"ROLE OF DNMT IN CANCER"

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

MASTER OF SCIENCE
IN
LIFE SCIENCE

SUBMITTED TO

NATIONAL INSTITUTE OF TECHNOLOGY, ROURKELA

BY

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CERTIFICATE

This is to certify that the thesis entitled "Role of DNMT in Cancer" which is being submitted by Mr. Suryanarayan Biswal, Roll No. 409LS2034, for the award of the degree of Master of Science in Life Science from National Institute of Technology, Rourkela, is a record of bonafide research work, carried out by him under my supervision. The results embodied in this thesis are new and have not been submitted to any other university or institution for the award of any degree or diploma.

To the best of my knowledge, Mr. Biswal bears a good moral character and is mentally and physically fit to get the degree.

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DECLARATION

I, Suryanarayan Biswal hereby declare that this project report entitled **"**Role of DNMT in Cancer**"** is the original work carried out by me under the supervision of Associate Prof. Samir K. Patra, Department of Life Science, National Institute of Technology Rourkela (NITR), Rourkela and the present work or any other part thereof has not been presented to any other University or Institution for the award of any other degree.

Suryanarayan Biswal

ACKNOWLEDGEMENTS

I wish to express my deepest sense of gratitude to my supervisor Dr. Samir K. Patra, Associate Professor, Department of Life Science, National Institute of Technology, Rourkela for his valuable guidance, assistance and time to time inspiration throughout my project.

I am very much grateful to Prof. P. C. Panda, Director, National Institute of Technology, Rourkela for providing excellent facilities in the Institute for carrying out research.

I would like to take the opportunity to acknowledge quite explicitly with gratitude my debt to all the Professors and Staff, Department of Life Science, National Institute of Technology, Rourkela for his encouragement and valuable suggestions during my project work.

I would like to give heartfelt thanks to Ms. Moonmoon Deb, all the other PhD scholars and second yr. friends for their inspirative support throughout my project work

Finally I was highly great full to my parent for their continued moral support.

And to all mighty, who made all things possible……..

(SURYANARAYAN BISWAL)

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ABSTRACT

DNA methylation is a biochemical process catalyzed by enzyme DNA Methyltransferases (DNMTs). In most of the carcinogenesis DNMTs are over expressed and aberrant genomic DNA methylation pattern (genome wide hypomethylation and regional hypermethylation) are being observed. One of such consequences results in hypermethylation of Tumor Suppressor Genes. In different cancerous, different DNMTs show significantly elevated expression than their normal cells. In our study we have investigated relative expression of DNMTs and TSGs in normal and cancerous tissue. We found that DNMT3A expression level is comparatively more than the other DNMTs and it may cause the transcriptional repression of TSGs, particularly in lymph Node cancer.

Key Words: DNA Methylation, DNA Methyltransferases, Tumor Suppressor Genes, Lymph Node Cancer.

PREFACE-"DNA: THE SECRET OF LIFE"

On February the 28th 1953, James Watson and Francis Crick deciphered the structure of deoxyribonucleic acid, DNA[1], which was later published in the journal *Nature* [1]. The DNA X-ray diffraction pictures made by Rosalind Franklin were essential for this discovery, as they provided several of the vital helical parameters [2]. The four separate building blocks of the DNA molecule, the nucleotides adenine, cytosine, guanine, and thymine, had been isolated and characterized several years before this immensely important discovery. The modified cytosine base, 5-methylcytosine, was first recognized in 1948 [3] and was later identified as a central element in the field of epigenetics[2]. The DNA contains the genetic instruction specifying how to assemble protein molecules, which are the building blocks of each phenotype. Indeed, Crick described the DNA molecule as "the secret of life", and today several fields of research address DNA directly or indirectly. The most recent breakthrough in the history of DNA research has been the sequencing of the human genome [4, 5], which has heralded a new era for genetic as well as epigenetic research. The challenge now, is to understand the molecular mechanisms that allow specific genes and gene families to be selectively expressed in normal development and how aberrations in this process can lead to disease. In addition to well-described genetic mechanisms, imbalances in the epigenetic control of gene expression can profoundly alter this finely tuned machinery. Epigenetic changes are now recognized to have a lead role in cancer development [6]. Simultaneously, such changes have been hypothesized to be a master key to more effective ways of diagnosing, monitoring, and treating cancer [7]. On our way to molecular assisted medicine, we need to explore this in detail in order to get a better understanding of the role of epigenetic in cancer development, which is necessary to fully master these new tools.

INTRODUCTION

What is a Cancer?:

Organisms are maintained by homeostasis, a finely tuned balance between cell proliferation and cell death. When the homeostasis is disturbed, either by an increased proliferation rate or a decrease in cell death, a tumor might occur, which can further progress into a cancer. Tumor development is most commonly described as natural selection followed by clonal expansion, resulting in monoclonal tumors originating from the progeny of a single cell [8]. Aberrations that confer growth advantages to the cell will accumulate during the clonal selection process. These changes are consequences of several processes: 1) activation of proto-oncogenes, rendering the gene constitutively active or active under conditions in which the wild type gene is not, 2) inactivation of tumor suppressor genes, reducing or abolishing the activity of the gene product, 3) alteration of repair genes, which normally keep genetic alterations to a minimum. Genomic analyses focusing on structural and numerical aberrations of chromosomes have long suggested that cancer is, in essence, a genetic disease [9].

The first cancer-specific genetic aberration described was the Philadelphia chromosome in patients with chronic myeloid leukemia. This was initially identified in 1960 by Nowell and Hungerford and was later demonstrated to be the result of a translocation between chromosomes 9 and 22 [10]. Today, numerous mutations at the chromosome and DNA level have been described in hematological as well as solid tumors [10, 11]. The Mitelman Database of Chromosome Aberrations in Cancer lists the chromosomal aberrations of more than 47,000 tumors [12], and the IARC mutation database have recorded 21,587 somatic mutations of the tumor suppressor gene *TP53* [13].

During the last decades, several lines of evidence have proven the importance also of epigenetic modifications in tumorigenesis. Indeed, epigenetic changes are now recognized to be at least as common as genetic changes in cancer [6]. Moreover, epigenetic changes often precede and appear to be essential for several genetic events that drive tumor progression.

REVIEW OF LITRETURE

Epigenetics:

The term 'epigenetic' was coined by Conrad Waddigton in 1940s to describe "the interactions of genes with their environment, which bring the phenotype into being" [14]. This early usage of the term has been effectively displaced during the last decades and today epigenetic inheritance is defined as cellular information, other than the DNA sequence itself, that is heritable during cell division i.e. "The sum of the alterations to the chromatin template that collectively establish and propagate different patterns of gene expression (transcription) and silencing from the same genome." [15]. Epigenetics affect the transcription in the cell, thereby controlling gene expression and abnormal epigenetic changes can have serious effects for the organism. Most epigenetic changes only occur within the course of one individual organism's lifetime, but, if a mutation in the DNA has been caused in gamete that result in fertilization, then some epigenetic changes are inherited from one generation to the next - "Lamarckism". We can very roughly divide epigenetics into three substantially overlapping categories: DNA methylation, genomic imprinting, and histone modification. Among these mechanisms, DNA methylation is the most studied, and is the main focus of this thesis.

DNA Methylation:

DNA methylation is a covalent modification of nucleotides and the most frequently methylated nucleotide in the human genome is cytosine subsequently followed by a guanine in the DNA sequence, constituting a CpG dinucleotide. The cytosine is methylated in the C-5 position by a family of DNA (cytosine-5) methyltransferases (DNMTs) using the universal methyl donor Sadenosyl- L-methionine (SAM) (Figure.1).

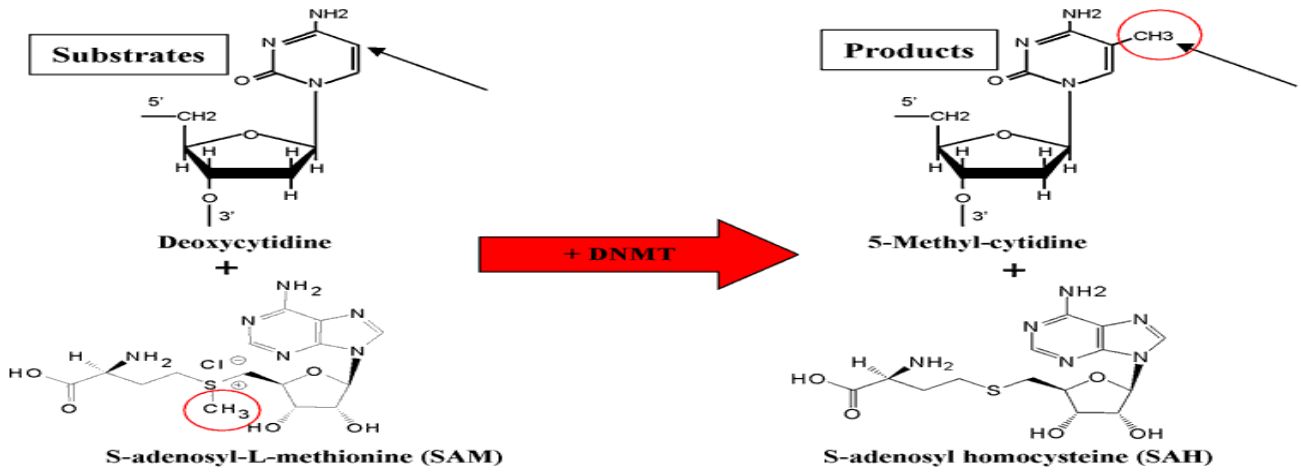


Figure.1: Methylation of cytosine catalyzed by DNMTs.

5 methylcytosines account for about 1% of total DNA bases in the human genome and affects 70-80% of the CpG sites in a human somatic cell [16]. Spontaneous deamination of 5-methylcytosine to thymine [17] has during the evolution led to a great under-representation of CpG dinucleotides in the human genome. When unmethylated cytosine deaminates to uracil it will be excised by the enzyme DNA-uracil glycosylase, and the original sequence is restored by DNA repair enzymes. However, the DNA repair machinery does not recognize the thymines resulting from 5-methylcytosine deaminations. Hence, the spontaneous deamination of 5-methylcytosine leads to a C to T transition mutation in the genome [17] (Figure.2). A substantial fraction of the CpG dinucleotides left in the genome are located in CpG islands, which are GC-rich regions that possess high relative densities of CpG. They are mainly positioned at the 5' ends of many human genes and are usually unmethylated regardless of the expression status of the associated gene [18]. Recent reports using computational analyses suggests that there are at least 29,000 CpG islands in the human genome [5, 19]. Several CpG sites are also found within repetitive or parasitic intragenomic elements. In contrast to the CpG islands, the CpG sites located here are largely methylated [20], as are the majority of the remaining CpG sites scattered in the human genome.

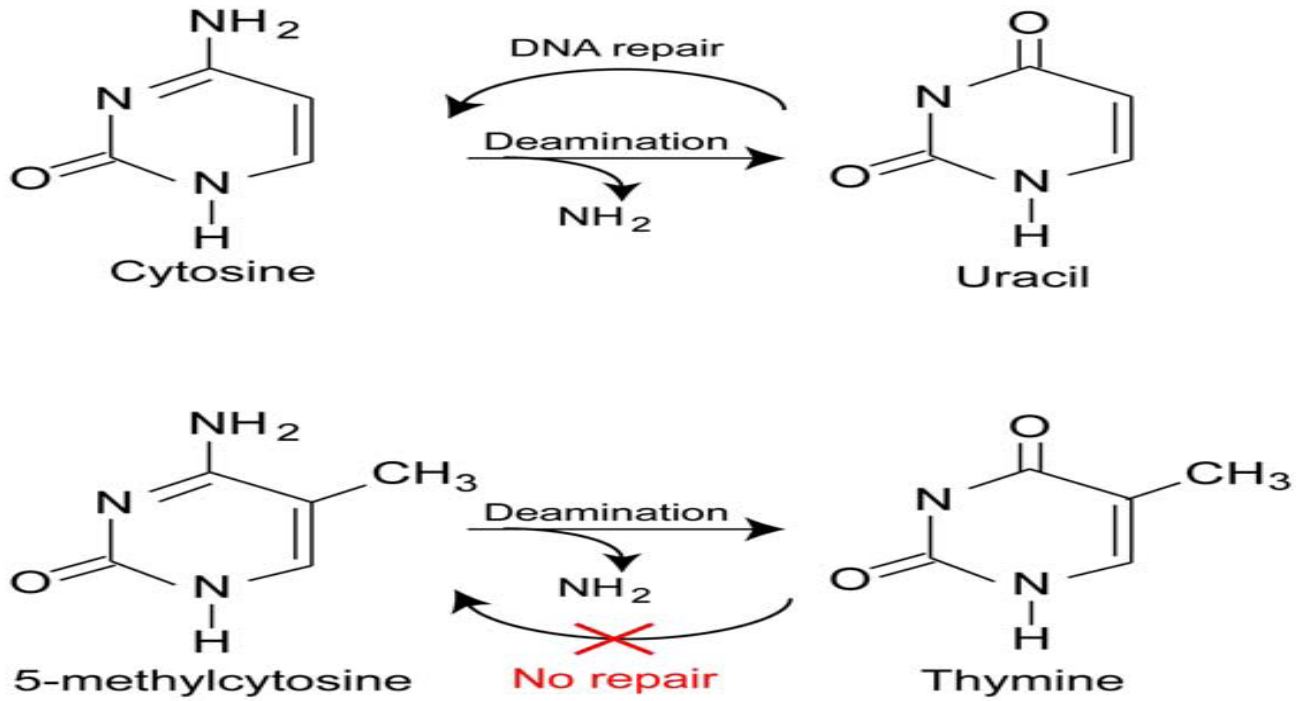


Figure.2: Introduction of mutation by spontaneous deamination of 5- methylcytosine.

DNA methylation as a gene silencing mechanism:

The first connection between DNA methylation and gene expression was published more than 25 years ago [21]. Two mechanisms have been proposed to account for transcriptional repression via DNA methylation. In the first mechanism, DNA methylation directly inhibits the binding of transcription factors (TFs) such as AP-2, c-Myc/Myn, E2F and NFkB to their binding sites within promoter sequence. In this mechanism, CpG dinucleotides have to be present within the binding site of TFs, which are sensitive to methylation of CpG dinucleotides (Figure.3: A & B).

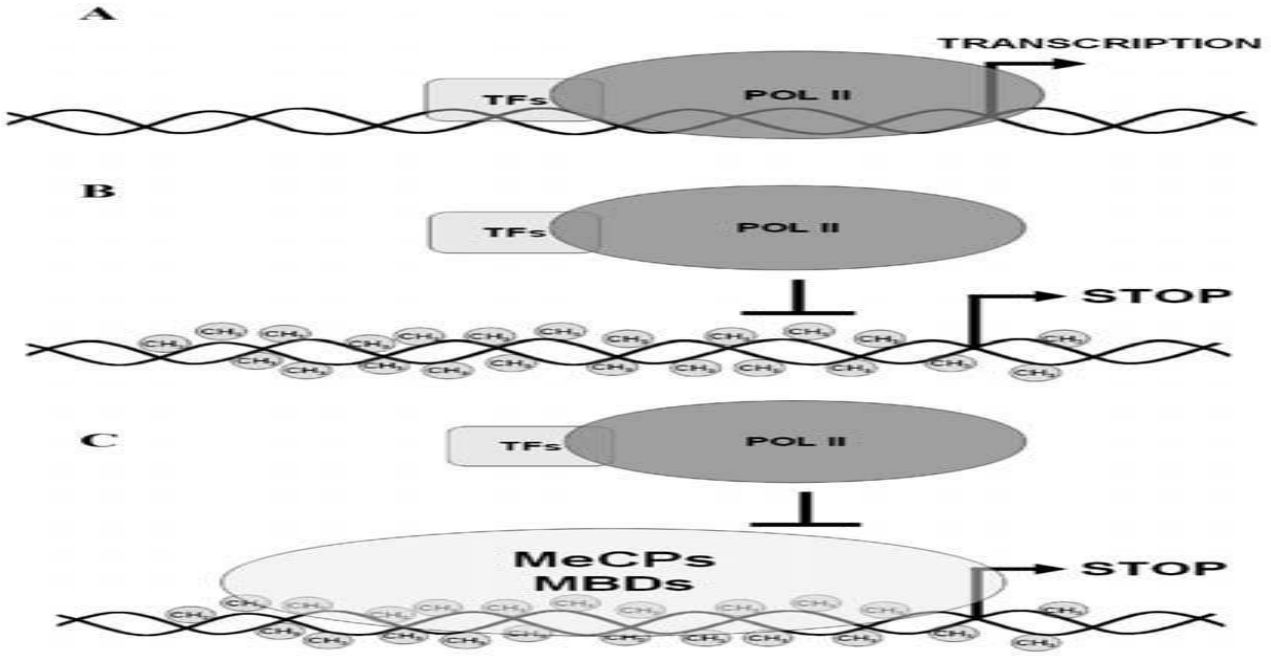


Figure.3: Repression of transcription via CpG dinucleotide methylation. (Fujita N *et.al.*2000)

The second mode of repression includes a binding of proteins specific for m5CpG dinucleotides to methylated DNA. Methylated DNA recruits m5CpG-binding (MeCP) and m5CpG-binding domain (MBD) proteins. MeCP1 and MeCP2 bind specifically to methylated DNA in whole genome and form spatial obstacle that unable binding of TFs to promoter sequences (Figure.3: C). MeCP1 represses transcription of specific genes, which are controlled by densely methylated promoters containing more than ten m5CpG dinucleotides. MeCP2 can bind to a single symmetrically located m^5CpG pair in two DNA strands [22].

These changes were inherited by the next generation of cells and it became obvious that reducing DNA methylation reactivated certain genes, allowing the development of new cells from the original embryo. Today, two different pathways have been described for the inactivation of gene transcription by DNA methylation:

1) Methyl-CpGs can repel transcription factors directly by being present in the transcription factor binding sequence. Although regulation by such a mechanism *in vivo* is relatively rare, some transcription factors, like Ets-1 [23] and the boundary element factor CTCF [24] are unable to bind DNA if the cytosines in their recognition sites are methylated.

2) DNA methylation can recruit proteins that bind methylated CpGs and subsequent inhibit transcription by remodelling the chromatin structure.

DNA Methyltransferases (DNMTs):

The mammalian DNMTs family encompasses DNMT1, DNMT2, DNMT3A and DNMT3B. This family is divided into maintenance and *de novo* Methyltransferases. Maintenance DNMT1 binds methyl groups to the hemimethylated DNA during replication, whereas *de novo* DNMT3A and DNMT3B add methyl groups to CpG dinucleotides of unmethylated DNA (Figure.4).

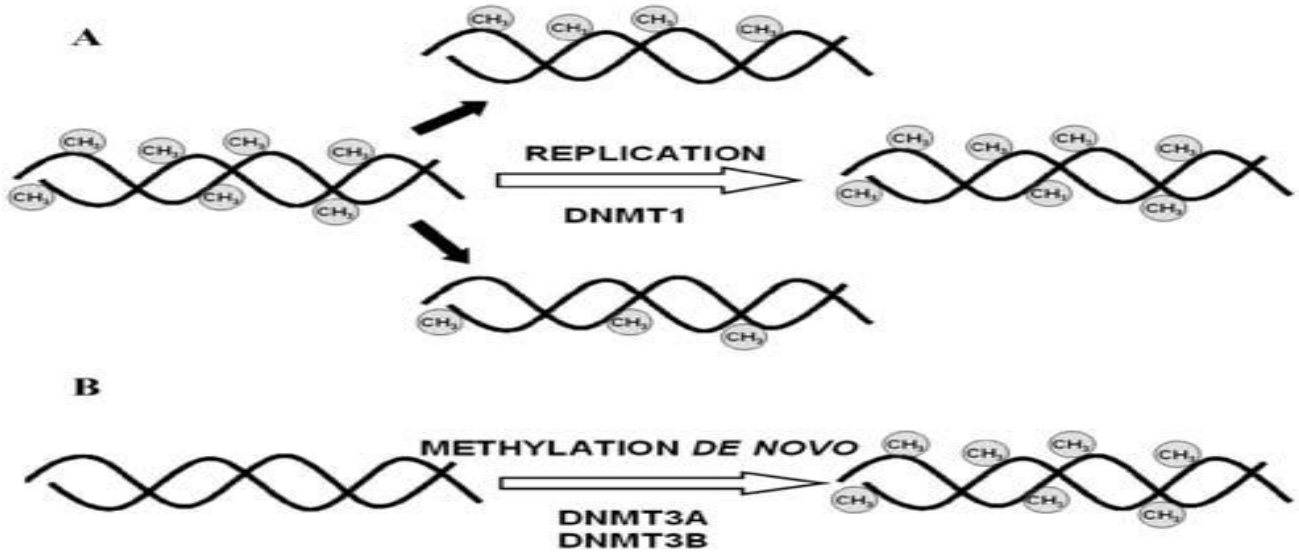


Figure.4: Methylation Pattern in genomic DNA. T.H. (Bestor *et.al*-1988, Xie S *et.al*.-1999)

DNMT1, DNMT3A and DNMT3B are required for formation of the established pattern of methylation in promoters and first exons of human genomic DNA [25]. In somatic cells, the pattern of DNA methylation is highly conservative and during cell division is kept by maintenance DNA methyltransferase (DNMT1) [25, 26, 27]. This enzyme is a component of DNA replication complex[28] and maintains the DNA methylation via addition of a methyl group to the 5-position of the cytosine ring within the CpG dinucleotides of newly synthesized DNA strand. DNMT1 forms three isoforms, which were found in somatic cells, pachytene spermatocytes, oocytes and preimplantation embryos. These transcript isoforms are produced by alternative usage of multiple first exon of DNMT1 primary transcript [29].

DNMT3A and DNMT3B enzymes are responsible for establishment of new methylation pattern in genomic DNA (Figure.4.B) [30, 31 & 25].

Mammalian DNMT1, DNMT3A and DNMT3B are composed of the N-terminal regulatory and the C-terminal catalytic domains that are linked by a short fragment of repeated GK dipeptides (Figure.5). The N-terminal domains of DNMT1 and DNMT3B do not exhibit extensive homolog of primary structure. These differences are responsible for distinct functions of N-regions in these enzymes. The DNMT1 requires interaction between the N- and C-terminal domains for catalytic activity. Separated C-terminal domain of DNMT1 is catalytically inactive despite the presence of the highly conserved sequence motifs typical of active DNMTs. In contrast to DNMT1, C-terminal domain of DNMT3A and DNMT3B is active without interaction with their N-regulatory regions. These differences between DNMT1 and *de novo* DNMTs indicate significantly disparate mechanism that regulate methylation activity of these enzymes.

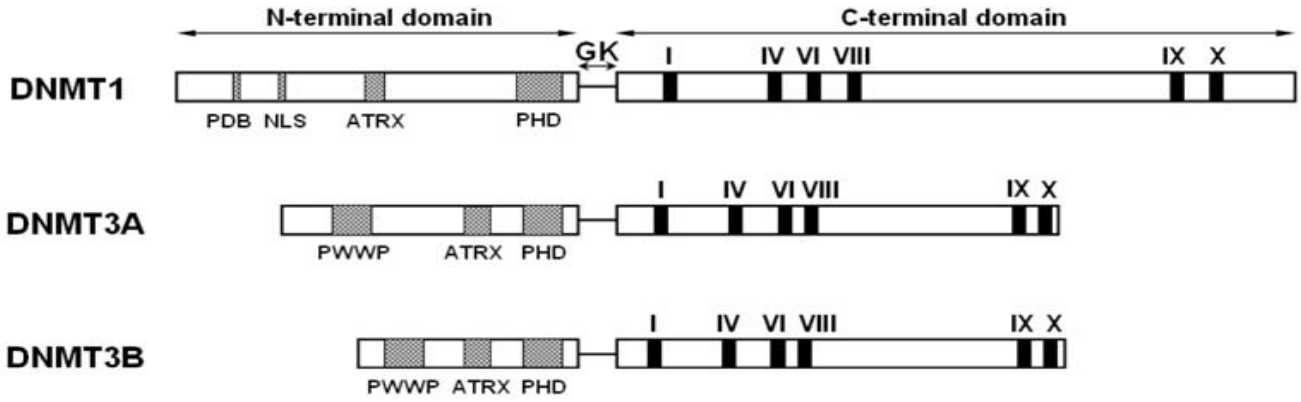


Figure.5: Members of mammalian DNMTs family. (Weisenberger DJ, *et.al*.-2004)

The N-terminal domain possesses nuclear localization signal sequence (NLS) responsible for localization of DNMTs in the nucleus. The N-fragment of DNMTs also contains proliferating cell nuclear antigen binding domain (PDB), a cysteine rich zinc finger DNA binding motif (ATRX), and polybromo homology domain (PHD) targeting DNMTs to the replication foci. However, PWWP tetrapeptide is only present in N-terminal domains of DNMT3A and DNMT3B and interact with histones [32]. The C-terminal domain contains six conservative motifs I, IV, VI, VIII, IX and X. Motifs I and X form S-adenosylomethionine binding site, motif IV binds cytosine at the active site, motif VI possesses glutamyl residue donating protons, and motif IX maintains the structure of the target recognition domain (TRD) usually located between motifs VIII and IX, that makes base-specific contacts in the major groove of DNA [33, 34, 35, 36].

DNMTs & TUMOR SUPRESSOR GENES (TSGs) IN CANCER:

DNA hypermethylation is responsible for epigenetic inactivation of TSGs expression in cancer cells. Increase in mRNA and protein biosynthesis of DNMT1 and DNMT3B in various cancer types significantly correlates with hypermethylation of CpG islands located in the promoter regions of various TSGs (Table.1). Methylation of these TSG promoters is associated with the complete loss of TSG protein products in cancer cells and development of malignant phenotype [37].

Etoh *et al.* [38] showed in gastric cancer cells significantly reduced expression of the *CDKN2A*, *MLH1*, and cell-cell adhesion protein encoded by *CDH1* gene. Loss of expression of mRNAs and proteins of TSGs correlated with intracellular elevation of DNMT1 content and complete methylation of the *CDKN2A*, *MLH1* and *CDH1* promoters in gastric cancer cells. Similar relationship between DNMT1 and DNMT3B expression and *CDH1* promoter methylation was observed by Girlaut *et al.* [39] in breast carcinoma. Increased expression of DNMT1 and DNMT3B were also correlated with the increase in breast cancer aggressiveness [39]. The epigenetic inactivation of *CDKN2A* and *MLH1* genes expression was also associated with increased level of DNMT1 and DNMT3B contents in colon cancer cells [40].

TSGs	**Functions**	**Cancer type with identified hypermethylation**	**References**
BRCA1	DNA damage repair	Breast, Ovarian	[41]
CDH1	Cell-Cell adhesion	Breast, Prostrate, Colorectal	[42, 43]
CDKN2A	Cyclin dependent kinase inhibitor	Lymphoma	[44]
CDKN2B		Leukemia	[45]
RB1	Represses the transcription of cellular genes	Retinoblastoma	[46]
TP53	Cell cycle regulation	Leukemia	[47]

Table.1: TSGs promoter methylation pattern in cancer.

OBJECTIVE

To clarify the role of DNMTs in the aberrant promoter hypermethylation of TSGs in various human cancers, the expression levels of a number of DNMTs in cancer tissues were examined and were correlated with the findings of the promoter methylation status of p53 TSGs which is commonly involved in cellular regulatory pathways. The expressions of the DNMTs, DNMT1, DNMT2, DNMT3A and DNMT3B were examined, since these enzymes have a reported role in the maintenance of the genome methylation status integrity and have been implicated in the transcriptional regulatory changes of human cancer.

So our main objective was "**Comparative Analysis of DNA Methyltransferase (DNMTs) Expression and Promoter Hypermethylation of Tumor Suppressor (TSGs) Genes in Normal and various Human Cancer Tissues"**.

MATERIALS AND METHODS

SAMPLE COLLECTION:

To achieve our objective, blood was collected from CWS Hospital, Rourkela as normal human tissue and cancer tissues (Gall Bladder and Lymph Node) from CMC, Kolkata.

TOTAL RNA ISOLATION:

Chemical Reagents and Buffer:-

- TRIzol Reagents (Sigma),
- Choloroform,
- Isopropanol,
- Ethanol (70%),
- Denaturation Buffer- 50 % deionized formamide,

, 2.2 M formaldehyde,

MOPS buffer (pH 7.0),

6.6 % glycerol,

0.5 % bromphenol,

- Ethidium Bromide,
- Agarose,

Protocol:-

- Transfered 50-100 mg of frozen tissue in a 2 ml tube with 1 ml TRIzol.
- Homogenized for 60 sec in the polytron
- Added 200 µl chloroform
- Mixed by inverting the tube for 15 sec
- Incubated for 3 min at room temperature
- Centrifuged at 12.000 g for 15 min
- Transfered the aqueous phase into a fresh Eppi tube
- Added 500 µl isopropanol
- Centrifuged at max. 12.000 g for 10 min in the cold room

- Washed the pellet with 500 µl 70 % ethanol
- Centrifuged at max. 7.500 g for 5 min in the cold room
- Dried the pellet on air for 10 min
- Disolved the pellet in 50-100 µl DEPC-H_2O
- Incubated for 10 min at 60° C
- Took spectrophotometer reading
- And analysed the RNA on a MOPS gel:

  -Disolved 1-3 µg RNA in 11 µl denaturation buffer

  - Added 1 µl ethidium bromide (1mg/ml) and denaturate at 65° C for 15 min

  - Loaded a 1 % agarose gel in MOPS buffer plus 5 % formaldehyde

  - Run the gel at 40 V for 4 h

cDNA SYNTHESIS:

Chemical Regents and Buffer:-

- 5X First Strand Buffer
- 10mM dNTP Set
- 0.1M DTT
- Random Primers
- RNaseOUT Ribonuclease Inhibitor
- SuperScript II RNase H- Reverse Transcriptase

Protocol:-

- Took 8µl of total RNA.
- Then added 3 µl Random Primers.
- Added 1 µl dNTP mix.
- Vortex and then spin down tube.
- Incubated at 65°C for 5 min.
- Placed tube on ice.

- Added 4 µl 5X Buffer, 2 µl DTT and 1µl RNAseOut.
- Vortex and then spin down tube.
- Incubated at 42°C for 1 min.
- Added 1µl SuperScript II RNase H- Reverse Transcriptase.
- Incubated at 42°C for 60 min.
- Incubated at 70°C for 15 min.
- Added 180 µl molecular grade water.
- Store at -80°C.

GENE SPECIFIC PCR:

Primers:-

Gene Name	Sense Primer	Antisense Primer	Tm
DNMT1	ACCAAGCAAGAAGTGAAGCC	GCTTCCTGCAGAAGAACCTG	63.3 & 64.0
DNMT3A	CACACAGAAGCATATCCAGGAGTG	AGTGGACTGGGAAACCAAATACCC	66.7 & 68.5
DNMT3B	AATGTGAATCCAGCCAGCCAGGAAAGGC	ACTGGATTACACTCCAGGAACCGT	77.9 & 67.6
p53	GTCACTGCCATGGAGGAGCCGCA	GACGCACACCTATTGCAAGCAAGGGTTC	78.2 & 76.1
B-Actin	TCTACAATGAGCTGCGTGTG	ATCTCCTTCTGCATCCTGTC	62.7 & 60.8

[Patra *et.al*, 2002. Dolidrup *et.al*, 2007]

PCR Mixture:- (Total 25µl)

- 0.2 µM dNTP- 0.5µl
- 1.5 mM $MgCl_2$- 1.5µl
- 1x PCR Buffer- 2.5µl
- Taq Polymearse (5U/µl)- 0.5µl
- Primers (0.2µM)- 0.5µl & 0.5µl
- cDNA- 2µl
- MQ Water- 17µl

PCR Condition:-

$94^oC_{1:00}[94^o\ C_{0:20}; 57^\circ C_{0:20}; 72^oC\ _{0.30}]_{30}; 72^oC\ _{5:00}$ for DNMT3B.

$94^oC_{1:00}[94^o\ C_{0:20}; 65^\circ C_{0:20}; 72^oC\ _{0.30}]_{30}; 72^oC\ _{5:00}$ for DNMT3A.

$94^oC_{1:00}[94^o\ C_{0:20}; 58^\circ C_{0:20}; 72^oC\ _{0.30}]_{30}; 72^oC\ _{5:00}$ for DNMT1.

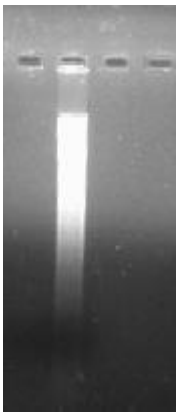
$94^oC_{1:00}[94^o\ C_{0:30}; 60^\circ C_{0:45}; 72^oC\ _{1.30}]_{30}; 72^oC\ _{5:00}$ for p53.

RESULTS & DISCUSSON

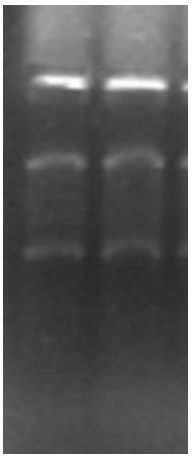
From Normal Tissue (Blood):

Product	Conc^{n}. (µg/ml)	Purity	
		260/280	260/230
Total RNA	570.32	1.34	0.82

Table.2: Spectrophotometer results of total RNA from blood tissue



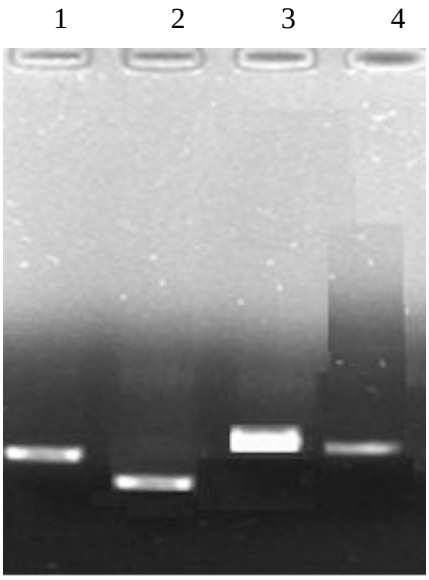
[Total RNA in 1% agarose gel]



[Total RNA in denaturation gel]

Gene	Conc^{n}. (µg/ml)	Purity	
		260/280	260/230
DNMT1	263.25	1.71	1.03
DNMT3A	286.69	1.79	0.96
DNMT3B	301.58	1.83	0.89
p53	276.23	2.01	0.86
B-Actin	401.35	1.93	1.02

Table.3: Spectrophotometer results of gene specific amplification product from blood



Lane.1- β-actin

Lane.2- DNMT3A

Lane.3-DNMT3B

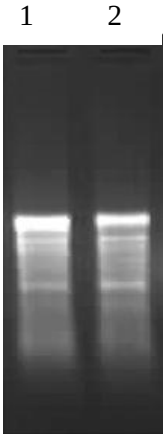
Lane.4-p53

[Gene specific PCR amplification]

From Cancerous Tissue:

Tissue	**Conc^{n}.** (µg/ml)	**Purity**	
		260/280	**260/230**
Gall Bladder Cancer	234.67	1.03	0.65
Lymph Node Cancer	478.51	1.61	1.02

Table.4: Spectrophotometer results of total RNA from cancerous tissue



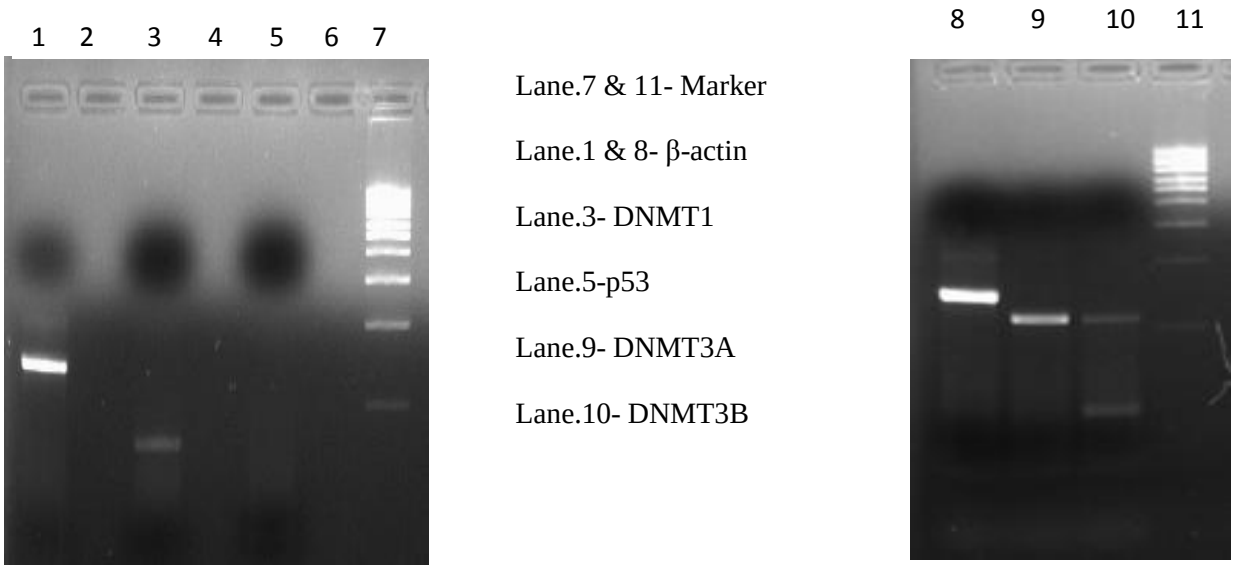
Lane.1: Lymph Node Cancer

Lane.2: Gall Bladder Cancer

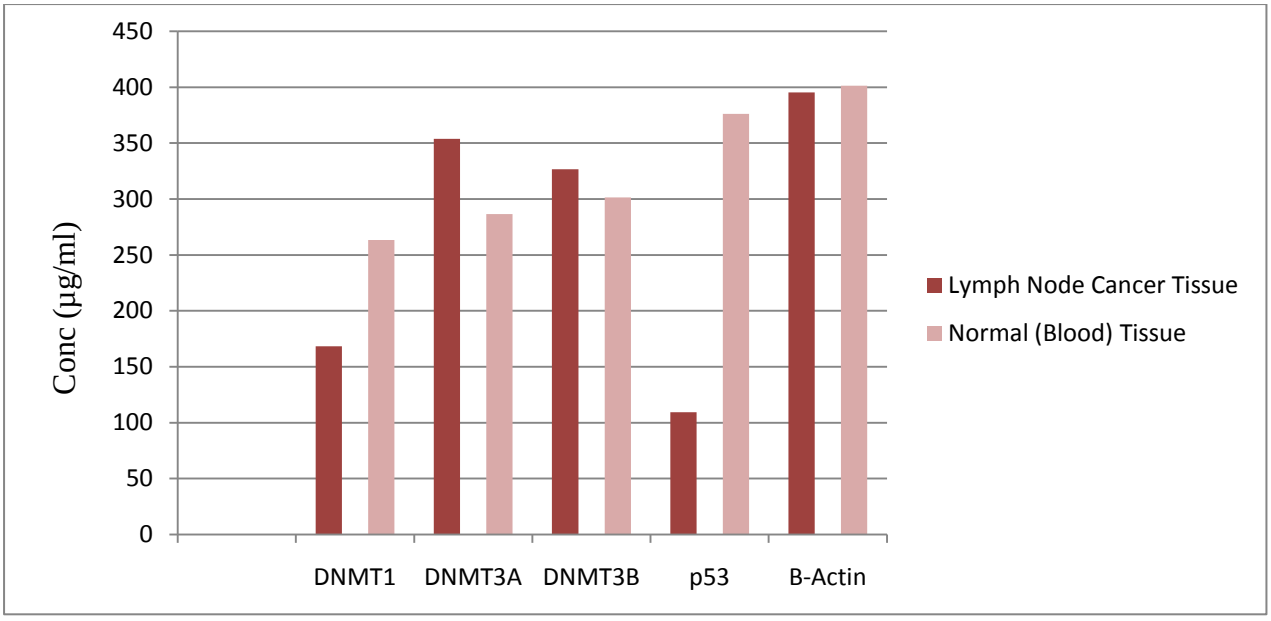
[Total RNA in denaturation gel]

Gene	Conc^{n}. (µg/ml)	Purity	
		260/280	260/230
DNMT1	168.23	1.74	1.28
DNMT3A	353.78	1.87	1.31
DNMT3B	326.69	1.69	1.25
p53	65.21	1.57	0.92
B-Actin	395.35	1.73	1.19

Table.5: Spectrophotometer results of gene specific amplification product from lymph node cancer tissue



[Gene specific PCR amplification]



[Comparative Study of Expression Level in both Normal & Cancer Tissue]

After isolation the total RNA from normal blood and Cancerous tissue, we checked their concentration by taking its OD in spectrophotometer, in case of Gall Bladder the concentration was found to be very low i.e. 234.67µg/ml, which was very low compare to the other samples reading. Because it was took a lot of time to processing after collecting the sample and also cDNA was not synthesize from the total RNA by rt-PCR method due to some unsuitable condition.

It was observed, after gene specific amplification there amazing results were found. These were the concentration of DNMT3A and DNMT3B i.e. 286.69 and 301.58 respectively in normal blood cells, but in cancerous tissue the concentration were found to increase i.e. 312.78 and 353.69 respectively. And TSG i.e p53 concentration was 376.23 µg/ ml in normal blood cells, but in cancerous tissue it was so much lowered i.e.109.21 µg/ml.

CONCLUSION

As observed from the above results, DNMTs are over express in Lymph Node cancer tissue. So from this we can hypothesize that these over expression of DNMTs leads to hypermethylation of TSG (for example, p53), which in turn diminish the expression of p53 and causing the cancer.

FUTURE WORK

The role of DNMTs in causing cancer through its effects on TSGs can be further confirmed by carrying out by bisulphate modification and methylation specific-PCR (MS-PCR) of the respective genes. This can help to locate the exact methylation site on the TSGs promoters.

REFERENCES

1. JD Watson, FH Crick: **Molecular structure of nucleic acids: a structure for deoxyribose nucleic acid.** *Nature* 1953, **171:** 737-738.

2. JD Watson: **The double helix**, 1 edn. Weidenfeld & Nicolson; 1968.

3. RD Hotchkiss: **The quantitative separation of purines, pyrimidines, and nucleosides by paper chromatography.** *J Biol Chem* 1948, **175:** 315-332.

4. JD McPherson, M Marra, L Hillier, RH Waterston, A Chinwalla, J Wallis, M Sekhon, K Wylie, ER Mardis, RK Wilson et al.: **A physical map of the human genome.** *Nature* 2001, **409:** 934-941.

5. JC Venter, MD Adams, EW Myers, PW Li, RJ Mural, GG Sutton, HO Smith, M Yandell, CA Evans, RA Holt et al.: **The sequence of the human genome.** *Science* 2001, **291:** 1304-1351.

6. PA Jones, SB Baylin: **The fundamental role of epigenetic events in cancer.** *Nat Rev Genet* 2002, **3:** 415 428.

7. PW Laird: **The power and the promise of DNA methylation markers.** *Nat Rev Cancer* 2003, **3:** 253- 266.

8. PC Nowell: **The clonal evolution of tumor cell populations.** *Science* 1976, **194:** 23-28.

9. B Vogelstein, KW Kinzler: **Cancer genes and the pathways they control.** *Nat Med* 2004, **10:** 789-799.

10. JD Rowley: **Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining.** *Nature* 1973, **243:** 290-293.

11. MR Teixeira, S Heim: **Multiple numerical chromosome aberrations in cancer: what are their causes and what are their consequences?** *Semin Cancer Biol* 2005, **15:** 3-12.

12. F Mitelman, B Johansson, F Mertens. Mitelman Database of Chromosome Aberrations in Cancer. http://cgap.nci.nih.gov/Chromosomes/Mitelman. 2005. Ref Type: Electronic Citation

13. M Olivier, R Eeles, M Hollstein, MA Khan, CC Harris, P Hainaut: **The IARC TP53 database: new online mutation analysis and recommendations to users.** *Hum Mutat* 2002, **19:** 607-614.

14. C Waddington: **The Epigenotype.** *Endeavour* 1942, **1:** 18-20.

15. AP Feinberg, B Tycko: **The history of cancer epigenetics.** *Nat Rev Cancer* 2004, **4:** 143-153.

16. M Ehrlich, MA Gama-Sosa, LH Huang, RM Midgett, KC Kuo, RA McCune, C Gehrke: **Amount and distribution of 5 methylcytosine in human DNA from different types of tissues of cells.** *Nucleic Acids Res* 1982, **10:** 2709-2721.

17. C Coulondre, JH Miller, PJ Farabaugh, W Gilbert: **Molecular basis of base substitution hotspots in Escherichia coli.** *Nature* 1978, **274:** 775-780.

18. AP Bird: **CpG-rich islands and the function of DNA methylation.** *Nature* 1986, **321:** 209-213.

19. ES Lander, LM Linton, B Birren, C Nusbaum, MC Zody, J Baldwin, K Devon, K Dewar, M Doyle, W FitzHugh et al.: **Initial sequencing and analysis of the human genome.** *Nature* 2001, **409:** 860-921.

20. F Larsen, G Gundersen, R Lopez, H Prydz: **CpG islands as gene markers in the human genome.** *Genomics* 1992, **13:** 1095-1107.

21. SM Taylor, PA Jones: **Multiple new phenotypes induced in 10T1/2 and 3T3 cells treated with 5- azacytidine.** *Cell* 1979, **17:** 771-779.

22. Hendrich B, Bird A: **Identification and characterizationof a family of mammalian methyl-CpG binding proteins**. *Mol Cell Biol* 1998, **18**: 6538-6547.

23. H Maier, J Colbert, D Fitzsimmons, DR Clark, J Hagman: **Activation of the early B-cell-specific mb-1 (Ig-alpha) gene by Pax-5 is dependent on an unmethylated Ets binding site.** *Mol Cell Biol* 2003, **23:** 1946-1960.

24. AC Bell, G Felsenfeld: **Methylation of a CTCF-dependent boundary controls imprinted expression of the Igf2 gene.** *Nature* 2000, **405:** 482-485.

25. Das PM, Singal R: **DNA methylation and cancer.** *J Clin Oncol* 2004, **22**: 4632-4642.

26. Momparler RL: **Cancer epigenetics**. *Oncogene* 2003, **22**: 6479-6483.

27. Robertson KD, Jones PA: **DNA methylation: past, present and future directions.** *Carcinogenesis* 2000, **21**: 461-467.

28. Szyf M, Pakneshan P, Rabbani SA: **DNA methylation and breast cancer**. *Biochem Pharmacol* 2004, **68**: 1187-1197.

29. Mertineit C, Yoder JA, Taketo T, Laird DW, Trasler JM, Bestor TH: **Sex-specific exons control DNA methyltransferase in mammalian germ cells**. *Development* 1998, **125**: 889-897.

30. Brown R, Strathdee G: **Epigenomics and epigenetic therapy of cancer**. *Trends Mol Med* **8**, 2002, Suppl 4: S43-S48.

31. Wang YM, Wang R, Wen DG, Li Y, Guo W, Wang N, Wei LZ, He YT, Chen ZF, Zhang XF, Zhang JH: **Single nucleotide polymorphism in DNA methyltransferase 3B promoter and its association with gastric cardiac adenocarcinoma in North China.** *World J Gastroenterol* 2005, **11**: 3623-3627.

32. Hermann A, Gowher H, Jeltsch A: **Biochemistry and biology of mammalian DNA methyltransferases**. *Cell Mol Life Sci* 2004, **61**: 2571-2587.

33. Bestor TH: **The DNA methyltransferases of mammals**. Hum Mol Genet 2000, **9**: 2395-2402.

34. Robertson KD, Uzvolgyi E, Liang G, Talmadge C, Sumegi J, Gonzales FA, Jones PA: **The human DNA Methyltransferases (DNMTs) 1, 3a and 3b: coordinate mRNA expression in normal tissues and overexpression in tumors**. *Nucleic Acids Res* 1999, **27**: 2291-2298.

35. Weisenberger DJ, Velicescu M, Cheng JC, Gonzales FA, Liang G, Jones PA: **Role of the DNA methyltransferase variant DNMT3b3 in DNA methylation**. *Mol Cancer Res* 2004, **2**: 62-72.

36. Xie S, Wang Z, Okano M, Nogami M, Li Y, He WW, Okumura K, Li E: **Cloning, expression and chromosome locations of the human DNMT3 gene family**. *Gene* 1999, **236**: 87-95.

37. Mizuno S, Chijiwa T, Okamura T, Akashi K, Fukumaki Y, Niho Y, Sasaki H: **Expression of DNA Methyltransferases DNMT1 , 3A, and 3B in normal hematopoiesis and in acute and chronic myelogenous leukemia.** *Blood* 2001, **97**: 1172-1179.

38. Etoh T, Kanai Y, Ushijima S, Nakagawa T, Nakanishi Y, Sasako M, Kitano S, Hirohashi S: **Increased DNA methyltransferase 1 (DNMT1) protein expression correlates significantly with poorer tumor differentiation and frequent DNA hypermethylation of multiple CpG islands in gastric cancers.** *Am J Pathol* 2004, **164**: 689-699.

39. Girault I, Tozlu S, Lidereau R, Bieche I: **Expression analysis of DNA methyltransferases 1, 3A, and 3B in sporadic breast carcinomas.** *Clin Cancer Res* 2003, **9**: 4415-4422.

40. Kanai Y, Ushijima S, Kondo Y, Nakanishi Y, Hirohashi S: **DNA methyltransferase expression and DNA methylation of CpG islands and peri-centromeric satellite regions in human colorectal and stomach cancers.** *Int J Cancer* 2001, **91**: 205-212.

41. Esteller M, Silva JM, Dominguez G, Bonilla F, Matias-Guiu X, Lerma E, Bussaglia E, Prat J, Harkes IC, Repasky EA, Gabrielson E, Schutte M, Baylin SB, Herman JG: **Promoter**

hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. *J Natl Cancer Inst* 2000, **92**: 564-569.

42. Darwanto A, Kitazawa R, Maeda S, Kitazawa S: **MeCP2 and promoter methylation cooperatively regulate E-cadherin gene expression in colorectal carcinoma**. *Cancer Sci* 2003, **94**: 442-447.

43. Graff JR, Herman JG, Lapidus RG, Chopra H, Xu R, Jarrard DF, Isaacs WB, Pitha PM, Davidson NE, Baylin SB: **E-cadherin expression is silenced by DNA hypermethylation in human breast and prostate carcinomas**. *Cancer Res* 1995, **55**: 5195- 5199.

44. Herman JG, Merlo A, Mao L, Lapidus RG, Issa JP, Davidson NE, Sidransky D, Baylin SB: **Inactivation of the CDKN2/p16/MTS1 gene is frequently associated with aberrant DNA methylation in all common human cancers**. *Cancer Res* 1995, **55**: 4525-4530.

45. Melki JR, Vincent PC, Clark SJ: **Concurrent DNA hypermethylation of multiple genes in acute myeloid leukemia**. *Cancer Res* 1999, **59**: 3730 3740.

46. Stirzaker C, Millar DS, Paul CL, Warnecke PM, Harrison J, Vincent PC, Frommer M, Clark SJ: **Extensive DNA methylation spanning the Rb promoter in retinoblastoma tumors.** *Cancer Res* 1997, **57**: 2229-2237.

47. Agirre X, Novo FJ, Calasanz MJ, Larrayoz MJ, Lahortiga I, Valganon M, Garcia-Delgado M, Vizmanos JL: **TP53 is frequently altered by methylation, mutation, and/or deletion in acute lymphoblastic leukaemia**. *Mol Carcinog* 2003, **38**: 201-208.

48. Patra S. K, Patra A, Zhao H, Dahiya R: **DNA Methyltransferase and Demethylase in Human Prostate Cancer.** *Mol Carcinog* 2002, **33**: 163-171.

49. Luczak M. W, Jagodzinski P. P: **The role of DNA methylation in cancer development.** *Folia Histo Et Cyto* 2006, **44** (3): 143-154.