DNA Methylation in Cancer: Review

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ABSTRACT: Mammalian genome has a profound effect of DNA methylation or covalent addition of a methyl group to cytosine with reference to CpG dinucleotide. These modifications are very stable but reversible. The effect of mutation of a number of Tumor suppressor gene (TSGs) can be mimicked by the change in methylation of promoter. Hypomethylation and hypermethylation of promoter of proto-oncogenes are aberrations of genomic DNA which can result in carcinogenesis. Proper embryonic development also requires DNA methylation. Global hypomethylation has been found to disrupt the normal methylation pattern in cancer cells along with region specific hypermethylation. If the promoter of a TSG are involved in hypermethylation event then silencing of that gene occurs which in turn gives cell a growth advantage in a manner similar to mutations. However the transcription of retrotransposons, protooncogene and gene involved in the malignant cell metastasis has been observed to be activated if the event of hypomethylation occurs. A high level of DNA methyltransferase DNMT1 and DNMT3B which plays a crucial role in the catalysis of methylation of genomic DNA in malignant cells is present in different types of cancers. This review will provide an overview of cancer, methylation, its various types, what leads to the malfunction in certain gene due to methylation and various methods how DNA methylation occurs which leads to oncogenesis. © 2015 iGlobal Research and Publishing Foundation. All rights reserved.

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INTRODUCTION

Cancer has become one of the most far reaching diseases human society has ever faced. Today there are a huge number of people worldwide which are affected by some form of cancer. According to National cancer Institute nearly 12 million Americans with a history of cancer were alive in January, 2008. According to this report the number of cancer cases to be diagnosed in 2012 is 1,638,910. Carcinoma in situ (noninvasive cancer) of any site except urinary bladder, basal and squamous cell skin cancer is not included in this approximation. More than 1500 people per day, about 577,190 Americans are expected to die of cancer in 2012 [1]. Factors which are making cancer cure difficult are: Lack of early detection methods and efficient treatments methods. Estimated new cases and deaths from cancer in the United States in 2013:
•   New cases: 1,660,290 (does not include non-melanoma skin cancers)
•   Deaths: 580,350 [2]

Defining the disease: What is Cancer?
According to National Cancer Institute,U.S.,Cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues. Cancer is not just one disease but many diseases.

According to Cancer research UK, cancer is a disease caused by normal cells changing so that they grow in an uncontrolled way. The uncontrolled growth causes a lump called a tumor to form. If not treated, the tumor can cause problems in one or more of the following ways:
•   Spreading into normal tissues nearby
•   Causing pressure on other body structures
•   Spreading to other parts of the body through blood stream.

According to Indian Society of Cancer, Cancer is an abnormal growth of body cells. Each individual is born with

a potential for cancer. One cannot catch it as one would an infection or cold. When the programming of a cell or a group of cells is affected, growth may become uncontrolled. Some of the factors that can alter the code are chronic irritation, tobacco, smoke and dust, radioactive substances, age, sex, race and heredity.

DNA methylation

All the genes which are needed to produce a human have nearly been enlisted due to the completion of Human Genome Project. However the situation in which we need to understand when and where a particular gene will be expressed during development is far more complex. This complete information has been imprinted on DNA in the form of epigenetic marks. These marks are heritable during cell division but play no role in alteration of DNA sequence. In mammals the only known epigenetic modification of DNA is methylation of cytosine at position C5 in CpGdinucleotides [3] (Fig 1). A significant role has been played by Epigentic events in the development and progression of cancer. The heritable changes in gene expression which do not come from alteration in the gene nucleotide sequence have been referred to as Epigenetics [4].When DNA methylation is present in the promoter region of transcription initiated sites, genes becomes inactivated or silent, a characteristic of tumor cells. The normal tumor suppressor function of cells becomes silent, a phenomon known as "epigenetic silencing" due to these aberrant methylation.

The addition of methyl group to cytosine or adenine nucleotide through a biochemical process is defined as DNA methylation or DNA methyltransferase (DMNTs) catalyzed covalent chemical modification of DNA is termed as DNA methylation [5]. The expression of genes in cell as they are differentiating from embryonic stem cells is altered by this process, which can be permanent and unidirectional. DNA methylation generally occurs in CpG dinucleotide; non-CpG methylation can be seen in embryonic stem cells [6].

CpG islands are the regions of high CG density are usually found in 5' promoter ends of genes and are un-methylated [9].

Approximately 4% of genomic DNA carries 5'-methylcytosine in mammalian DNA which is primarily cytosine-guanosinedinuleotides (CpGs) sites. These sites are present more frequently in a small stretches of DNA termed as CpG islands, which are present in or near promoter region

of genes, where transcription is initiated. This sites/island of CpGs remains unmethylated as compared to the CpG sites present in genomic DNA which are highly methylated which in turn allows gene expression [4].

An important component of cancer development has been recognized as alteration in DNA methylation [13].

Types of DNA methylation

There are two types of alteration

(1) Hypermethylation: In this methylation the region which is to be methylated has experienced an over activity of enzymes which causes methylation or an increase in the epigenetic methylation of cytosine and adenosine residues in DNA.

(2) Hypomethylation: In this methlyation the region which is to be methylated has experienced a lower activity of enzymes which causes methylation or a decrease in the epigenetic methylation of cytosine and adenosine residues in DNA. Chromosomal instability and loss of imprinting has been attributed to Hypomethylation whereas the alteration which is associated with promoters and which arises secondary to gene silencing is Hypermethylation [13]. The reason why some CpG islands are hypermethylated or hypomethylated in certain cancer is not currently known.

The transcription of genes can be affected by DNA methylation in two ways. First, impediment is the binding of transcriptional proteins to the gene by methylation of DNA (Mun Choy et al, 2010) and second the binding of methyl-CpG-binding-domain (MBDs) proteins to methylated DNA. Histone deacetylase and other chromatin remodeling proteins are then recruited to the locus which can modify histones, which results in the formation of inactive chromatin, called heterochromatin. DNA methylation also regulates the storage of long term memory in humans [5].

DNA methylation and cancer

An intense topic of clinical investigators of cancer has recently been shifted to DNA methylation. The DNA methylation sequence has been disrupted in cancerous cell as compared to normal cells [16]. Hypermethylation generally occurs in CpG islands and Hypomethylation involves repetitive DNA sequence [17] (Fig 2).

Hypermethylation in cancer

Cancer caused by Hypermethylation has been found in huge number of patients as compared to Hypomethylation.Hypermethylation in CpG islands has been prevented by a number of mechanisms. Active transcription, active demethylation, replication timing, and local chromatin structure are few processes which prevents access to DNA methyltransferases (DNMT) [18].

A huge number of genes undergo hypermethylation which causes cancer (Table 2).

The Genes of cell cycle regulation (p16INK4a, p15INK4a, Rb, p14ARF), DNA repair (BRCA1, MGMT), drug resistance, metastasis are susceptible for being hypermethylated during cancer. (Table1).

RASSF1A and p16 are the most commonly methylated genes in cancer cells in various cancers others but some cancer are specific to the methylation of some genes. For example in prostate cancer gene GSTP1 is hypermethylated in approximately 91% but this same gene is acute myeloid leukemia is unmethylated [19-20].

More than one gene can undergo hypermethylation in some tumors (Table1). Lung cancer has been studied in details to explain the above phenomenon. In Lung cancer there was some kind of difference in DNA methylation pattern in more than 40 genes. RARβ, CDNK2A, and APC are few genes which are recorded to be hypermethylated in a Lung cancer [21].

Hypermethylation has also been found in various hematological diseases. In various hematologic cancer, calcitonin gene, p15INK4B, p21Cip1/Waf1, the ER gene, and SDC4 are hypermethylated [22].

Hypomethylation in cancer

In various malignancies a second variety of DNA methylation occurs i.e. Hypomethylation [23-24].Few examples in which DNA hypomethylation causes cancer are: cervical cancer, prostate cancer, B-cell chronic lymphocytic leukemia. Patients with immunodeficiency, centomeric instability and cancer have pericentic heterochromatin region of chromosome 1 and 16 hypomethylated. DNMTs have also been found mutated in the patients of immunodeficiencies, centomeric instability which causes the instability of the chromatin[25].Oncogene such as cMYC and H-RAS has been activated by hypomethylation in various cancers [23].

Hypomethylation has also been found contributing in the development of cancer by activating latent retrotransposons or by chromosome instability [27-29].

An overview of the Folic acid pathway (Fig 3) shows that Folic acid is involved in the synthesis of Purines. This pathway also produces 5'-methylocytosine which is demethylated by various agents like Zebularine and DNMTs which in turn recruits MBDs and histone deacetylase which prevents the binding of transcription factors. Gene silencing is the end result of this whole process.

DNMTs

The family of enzymes which catalyzes the reaction in which a methyl group is transferred to DNA is known as DNA methyltransferases (DNA MTase). A wide variety of biological function has been served by DNA methylation.

DNMT1, DNMT2, DNMT3A and DNMT3B makes up a family of enzymes called DNMTs family in mammals. Maintenance and de novo Methyltransferases are two sub-categories of this family. DNMT1, maintenance enzyme performs the task of binding methyl groups to the hemimethylated DNA during replication, whereas DNMT3A and DNMT3B, both de novo DNMT play a role in the methylation of CpG dinucleotides of unmethylated DNA (Figure).

Mammalian DNA methyltransferase (DNMT)

In mammals three active DNA methyltransferase have been identified namely DNMT1, DNMT3Aand DNMT3B. Previously DNMT2 was also considered as a DNA methyltransferase but now it not included in this list.

DNMT1

This enzyme is coded by DNMT1 gene in humans. It complete name is DNA (cytosine-5)-methyltransferase 1[30][31]The length of this enzyme is 1,620 amino acid. The regulatory domain consists of 1,100 amino acid and the catalytic domains comprises of the remaining amino acid. The presence of both domains is compulsory for the catalytic function of the enzyme.

It is the most abundant and a key player in the maintenance of methyltransferases in mammals is DNMT1. The hemimethylated CpG dinucleotide region are its main site of action. It has a role in the establishment and regulation of tissue-specific patterns of methylated cytosine residues [32].
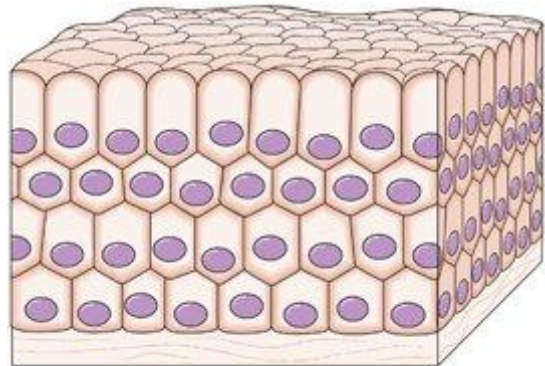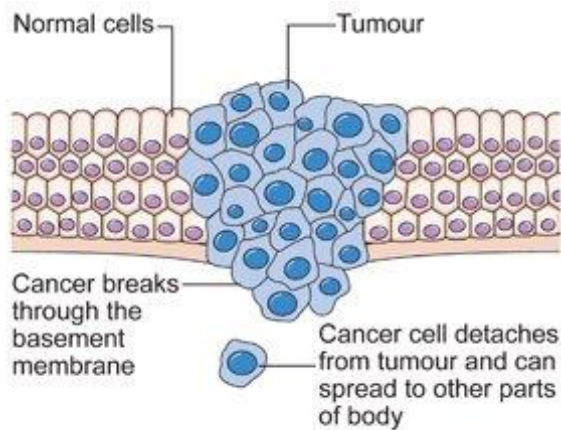
Table 1

Normal Cell	Cancer Cell
Stop reproducing at the right time	Cancer cells don't stop reproducing
Stick together in the right place	Cancer cells don't stick together
Self-destruct if they are damaged	Cancer cells don't obey signals from other cells
Become specialized or 'mature'	Cancer cells don't specialize, but stay immature

Diagram showing how normal cells make up the tissue in our body

Normal cells — Tumour

Cancer breaks through the basement membrane

Cancer cell detaches from tumour and can spread to other parts of body

Diagram showing a malignant tumour

Table 2

Gene	Role in Tumor Development	Site of Tumor	Reference
APC	Deranged regulation of cell proliferation, cell migration, cell adhesion, cytoskeletal reorganization, and chromosomal stability	Breast Lung Esophageal	[45] [46]
BRCA1	Implicated in DNA repair and transcription activation	Breast Ovarian	[47] [48]
CDKN2A/p16	Cyclin-dependent kinase inhibitor	GIT Head and neck NHL Lung	[49] [50] [51]
DAPK1	Calcium/calmodulin-dependent enzyme that phosphorylates serine/threonine residues on proteins; Suppression of apoptosis	Lung	[52]

E-cadherin	Increasing proliferation, invasion, and/or metastasis	Breast	**[53]**
		Thyroid	**[54]**
		Gastric	
ER	Hormone resistance	Breast	**[56]**
		Prostate	**[57]**
GSTP1	Loss of detoxification of active metabolites of several carcinogens	Prostate	**[58]**
		Breast	**[59]**
		Renal	**[59]**
hMLH1	Defective DNA mismatch repair and gene mutations	Colon	**[60]**
		Gastric	**[55]**
		Endometrium	**[61]**
		Ovarian	**[62]**
MGMT	*p53*-related gene involved in DNA repair and drug resistance	Lung	**[52]**
		Brain	**[59]**
p15	Unrestrained entry of cells into activation and proliferation	Leukemia	**[64]**
		Lymphoma	**[65]**
		Squamous cell carcinoma, lung	[66]
RASSF1A	Loss of negative regulator control of cell proliferation through inhibition of G_1/S-phase progression	Lung	**[67]**
		Breast	**[67]**
		Ovarian	**[67]**
		Kidney	**[68]**
		Nasopharyngeal	**[69]**
Rb	Failure to repress the transcription of cellular genes required for DNA replication and cell division	Retinoblastoma	**[70]**
		Oligodendroglioma	
VHL	Altered RNA stability through and erroneous degradation of RNA-bound proteins	Renal cell cancer	**[68]**

Abbreviations: *APC*, adenomatous polyposis coli; *BRCA1*, breast cancer 1; *CDKN2A/p16*, cyclin-dependent kinase 2A; *DAPK1*, death-associated protein kinase 1; *ER*, estrogen receptor; *GSTP1*, glutathione S-transferase Pi 1; *hMLH1*, Mut L homologue 1; *MGMT*, O-6 methylguanine-DNA methyltransferase; *RASSF1A*, Ras association domain family member 1; *Rb*, retinoblastoma; *VHL*, von Hippel-Lindau; GIT, gastrointestinal tract; NHL, non-Hodgkin's lymphoma.

Table 3: Genes Commonly Methylated in Human Cancer and Their Role in Tumor Development

Tumor suppressor gene	Tumor type(s)	Reference
pRb	Retinoblastoma	**[71]**
VHL	Renal carcinoma	**[72]**
p16INK4a	Melanoma and many others	**[73]**
p15INK4b	Hematologic malignancies	**[74]**

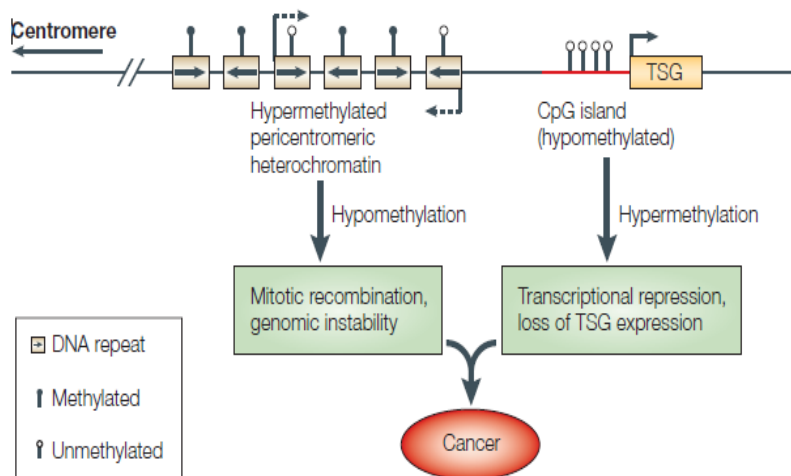| hMLH1	Colorectal carcinoma	[75]
APC	Colorectal carcinoma	[76]
BRCA1	Breast cancer	[77]

Figure 1 DNA Methylation and cancer. The diagram shows a representative region of genomic DNA in a normal cell. The region shown contains repeat-rich, hypermethylatedpericentromeric heterochromatin and an activelytranscribed tumor suppressor gene (TSG) associated with a hypomethylatedCpG island (indicated in red). In tumor cells, repeat- rich heterochromatin becomes hypomethylated and this contributes to genomic instability, a hallmark of tumor cells, through increased mitotic recombination events. *De novo* methylation of CpG islands also occurs in cancer cells, and can result in the transcriptional silencing of growth-regulatory genes. These changes in methylation are early events in tumorigenesis [3].
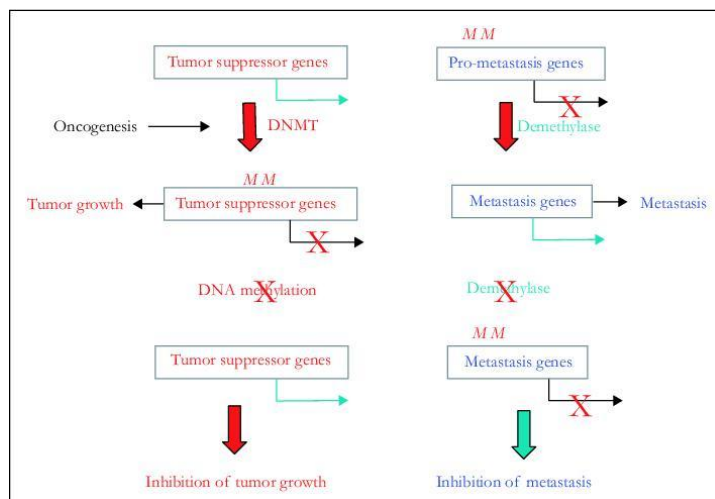

Figure 2 Hypo- and hypermethylation in cancer. In cancer cells two classes of methylation changes occur. Genes who block uncontrolled growth such as tumor suppressor genes are unmethylated in normal cells, but oncogenic transformation results in methylation (M) of control regions on these genes. This causes silencing of genes, which impede tumor growth. Thus in turn it allows the cancer cell to bypass growth arrest signals and promote growth. On the other hand other genes, which are normally methylated such as genes required for invasion and metastasis, are methylated in normal and in noninvasive cancerous cells. During transformation to a metastatic state demethylasedemethylates the pro-

metastatic gene resulting in its activation and promotion of tumor invasion and metastasis. These processes offer us therapeutic targets. Inhibition of DNMT would result in demethylation of the tumor suppressor gene its activation and arrest of tumor growth. Inhibition of demethylase would result in remethylation silencing of pro-metastatic genes and inhibition of metastasis.
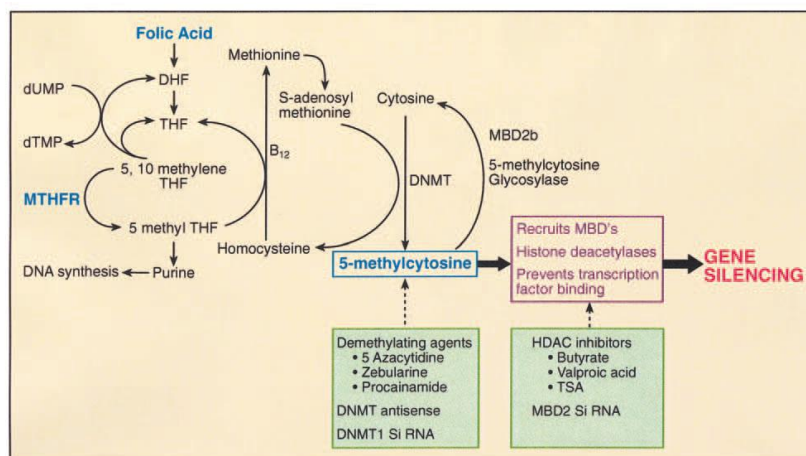

Fig 3. An overview of the folic acid pathway, cytosine methylation, and gene silencing. The mechanism of action of demethylating agents, HDAC inhibitors,and other agents is also shown. dUMP, deoxyuridine monophosphate; dTMP, deoxythymidine monophosphate; DHF, dihydrofolate; THF, tetrahydrofolate; MTHFR, methyelenetetrahydro folate reductase; DNMT, DNA methyltransferase; MBD, methyl CpG binding domain; TSA, trichostatin A [34].
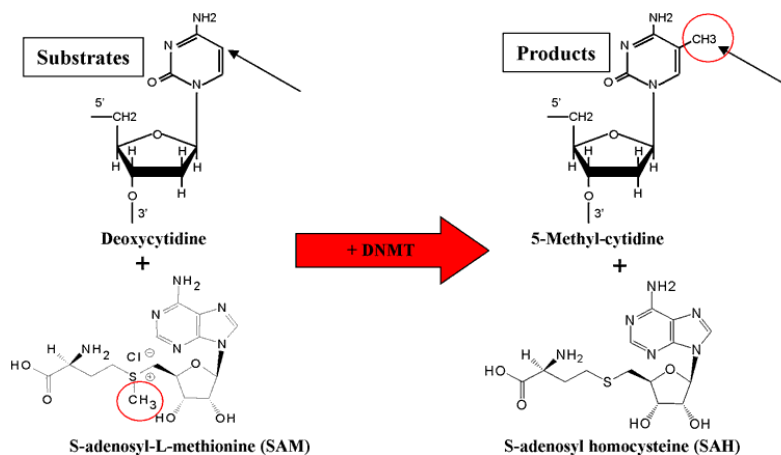

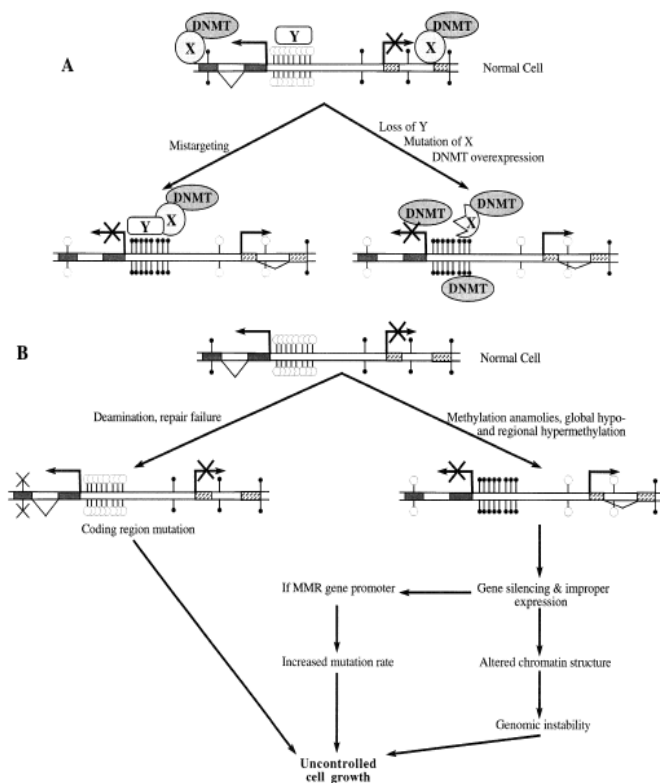Figure 4: Methylation of cytosine catalyzed by DNMTs.

Figure 5 (A) Schematic diagram showing how interaction of the various DNMTs (DNMT1, 3a or 3b in this case) with other cellular proteins may target methylation to the proper regions (shown by interaction with hypothetical protein X) and protect CpGislands from *de novo* methylation (shown by hypothetical protein Y blocking access of DNMT to DNA). Aberrant methylation patterns in tumors may result from mistargeting of DNMTs or improper expression during the cell cycle (left) or through any one or a combination of loss of Y, mutation in X, and over expression of one or more of the DNMTs (right). (B) Mechanism by which CpG methylation may contribute to tumor genesis. The presence of a methylated CpG within the coding region of a gene may predispose that region to mutation due to deamination and failure to repair resulting in a point mutation (left). Alternatively alteration in the normal cellular methylation patterns, by mechanism that remains unknown, results in gene silencing and altered chromatin structure (right). If *de novo* methylation occurs within the promoter region of tumor suppresser gene or a gene involved in maintaining genome stability then that cell may gain a growth advantage. Black lollypops are methylated CpGs and open lollypops are unmethylatedCpGs. An 'X' represents a point mutation or promoter silence. Filed boxes are exons and bent arrows are promoters [36].

DNMT2

DNMT2 is similar to 5-methyltransferase of both eukaryotes and prokaryotes but it does not methylated DNA rather it methylates aspartic acid transfer RNA [33]. Hence the name of this enzyme has been changed to TRDMT1 (tRNA aspartic acid methyltransferase 1)

DNMT3

The methylation of hemimethylated and unmethylatedCpG at the same rate can be catalyzed by DNMT3 family. It has three members: DNMT3a, 3b, and 3L.

DNMT3a and DNMT3b can control gene repression without methylation. CpG methylation of CpA, CpT and CpC is preferred by DNMT3a.

During the time of gametogenesis, genomic imprinting takes place and DNMT3L is expressed. Bi-allelic expression of genes is seen in the case of lossof DNMT3L which is not normally expressed by the maternal allele. DNMT3a and DNMT3b are found to be intermingled by DNMT3L, which is co-localized in the nucleus.

Role of DNMTs In cancer

The methylation of cytosine is carried out with the help of DNMTs. These DNMTs generally transfer the methyl group of S-adenoyl-L-methionine (SAM)(FIG 4). For mentioning the direct role of DNMTs in carcinogenesis there has been a debate. When the hypermethylatedalleles of TSG mentioned in the table 3 were examined then it is concluded that the maintenance of methylation – free CpGisland is somehow defective (Fig 5-A).

Several Line of evidence point to a direct role of DNA methylation in tumorigenesis. 1st is that reduced DNA methylation suppresses the formation of intestinal polyps in APCMin/+[37].Secondly in many cases of unilateral retinoblastoma [38] and renal cancer [39], the role of promoter region methylation of the retinoblastoma (pRB) gene and the Von Hippel Lindau (VHL) gene have been acknowledged respectively. Lastly hypermethylation has also been documented to silence one copy of a tumor suppressor gene in wild type, however the second copy is either mutated or lost,and this supported the motion of DNA hypermethylation as one of the prime, inactivating events that lead to tumorigenesis [40].

In general 30% of all point mutations in germline are advocated to be contributed by CpG site, which have been found to play as hotspot sites for mutations [41]. Tallying to it, CpG sites in coding region of TSGs are found to be strong hotspotsfor acquired somatic mutations leading to cancer [42-43]. For an instance, in all the human tissues studied the CpG sites of the p53 coding region are found to be methylated [44]and contribute to as many as 50% of all inactivating mutations in colon cancer and 25% in cancer in general [45](Fig 5B).

CONCLUSION

DNA methylation, its biological significance in the regulation of gene expression and role in cancer is recognized at a much fast rate. We have attempted to provide an overview of cancer, methylation, its various types, what leads to the malfunction in certain gene due to methylation and various methods how DNA methylation occurs which leads to oncogenesis. But still a huge number of questions are unanswered like what is the basis of the establishment and alteration of methylation patterns in DNA. The relationship between DNA methylation events at molecular level and its clinical application if understood in greater detail then it can provide the platform on which the treatment of cancer can depend.

ACKNOWLEDGEMENT

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