Lung Cancer 66 (2009) 319–326

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

The positive correlation between gene expression of the two angiogenic factors: VEGF and BMP-2 in lung cancer patients

Magdalena Bieniasz[a,*], Katarzyna Oszajca[a], Mak Eusebio[a], Jacek Kordiak[b], Jacek Bartkowiak[a], Janusz Szemraj[a]

[a] Department of Medical Biochemistry, Medical University of Lodz, 6/8 Mazowiecka, Lodz 92215, Poland
[b] Department of Surgery, Medical University of Lodz, 4 Zeromskiego, Lodz 93509, Poland

ARTICLE INFO

Article history:
Received 2 October 2008
Received in revised form 17 February 2009
Accepted 22 February 2009

Keywords:
Lung cancer
Non-small cell lung cancer
Small cell lung cancer
Angiogenesis
VEGF
BMP-2
BMP-4
Polymorphism

ABSTRACT

Lung cancer is a particular challenge in oncology. More than 1 million new cases occur worldwide every year and despite many clinical trials and modern diagnostic techniques, long-term survival rate has only marginally improved. The aim of the current research is to explore new molecular prognostic factors and identify new targets for anticancer therapy. Current evidence shows that angiogenesis is controlled by several angiogenic factors including VEGF and BMP-2. It has been also demonstrated that VEGF plays a key role in this process that is essential in carcinogenesis. Our study has shown that the expressions of the VEGF, BMP-2 and BMP-4 mRNAs were significantly higher (7.1-fold, 25.6-fold and 2.3-fold, respectively) in lung cancer samples than in adjacent normal lung tissues (real-time RT-PCR). Analysis based on the Pearson's correlation coefficient indicated the positive correlation between *VEGF* and *BMP-2* gene expression, whereas no significant correlation between *VEGF* and *BMP-4* gene expression was found. The mean ± standard deviation serum level of VEGF was 423 ± 136 pg/ml. Significant differences in the serum levels of VEGF between patients with T1 tumors and patients with T2, T3 or T4 tumors were observed. Patients with T2, T3 and T4 tumors, respectively, had 1.6-fold, 1.8-fold and 2.3-fold greater serum levels of VEGF than their peers with T1 tumors. In current study patients homozygous for the 936T allele of the +936C/T *VEGF* gene polymorphism had 12-fold lower *VEGF* gene expression and 1.3-fold lower VEGF serum level than patients homozygous for the 936C allele. In conclusion, our findings underline the importance of the two angiogenic factors namely VEGF and BMP-2 as well as +936C/T *VEGF* gene polymorphism in the evaluation of lung cancer patients.

1. Introduction

1.1. Lung cancer

Lung cancer is the leading cause of death among malignant tumors worldwide [1]. Unfortunately, its prognosis is poor, the disease is rarely curable with an overall 5-year survival rate of about 15% [2]. The cure rates of lung cancer have been relatively unaltered during the past 40 years. Therefore, new strategies for screening and treatment of this disease are necessary for the improvement of patients' outcome [3,4].

It has been shown that angiogenesis, a process whereby new blood vessels are formed by sprouting from a preexisting vasculature, is a relatively early event of carcinogenesis [5,6]. Neovascularization is necessary for tumors to be able to growth beyond 2 mm^3, and is essential for adequate supply of oxygen and nutrients to tissues [7]. VEGF is the most important growth factor controlling angiogenesis in normal and tumor cells. It binds to different vascular endothelial growth factor receptors (VEGFR) that belong to the tyrosine-kinase receptor family [6–10]. *VEGF* gene expression is regulated by several factors, including hypoxia, growth factors, cytokines and other extracellular molecules [7,8].

It is suggested that VEGF activates several critical gene products, which are involved in VEGF-induced progression and metastasis of lung cancer [9,11]. Several studies have demonstrated that the VEGF mRNA expression [11–18] and the serum level of VEGF [14,19–24] are increased in patients with lung cancer as compared to healthy individuals. Other studies have shown the association between increased tumor or serum VEGF levels and poorer survival [16,18,23,25–33], higher stage of the lung cancer [9,23,25] and greater tumor size [30,34]. Furthermore, VEGF serum level is considered to be a prognostic factor in patients with lung malignancy [11,18,27–30,34,35]. It has been also reported that tumor angiogenesis, tumor growth and metastases are suppressed by the inhibition of VEGF signal transduction [36]. The expression of VEGF may therefore reflects the angiogenic potential and biological aggressiveness

* Corresponding author. Tel.: +48 42 678 06 20; fax: +48 42 678 24 65.
E-mail address: Magda.Bieniasz@gmail.com (M. Bieniasz).

0169-5002/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.lungcan.2009.02.020

of a tumor and may be an effective target for therapy to improve the prognosis of lung cancer [37,38].

Bone morphogenetic proteins (BMP) represent about one-third of the TGF-β superfamily composed of growth and differentiation factors [39–41]. They bind serine kinase receptors (BMPR I and BMPR II) and regulate signaling predominately through Smad proteins [42–44]. BMPs have been recognized as critical in the control of multiple organogenic processes including the development of lungs where BMP-2 and BMP-4 play an important role [40,41,45]. Several studies also claimed that BMP-2 promotes angiogenesis by activating endothelial cells through the stimulation of Smad 1/5, Erk 1/2, and Id expression [43,46]. The Id transcription factors which are very important in neovascularization have been identified as one of the main targets of the BMP-2 signaling pathway [47]. Other studies have shown that BMP-2 and BMP-4 induce the production of VEGF by different cell lines or tumor cells, which contributes to the angiogenic response [46,48,49]. Furthermore, BMP-2 may enhance angiogenesis by serving as a chemotactic factor for monocytes [50]. Monocytes are present in lung tumors, which can secrete cytokines that promote blood vessels formation [51].

It has been demonstrated that the *BMP-2* gene is highly expressed in non-small cell lung cancer (NSCLC) when compared to non-neoplastic lung tissue [43,52]. Several reports provide evidence that BMP-2 stimulates the growth and progression of lung tumor [46]. BMP-4, a close relative of BMP-2, has not been extensively studied in lung cancer and its role is not established in this malignancy. Further studies are needed to clarify the role of bone morphogenetic proteins in lung cancer development and establish a probable relationship between these molecules and vascular endothelial growth factor in the promotion of tumor angiogenesis.

Although cigarette smoking is the major cause of lung cancer [2], only a small fraction of smokers suffer from this disease, which suggests the influence of some genetic factors in lung cancer development. Several studies indicate that the genetic susceptibility to lung cancer may result from a combination of low-penetrance gene polymorphisms [53,54]. DNA sequence variations in the *VEGF* gene may lead to altered VEGF production and/or activity, thereby causing interindividual variability in the susceptibility to lung cancer development and progression [55]. VEGF gene polymorphisms such as +405G/C localized in the 5′-untranslated region (5′-UTR) and +936C/T localized in the 3′-untranslated region (3′-UTR) (transcription start continued as +1) have been associated with variations in VEGF protein production. These polymorphisms have been involved in the susceptibility to several disorders, including lung cancer, in which angiogenesis may play a critical role [55–61].

The purposes of this study were: (1) to evaluate gene expression of angiogenic factors such as *VEGF*, *BMP-2* and *BMP-4* in lung cancer tissue and its surrounding healthy tissue and establish probable association between these factors in 88 preoperative lung cancer patients; (2) to determine the correlation between VEGF serum level and clinicopathological characteristics including T stage of the tumor, the involvement of lymph nodes and the histological type of lung cancer; (3) to determine the link between any of these two polymorphisms (+405G/C and +936C/T) and *VEGF* gene or VEGF protein expression in patients with lung cancer.

Gene and/or protein expression of VEGF and BMP-2, BMP-4 associated with common polymorphisms (+405G/C and +936C/T) of the *VEGF* gene may be useful genetic markers of angiogenesis-linked pathologic processes leading to the progression of the lung cancer. Furthermore, the results of our study may contribute to a better understanding of the role of VEGF, BMP-2 and BMP-4 in angiogenesis and in the biological behavior of this tumor. In addition it will provide potential molecular targets for the management of lung malignancies.

Table 1
Characteristics of patients with lung cancer.

	No. of cases	Percentage
Age (years)		
Mean age	65	N/A
Range	51–82	N/A
Sex		
Male	68	77.3%
Female	20	22.7%
Histological type		
AD	31	35.2%
SCC	36	41.0%
SCLC	21	23.8%
T stage of the tumor		
T1	16	18.2%
T2	37	42.0%
T3	31	35.2%
T4	4	4.6%
Lymph nodes involvement		
N0	18	20.4%
N1	38	43.2%
N2	32	36.4%

2. Materials and methods

2.1. Patient characteristic

The study included 88 non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) patients diagnosed between 2005 and 2007 in the Chest and Surgical Oncology Clinic of the Medical University of Lodz. There were 68 men and 20 women. The median age of the lung cancer patients was 65 years (range: 51–82 years). Details of the patient characteristics are listed in Table 1. Venous blood was taken from each patient and centrifuged at $3000 \times g$ for 5 min at 4 °C. Supernatant was transferred into microtubes and stored at −70 °C until use. Fresh tissues from the carcinomas and non-neoplastic fragments from surrounding lung tissues were snap frozen in liquid nitrogen and stored at −70 °C until use. All samples were collected before treatment. Tumor fragments were analyzed according to WHO histological classification (World Health Organization, 1982). Pathological stage was determined according to the TMN staging system of lung cancer [62]. Prior to sampling, all patients gave informed consent and the study has been reviewed and approved by the Bioethical Committee of the Medical University of Lodz, no.: RNN/211/07/KE.

2.2. RNA extraction and reverse transcription

Total RNA was extracted from resected lung tissues using a RNA extraction reagent, TRIZOL (Invitrogen Life Technologies), according to the standard acid–guanidinium–phenol–chlorophorm method [63]. The extracted RNA was analyzed by agarose gel electrophoresis and only cases with preserved 28S, 18S and 5S ribosomal RNA bands indicating good RNA quality were used in the study. Total RNA was digested with DNase (GIBCO) at room temperature for 15 min. Five micrograms of digested RNA were reverse transcribed at 42 °C for 60 min in a total of 20 μl reaction volume using the ImProm-II™ Reverse Transcription System kit (Promega, USA). Obtained cDNA was used in real-time PCR reaction.

2.3. Detection of gene expression using real-time RT-PCR method

Real-time PCR based on TaqMan™ technology was performed using master mix prepared according to the FastStart Universal Probe Master (ROX) from Roche Applied Science. Probes and primers were designed using the online Universal ProbeLibrary