Prognostic Factors Including the Expression of Thyroid Transcription Factor 1 (TTF1) in Patients Irradiated for Limited-disease Small Cell Lung Cancer

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Abstract. *Aim: To investigate the impact of tumor-cell expression of thyroid transcription factor 1 (TTF1) on outcomes after irradiation of limited-disease small cell lung cancer (LD-SCLC). Patients and Methods: TTF1 expression plus eight factors were evaluated in 32 patients for locoregional recurrence-free survival (LRFS), metastases-free survival (MFS) and overall survival (OS). These factors included age, gender, Karnofsky performance score (KPS), T- and N-category, respiratory insufficiency, smoking during radiotherapy and hemoglobin levels. Results: On univariate analysis of LRFS, no factor achieved significance. Improved MFS was associated with KPS >70 (p=0.029), N0-2 (p=0.003), no respiratory insufficiency (p=0.029) and non-smoking states (p<0.001) on univariate analysis, and N-category (p=0.015) and non-smoking (p<0.001) on multivariate analysis. On univariate analysis, OS was associated with KPS >70 (p<0.001), N0-2 (p=0.002), no respiratory insufficiency (p=0.008) and non-smoking (p=0.022). On multivariate analysis, KPS (p=0.008) and N-category (p=0.018) remained significant. Conclusion: In contrast to other factors, TTF1-expression had no significant impact on outcomes after irradiation of LD-SCLC.*

Lung cancer remains the leading cause of cancer deaths in the United States (1). Small cell lung cancer (SCLC) represents 10% to 25% of all lung cancer and is characterized by rapid growth, early locoregional recurrence and early distant metastases (1-3). The prognosis of many patients with SCLC is poor and needs to be considerably improved. This may be achieved with new anticancer drugs, drug combinations and new radiotherapy techniques, and additionally with the administration of very personalized treatment approaches. Since such approaches should always consider the patient's prognosis, a profound knowledge of prognostic factors and identification of new predictors are important.

Recent studies investigated the prognostic value of thyroid transcription factor 1 (TTF1) expression in non-small cell lung cancer (NSCLC) (4-7). This nuclear transcription factor plays an important role in embryologic development of the lung, as well as carcinogenesis of lung cancer. In NSCLC, the expression of TTF1 has been shown to inhibit cell migration and invasion (7). Particularly in patients presenting with adenocarcinoma, tumor-cell expression of TTF1 was associated with improved overall survival (6). The prognostic role of TTF1 expression in SCLC contributing to patient prognosis and treatment effects has not yet been properly investigated and, therefore, needs clarification. The present study was performed to investigate the effect of TTF1 expression in tumor cells on locoregional recurrence-free survival (LRFS), metastasis-free survival (MFS), and overall survival (OS) in patients irradiated for limited disease SCLC (LD-SCLC).

Patients and Methods

Thirty-two patients irradiated for LD-SCLC at the University of Lubeck between 2006 and 2014 were included in this retrospective study. The expression levels of TTF1 were evaluated in tumor samples obtained before radiotherapy by transbronchial biopsy. SCLC tissues were fixed in 10% buffered formalin (pH 7.0) and subsequently embedded in paraffin. The patient characteristics are summarized in Table I.

Immunohistochemistry. TTF1 immunostaining was performed prospectively using a standardized immunohistochemistry protocol (Antibody Clone TTF-1 8G7G3/1, manufactured by NeoMarkers for Lab Vision Corporation, Freemont, CA, USA) and evaluating each specimen in relation to a standard positive sample. Tumor cells were

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Key Words: Small cell lung cancer, TTF1 expression, irradiation, outcomes, predictive factors.

considered positive only if distinct nuclear staining was identified (5, 8). According to a previous report, the tumor samples were considered TTF1-positive if at least 5% of the tumor cells exhibited nuclear expression (5).

Treatment. Radiotherapy was performed following computed tomography-based three-dimensional treatment planning with a linear accelerator and 6- to 18-MV photons. The target volume included the primary tumor and the loco-regional lymph nodes with a margin of 2 cm. The total dose given as the equivalent dose in 2-Gy fractions (EQD2) ranged between 40 Gy and 64 Gy (median dose=56 Gy) and depended on the treatment schedule favored at our institution during different periods of time.

The EQD2 was calculated with the equation $EQD2=D\times[(d+\alpha/\beta)/(2\ Gy+\alpha/\beta)]$, where D=total dose, d=dose per fraction, α=linear component of cell killing, β=quadratic component of cell killing, α/β ratio=dose at which both components are equal (9). The α/β-ratio for tumor cell kill was assumed to be 10 Gy. Thoracic radiotherapy was combined with 2-6 cycles of platin-based chemotherapy (cisplatin or carboplatin plus etoposide), of which two cycles were administered concurrently with thoracic radiotherapy. Follow-up evaluations were regularly performed every 3-4 months during the first year and every 6 months thereafter. Additional follow-up visits were performed if patients developed any symptoms. Twenty patients received prophylactic cranial irradiation after thoracic radiotherapy.

Potential prognostic factors. In this study, we evaluated the prognostic impact of tumor cell expression of TTF1 (no *vs*. yes) and eight additional potential factors with respect to LRFS, MFS, and OS. The factors included age (≤65 *vs*. >65 years), gender, Karnofsky performance score (≤70 *vs*. >70), T-category (T1-3 *vs*. T4), N-category (N0-2 *vs*. N3), respiratory insufficiency before radiotherapy (no *vs*. yes), smoking during radiotherapy (no *vs*. yes), and median weekly hemoglobin level during radiotherapy (<12 *vs*. ≥12 g/dl). Four to six determinations of hemoglobin level were obtained during the course of radiotherapy. The majority of hemoglobin levels during radiotherapy were either consistently <12 g/dl or ≥12 g/dl. The situation that three or fewer out of six measurements or two or fewer out of four measurements were <12 g/dl or ≥12 g/dl did not occur.

Statistical analyses. LRFS, MFS, and OS were calculated using Kaplan–Meier method (10). Differences between the Kaplan–Meier curves were calculated with log-rank test (univariate analysis). Results were considered significant when $p<0.05$. The potential prognostic factors found to be significant were additionally evaluated in a multivariate analysis using the Cox proportional hazards model. Time to locoregional failure, time to distant failure, and time to death were referenced from the last day of radiotherapy. Locoregional failure was identified by endoscopy and serial computed tomography scans. Patients were followed-up until death or for a median of 84 months (range=7-106 months) in those alive at the last follow-up visit.

Results

Locoregional failure occurred in eight patients after a median of 9 months. The LRFS rates at 1 and 2 years were 72% and 69%, respectively. On univariate analysis of LRFS, none of the investigated factors achieved significance (Table II). This also applied to the tumor cell expression of TTF1 ($p=0.35$).

Table I. *Patients' characteristics*.

Factor	Number of patients	Proportion (%)
TTF1 expression		
Yes	19	59
No	13	41
Age		
≤65 years	16	50
>65 years	16	50
Gender		
Female	12	40
Male	20	60
Karnofsky performance score		
≤70	10	31
>70	22	69
T-Category		
1-3	18	56
4	14	44
N-Category		
0-2	26	81
3	6	19
Smoking during radiotherapy		
Yes	7	22
No	25	78
Hemoglobin during radiotherapy		
≤12 mg/dl	7	22
>12 mg/dl	25	78
Respiratory insufficiency		
No	26	81
Yes	6	19

TTF1: Thyroid transcription factor-1.

Distant metastases occurred in nine patients after a median of 9 months after irradiation. The MFS rates at 1 and 2 years were 72% and 63%, respectively. On univariate analysis, improved MFS was significantly associated with KPS >70 ($p=0.029$), N-category 0-2 ($p=0.003$) and non-smoking status during radiotherapy ($p<0.001$), whereas tumor cell expression of TTF1 had no significant impact on MFS ($p=0.47$). The results of univariate analysis of MFS are summarized in Table III. On multivariate analysis of MFS, independent positive prognostic factors were N-category [hazard ratio (HR)=5.26; 95% confidence interval (CI)=1.44-18.94; $p=0.012$] and non-smoking during radiotherapy (HR=9.50; 95% CI=2.57-35.11; $p=0.001$).

Median survival time in the entire cohort was 23 months. OS rates at 1 and 2 years were 66% and 34%, respectively. On univariate analysis, OS was better in patients with a KPS >70 ($p<0.001$), N-category 0-2 ($p=0.002$), absence of respiratory insufficiency ($p=0.008$) and non-smoking status during radiotherapy ($p=0.022$). In contrast, the tumor cell

Table II. *Univariate analysis of locoregional recurrence-free survival.*

Factor	At 1 year (%)	At 3 years (%)	*p*-Value
TTF1 expression			
Yes	42	11	
No	31	15	0.35
Age			
≤65 years	38	13	
>65 years	38	13	0.11
Gender			
Female	42	0	
Male	35	20	0.08
Karnofsky performance score			
≤70	10	0	
>70	50	18	0.16
T-Category			
1-3	39	11	
4	36	14	0.56
N-Category			
0-2	46	15	
3	0	0	0.29
Smoking during radiotherapy			
Yes	0	0	
No	48	16	0.14
Hemoglobin during radiotherapy			
≤12 mg/dl	38	23	
>12 mg/dl	37	5	0.71
Respiratory insufficiency			
No	42	15	
Yes	17	0	0.78

TTF1: Thyroid transcription factor-1.

Table III. *Univariate analysis of metastases-free survivial.*

Factor	At 1 year (%)	At 3 years (%)	*p*-Value
TTF1 expression			
Yes	58	11	
No	54	23	0.47
Age			
≤65 years	50	13	
>65 years	63	19	0.89
Gender			
Female	50	0	
Male	60	25	0.71
Karnofsky performance score			
≤70	10	0	
>70	77	23	0.029
T-Category			
1-3	67	17	
4	43	14	0.36
N-Category			
0-2	65	19	
3	17	0	0.003
Smoking during radiotherapy			
Yes	14	0	
No	68	20	<0.001
Hemoglobin during radiotherapy			
≤12 mg/dl	62	23	
>12 mg/dl	53	11	0.60
Respiratory insufficiency			
No	62	19	
Yes	33	0	0.09

TTF1: Thyroid transcription factor-1.

expression of TTF1 had no significant impact on OS (p=0.99). The results of univariate analysis of OS are summarized in Table IV. On multivariate analysis of OS, KPS (HR=0.21; 95% CI=0.06-0.72; p=0.008) and N-category (HR=3.61; 95% CI=1.07-12.24; p=0.018) achieved significance.

Discussion

The importance of identifying preclinical biomarkers has emerged in recent years. Consequently, prognostic subgroups of patients or potential candidates for clinical trials may be identified with the help of such markers. Furthermore, a better understanding of prognostic factors can guide the physician to appropriately select therapy regimens and options for the individual patient. In recent studies, in addition to the prognostic impact of comorbidities, several preclinical markers have been identified in patients with lung cancer, such as nuclear survivin expression and expression of the alternatively spliced actinin-4 variant (3, 11-13).

The role of TTF1 tumor cell expression in determining tumor outcomes remains controversial. A meta-analysis of 17 studies with 2,235 patients with NSCLC suggested that TTF1 overexpression had a favorable impact on patient survival. The hazard ratio was 0.49 (95% confidence interval=0.42 to 0.55) in the entire cohort, 0.46 (0.38-0.54) in Asian patients, and 0.52 (0.42-0.63) in non-Asian patients, respectively (6).

Until now, the impact of the tumor cell expression of TTF1 has not yet been investigated in patients with SCLC. Therefore, the present study was performed to evaluate the prognostic role of TTF1 expression in patients receiving radiotherapy for LD-SCLC. According to our results, the expression of TTF1 was not significantly associated with treatment outcomes in terms of LRFS, MFS and OS. When interpreting these results, the retrospective nature of the data and the relatively small number of patients should be considered. However, prospective studies regarding the potential prognostic impact of tumor expression of TTF1 are not available.

With respect to the expression of TTF1, our findings agree with those of Misch *et al*. (5). In that retrospective study of 221 evaluable patients with locally advanced and extensive

Table IV. *Univariate analysis of overall survival.*

Factor	At 1 year (%)	At 3 years (%)	*p*-Value
TTF1 expression			
Yes	61	23	
No	68	11	0.99
Age			
≤65 years	56	13	
>65 years	75	19	0.92
Gender			
Female	67	0	
Male	65	25	0.05
Karnofsky performance score			
≤70	20	0	
>70	86	23	<0.001
T-Category			
1-3	67	17	
4	64	14	0.83
N-Category			
0-2	73	19	
3	33	0	0.002
Smoking during radiotherapy			
Yes	29	0	
No	76	20	0.022
Hemoglobin during radiotherapy			
≤12 mg/dl	69	23	
>12 mg/dl	63	11	0.65
Respiratory insufficiency			
No	73	16	
Yes	33	0	0.008

TTF1: Thyroid transcription factor-1.

SCLC, TTF1 was also not of prognostic relevance for OS. Patients with TTF-1-positive SCLC had a median OS of 12.5 months compared to 9.7 months in those patients with TTF1-negative SCLC (p=0.25)

In contrast to the expression of TTF1, several factors were significantly associated with treatment outcomes in the present study. The prognostic impact of the performance status, the T-category and the N-category has been reported for several cancer types including SCLC (14, 15). Improvement of OS and MFS in patients, who have stopped smoking before radiotherapy, has been reported for patients with NSCLC and those with SCLC (16, 17). In a series of 215 patients with SCLC, Videtic *et al*. found an improved median survival for patients who quit smoking prior to radiotherapy when compared to those patients who continued smoking during their radiation treatment (18 *vs*. 13.6 months, p=0.002) (16).

In conclusion, several independent predictors for OS and MFS in patients with LD-SCLC were identified in the present study. In contrast, TTF1 did not appear to be associated with treatment outcomes in terms of LRFS, MFS, and OS. Further investigation, ideally prospective studies, is needed to better define the role of the tumor cell expression of TTF1 in this group of cancer patients.

Conflicts of Interest

On behalf of all Authors, the corresponding Author states that there is no conflict of interest related to this study.

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Received April 15, 2016
Revised May 26, 2016
Accepted May 31, 2016