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Promoter Hypermethylation of Genes In Aging

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ABSTRACT

Hypermethylation is an important process of gene regulation. Gene silencing through methylation occurs through the activity of DNA methyltransferases, enzymes that transfer a methyl group from *S*-adenosyl-L-methionine to the carbon 5 position of cytosine. DNA methylation levels and aging are strongly linked. The general trend seems to be the establishment of global hypomethylation and regions of CpG-island hypermethylation with age. Speculatively, this overall decrease in 5mC content could lead to less efficient gene regulation, and the CpG island hypermethylation could cause inappropriate silencing of specific genes. Aging and age-related diseases include defined changes in 5-methylcytosine content and are generally characterized by genome-wide hypomethylation and promoter-specific hypermethylation. In this review promoter hypermethylation in different aging genessuch as GSTP1,p16, p14 RASSF1A,ESR1,GSTP1,NKX2-5 and APC has been discussed.

INTRODUCTION

Methylation of DNA is one of an important mechanism of gene regulation.Promoter hypermethylation plays a key role in the inactivation of tumor suppressor[1]-[3] and metabolic genes during the tumorigenesis. The promoter methylation of several tumor suppressor genes have been reported in various cancers such as prostate cancer, colorectal cancers and lung cancer and other cancers cells also. The most common targets for abnormal methylation in tumors (like in lung cancer) are the promoter regions of the genes like GSTP1, p14, p16, and RASSF1. ESR1gene occurs when there is some addition and removal of chemical that tags to DNA-associated proteins and to the DNA itself. DNA methylation targets latter and includes the addition of a methyl group to the carbon 5 at the position of cytosine which leads to gene silencing.[4]

Although only four bases — adenine, guanine, cytosine, and thymine — spell out the primary sequence of DNA, there is a covalent modification of post replicative DNA(i.e., DNA that has replicated itself in a dividing cell) that produces a "fifth base." Reactions Using *S* -adenosyl-methionine as a methyl donor and catalyzed by enzymes called DNA methyltransferases (DNMTs) add a methyl group to the cytosine ring to form methyl cytosine. [5]

Hypermethylation has been identified with age for numbers of CpG islands, with affected genes which includes such as tumor-suppressor, DNA-repair/detoxification genes, metastasis/angiogenesis-related, growth/differentiation, and others.[6] The KRAS oncogene has chosen because hypermethylation of it is promoter region is one of the most frequent genetic aberrations observed in lung cancer and can even be used as a screening marker for early tumor detection. [7] These results shows that *DNMT3A*, through silencing of cancer-promoting genes, may fulfill the role of a tumor suppressor.

Aging strongly corresponds with the changes in DNA methylation. The DNA methylation and epigenetic alterations are linked to longevity in a wide array of organisms, ranging in complexity from yeast to humans.[8] DNA methylation levels and aging are strongly linked. The general trend seems to be the establishment of global hypomethylation and regions of CpG-island hypermethylation with age. Speculatively, this overall decrease in 5mC content could lead to less efficient gene regulation, and the CpG island hypermethylation could cause inappropriate silencing of specific genes.[9] The number of cancer-related genes affected by epigenetic inactivation equals or exceeds the number that are inactivated by mutation.[10]-[14]

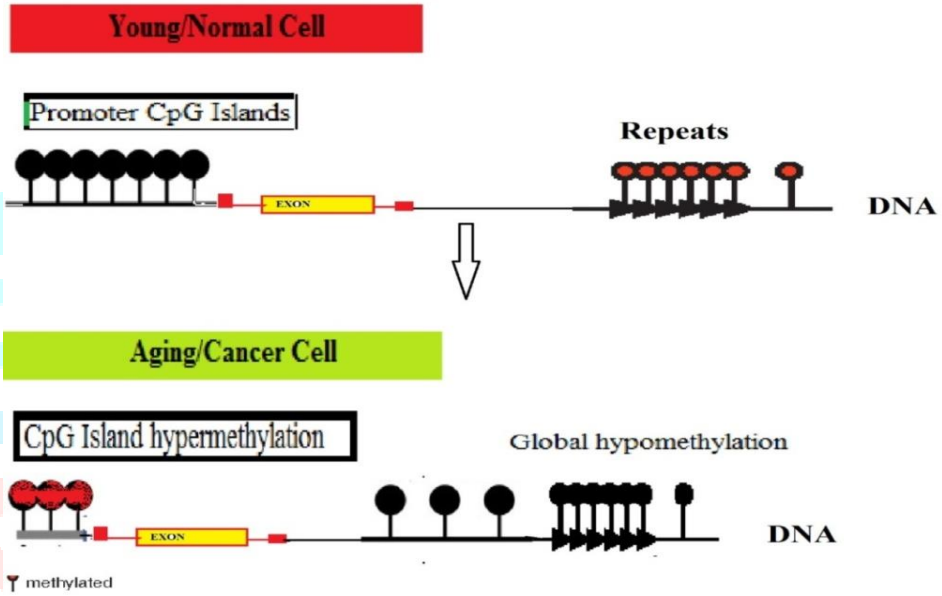


Fig1:DNA-methylation-pattern-promoter in young and aging cell

DNA methylation can maintain the large amount of non-coding DNA in a transcriptionally inert state of the cells of higher organisms. It is true that the large amount of methylation in human DNA that occurs in the large portion of the DNA and does not encode for genes (non-coding DNA). Later it replicates and then non-methylated DNA which shows that methylation helps to ensure late replication of large amount of the genome. The association of Late replication occurs with the formation of inactive chromatin, which can facilitate the transcriptional silencing of non-coding regions. This process helps to prevent the transcription of large parts of the genome that consist of repeat elements, inserted viral sequences, and transposons. Transcription of these elements might be harm cells.[15]

Hypermethylation of CpG sites in promoters or enhance typically leads to transcriptional silencing, where as hypomethylation of CpG sites in a gene body frequently results in an increase in gene expression.[16][17]

ROLE OF HYPERMETHYLATED AGING GENES

1 .Glutathione StransferasePi gene (*GSTP1*)

GSTP1 gene is located on chromosome 11q13.[20][21] a metabolic gene, plays a key role in detoxification by catalyzing the conjugation of many hydrophobic and electrophilic compounds with reduced glutathione .(18)-(19) ***GSTP1*** represents a typical example of an ideal epigenetic biomarker, as its methylation is involved in a number of diseases, in particular in PCa.Despite***GSTP1*** methylation in breast cancer is associated with a poorer patient's outcome, individuals with high methylation seem to have a better response to doxorubicin, compared to those without gene methylation. In this specific context, ***GSTP1*** seems to have a role as predictive biomarker [22].

The glutathione Stransferase Pi gene (*GSTP1*) is a polymorphic gene encoding active, functionally different *GSTP1* variant proteins that are thought to function in xenobiotic metabolism and play a role in susceptibility to cancer, particularly more in prostate cancer and other diseases [23]-[25].

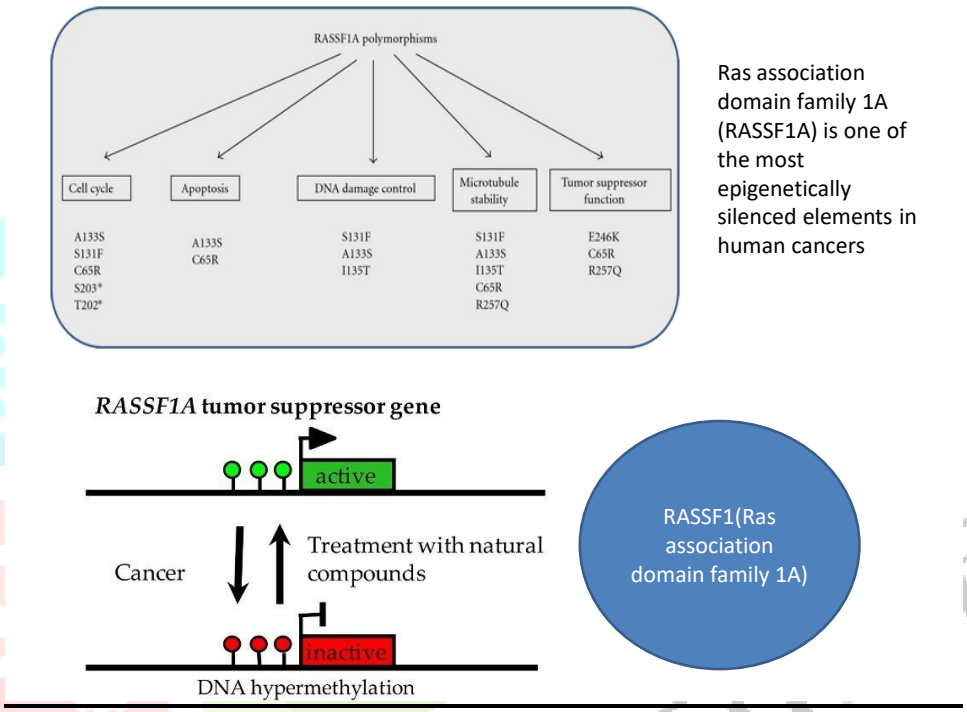
2. p16

p16 (p16INK4a/CDKN2A), a tumour suppressor gene, is a cell cycle regulator that is frequently inactivated in different types of malignancies, including lung cancer The gene product of p16 protein is a molecular component of the retinoblastoma protein (pRB) regulatory pathway which inhibits G1 cyclin-dependent kinases .[26]-[27]

3. **p14 gene**

p14 gene (p14ARF/ARF) is an alternatively spliced variant of p16 gene encoding a distinct but important regulatory protein functioning in the p53 pathway .[28] This gene is known to be an important tumor suppressor gene

4. ***Ras association domain family 1A gene* (RASSF1A)**



Ras association domain family 1A (RASSF1A) is one of the most epigenetically silenced elements in human cancers

Fig 2

RASSF1A (Ras association domain family 1A) gene is a tumor suppressor gene .[29]-[30] The inactivation of this gene was found to be correlated with the hypermethylation of its CpG-island rich promoter region. Suppression of RASSF1A expression has been reported in progression of lung cancer. Depletion of RASSF1A and hypermethylation of the *RASSF1A* promoter are common epigenetic alterations in renal cell carcinoma. *RASSF1A* becomes progressively methylated with age and increased adiposity two known risk factors for renal cancer.[31] Methylation of *RASSF1A* also correlates highly with breast cancer risk, atypical cytology, and benign breast disease requiring biopsy.[32]

5. ***Estrogen receptor 1* (ESR1)**

Early studies of epigenetic regulation of *ESR1* found that its gene expression is either absent or heavily diminished in colorectal tumors. This gene suppression was accompanied by significant hypermethylation compared to nontumor controls. Exogenously expressing the *ESR1* gene in cultured colon carcinoma cells significantly suppressed growth, suggesting that ESR1 likely functions as a tumor suppressor. *ESR1*, which becomes hypermethylated with age in human colonic mucosa, exhibits further age-dependent methylation in atherosclerosis and prostate cancer.[33]-[35]

6.* NKX2-5 *genes

NKX2-5, a cardiac transcription factor, has received much attention for its role in cardiac dysmorphogenetic.[36] It functions as a key regulator in cardiac morphogenesis, regulating the transcription of

various genes involved in the process. Mutations in the Tinman in Drosophila embryos results in the loss of heart formation.

NKX2-5 has a role in nearly almost all phases of heart. Development, including the regulation of number of cardiac progenitor cells, conduction system development, valve formation, and septation. Interestingly, NKX2-5 acts in combination with other transcription factors that are highly-conserved to organize cardiogenesis. Genome–wide expression analysis has begun to investigate NKX2-5 dependent genes [37]-[38]

Molecular analysis of protein products resulting from Nkx2-5 point mutations identifed in these studies has demonstrating altere DNA binding affnity compared to wild-type, indicating that developmental pathology likely results from abnormal regulation of Nkx2-5 target genes during heart development.[39]- [42]

7. Adenomatous polyposis coli gene (APC gene)

APC gene is firstly identified by positional cloning of the FAP (familial adenomatous polyposis) locus [43]-[44]. The *APC* gene provides instructions for making the APC protein, which plays a critical role in several cellular processes. The APC protein acts as a tumor suppressor, which means that it keeps cells from growing and dividing too fast or in an uncontrolled way. It helps control how often a cell divides, how it attaches to other cells within a tissue, and whether a cell moves within or away from a tissue. This protein also helps ensure that the number of chromosomes in a cell is correct following cell division.

One protein with which APC associates is beta-catenin. Beta-catenin[45]-[46] also helps cells attach to one another and is important for tissue formation. Association of APC with beta-catenin signals for beta-catenin to be broken down when it is no longer needed.

8. p73

p73 is a protein related to the p53 tumor protein.[47] p73, also known as tumor protein 73 (TP73), protein was the first identified homologue of the tumor suppressor gene,p53.[48] Like p53, p73 has several variants. p73 is present in early stages of neurological development and neuronal apoptosis by blocking the pro-apoptotic function of p53.[49]

BASIC MECHANISMS OF HYPERMETHYLATED AGING GENES

DNA methylation

The methylation reaction is catalyzed by DNMTs, enzymes that transfer a methyl group from *S*-adenosyl-L-methionine (SAM) to the C5 of a cytosine. This reaction features SAM as the electrophile methyl donor and C5 as a weak nucleophile unable to interact with SAM on its own.

However, a nucleophile from a DNMT can attack the carbon-6 of cytosine, covalently binding the enzyme to the DNA. This activates the nucleophilic character of C5, facilitating the transfer of a methyl group from SAM. The enzyme nucleophile is consequently eliminated and deprotonation at C5 separates the nucleotide–DNMT complex.[50] In normal human prostate tissue procured from 45 organ donors, hypermethylation was observed as a function of age for CpG islands in *RARβ2*, *RASSF1A*, *GSTP1*, *NKX2-5*, and *ESR1* genes— all genes in which mutations are believed to be a risk factor for cancer.[51]

Silencing Pathways

There are multiple routes to gene silencing via methylation, the most direct of which involves interfering with transcription factors or basal transcriptional machinery that interact with cytosines in the major groove of double helices. Because the majority of mammalian transcription factors possess DNA recognition elements containing CpG-rich motifs as well as GC-rich binding sites, DNA methylation can obstruct or eliminate their ability to act on many important regulatory sites. Alternatively, transcriptional machinery can be directly excluded from methylated promoter DNA by altering nucleosome stability or position. [52]

Demethylation and hydroxy methylation

Multiple mechanisms for active DNA demethylation have been proposed, including base excision repair, enzymatic removal of the methyl group, and oxidative demethylation of 5mC.22 The most compelling demethylating scheme involves the ten eleven translocation (TET) family, enzymes that iteratively oxidize 5mC to 5-hydroxymethylcytosine (5hmC), to 5-formylcytosine, and finally to 5-carboxylcytosine (5caC). 5caC has

been theorized to be decarboxylated by an unknown decarboxylase, providing an elegant means of regenerating normal cytosine. [53]

Regulation of DNMTs

Regulation of DNA methylation appears to be quite extensive because DNMT1 is targeted by multiple transcription factors and can be methylated, phosphorylated, acetylated, and ubiquitinated. The maintenance methyltransferase can also be modified by covalent attachment of small ubiquity in like modifier (SUMO) proteins, a process referred to asSUMOylation. [54]

BIOLOGICAL EFFECTS OF PROMOTER HYPERMETHYLATION

The biologic effects of the loss of gene function caused by promoter hypermethylation and by coding- region mutations are similar.[55]-[57] For example, both mutations and promoter hypermethylation of *VHL* occur almost exclusively in renal cancers; either mutation or epigenetic silencing of *MLH1* causes the form of genomic instability termed microsatellitein stability in colon cancer and other tumors, and both genetic and epigenetic changes in *BRCA1,* a breast-cancer susceptibility gene, produce similar DNA-microarray patterns of gene expression in breast cancer.[55][57]-[59] For example, *RASSF1A,* a candidate tumor-suppressor gene related to the *ras* oncogene,[60] –[62] is hypermethylated in many types of tumors; *p73,* which is related to the tumor-suppressor gene *p53,* is silenced in acute lymphocytic leukemia.[63] However, an epigenetic change alone does not necessarily indicate that the affected gene is a tumor-suppressor gene.
DNA from cancer cells is being screened for hypermethylated and silenced genes without knowledge of the function of the affected genes.(11) ,[64]-[66]

CONCLUSION

In this review promoter hypermethylation (an important pathway for the repression of gene transcription in cancer) related with aging in different genes has been summarized. For example Glutathione S transferase Pi gene (GSTP1), Adenomatous polyposis coli gene (APC gene), p14, p16,Ras association domain family 1A gene (RASSF), *p73*,NKX2-5 *genes and* Estrogen receptor 1 (ESR1) gene. Variety of ageing gene play a very important role in cancer It is difficult to judge the importance of promoter hypermethylation in genes that are rarely, if ever, disrupted by mutation in cancer cells. In some cases, a clue is provided by the function of the hypermethylated gene. For example, *RASSF1A,* a candidate tumor-suppressor gene related to the *ras* oncogene, is hypermethylated in many types of tumors; *p73,* which is related to the tumor-suppressor gene *p53,* is silenced in acute lymphocytic leukemia. 38 However, an epigenetic change alone does not necessarily indicate that the affected gene is a tumor-suppressor gene.
DNA methylation changes occurring early could be an effective means of predicting cancer risk. The specific functions of DNMT1 that are involved in tumori genesis must be isolated from the functions involved in metastasis. The genes involved in aging gene are following four basic mechanisms are given below – DNA methylation, silencing pathway, demethylation and hydroxy methylation and regulation of DNMTs.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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