

Received: 2014-03-16
Revised: 2014-07-30
Accepted: 2014-08-22

Serum Levels of Fatty Acid Binding Protein 5 Decreased in Squamous Cell Lung Carcinoma

Mohammad Ali Ghayyoumi^{1,2✉}, Zahra Mojtahedi¹, Mahsa Mansouri¹, Narges Montazari¹, Hamidreza Sahraeian¹, Ghasem Ghalamfarsa³

¹Cancer Proteomics and Biomarkers Lab, Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.

²Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Yasuj, Iran.

³Department of Internal Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.

Abstract

Background: The aberrant expression of Dickkopf-1 (DKK1), triose phosphate isomerase (TPI) and fatty acid binding-protein 5 (FABP5) in lung cancer tissues and their presence in secretome of lung cancer cell lines were reported. Here, serum levels of these molecules in lung cancer patients were compared to those in a healthy control group. **Materials and Methods:** The patients' group consisted of 50 newly diagnosed male patients with a mean age of 65.3±11.5 years (27 squamous cell carcinoma (SCC), 7 adenocarcinoma, and 16 small cell carcinoma). Thirty eight apparently healthy men (mean aged 65.1±11.4) served as the control group. ELISA was employed for quantification. **Results:** Comparison between patients and controls revealed no significant difference, although FABP5 level was lower in patients (3.2 vs. 4.8; p=0.09). Analysis of each type of lung cancer with controls indicated that patients with SCC had significantly lower levels of FABP5 than in controls (2.7 vs. 4.8 ng/ml; P=0.03). **Conclusion:** Comparison between patients and controls revealed no significant difference, although FABP5 level was lower in patients (3.2 vs. 4.8; p=0.09). Analysis of each type of lung cancer with controls indicated that patients with SCC had significantly lower levels of FABP5 than in controls (2.7 vs. 4.8 ng/ml; P=0.03). [GMJ. 2014;3(4):222-7]

Key Words: DKK1; FABP5; Lung Cancer; Serum; Squamous Cell Carcinoma; TPI

Introduction

Lung cancer is one of the leading causes of malignancies in most countries including Iran [1,2]. Its current serological markers, e.g. carcinoembryonic antigen, cytokeratin 19 fragment (CYFRA 21-1), and neuron-specific enolase (NSE), lack enough sensitivity and specificity in detecting the disease at an early stage, in follow up, and as a treatment guide-

line [1,3]. Identification of novel biomarkers is under intensive research for improving survival. Candidate serological biomarkers for further research are particularly those aberrantly expressed in lung cancer and/or present in lung cancer secretome [4].

Dickkopf-1 (DKK1) is a secretory protein whose presence in the secretome of lung cancer cell lines has been reported. It is known as a negative regulator of the Wnt signaling,

GMJ

©2013 Galen Medical Journal
Fax: +98 731 2227091
PO Box 7461686688
Email: info@gmj.ir



✉ **Correspondence to:**

Mohammad Ali Ghayumi, Department of Internal Medicine, Namazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran
Telephone Number: (+98) 711 2304952
Email Address: ghayyoumim@sums.ac.ir

a pathway which is linked to critical steps during tumorigenesis, such as proliferation, differentiation, survival, apoptosis, and cell motility. In addition to the role of DKK1 as a good molecule or bad molecule in cancer, its increase or decrease in sera from cancer patients is also the subject of controversy [5-7]. For example, in the study conducted by Sheng *et al.*, DKK-1 level was significantly lower in patients' sera with gastric cancer compared with healthy people [6]; this is in contrast to the results of Lee *et al.*, who found a significant increase of serum DKK1 levels in gastric cancer [7]. In lung cancer, the role of DKK1 is the subject of controversy as well. It has been shown that tobacco smoke, the most culprit factor related to lung cancer, induces repression of DKK1 in lung cancer cells, and enhances the malignant phenotype. In fact under-expression of DKK1 has been suggested as a potential mechanism by which smoking could negatively influence survival in lung cancer patients [8]. However, the over-expression of DKK1 in lung tumor tissues has been reported. Serologic analysis also revealed a significant increase of DKK-1 level in lung cancer patients' sera compared to healthy people in two studies from China and Japan [6,9], the findings which should be confirmed in other populations.

Triose Phosphate Isomerase (TPI) is a glycolytic enzyme that catalyzes the interconversion of Dihydroxyacetone Phosphate and Glyceraldehyde-3-Phosphate. Its overexpression has been linked to increased glycolysis and aggressiveness in cancer [10]. TPI presence in culture media of lung cancer cell lines and upregulation in tumor tissue have been frequently detected [11,12]. An elevated serum level of TPI in lung cancer was reported from Korea and China [11,13].

Fatty acid binding-proteins (FABPs) bind to long-chain fatty acids, and are thought to be involved in fatty acid uptake, transport, and metabolism. FABP5 also called epidermal fatty-acid-binding protein and psoriasis-associated fatty-acid-binding protein was first identified as an upregulated protein in psoriasis, a skin disease characterized by abnormal keratinocyte differentiation [14-16]. Later, it was found that FABP5 was highly expressed in several types of cancer, e.g., skin, prostate,

and oral squamous cell carcinoma (SCC) [16,17]. By increasing or silencing FABP5 expression in oral SCC cells, FABP5 levels were found to be positively correlated with in vitro cell growth, MMP-9 expression, and invasiveness [16]. In lung cancer, controversy exists about over-expression or under-expression of FABP5 [18]. Its presence in lung cancer secretome has been reported [12]. To the best of our knowledge, serologic value of this molecule in lung cancer has not been investigated before. A few publications are available regarding serum levels of FABP5 in pathological conditions. An example is type 2 diabetes, in which increased level of FABP5 was reported to be related to the disease complications [19].

Although there are reports to show the increased serum DKK1 and TPI levels in lung cancer, verification is required in distinct populations. On the other hand, no data are available about serum FABP5 levels and lung cancer. Accordingly, the aims of the present study were to investigate the possible difference between serum levels of DKK1, TPI and FABP5 in Iranian lung cancer patients and healthy individuals.

Materials and Methods

Serum Samples

The study was approved by the Ethic Committee of Shiraz University of Medical Sciences. The patients' group consisted of 50 newly diagnosed male patients (mean age 65.3 ± 11.5 , age range from 40 to 83 years) with pathologically confirmed lung cancer; of them, 27 had SCC, 7 had adenocarcinoma, and 16 had small cell carcinoma. In 26 patients, tumor stage at diagnosis and smoking status were recorded in their files. Three patients were in stage I/II of disease and 23 were in stage III/VI of disease. 26 patients with recorded smoking status were current smokers.

Thirty eight apparently healthy men (mean age 65.1 ± 11.4 , age range from 40 to 83 years) with no history of a malignancy, an autoimmune disease or a serious infectious disease also participated in our study serving as the control group. All control individuals were never smokers.

Serum ELISA Assays

The blood samples within 2 hours of collection were centrifuged at 1000xg for 15 minutes at 4°C, and the sera were collected, aliquoted and stored at -70°C until use. Time interval from storage to assay was less than 2 years for all sera.

The Enzyme linked Immunosorbent Assay (ELISA) kits for TPI and FABP5 were purchased from USCNK (Wuhan, China). The minimum detectable limit of TPI was 0.32 ng/mL and that of FABP5 was 0.124 ng/mL. The detectable limits of the other ELISA kit, DKK1 (Abcam, Cambridge, UK), was 100 pg/ml. The serum levels of all measured molecules were determined in duplicate according to the manufacturer's protocols.

Data Analysis

The data were analyzed using SPSS software (version 11.5.0; SPSS, Chicago, IL, USA). Analysis of variance (ANOVA) and t-test, where appropriate, were used for analyses of serum DKK1, TPI and FABP5 levels. Levene's test for equality of variances, which indicates whether an assumption of the t-test or ANOVA has been met, was done for all the analyses. Whenever Levene's test was significant, the P value corresponding to the row labeled "Equal variances not assumed" was presented. The relation of serum levels of the analyzed molecules with packs of cigarettes used per year was examined using the Spearman Correlation coefficient. Findings were considered statistically significant at a P value less than 0.05.

Results

In the present study, we measured serum FABP5, TPI, and DKK1 levels in lung cancer patients and in a healthy control group. The serum levels of the three molecules in the whole group of the patients did not significantly differ from those in the healthy control group, although the level of FABP5 was lower in patients than in controls with a P value near statistical significance (3.2 vs. 4.8; P=0.09) (Table 1). A significant difference was appeared when lung cancer patients were analyzed based on their histological types. Patients with SCC had significantly lower

levels of FABP5 than in healthy controls (2.7 vs. 4.8 ng/ml; P=0.03). Comparisons of other histological types with healthy control group regarding FABP5 level showed no significant difference. Moreover, DKK1 and TPI levels were not significantly associated with types of lung cancer studied here (Table 2).

ANOVA revealed no statistically significant difference among different histological types of lung cancer (SCC, adenocarcinoma, and small cell carcinoma) according to the serum levels of TPI (P=0.80), FABP5 (P=0.21), and DKK1 (P=0.62). Moreover, there was no correlation between serum levels of these molecules and packs of cigarettes used per year by patients (P>0.05).

Discussion

Lung cancer patients are usually diagnosed at a late clinical stage, and the 5-year survival rate remains poor [1]. Identification of novel serological biomarkers for lung cancer is under intensive research. Here, we compared serum DKK1, TPI and FABP5 levels in lung cancer patients with those in healthy people. We did not observe any significant association of serum DKK1 and TPI levels with lung cancer, the two molecules that a few prior publications reported as a positive association [6,9]. For example, Sheng *et al.* investigated the serum levels of DKK1 in several types cancer in China. Serum DKK1 levels were significantly higher in patients with non-small cell lung cancer than in patients

Table 1. Mean Serum Levels of FABP5, TPI, and DKK1 in Lung Cancer Patients and Healthy Controls

Cytokine level	Lung cancer (n=50)*	Healthy controls (n=38)*	P value
FABP5 (ng/mL) Mean ± SD	3.2±2.3	4.8±5.5	0.09
TPI (ng/mL) Mean ± SD	43.5±3.6	43.1±4.2	0.76
DKK1 (pg/ml) Mean ± SD	206.7±24.8	208.6±29.2	0.74

*The OD of TPI in 20 patients and 20 controls were read as overflow and therefore ignored in analysis.

Table 2. Mean Serum Levels of FABP5, TPI, and DKK1 in Lung Cancer Patients According to Tumor Types

Cytokine level	Squamous Cell Carcinoma (N=27)	Adenocarcinoma (N=7)*	Small Cell Carcinoma (N=16)*	Healthy controls (N=38)†
FABP5 (ng/mL)				
Mean ± SD	2.7±1.5	4.4±2.6	3.5±3.2	4.8±5.5
P value	0.03	0.83	0.39	
TPI (ng/mL)				
Mean ± SD	43.6±3.6	44.7±3.0	43.0±4.3	43.1±4.2
P value	0.7	0.61	0.93	
DKK1 (pg/ml)				
Mean ± SD	209.7±24.7	205.8±33.06	202.1±21.7	208.6±29.2
P value	0.87	0.82	0.42	

*The OD of TPI in 15 patients with squamous cell carcinoma, and 5 patients with small cell carcinoma were read as overflow and therefore were excluded from analysis.

†The OD of TPI in 20 controls were read as overflow and ignored in analysis.

with other malignant tumors and healthy controls. Moreover, DKK1 levels were positively associated with stage, lymph node involvement and distant metastases in lung cancer. However, serum levels of DKK1 decreased significantly in groups of patients with gastric cancer, colorectal cancer, ovarian cancer, and cervical adenocarcinoma compared with healthy controls [6]. In contrast, Lee *et al.*, reported elevated levels of DKK1 in Japanese gastric cancer patients compared to healthy people [7]. Kim *et al.*, and Zhang *et al.* reported a significant increase of serum TPI levels in Korean and Chinese patients with lung cancer [11,13]. These two groups investigated serum peroxiredoxin-6 levels in lung cancer as well, but Kim *et al.*, found no significant association but Zhang *et al.* found a significant association between peroxiredoxin-6 and lung cancer [11,13]. The varying results might partly be due to the multifunctional nature of these molecules. The overtly conflicting reports of DKK1 in cancer contributed to cancer type-specific and patient-specific role of the molecule [5].

We found significant decrease of FABP5 in patients with SCC of the lung compared with healthy individuals. FABP5 has been demonstrated to be highly expressed by keratinocytes and endothelial cells, but could not be detected in transformed endothelial cells

implying its expression is lost during transformation, and that FABP5 expression might be regulated by processes implicated in cell differentiation [15]. Evidence suggests that the lung might be the only organ in which cell types other than endothelial cells and keratinocytes contain E-FABP in physiological conditions [15]. In smokers with chronic obstructive pulmonary disease (COPD) compared with smokers without COPD, FABP5 was reduced in bronchial epithelial cells [20]. Interestingly, smokers with COPD have been shown a significantly higher incidence of lung cancer compared with the latter group [21]. Both over-expression and under-expression of FABP5 have been reported in lung cancer by proteomic approaches [18] which need to be verified with other techniques such as Western blotting [10].

In conclusion, our study shows a significant association of serum FABP5 with lung cancer, but not DKK1 and TPI. Our report represents the first serological analysis of FABP5 in lung cancer. Serum level of FABP5 was found to be decreased in SCC of the lung. It remains to be identified the expression of FABP5 in lung cancer by precise techniques and the consequence of FABP5 alteration in lung cancer. Further studies in other populations are also warranted to analyze FABP 5 levels in serum of lung cancer patients.

Acknowledgments

This research was funded by grants from Shiraz University of Medical Sciences (90-01-01-3113, 90-01-01-3046, 90-01-01-3112) and Shiraz Institute for Cancer Research (ICR-87-503), and was partly based on the MD theses

(M Mansoori, N Montazari, H Sahraeian) submitted to the Shiraz University of Medical Sciences. No conflict of interest is declared by all authors.

References

- Gabrielson E. Worldwide trends in lung cancer pathology. *Respirology*. 2006;11(5):533-8.
- Masoompour SM, Yarmohammadi H, Rezaianzadeh A, Lankarani KB. Cancer incidence in southern Iran, 1998-2002: results of population-based cancer registry. *Cancer Epidemiol* 2011;35(5):e42-7.
- Merle P, Janicot H, Filaire M, Roux D, Bailly C, Vincent C, et al. Early CYFRA 21-1 variation predicts tumor response to chemotherapy and survival in locally advanced non-small cell lung cancer patients. *Int J Biol Markers*. 2004;19(4):310-5.
- Planque C, Kulasingam V, Smith CR, Reckamp K, Goodglick L, Diamandis EP. Identification of five candidate lung cancer biomarkers by proteomics analysis of conditioned media of four lung cancer cell lines. *Mol Cell Proteomics*. 2009;8(12):2746-58.
- Menezes ME, Devine DJ, Shevde LA, Samant RS. Dickkopf1: a tumor suppressor or metastasis promoter? *Int J Cancer*. 2012;130(7):1477-83.
- Sheng SL, Huang G, Yu B, Qin WX. Clinical significance and prognostic value of serum Dickkopf-1 concentrations in patients with lung cancer. *Clin Chem*. 2009;55(9):1656-64.
- Lee HS, Lee HE, Park do J, Kim HH, Kim WH, Park KU. Clinical significance of serum and tissue Dickkopf-1 levels in patients with gastric cancer. *Clin Chim Acta*. 2012;413(21-22):1753-60.
- Hussain M, Rao M, Humphries AE, Hong JA, Liu F, Yang M, et al. Tobacco smoke induces polycomb-mediated repression of Dickkopf-1 in lung cancer cells. *Cancer Res*. 2009;69(8):3570-8.
- Yamabuki T, Takano A, Hayama S, Ishikawa N, Kato T, Miyamoto M, et al. Dickkopf-1 as a novel serologic and prognostic biomarker for lung and esophageal carcinomas. *Cancer Res*. 2007;67(6):2517-25.
- Mojtahedi Z, Safaei A, Yousefi Z, Ghaderi A. Immunoproteomics of HER2-positive and HER2-negative breast cancer patients with positive lymph nodes. *OMICS*. 2011;15(6):409-18.
- Kim JE, Koo KH, Kim YH, Sohn J, Park YG. Identification of potential lung cancer biomarkers using an in vitro carcinogenesis model. *Exp Mol Med*. 2008;40(6):709-20.
- Yousefi Z, Sarvari J, Nakamura K, Kuramitsu Y, Ghaderi A, Mojtahedi Z. Secretomic analysis of large cell lung cancer cell lines using two-dimensional gel electrophoresis coupled to mass spectrometry. *Folia Histochem Cytobiol*. 2012;50(3):368-74.
- Zhang XZ, Xiao ZF, Li C, Xiao ZQ, Yang F, Li DJ, et al. Triosephosphate isomerase and peroxiredoxin 6, two novel serum markers for human lung squamous cell carcinoma. *Cancer Sci*. 2009;100(12):2396-401.
- Madsen P, Rasmussen HH, Leffers H, Honore B, Celis JE. Molecular cloning and expression of a novel keratinocyte protein (psoriasis-associated fatty acid-binding protein [PA-FABP]) that is highly up-regulated in psoriatic skin and that shares similarity to fatty acid-binding proteins. *J Invest Dermatol*. 1992;99(3):299-305.
- Masouye I, Hagens G, Van Kuppevelt TH, Madsen P, Saurat JH, Veerkamp

- JH, et al. Endothelial cells of the human microvasculature express epidermal fatty acid-binding protein. *Circ Res.* 1997;81(3):297-303.
16. Fang LY, Wong TY, Chiang WF, Chen YL. Fatty-acid-binding protein 5 promotes cell proliferation and invasion in oral squamous cell carcinoma. *J Oral Pathol Med.* 2010;39(4):342-8.
17. Morgan E, Kannan-Thulasiraman P, Noy N. Involvement of Fatty Acid Binding Protein 5 and PPARbeta/delta in Prostate Cancer Cell Growth. *PPAR Res.* 2010;2010.
18. Buhrens RI, Amelung JT, Reymond MA, Beshay M. Protein expression in human non-small cell lung cancer: a systematic database. *Pathobiology.* 2009;76(6):277-85.
19. Yeung DC, Xu A, Tso AW, Chow WS, Wat NM, Fong CH, et al. Circulating levels of adipocyte and epidermal fatty acid-binding proteins in relation to nephropathy staging and macrovascular complications in type 2 diabetic patients. *Diabetes care.* 2009;32(1):132-4.
20. Green RM, Gally F, Keeney JG, Alper S, Gao B, Han M, et al. Impact of cigarette smoke exposure on innate immunity: a *Caenorhabditis elegans* model. *PloS one.* 2009;4(8):e6860.
21. Miller YE, Blatchford P, Hyun DS, Keith RL, Kennedy TC, Wolf H, et al. Bronchial epithelial Ki-67 index is related to histology, smoking, and gender, but not lung cancer or chronic obstructive pulmonary disease. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2007;16(11):2425-31.