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Original Article

Increased expression of proline-, glutamic acid- and leucine-rich protein PELP1 in non-small cell lung cancer

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ABSTRACT

It has been demonstrated that estrogens are able to enhance lung tumorigenesis by estrogen receptor (ER) pathway. ER signaling is a highly complex process that requires a number of different coactivators, including proline-, glutamic acid- and leucine-rich protein-1 (PELP1). We studied PELP1 transcript and protein levels in cancerous and histopathologically unchanged lung tissues obtained from 73 patients diagnosed with non-small cell lung cancer (NSCLC). We observed increased levels of PELP1 transcript ($P = 0.00001$) and protein ($P = 0.00001$) in tumor tissues compared to adjacent histopathologically unchanged tissues. Significant increase of PELP1 transcript/protein level was found in all patients, regardless of gender (males: $P = 0.0003/P = 0.000003$; females: $P = 0.0005/P = 0.02$), age (≤ 60 patients: $P = 0.042/P = 0.016$; >60 patients: $P = 0.00001/P = 0.00001$) or histopathological type of tumor (adenocarcinoma [ADC]: $P = 0.004/P = 0.0006$; squamous cell carcinoma [SSC]: $P = 0.0009/P = 0.0008$). Increased PELP1 transcript/protein levels were also correlated with some lung cancer stage (1a: $P = 0.07/P = 0.02$; 1b: $P = 0.001/P = 0.03$; 2a: $P = 0.012/P = 0.001$), tumor size (T2a: $P = 0.0006/P = 0.001$) and lymph node metastasis (N0: $P = 0.0003/P = 0.0006$; N1: $P = 0.017/P = 0.003$). Moreover, significant increase in PELP1 transcript level in cancer stage 1a ($P = 0.02$) was observed. PELP1 protein content was higher in tumor tissues of patients with cancer stage 3a ($P = 0.04$) and in T1a tumor size ($P = 0.03$). Our studies demonstrate significantly higher amounts of PELP1 transcript and protein in tumor tissues in patients with NSCLC. Moreover, we also determined the association of PELP1 transcript and protein level with some clinicopathological features of NSCLC.

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1. Introduction

Lung cancer (LC) is one of the most frequent tumors, which annually affects a lot of individuals in the world. Despite recent advances in molecular diagnostics and treatment strategies, LC remains one of the most common causes of death among men and women, from malignant diseases, worldwide [1–3]. Low survival rate among patients afflicted with LC is caused by the late detection, which results in inability of surgical intervention and delayed implementation of treatment [3].

There are two main histological types of LC. The first type – small cell lung cancer (SCLC) occurs less frequently (20%) and the second one – non-small cell lung cancer (NSCLC) is recognized in 80% of cases. NSCLC includes few subtypes, but adenocarcinoma (ADC), squamous cell carcinoma (SSC) and large cell carcinoma are the most common types, derived from epithelial cells [4]. SSC often affects men and its pathogenesis is mainly related to tobacco combustion, while ADC occurs more frequently in women. LC is a complex disease, depending on many environmental factors, genetic mutations and chromosomal rearrangements. Recent studies have provided evidence that it may also be affected with gender-dependant factors, especially with estrogens. This problem mainly applies to premenopausal women, because of physiologically high level of circulating 17-beta-estradiol (E2) [5–9].

Estrogens may affect cells by two ways: genomic and non-genomic pathway. In the genomic pathway, estrogens bind to estrogen receptors (ERα, ERβ) [5]. This action leads to the dimerization of the receptors, their translocation to the nucleus and binding with DNA regions known as estrogen response elements (ERE) [6]. Subsequently, the transcription of estrogen-dependent genes is promoted. In the second, non-genomic

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pathway, estrogens bind with cell membrane isoforms of ERs, which causes immediate effect, such as activation of mitogen activated protein kinases (MAPKs), non-receptor tyrosine kinases (Src), phosphatidylinositol-3 kinase (PI3K) or releasing of intracellular calcium ions [6,10,11].

Previous studies indicated that the proline, proline-, glutamic acid- and leucine-rich protein-1 (PELP1), also known as MNAR, plays significant role in estrogen response. PELP1 acts as coactivator of both, ERα and ERβ, and modulates their function [12–17].

Moreover, PELP1 was identified as protooncogene, which is overrexpressed in many estrogen-dependent tumors [15,18]. However, little is known about the role of PELP1 in LC tumorigenesis. Therefore, we studied the PELP1 expression at both, mRNA and protein levels in primary lung cancer tissues and histopathologically unchanged lung tissues obtained from patients diagnosed with NSCLC.

2. Material and methods

2.1. Patient material

Lung cancer tissues were obtained from 73 patients with diagnosed NSCLC who underwent surgery at the department of thoracic surgery, Poznań University of Medical Sciences, Poland. All patients were not treated by chemo- or radiotherapy before surgical intervention. Histopathologically unchanged lung tissues were collected from the same patient, from macroscopically unchanged place, which was located at least 10–20 cm away from tumor foci. Type of neoplastic lesion has been detected and confirmed by histopathological examination. After surgical removal, tissue specimens were frozen in liquid nitrogen and stored at −80 °C until isolation of RNA and protein. All patients provided written and oral consent to participate in this study under a protocol approved by the Local Ethical Committee of Poznań University of Medical Sciences.

2.2. RNA isolation, reverse transcription and real-time quantitative polymerase chain reaction (RQ-PCR)

Total RNA was isolated according to the method of Chomczyński and Sacchi [19]. The concentration of RNA was determined spectrophotometrically and agarose gel electrophoresis was carried out to check the RNA integrity. The isolated RNA was stored at −80 °C until further analysis.

Complementary DNA (cDNA) was synthesized by using MLV reverse transcriptase, according to manufacturer's protocol (Invitrogen, Life Technologies, Grand Island, NY). RQ-PCR reaction was performed in a Light Cycler®480 Real-Time PCR System, Roche Diagnostics GmbH (Mannheim, Germany) using SYBR® Green I as detection dye. Standard curves were generated to calculate the PCR efficiency using serial dilution of cDNA calibrator. For calibrator, cDNA mix from all of patients' samples was prepared. For amplification, 1 ul of (total 20 ul) cDNA was added to 9 ul of Light Cycler® SYBR Green I master mix, Roche Diagnostics GmbH (Mannheim, Germany). The negative, no-template control was included in all runs. The quantity of *PELP1* transcripts in each sample was standardized by the geometric mean of porphobilinogen deaminase (*PBGD*) and human mitochondrial ribosomal protein L19 (*hMRPL19*) cDNA levels. The *PELP1* transcript levels were expressed as a multiplicity of these cDNA concentrations in the calibrator. For amplifications, the following primer sequences were employed: *PELP1*; F: 5′-GAGCATTCAGCAGGTGTTAC-3′; R: 5′-AGGTGGTTCATGGAGATGTC-3′; *PBGD*; F: 5′-GCCAAGGACCAGGACATC-3′; R: 5′-TCAGGTACAGTTGCCCATC-3′; *hMRPL19*: F: 5′-ACTTTATAAATCCTCGGGTC-3′; R: 5′-ACTTTCAGCTCATTAAGAG-3′.

2.3. Protein isolation and Western Blotting analysis

For protein isolation, tissues from NSCLC patients were treated with RIPA lysis buffer, Sigma–Aldrich Co. (St Louis, MO). For 10 minutes, 30 ug of protein was resuspended in sample loading buffer, incubated at 99 °C and separated on 8% Tris-glycine gel using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Gel proteins were transferred to a nitrocellulose membrane, which was blocked with 5% milk in Tris/HCl saline/Tween buffer. Immunodetection of protein

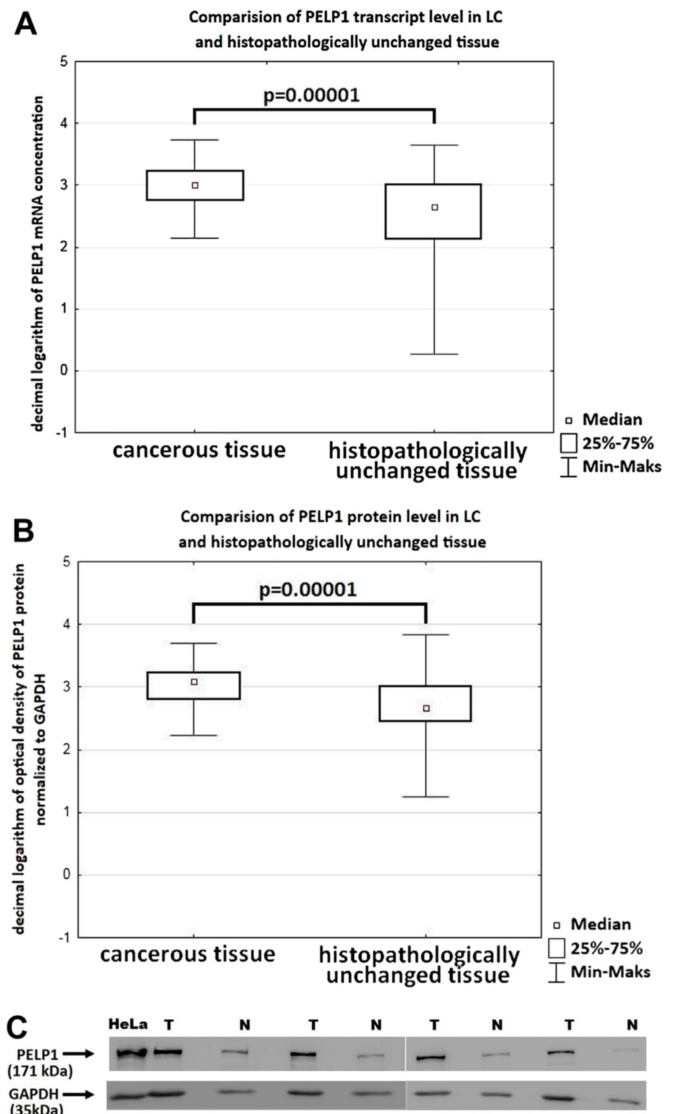

Fig. 1. PELP1 transcript (A) and protein (B) levels and representative image of Western Blot analysis (C) of PELP1 presence in lung cancer (T) and histopathologically unchanged tissue (N). Tumoral and nontumoral material was obtained from 73 patients with diagnosed non-small cell lung cancer disease (NSCLC) and was used for RNA and protein isolation. Total RNA was reverse-transcribed and cDNA was examined by RQ-PCR analysis. PELP1 transcript levels were standardized by geometric mean of *PBGD* and *hMRPL19* cDNA levels. Standard curves were generated to calculate the PCR efficiency using serial dilution of cDNA mix from all patients' samples. Proteins were isolated by using RIPA lysis buffer and separated by 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), then transferred to nitrocellulose membrane and immunobloted with polyclonal rabbit anti-PELP/MNAR Ab, followed by incubation with goat anti-rabbit HRP-conjugated Ab. After stripping, membranes were reblotted with rabbit polyclonal anti-GAPDH Ab and goat anti-rabbit HRP-conjugated Ab The amount of PELP1 proteins was presented as PELP1-to-GAPDH band optical density ratio. The *P*-value was evaluated by Mann-Whitney test.

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