K. and Plass, C. (2000) Aberrant CpG-island methylation has non-random and tumour-type-specific patterns. *Nat. Genet.*, **24**, 132–138.
56. Esteller, M., Corn, P.G., Baylin, S.B. and Herman, J.G. (2001) A gene hypermethylation profile of human cancer. *Cancer Res.*, **61**, 3225–3229.
57. Esteller, M., Fraga, M.F., Guo, M., Garcia-Foncillas, J., Hedenfalk, I., Godwin, A.K., Trojan, J., Vaurs-Barriere, C., Bignon, Y.J., Ramus, S., Benitez, J., Caldes, T., Akiyama, Y., Yuasa, Y., Launonen, V.,

Canal, M.J., Rodriguez, R., Capella, G., Peinado, M.A., Borg, A., Aaltonen, L.A., Ponder, B.A., Baylin, S.B. and Herman, J.G. (2001) DNA methylation patterns in hereditary human cancers mimic sporadic tumorigenesis. *Hum. Mol. Genet.*, **10**, 3001–3007.
58. Smiraglia, D.J., Rush, L.J., Fruhwald, M.C., Dai, Z., Held, W.A., Costello, J.F., Lang, J.C., Eng, C., Li, B., Wright, F.A., Caligiuri, M.A. and Plass, C. (2001) Excessive CpG island hypermethylation in cancer cell lines versus primary human malignancies. *Hum. Mol. Genet.*, **10**, 1413–1419.
59. Paz, M.F., Fraga, M.F., Avila, S., Guo, M., Pollan, M., Herman, J.G. and Esteller, M. (2003) A systematic profile of DNA methylation in human cancer cell lines. *Cancer Res.*, **63**, 1114–1121.
60. Esteller, M. and Herman, J.G. (2004) Generating mutations but providing chemosensitivity: the role of O6-methylguanine DNA methyltransferase in human cancer. *Oncogene*, **23**, 1–8.
61. Hedenfalk, I., Duggan, D., Chen, Y., Radmacher, M., Bittner, M., Simon, R., Meltzer, P., Gusterson, B., Esteller, M., Kallioniemi, O.P., Wilfond, B., Borg, A., Trent, J., Raffeld, M., Yakhini, Z., Ben-Dor, A., Dougherty, E,. Kononen, J., Bubendorf, L., Fehrle, W., Pittaluga, S., Gruvberger, S., Loman, N., Johannsson, O., Olsson, H. and Sauter, G. (2001) Gene-expression profiles in hereditary breast cancer. *N. Engl. J. Med.*, **344**, 539–548.
62. Chen, W.Y. (2003) Heterozygous disruption of Hic1 predisposes mice to a gender-dependent spectrum of malignant tumors. *Nat. Genet.*, **33**, 197–202.
63. Ropero, S., Setien, F., Espada, J., Fraga, M.F., Herranz, M., Asp, J., Benassi, M.S., Franchi, A., Patino, A., Ward, L.S., Bovee, J., Cigudosa, J.C., Wim, W. and Esteller, M. (2004) Epigenetic loss of the familial tumor-suppressor gene exostosin-1 (EXT1) disrupts heparan sulfate synthesis in cancer cells. *Hum. Mol. Genet.*, **13**, 2753–2765.
64. Agrelo, R., Cheng, W.H., Setien, F., Ropero, S., Espada, J., Fraga, M.F., Herranz, M., Paz, M.F., Sanchez-Cespedes, M., Artiga, M.J., Guerrero, D., Castells, A., von Kobbe, C., Bohr, V.A. and Esteller, M. (2006) Epigenetic inactivation of the premature aging Werner syndrome gene in human cancer. *Proc. Natl. Acad. Sci. USA*, **103**, 8822–8827.
65. Keshet, I., Schlesinger, Y., Farkash, S., Rand, E., Hecht, M., Segal, E., Pikarski, E., Young, R.A., Niveleau, A., Cedar, H. and Simon, I. (2006) Evidence for an instructive mechanism of *de novo* methylation in cancer cells. *Nat. Genet.*, **38**, 149–153.
66. Feltus, F.A., Lee, E.K., Costello, J.F., Plass, C. and Vertino, P.M. (2006) DNA motifs associated with aberrant CpG island methylation. *Genomics*, **87**, 572–579.
67. Shu, J., Jelinek, J., Chang, H., Shen, L., Qin, T., Chung, W., Oki, Y. and Issa, J.P. (2006) Silencing of bidirectional promoters by DNA methylation in tumorigenesis. *Cancer Res.*, **66**, 5077–5084.
68. Ying, J., Li, H., Cui, Y., Wong, A.H., Langford, C. and Tao, Q. (2006) Epigenetic disruption of two proapoptotic genes MAPK10/JNK3 and PTPN13/FAP-1 in multiple lymphomas and carcinomas through hypermethylation of a common bidirectional promoter. *Leukemia*, **20**, 1173–1175.
69. Graff, J.R., Herman, J.G., Myohanen, S., Baylin, S.B. and Vertino, P.M. (1997) Mapping patterns of CpG island methylation in normal and neoplastic cells implicates both upstream and downstream regions in *de novo* methylation. *J. Biol. Chem.*, **272**, 22322–22329.
70. Frigola, J., Song, J., Stirzaker, C., Hinshelwood, R.A., Peinado, M.A. and Clark, S.J. (2006) Epigenetic remodeling in colorectal cancer results in coordinate gene suppression across an entire chromosome band. *Nat. Genet.*, **38**, 540–549.
71. Bernstein, B.E., Mikkelsen, T.S., Xie, X., Kamal, M., Huebert, D.J., Cuff, J., Fry, B., Meissner, A., Wernig, M., Plath, K., Jaenisch, R., Wagschal, A., Feil, R., Schreiber, S.L. and Lander, E.S. (2006) A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell*, **125**, 315–326.
72. Azuara, V., Perry, P., Sauer, S., Spivakov, M., Jorgensen, H.F., John, R.M., Gouti, M., Casanova, M., Warnes, G., Merkenschlager, M., Fisher, A.G. (2006) Chromatin signatures of pluripotent cell lines. *Nat. Cell. Biol.*, **8**, 532–538.
73. Feinberg, A.P., Ohlsson, R. and Henikoff, S. The epigenetic progenitor origin of human cancer. *Nat. Rev. Genet.*, **7**, 21–33.
74. Crawford, Y.G., Gauthier, M.L., Joubel, A., Mantei, K., Kozakiewicz, K., Afshari, C.A. and Tlsty, T.D. (2004) Histologically normal human mammary epithelia with silenced p16(INK4a) overexpress COX-2, promoting a premalignant program. *Cancer Cell*, **5**, 263–273.
75. Weber, M., Davies, J.J., Wittig, D., Oakeley, E.J., Haase, M., Lam, W.L., Schubeler, D. (2005) Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. *Nat. Genet.*, **37**, 853–862.
76. Suzuki, H., Gabrielson, E., Chen, W., Anbazhagan, R., van Engeland, M., Weijenberg, M.P., Herman, J.G., Baylin, S.B. (2002) A genomic screen for genes upregulated by demethylation and histone deacetylase inhibition in human colorectal cancer. *Nat. Genet.*, **31**, 141–149.
77. Yamashita, K., Upadhyay, S., Osada, M., Hoque, M.O., Xiao, Y., Mori, M., Sato, F., Meltzer, S.J. and Sidransky, D. (2002) Pharmacologic unmasking of epigenetically silenced tumor suppressor genes in esophageal squamous cell carcinoma. *Cancer Cell*, **2**, 485–495.
78. Esteller, M. (2000) Epigenetic lesions causing genetic lesions in human cancer: promoter hypermethylation of DNA repair genes. *Eur. J. Cancer*, **36**, 2294–2300.
79. Esteller, M., Hamilton, S.R., Burger, P.C., Baylin, S.B. and Herman, J.G. (1999) Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. *Cancer Res.*, **59**, 793–797.
80. Hisama, F.M., Bohr, V.A. and Oshima, J. (2006) WRN's tenth anniversary. *Sci. Aging Knowledge Environ.*, **28**, pe18.
81. Di Croce, L., Raker, V.A., Corsaro, M., Fazi, F., Fanelli, M., Faretta, M., Fuks, F., Lo Coco, F., Kouzarides, T., Nervi, C., Minucci, S. and Pelicci, P.G. (2002) Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor. *Science*, **295**, 1079–1082.
82. Esteller, M., Fraga, M.F., Paz, M.F., Campo, E., Colomer, D., Novo, F.J., Calasanz, M.J., Galm, O., Guo, M., Benitez, J. and Herman, J.G. (2002) Cancer epigenetics and methylation. *Science*, **297**, 1807–1808.
83. Brenner, C., Deplus, R., Didelot, C., Loriot, A., Vire, E., De Smet, C., Gutierrez, A., Danovi, D., Bernard, D., Boon, T., Pelicci, P.G., Amati, B., Kouzarides, T., de Launoit, Y., Di Croce, L. and Fuks, F. (2005) Myc represses transcription through recruitment of DNA methyltransferase corepressor. *EMBO J.*, **24**, 336–346.
84. Weisenberger, D.J., Siegmund, K.D., Campan, M., Young, J., Long, T.I., Faasse, M.A., Kang, G.H., Widschwendter, M., Weener, D., Buchanan, D., Koh, H., Simms, L., Barker, M., Leggett, B., Levine, J., Kim, M., French, A.J., Thibodeau, S.N., Jass, J., Haile, R. and Laird, P.W. (2006) CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. *Nat. Genet.*, **38**, 787–793.
85. Ershler, W.B. and Longo, D.L. (1997) Aging and cancer: issues of basic and clinical science. *J. Natl. Cancer Inst.*, **89**, 1489–1497.
86. Agrelo, R., Setien, F., Espada, J., Artiga, M.J., Rodriguez, M., Perez-Rosado, A., Sanchez-Aguilera, A., Fraga, M.F., Piris, M.A. and Esteller, M. (2005) Inactivation of the lamin A/C gene by CpG island promoter hypermethylation in hematologic malignancies, and its association with poor survival in nodal diffuse large B-cell lymphoma. *J. Clin. Oncol.*, **23**, 3940–3947.
87. Hutchinson, C.J. and Worman, H.J. (2004) A-type lamins: guardians of the soma? *Nat. Cell. Biol.*, **6**, 1062–1067.
88. Broers, J.L., Ramaekers, F.C., Bonne, G., Yaou, R.B. and Hutchison, C.J. (2006) Nuclear lamins: laminopathies and their role in premature ageing. *Physiol. Rev.*, **86**, 967–1008.
89. Stadelmann, B., Khandjian, E., Hirt, A., Luthy, A., Weil, R. and Wagner, H.P. (1990) Repression of nuclear lamin A and C gene expression in human acute lymphoblastic leukemia and non-Hodgkin's lymphoma cells. *Leuk. Res.*, **14**, 815–821.
90. Epstein, C.J., Martin, G.M., Schultz, A.L. and Motulsky, A.G. (1966) Werner's syndrome a review of its symptomatology, natural history, pathologic features, genetics and relationship to the natural aging process. *Medicine (Baltimore)*, **45**, 177–221.
91. Opresko, P.L., Cheng, W.H., von Kobbe, C., Harrigan, J.A. and Bohr, V.A. (2003) Werner syndrome and the function of the Werner protein; what they can teach us about the molecular aging process. *Carcinogenesis*, **24**, 791–802.
92. Hickson, I.D. (2003) RecQ helicases: caretakers of the genome. *Nat. Rev. Cancer*, **3**, 169–178.
93. He, L. and Hannon, G.J. (2004) MicroRNAs: small RNAs with a big role in gene regulation. *Nat. Rev. Genet.*, **5**, 522–531.
94. Miska, E.A. (2005) How microRNAs control cell division, differentiation and death. *Curr. Opin. Genet.*, **5**, 563–568.

95. Lu, J., Getz, G., Miska, E.A., Alvarez-Saavedra, E., Lamb, J., Peck, D., Sweet-Cordero, A., Ebert, B.L., Mak, R.H., Ferrando, A.A., Downing, J.R., Jacks, T., Horvitz, H.R., Golub, T.R. (2005) MicroRNA expression profiles classify human cancers. *Nature*, **435**, 834–838(Baltimore).
96. Calin, G.A. and Croce, C.M. (2006) MicroRNA signatures in human cancers. *Nat. Rev. Cancer*, **6**, 857–866.
97. Johnson, S.M., Grosshans, H., Shingara, J., Byrom, M., Jarvis, R., Cheng, A., Labourier, E., Reinert, K.L., Brown, D. and Slack, F.J. (2005) RAS is regulated by the let-7 microRNA family. *Cell*, **120**, 635–647.
98. Cimmino, A., Calin, G.A., Fabbri, M., Iorio, M.V., Ferracin, M., Shimizu, M., Wojcik, S.E., Aqeilan, R.I., Zupo, S., Dono, M., Rassenti, L., Alder, H., Volinia, S., Liu, C.G., Kipps, T.J., Negrini, M. and Croce, C.M. (2005) miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc. Natl. Acad. Sci.*, **102**, 13944–13949.
99. Thomson, J.M., Newman, M., Parker, J.S., Morin-Kensicki, E.M., Wright, T. and Hammond, S.M. (2006) Extensive post-transcriptional regulation of microRNAs and its implications for cancer. *Genes Dev.*, **20**, 2202–2207.