Correlation between RASSF1A, P16, DAP Kinase Promoter Hypermethylation and Lung Cancer: Relation with Smoking Status
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Abstract
Epigenetic silencing of tumor suppressor genes (TSG) is a fundamental incident in the pathogenesis of human cancer. Inactivation of TSG is accomplished by aberrant chromatin modifications including DNA hypermethylation of the gene promoter. Of the most frequently hypermethylated TSG, Ras Association Domain Family1 (RASSF1A), P16 and Death Association Protein kinase (DAPK) genes. Aberrant hypermethylation of these genes have been correlated with non small cell lung cancer (NSCLC) promoting disease recurrence and remote metastasis. This review aims to provide the readers with a precise description of the research to date in the field of epigenetics and its impact on people with NSCLCs. The focus of this study will be on promoter hypermethylation of three different lung cancer associated genes P16, RASSF1A and DAPK and how these genes inactivated and contribute to the pathogenicity of human malignancies. Moreover, The study aims to investigate the impact of tobacco smoke on the hypermethylation frequency of the mentioned genes. Thus, the promoter hypermethylation frequency could be a promising biomarker to improve NSCLC diagnosis and screening.

Keywords: Non small cell lung cancer, DNA methylation; Tumor suppressor genes; RASSF1A; P16, DAPK.

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INTRODUCTION
Lung cancer is responsible for cancer related deaths and one of most common malignancies worldwide [1]. Millions of people died due to lung cancer each year, and more than a million of people are annually diagnosed with lung cancer [2]. In addition, the survival rate is about 16% for most of the cases diagnosed with lung cancer [3]. These percentages highlighted the disease as a major public health panic. On the other hand, an early detection of the disease predisposes for a significant increase in survival rate [4]. Approximately half of the patients with non-small cell lung cancer (NSCLC) were not diagnosed at an early stages, where treatment is curative [3]. Lung cancer is divided into two major groups non small cell lung cancer (NSCLC) and small cell lung cancer, NSCLC is account for approximately 85% of lung cancer, and sub divided into adenocarcinoma, squamous cell carcinoma and large cell carcinoma [5].

Effects of Environmental Carcinogenesis on Epigenetics
Epigenetic patterns found to be altered by several epimutagens in the environment and diet [6]. Numerous environmental factors including chemicals found in cigarette smoke and alcohol consumption as well as dietary contaminants play a crucial role in the etiology of human cancer [7, 8]. Other life style factors such as plutonium or radon exposure and helicobacter pylori infections are thought to be involved in the development of broad spectrum of human cancers including NSCLC [9]. More importantly, tobacco smoking is considered to be the most life style that contribute to cancer development, and reported to be the most common cause of lung cancer worldwide [10]. It is argued that the overwhelming majority of patients with lung cancer are indicated to have smoking status [11], estimating that as many as 69 of 4800 identified compounds of a cigarette may possibly be carcinogens [12].

Challenges Faced the Efficiency of Lung Cancer Treatment
It is widely accepted that lung cancer faced noticeable challenges to diagnose early and treat efficiently regardless of the reality that most of the causes of lung cancer are well recognized, in addition to the remarkable advance in the molecular mechanisms underlying lung carcinogenesis [9]. Lung cancer is a deadly disease and the five year survival rates at approximately 17%, despite the advanced diagnostic strategies and development of innovative treatment
[13]. More importantly, treatment failure and death for NSCLC patients are mainly due to disease recurrence and remote metastasis [14]. It is therefore of critical urgency to detect lung cancer at an early stage with the current innovative technology using suitable molecular markers as an alternative approach. These specific biomarkers could play a significant role in determining pathological changes at an early stage and facilitate clinical intervention of the lung cancer [4].

Role of Epigenetics in Lung Cancer

It is commonly known that lung cancer is a genetic disease, however; etiology of lung cancer is not only affected by genetic factors such as mutations and genetic polymorphisms but also the effect of epigenetic changes has been implicated in lung cancer [9]. Determining where and when a specific gene to expressed during development is influenced by epigenetic modifications, which are heritable during cell division without alterations in DNA sequence [15]. Therefore, the initiation and progression of cancer triggered by acquired epigenetic alterations through modulating gene expression [16]. Methylation of genomic promoter region is the most studied epigenetic mechanisms whereby cytosines at position 5 in CpG dinucleotides are methylated [17]. Promoter methylation of tumor suppressor genes (TSG) predisposes for frequent alterations on several types of human cancers including NSCLC [9].

Epigenetic silencing of tumor suppressor genes is a fundamental incident in the pathogenesis of human cancer [18]. One of the main mechanisms to inactivate TSG is CpG promoter hypermethylation of a gene [19]. Such alterations are sensitive and specific biomarkers to a wide variety of tumor cells [20]. Furthermore, aberrant DNA methylation of normally unmethylated CpG-islands within or near the promoter region is associated with the transcription silencing of tumor suppressor genes in human cancers including lung cancer. Additionally, inactivation of TSG is accomplished by aberrant chromatin modifications including hypermethylation of the promoter region of a gene [18]. Several studies have illustrated the diagnostic effectiveness of DNA hypermethylation of a broad spectrum of well known tumor related genes such as p16, RASSF1, APC, MGMT, DAPK, GATA5, and HOX9, in various biofluids, including bronchial aspirates, sputum, serum, plasma, and cell free circulating DNA [21]. Hypermethylation of three different lung cancer associated genes P16, RASSF1A and DAPK will be discussed in the following paragraphs.

Hypermethylation of Ras Association Domain Family 1 (RASSF1A) Gene in Lung Cancer

RASSF1A is a tumor suppressor gene that are epigenetically inactivated and contribute to pathogenesis and prognosis of human tumours [22]. Hypermethylation of CpG promoter region is a common cause of RASSF1A gene expression inactivation [23]. Inactivation of the gene expression is significantly affected by promoter hypermethylation rather than mutation of RASSF1A gene [24]. Moreover, methylation of RASSF1A gene is rarely expressed in normal tissue, whereas the frequency of CpG methylation in the promoter region rises dramatically in tumor tissues [24]. It is found that the frequency of DNA hypermethylation of CpG islands ranges from 99% in tumor tissue compared 0% in normal tissues, with the highest frequency of approximately 88% in lung cancer [25]. Hence, RASSF1A gene is one of the most frequently hypermethylated tumor suppressor genes in NSCLC and could be a significant biomarker for cancer detection.

Hypermethylation of P16 Gene in Lung Cancer

P16 is an essential tumor suppressor gene that play a potential role in cell cycle regulation, and the CPG promoter hypermethylation of P16 is a frequent occurrence in human tumors [26]. The higher frequency of P16 hypermethylation was reported in various types of human cancers including NSCLC, in which approximately 40% of the lung cancers exhibited p16 methylation [27]. In addition, about 22–60% loss expression of p16 is correlated with several mechanisms together with promoter hypermethylation [28]. In 22 cases with p16 gene hypermethylation that found in tumor tissues, only 4 (18.1%) cases exhibited hypermethylation status in normal tissues, suggesting that tumor cells show more p16 hypermethylation frequency than that in neighboring normal cells [29]. It is therefore of important value to use gene promoter methylation of P16 detected in serum or sputum as a biomarker for NSCLC diagnosis [30].

Hypermethylation of Death Association Protein Kinase (DAPK) in Lung Cancer

A significant correlation between the death association protein kinase (DAPK) hypermethylation and NSCLC has been reported in several studies. Tang et al., illustrated that 44% of the examined tumors were hypermethylated at the CPG region of DAP- kinase [31]. Additionally, Yang et al., reported that the frequency of DAPK methylation was considerably higher in NSCLC than peficancerous tissue [32]. Furthermore, in 2148 patients with NSCLC pooled from 28 studies, 870 cases were reported with DAP kinase CPG promoter hypermethylation, the rate of hypermethylation was 40.50%, and about 5.69% times higher than the one in normal lung tissues. Consequently, the risk of NSCLC was significantly correlated with DAPK promoter hypermethylation [33]. Promoter hypermethylation of DAPK in an early stage of tumor cells confirms the importance of this epigenetic abnormality in NSCLC [31].
Silencing of the tumor suppressor gene DAPK through methylation plays an essential role in lung cancer pathogenesis, which could be due to the higher frequency rate of methylation found in malignant lesions [5]. These findings indicated the previously reported data by Yanagawa et al., in which methylation of DAPK gene exhibited a higher frequency rate in NSCLCs than in non neoplastic lung tissue [2]. The 5 year survival rate of lung cancer patients with DAP kinase methylation is lower than that in patients with unmethylated DAP kinase gene [21]. Inactivation of DAPK may be a remarkable biomarker for the molecular classification of stage I NSCLC [31].

Impact of Tobacco Smoke on Methylation Levels of RASSF1A, P16 and Dap kinase Genes

Several recent studies demonstrated the correlation between promoter hypermethylation of a number of genes and smoking behavior [6]. RASSF1A is one of the genes that frequently altered by CpG promoter methylation in smokers with lung cancer [34]. These results were consistent with another study which showed a higher methylation density of RASSF1A gene of former smokers compared to both current smokers and non smokers, indicating significant responsive of RASSF1A methylation to smoking status [24]. Also, Kim et al., found that hypermethylation of RASSF1A promoter occurred more frequently in patients who started smoking early in their life. Moreover, hypermethylation could affect the survival of patients with NSCLC [10].

P16 gene also found to be a fundamental target to genetic changes in lung cancer pathogenesis of smokers [35]. Hypermethylation of CpG promoter region of P16 appeared to be influenced by smoking status with a significant higher frequency compared to non smokers [36]. In addition, Jarmalaite et al., reported an interesting finding in which the frequency of p16 methylation of former smokers was significantly higher than that of current smokers [27]. Moreover, a remarkable association was shown between smoking characteristics such as duration of smoking or time since quitting and P16 methylation, which may contribute to the increased incidence of NSCLC [37]. However, Jarmalaite et al. demonstrated that no significant correlation was found between cumulative exposure to tobacco or duration of smoking and methylation [27]. Another noticeable finding exhibited that the occurrence of P16 hypermethylation was similar for individuals who started smoking before 19 years of age and individuals who begun smoking after 19 years [10]. On the other hand, the hypermethylation prevalence of RASSF1A gene was shown to be more frequent in those who started smoking while young [10]. The carcinogenic effect of tobacco smoke exerts by silencing tumor suppressor pathways through genetic and epigenetic mechanism [6].

Accumulating data reported no correlation between DAP kinase promoter methylation and smoking behavior. Soria et al., showed no correlation between CpG promoter methylation and the smoking behavior [19]. Consistently, Yanagawa et al., argued that the frequency of alterations of DAP Kinase methylation did not change with the smoking status compared to RASSF1A and P16 [34]. Moreover, no association was noted between DAPK promoter hypermethylation and tobacco smoke despite the reality that hypermethylation is an early incident of carcinogenesis and the significant role of smoking on development of NSCLC [33]. Further studies to gain a precise insight about the correlation between promoter methylation of these genes and smoking status are required.

It is believed that hypermethylation of a gene in individuals who started smoking early in life occurred in a gene specific manner, also; the nonrandom changes in the environment around a gene could influence the susceptibility to hypermethylation [10]. The raised activity of DNA methyltransferase is a common factor for the increased susceptibility to the promoter hypermethylation of a specific gene in those who started smoking while young [38]. Cigarette smoke contains abundant carcinogens such as nitrosamine 4-(methylnitro-samino)-1-(3-pyridyl)-1-butane (also known as nicotine-derived nitrosamine ketone; NNK), these ingredients systematically stimulate lung tumors [39]. NNK triggers methyltransferase accumulation and the subsequent promoter hypermethylation of TSG will possibly lead to tumorigenesis and offers a significant association between tobacco smoking and lung cancer [40]. Clinical studies described a potential correlation between promoter hypermethylation and tobacco smoke at approximately 20 TSGs in lung tumors [41].

Conclusion

The prevalence of hypermethylation of CpG promoter region of the described genes was consistent. Correlation between promoter methylation of these genes and lung cancer has been indicated since the promoter hypermethylation was crucially different between cancer and normal control tissues. Hypermethylation of both P16 and RASSF1A genes was significantly correlated with tobacco smoke and drives a potential impact on patients with NSCLC. Understanding the accurate epigenetic signal at promoter region will certainly be of great clinical benefit. Hence, these findings recommend that methylation of P16, RASSF1A and DAP kinase could be a promising biomarker to improve the early detection of NSCLC and subsequent management of individuals with diagnosed malignancy. Further research to establish the underlying mechanisms for promoter hypermethylation in tobacco induced cancer, and to overcome challenges facing development of epigenetic
biomarkers that will improve prognostication and direct for the appropriate use of cancer therapies.

REFERENCES


