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Correlation of a Serological Proteome and Lung Cancer Prognosis

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Abstract

Background

To study the correlation of the lost goodwill target (LGT) proteome and the prognosis in patients with lung cancer and explore whether the LGT proteome can be used as an accurate and reliable prognostic biomarker for lung cancer.

Methods

One hundred eighty eight patients with lung cancer were enrolled in the Shanxi Cancer Hospital, China. For each patient, LGT test in serum was performed using the technique SELDITOF-MS after the pathological diagnosis. Kaplan-Meier survival analysis, Log-rank test and multivariate Cox proportional hazards regression analysis were performed to explore the influence of LGT different expression on the prognosis.

Results

The median survival times were 865 and 514 days in the LGT negative and LGT positive groups, respectively. There was statistically significant difference between the two survival curves, and the survival rate of the LGT negative group was higher than that of the LGT positive (χ^2=5.757, P=0.016). Multivariate Cox proportional hazards regression analysis confirmed that the LGT proteome (RR=1.5, 95% CI 1.075~2.196, P=0.019) predicted for death.

Conclusion

Our results showed that the prognosis of lung cancer is related to LGT proteome expression, suggesting that LGT may be regarded as one of the serological protein that signs a poor prognosis in lung cancer and has important clinical significance in predicting illness development.

Keywords: Lung cancer prognosis; Loss of goodness target (LGT) proteome; Survival analysis

Introduction

Lung cancer ranks as the leading cause of cancer in many countries [1]. For example, it accounts for 30% and 22.7% of cancer-related mortality in the United States [2] and China [3], respectively. Both biologically and clinically, lung cancer is a highly heterogeneous disease. Approximately 15% of lung cancer is small-cell lung cancer (SCLC), which is found to be highly responsive to chemotherapy and radiation therapy, but is often widely disseminated by the time of diagnosis [4]. The remaining lung cancers, referred to as non-small-cell lung cancer (NSCLC), include adenocarcinoma, large-cell carcinoma, and squamous-cell carcinoma, and most show a strong primary resistance to anticancer drugs [4]. Different therapeutic strategies are needed for patients diagnosed at different stages. It is highly desirable to identify new biomarkers for early diagnosis and accurate prognosis that open the way for developing novel therapeutic strategies of lung cancer. Examples of known potential biomarkers include alterations in expression of cytokeratin-19 fragment, neuron-specific enolase and cancer antigen-125 [5,6]. Most of these biomarkers have low sensitivity, specificity, or reproducibility [1]. However, a recently identified blood marker, named tumor liberated protein, has been shown to be potentially promising for early diagnosis of lung cancer [6,7].

Survival of patients cannot be solely predicted based on the tumor stage. Even patients diagnosed with stage 1 lung cancer have a surprisingly low survival. Prognostic biomarkers are of great importance for identifying the high risk patients and improving their clinical management. Proteomics is an important tool for the identification of biomarkers for cancer diagnosis and prognosis [8,9]. Examples of known prognostic biomarkers include annexin A3 [10], S100A11 [11], S100A6 [12,13], CK18 [14], and phosphohistidine phosphatase (PHP14) [15]. Most of these biomarkers are related to cancer metastasis via promoting angiogenesis. Recently, eleven components of the glycolysis pathway that were identified by proteomics [16] have been found to be significantly associated with poor survival of lung adenocarcinoma, the most commonly diagnosed early stage lung cancer. However, cell line studies show that further exploration is needed before these markers can be effectively used as prognostic biomarkers [17].

A protein group in serum has recently been identified from patients with tumor using the SELDITOF-MS technique [18]. This group of proteins, referred to as the lost goodwill target (LGT), can be regulated and controlled, and is found to be closely related to the death of cancer patients. In this study, we investigate the correlation of LGT proteome and the prognosis in patients with lung cancer. We followed

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up 188 recently diagnosed patients with lung cancer to explore the relationship between the LGT proteome and survival of these patients, in order to provide evidence on whether the LGT proteome can be used as an accurate and reliable prognostic biomarker for lung cancer.

Material and Methods

Patients

Consecutive cases of 188 patients with lung cancer were enrolled in the Shanxi Cancer Hospital from January 2008 to May 2009. All cases were confirmed with lung cancer by pathology. Institutional Review Board approval from the Ethics Committee of Shanxi Cancer Hospital was obtained for this study. Consent form was obtained from all subjects.

Proteomics analysis

For each patient, LGT test in serum was performed using the technique SELDITOF-MS after the pathological diagnosis without any chemotherapy. Each sample was collected into a 4 ml serum separator vacutainer tube and laid up at 4°C for 3 h, and then centrifuged for 5 min at 3000 rpm. The serum was stored frozen at -80°C until analysis. A total of 188 serum specimens were collected.

The expression of the LGT proteomic was recorded as positive if there was a single cluster in the M/Z spectrum between11 100+H and 11 900+H with maximum intensity ≥ 20%, minimum intensity ≥ 5%, and there were no peak in the nearby region of 1000 mass units. Otherwise the expression of LGT was recorded as negative. All cases were followed up by telephone to record the treatment, survival condition, death time and other information.

Quality control

Before the project started, all interviewers were trained. In each case, the questionnaire was inspected for accuracy and completeness. If the questionnaire was found to be incomplete or have errors, corrections were done immediately.

Statistical analyses

Kaplan-Meier survival analysis was performed to compare the survival difference between patients with LGT positive and LGT negative, with different initial tumor locations, with different lymph node metastasis, and with or without distant metastasis. Multivariate Cox proportional-hazards regression analysis was also performed with the covariates including gender, age, pathological staging, treatment procedure, subtype of the lung cancer, initial tumor locations, lymph node metastasis, and distant metastasis. Log-Rank tests were conducted to compare different survival functions obtained from Kaplan-Meier analysis. Visual FoxPro was used to establish a database and double entry check, and the SPSS13 software was used to perform statistical analysis. A probability of less than 5% ($P<0.05$) was considered statistically significant.

Result

Descriptive statistics

In total, 188 lung cancer patients were recruited. Demographic and clinical data are summarized in Table 1. These 188 cases of lung cancer included 145 males and 43 females. The age of these patients ranged from 26 to 91 years with mean age 61.17 ± 10.80 years. There were 65 cases of squamous cell carcinoma cases and 57 cases of adenocarcinoma. A total of 158 cases were at pathological stages III and IV, accounting for 84.04% of the total cases. The treatment included chemotherapy, surgery, and radiotherapy. Chemotherapy cases accounted for 80.31% of all cases. LGT group protein expressed positive in 93 cases, and negative in 95 cases.

Survival analysis

Kaplan-Meier analysis showed that the median survival time for LGT (+) and LGT (-) patients were 514 and 865 days, respectively, as shown in Table 2. Figure 1 shows the Kaplan-Meier estimates of the survival functions for patients with positive and negative LGT scores. Log-rank test showed that difference between the survival functions of these two groups of patients was statistically significant (χ^2=5.757, P=0.016). Table 2 also shows that survival time of the patients decreased as the degree of primary tumor invasion depth, lymph node metastasis increase in the number and distant metastasis. Figures 2 and 3 show the Kaplan-Meier plots comparing different survival functions of patients with different degree of primary tumor invasion depth, and with and without metastasis, respectively. Corresponding Log-rank tests showed that these differences were also statistically significant: χ^2 =11.025 P=0.004 for primary tumor T1-2, T3, and T4; χ^2=8.093, P=0.004 for without or with distant metastasis, respectively. In addition we also compared the survival functions among different number of lymph node metastasis 0,1,2,3 and found that χ^2=9.589 P=0.022.

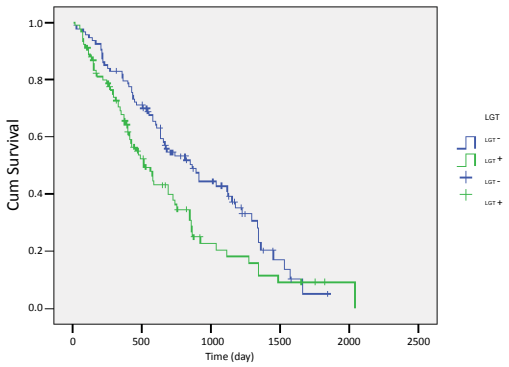

Figure 1: The survival curve comparison of patients with lung cancer between LGT positive group and LGT negative group.

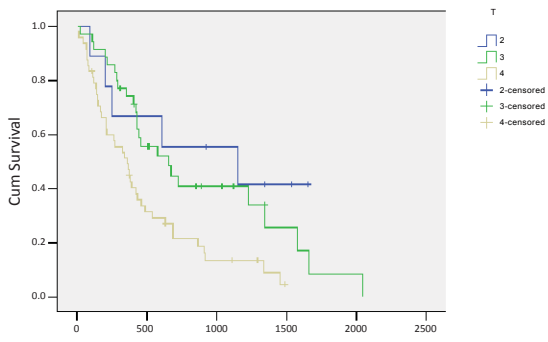

Figure2: The survival curve comparison of patients with lung cancer among different primary tumor types.

Basic Characteristics		LGT		total
		LGT(+)	LGT(-)	
Sex	male	74	71	145
	female	19	24	43
Profession	farmer	13	15	28
	worker	23	31	54
	office worker	49	36	85
primary tumor	T_1	1	7	8
	T_2	15	19	34
	T_3	15	28	43
	T_4	30	12	42
	T_X	2	3	5
Clinical stage	I	2	7	9
	II	9	12	12
	III	37	48	85
	IV	38	35	73
Pathological diagnosis typing	Squamous carcinoma	36	29	65
	adenocarcinoma	23	34	57
	Others	34	32	66
Treatment	surgery	22	42	64
	chemotherapy	80	71	151
	radiotherapy	36	22	58
Age	≤ 50	13	14	27
	51-60	15	18	33
	61-70	27	32	59
	≥ 71	38	31	69
Total		93	95	188

Table 1: Descriptive statistics of lung cancer patients with positive and negative LGT scores

	Median survival time	Standard deviation	95%CI	
			lower	upper
LGT (+)	514	61	382	646
LGT (-)	865	67	641	1089
total	673	114	553	793
N 0	1153	881	0	2880
1	673	258	167	1179
2	455	216	33	877
3	292	63	169	415
M 0	673	355	0	1369
1	394	45	306	482
T T1-T2	1153	676	0	2478
T3	656	174	315	997
T4	361	67	230	492

N: number of lymph node metastasis; M: tumor metastasis; T: primary tumor type.

Table 2: LGT different expression of the survival time of patients with lung cancer (days).

Index	Regression coefficient	SD	Waldχ^2	P	RR	RR95%CI
LGT	0.0429	0.182	5.547	0.019	1.536	(1.075, 2.196)
T	-0.290	0.142	4.184	0.041	0.749	(0.567, 0.988)
N	0.407	0.145	7.865	0.005	1.502	(1.130, 1.995)
M	0.501	0.237	4.489	0.034	1.651	(1.038, 2.624)

Table 3. Results of multivariate Cox regression analysis

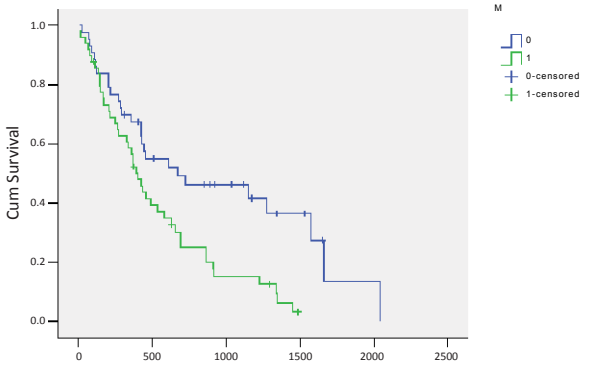

Figure 3: The survival curve comparison of patients with lung cancer with and without metastasis.

Because Kaplan-Meier analysis showed that, in addition to the LGT score, several other factors had statistically significant effects on the survival of lung cancer patients, we conducted a multivariate Cox proportional-hazards regression analysis in order to rule out possible confounding effects. The covariates we considered include gender, age, pathological staging, treatment procedure, subtype of the lung cancer, initial tumor locations, lymph node metastasis, and distant metastasis. Our result (Table 3) showed that indeed patients with positive LGT scores had shorter survival time than those with negative LGT scores (RR=1.536, 95% CI 1.075~2.196 P=0.019), after adjusting for the covariates. As shown in Table 3, primary tumor type, tumor metastasis, and number of lymph node metastasis were also significantly associated to the survival time of lung cancer patients. We did not observe a significant effect of treatment procedures on the patients' survival times.

Discussion

Application of advanced technology in proteomics, such as surface-enhanced laser desorption / ionization-time of flight-mass spectrometer (SELDI-TOF-MS), has opened up broad prospects and provides a new effective method for the study of disease at the protein level. By combining the specific protein chip system and mass spectrometer, this technology generates maps that are simple and repeatable, and is thus extremely useful in detecting low intensity proteins or peptides which accounts for a large proportion of serum [9]. The LGT protein group identified by Pei et al. [18] is a good example of application of proteomics in identifying biomarkers for cancer prognosis.

In this study, we showed that the survival rate of lung cancer patients is associated with the intensity of the LGT group. From 188 cases of mass spectrometry in serum proteomic of patients with lung cancer, we observed that there is an isolated mass spectrum peak cluster between (M/Z) 11100 + H and 11900 + H, where the LGT was discovered, which is combined with three or more peaks. The boundary can be clearly distinguished from its upstream and downstream protein groups. We emphasize that the LGT protein group can also be identified in serum proteomic of patients with lung cancer. Our results therefore provided strong evidence that the LGT group may be used as a promising biomarker for the prognosis of lung cancer. Specifically, we propose that the LGT group serve as serum protein markers to show poor prognosis of lung cancer patients: LGT positive status predicts a

shorter time to patients' death with lung cancer and LGT negative is the early diagnostic marker of survival.

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