Biomarkers, 2010; 15(2): 104–110

informa
healthcare

ORIGINAL ARTICLE

SLP-2 overexpression is associated with tumour distant metastasis and poor prognosis in pulmonary squamous cell carcinoma

Dong Chang[1], Kai Ma[1], Min Gong[1], Yong Cui[1], Zhi-hua Liu[2], Xiao-ge Zhou[3], Chuan-nong Zhou[2], and Tian-you Wang[1]

[1]Department of Thoracic Surgery, Beijing Friendship Hospital, Capital Medical University, Beijing, People's Republic of China, [2]State Key Laboratory of Molecular Oncology, Cancer Institute and Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, People's Republic of China and [3]Department of Pathology, Beijing Friendship Hospital, Capital Medical University, Beijing, People's Republic of China

Abstract

Objective: To investigate the role of stomatin-like protein 2 (*SLP-2*), a novel cancer-related gene, in pulmonary squamous cell carcinoma (PSCC) and its implications.

Methods: Immunohistochemical detection of *SLP-2* was performed on 96 cases of PSCC with a tissue microarray.

Results: SLP-2 was overexpressed in lung cancer compared with normal lung tissue ($p < 0.001$). High-level *SLP-2* expression was significantly correlated with distant metastasis ($p = 0.025$), decreased overall survival ($p = 0.018$) and disease-free survival ($p = 0.017$). *SLP-2* overexpression was an independent prognostic factor in multivariate analysis using the Cox regression model ($p < 0.05$).

Conclusion: SLP-2 overexpression is associated with tumour distant metastasis and poor prognosis in PSCC. *SLP-2* could be regarded as a new significant prognostic biomarker for patients with PSCC.

Keywords: *Pulmonary squamous cell carcinoma; SLP-2; tissue micro-array; prognosis*

Introduction

Lung cancer is currently the leading cause of cancer death worldwide (Parkin et al. 2002) and mean relative 5-year survival is only 12.6% in Europe (Berrino et al. 2007). In China, the incidence and mortality of lung cancer have been showing a dramatically rising trend during the past decades and the average survival is only about 10% (Yang L et al. 2004). Surgical resection remains a main treatment choice for patients with non-small cell lung cancer (NSCLC). Despite advances in treatment, the prognosis remains poor with recurrence rates as high as 50% after surgical resection alone. In recent years, there have been major improvements in the role of chemotherapy–radiotherapy in the treatment of NSCLC, which combined with surgery increased the 5-year survival to nearly 30% (Horn & Sandler 2007, Gkiozos et al. 2007). Nevertheless, these adjuvant or neoadjuvant therapies are associated with significant morbidity and mortality needing appropriate patient selection, i.e. the patients who will most benefit from these approaches. In this setting, prognostic factors could be of potential value. Some clinical prognostic markers such as clinical stage, lymph node metastasis and histopathological grade have already been identified, but their discriminant value is insufficient to allow a decision alone.

Recent researches have focused on the potential role of new biological factors involved in the carcinogenic process, as prognostic markers for survival in

Address for Correspondence: Dong Chang or Prof. Tian-you Wang, Department of Thoracic Surgery, Beijing Friendship Hospital, Capital Medical University, 95 Yong An Road, Beijing 100050, P. R. China. Tel: 86-10-63138767. E-mail: changdongj@yahoo.com.cn; articlefriendship@hotmail.com

(Received 21 July 2009; revised 03 September 2009; accepted 04 September 2009)

ISSN 1354-750X print/ISSN 1366-5804 online © 2010 Informa UK Ltd
DOI: 10.3109/13547500903311910

patients with NSCLC. In our previous studies, *SLP-2* (human stomatin-like protein 2) was first identified as a differentially expressed cancer-related gene through cDNA microarray analysis, which showed that it was expressed over six times in esophageal squamous cell carcinoma (ESCC) tissues compared with their normal counterparts (Luo et al. 2004, Lu et al. 2001, Zhi et al. 2003). *SLP-2* is a novel and unusual member of the stomatin gene superfamily, with unknown functions, has been cloned and characterized by Wang & Morrow (2000). Our previous studies revealed that *SLP-2* was overexpressed in many human cancer tissues, including ESCC, lung cancer, endometrium adenocarcinoma and laryngeal squamous cell carcinoma (LSCC). Furthermore, antisense *SLP-2* showed decreased cell growth, tumorigenicity and cell adhesion in ESCC (Zhang et al. 2004, 2005, 2006, Cui et al. 2007). High expression of SLP-2 protein could contribute to the prognostic characteristics of lymph node metastasis in LSCC (Cao et al. 2007b) and in breast cancer (Cao et al. 2007a). In addition, SLP-2 high expression was associated with significantly decreased breast cancer patient survival (Cao et al. 2007a). It seems that SLP-2 overexpression is a very common event in many kinds of cancer development, and SLP-2 expression may be a new valuable prognostic biomarker.

In order to further evaluate the role of SLP-2 protein in pulmonary squamous cell carcinoma (PSCC), a main subtype of human lung cancer, on the basis of our previous work, we performed immunohistochemistry (IHC) studies on primary tumour tissue microarray (TMA) from a group of patients with sufficient follow-up data. Then, we investigated whether the increased SLP-2 expression could predict the prognosis of the patients. We also investigate the relationships between SLP-2 expression and other clinicopathological parameters, including tumour site, tumour grade, lymph node metastasis, and clinical stage.

Materials and methods

Patients and clinical data

Ninety-six patients with PSCC who were treated surgically between June 1999 and November 2004 at the Department of Thoracic Surgery, Beijing Friendship Hospital, Capital Medical University (BFH-CMU), enrolled in this study. None of the patients had received radiotherapy or chemotherapy before surgery. The use of human tissues was approved by the local ethics committee. Only patients who agreed with the aim and contents of this study and who gave written informed consent to participate in it were included in this study.

Patient characteristics, including age (33–76 years; median 63.5 years), clinical stage (classified according to the 2002 TNM classification of the International Union Against Cancer), along with information on distant metastasis, recurrence (local/regional) and survival are shown in Table 1. The median follow-up period was 53 months (range 1–100); all patients had follow-up of more than 36 months unless they died of any cause. Cases lost to follow-up were excluded from the study. Local recurrence occurred in five cases (5.2%). Distant metastasis occurred in 30 cases (31.3%), totalling 38 organs, including 17 cases to bone, seven cases to the mediastinal, four cases to the pleura, three cases to the lung, two cases to the liver, two cases to the brain, two cases to the adrenal, one case to the chest

Table 1. Patient and tumour characteristics in 96 cases of pulmonary squamous cell carcinoma.

Characteristic	No. (%) of cases
Age (years)	
<50	13 (13.5)
50–70	67 (69.8)
>70	16 (16.7)
Gender	
Male	76 (79.2)
Female	20 (20.8)
Smoking status	
Yes	46 (47.9)
No	50 (52.1)
Tumour stage	
pT1	15 (15.6)
pT2	58 (60.4)
pT3	21 (21.9)
pT4	2 (2.1)
Lymph node status	
pN0	56 (58.3)
pN1	28 (29.2)
pN2	12 (12.5)
Differentiation/histological grade	
Well	15 (15.6)
Moderate	68 (70.8)
Poor	13 (13.5)
TNM stage	
I	49 (51.0)
II	26 (27.1)
III	21 (21.9)
Distant metastasis	
Absent	66 (68.8)
Present	30 (31.3)
Any recurrence (local or regional)	
Absent	91 (94.8)
Present	5 (5.2)
Survival	
Alive	56 (58.3)
Died of lung cancer	37 (38.5)
Died of other cause	3 (3.1)

wall. Tumour characteristics, including histological grade and lymph node status were routinely assessed by pathologists (Table 1).

Preparation of TMAs

Ninety-six polyformaldehyde fixed and paraffin-embedded PSCC tissue blocks were obtained from the stored files of the Department of Pathology, BFH-CMU. Twenty control samples from matched normal lung tissues in the same resected lobes and 20 metastatic lymph nodes from paired PSCCs were also collected. Thus, we had in total 136 specimens used for making TMA. Normal tissues were taken from the distal resection margin. Histopathologically representative tumour regions and the normal tissues were defined from haematoxylin and eosin (HE)-stained sections and marked on the donor blocks.

The TMA block was constructed with a Beecher Instruments Tissue Array (Beecher Instruments, Silver Spring, MD, USA). Recipient blocks were made with purified agar in frames. Holes of 0.6 mm were cut in the recipient blocks with a core needle, and the agar core was discarded. After identifying the representative tumour areas in each whole-mount slide, two representative portions of each case of cancer or normal samples were transplanted to the recipient blocks using a 0.6-mm core needle. Recipient blocks were framed in the mold used to frame conventional paraffin blocks, and then paraffin was added. Consecutive 4- μm thick sections were cut from the recipient blocks to poly-L-lysine-coated glass slides and processed for IHC analysis.

Immunohistochemistry

Immunohistochemical staining was performed using a method described previously (Chang et al. 2007). The prepared TMA section were dewaxed with xylene and rehydrated through gradient ethanol into water. After endogenous peroxidase activity had been quenched with 3% H_2O_2 for 20 min, antigen retrieval was achieved by using microwave heating in 1 mM of ethylenediaminetetraacetic acid (EDTA) (pH 8.0) at 97 °C for 15 min, followed by a 60-min cool-down period at room temperature. After washing with phosphate-buffered saline (PBS), sections were incubated with the rabbit polyclonal primary antibody against SLP-2 at a 1:200 dilution (Proteintech Group, Inc., USA) at 4 °C overnight. Subsequently, IHC staining was performed using the PV-9000 kit (Golden Bridge International Inc., Mulkiteo, WA, USA) according to the manufacturer's instructions. Briefly, sections were incubated with polymer helper for 20 min at room temperature. Then, sections were incubated with a secondary antibody named polyperoxidase-antirabbit IgG at a 1:200 dilution (Golden Bridge International Inc.) for 30 min at room temperature. The immune complexes were visualized by incubation with 3,3′-diaminobenzidine (Sigma, St Louis, MO, USA) for 5 min. Sections were then washed in running tap water and lightly counterstained with haematoxylin, followed by dehydration and coverslip mounting. For negative controls, the primary antibody was replaced by PBS. For positive controls, a section of human ESCC, known to be positive for SLP-2 (Zhang et al. 2004, 2005, 2006, Cui et al. 2007), was used.

TMA immunostaining was scored semiquantitatively in a blinded manner for intensity and extent, by two independent pathologists (Dr Zhou Chuan-nong and Dr Zhou Xiao-ge). Discordant scores were re-evaluated by the investigators, and the consensus score was used for further analysis. The criterion was almost based on our previous work (Cao et al. 2007, Chang et al. 2007). The intensity of the immunostaining was defined in four categories: 0, no yellow particles in the tumour cell cytoplasm or plasma membrane; 1, light yellow particles present (weak); 2, general yellow particles present (moderate); and 3, deep yellow particles present (strong). The percentage of positive cells and the extent of immunostaining were determined and classified into five groups: 0, fewer than 5% positive tumor cells; 1, 5% to 25% positive tumor cells; 2, 26% to 50% positive tumour cells; 3, 51% to 75% positive tumour cells; and 4, more than 75% positive tumour cells. For each case, the immunostaining score (ranges 0–12) also known as the staining index (SI), was the product of the two scores for the separately assessed samples. In this study, we defined high-level expression of SLP-2 protein as an SI of 6 or more; an SI from 0 to 5 denoted low or negative SLP-2 expression.

Statistical analysis

Statistical analysis was performed using the SPSS statistical software (version 13.0, SPSS Inc, Chicago, IL, USA). The difference was considered significant when the p-value was <0.05. The correlations between SLP-2 expression and clinicopathological variables were analysed using Spearman's correlation test and the Phi correlation test. The difference of SLP-2 expression between PSCC and normal lung tissues was analysed using the Mann–Whitney U test. Survival curves were plotted using the Kaplan–Meier method, and statistical significance of survival between different groups was compared by the log rank test. Univariate and multivariate analyses in Cox proportional hazards models were used for SLP-2 scores and other recognized clinicopathological predictors of outcome to evaluate the independent prognostic variables on overall survival.

Results

Expression of SLP-2 in human PSCC

SLP-2 protein expression was localized mainly in the cytoplasm (Figure 1). Weak or absent staining (SI <6) was noted in 69 cases (71.9%), and strong staining (SI ≥6) was shown in 27 cases (28.1%). The mean SIs of normal lung tissue and lung cancer tissue were 1.15 and 4.84, respectively. By the Mann–Whitney *U* test, the mean rank for normal lung tissue was 28.39, and for PSCC sample 55.82. Compared with normal lung tissue, SLP-2 expression in PSCC was significantly high (p <0.001) (Table 2). All cancer cells in metastatic lymph nodes of 20 cases PSCC showed strong expression of SLP-2, whether their primary tumours were SLP-2 high expression or low expression.

Association of SLP-2 expression with clinicopathological characteristics

After choosing appropriate cut-off points for each parameter, we analysed the relationship between SLP-2 expression and clinicopathological parameters, according to the SLP-2 high-level expression (n = 27) group and the SLP-2 low/negative expression group (n = 69) (Table 3). SLP-2 high-level expression showed a significant correlation with distant metastases (p = 0.025). However, no significant associations were observed between SLP-2 expression and other clinicopathological characteristics such as age, gender, smoke status, tumour differentiation, lymph node metastasis, tumour stage, TNM stage and cancer recurrence (Table 3).

Survival analysis

Statistics analysis on survival data of these PSCC patients, with mean follow-up of 46 months (median follow-up of 53 months), showed significant differences in the overall survival (OS) and disease-free survival (DFS) between patients with high SLP-2 expression versus patients with low SLP-2 expression. Namely, elevated SLP-2 expression was significantly associated with worse OS (χ^2 = 5.573, p = 0.018) and DFS (χ^2 = 5.680, p = 0.017) (Table 4). Kaplan–Meier survival curves were generated on the basis of low and high expression of SLP-2 protein (Figures 2 and 3).

In univariate survival analysis, only tumour stage (T stage) was associated with DFS, all other clinicopathological parameters including age, gender, smoke

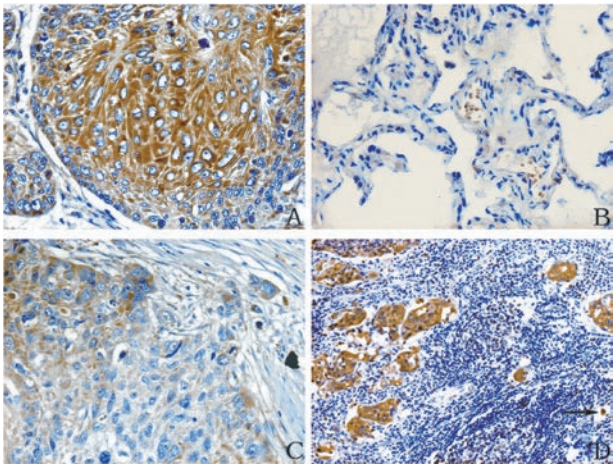

Figure 1. Immunohistochemical staining of SLP-2 protein expression in series sections (200×). A pulmonary squamous cell carcinoma sample shows strong SLP-2 expression (A), and the corresponding normal lung tissue with negative SLP-2 expression (B). Another squamous cell carcinoma sample shows weak SLP-2 expression (C), but its metastatic lymph node has strong SLP-2 expression (100×). The black arrow shows a single cancer cell with strong SLP-2 expression (D).

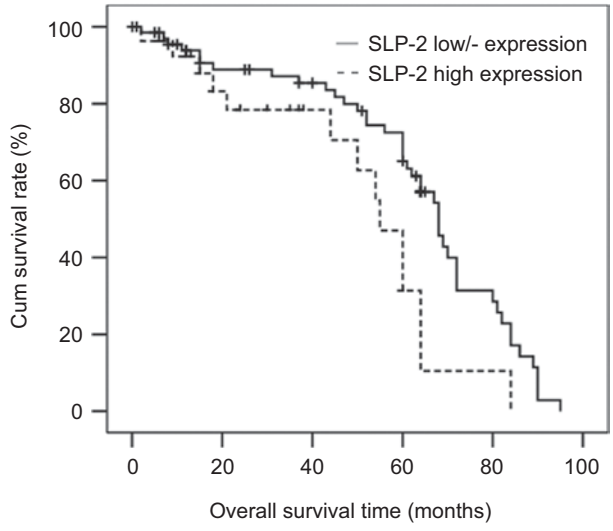

Figure 2. Overall survival curve for SLP-2 high expression (n = 27) and low expression (n = 69) in 96 patients with pulmonary squamous cell carcinoma (χ^2 = 5.573, p = 0.018).

Table 2. Expression of SLP-2 in normal vs malignant lung tissue.

	n	SI (mean ± SE)	Mean rank	*U*[a]	*p*-Value
Normal lung tissue	20	1.15 ± 0.31	28.39	32.51	<0.001
Lung cancer (PSCC)	20	4.84 ± 0.28	55.82		

PSCC, pulmonary squamous cell carcinoma, [a]Mann-Whitney *U* test.

Table 3. Association of SLP-2 expression with clinicopathological and prognostic characteristics.

Characteristic	*n*	SLP-2 expression Low/negative (*n* = 69)	SLP-2 expression High (%) (*n* = 27)	Correlation test	Coefficient	*p*-Value
Gender						
Male	76	53	23	Phi	−0.093	0.364
Female	20	16	4			
Age (years)						
<50	13	11	2	Phi	0.210	0.119
50–70	67	44	23			
>70	16	14	2			
Smoking status						
No	50	36	14	Phi	0.003	0.977
Yes	46	33	13			
Tumour stage						
T1	14	11	3	Spearman	0.147	0.152
T2	60	45	15			
T3	20	12	8			
T4	2	1	1			
Lymph node status						
pN0	57	45	12	Phi	0.190	0.062
pN1, pN2	39	24	15			
Differentiation						
Well	15	10	5	Spearman	−0.019	0.851
Moderate	68	50	18			
Poor	13	9	4			
TNM stage						
I	50	39	11	Spearman	0.178	0.083
II	26	19	7			
III	20	11	9			
Distant metastasis						
Absent	66	52	14	Phi	0.228	**0.025***
Present	30	17	13			
Any recurrence						
Absent	93	68	25	Phi	0.166	0.103
Present	3	1	2			

*p <0.05

Table 4. Univariate analysis of the factors that influenced the overall and disease-free survival.

Factors	Overall survival χ^2	Overall survival *p*-Value	Disease-free survival χ^2	Disease-free survival *p*-Value
Age	1.112	0.573	0.232	0.890
Gender	2.976	0.085	0.114	0.735
Smoking status	1.449	0.229	0.229	0.632
Tumour stage	1.799	0.615	5.254	**0.022***
Lymph node status	0.090	0.764	1.267	0.202
Differentiation	0.750	0.687	0.503	0.778
TNM stage	1.007	0.604	3.279	0.070
SLP-2 expression	5.573	**0.018***	5.680	**0.017***

*p <0.05

status, tumour differentiation, lymph node metastasis and TNM stage were not associated with OS and DFS (Table 4). A multivariate survival analysis was performed to evaluate the independent prognostic role of SLP-2 expression. In order to avoid analysis bias, we used three different Cox proportional hazards models (Table 5). In all the three models, SLP-2 expression level was an independent prognostic factor for PSCC patients (p <0.05).

Discussion

SLP-2 is a member of the highly conserved stomatin family of proteins whose homologues span from archae to humans and include stomatin, SLP-1, and SLP-3 (Seidel & Prohaska 1998, Goldstein et al. 2003, Wang & Morrow 2000). SLP-2 shares a central SPFH (stomatin/prohibitin/flotillins/HflK-HflC) domain characteristic of this family that may mediate interactions with membrane proteins. However, SLP-2 is unique among stomatins in that it does not have a putative

transmembrane domain, but has six myristoylation/palmitoylation sites and an N terminal mitochondria targeting sequence. The function of stomatins, including SLP-2, is unknown. It has been suggested that they are involved in the organization of the peripheral cytoskeleton and sphingolipid and cholesterol-rich lipid rafts, and in the assembly of ion channels and mechanosensation receptors (Hajek et al. 2007). Our previous studies (Zhang et al. 2006, Cui et al. 2007, Cao et al. 2007a, b) suggested that SLP-2 could be regarded as a novel cancer-related gene and a new prognostic biomarker.

In this study, we chose 96 cases of PSCC to investigate the SLP-2 protein expression status and the correlation between SLP-2 expression and tumour clinicopathological characteristics, and prognosis. The results showed obvious overexpression of SLP-2 in human PSCC tissues compared with normal lung tissues (p <0.001). SLP-2 protein expression was localized mainly in the cytoplasm. In total 96 cases, there existed 27 cases (28.1%) with SLP-2 high expression and 69 cases (71.9%) showed low expression of SLP-2 protein. All cancer cells in metastatic lymph nodes of 20 cases PSCC showed strong expression of SLP-2, whether their primary tumours were SLP-2 high expression or low expression. Therefore, we came to the conclusion that SLP-2 may be involved in human PSCC formation and progression.

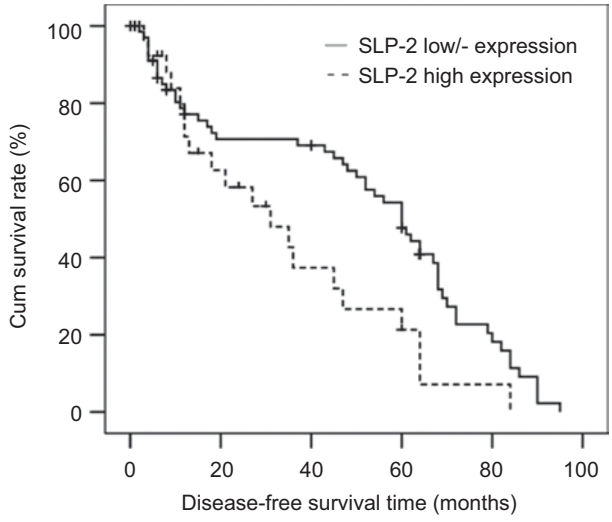

Figure 3. Disease-free survival curve for SLP-2 high expression (n =27) and low expression (n = 69) in 96 patients with pulmonary squamous cell carcinoma (χ^2 = 5.680, p = 0.017).

Association analysis of SLP-2 expression with clinicopathological characteristics indicated that a high level of SLP-2 expression had an obvious relationship with distant metastasis. However, other clinicopathological characteristics such as age, gender, smoke status, tumour differentiation, lymph node metastasis, tumour stage, TNM stage and cancer recurrence showed no association with SLP-2 protein high expression. The results are a little different from our previous study on LSCC and breast cancer (Cao et al. 2007a, b), in which we found significant associations between SLP-2 expression and lymph node metastasis and clinical stage. However, all cancer cells in metastatic lymph nodes showed strong expression of SLP-2, which indicated the potential predicting value of SLP-2 in cancer metastasis. Taking these data together, we concluded that SLP-2 expression in human cancers could be of tissue specific. The possible role of SLP-2 in the progression of human tumours is not clear. SLP-2 high expression correlated significantly with distant metastases in PSCC; such malignant biological behaviour and the death-threatening factor, means that detection of the level of SLP-2 expression is of important long-term prognostic significance.

Moreover, we analysed the overall and disease-free survival rates in PSCC with low and high SLP-2 expression, finding a statistical association between high SLP-2 expression and poor prognosis. In this study, the median overall survival time for SLP-2 high expression

Table 5. Multivariate analysis of the factors for overall survival using Cox regression model.

Prognostic factors	Model 1				Model 2				Model 3			
	Wald	*p*-Value	*RR*	95% CI	Wald	*p*-Value	*RR*	95% CI	Wald	*p*-Value	*RR*	95% CI
Tumour stage	1.421	0.233	1.457	0.785-2.705	0.704	0.401	1.213	0.772-1.906	-	-	-	-
Lymph node status	0.518	0.472	1.605	0.442-5.824	0.028*	0.867	0.948	0.504-1.783	-	-	-	-
TNM stage	0.810	0.368	0.641	0.243-1.698	-	-	-	-	0.014	0.907	1.021	0.722-1.443
Differentiation	0.106	0.744	0.908	0.509-1.620	0.080	0.777	-0.923	0.530-1.608	0.095	0.758	0.918	0.533-1.580
SLP-2 expression	4.056	**0.044***	1.983	1.018-3.860	4.231	**0.040***	2.014	1.034-3.925	1.708	**0.030***	2.085	1.074-4.050
	−2 Log Likelihood = 373.044 χ^2 = 16.930 p = 0.0022				−2 Log Likelihood = 373.044 χ^2 = 16.186 p = 0.0018				−2 Log Likelihood = 373.044 χ^2 = 15.446 p = 0.0014			

*p <0.05, RR, relative risk; CI, confidence interval.

and low expression were 68 (95% confidence interval (CI) 63–73) months and 55 (95% CI 47–63) months, respectively. The median disease-free survival time for SLP-2 high expression and low expression were 60 (95% CI 51–69) months and 31 (95% CI 12–50) months, respectively. In our series, Cox proportional hazards models showed that high SLP-2 expression maintained its independent prognostic impact on overall and disease-free survival. SLP-2 seems to be a strong predictor of poor prognosis for PSCC, in keeping with the findings in breast cancer, the only related study (Cao W et al. 2007a). Overexpression of SLP-2 independently predicted poor survival for patients with PSCC. The mechanisms leading to SLP-2 overexpression in human tumours are not well known. Possible mechanisms include point mutation, gene amplification, gene rearrangement and insertion of strong promoter or enhancer. Epigenetic modifications including demethylation and deacetylation may also be responsible. In our previous study (Zhang et al. 2006), no mutation was found within the open-reading frame of SLP-2 by using the PCR technique. However, the levels of SLP-2 protein expression correlated well with the levels of SLP-2 mRNA expression in a small cohort of PSCC cases. Further studies to investigate the mechanism of SLP-2 overexpression and the up-/downstream cellular signal pathways are ongoing.

There are few studies investigating the prognostic factors of PSCC alone, although there are many more studies on NSCLC (including both PSCC and lung adenocarcinoma). Therefore, the prognostic factors, especially clinical factors (such as T, N, and TNM stage) of PSCC are still controversial. In the present study, only 'tumour stage' was associated with DFS for PSCC patients. To our knowledge, the present study is the first report investigating the expression of SLP-2 in patients with PSCC to include prognostic significance. In conclusion, we have shown that the expression of SLP-2 is upregulated in PSCC compared with normal lung tissues. SLP-2 overexpression correlated with distant metastases, and both poor overall and disease-free patient survival, suggesting that it may contribute to the malignant potential of PSCC. SLP-2 could serve as a valuable new biomarker for predicting prognosis and direct clinical decision-making for patients with PSCC.

Acknowledgements

We thank M.M. Xiao-dan Hu for his help with the patients' follow-up.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Berrino F, Angelis de R, Sant M. (2007). Survival for eight major cancers and all cancers combined for European adults diagnosed in 1995-99: results of the EUROCARE-4 study. *Lancet Oncol* 8:773–83.

Cao W, Zhang B, Liu Y. (2007a). High-level SLP-2 expression and HER-2/neu protein expression are associated with decreased breast cancer patient survival. *Am J Clin Pathol* 128:430–6.

Cao WF, Zhang LY, Liu MB. (2007b). Prognostic significance of stomatin-like protein 2 overexpression in laryngeal squamous cell carcinoma: a clinical, histological and immunohistochemistry analysis with tissue microarray. *Hum Pathol* 38:747–52.

Chang D, Wang TY, Li HC. (2007). Prognostic significance of PTEN expression in esophageal squamous cell carcinoma from Linzhou City, a high incidence area of northern China. *Dis Esophagus* 20:491–6.

Cui Z, Zhang L, Hua Z. (2007). Stomatin-like protein 2 is overexpressed and related to cell growth in human endometrial adenocarcinoma. *Oncol Rep* 17:829–33.

Gkiozos I, Charpidou A, Syrigos K. (2007). Developments in the treatment of non-small cell lung cancer. *Anticancer Res* 27:2823–7.

Goldstein BJ, Kulaga HM, Reed RR. (2003). Cloning and characterization of SLP-3: a novel member of the stomatin family expressed by olfractory receptor neurons. *J Assoc Res Otolaryngol* 4:74–82.

Hajek P, Chomyn A, Attardi G. (2007). Identification of a novel mitochondrial complex containing mitofusin 2 and stomatin-like protein 2. *J Biol Chem* 282: 5670–81.

Horn L, Sandler A. (2007). Lung cancer adjuvant therapy. *Cancer J* 13:210–16.

Lu J, Liu Z, Xiong M. (2001). Gene expression profile changes in initiation and progression of squamous cell carcinoma of esophagus. *Int J Cancer* 91:288–94.

Luo A, Kong J, Hu G. (2004). Discovery of Ca2+-relevant and differentiation-associated genes downregulated in esophageal squamous cell carcinoma using cDNA microarray. *Oncogene* 23:1291–9.

Parkin DM, Bray F, Ferlay J. (2002). Global Cancer Statistics, 2002. *CA Cancer J Clin* 55:74–8.

Seidel G, Prohaska R. (1998). Molecular cloning of hSLP-1, a novel human brain-specific member of the band 7/MEC-2 family similar to *Caenorhabditis elegan*s UNC-24. *Gene* 225:23–9.

Wang Y, Morrow JS. (2000). Identification and characterization of human SLP-2, a novel homologue of stomatin (Band 7.2b) present in erythrocytes and other tissues. *J Biol Chem* 275:8062–71.

Yang L, Parkin DM, Li LD. (2004). Estimation and projection of the national profile of cancer mortality in China: 1991–2005. *Br J Cancer* 90:2157–66.

Zhang L, Ding F, Cao W. (2006). Stomatin-like protein 2 is overexpressed in cancer and involved in regulating cell growth and cell adhesion in human esophageal squamous cell carcinoma. *Clin Cancer Res* 12:1639–46.

Zhang LY, Ding F, Liu ZM. (2005). Effect of stomatin-like protein 2 (SLP-2) gene on growth and proliferation esophageal squamous cell carcinoma cell line TE12. *Ai Zheng* 24:155–9.

Zhang LY, Wang T, Ding F. (2004). Differential expression and bioinformatics analysis of SLP-2 gene in esophageal squamous cell carcinoma. *World Chin J Digestol* 12:1517–21.

Zhi H, Zhang J, Hu G. (2003). The deregulation of arachidonic acid metablism-related genes in human squamous cell carcinoma. *Int J Cancer* 106:327–33.