From aging to cancer: a DNA methylation journey

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Abstract

Epigenetic gene silencing through DNA promoter hypermethylation is now recognized as a major step in the neoplastic transformation of the cell. The methylation levels of several genes increase with age in normal tissues such as the prostate or colon. Genes like *WRN* or *LMNA* are involved in progeria, a premature aging disease. *WRN* and *LMNA* are epigenetically inactivated in cancer. In both aging and cancer, global DNA methylation decreases, potentially accounting for the characteristic genomic instability of these processes. In this review, we will focus on how the accumulation of changes in DNA methylation during aging impact tumorigenesis.

Introduction

Epigenetic changes that take place during the neoplastic process in cells have been a focus of study for more than a decade.[1] Moreover, epigenetic changes, including DNA methylation, acetylation, ubiquitination and phosphorylation of histone tails, could also play an important role in cellular senescence and organism aging.[2]

In cancer, DNA methylation of promoters at CpG islands (CGIs) plays a critical role in silencing expression of some genes, including tumor-suppressor genes.[1] There is also a decrease in global DNA methylation that may cause the genomic instability that is characteristic of the cancer cell.[1]

Both an increase in DNA methylation of promoters and a decrease in global DNA methylation have been reported in aged tissues.[3,4] These aging-related methylation changes may cause the well-known epigenetic changes observed in cancer, such as gene silencing, associated with CGIs promoter hypermethylation and genome instability.

Furthermore, certain genes that play a dual role in cancer and aging, such as *WRN* and *LMNA*, both of which are involved in the pathogenesis of progeria, are epigenetically silenced through the methylation of their promoters.[5,6] *WRN* is silenced in a wide variety of tumors, while *LMNA* is silenced in lymphoma and leukemia.[5,6] Moreover, recent evidence suggests that *WRN* can be subject to alterations in DNA methylation in different aged tissues, as has been previously proposed.[2]

DNA methylation: an overview

DNA methylation is probably the best documented example of epigenetic modification. This modification involves the attachment of a covalent methyl (CH3) group to position C5 of the cytosine ring of Cytosine-Guanine pairs (CpGs) in the genome (5meC).[1] It is estimated that, in mammals, about 60-90% of cytosines within CpGs are constitutively methylated with the exception of CGIs CGIs. CGIs are dense clusters of CpG dinucleotides, at least 200 base pairs (bp) long that are situated in or near gene promoters and often mark transcription initiation sites. In normal cells, CpG methylation plays a critical role in maintaining gene silencing. This process of gene silencing is necessary for a wide variety of cell functions, including tissue- and development-specific gene expression, X-chromosome silencing, genomic imprinting, and protection against expression of intragenomic parasite elements.[1]

Methylated CpGs (mCpGs)

In normal mammalian cells, the CpG dinucleotide is underrepresented due to spontaneous deamination of methylated cytosines and inaccurate repair to thymines.[7,8]

mCpGs are distributed all over the genome, either in gene bodies, intergenic regions or non-coding repetitive elements.[9]

More than a third of the human genome is composed of gene bodies, or the transcribed portions of genes. CpG pairs of gene bodies are methylated, as described previously,[10] accounting for the majority of the methylated genome CpGs.[10-12] (Figure 1A)

About 20% of all internal exons are densely methylated across their entire length, while only small regions within introns are methylated.[13] The significance of this difference still needs to be clarified,[14] but one possibility is that this contributes to splicing regulation.[15]

Overall, it can be argued that gene-body methylation may specifically protect transcriptionally-active regions from deleterious processes, such as initiation of improper pseudo gene-body transcription, incorrect expression of the parent gene and/or chromosomal aberrations.[16] Gene-body methylation may also help to regulate transcription elongation rates[16] and control alternative promoter usage[16,17] (Figure 1A). Notably, some intragenic CpGs colocalize with sites of antisense non-coding RNA (*ncRNA*) transcription initiation, such as *Air*, and *Tsix*, which negatively regulate the expression of the sense transcript.[18-20] HOTAIR, an *ncRNA* transcribed from the *HOXC* locus, can repress in trans the expression of the *HOXD* cluster. In all the aforementioned cases, methylation can repress the respective non-coding RNA, consequently derepressing the target gene (s).[21]

Moreover, some methylated CpG clusters can regulate replication origin activity, as in the case of ori-β in the dihydrofolate reductase (*DHFR*) locus of Chinese hamster ovary (CHO) cells.[22,23]

The role of intergenic hypermethylated CpGs in gene transcription is less clear. For instance, parent-specific methylation at an upstream intergenic CpG cluster in the H19/IGF2 imprinted locus prevents the association of the insulator element CTCF and determines expression of the imprinted locus.[24]

Finally, 35-50% of the human genome is composed of interspersed transposon-derived repeats, such as Long Interspersed Nuclear Elements (LINE-1) and Alu elements[25] (Table 1). These interspersed repeats are often rich in CpG regions, which are methylated very early during embryonic development.[25-29]

Heterochromatic regions of telomeres (subtelomeric regions) are mostly composed of minisatellite extragenic variable number tandem repeats (minisatellite-9-64pb-VNTRs) (Table 1).[4] Some VNTRs have a high content of methylated CpGs[4] (Table 1). Although the role of epigenetic regulation of VNTRs still needs to be clarified,[30] the involvement of macrosatellite VNTR (several kb) in certain diseases, such as

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Fragile X syndrome and Facioscapulohumeral Dystrophy (FSHD), has shed some light on this phenomenon. In Fragile X syndrome, an expansion of the promoter CpGs[31] of the *FMR1* gene triggers abnormal DNA methylation, which in turn leads to *FMR1* silencing.[32-34] On the other hand, FSHD is thought to be caused by reduced methylation of the D4Z4 macrosatellite[35] due to the reduced VNTR length present in the disease.[36,37]

What is more, pericentromeric regions are partly composed of methylated GC-rich Satellite II and III[4] (Table 1).

CGIs and CpG shores

In contrast to other genome regions, CGIs are regions of permissive chromatin state and they are typically 200-3000bp in length. CGIs are defined by sequence characteristics[1] because they constitute a high proportion of repetitive CpGs . CGIs surround gene promoters, Transcription Start Sites (TSSs), and/or first exons, and remain constitutively free of DNA methylation in normal cells.

In approximately 60-70% of human genes, including almost all housekeeping genes and some individual genes, promoters overlap with non-methylated CGIs for a length of 1kb.[38]

Although not all these CGIs localize to annotated TSSs, it is likely that all CGIs represent transcriptional initiation sites that have not yet been characterized. Interestingly, recent reports have found that almost 50% of replication origins are located in or close to CGIs.[39-41]

In fact, it was proposed earlier that CGIs may be footprints of promoters that are associated with replication origins.[42,43]

Recently, a new class of intragenic CpGs has been defined: the CpG *shores*.[44]

CpG shores are low density CpG regions in close proximity (approximately 2kb) to classical CGIs that also extend beyond the flanks of the classical CGIs (Figure1). Their methylation is tightly linked with transcriptional inactivation, and it has been shown that most of the tissue-specific intragenic CpG methylation occurs at these CpG shores instead of at classical CGIs[45,46] (Figure 1B).

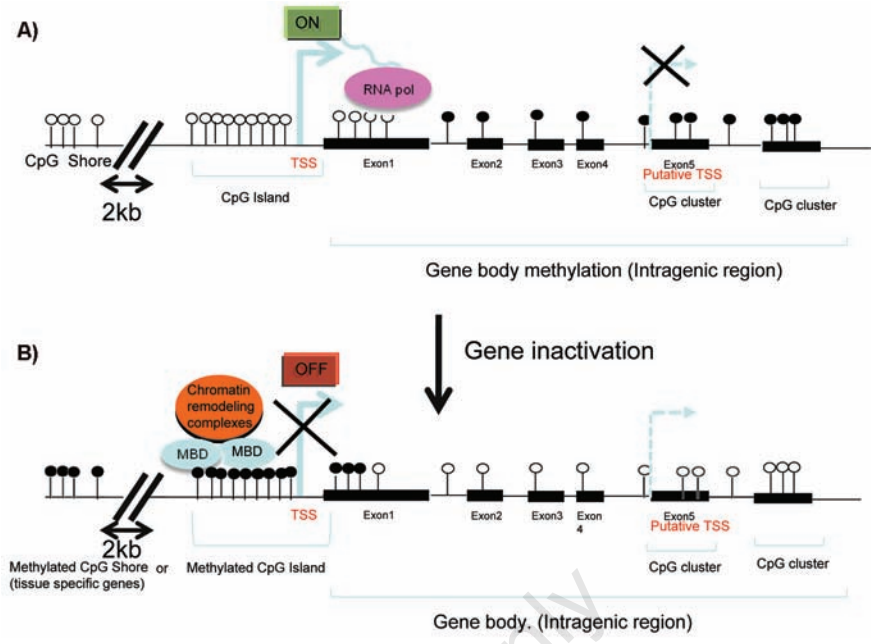

Figure 1. Gene inactivation due to methylation. (A) Gene representation showing genomic features, including CpG shores and CpG Islands at the promoter region and the intergenic region. A CpG shore in an unmethylated state, located about 2kb from the transcription start site (TSS - sky blue arrow) is shown. A CpG island located at the promoter and part of the first exon is represented. The gene body or intragenic region is methylated. Locations where CpG clusters could coincide with putative TSS CpGs are represented as white lollipops. (B) CpG shores can become methylated and inactivate a gene that is located 2kb away. This phenomenon is particularly seen in tissue-specific genes. Methylation of a CpG island can turn off transcription by recruiting MBD proteins and chromatin-remodeling factors. On the other hand, demethylation of intragenic CpG clusters could potentially activate putative TSSs.

DNA methylation machinery and function

DNA methylation is catalyzed by the DNA methyltransferase (DNMTs) family of enzymes, which transfer the methyl group from S-adenosyl methionine (SAM) to the cytosine ring.[1,7]

Thus far, five types of DNMTs have been identified in mammals: DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3L. Curiously, only three of the members of this family possess methyltransferase activity: DNMT1, DNMT3a and DNMT3b.[1,7] These enzymes are classified by their role in the methylation process. For example, DNMT3a and DNMT3b are *de novo* DNMTs and DNMT1 is a maintenance DNMT.[1,7]

DNA methylation patterns are established during embryogenesis by DNMT3a and DNMT3b, and are maintained throughout successive cell divisions by DNMT1. Therefore, DNMT1 catalyzes methylation patterns from a template to newly synthesized DNA strands following DNA replication[1,7] In the absence of this enzyme, the methylation patterns are gradually erased through cell divisions. This is a process referred to as *passive demethylation*.[14]

DNMT1 is the most represented DNMT in the cell and is mainly transcribed during the S phase of the cell cycle.[14] Even though this enzyme may exhibit *de novo* activity in certain circumstances, its main role in the cell is to methylate the hemimethylated sites that are produced during semi-conservative DNA replication.[14] The N-terminal domain of DNMT1 (and DNMT3a)[47,48] can bind to histone deacetylases (HDACs), which promote histone deacetylation, which ultimately suppress gene expression.[48] Interestingly, gene-specific promoter methylation is abolished in cells with a mutated DNMT3b gene.[14] Thus, DNMT3b may act as a transcription-coupled methyltransferase in somatic cells, in addition to its role as a *de novo* methyltransferase during embryogenesis. Given its limited expression level in somatic cells,[14] coupling with the transcriptional machinery may focus its activity at particularly important targets.[14]

Despite being catalytically inactive, DNMT3L is expressed during gametogenesis and is required for the establishment of maternal genomic imprinting[49] and regulating DNMT3a and DNMT3b.[50] On the other hand, DNMT2 has been reported to be responsible for tRNAAsp methylation.[51] Methylation of the promoter CGIs can lead to gene suppression by at least three main mechanisms. First, it can prevent the binding of transcriptional machinery or other *cis* regulatory elements, such as AP-2[52], ATF/CREB[53], c-myc[54] or Sp1/Sp3,[55,56] to their specific sequences within the promoters regions.[57] Second, methylcytosine-binding proteins (MBP), such as MeCP2 and other members of the methyl-binding domain (MBD) family of proteins[57] can exclude the transcriptional machinery from the promoter region. Three families of MBP have been identified; the MBD family which include MBD1-4 and MeCP2, the zinc finger proteis Kaiso (a

methyl-cytosine binding protein composed of a POZ-domain and C2H2 zinc finger-domain), ZBT4, ZBTB38, and the SET-and-RING finger-associated proteins UHRF1 and UHFR2.[44] Through interactions with histone deacetylases, histone methyltransferases (*e.g.* HP1), and ATP-dependent chromatin remodeling enzymes, the MBDs can translate methylated DNA into a compacted chromatin environment that is repressive for transcription.[44] For example, loss of MBD2 and KAISO in APC min/+ mice suppresses intestinal carcinogenesis and induces tumor-suppressor gene expression in cancer cell lines.[58,59]

Finally, as already mentioned, these proteins can recruit chromatin inactivation complexes, such as histone deacetylases and histone methylases,[60] which lead to a condensed chromatin status at the promoter region. This results in stable transcriptional repression[7,38] and correlates with tissue-specific gene silencing, as in the case of GATA2.[61]

Briefly, when transcriptionally active, genes are enriched with di- and trimethylated histone H3 lysine4 (H3K4me2/me3) and have acetylation of key H3 and H4 amino acids. When genes are inactive, they lack or have minimum levels (bivalent chromatin) of these patterns but are enriched in trimethylated histone H3 lysines 9 and 27 (H3K9me3), and (H3K27me3) and trimethylation of H4K20(H4K20me3).[44] Bivalent chromatin is mediated by the polycomb group (PcG) of proteins. PcG proteins are composed of two repressive complexes: the repressive complex 1 (PRC1) and 2 (PRC2). PRC1 contains Bmi1/Mel18, MPH 1/2, Ring1a/b and CBX, whereas PRC2 contains EED, Suz12 and lysine methyltransferase EZH1/2.[62]

While PRC2 initiates long-term repression of target genes by forming H3K27me3, and to a lesser extent H3K9me3, PRC1 recognizes H3K27 and maintains a silenced state by monoubiquitination of H2AK119.[62] It has been suggested that PRC-mediated transcriptional silencing predisposes PcG target genes to hypermethylation of the CpG island. Recently, EZH2 and CBX7 have been linked to DNMTs, suggesting a role for these proteins in inducing and targeting methylation to CpG islands.[63,64]

It is well-established that CGI methylation is intimately associated with gene silencing. In normal cells, DNA methylation plays an important role in imprinting, where hypermethylation at the locus of one of the parents determines monoallelic expression.[65-67]

Moreover, DNA methylation is involved in X-chromosome inactivation (maintenance phase) in female cells.[65]

Tissue-specific genes can also be regulated by methylation, as is the case for genes involved in developmental processes, such as PAX6, OSR1 and the Homeobox Ho x superfamily.[67-70]

However, further study is required to test the hypothesis that tissue-specific DNA methylation in certain genes plays a role in cell identity.

Importantly, DNA methylation in repetitive elements can protect the genome from reactivation of endoparasitic sequences.

Interestingly, several genome-wide studies have analyzed DNA replication timing and found a tight coordination between gene regulation and replication timing, in that GC-rich regions replicate early and AT-rich regions replicate late.[41]

DNA hypomethylation: from aging to cancer

It is well-known that advancing age is one of the major risk factors for developing tumors in humans.[71] Cancer and aging are two processes that are characterized by global hypomethylation and focal hypermethylation of CpG islands.[23,72] The term hypomethylation refers to a decrease from the *normal* methylation level of DNA.[72]

Early studies have indicated that DNA methylation patterns may be of significance in the aging process. For example, as opposed to

Table 1. Classification of silenced repeat elements during the aging and cancer processes.[26]

Non-transposable tandem arrays of short sequences			
Macrosatellites are blocks of hundreds of kb with a repeat size of several kb. They are VNTRs and can be rich in GC content.	Satellites have sequences greater than 100kb and are composed of repeats from 5 to 200bp in length. Centromeric alphoid or Satellite alpha is composed of 171bp sequence that is repeated many times, resulting in a region of about 500kb. Satellite beta (Sau3A family) is composed of a 68bp sequence that is repeated many times. Pericentromeric regions are composed of Sat1 (25-48bp), 2 and 3 (5bp). Sat2 and 3 are more abundant than Sat1 and can undergo demethylation during aging.	Mini-Satellites are members of the Telomeric family. The sequences are less than 20kb long and are composed of repeats less than 25bp in length. Telomeres have tandem repeats of 6bp (TTAGG) that are not subject to hypomethylation. Subtelomeric family (9-64bp): sequences are located in subtelomeric regions. They can can suffer from hypomethylation. The number of repeats varies among individuals, so they are known as variable numbers of tandem repeats, or VNTRs.	Microsatellites are normally less than 150bplong and are composed of 1-4bp sequences. They are also VNTRs .Microsatellites rich in GC content can suffer from hypomethylation
Interspersed transposon-derived repeats			
Active DNA transposons are 2-3kb in length and contain the transposase gene flanked by terminal repeat sequences. Inactive transposons have inverted repeats but lack the transposase gene. They spread using a copy-paste mechanism and represent 3% of the human genome.	LTR retrosposons or Retroposons with a long terminal repeat (LTR). Retrosposons are retrovirus-like elements and contain long terminal repeats and necessary signals to allow transposition within the genome. This process requires transcription to form a DNA copy which then integrates into the genome. They transpose via an intermediate RNA and represent 8% of the genome.	LINEs or Long interspersed elements are retroelements of 6-8 kb in length that do not have the LTR sequence. They encode two proteins involved in the transposition of the sequence within the genome. The mechanism used during transposition is inefficient and frequently results in truncated forms of the LINEs being transposed. They transpose via RNA intermediates and represent 20% of the genome.	SINEs or Short interspersed elements are short sequences of DNA about 100-300bp long. They cannot transpose without using functions provided by LINE elements. The most abundant SINE is the Alu element which has more than 1×106 copies in the genome and is 300bp in length. Alu elements transpose via an intermediate RNA and represent 20% of the genome.

immortal cell lines, there is a decrease in the 5meC content of DNA in cultured normal diploid mouse, hamster or human fibroblasts as their number of cell divisions increases.[73]

Because of these findings, in 1985 Holliday proposed that the loss of 5meC may function as a mechanism to monitor the number of times a cell is capable of dividing during its lifetime.[74] Subsequently, multiple studies established that normal aging cells derived from mice, rats and humans show a progressive loss of 5meC.[75-78]

Age-related methylation was also observed in lymphocytes, in addition to fibroblasts.[79]

Indeed, a developmental analysis of 5meC content in various mouse tissues of different ages has demonstrated that the rate of loss of methylated cytosines is inversely correlated to life span.[75]

Moreover, *in vitro* studies of human fibroblasts (MRC-5) showed that artificially-induced DNA demethylation, with the demethylating agent 5-aza-2'-deoxycytidine (5-aza-Dc), substantially shortened the life span of the cells.[80,81]

Because much of the human genome (about 55%) consists of repetitive elements, which contain the LINE-1 and Alu elements (representing approximately 30% of the total),[25] and because the majority of cytosine methylation take place within the CG-rich repetitive DNA sequences of the genome,[25,82,83] it is not surprising that interspread transposons as well as minisatellite and tandem repeats[84] become hypomethylated over time,[85,86] and contribute significantly to the global genetic demethylation that comes with age (Figure 2).[2]

In addition to the decreased methylation status of the repetitive sequences of the genome, there is also a decrease in the methylation status and activation of numerous single-copy genes with age,[87-90] such as c-myc in mouse spleen, NR2B in mouse brain, and the estrogen receptor I gene methylation in rat mammary glands.[87,88,90]

DNA hypomethylation with age has been found to reactivate imprinted genes, such as IGF2 in mouse prostatic tissue.[91]

A model of cellular aging led to the hypothesis that age-dependent DNA hypomethylation could affect the replication activity of origins located in CpG clusters (not associated with promoters), which are active when the clusters are methylated, reducing the DNA replication capacity of the cell.[22,23] In support of this hypothesis, the demethylation of ori-β in the *DHFR* locus of CHO cells by 5-aza led to a reduction in its activity.[22]

Cells approaching senescence exhibit decreased DNMTA-methyltransferase activity, leading to a global decrease in DNA methylation.[92,93] DNMT1 has been found to be downregulated in aged cultured fibroblasts. This observation was confirmed *in vitro* and *in vivo*, proving that expression of DNMT1 and DNMT3a decreased with aging.[92,93]

The exact mechanism of DNA hypomethylation is still unclear, but it has been proposed that the loss of methylation may reflect the inefficiency of DNMT1 to maintain the hypermethylated status of repeated heterochromatic DNA.[92,93] Consistently, a deletion of DNMT1 in mice shortens their lifespan.[94] Finally, the increased activity of DNMT3b that was observed in aged cultured fibroblasts[93] may reflect a mechanism to compensate for the loss of methylation in repetitive DNA.[2]

DNA hypomethylation is a central feature of both human aged cells and cancer cells. Therefore, there are several ways in which hypomethylation can contribute to the neoplastic process. First, hypomethylation could contribute to carcinogenesis when silenced transposons (such as Line-1 and Alu) are transcribed in or relocated to other genomic regions, resulting in a disruption of the function of cellular genes. (Figure 2)

Reactivated LINE-1 transcripts may insert themselves into functional sequences, participating in homologous recombination and deregulation of protein expression, which may contribute to carcinogenesis.[95]

Individuals with LINE-1 hypomethylation showed increased risk of developing cancers.[95]

Regarding cancer prognosis, hypomethylation of LINE-1 elements in human tumor tis-

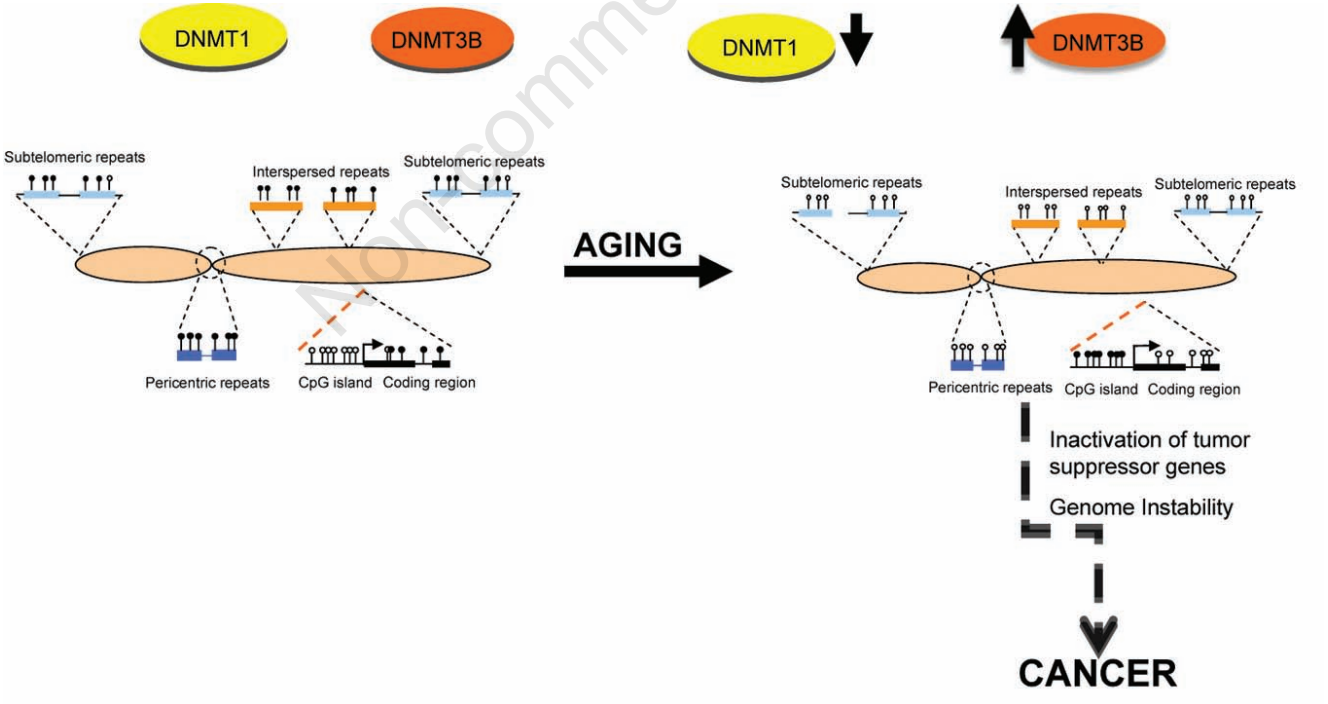

Figure 2. During the aging process, there is an increase in DNMT1 levels (yellow) that may cause demethylation of interspersed repeats (orange), subtelomeric repeats (skyblue) and pericentric repeats (blue). As a result, an increase in the DNMT3b levels (orange) may cause hypermethylation of gene promoters. These changes in the methylation patterns during the aging process can produce genome instability. This could be due to global hypomethylation or inactivation of tumor-suppressor genes by gene promoter hypermethylation, ultimately leading to cancer.

sues or serum has been associated with poorer survival of patients with cancer[96,97] and lower methylation of LINE-1 together with, lower methylation of Alu elements was shown to be associated with increased cancer mortality.[95] It has been postulated that repetitive DNA element hypomethylation can be a preceding cause for cancer occurrence.[95]

Moreover, hypomethylation can trigger genomic instability by inducing genomic recombination, resulting in chromosome breaks, translocation or allelic loss[2] In particular, hypomethylation at pericentromeric regions of chromosomes can cause chromosome breakage at those regions, and aneuploidy.[2]

For example, patients with ICF (Immunodeficiency, Centromere instability and Facial anomalies) syndrome have a significant number of chromosomal aberrations.[98]

This syndrome is caused by a mutation in the DNA-methyltransferase-3b (DNMT3b) gene which is associated with hypomethylation at pericentromeric regions.[98]

These patients also have hypomethylated subtelomeric regions, and telomeres are abnormally short in both the telomerase-positive and -negative cells[99] with this phenomena probably mediated by TERRA, a long non-coding RNA.[99]

These findings are in contrast to other mouse studies that showed that DNA hypomethylation in subtelomeric regions of cells lacking the enzymes responsible for DNA methylation both increased Telomere Sister Chromatid Exchange (T-SCE) and telomere elongation, independently of heterochromatic marks.[100]

Second, DNA hypomethylation can cause loss of normal imprinting patterns. Indeed, studies in mice have demonstrated that this loss could itself be tumorigenic.[101] For example, IGF2 is hypomethylated in several tumor types, including breast, liver, lung and colon.[102]

Third, hypomethylation at specific promoters can activate expression of oncogenes. This seems to be the case for *MASPIN*, which becomes hypermethylated in epithelial cells of the prostate and breast,[103] but is hypomethylated in other tumor types where its expression is correlated with the degree of dedifferentiation.[104,105] Other examples include S100P in pancreatic cancer, SNCG in breast and ovarian cancer, DPP6 and oncogenic micro-RNAs.[106] The melanoma-associated gene (*MAGE1-A*) represents an interesting case because it is located on the X-chromosome.[106]

Lastly, there is substantial evidence to support the idea that global hypomethylation correlates with different stages of cancer progression and metastasis in various tumor types, including brain, prostate, hepatocellular, and cervical cancers.[107-110] Furthermore, hypomethylation may appear at earlier stages of cell transformation, such as in pre-invasive colon polyps.[111]

DNA hypermethylation: from aging to cancer

During the last decade, great progress has been made to support the hypothesis that DNA gene promoter methylation during aging contributes to cancer. As mentioned before, the expression of DNMT3b is up-regulated in aged normal diploid fibroblasts, probably as a natural response to loss of methylation in repetitive sequences. It is possible that upregulation of DNMT3b leads to aberrant promoter hypermethylation in CpG islands that are not methylated in normal cells, although this has not been demonstrated.[2]

The first gene identified as becoming hypermethylated with age was the proto-oncogene c-myc in the liver of aged mice.[112] It was also demonstrated that c-fos, another proto-oncogene, is progressively methylated in aged mouse and human liver.[113] However, methylation of c-myc, even though there was a decrease in the transcript with age, was located distant to the promoter. This was also true for c-fos.

Importantly, a set of gene promoters were identified to be hypermethylated with age in the human liver. These included RAS association family 1 (*RASSF1A*) , adematosis polyposis coli (*APC*), *p16*, and glutathione S-transferase p1 (*GSTP1*), among others.[114]

The gastrointestinal tract has also been intensively studied. In the duodenum, the following genes have been investigated: helicase-like transcription factor (*HLTF*), P-caherin (*CDH3*), LIM homeobox protein 1 (*LHX1*), O-6-methylguanine-DNA methyltransferase (*MGMT*), ubiquitin carboxyl-terminal esterase L1(*UCHL1*), suppression of tumourigenicity 14 (*ST14*), serum deprivation response factor-related gene product that binds to c-kinase (*SRBC*), *HPP1* and *SOCS1*.[115,116]

In the duodenum, pancreas and gallbladder, it was found that methylation levels of cyclin D2 increased with age.[117]

Overall, the methylation of various promoter regions of genes (*hMLH1*, *DAP-kinase*, *E-cadherin*, *GSTP1*, *RASSF1A*, *AP*, *p16*, *RUNX3*) were described in a number of organs, including the lung, kidney and liver.[118]

In the human colon, the oestrogen receptor (ER) gene,[119] insulin-like growth factor II,[120] *N33* and *MYOD*[121] were methylated with age.

It has also been proposed that promoter hypermethylation of CpG islands in aging can change the replication timing of the replication origins located in these islands when methylated.[40]

Methylation of CpG islands in gene promoter regions is tightly associated with aberrant silencing of transcription, and along with mutations, is one of the best-characterized mechanisms contributing to the inactivation of tumor-suppressor genes in cancer. In fact, hypermethylation affects more genes than do DNA sequence mutations.[122] Epigenetic silencing could inactivate one or both alleles of a gene or could coexist with a mutation or deletion in the opposite allele.[123] Epigenetic silencing of specific genes can affect virtually all of the mechanisms and stages of cancer development. Some of these include, among others, the DNA repair pathway (*hMLH1*, *MGMT*, *WRN*, *BRCA1*), Ras signaling (*RASSFIA*, *NOREIA*), the cell cycle (*p16*, *p15*, *RB*) and the p53 pathway (*p14*, *p73*, *HIC1*).[1]

Hypermethylation patterns have been found to be tumor type-specific, but it is still unknown why some regions become hypermethylated. Current studies suggest that hypermethylation in cancer is the result of non-specific *de novo* methylation of particular genes that confers some advantage to the cancer cells, resulting in clonal selection. The second possibility is that highly methylated sequences can be methylation *seeds* that spread to the surrounding genomic regions. Interestingly, it has also been suggested that CGIs promoter hypermethylation is pre-programmed by PcG that are normally involved in the regulation of embryonic genes.[124]

Very recently, genomic architecture has been found to be an important factor in promoting CpG island methylation. In the reported study, a remote location from SINE and LINE was found to be associated with the susceptibility of promoter CGIs to aberrant hypermethylation during carcinogenesis. This was independent of epigenetic factors such as RNA polII and the repressive histone mark H3K27me3.[125] Moreover, a link between high order chromatin folding PcG repression and CGIs methylation has been established in colorectal cancer. One study found PcG/H3K27me3-enriched elements at the base of a chromatin loop intersection point over a 100kb region at a *GATA4* non-transcribed locus, but this structure was not present in cells that expressed *GATA4*.[126] These data support the idea that PcG is involved in determining a three-dimensional repressive chromatin structure.[126]

Interestingly, transcription requires the removal of nucleosomes fromTSS. Therefore, promoter hypermethylation results in the occupation of the transcription start site by a nucleosome, as has been described for *hMLH1* in colon cancer.[127,128]

Recently, a phenomenon named Long Range Epigenetic Silencing (LRES) was described. Epigenetic silencing can span large chromosomal regions that contain a significant number of genes. Several tumor-suppressor genes can be methylated simultaneously.[129] For example, LRES has been reported for a 4/MB region on chromosome 3p22 that includes the MLH1 gene in Microsatellite Instability Colorrectal Cancers (*MSI*-H-CRC).[130]

It is also well-known that in certain subsets of cancers, multiple tumor-suppressor genes could be systematically disrupted.

This alteration is known as CpG island methylator phenotype (CIMP), and in a subset of colon cancers, this has been associated with a genotype that includes mutations of the *BRAF* oncogene and microsatellite instability (*MSI*) due to promoter hypermethylation of *hMLH1*.[131]

To conclude, it is important to remember that some genes, such as Ras association gene (*RASSF1A*), *p14*, *p16* and *hMLH1*, are suppressed by hypermethylation in cancer and are also subject to age-related methylation, linking both processes (Figure 3).

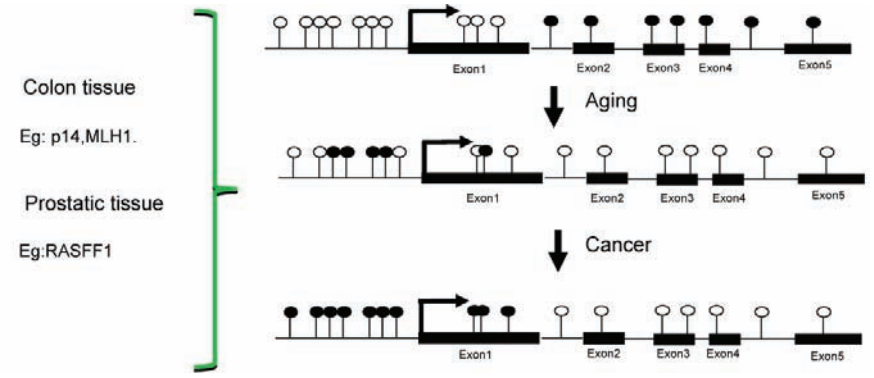

Figure 3. Genes involved in DNA repair and tumor-suppressor genes can be inactivated by gene promoter hypermethylation in the aged tissue, and this suppression can contribute to neoplastic transformation of the cell. For example, this could happen in colon tissue or prostatic tissue.

WRN and *LMNA*: epigenetically silenced progeroid genes

Because aging is the main biological phenomenon associated with the risk of developing cancer, it is not surprising to find that genes which are involved in *safeguarding* the genome against aging are inactivated in cancer cells.

The first example is the epigenetic silencing of the *WRN* gene. The mutation of this gene causes adult progeria, or Werner syndrome (WS).

WS is a very rare autosomal recessive human disorder that is characterized by the appearance of premature aging.[132,133] WS patients often suffer from type 2 diabetes, arteriosclerosis, cataracts, and osteoporosis. They also exhibit a short stature, premature graying and loss of hair, skin problems such as scleroderma, characteristic pitched voice and regional subcutaneous fat atrophy. The two main causes of death in WS patients are arteriosclerosis (44%) and neoplasia (39.5%).[133,134] Moreover, the type of neoplasms in WS patients is not the same as in cancer patients without this syndrome; the ratio of mesenchymal to epithelial cancers is 1:1 in WS patients[134,135] and 1:10 in the normally aged population. However, almost all types of tumors are found in WS patients.[136] WS cells show increased chromosomal instability, with deletions, reciprocal translocations and inversions, in what is known as variegated translocated mosaicism.[137]

WRN is a member of the RecQ helicase family. This family includes the genes RecQ, RecQ5, and RecQ4, which is associated with Rothmund Thompson syndrome, and BLM, which is associated with Bloom syndrome. *WRN* possesses DNA-dependent ATP-ase activity and 3'-5' helicase and exonuclease activity.[138-140]

WRN participates in diverse pathways, including repair, replication, telomere metabolism, and p53-mediated pathways.[139,140]

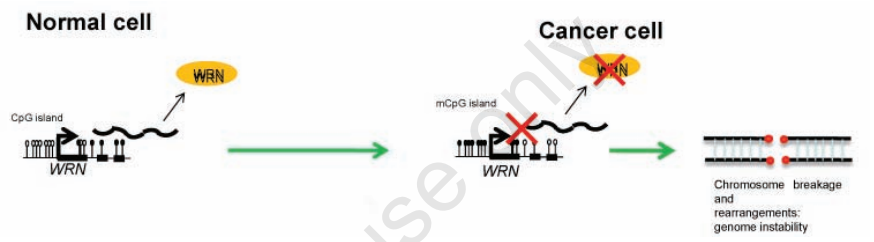

Figure 4. *WRN* can be epigenetically silenced in a wide variety of human cancers, contributing to cell transformation due to genome instability. *WRN* is involved in several pathways of DNA repair, and its absence could produce chromosome breaks. *WRN* could be considered a type of *caretaker* of tumor-suppressor gene.

WRN has been found to be inactivated by CpG island promoter hypermethylation in a wide variety of tumors of mesenchymal and epithelial origin.[5]

The re-introduction of the *WRN* gene into tumor cells with the epigenetically inactivated *WRN* produces tumor-suppressor-like features. Moreover, when tumor cells whose *WRN* promoter had been silenced by hypermethylation were treated with 5-AZA, the exonuclease activity of the *WRN* protein was restored.[5] Notably, upon exposure to DNA-damaging agents, cancer cells with *WRN* promoter hypermethylation and cells from WS patients were highly sensitive to these drugs, with a high frequency of chromomosomal breakage, in striking contrast to cancer cells with unmethylated *WRN* promoters. This sensitivity was abolished in *WRN*-methylated cells, upon transfection of *WRN* (Figure 4).[5]

Interestingly, significant age-related methylation alterations in *WRN* in different tissues were recently found.[141]

The second example of this type of gene is *LMNA*.

LMNA-type proteins, together with B-type lamins and lamin-associated proteins, are components of the nuclear lamina.[142,143]

The nuclear lamina is a network of filaments and proteins that is located on the inner side of the nuclear membrane. Nuclear lamins are extremely dynamic, suggesting their role in the non-random positioning of subchromosome domains.[142,143]

They are also involved in cell cycle regulation, DNA replication, differentiation, and apoptosis.[142,143]

Lamins can be classified into types B and A. Type B are essential for cell viability and expressed in all cell types, while type A are expressed in most differentiated somatic cells.[142,143]

The lamin A/C gene encodes lamins A and C, which are two isoforms that arise as a consequence of alternative RNA splicing. Mutations in human *LMNA* cause a plethora of pathologies called laminopathies. These include atypical WS and Hutchinson Guilford Progeria (HGPS).[143]

CpG island hypermethylation of the lamin A/C gene promoter has been found in leukemias and lymphomas, and is a predictor of poor outcome in these patients. This is particularly true for diffuse large B-cell lymphomas (DLBCL).[6]

Because lamin A/C is involved in the chromatin reorganization and reprogramming necessary for differentiation, one possibility is that blocking it can facilitate an undifferentiat-

ed phenotype that could be advantageous for tumor progression.[144]

The second possibility is that its absence could destabilize RB protein, promoting cell cycle progression through E2F1, as is the case for *LMNA-/-* embryonic mouse fibroblasts (MEFS).[145]

However, both possibilities are not mutually exclusive.

Concluding remarks

We provide an overview of DNA methylation and its associated machinery to explore the role of methylation in the aging process and neoplastic transformation, and to demonstrate how both these processes are linked. In particular, we have focused on what is known about the global loss of DNA methylation in relation to DNMT1 downregulation in aging cells, and the consequences for cancer development.[92,93] Genes that are methylated during aging, probably as a consequence of DNMT3b upregulation, are hypermethylated in cancer, which favors neoplastic transformation.[2] Importantly, *WRN* and *LMNA*, two genes involved in the aging process with roles in human progeria and tumor suppression, were found to be hypermethylated in human neoplasia.[5,6]

Current technological development has reached a point where whole genome methylation scans are now possible, and these are starting to shed light on the intricacies of DNA methylation phenomena. However, one caveat of these studies in relation to aging epigenomes is that changes in aging are sometimes subtle and less frequent than in cancer epigenomes. It should be noted that, together with methylome changes, the role for transcriptional networks in controlling these changes should be studied.

Several large scale studies have revealed in both human tissue[146] and mouse intestinal tissue[147] that methylation is associated with promoters of cancer-relevant genes which are methylated during the aging process. It has been suggested that age-dependent DNA methylation changes begin before adulthood[148] and that different patterns of epigenetic dysregulation occur within each tissue over time.[148]

Moreover, genes methylated during aging can be polycomb targets that are suppressed in stem cells.[150,151]

A recent observation using cultured Mesenchymal Stromal Cells (MSC), which are precursors for mesodermal cell lineages, found a concordance between methylation changes upon long-term cell cultured samples, aging samples and ovarian cancer samples.[152,153] Following these results, it has been suggested that changes in the methylation patterns observed in the aging and replicative senescence process could represent a developmental program. This is opposed to stochastic events, as we discussed earlier in this review.[148] However, it is more likely that both stochastic events and a developmental program could coexist during aging to some extent.

Using large-scale approaches, it has also been demonstrated that loci in CpG island gained methylation with age, while loci at non-CpG islands lost methylation with age. As previously mentioned, *WRN* has been found to have altered methylation in different tissues according to age.[141]

To conclude, it should be possible in the near future to address several unanswered questions. i) it will be necessary to dissect the mechanism that targets and initiates DNA methylation, as well as the mechanism that causes loss of global methylation in aging and cancer; ii) the recent discovery of CpG shores makes them an important target for the study of methylation in aging; iii) although the contribution of hypermethylation to the suppression of non-coding RNAs in cancer has been demonstrated,[1] it will be interesting to evaluate its role in the aging process; iv) we need a better understanding of how and in what cell context PcG complexes pre-program CGIs for methylation; v) it is of fundamental importance to improve our understanding of the role nuclear structure may play in senescent cells, as well as its interplay with epigenetic markers;[6,154] vi) it is important to determine if the epigenetic regulation of DNA replication origins has an important role in the aging process, as previously suggested in a model of cellular aging.[23]

Tackling these questions will lead to a better understanding of DNA methylation in aging and cancer.

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