Chapter 7
DNA Hypomethylation and Activation of Germline-Specific Genes in Cancer

Charles De Smet and Axelle Loriot

Abstract DNA methylation, occurring at cytosines in CpG dinucleotides, is a potent mechanism of transcriptional repression. Proper genomic methylation patterns become profoundly altered in cancer cells: both gains (hypermethylation) and losses (hypomethylation) of methylated sites are observed. Although DNA hypomethylation is detected in a vast majority of human tumors and affects many genomic regions, its role in tumor biology remains elusive. Surprisingly, DNA hypomethylation in cancer was found to cause the aberrant activation of only a limited group of genes. Most of these are normally expressed exclusively in germline cells and were grouped under the term "cancer-germline" (CG) genes. CG genes represent unique examples of genes that rely primarily on DNA methylation for their tissue-specific expression. They are also being exploited to uncover the mechanisms that lead to DNA hypomethylation in tumors. Moreover, as CG genes encode tumor-specific antigens, their activation in cancer highlights a direct link between epigenetic alterations and tumor immunity. As a result, clinical trials combining epigenetic drugs with anti-CG antigen vaccines are being considered.

7.1 Introduction

Although DNA hypomethylation was the first epigenetic alteration to be described in human cancers, its effect on gene expression programs and tumor biology has remained enigmatic. Initial examination of cancer genomes identified most losses of DNA methylation in repeated elements [29]. This is not surprising, since these DNA elements are highly abundant and comprise most of the CpG sites that are normally methylated in healthy somatic tissues. A crucial question was whether

C. De Smet (✉) • A. Loriot
Laboratory of Genetics and Epigenetics, de Duve Institute,
Catholic University of Louvain, Brussels, Belgium
e-mail: Charles.Desmet@uclouvain.be

A.R. Karpf (ed.), *Epigenetic Alterations in Oncogenesis*, Advances in Experimental Medicine and Biology 754, DOI 10.1007/978-1-4419-9967-2_7,

DNA hypomethylation also affected protein-encoding genes, leading to their aberrant expression in tumor cells. It appeared, however, that genome hypomethylation in tumors is not generally associated with the ectopic activation of a multitude of genes [5]. A plausible explanation for this is that most tissue-specific genes use other regulatory mechanisms, including histone modifications, and that DNA methylation, if present, serves merely as secondary layer of repression. Losses of DNA methylation within such genes would therefore not be sufficient to trigger transcriptional activation.

Later work, aiming at isolating genes that code for tumor-specific antigens, led to the identification of a particular group of genes, which are normally expressed exclusively in germline cells but become aberrantly activated in a wide variety of tumors [86]. Given this expression profile, these genes were termed "cancer-germline" (CG) genes. Interestingly, CG genes were found to rely primarily on DNA methylation for repression in normal somatic tissues, and their activation in tumors was shown to be a direct consequence of genome hypomethylation [22]. These observations highlighted an unexpected link between epigenetic alterations in tumors and cancer immunity. They also provided clear examples of genes that owe their tissue-specific expression to DNA methylation. Moreover, CG genes are being exploited to try to uncover the molecular mechanisms underlying genome hypomethylation in tumors, as this epigenetic process remains largely unexplained.

7.2 Characterization of CG Genes

Human tumors express specific antigens, as evidenced by the existence in the blood of cancer patients of cytolytic T lymphocytes (CTL) that recognize antigens present on their tumor cells but not on normal cells [10]. Using a gene library transfection approach and a CTL clone isolated from a melanoma patient, Boon and colleagues identified the first human tumor antigen-encoding gene [85]. The gene was named melanoma antigen 1 or *MAGE-1* (later renamed *MAGEA1*). *MAGEA1* expression was not found in normal tissues except for testis, but was instead detected in a significant fraction of melanoma samples, as well as in various other tumor types [20, 23]. The same genetic approach led to the identification of other melanoma antigen genes, namely *BAGE*, *GAGE*, and *MAGEA3*, a gene closely related to *MAGEA1* [9, 34, 84]. For these genes too, expression among normal tissues was restricted to testis, and activation in tumors was detected among various cancer types. Additional tumor antigen genes were subsequently identified, using an alternative cloning approach, called SEREX (serological analysis of recombinant tumor cDNA expression libraries), and based on the presence of high titers of antitumor IgGs in the blood of tumor-bearing patients [73]. Again, several of the identified genes, including *SSX2* and *NY-ESO-1*, had their normal expression restricted to testis and were activated in a percentage of different tumor types. Later studies indicated that the normal expression of most isolated genes was confined to the germ cells in both testis and fetal ovary [44, 52, 82].

Together, these findings led to the important notion that specific antigens in tumors arise from the aberrant activation of genes that are normally transcribed exclusively in the germline. From an immunological point of view, this dual expression pattern is understandable. Unlike most somatic cells, germ cells lack MHC class I molecules, which are required to present antigenic peptides at the cell surface [37]. Activation of germline-specific genes in tumor cells therefore leads to the expression of truly tumor-specific antigens, which can be recognized as nonself by the immune system.

Further studies using cDNA subtraction procedures or database mining have permitted the identification of additional genes expressed in germ cells and cancer but not in normal somatic tissues [56, 60, 63, 75]. Some genes identified in this way were subsequently shown to encode tumor-specific antigens recognized by CTLs [86]. Altogether about 50 human genes or gene families were identified, which displayed specific expression in the germline and activation in a significant proportion of cancers [2]. These genes appear to exert a variety of cellular functions, but on the basis of their common expression pattern they were grouped under the term cancer-germline (CG) genes. CG genes are dispersed on several chromosomes, with a marked preference for the X chromosome. In human cancers, CG genes are expressed more frequently in specific tumor types, like for instance lung cancer, head and neck cancer, bladder cancer, and melanoma [76]. Other tumor types like colon cancer, renal cancer, and leukemia only rarely show activation of CG genes. An important feature of CG genes is their frequent co-activation in tumors [74]. It was observed indeed that positive tumors often express several CG genes. Clearly, the widespread and concerted expression of CG genes in tumors indicates that their activation in cancer results from a global gene activation process, rather than stochastic individual events.

7.3 DNA Demethylation in the Activation of CG Genes in Tumors

The marked tendency of CG genes to become co-expressed in tumors suggested that these genes share, at least in part, a common mechanism of transcriptional activation. Initial studies were performed with the *MAGEA1* gene in order to identify essential promoter elements and corresponding transcription factors that may contribute to the cell-type-specific expression of the gene. Surprisingly, however, transfection experiments revealed that all cells, including those that do not express *MAGEA1*, contain transcription factors capable of inducing significant *MAGEA1* promoter activity [24]. Transfection experiments with other CG gene promoter constructs led to similar results [17, 89]. This implied that nonexpressing cells have a repression mechanism, probably operating at the chromatin level that protects CG gene promoters against spurious activation.

The initial observation by Weber and colleagues that *MAGEA1* could be induced in nonexpressing melanoma cell lines following treatment with the DNA

methylation inhibitor 5-aza-2′-deoxycytidine provided a first hint that DNA methylation may contribute to the transcriptional regulation of this gene [91]. This was confirmed by studies showing that the promoter of *MAGEA1* is invariably methylated in all normal somatic tissues and instead unmethylated in germ cells [26]. Likewise, activation of the *MAGEA1* gene in tumors was strictly correlated with demethylation of its promoter [26]. Further studies showed that DNA methylation was similarly involved in the regulation of other CG genes [17, 26, 52, 56, 89]. Altogether, these observations indicated that CG genes rely on DNA methylation for repression in somatic tissues, and that aberrant activation of these genes in tumors results from demethylation of their promoter.

Interestingly, demethylation and activation of CG genes in tumors was found to correlate with global genome hypomethylation [14, 25, 45]. This association was further confirmed by a study on microdissected tumor samples, revealing that intratumor heterogeneity of CG gene expression also correlates with global genome hypomethylation levels [96]. These observations provided therefore the first clear evidence that the process of genome-wide demethylation, common to many cancers, not only affects repeated sequences but also single copy genes, and can lead to aberrant gene activation. The frequent co-activation of CG genes in tumors likely reflects the global process of DNA demethylation, which can simultaneously affect many loci across the cancer genome.

7.4 DNA Methylation in the Regulation of Germline Genes

Considering the potent effect of DNA methylation on transcriptional repression, it was originally proposed that this DNA modification might serve as a general mechanism to control the programmed expression of tissue-specific genes [39, 72]. Evidence, however, indicates that most tissue-specific genes rely on mechanisms other than DNA methylation for repression in nonexpressing cells [8, 88]. This may be ascribed to the distribution of CpG sequences, where cytosine methylation can occur. Vertebrate genomes show a general depletion of CpG dinucleotides, which was attributed to the high mutability of methylated cytosines, and hence the progressive disappearance of this sequence during evolution [7]. Discrete genomic regions however, which appear generally free of CpG methylation, maintained a high density of CpG sites. These so-called CpG islands often overlap gene promoters [19]. Many tissue-specific genes contain a methylation-free CpG island within their promoter and can therefore not rely on DNA methylation for repression in nonexpressing tissues. On the other hand, genes with few CpG sites within their promoter are only little affected by DNA methylation, and often show an inconstant relationship between promoter methylation and transcriptional silencing [12]. It was therefore proposed that DNA methylation in vertebrates is solely involved in the control of retrotransposable elements, monoallelically expressed imprinted genes, and X chromosome inactivation, the only cases where consistent methylation of CpG-rich regions appeared to exist [101].

This view was challenged by the discovery of CG genes, which were found to be characterized by the presence of a high density of CpG sites within their promoter [26]. Yet, unlike classical CpG islands, CpG-rich promoters of CG genes are methylated in all normal somatic tissues. CG gene promoters appear therefore favorably disposed to DNA methylation-mediated regulation. Consistently, transfection experiments with in vitro methylated CG gene constructs indicated that DNA methylation was sufficient to repress transcription, even in cells that express the corresponding endogenous CG gene, and therefore obviously contain appropriate transcriptional activators [17, 26, 27, 78, 89]. This and the above-mentioned observation that unmethylated CG gene promoters are transcriptionally active in nonexpressing cells provided strong evidence that DNA methylation is an essential component of the repression of this group of germline-specific genes in somatic cells.

More recently, genome-wide studies were conducted in order to identify the distribution of differentially methylated CpG sites across the genome of distinct types of human cells [77, 93]. These studies revealed the existence of novel sets of genes with a CpG-rich promoter that was densely methylated in somatic tissues (in addition to the previously characterized CG genes). Remarkably, most of these genes were specifically demethylated and expressed in testis. It appears therefore that DNA methylation has a particular role in the regulation of germline-specific genes.

Why would DNA methylation be particularly suitable for the regulation of genes with specific expression in germline cells rather than in other cell types? A plausible explanation may be that methylation-dependent germline genes have the advantage of being little exposed to the evolutionary loss of methylated CpGs, because they are unmethylated precisely in the cells that transmit their genome to the offspring. As a result, such genes maintain a high density of CpG sites within their promoter and remain therefore fully responsive to DNA methylation.

7.5 Mechanisms Leading to Hypomethylation of CG Genes in Cancer

CG genes have served as model sequences to investigate the distribution and dynamics of methylation losses in tumor genomes. Detailed analysis of the *MAGEA1* locus revealed preferential hypomethylation of a restricted region surrounding the transcription start site of the gene in expressing tumor cells, suggesting that hypomethylated CpG sites are unevenly distributed across cancer genomes [27]. Consistently, recent genome-wide DNA methylation studies confirmed that DNA hypomethylation in tumors adopts mosaic patterns, with defined hypomethylated domains (between one kilobase and several megabases in size) surrounded by normally methylated regions [66, 71, 92]. These observations indicate that certain genomic regions, including CG promoters, are particularly susceptible to DNA hypomethylation in tumors.

The possibility that *MAGEA1*-expressing tumor cells possess a DNA demethylation activity targeted towards the 5′-region of the gene was investigated [27, 58].

Thus, a large genomic fragment comprising the *MAGEA1* gene was methylated in vitro and then stably transfected into several human tumor cell lines, where the endogenous *MAGEA1* gene is hypomethylated and active. The newly integrated *MAGEA1* transgenes did not undergo demethylation, indicating that the process that once led to demethylation of the endogenous *MAGEA1* gene was not preserved in these cells. Remarkably, when unmethylated *MAGEA1* constructs were introduced into such cells, de novo methylation of the transgenes occurred except in a region overlapping the *MAGEA1* promoter [27]. This mechanism of protection against de novo DNA methylation was lost when mutations that impair the *MAGEA1* promoter activity were introduced into the transgene, or when the transgene was transfected into tumor cells that induce only little *MAGEA1* promoter activity. Altogether, these data suggest that site-specific hypomethylation of *MAGEA1* in tumors results from a past event of transient DNA demethylation and is maintained locally by the presence of potent transcriptional activators that prevent remethylation.

In vivo studies, evaluating global genome methylation levels in colon and breast cancers, demonstrated that DNA hypomethylation is present in the early stages of the disease, and does not progress towards later stages, adding support the transient nature of the DNA demethylation process [30, 41]. Other studies, however, reported a higher prevalence of genome hypomethylation and an increased frequency of CG gene activation in more advanced tumor stages [53, 100]. This was interpreted as an indication that DNA demethylation might instead be a continuous process leading to progressive methylation losses with tumor development. Other interpretations for the increased hypomethylation in advanced tumor genomes, which implicate a transient DNA demethylation process, are however possible: (1) transient demethylation would initially produce a mixed population of precancerous cells with varying levels of DNA hypomethylation, and cells with the most hypomethylated genome would later be selected to contribute to the more advanced stages of the disease; or (2) the transient demethylation process could occur at varying time points during tumor progression and would therefore be more likely to have already occurred in late stage tumor samples [22]. Additional support for a transient DNA demethylation process comes from the observation that tumor cell lines with a hypomethylated genome do not show further CpG methylation losses during culturing [32, 55, 94]. Of note, many tumor cells display instead de novo methylation activities [3, 43].

Considering the suggested dynamics of DNA demethylation in tumors, it is reasonable to propose that hypomethylation of CG genes in tumors is mediated by two groups of factors: those that contribute to the transient DNA demethylation process and those that are required to protect the CG gene promoter region against subsequent remethylation.

7.5.1 Process of DNA Demethylation

Factors contributing to the DNA demethylation process during cancer development remain unknown. The apparent transient nature of this process suggests that activation of such demethylation-inducing factors might occur in association with

one (or several) of the multiple steps through which precancerous cells are progressing before acquiring full malignancy. Interestingly, a recent study evaluating genome methylation levels in an isogenic series of human mammary epithelial cell cultures transitioning from normal to malignantly transformed revealed that most losses of DNA methylation occurred at the stage of acquisition of indefinite lifespan [67]. Another study reported that genome hypomethylation and CG gene activation is more prevalent in tumors displaying the alternative telomere (ALT) maintenance phenotype rather than telomerase activation, the two possible mechanisms by which cancer cells stabilize their telomeres and acquire immortality [83]. These observations establish therefore a possible link between DNA demethylation and cellular immortalization. Underlying molecular mechanisms remain, however, to be identified.

Theoretically, DNA demethylation in tumor cells could possibly occur through two distinct processes commonly referred to as active demethylation and passive demethylation [16]. Active demethylation would involve the activation of demethylating enzymes, which can remove methylation marks from the DNA in a replication-independent manner. Enzymes contributing to active DNA demethylation in animal cells are beginning to be characterized [16], but their potential involvement in cancer genome demethylation has not yet been reported. Passive demethylation on the other hand, would rely on the inhibition of DNA methyltransferases, which normally preserve the DNA methylation marks through the successive replication cycles. Three DNA methyltransferases exist in mammals: DNMT1, DNMT3A, and DNMT3B [6]. DNMT1 is primarily involved in DNA methylation maintenance, as it appears to be specialized in copying preexisting methylation sites onto the newly synthesized strand during replication. DNMT3A and DNMT3B instead have de novo DNA methylation activity and are responsible for the establishment of new DNA methylation marks in the developing embryo. For CG genes in particular, studies based on targeted depletion of the distinct DNMTs indicate that DNMT1 is the principal enzyme for methylation maintenance [42, 57]. It is therefore likely that passive DNA demethylation of CG genes in tumors would necessarily involve factors that decrease the amount or impair proper functioning of DNMT1. In certain tumor cells, however, combined depletion of DNMT1 and DNMT3 enzymes was required to obtain efficient demethylation and activation of CG genes [42, 95]. This indicates that de novo methyltransferases can be targeted to these genes, where they might restore lost methylation sites, and underscores the importance of acquiring mechanisms of protection against remethylation for long-term activation.

7.5.2 Factors that Protect Against Remethylation

Studies with the *MAGEA1* promoter suggest that protection of the promoter against DNA remethylation is dependent on the level of transcriptional activation [27]. It is therefore likely that maintenance of CG gene promoter hypomethylation in tumor cells relies on the presence of appropriate transcription factors, as well as on the activation of such factors by upstream signaling pathways.

Several DNA-binding factors have been identified, which appear to induce activation of CG gene promoters. Transcriptional activation of several genes of the *MAGEA* family has been shown to depend on the binding of ETS transcription factors within their promoter [21, 24]. Interestingly, ETS-binding sequences in *MAGEA* promoters contain a CpG site, and it was shown that methylation of this site inhibits binding of the corresponding factor [25]. In the promoter of *MAGEA1*, two ETS-binding sites were shown to be essential to maintain hypomethylation of the promoter in expressing tumor cells, as evidenced by remethylation of transfected *MAGEA1* constructs containing mutations within these two essential promoter elements [27]. The ETS family of transcription factors comprises about 30 members in humans, which all bind a similar DNA motif with a central GGAA/T sequence [68]. The precise member(s) involved in the regulation of *MAGEA* genes remain(s) to be characterized.

SP1 is another transcription factor, which was shown to contribute to the activation of several *MAGEA* genes, as well as the *CTAG1* gene (also termed *NY-ESO-1*) [24, 46]. The ubiquitously expressed SP1 factor acts as a transcriptional activator and recognizes a consensus DNA sequence (GC box element), which includes a CpG site [80]. SP1-binding elements are therefore often present in CG-rich promoter sequences. Binding of SP1 to the *CTAG1* gene was shown to occur only in cells where the promoter is unmethylated [46]. Interestingly, SP1-binding elements were previously shown to be involved in preserving the methylation-free status of classical CpG-island promoters [13, 62]. It is therefore likely that, once bound to the demethylated promoter of CG genes, SP1 proteins contribute to protect the region against remethylation.

BORIS (also known as CTCFL) is a testis-specific paralog of the ubiquitously expressed DNA-binding protein CTCF, which is involved in various aspects of epigenetic regulation, including gene imprinting and X chromosome inactivation [59]. Both proteins share a highly similar central DNA-binding domain, and recognize therefore overlapping DNA sequences, but contain divergent amino- and carboxy-terminal domains. The gene-encoding BORIS belongs to the CG group of genes, as its expression is regulated by DNA methylation and becomes activated in a wide variety of tumors [38, 49, 87, 95]. Remarkably, it has been demonstrated that in expressing tumors cells, BORIS is targeted to the promoters of other CG genes, namely *MAGEA1* and *CTAG1*, where its recruitment coincides with loss of CTCF binding [40, 87]. BORIS exerts transcriptional activation of CG genes, possibly in cooperation with SP1 transcription factors [46, 87]. In one study, forced overexpression of BORIS led to demethylation (albeit only partially) and activation of various CG genes in normal human fibroblasts, suggesting that BORIS activation in tumors might represent a primary triggering event for the epigenetic de-repression of other CG genes [87]. However, similar experiments from other groups did not confirm CG gene demethylation and activation resulting from BORIS overexpression [49, 97]. Moreover, it was found that many tumors display activation of various CG genes in the absence of BORIS expression. It is therefore unlikely that BORIS is a necessary factor for the derepression of other CG genes in tumors. Its presence in certain tumor cells may, however, facilitate maintenance of the hypomethylated and active state of CG gene promoters.

Many more transcription factors involved in CG gene regulation remain to be identified, and it is likely that each particular CG gene is controlled by a distinct combination of transcription factors. Tissue-specific differences in the content of transcription factors probably account for the fact that, while CG genes tend to be co-activated in hypomethylated tumors, some of them nevertheless show preferential activation in specific tumor types [36, 56].

Cell signaling through tyrosine kinase receptors appears to represent an additional level of control of CG gene regulation. A study in mast cell lines reported that signaling through KIT, an oncogenic receptor hyper-activated in several types of cancers, increases transcription of *MAGE* genes [99]. Other studies revealed that signaling through FGFR2, an FGF receptor often down-regulated in thyroid and pituitary cancers, exerts a negative effect on *MAGEA3* and *MAGEA6* transcription [51, 102]. It is therefore possible that particular dysregulations in cancers, such as those affecting cell signaling pathways, increase the activity of transcription factors that target CG genes, and thereby facilitate long-term activation of these genes in hypomethylated tumor cells. This may partially explain the observation that experimental DNA demethylation, by the use of DNMT inhibitors, often induces CG gene activation more efficiently in tumor cells than in normal cells [47].

7.5.3 Histone Modifications

Active CG gene promoters in tumors usually display a hypomethylated region that comprises one to several kilobases [27]. It is therefore likely that the protective influence of transcription factors against DNA remethylation extends beyond their narrow-binding site. Consistently, impaired binding of ETS transcription factors to *MAGEA1* transgenes, as caused by mutations in their recognition sites, resulted in de novo methylation of CpG sites within the entire promoter region, not just those located nearby the mutated ETS-binding sites [27]. This regional, rather than site-specific effect, might be related to the presence of modifications on the chromatin, such as histone modifications, which after being initiated by specific transcription factors often propagate themselves over larger domains [31]. Histone modifications can indeed influence DNA methylation states [15]. Repressive histone marks, such as methylation of lysine 9 and 27 of histone H3 (H3K9 and H3K27), favor local DNA methylation, whereas active marks, such as histone acetylation or methylation of lysine 4 of histone H3 (H3K4), appear to exclude the DNA methylation machinery. Studies from several groups have shown that demethylation and activation of CG genes in tumor cells is always associated with gains in histone acetylation and H3K4 methylation [42, 70]. The repressed state of human CG genes instead has been associated to a certain extent with the presence of H3K27 and H3K9 methylation marks [42, 70]. The exact relationship between histone modifications changes and DNA demethylation in CG gene promoters remains unclear. A crucial question is whether the varying histone modifications in CG gene promoters are a cause or a consequence of DNA methylation alterations. Studies using inhibitors of histone-modifying enzymes showed that these were on their own unable to induce significant

demethylation and activation of CG genes. Only in combination with inhibitors of DNA methylation, did they significantly modulate the level of activation of CG genes [35, 54, 70]. These observations support the notion that DNA methylation exerts a dominant role in the epigenetic repression of CG genes. But it remains possible that histone modifications assume the responsibility of maintaining the active status of the promoter following its demethylation.

7.5.4 Multiple Factors Determining CG Gene Activation in Tumors

Considering the above, it appears that activation of a particular CG gene in a tumor cell will depend on several factors: (1) the extent of CpG methylation losses resulting from the transient DNA demethylation process; (2) the level of de novo DNA methylation activities in the cell, which might induce remethylation of the promoter; (3) the presence of transcriptional activators and histone-modifying enzymes capable of counteracting remethylation activities. The likelihood that a CG gene becomes activated in a tumor cell probably depends on a complex balance between these different factors (Fig. 7.1).

7.6 Oncogenic Function of CG Genes

Activation of CG genes in tumor cells raises the possibility that their proteins might have oncogenic activities. The biological function of most of these genes, which encode very diverse proteins, remains however poorly understood. One extreme possibility is that the main contribution of DNA hypomethylation to tumor progression resides in its repercussions on genomic instability [33], and that the accompanying activation of CG genes is merely a side effect with no impact on malignancy (other than inducing the expression of tumor antigens). Another possibility has been proposed, in which the concerted expression of CG genes in cancer would correspond to the activation of a gametogenic program, thereby bestowing tumor cells with germ cell properties, including the capacity to self-renew (a feature of spermatogonial stem cells) and increased motility (a feature of sperm cells) [79]. Activation of CG genes in tumors is however only partial, making it very unlikely that all genes necessary for inducing a gametogenic program become expressed at the same time. Nevertheless, it remains possible that some CG genes contribute to tumor progression. Several MAGE proteins were found to inhibit p53 transactivation function, thereby exerting antiapoptotic properties [28, 64, 98]. GAGE proteins were also shown to render cells resistant to apoptosis [18]. Other studies reported that MAGEA11 serves as a co-stimulator for the androgen receptor and might therefore contribute to the development of prostate tumors that have become independent of the presence of androgen for their growth [4, 48]. Moreover, it was noted that

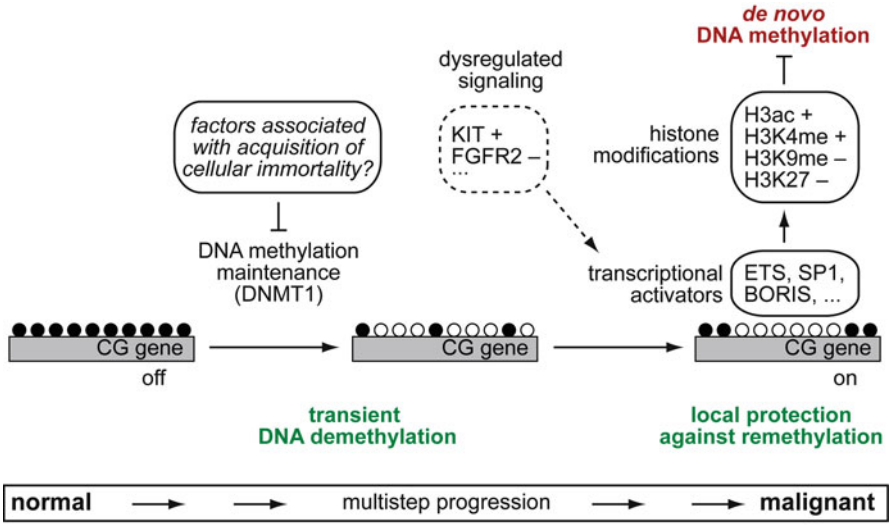

Fig. 7.1 Proposed model of demethylation and activation of CG genes during tumor development. The activation of CG genes in tumors depends on several factors: the extent of the transient DNA demethylation process, occurring at some step of tumor development; the level of counteracting de novo methylation activities in the cell; and the presence of transcriptional activators that protect the CG gene promoter against remethylation, for instance by increasing (+) or decreasing (–) distinct histone marks locally. *Filled circles* represent methylated CpG, *empty circles* unmethylated cytosines

several CG genes, including *BORIS*, *BRDT,* and *ATAD2*, encode nuclear proteins that have a potential impact on chromatin structures and might therefore be involved in the epigenetic alterations commonly affecting cancer genomes [90]. Altogether, these observations support the notion that the activation of several CG genes in tumors, resulting from DNA demethylation, might be associated with the acquisition of oncogenic properties.

Surprisingly, however, two independent studies indicate that *MAGEA4* displays instead tumor-suppressor functions. In one study, MAGEA4 was shown to interact with gankyrin and to inhibit anchorage-independent growth in vitro and tumor formation in mice [65]. In the other study, MAGEA4 was found to promote tumor cell death and to increase their sensitivity to apoptotic stimuli [69]. Clearly, more studies will be required before we can evaluate the full spectrum of consequences of CG gene activation in tumors.

7.7 DNA Hypomethylation in Cancer: An Immunological Paradox

There is now compelling evidence that the immune system is able to identify and destroy tumor cells [81]. This immune surveillance of cancer is believed to provide a barrier to cancer development, even though progressing tumors eventually escape

this obstacle by activating a variety of immune evasion strategies. Evidence for the existence of such surveillance of cancer by the immune system is provided for instance by the observation that solid tumors are often infiltrated by lymphocytes. Not surprisingly, several of these tumor-infiltrating lymphocytes were shown to be directed against antigens encoded by CG genes [50]. This suggests therefore that DNA hypomethylation and the consequent activation of CG genes has, at least at some stage of oncogenesis, a detrimental effect on tumor development. Yet, DNA hypomethylation is observed in most tumors, suggesting that it must otherwise have a strong tumor-promoting effect that outweighs this negative immunogenic effect.

7.8 Epigenetically Assisted Cancer Immunotherapy

Clinical trials of therapeutic vaccination of cancer patients using antigens encoded by CG genes are underway. Noticeable clinical responses were observed, albeit in only a fraction of the treated patients [11]. An interesting possibility to increase vaccination efficiencies would be the use of epigenetic drugs, such as the DNA methylation inhibitor decitabine, which should increase the number of expressed CG genes in the tumors, thereby rendering them more visible to the immune system. Importantly, decitabine is expected to induce reactivation of epigenetically silenced tumor-suppressor genes as well, and hence to reduce the growth rate of the tumors at the same time. Clinical trials combining decitabine and vaccination against antigens encoded by CG gene have been initiated [1].

There are, however, several points concerning the efficiency and safety of such approaches, which remain to be addressed. The first point concerns the specificity of decitabine-induced expression of CG genes in tumor cells rather than normal cells. Although studies have found that tumor cells are more sensitive to decitabine [47], it is obvious that the drug also induces CG genes in normal cell cultures, including fibroblasts and blood lymphocytes [25, 56, 61]. It will therefore be crucial to monitor decitabine/vaccine-treated patients for potential autoimmune reactions directed against their healthy tissues. Another concern relates to the duration of CG gene expression following decitabine treatment. Several studies have shown that CG gene expression in tumor cells was only transient following exposure to decitabine [26, 91]. This may be related to the absence of appropriate transcription factors, and hence lack of protection of the promoters against remethylation. The duration of CG gene expression in tumor cells may be critical to allow complete rejection by the immune cells. In this particular immune context, tumor cells that lose CG gene expression might be strongly selected. Prolonged decitabine treatment or combination with another epigenetic drug favoring protection of CG promoters against remethylation (e.g., drugs affecting histone marks) might be a solution to the problem. Finally, as genome hypomethylation is obviously associated with tumor development, there is a concern that decitabine treatment may generate strongly hypomethylated tumor cells with increased malignancy [33]. This is particularly problematic if it is confirmed that CG genes themselves exert oncogenic functions.

Clearly, a better understanding of the mechanisms of activation and of the biological functions of CG genes should help to resolve these questions, and may help to design the most efficient and safest ways to epigenetically augment tumor immunogenicity, thereby rendering cancer cells more vulnerable to vaccination.

References

1. Akers SN, Odunsi K, Karpf AR (2010) Regulation of cancer germline antigen gene expression: implications for cancer immunotherapy. Future Oncol 6(5):717–732
2. Almeida LG, Sakabe NJ, deOliveira AR, Silva MC, Mundstein AS, Cohen T, Chen YT, Chua R, Gurung S, Gnjatic S, Jungbluth AA, Caballero OL, Bairoch A, Kiesler E, White SL, Simpson AJ, Old LJ, Camargo AA, Vasconcelos AT (2009) CTdatabase: a knowledge-base of high-throughput and curated data on cancer-testis antigens. Nucleic Acids Res 37(Database issue):D816–819
3. Antequera F, Boyes J, Bird A (1990) High levels of de novo methylation and altered chromatin structure at CpG islands in cell lines. Cell 62(3):503–514
4. Bai S, He B, Wilson EM (2005) Melanoma antigen gene protein MAGE-11 regulates androgen receptor function by modulating the interdomain interaction. Mol Cell Biol 25(4):1238–1257
5. Baylin SB, Herman JG, Graff JR, Vertino PM, Issa JP (1998) Alterations in DNA methylation: a fundamental aspect of neoplasia. Adv Cancer Res 72:141–196
6. Bestor TH (2000) The DNA methyltransferases of mammals. Hum Mol Genet 9(16):2395–2402
7. Bird AP (1980) DNA methylation and the frequency of CpG in animal DNA. Nucleic Acids Res 8:1499–1504
8. Bird A (2002) DNA methylation patterns and epigenetic memory. Genes Dev 16(1):6–21
9. Boël P, Wildmann C, Sensi M-L, Brasseur R, Renauld J-C, Coulie P, Boon T, van der Bruggen P (1995) BAGE, a new gene encoding an antigen recognized on human melanomas by cytolytic T lymphocytes. Immunity 2:167–175
10. Boon T, Cerottini J-C, Van den Eynde B, van der Bruggen P, Van Pel A (1994) Tumor antigens recognized by T lymphocytes. Annu Rev Immunol 12:337–365
11. Boon T, Coulie PG, Van den Eynde BJ, van der Bruggen P (2006) Human T cell responses against melanoma. Annu Rev Immunol 24:175–208
12. Boyes J, Bird A (1992) Repression of genes by DNA methylation depends on CpG density and promoter strength: evidence for involvement of a methyl-CpG binding protein. EMBO J 11(1):327–333
13. Brandeis M, Frank D, Keshet I, Siegfried Z, Mendelsohn M, Nemes A, Temper V, Razin A, Cedar H (1994) Sp1 elements protect a CpG island from de novo methylation. Nature 371(6496):435–438
14. Cadieux B, Ching TT, VandenBerg SR, Costello JF (2006) Genome-wide hypomethylation in human glioblastomas associated with specific copy number alteration, methylenetetrahydrofolate reductase allele status, and increased proliferation. Cancer Res 66(17):8469–8476
15. Cedar H, Bergman Y (2009) Linking DNA methylation and histone modification: patterns and paradigms. Nat Rev Genet 10(5):295–304
16. Chen ZX, Riggs AD (2011) DNA methylation and demethylation in mammals. J Biol Chem 286(21):18347–18353
17. Cho B, Lee H, Jeong S, Bang YJ, Lee HJ, Hwang KS, Kim HY, Lee YS, Kang GH, Jeoung DI (2003) Promoter hypomethylation of a novel cancer/testis antigen gene CAGE is correlated with its aberrant expression and is seen in premalignant stage of gastric carcinoma. Biochem Biophys Res Commun 307(1):52–63

18. Cilensek ZM, Yehiely F, Kular RK, Deiss LP (2002) A member of the GAGE family of tumor antigens is an anti-apoptotic gene that confers resistance to Fas/CD95/APO-1, Interferon-gamma, taxol and gamma-irradiation. Cancer Biol Ther 1(4):380–387
19. Cross SH, Bird AP (1995) CpG islands and genes. Curr Opin Genet Dev 5:309–314
20. De Plaen E, Arden K, Traversari C, Gaforio JJ, Szikora J-P, De Smet C, Brasseur F, van der Bruggen P, Lethé B, Lurquin C, Brasseur R, Chomez P, De Backer O, Cavenee W, Boon T (1994) Structure, chromosomal localization and expression of twelve genes of the MAGE family. Immunogenetics 40:360–369
21. De Plaen E, Naerhuyzen B, De Smet C, Szikora J-P, Boon T (1997) Alternative promoters of gene MAGE4a. Genomics 40:305–313
22. De Smet C, Loriot A (2010) DNA hypomethylation in cancer: epigenetic scars of a neoplastic journey. Epigenetics 5(3):206–213
23. De Smet C, Lurquin C, van der Bruggen P, De Plaen E, Brasseur F, Boon T (1994) Sequence and expression pattern of the human MAGE2 gene. Immunogenetics 39:121–129
24. De Smet C, Courtois SJ, Faraoni I, Lurquin C, Szikora JP, De Backer O, Boon T (1995) Involvement of two Ets binding sites in the transcriptional activation of the MAGE1 gene. Immunogenetics 42(4):282–290
25. De Smet C, De Backer O, Faraoni I, Lurquin C, Brasseur F, Boon T (1996) The activation of human gene MAGE-1 in tumor cells is correlated with genome-wide demethylation. Proc Natl Acad Sci USA 93(14):7149–7153
26. De Smet C, Lurquin C, Lethé B, Martelange V, Boon T (1999) DNA methylation is the primary silencing mechanism for a set of germ line- and tumor-specific genes with a CpG-rich promoter. Mol Cell Biol 19:7327–7335
27. De Smet C, Loriot A, Boon T (2004) Promoter-dependent mechanism leading to selective hypomethylation within the 5′ region of gene MAGE-A1 in tumor cells. Mol Cell Biol 24(11):4781–4790
28. Doyle JM, Gao J, Wang J, Yang M, Potts PR (2010) MAGE-RING protein complexes comprise a family of E3 ubiquitin ligases. Mol Cell 39(6):963–974
29. Ehrlich M (2002) DNA methylation in cancer: too much, but also too little. Oncogene 21(35):5400–5413
30. Feinberg AP, Gehrke CW, Kuo KC, Ehrlich M (1988) Reduced genomic 5-methylcytosine content in human colonic neoplasia. Cancer Res 48(5):1159–1161
31. Felsenfeld G, Groudine M (2003) Controlling the double helix. Nature 421(6921):448–453
32. Flatau E, Gonzales FA, Michalowsky LA, Jones PA (1984) DNA methylation in 5-aza-2′-deoxycytidine-resistant variants of C3H 10T1/2C18 cells. Mol Cell Biol 4(10):2098–2102
33. Gaudet F, Hodgson JG, Eden A, Jackson-Grusby L, Dausman J, Gray JW, Leonhardt H, Jaenisch R (2003) Induction of tumors in mice by genomic hypomethylation. Science 300(5618):489–492
34. Gaugler B, Van den Eynde B, van der Bruggen P, Romero P, Gaforio JJ, De Plaen E, Lethé B, Brasseur F, Boon T (1994) Human gene MAGE-3 codes for an antigen recognized on a melanoma by autologous cytolytic T lymphocytes. J Exp Med 179:921–930
35. Goodyear O, Agathanggelou A, Novitzky-Basso I, Siddique S, McSkeane T, Ryan G, Vyas P, Cavenagh J, Stankovic T, Moss P, Craddock C (2010) Induction of a CD8+ T-cell response to the MAGE cancer testis antigen by combined treatment with azacitidine and sodium valproate in patients with acute myeloid leukemia and myelodysplasia. Blood 116(11):1908–1918
36. Grunwald C, Koslowski M, Arsiray T, Dhaene K, Praet M, Victor A, Morresi-Hauf A, Lindner M, Passlick B, Lehr HA, Schafer SC, Seitz G, Huber C, Sahin U, Tureci O (2006) Expression of multiple epigenetically regulated cancer/germline genes in nonsmall cell lung cancer. Int J Cancer 118(10):2522–2528
37. Haas GG Jr, D'Cruz OJ, De Bault LE (1988) Distribution of human leukocyte antigen-ABC and -D/DR antigens in the unfixed human testis. Am J Reprod Immunol Microbiol 18(2):47–51
38. Hoffmann MJ, Muller M, Engers R, Schulz WA (2006) Epigenetic control of CTCFL/BORIS and OCT4 expression in urogenital malignancies. Biochem Pharmacol 72(11):1577–1588

39. Holliday R, Pugh JE (1975) DNA modification mechanisms and gene activity during development. Science 186:226–232
40. Hong JA, Kang Y, Abdullaev Z, Flanagan PT, Pack SD, Fischette MR, Adnani MT, Loukinov DI, Vatolin S, Risinger JI, Custer M, Chen GA, Zhao M, Nguyen DM, Barrett JC, Lobanenkov VV, Schrump DS (2005) Reciprocal binding of CTCF and BORIS to the NY-ESO-1 promoter coincides with derepression of this cancer-testis gene in lung cancer cells. Cancer Res 65(17):7763–7774
41. Jackson K, Yu MC, Arakawa K, Fiala E, Youn B, Fiegl H, Muller-Holzner E, Widschwendter M, Ehrlich M (2004) DNA hypomethylation is prevalent even in low-grade breast cancers. Cancer Biol Ther 3(12):1225–1231
42. James SR, Link PA, Karpf AR (2006) Epigenetic regulation of X-linked cancer/germline antigen genes by DNMT1 and DNMT3b. Oncogene 25(52):6975–6985
43. Jones PA, Wolkowicz MJ, Rideout WM III, Gonzales FA, Marziasz CM, Coetzee GA, Tapscott SJ (1990) De novo methylation of the MyoD1 CpG island during the establishment of immortal cell lines. Proc Natl Acad Sci USA 87(16):6117–6121
44. Jungbluth AA, Chen YT, Stockert E, Busam KJ, Kolb D, Iversen K, Coplan K, Williamson B, Altorki N, Old LJ (2001) Immunohistochemical analysis of NY-ESO-1 antigen expression in normal and malignant human tissues. Int J Cancer 92(6):856–860
45. Kaneda A, Tsukamoto T, Takamura-Enya T, Watanabe N, Kaminishi M, Sugimura T, Tatematsu M, Ushijima T (2004) Frequent hypomethylation in multiple promoter CpG islands is associated with global hypomethylation, but not with frequent promoter hypermethylation. Cancer Sci 95(1):58–64
46. Kang Y, Hong JA, Chen GA, Nguyen DM, Schrump DS (2007) Dynamic transcriptional regulatory complexes including BORIS, CTCF and Sp1 modulate NY-ESO-1 expression in lung cancer cells. Oncogene 26(30):4394–4403
47. Karpf AR, Lasek AW, Ririe TO, Hanks AN, Grossman D, Jones DA (2004) Limited gene activation in tumor and normal epithelial cells treated with the DNA methyltransferase inhibitor 5-aza-2′-deoxycytidine. Mol Pharmacol 65(1):18–27
48. Karpf AR, Bai S, James SR, Mohler JL, Wilson EM (2009) Increased expression of androgen receptor coregulator MAGE-11 in prostate cancer by DNA hypomethylation and cyclic AMP. Mol Cancer Res 7(4):523–535
49. Kholmanskikh O, Loriot A, Brasseur F, De Plaen E, De Smet C (2008) Expression of BORIS in melanoma: lack of association with MAGE-A1 activation. Int J Cancer 122(4):777–784
50. Khong HT, Wang QJ, Rosenberg SA (2004) Identification of multiple antigens recognized by tumor-infiltrating lymphocytes from a single patient: tumor escape by antigen loss and loss of MHC expression. J Immunother 27(3):184–190
51. Kondo T, Zhu X, Asa SL, Ezzat S (2007) The cancer/testis antigen melanoma-associated antigen-A3/A6 is a novel target of fibroblast growth factor receptor 2-IIIb through histone H3 modifications in thyroid cancer. Clin Cancer Res 13(16):4713–4720
52. Koslowski M, Bell C, Seitz G, Lehr HA, Roemer K, Muntefering H, Huber C, Sahin U, Tureci O (2004) Frequent nonrandom activation of germ-line genes in human cancer. Cancer Res 64(17):5988–5993
53. Lin CH, Hsieh SY, Sheen IS, Lee WC, Chen TC, Shyu WC, Liaw YF (2001) Genome-wide hypomethylation in hepatocellular carcinogenesis. Cancer Res 61(10):4238–4243
54. Link PA, Gangisetty O, James SR, Woloszynska-Read A, Tachibana M, Shinkai Y, Karpf AR (2009) Distinct roles for histone methyltransferases G9a and GLP in cancer germ-line antigen gene regulation in human cancer cells and murine embryonic stem cells. Mol Cancer Res 7(6):851–862
55. Lorincz MC, Schubeler D, Goeke SC, Walters M, Groudine M, Martin DI (2000) Dynamic analysis of proviral induction and de novo methylation: implications for a histone deacetylase-independent, methylation density-dependent mechanism of transcriptional repression. Mol Cell Biol 20(3):842–850
56. Loriot A, Boon T, De Smet C (2003) Five new human cancer-germline genes identified among 12 genes expressed in spermatogonia. Int J Cancer 105(3):371–376

57. Loriot A, De Plaen E, Boon T, De Smet C (2006) Transient down-regulation of DNMT1 methyltransferase leads to activation and stable hypomethylation of MAGE-A1 in melanoma cells. J Biol Chem 281(15):10118–10126
58. Loriot A, Sterpin C, De Backer O, De Smet C (2008) Mouse embryonic stem cells induce targeted DNA demethylation within human MAGE-A1 transgenes. Epigenetics 3(1):38–42
59. Loukinov DI, Pugacheva E, Vatolin S, Pack SD, Moon H, Chernukhin I, Mannan P, Larsson E, Kanduri C, Vostrov AA, Cui H, Niemitz EL, Rasko JE, Docquier FM, Kistler M, Breen JJ, Zhuang Z, Quitschke WW, Renkawitz R, Klenova EM, Feinberg AP, Ohlsson R, Morse HC III, Lobanenkov VV (2002) BORIS, a novel male germ-line-specific protein associated with epigenetic reprogramming events, shares the same 11-zinc-finger domain with CTCF, the insulator protein involved in reading imprinting marks in the soma. Proc Natl Acad Sci USA 99(10):6806–6811
60. Lucas S, De Smet C, Arden KC, Viars CS, Lethe B, Lurquin C, Boon T (1998) Identification of a new MAGE gene with tumor-specific expression by representational difference analysis. Cancer Res 58(4):743–752
61. Lurquin C, De Smet C, Brasseur F, Muscatelli F, Martelange V, De Plaen E, Brasseur R, Monaco AP, Boon T (1997) Two members of the human MAGEB gene family located in Xp21.3 are expressed in tumors of various histological origins. Genomics 46(3):397–408
62. Macleod D, Charlton J, Mullins J, Bird AP (1994) Sp1 sites in the mouse aprt gene promoter are required to prevent methylation of the CpG island. Genes Dev 8(19):2282–2292
63. Martelange V, De Smet C, De Plaen E, Lurquin C, Boon T (2000) Identification on a human sarcoma of two new genes with tumor-specific expression. Cancer Res 60(14):3848–3855
64. Monte M, Simonatto M, Peche LY, Bublik DR, Gobessi S, Pierotti MA, Rodolfo M, Schneider C (2006) MAGE-A tumor antigens target p53 transactivation function through histone deacetylase recruitment and confer resistance to chemotherapeutic agents. Proc Natl Acad Sci USA 103(30):11160–11165
65. Nagao T, Higashitsuji H, Nonoguchi K, Sakurai T, Dawson S, Mayer RJ, Itoh K, Fujita J (2003) MAGE-A4 interacts with the liver oncoprotein gankyrin and suppresses its tumorigenic activity. J Biol Chem 278(12):10668–10674
66. Novak P, Jensen T, Oshiro MM, Watts GS, Kim CJ, Futscher BW (2008) Agglomerative epigenetic aberrations are a common event in human breast cancer. Cancer Res 68(20):8616–8625
67. Novak P, Jensen TJ, Garbe JC, Stampfer MR, Futscher BW (2009) Stepwise DNA methylation changes are linked to escape from defined proliferation barriers and mammary epithelial cell immortalization. Cancer Res 69(12):5251–5258
68. Oikawa T, Yamada T (2003) Molecular biology of the Ets family of transcription factors. Gene 303:11–34
69. Peikert T, Specks U, Farver C, Erzurum SC, Comhair SA (2006) Melanoma antigen A4 is expressed in non-small cell lung cancers and promotes apoptosis. Cancer Res 66(9): 4693–4700
70. Rao M, Chinnasamy N, Hong JA, Zhang Y, Zhang M, Xi S, Liu F, Marquez VE, Morgan RA, Schrump DS (2011) Inhibition of histone lysine methylation enhances cancer-testis antigen expression in lung cancer cells: implications for adoptive immunotherapy of cancer. Cancer Res 71(12):4192–4204
71. Rauch TA, Zhong X, Wu X, Wang M, Kernstine KH, Wang Z, Riggs AD, Pfeifer GP (2008) High-resolution mapping of DNA hypermethylation and hypomethylation in lung cancer. Proc Natl Acad Sci USA 105(1):252–257
72. Riggs AD (1989) DNA methylation and cell memory. Cell Biophys 15(1–2):1–13
73. Sahin U, Tureci O, Schmitt H, Cochlovius B, Johannes T, Schmits R, Stenner F, Luo G, Schobert I, Pfreundschuh M (1995) Human neoplasms elicit multiple specific immune responses in the autologous host. Proc Natl Acad Sci USA 92:11810–11813
74. Sahin U, Tureci O, Chen YT, Seitz G, Villena-Heinsen C, Old LJ, Pfreundschuh M (1998) Expression of multiple cancer/testis (CT) antigens in breast cancer and melanoma: basis for polyvalent CT vaccine strategies. Int J Cancer 78(3):387–389

75. Scanlan MJ, Gordon CM, Williamson B, Lee SY, Chen YT, Stockert E, Jungbluth A, Ritter G, Jager D, Jager E, Knuth A, Old LJ (2002) Identification of cancer/testis genes by database mining and mRNA expression analysis. Int J Cancer 98(4):485–492
76. Scanlan MJ, Simpson AJ, Old LJ (2004) The cancer/testis genes: review, standardization, and commentary. Cancer Immun 4:1
77. Shen L, Kondo Y, Guo Y, Zhang J, Zhang L, Ahmed S, Shu J, Chen X, Waterland RA, Issa JP (2007) Genome-wide profiling of DNA methylation reveals a class of normally methylated CpG island promoters. PLoS Genet 3(10):2023–2036
78. Sigalotti L, Coral S, Nardi G, Spessotto A, Cortini E, Cattarossi I, Colizzi F, Altomonte M, Maio M (2002) Promoter methylation controls the expression of MAGE2, 3 and 4 genes in human cutaneous melanoma. J Immunother 25(1):16–26
79. Simpson AJ, Caballero OL, Jungbluth A, Chen YT, Old LJ (2005) Cancer/testis antigens, gametogenesis and cancer. Nat Rev Cancer 5(8):615–625
80. Suske G (1999) The Sp-family of transcription factors. Gene 238(2):291–300
81. Swann JB, Smyth MJ (2007) Immune surveillance of tumors. J Clin Invest 117(5):1137–1146
82. Takahashi K, Shichijo S, Noguchi M, Hirohata M, Itoh K (1995) Identification of MAGE-1 and MAGE-4 proteins in spermatogonia and primary spermatocytes of testis. Cancer Res 55(16):3478–3482
83. Tilman G, Loriot A, Van Beneden A, Arnoult N, Londono-Vallejo JA, De Smet C, Decottignies A (2009) Subtelomeric DNA hypomethylation is not required for telomeric sister chromatid exchanges in ALT cells. Oncogene 28(14):1682–1693
84. Van den Eynde B, Peeters O, De Backer O, Gaugler B, Lucas S, Boon T (1995) A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. J Exp Med 182:689–698
85. van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, Knuth A, Boon T (1991) A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science 254(5038):1643–1647
86. Van Der Bruggen P, Zhang Y, Chaux P, Stroobant V, Panichelli C, Schultz ES, Chapiro J, Van Den Eynde BJ, Brasseur F, Boon T (2002) Tumor-specific shared antigenic peptides recognized by human T cells. Immunol Rev 188(1):51–64
87. Vatolin S, Abdullaev Z, Pack SD, Flanagan PT, Custer M, Loukinov DI, Pugacheva E, Hong JA, Morse H III, Schrump DS, Risinger JI, Barrett JC, Lobanenkov VV (2005) Conditional expression of the CTCF-paralogous transcriptional factor BORIS in normal cells results in demethylation and derepression of MAGE-A1 and reactivation of other cancer-testis genes. Cancer Res 65(17):7751–7762
88. Walsh CP, Bestor TH (1999) Cytosine methylation and mammalian development. Genes Dev 13(1):26–34
89. Wang Z, Zhang J, Zhang Y, Lim SH (2006) SPAN-Xb expression in myeloma cells is dependent on promoter hypomethylation and can be upregulated pharmacologically. Int J Cancer 118(6):1436–1444
90. Wang J, Emadali A, Le Bescont A, Callanan M, Rousseaux S, Khochbin S (2011) Induced malignant genome reprogramming in somatic cells by testis-specific factors. Biochim Biophys Acta 1809(4–6):221–225
91. Weber J, Salgaller M, Samid D, Johnson B, Herlyn M, Lassam N, Treisman J, Rosenberg SA (1994) Expression of the MAGE-1 tumor antigen is up-regulated by the demethylating agent 5-aza-2′-deoxycytidine. Cancer Res 54(7):1766–1771
92. Weber M, Davies JJ, Wittig D, Oakeley EJ, Haase M, Lam WL, Schubeler D (2005) Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. Nat Genet 37(8):853–862
93. Weber M, Hellmann I, Stadler MB, Ramos L, Paabo S, Rebhan M, Schubeler D (2007) Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. Nat Genet 39(4):457–466
94. Wilson VL, Jones PA (1983) DNA methylation decreases in aging but not in immortal cells. Science 220(4601):1055–1057

95. Woloszynska-Read A, James SR, Link PA, Yu J, Odunsi K, Karpf AR (2007) DNA methylation-dependent regulation of BORIS/CTCFL expression in ovarian cancer. Cancer Immun 7:21
96. Woloszynska-Read A, Mhawech-Fauceglia P, Yu J, Odunsi K, Karpf AR (2008) Intertumor and intratumor NY-ESO-1 expression heterogeneity is associated with promoter-specific and global DNA methylation status in ovarian cancer. Clin Cancer Res 14(11):3283–3290
97. Woloszynska-Read A, James SR, Song C, Jin B, Odunsi K, Karpf AR (2010) BORIS/CTCFL expression is insufficient for cancer-germline antigen gene expression and DNA hypomethylation in ovarian cell lines. Cancer Immun 10:6
98. Yang B, O'Herrin SM, Wu J, Reagan-Shaw S, Ma Y, Bhat KM, Gravekamp C, Setaluri V, Peters N, Hoffmann FM, Peng H, Ivanov AV, Simpson AJ, Longley BJ (2007) MAGE-A, mMage-b, and MAGE-C proteins form complexes with KAP1 and suppress p53-dependent apoptosis in MAGE-positive cell lines. Cancer Res 67(20):9954–9962
99. Yang B, Wu J, Maddodi N, Ma Y, Setaluri V, Longley BJ (2007) Epigenetic control of MAGE gene expression by the KIT tyrosine kinase. J Invest Dermatol 127(9):2123–2128
100. Yegnasubramanian S, Haffner MC, Zhang Y, Gurel B, Cornish TC, Wu Z, Irizarry RA, Morgan J, Hicks J, DeWeese TL, Isaacs WB, Bova GS, De Marzo AM, Nelson WG (2008) DNA hypomethylation arises later in prostate cancer progression than CpG island hypermethylation and contributes to metastatic tumor heterogeneity. Cancer Res 68(21):8954–8967
101. Yoder JA, Walsh CP, Bestor TH (1997) Cytosine methylation and the ecology of intragenomic parasites. Trends Genet 13(8):335–340
102. Zhu X, Asa SL, Ezzat S (2008) Fibroblast growth factor 2 and estrogen control the balance of histone 3 modifications targeting MAGE-A3 in pituitary neoplasia. Clin Cancer Res 14(7):1984–1996