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Decrease of PDSS2 expression, a novel tumor suppressor, in non-small cell lung cancer

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ABSTRACT

Background: Prenyl diphosphate synthase subunit 2 (PDSS2) gene has recently been reported as a potential tumor suppressor. The association of PDSS2 and non-small cell lung cancer (NSCLC) has not been known. *Methods*: To investigate its association with NSCLC, we examined the expression level of PDSS2 in 28 paired clinical samples of non-small cell lung cancer tissues and surrounding normal tissues. *Results*: PDSS2 was constitutionally expressed in normal lung tissues regardless of sex, race and smoking history. An overall decreased PDSS2 expression was found in the tumor tissues compared to surrounding normal tissues. Decrease in PDSS2 expression was more severe in poorly and poor-to-moderately differentiated lung cancers, while the decrease was not significant in moderately to well-differentiated tumors. Moreover, the expression of PDSS2 decreased more in higher pathological stage, and in patients with lymph node metastasis. The decrease in PDSS2 expression in tumor tissues was not related to sex or histological type of NSCLC, but was related to smoking history. No correlation has been found between PDSS2 and the clinical factors of EGRF, Ki-67 and p53. *Conclusion*: Taken together, decreased expression of PDSS2 in NSCLC was evident. This is an initial report for the expression of PDSS2 in relation to different factors in lung cancer. Loss of PDSS2 could serve as a potential biomarker in NSCLC development. The role of PDSS2 as a tumor suppressor, and the mechanism of its potential anti-tumor action in NSCLC warrant further investigation.

1. Introduction

Prenyl diphosphate synthase subunit 2 (PDSS2) is the essential but non-catalyzing subunit of prenyl diphosphate synthase, the first enzyme in CoQ10 biosynthesis [1,2]. PDSS2 gene locates at the chromosome 6q21 and is constitutionally expressed in normal lung tissues at a high level (2.1 times of average genes, by AceView) [3,4]. This may due to its important function in CoQ10 biosynthesis, which is important for mitochondria function and cellular antioxidant defense.

In lung cancers, loss of heterozygosity (LOH) in the region of 6q16.3-21 occurs frequently, causing a possible loss of function of genes located in this region, which may in turn contribute to the multistep mechanisms for lung cancer development [5,6]. The 6q LOH has been found in 47% of primary SCLCs (small cell lung cancer) [7], and 6q16.3-21 LOH has been found in 78% of NSCLCs (non-small cell lung cancer) [8]. PDSS2 gene is located in the frequent LOH region of 6q16.3-21, implying for its potential role as tumor suppressor [9,10]. In fact, PDSS2 was proposed to be a target for chromosome 6q deletion and rearrangement in malignant cells, and its deficiency has been found in melanoma and gastric cancers [9–11]. Forced over-expression of this gene caused apoptosis in melanoma and gastric cancer cell lines [9,11]. However, the mechanism of PDSS2 tumor suppressing is not known to date. The association of PDSS2 with cancer development, differentiation, and prognosis has not been studied. Other studies on PDSS2 have been focused on the effect of its prenyl diphosphate synthase activity [2] and mutations of PDSS2 in patients with CoQ10 deficiency [12–17]. The role that PDSS2 plays in lung cancers, as well as in many other cancers, has not been reported to our knowledge.

Utilizing a tissue bank of 28 NSCLC patients, we investigated PDSS2 expression in paired normal and tumor samples using

Abbreviations: PDSS2, prenyl diphosphate synthase subunit 2; LOH, loss of heterozygosity; CoQ10, coenzyme Q10; EGFR, epithelial growth factor receptor; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer.

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quantitative RT-PCR and western blots. The goal is to investigate the association of PDSS2 with the development of NSCLC. We hypothesized that PDSS2 expression level is decreased in NSCLC, and the decrease is associated with pathological staging and differentiation.

2. Materials and methods

2.1. Tissue samples

The study was reviewed by the Human Subjects Committee/Institutional Review Board at the University of Kansas Medical Center and categorized as an exempt study. All tissue samples were provided by the Biospecimen Shared Resource, University of Kansas Cancer Center. Samples were collected when Wedge resection or lobectomy was taking place, with the only exception for sample #1525 which was collected at pneumonectomy. All samples were de-identified and were stored in liquid nitrogen till use. Twenty-eight pairs of NSCLC tissues and surrounding normal lung tissues were obtained, with independent surgical pathology reports and histological records. Sample related information was shown in Table 1.

2.2. Quantitative RT-PCR

Total RNA was isolated using RNeasy® Mini Kit (Qiagen Science, Valencia, CA) and checked purity at OD 260/280 and OD 260/230. cDNA was synthesized by using Qiagen's Omniscript RT kit with random primer (Invitrogen, Carlsbad, CA). Real-time PCR was performed on a BioRad I cycle IQ5 real-time PCR station. Master mixture was made with IQ SYBR Green Supermix (BioRad, Hercules, CA).

The primers for PDSS2 were designed using the online primer design tool ProbeFinder ver 2.45 of Universal Probelibrary, provided by Roche Applied Science (Indianapolis, IN). The forwarding sequence was 3′-ggcactcagcaccctctg-5′, and the reverse sequence was 3′-ccctcaactggaggctatt-5′. All Ct values were normalized to the Ct values of respective18s rRNA. Relative expression level was represented as $2^{-\Delta Ct}$.

2.3. Western blot

Whole proteins were isolated and solubilized using RIPA lysis buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Igepal Ca 630, 0.1% SDS, and 0.5% sodium deoxycholate), and phosphatase inhibitor cocktails (Sigma–Aldrich, MO). Sixty milligrams of protein was loaded for SDS-polyacrylamide gel electrophoresis. Anti-PDSS2 antibody was from Santa Cruz Biotechnology, Inc., CA. Anti-β-tubullin (Neomarkers Inc., Fremont, CA) was used as control.

2.4. Statistical analysis

Paired difference test was used for comparison of PDSS2 expression levels from paired samples. Welch's *t*-test was used when the variations in two compared populations were unequal. Dependence and correlation were detected using Pearson product-moment correlation coefficient. When the variables are

Table 1
PDSS2 relative expression levels and sample information of paired normal and cancerous lung tissues from 28 NSCLC patients.

# Sample	PDSS2 relative expression ($2^{-\Delta Ct}$)		Sub-type[a]	Differentiation[b]	PT stage	Metastasis to lympho nodes[c]	EGRF (%)	Ki-67 (%)	P-53 (%)	Sex	Race	Prior treatment	Smoking history
	Normal	Tumor											
986	3.03E−06	4.63E−06	1	3	0	0	20	52	1	F	Caucasian	NA	Quit, 15y
1054	1.70E−06	1.19E−06	1	1	1	0	97	84	83	F	Caucasian	NA	
1057	2.86E−06	3.38E−06	1	3	1		95	14	0	F	Caucasian	NA	No
1060	4.80E−06	3.96E−06	3	1	2	0	18	99	0	F	Caucasian	NA	Quit, 16y
1076	4.42E−06	4.19E−06	1	2	2	1	91	3	15	F	Caucasian	Chemo, Rad	
1087	3.27E−E−06	2.51E−06	1	1	1	1	100	3	2	M	Caucasian	Chemo, Rad	Yes
1088	4.73E-06	3.44E−06	2	2	2		97	100	100	M	African American	NA	
1089	3.48E−06	2.27E−06	2	1	1	0	25	89	6	F	Caucasian	NA	Quit, 2y
1100	4.26E−06	4.31E−06	3	2	1	0	89	18	5	F	Caucasian	NA	
1106	4.38E−06	6.64E−06	1	3	1	0	48	9	1	F	Caucasian	NA	
1153	4.90E−06	6.36E−06	1	4	1	0				F	Caucasian	NA	No
1175	7.99E−06	7.22E−06	2	3	2	1	95	99	0	M	Caucasian	NA	
1187	5.81E−06	3.34E−06	1	1	2	1	98	88	99	F	Caucasian	NA	Yes, 2ppd
1206	5.51E−06	5.90E−06	1	4		0	20	34	95	M	Caucasian	NA	Yes, 1ppd
1233	3.19E−06	2.34E−06	1	1	2	1				F	Caucasian	NA	
1351	6.86E−06	3.53E−06	2	3	2	0				M	Caucasian	NA	
1363	6.13E-06	6.46E−06	1	3	2	0	15	4	1	F	Caucasian	NA	
1385	4.94E−06	6.84E−06	1	3	2	1	14	20	0	M	Caucasian	NA	Quit, 1m
1398	7.40E−06	3.04E−06	2	1	2	0				F	Caucasian	NA	
1406	5.85E−06	3.35E−06	2	3	2	0	100	77	64	M	Caucasian	NA	Quit, 24y
1412	3.22E−06	2.29E−06	2	3						M	Caucasian	NA	
1452	4.54E−06	5.51E−06	2	3	1	0	95	8	2	M	Caucasian	NA	Quit, 8y
1461	4.92E−06	4.20E−06	2	1	2	0				M	Caucasian	Rad	
1475	1.02E−05	6.13E−06	1	3	2	1	97	55	58	F	African American	NA	No
1525	8.14E−06	3.63E−06	2	1	2	1				M	African American	Chemo	
1586	4.37E−06	3.09E−06	2	1	2	0				F	Caucasian	NA	Quit, 17y
1648	3.77E−06	3.26E−06	1	1	1					M	Caucasian	NA	Yes
1968	3.79E−06	3.34E−06	1	2	2	1				F	Caucasian	NA	Quit, 27y

[a] Subtype: subtypes of NSCLC: 1, adenocarcinoma; 2, squamous carcinoma; 3, other sub-types.
[b] Differentiation: 1, poorly; 2, poor-moderately; 3, moderately; 4, well differentiated.
[c] Metastasis to lymph nodes: 0, no; 1, yes.