

**HYPERMETHYLATED DNA AS BIOMARKER FOR NASOPHARYNGEAL CANCER DETECTION**THUAN DUC LAO<sup>1</sup>, THUY AI HUYEN LE<sup>2\*</sup>

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**ABSTRACT**

**Background:** Nasopharyngeal carcinoma (NPC) is a malignancy with remarkable geographic and distribution worldwide, towards in Southern China and Southern Asia. In addition to Epstein-barr virus infection, environmental carcinogens, the development of NPC involves the cumulative genetic as well as epigenetic alteration. More recently, it has been reported that DNA hypermethylation, an epigenetic mechanism, that occurred by the addition of a methyl group at 5' position of the pyrimidine ring of Cytosine residues at CpG islands, has been considered as the cause of nasopharyngeal tumorigenesis. In recent years, many reports have focused on the identification, evaluation of aberrant methylation of target tumor suppressor genes' promoters, such as *RASSF1A*, *Blu*, *DLEC*, *RARβ*, *p16*, *p15*, *p14*, *MGMT*, etc. in the NPC development.

**Objective and Method:** In this review, we summarized and focused on the description and exemplification of the DNA hypermethylation changes, particularly, highlight the DNA hypermethylation as a potential biomarker applied in monitoring, screening, early diagnosis for cancer of nasopharynx.

**Conclusion:** Measuring and detecting the hypermethylation status of TSGs could be served as potential, and promising biomarkers for monitoring, early screening and diagnosis of human cancers.

**Keywords:** DNA hypermethylation, Epigenetic, Tumor suppressor gene, Biomarkers.

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**INTRODUCTION**

Nasopharyngeal carcinoma (NPC), arises from the epithelium of the nasopharynx, is the most common malignant tumor of head and neck cancer with a remarkable geographic and racial distribution worldwide [1-3]. There is a striking distribution of NPC, which encounters in Southern China, Singapore, Vietnam, Malaysia, and the Philippines. On the contrary, it is also fairly common in Europe, USA, Northwest Canada, and Greenland [1,4,5]. According to the etiological factors of NPC, many previous studies suggested that there are several cofactors that are associated with NPC development. The major etiological factors proposed for NPC pathogenesis include the dietary factors, the infection with an oncovirus, named Epstein-Barr virus (EBV), a gamma herpes virus discovered in 1964 by Epstein *et al.* [5,6], and genetic-epigenetic factors, in a multistep carcinogenic process [3,7,8]. The remarkable racial and geographic distribution of NPC suggests the strong association of NPC with genetic susceptibility and environmental factors [7]. Based on the previous studies, a collaborative model for NPC tumorigenesis driven by specific genetic alteration, EBV infection, and environmental factors is proposed (Fig. 1). The viral risk factors and environmental factors for NPC discussed separately in many previous studies; therefore, this article will focus primarily on the epigenetic alterations, including the promoter hyper methylation, in NPC tumorigenesis.

**DNA HYPERMETHYLATION AND CANCER**

In contrast to the genetic alterations, epigenetic regulations, which were first coined by Waddington in 1942, means as "outside conventional genetics," refer to the heritable, reversible changes in gene expression that does not change the sequence of genome [9]. Recent advances in the field of epigenetics have shown that epigenetic modification is the natural processes and essential for mammalian development and cell proliferations that affect gene products at multiple levels, including transcriptional level, post-transcriptional regulation, leading to the great diversity to the gene regulation network [9,10]. In the case of improperly occurred, they could

be the major cause of health and normal development. Three main types of epigenetic modification systems, including DNA methylation, histone covalent modification, and non-coding RNA modification, leading to associated-gene disrupted, have been identified [11-13]. The pattern of hypermethylation, one of the two aberrant methylations, at the specific sites, namely, the CpG islands (CGIs) at the tumor suppressor genes (TSGs) promoter region, leads to the increasing of level of methyl group modification, is an important mechanism of a number of TSG inactivation [14-17]. This methylation process is catalyzed by DNA (cytosine-5)-methyltransferases (DNMTs). In mammalian, DNMTs are a highly conserved family protein encompassing DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L, which could be distinguished by their function [18-20]. DNMT1, which functions as the major maintenance of the existence of methylation patterns following DNA replication on the newly synthesized, was the first methyltransferase to be discovered. DNMT3A and DNMT3B perform *de novo* methylation by adding the methyl groups to unmethylated CpG, which is responsible for the establishment of new methylation pattern in genomic [20-22]. DNMT3L has no catalytic activity, and it has been shown to act as a general stimulatory factor for *de novo* methylation and facilitate methylation of DNMT3A and DNMT3B [23-25]. The hypermethylated CGIs at promoter region prevent the binding of RNA polymerase and transcription factors, such as AP-2, c-Myc/Myn, E2F, and NF-κB, as the results inhibit the TSGs transcription [10,20] (Fig. 2). The hallmark of cancer involves the loss of the function of TSGs through the silencing genetic information. The silencing of TSGs by the high levels of 5-methylcytosine in their CGI promoter regions, considered as the "first and second hit," is equivalent to mutation and translocations, in Knudson's two-hit model of tumorigenesis [26,27]. Thus, the presence of m5CpG dinucleotide in TSGs' promoters is recognized as an important event in many human cancers.

**DNA HYPERMETHYLATION IN NPC: BIOMARKERS FOR NPC DIAGNOSIS AND PROGNOSIS**

Previous studies suggested that NPC is associated with the accumulation of many epigenetic alterations on the particular chromosomal regions

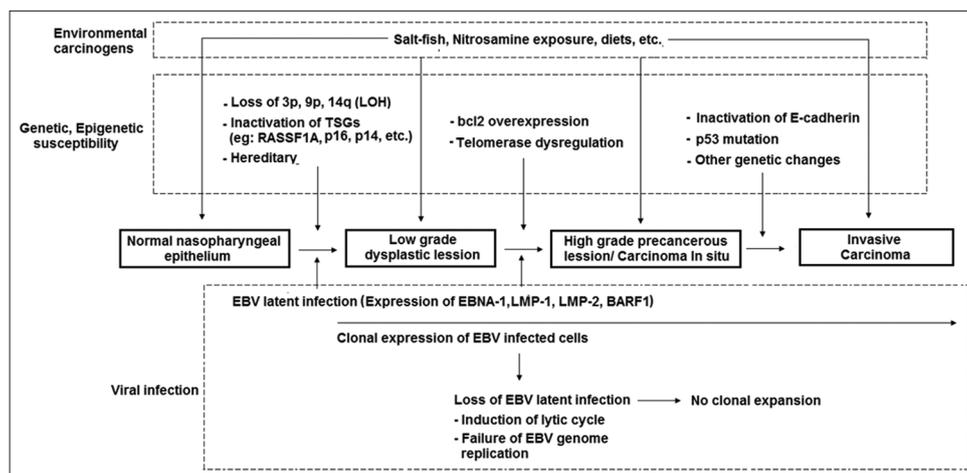


Fig. 1: Pathogenesis model for nasopharyngeal carcinoma

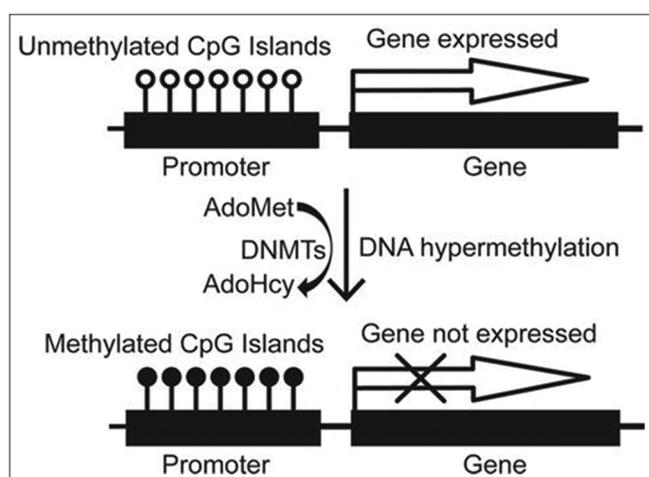


Fig. 2: The typical CpG island of a tumor suppressor gene is represented in a normal and a tumor cell [20]. White dots: Unmethylated CpG; black dots: Methylated CpG. AdoMet: S-adenosylmethionine. AdoHcy: S-adenosylhomocysteine. DNMTs: DNA (cytosine-5)-methyltransferases

and genes, including the alterations involve both TSGs and proto-oncogenes on multiple cellular pathways, which further contribute to the malignant cancer hallmarks [2,8,10]. Interestingly, recent studies confirm that epigenetic alterations, including the hypermethylation, are also one of the crucial factors that are highly associated with NPC development. According to the research of Dai *et al.*, in their comparative methylome study, as the compared to nine other human cancer types, including liver, head and neck, colon, lung, thyroid, kidney, breast, pancreatic, and prostate cancer, they found that NPC had the highest hypermethylation frequency [28]. Many studies demonstrated that the inactivation of TSGs located on chromosome 3p, 9p, 9q, 11q, 13q, 14q, and 16q is the common and important events in the NPC tumorigenesis and development, as summarized in Table 1 [8,29-32]. Representatively, the most frequently hypermethylation is reported in critical regions on chromosome 3p in NPC, as noted in Table 1.

Previous studies have shown that the inactivation of TSGs, which were located in chromosome 3, was significant associated with the development of NPC. Given the aberrant methylation frequently observed in NPC, we, therein, evaluated the status of methylation biomarkers for NPC detection, as summarized in Table 2. Based on Table 2, it suggested that *ZMYND10*, *RASSF1A*, *DLEC1*, and *RARβ* promoter hypermethylation are more frequent in NPC than in non-cancerous samples. Given examples, the well-known suppressor gene,

*RASSF1A*, located at 3p21.31, according to the systematical analysis of *RASSF1A* promoter methylation in NPC, Ye *et al.* suggested that, comprised 11 studies forming a large population, *RASSF1A* promoter methylation is significantly increased and notably common in NPC tissue samples compared with non-tissue samples, which indicated that hypermethylation of the *RASSF1A* promoter is closely linked to NPC tumorigenesis. In addition, they found that the pooled specificity and AUC of *RASSF1A* promoter hypermethylation were very good in tissue, brushing, and blood samples in the cases of NPC patients versus corresponding non-tumor samples [45]. Up to now, many studies relevant to the determination of combination many TSGs inactivated by promoter hypermethylation were carried. As the report of Kwong *et al.* [40], they have analyzed the promoter hypermethylation pattern of panel of eight TSGs, including *RASSF1A* (3p21.31), *RARβ2* (3p24.2), *DAPK* (9p21.33), *p16* (9p21.3), *p15* (9p21.3), *p14* (1q42.13), *MGMT* (10p26.3) and *GSTP1* (11q13.2). They reported that the high frequency of promoter hypermethylation of multiple cancer-related genes as well as at least one of eight genes showed aberrant methylation in all samples, provides an opportunity that the promoter hypermethylation may be used for the clinical diagnosis of NPC. In addition, more recent work has used next-generation sequencing (NGS) techniques, which has emerged as a powerful method to characterize the methyl changes in high resolution as well as to profile DNA hypermethylation across a whole genome or large regions of a genome, which is expected to be affordable for most research centers in the near future [46,47]. The advent of NGS techniques opens the possibility of discovery studies that quantify DNA methylation patterns and differences in DNA methylation could be served as potential biomarkers for the clinical diagnosis and screening of NPC.

#### METHYLATED CIRCULATING DNA AS A NASOPHARYNGEAL CANCER BIOMARKER

Recent advance technologies in the detection and characterization circulating tumor DNA (ctDNA) may address the great promise for the early detection and management of human cancers. Even in the early stage, ctDNA is considered to be easily detected in plasma of cancer patients [48,49]. In comparison to application of tissue biopsies in cancer detection, ctDNA, which fully representing tumors and is released into circulation by various pathologic and mechanism, represents a non-invasive, high specificity, sensitivity method for tumor diagnosis, and monitoring [48,50]. In addition, ctDNA could be distinguished from circulating DNA derived from healthy cells by the presence of genomic aberrant modifications [51]. The feasibility of using ctDNA in cancer detection has been demonstrated in many human cancers, including NPC. Given example, according to Wong *et al.*, 2004, they evaluated the proposed diagnosis value of quantitative measurement of plasma DNA concentration and hypermethylated DNA markers, including panel of genes, *CDH1*, *DAPK1*, *p15*, *p16*, *RASSF1A*,

**Table 1: TSG located on chromosome 3 frequently hypermethylated in NPC**

Gene	Full name	Location	Functions	References
<i>ZMYND10</i>	Zinc finger MYND-type containing 10	3p21.31	Environmental stress-response, cell cycle progression.	[33-38]
<i>RASSF1A</i>	Ras association Domain family member 1	3p21.31	Cell cycle arrest, apoptosis, proliferation.	[39-42]
<i>RARβ</i>	Retinoic acid receptors beta	3p24.2	Thyroid hormone receptor, transcription regulator.	[40,41,43]
<i>DLEC1</i>	Deleted in lung and esophageal cancer 1	3p21.3	Signaling transduction.	[38,44]

TSG: Tumor suppressor genes, NPC: Nasopharyngeal carcinoma

**Table 2: Summary of hypermethylation status based on previous studies**

Gene	Reports	Sample type	Method	Patients (%)	Controls (%)
<i>ZMYND10</i>	Qiu <i>et al.</i> , 2004	Primary tumor	MSP	66.0	0.0
	Ayadi <i>et al.</i> , 2008	Primary tumor	MSP	34.1	nd
	Agathangelou <i>et al.</i> , 2003	Tumor cell line	MSP	80.0	nd
	Yau <i>et al.</i> , 2006	Primary tumor	qRT-PCR	80.0	nd
		Tumor cell line		83.3	
<i>RASSF1A</i>	Qiu <i>et al.</i> , 2004	Primary tumor	MSP	74.0	0.0
	Wang <i>et al.</i> , 2009	Primary tumor	MSP, RT-PCR	71.05	0.0
	Lo <i>et al.</i> , 2001	Xenograft	MSP	100	nd
		Tumor cell line		75.0	
		Primary tumor		66.7	
	Kwong <i>et al.</i> , 2002	Xenograft	MSP	100	nd
		Tumor cell line		75.0	nd
<i>DLEC1</i>	Fendri <i>et al.</i> , 2009	Primary tumor	MSP	84.0	0.0
	Ayadi <i>et al.</i> , 2008	Primary tumor	MSP	91.0	0.0
	Loyo <i>et al.</i> , 2011	Primary tumor	MSP	86.3	nd
<i>RARβ</i>	Kwong <i>et al.</i> , 2002	Primary tumor	MSP	60.4	3.6
		Xenograft	MSP	50	nd
		Tumor cell line		100	nd
		Primary tumor		81.0	0.0
	Kwong <i>et al.</i> , 2005	Primary tumor	MSP	78.7	nd
Fendri <i>et al.</i> , 2009	Primary tumor	MSP	88.0	0.0	

nd: Not done

and *MLH1*, in undifferentiated NPC patients. As the results, they found that methylated DNA was detectable in plasma of NPC patients, and the frequencies of *CDH1*, *DAPK1*, *p15*, *p16*, and *RASSF1A* were 46%, 42%, 20%, 20%, and 5%, respectively. In contrast, the hypermethylation of *MLH1* was not detected in plasma of all of the NPC patients and normal individuals. Moreover, they found that aberrantly hypermethylated promoter DNA at least one of five genes detectable in 29 of 41 (counting for 71%) plasma of NPC patients. Thus, their results demonstrated that hypermethylated genes could be detected in the plasma of NPC patients, suggesting that hypermethylated gene might be used as a serological tumor marker in screening of primary NPC [52]. In summary, based on the circulating methylated gene promoter DNA is a possibly useful biomarker for diagnoses, prognoses, and guidance for treatments.

## CONCLUSION

NPC is characterized with multiple hallmarks including the EBV infection, dietary factors, and genetic-epigenetic factors. The hypermethylation of TSG promoter, epigenetic alteration, has been shown to be a specific event that interplays during NPC initiation and progression. Measuring and detecting the hypermethylation status of TSGs derived from tissue biopsies as well as the ctDNA dynamics in body fluids such as blood, or serum is a novel area and developing research, additionally, it is considerably served as potential, great versatility and promising biomarkers for monitoring, early screening, and diagnosis of human cancers, including NPC.

## CONFLICTS OF INTERESTS

The authors declared that they have no competing interests.

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