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Investigation of PTEN Promoter Methylation and Its Effect on Non-Small Cell Lung Carcinoma

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Abstract

PTEN is a tumor suppressor gene with important roles in apoptosis. This gene is located on chromosome 10q23 and is one of the most frequently inactivated genes in cancers. Severe underexpression of *PTEN* has been reported in several types of cancer, including endometrial, prostate, breast, and brain cancer. Also, this gene is epigenetically silenced through aberrant hypermethylation of CpG islands on its promoter. The present study investigated the promoter methylation and expression levels of *PTEN* in patients with Non-Small Cell Lung Carcinoma (NSCLC).

The study included 20 patients with NSCLC, whose bisulfite-treated DNA samples were investigated using the Methyl-Specific PCR (MSP) with primers specific for either methylated or non-methylated forms of *PTEN*. *PTEN* expression was also assessed using real-time PCR.

20 samples from NSCLC patients, 70% (n=14) showed *PTEN* underexpression, while 85% (n=17) had *PTEN* promoter methylation. Also, 12 of 17 (70%) samples with PTEN promoter methylation had concomitant *PTEN* underexpression.

According to our results, there was a significant correlation between the *PTEN* promoter methylation and NSCLC. *PTEN* underexpression and promoter methylation were also significantly correlated.

INTRODUCTION

Due to late diagnosis and limited therapeutic interventions available, lung cancers are the leading cause of malignancy-related death globally (1). This group of cancers consists of different subtypes with molecular and histological heterogeneity, of which Non-Small Cell Lung Carcinoma (NSCLC) and Small Cell Lung Carcinoma (SCLC) are the most common, accounting for 76% and 13% of the lung cancer cases in the United States, respectively (2). In general, NSCLCs are divided into 3 groups: adenocarcinomas, Squamous Cell Carcinomas (SqCC), and large cell carcinomas (3). According to the increasing evidence, even a single subtype of lung cancer includes diseases with different histological and molecular characteristics (4). The histopathological classification of lung cancer was revised and published by the World Health Organization (WHO) in 2015 (5). Due to recent discoveries in molecular pathology, this new classification of lung cancers includes several major revisions (6). New and comprehensive human projects on the molecular characterization of lung cancers have identified novel molecular characteristics and different subtypes even at the levels of DNA alteration and methylation, as well as mRNA, microRNA, and protein expressions (7). The genomic landscapes of numerous cancers have been defined using the whole genome sequencing technique, which is increasingly used nowadays.

At the end of the 1990s, phosphatase and tensin homolog deleted in chromosome 10 (*PTEN*) was discovered to be a tumor suppressor gene (8). It is one of the most frequently inactivated genes in cancers, and its expression is severely underexpressed in several types of cancer, including endometrial, prostate, breast, and brain cancer (9). This gene plays an important role in apoptosis and is also involved in cell migration, differentiation, and proliferation suppression, which is mediated by PIP3 inhibition. *PTEN* consists of nine exons that encode for a 403 aa protein that acts as a dual-specificity protein and lipid phosphatase. This protein is a major negative regulator of the PI3K/Akt/mTOR pathway (10). Different changes in *PTEN*, including mutations, homozygous deletions, promoter hypermethylation,

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and microsatellite instability, can affect the negative regulation of the PI3K/Akt signaling pathway, which is a downstream survival signal activated by the Epidermal Growth Factor Receptor (EGFR) (11). Signaling pathways activated by EGFRs have critical roles in the pathogenesis of lung cancers while activating mutations of the EGFR gene have been reported in patients with lung adenocarcinomas (12). Moreover, the *PTEN* is epigenetically silenced in different cancers through aberrant hypermethylation of CpG islands on its promoter. The present study investigated the *PTEN* promoter methylation and expression levels in patients with Non-Small Cell Lung Carcinoma (NSCLC).

METHODS AND MATERIALS

twenty patients with lung carcinoma were The present study included 20 patients with NSCLC whose histopathological diagnosis was confirmed by two independent pathologists based on the WHO criteria. At first, the samples, which were kept frozen in liquid nitrogen, underwent DNA extraction using the TaKaRa MiniBEST Universal Genomic DNA Extraction Kit Ver.5.0 (Takara, Japan). Then, the concentration of double-stranded DNA was assessed, and distilled water was added to reach the concentration of 5 µg/mL. Bisulfite conversion was performed using the EpiJET Bisulfite Conversion Kit (Thermo Fisher scientific, USA). This process converted the non-methylated cytosine to uracil, while the methylated cytosine remained unchanged. The bisulfite-treated DNA samples were then subjected to Methyl-Specific PCR (MSP) with primers specific for either methylated or non-methylated forms of *PTEN*. Methylated *PTEN* primer sequences were 5′-GTTTGGGGATTTTTTTTTCGC-3′ and 5′-AACCCTTCCTACGCCGCG-3′ for forward and reverse primers, respectively. For non-methylated *PTEN* primers, 5′-TATTAGTTTGGGGATTTTTTTTTTGT-3′ and 5′-CCCAACCCTTCCTACACCACA-3′ were used as forward and reverse primers, respectively (11). Both *PTEN* forms were amplified using the TEMPase Hot Start Master Mix BLUE (ampliqon, Denmark). Then, aliquots of the resulting products were analyzed on 2% agarose gel.

PTEN expression was assessed using real-time PCR. Total RNA was extracted using the TRIzol reagent (Invitrogen, Singapore) based on the manufacturer's instructions, while single-stranded complementary DNA (cDNA) was synthesized using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA). Then, the expressions were quantitatively measured using a Rotor Gene 6000 Real-Time PCR Machine (Qiagen, Germany) with the SYBR Green method (AccuPower Green Star qPCR Master Mix; Bioneer, Korea).

Gene expression assays were performed in duplicate on 10µl reaction mixtures using specific primers (F: CCAGGACCAGAGGAAACCT, R: GCTAGCCTCTGGATT TGA). The Cycle Threshold (CT) values provided by the RT-qPCR were used to calculate the relative fold changes of expression using the 2-ΔΔCT method. The IBM SPSS software version 16 was used for statistical analysis, along with the unpaired t-test and chi-square test. The significance level was considered as < 0.05.

RESULTS

We investigated the promoter methylation of *PTEN* and its potential effects on the gene expression in samples from patients with NSCLC. Of a total of 20 samples evaluated, 85% (n=17) had promoter methylation of *PTEN*. We used GAPDH as an appropriate housekeeping gene to normalize the expression in different samples and observed that 70% (n=14) of the samples showed PTEN underexpression. Also, 12 of 17 (70%) samples with PTEN promoter methylation had concomitant *PTEN* underexpression.

DISCUSSION

PTEN is a potent tumor suppressor gene that neutralizes several oncogenic stimuli. It has been shown that even partial loss of *PTEN* protein could initiate carcinogenesis (13). The most critical role of *PTEN* is in the negative regulation of the PI3K/mTOR/Akt oncogenic pathway. Other potential functions of this gene include inhibition of uncontrolled cell survival, growth, and migration, as well as other crucial anti-oncogenic functions (14). Interestingly, large cohort studies have found a *PTEN* underexpression or complete loss of expression in almost 40% of the samples from lung carcinoma patients (15). However, the genetic variants (mutations/deletion) of *PTEN* in lung cancer do not match the prevalence of protein loss. Therefore, it is likely that non-genomic mechanisms regulating *PTEN* expression should be present. Pre-clinical and clinical studies have reported epigenetic (miRNAs, methylation, acetylation) and post-translational (phosphorylation, oxidation) mechanisms involved in this underexpression (16).

study investigated the promoter methylation of *PTEN* and the expression of the related mRNA in samples from 20 patients with NSCLC. According to our results, *PTEN* promoter methylation was observed in 85% (n=17) of the samples, while 12 (70%) of these 17 samples had a concomitant loss of expression of *PTEN*. Therefore, there was a significant relationship between *PTEN* promoter methylation and NSCLC. Moreover, *PTEN* promoter methylation and underexpression of the related mRNA were also significantly related.

some studies, loss of *PTEN* expression is frequent in patients with NSCLC and is associated with shortened survival rates (18). Jiang and Wang reported the lower expression of *PTEN* in tumoral

tissue than in the normal tissue in patients with NSCLC (19). Moreover, some studies have found that lack of *PTEN* protein, along with PI3K and pAKT overexpressions, were associated with tumor development and poor prognosis in patients with NSCLC (20, 21). Also, Liao et al. reported that *PTEN* underexpression was correlated with tumorigenesis and lymph node metastasis, while no association between *PTEN* underexpression and the factors of pathologic type or tumor differentiation was found (22). Therefore, our results were compatible with other studies in this field, indicating that loss of *PTEN* expression is an effective factor in tumor development, recurrence, or progression.

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