

# Prognostic Significance of Pigment Epithelium-Derived Factor Expression in Patients with Non-Small-Cell-Lung Cancer

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## Abstract

The aims of this study were to examine prognostic significance of pigment epithelium-derived factor (PEDF) in patients with stage IA-IIIB non-small cell lung cancer (NSCLC). Using immunohistochemistry and multivariate analysis, we set out to investigate whether PEDF expression could provide prognostic information in NSCLC in a cohort of 69 patients who had undergone radical resection for NSCLC. The correlation between PEDF and the clinical pathological features of stage I-III NSCLC after radical surgery were analyzed as well as influence on long term survival. No correlation between PEDF intensity, PEDF area or PEDF area index and clinicopathologic parameters was seen. PEDF values showed a slight correlation to the tumor stage. There was a significant negative correlation ( $T = -0.288$ ,  $p = 0.002$ ) between pathologic T-stage and median PEDF area and vice versa a positive correlation ( $T = 0.227$ ,  $p = 0.016$ ) with median PEDF intensity. We could not detect any correlation between PEDF and long term survival. For PEDF analysis, there was only a slight correlation between expression and T-stage of the tumor.

## Keywords

NSCLC, PEDF, Neoangiogenesis

## 1. Background

Non-small-cell lung cancer (NSCLC) is one of the most common cancers worldwide

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and the leading cause of cancer related death in several countries. With the recent advances in screening, diagnosis, and treatment of this disease, guidelines are constantly being reviewed to provide the best in detection and therapy. Currently, the new TNM classification for lung cancer is being proposed by the International Association for the Study of Lung Cancer (IASLC), and the 8th edition of TNM staging system is expected to be released in 2016 [1]. However, especially for early stage NSCLC, prognostic biomarkers are still lacking.

NSCLC is characterized by uncontrolled growth and spread of abnormal cells and by tumor neoangiogenesis [2] [3]. Under physiological conditions, neoangiogenesis is the formation of new capillaries and blood vessels by growth and differentiation from existing ones. It occurs mainly during embryonic development, but also at wound sites [4]. In recent years, therapies selectively targeting neoangiogenesis pathways, such as VEGFR have both provided a better understanding of NSCLC and have been investigated as prognostic factors or targets for individualized therapy [5]-[7], but have not led to a significant therapy improvement.

A potentially significant molecule, in understanding NSCLC neoangiogenesis is Pigment Epithelium-Derived Factor (PEDF), also known as early population doubling level cDNA-1. PEDF is an endogenous 50-kDa glycoprotein that is first shown to be capable of inducing differentiation in Y-79 retinoblastoma cells [8]-[11]. Among its other functions, it inhibits the proliferation and migration of endothelial cells toward various angiogenic inducers and thereby suppresses angiogenesis [8]-[11]. In the context of tumors, PEDF exhibits an impressive tumor growth-suppressing activity by targeting tumor vasculature and tumor cells per se. Initially, the antitumor activity of PEDF was proven to be attributable to its antiangiogenic property [12] [13]. Furthermore, PEDF expression is significantly reduced in a wide range of tumor types, including pancreatic adenocarcinoma, glioblastoma and ovarian carcinoma [14] [15] and its recovered expression in these tumors delays the onset of primary tumors and decreases metastasis [14] [15].

Using immunohistochemistry and multivariate analysis, we set out to investigate whether PEDF expression could provide prognostic information in NSCLC patients. The correlation between PEDF and the clinical pathological features of stage I - III NSCLC after radical surgery were analysed.

## 2. Methods

### 2.1. Patient Inclusion and Tissue Samples

A total of 69 patients with a stage IA - III NSCLC (either adenocarcinoma ADC or squamous cell carcinoma SCC), who underwent radical surgical resection at the thoracic surgery department of Thoracic and Cardiovascular Surgery of the Göttingen University Medical Center were enrolled in this retrospective study. Exclusion criteria were NSCLC stage IV and unresectable tumor. The local ethical committee approved this study (#1-2-08). Preoperative interdisciplinary tumor board meetings were held and proposed curative resection surgery for each patient included. None of the patients

had received prior chemo or radiation therapy. No other previous or concomitant primary cancer was present. All procedures were conducted in accordance with institutional, state and federal guidelines.

WHO classification was used for histological assessment of each specimen and pathological staging of each tumor was determined using the renewed TNM staging system for NSCLC [8]. Histological types of lung cancer included: ADC in 27 patients (39.1%), and SCC in 42 patients (60.9%). **Table 1** summarizes further clinical characteristics.

All immunostained sections were reviewed by two investigators who had no knowledge of the patients' clinical status. A structured follow-up questionnaire was sent to oncologists, pulmonary physicians and general practitioners.

## 2.2. Tumor PEDF Immunohistochemistry

Formalin fixed and paraffin-embedded primary lung tumor tissue was selected by pathologists to allow clear definition of tumor tissue areas on haematoxylin-eosin (H&E) stained sections. A two-step indirect streptavidin-biotin technique was used for further

**Table 1.** Clinical characteristics of included lung cancer patients.

Histology	Adenocarcinoma (n = 27)	Squamous cell carcinoma (n = 42)
Age (years)	63.55 ± 10.8	66.2 ± 7.7
Gender	20 male (74.1%) 7 female (25.9%)	41 male (97.6%) 71 female (2.4%)
Operation type	Pneumectomy: 3 (11.1%) Lobectomy: 22 (81.5%) Bilobectomy: 2 (7.4%)	Pneumectomy: 10 (23.8%) Lobectomy: 27 (64.3%) Bilobectomy: 5 (11.9%)
Lymphnodes resected (n)	17.8 ± 6.8	20.9 ± 8.6
Pathologic Stage	IA: 3 (11.1%) IB: 13 (48.1%) IIA: 1 (3.7%) IIB: 4 (14.8%) IIIA: 6 (22.2%)	IA: 3 (7.1%) IB: 12 (28.6%) IIA: 9 (21.4%) IIB: 8 (19.0%) IIIA: 9 (21.4%) IIIB: 1 (2.4%)
T-Status	T1a: 17 (18.5%) T1b: 6 (22.2%) T2a: 10 (37.0%) T2b: 4 (14.8%) T3: 2 (7.4%)	T1a: 3 (7.1%) T1b: 7 (16.7%) T2a: 13 (31.0%) T2b: 9 (21.4%) T3: 7 (16.7%) T4: 3 (7.1%)
N-Status	N0: 17 (63%) N1: 16 (14.8%) N2: 6 (22.2%)	N0: 27 (64.3%) N1: 9 (21.4%) N2: 5 (11.9%) N3: 1 (2.4%)
Grading	1° Grade: 1 (3.7%) 2° Grade: 17 (63.0%) 3° Grade: 9 (33.3%)	2° Grade: 28 (66.7%) 3° Grade: 14 (33.3%)
Mean follow up time (month)	35 ± 27.9	51.5 ± 35.9

immunohistochemical staining. Following deparaffinisation and rehydration the peroxidase was rinsed with 3% H<sub>2</sub>O<sub>2</sub> (S2023, Dako, Hamburg, Germany) and samples were then washed with Tris buffered saline (TBS). Staining PEDF was carried out using monoclonal antibody JC70A (Dako, Hamburg, Germany) and monoclonal antibody SerpinF1/PEDF (R&B, Abingdon, Great Britain) respectively.

### 2.3. Evaluation of PEDF-Staining

Regions of interest were determined by the hot spot method and detection of vital tumour tissue and cancer cells by the H&E stained sections. These regions were analyzed at a magnification of  $\times 25$  by two separate investigators. Signal intensity was measured by semi quantitative scale (1, 1+, 2-, 2, 2+, 3-, 3, 3+) and median values were determined. As PEDF signalling appeared in spot like forms, a PEDF signalling area measurement was undertaken in  $\mu\text{m}^2$ . A product of the intensity index and area of PEDF was calculated (Area-intensity index).

### 2.4. Statistical Analysis

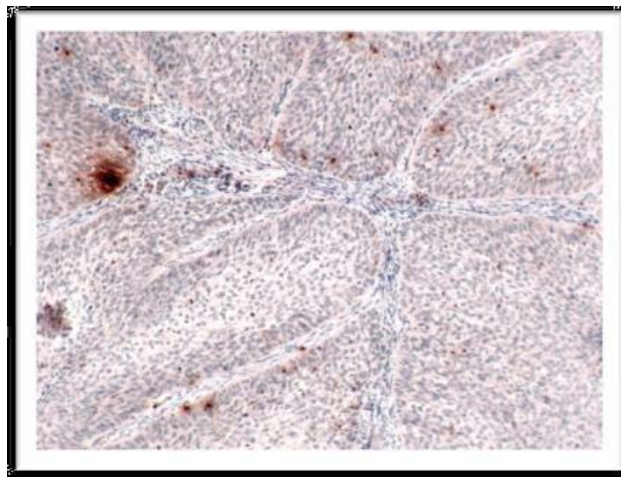
The following variables were examined and analyzed with regard to the findings of immunohistochemistry: gender, histopathology, UICC and pT stage, lymph node status, and grading. Categorical variables were expressed as proportions (%) or number (n) and continuous variables as mean  $\pm$  standard deviation (SD) and median with range. Long-term follow-up (survival or lung cancer related death at 90 days after surgery) was calculated (using the Kaplan-Meier method). Differences between groups were calculated and compared (using the log-rank test). Cox Regression to influence of clinicopathologic parameters analysis was used. Association analysis for linear correlation of median PEDF values was given as Pearson's correlation coefficient, whereas Kendall tau-b correlation coefficient T was provided for correlation with clinicopathologic parameters. Results were considered significant for p values below 0.05. Long term follow up, defined as survival or death after 90 days postoperative could be fully completed. To test the influence of PEDF expression on long-term survival, all patients with known lung cancer death or living were studied. Statistical modelling and testing were performed with SPSS® statistical software (Release 15.0.0).

## 3. Results

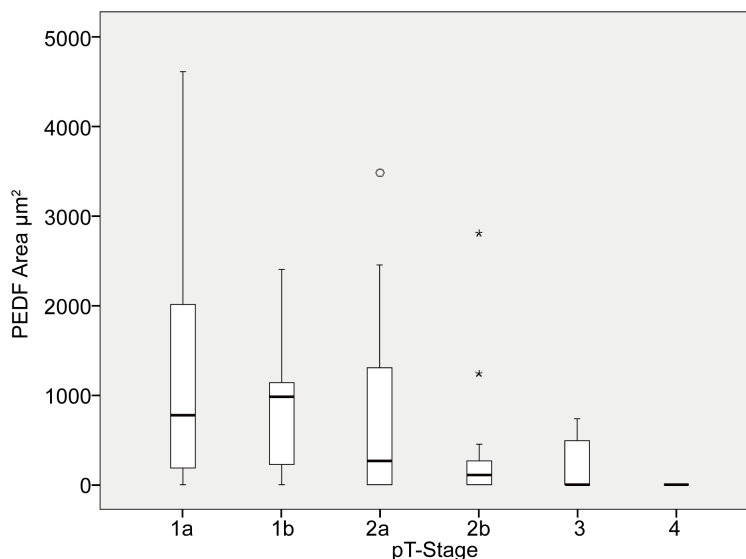
A total of 69 patients with available NSCLC tumor tissue blocks were included in the analysis. During a median follow-up of 48 months, patients were clinically evaluated and were referred for radiological assessment as per clinical indications. The socio-demographic and clinical characteristics of the patients are described in **Table 1**. At the time of last follow-up 25 patients were alive (36.2%). Overall median survival time was 45 month. Lung cancer related death occurred in 33 patients (47.8%); for unknown reasons in 6 (8.7%), due to a secondary tumor in 1 (1.5%), and for other non-tumorous reasons in 4 patients (5.8%).

In PEDF staining, the mean intensity of signalling was  $2.18 \pm 0.50$  (median 2.22,

range 1.1 - 3.2). The mean PEDF area was  $1012 \pm 1285 \text{ mm}^2$  (median  $682 \text{ mm}^2$ , range 0 -  $4617 \text{ mm}^2$ ), and the area intensity index was mean  $2142 \pm 2731$ , median 1437 (range 0 - 8601). PEDF staining did not correlate with gender, histopathology, lymph node status or grading. Nevertheless, there was a significant difference of median PEDF intensity values between UICC stage ( $T = 0.243$ ,  $p = 0.010$ ), as well as a slight but positive correlation ( $T = 0.240$ ,  $p = 0.010$ ) to pT stage (Figure 1). Vice versa, the median PEDF area showed a negative correlation with UICC stage ( $T = -0.224$ ,  $p = 0.013$ ) and pT-stage ( $T = -0.305$ ,  $p < 0.01$ ; Figure 2).



**Figure 1.** Staining of PEDF was carried out using monoclonal antibody monoclonal antibody SerpinF1/PEDF respectively. Regions of interest were determined by the hot spot method outlined above and detection of vital tumour tissue and cancer cells by the H&E stained sections. These regions were analysed at a magnification of 25 $\times$ .



**Figure 2.** The median PEDF area showed a negative correlation with UICC stage ( $T = -0.224$ ,  $p = 0.013$ ) and pT-stage ( $T = -0.305$ ,  $p < 0.01$ ).

## 4. Discussion

In this retrospective study we evaluated the prognostic value PEDF in patients with resected NSCLC of stage IA-III.

In 1989, Folkman hypothesized that tumor growth is dependent on successful angiogenesis and that methods that inhibit angiogenesis could be integrated into tumor therapy [2]. Neoangiogenesis is a complex phenomenon and solid tumors cannot grow beyond 1 - 2 mm in diameter without neoangiogenesis [16]. Expanding evidence suggests that neoangiogenesis is also an integral part of tumor cell dissemination and propensity for metastasize [2] [3]. Therefore detailed assessment of neoangiogenesis in tumors could not only lead to the development of new improved anti-angiogenic therapies, but also provide information that could help stratify patients into different treatment regimens and potentially predict response to chemotherapy [5]-[7]. Especially in patients with early stage lung cancer, reliable prognostic factors for risk stratification and decision upon adjuvant chemotherapy still need to be clinically established [17] [18].

Although, studies on the relationship of PEDF expression (particularly in NSCLC) are few in number more standardized methods of assessing neoangiogenesis should be agreed upon, if studies are to be compared and reliable conclusions drawn.

We looked at PEDF, which acts directly on endothelial cells and can halt new vessel formation by inducing apoptosis in endothelial cells stimulated during new vessel formation. PEDF inhibits the proliferation and migration of endothelial cells and is perhaps the most potent anti-angiogenic factor to be identified [19]. It has been shown to be reduced in NSCLC and gene therapy research in other forms of cancer e.g. DPEDF inhibiting human pancreatic cancer cells in mice has been promising and may provide a novel approach for treatment of pancreatic adenocarcinoma [20] [21]. We sought to build on these previous findings and investigate PEDF as a prognostic factor in NSCLC. After analysing tumour samples obtained in surgery, we found no correlation between PEDF staining and gender, histopathology, lymph node status or tumour grading in NSCLC patients. However, similarly to a previous study with 91 patients, a significant positive correlation of PEDF intensity values and pathologic T-stage was observed. PEDF expression has also been shown to be related to TNM staging and tumor size and a screening for expression of PEDF in liver metastasis (from ductal pancreatic adenocarcinoma) identified PEDF expression as an independent positive prognostic factor-in PEDF positive samples, patient survival was significantly longer than when PEDF was not expressed [22]. Whilst our study in NSCLC, did show a correlation with pathologic T-stage and PEDF expression, no actual influence of PEDF on survival was observed.

Nonetheless, although PEDF expression may not appear to be useful indicator of prognosis, it still could be of clinical importance. The past few years have seen rapid development and improvement of tumor inhibitors, and an increasing body of evidence suggests that selective inhibitors of growth factors (e.g. EGFR) provide useful therapeutic agents, particularly in advanced NSCLC.

## 5. Conclusion

In conclusion, we found that PEDF (a potential antiangiogenic factor) has no significance with long-term survival. Significant limitations to this study include that it is a retrospective study and that only patients who have undergone surgery are included. Additionally, although our findings may appear surprising or even paradoxical, previous studies on MVD have also been controversial and unequivocal. Nevertheless, even though studies on the subject continue to yield seemingly contradictory results, the role of this factor in antitumor therapy should continue to be researched and perhaps standardised prospective studies will elucidate matters.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## Author's Contributions

AE, BCD and FAS conceived of the study and participated in its design. AO participated in the design of the study and contributed to the immunohistochemical analysis. LF, HB and RWB participated in the interpretation of the pathologic data. AE and BCD drafted the manuscript. All authors read and approved the final manuscript.

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## Abbreviations

H&E: Hematoxylin-Eosin Staining

IHC: Immunohistochemistry

NSCLC: Non-Small Cell Lung Cancer

MVD: Microvessel Density

PECAM-1: Platelet Endothelial Cell Adhesion Molecule-1

SD: Standard Deviation, PEDF



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