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Downregulation of MTSS1 expression is an independent prognosticator in squamous cell carcinoma of the lung

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Background: The metastasis suppressor 1 (MTSS1) is a newly discovered protein putatively involved in tumour progression and metastasis.

Material and Methods: Immunohistochemical expression of MTSS1 was analysed in 264 non-small-cell lung carcinomas (NSCLCs).

Results: The metastasis suppressor 1 was significantly overexpressed in NSCLC compared with normal lung ($P=0.01$). Within NSCLC, MTSS1 expression was inversely correlated with pT-stage ($P=0.019$) and histological grading ($P<0.001$). NSCLC with MTSS1 downregulation ($<20\%$) showed a significantly worse outcome ($P=0.007$). This proved to be an independent prognostic factor in squamous cell carcinomas (SCCs; $P=0.041$), especially in early cancer stages ($P=0.006$).

Conclusion: The metastasis suppressor 1 downregulation could thus serve as a stratifying marker for adjuvant therapy in early-stage SCC of the lung.

Lung cancer is one of the leading causes of cancer-related deaths worldwide (Travis *et al*, 2004; Jemal *et al*, 2011, 2013). It includes a broad variety of histological subtypes classified either as small-cell lung carcinoma or non-small-cell lung carcinoma (NSCLC). Non-small-cell lung carcinoma accounts for more than 80% of lung cancers and can further be divided into squamous cell carcinoma (SCC), lung adenocarcinoma (LAC), and large-cell carcinoma (LCC). Despite evolving novel treatment regimens, prognosis of NSCLC remains poor and often only palliative treatment options can be offered to the patient. As the major cause of NSCLC-associated mortality is related to distant metastases, there is a need to identify biomarkers possibly predicting aggressive biological behaviour of NSCLC (Pao *et al*, 2009).

Metastasis suppressor 1 (MTSS1) gene was first described in 2002 by Lee *et al* who detected that the MTSS1 transcript was

missing in tumour cell lines derived from metastatic bladder cancer. Thus, it was first named 'missing in metastasis' (Lee *et al*, 2002). Initially, MTSS1 was proposed as a potential suppressor protein for metastasis. But functional studies revealed a correlation of MTSS1 to cytoskeleton and actin filament organisation (Mattila *et al*, 2003; Lee *et al*, 2007; Mattila *et al*, 2007). In addition, physiologic MTSS1 expression was recognised during organ development (Glassmann *et al*, 2007). These findings support that MTSS1 is involved in early carcinogenesis because of an increase in cell motility and invasiveness (Woodings *et al*, 2003). Using cell culture experiments, Callahan *et al* were able to show a new function of MTSS1, that is, MTSS1 is able to potentiate the transcription of effector genes of the sonic hedgehog signalling pathway (Callahan *et al*, 2004). Accordingly, it has been shown that MTSS1 serves as a co-transcription factor by binding to

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Gli proteins or the Gli-Sufu complex, thereby enhancing gene transcription (Callahan *et al*, 2004). These findings further support the pro-oncogenic potential of MTSS1. In solid carcinomas of the breast, liver, and bladder, MTSS1 expression has been shown to be negatively correlated with prognostic parameters including patient's survival (Hicks *et al*, 2006; Ma *et al*, 2007; Wang *et al*, 2007; Parr and Jiang, 2009). In order to elucidate the potential prognostic impact of MTSS1 in NSCLC, we investigated its expression in correlation with clinico-pathological parameters including survival. The results obtained from our large and well-characterised NSCLC cohort clearly indicate that MTSS1 expression is an independent prognostic factor in NSCLC and may serve as a new potential marker to stratify therapeutic decisions.

PATIENTS AND METHODS

Patient cohort and tissue multiarray (TMA). Two hundred and sixty-four patients were included in this study after approval by the Institutional Ethics Committee (EK-University Medical Center Freiburg 10/12). All patients were operated with curative intent between 1 January 1990 and 31 August 2007 at the Department of Thoracic Surgery, University Medical Center Freiburg. A summary of the clinico-pathological data is given in Table 1. Samples from malignant and non-neoplastic lung tissues were collected, formalin fixed (24–48 h in 4% buffered formalin) and paraffin embedded on routine basis. All specimens were evaluated, reclassified by three experienced lung pathologists (GK, AC, AzH) according to the current WHO classification (Travis *et al*, 2004) and re-staged using the latest UICC-TNM classification (7th edition; Sobin *et al*, 2009). As currently no specific system for the assessment of histological

grading is defined and recognised by the WHO (Travis *et al*, 2004), we assessed histological grade with respect to tumour cell pleomorphism, proliferative activity, and nuclear morphology. In SCC and LAC, a three-tiered grading system (G1–well differentiated, G2–moderately differentiated, G3–poorly differentiated) was applied. LCCs were graded as G3. If frequent atypical mitoses, giant and/or spindle cells were present in an appreciable amount, these LCCs were graded as G4.

Discordant cases were discussed and the in common defined consensus was used for subsequent statistical analyses. With respect to tumour heterogeneity three cores of each tumour with a diameter of 2 mm were transferred into TMA. The tumour cores of pulmonary adenocarcinomas were chosen with respect to heterogeneity of growth patterns encountered. A control set of 33 samples of non-neoplastic lung tissue of the same cohort, that is, alveolar and bronchial areas as well as small peribronchial glands, was also included.

Immunohistochemistry and scoring. Three-micrometre-thick tissue sections were mounted on coated glass slides (Superfrost, Langenbrinck, Emmendingen, Germany) dewaxed and rehydrated in a descending alcohol row. The slides were incubated in target retrieving solution (TRS, DAKO, Hamburg, Germany) for 20 min at pH 9. Subsequently, endogenous avidin-biotin was blocked by incubation in biotin-blocking system (DAKO) for 10 min. After washing twice with Tris/saline buffer, monoclonal mouse-anti human MTSS1 antibody (Abcam, Cambridge, UK, ab56780, dilution 1:600) was applied for 30 min. The sections were then rinsed with Tris/saline buffer and incubated with the secondary biotinylated antibody for 15 min. After activation by streptavidin-peroxidase (DAKO) for 10 min, visualisation was performed by 3-amino-5-ethylcarbazole. Finally, the slides were counterstained with haematoxylin.

The immunohistochemical stains were analysed as previously described (Kayser *et al*, 2010, 2011). Specific cytoplasmic MTSS1 expression was evaluated. In single cases, some nuclear positivity could be observed but due to combination with at least moderate cytoplasmic staining intensity it was not separated as a distinct different staining pattern. The total number of positive carcinoma cells within a single core and the total number of NSCLC cells within the same core were counted. For statistical analyses, the percentage of positive tumour cells out of these figures was calculated and rounded to the next decimal. Furthermore, we scored the staining intensity semi-quantitatively (0 = negative, no specific staining; 1 = weak specific staining; 2 = moderate specific staining; 3 = strong specific staining; Figure 1). To further evaluate immunohistochemical staining patterns, a combined H-score was calculated by multiplying the value of the staining intensity by the percentage of positive tumour cells. The range of the resulting H-score was between 0 and 300. Mean values of available TMA cores from each patient were used for statistical analyses.

Statistical analysis. For test assessment, distribution of MTSS1 expression was evaluated using the Kolmogorov-Smirnov test. As distribution of MTSS1 expression in our cohort did not prove to be normal, non-parametric statistics including Kruskal-Wallis test and Mann-Whitney U/Wilcoxon test were chosen for evaluation of significant changes of MTSS1 expression in different subgroups. Survival analysis included Kaplan-Meier survival curves and log-rank tests for evaluation of statistical significance, as well as multivariate Cox-regression analysis to test for independence of the prognostic value of downregulated MTSS1 expression. The latter included all parameters that proved to be of statistical significance for patient's survival, that is, histological grading, UICC stage, median age (65 years), and MTSS1 downregulation. Statistical analysis was performed using the software package SPSS version 17. Level of significance α was set to 5% (i.e., $P < 0.05$).

Table 1. Summary of the clinico-pathologic data of 264 lung cancer patients

Age	35–83 years Number	Median: 65 years Per cent (%)
Gender		
Male	189	72
Female	75	28
Histologic entity		
AC	91	34
SCC	126	48
LC	47	18
pT category		
pT1a	30	11.4
pT1b	35	13.2
pT2a	90	34.1
pT2b	30	11.4
pT3	62	23.5
pT4	17	6.4
pN category		
pNx	5	1.9
pN0	147	55.7
pN1	40	15.1
pN2	71	26.9
pN3	1	0.4
UICC stage		
Not assessable	5	1.9
1A	40	15.1
1B	51	19.3
2A	38	14.4
2B	31	11.8
3A	83	31.4
3B	7	2.7
4	9	3.4

Abbreviations: AC = adenocarcinoma; LCC = large-cell carcinoma; SCC = squamous cell carcinoma.

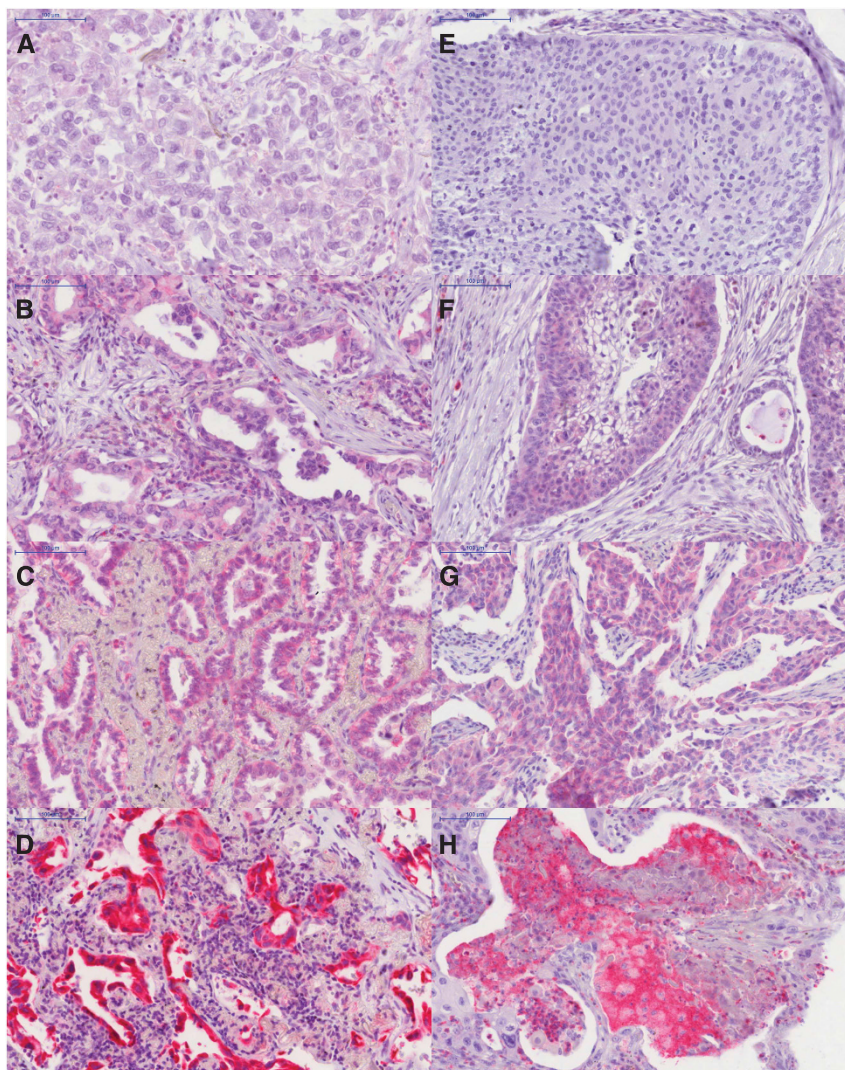


Figure 1. Immunohistological stainings of NSCLC for MTSS1. Left side (A–D): adenocarcinoma (LAC); right side (E–H): squamous cell carcinoma (SCC). (A) LAC without specific cytoplasmatic staining–intensity score = 0; (B) LAC with weak specific cytoplasmatic staining–intensity score = 1; (C) LAC with moderate specific cytoplasmatic staining–intensity score = 2; (D) LAC with strong specific cytoplasmatic staining–intensity score = 3; (E) SCC without specific cytoplasmatic staining–intensity score = 0; (F) SCC with weak specific cytoplasmatic staining–intensity score = 1; (G) SCC with moderate specific cytoplasmatic staining–intensity score = 2; (H) SCC strong specific cytoplasmatic staining–intensity score = 3. Immunostain with MTSS1, objective magnification: $\times 20$.

RESULTS

MTSS1 is differentially expressed in NSCLC and non-neoplastic lung tissue. Comparing the expression of MTSS1 in non-neoplastic lung tissues and NSCLC, the mean fraction of positive cells was $20 \pm 28\%$ (standard deviation (s.d.)) in normal controls and increased significantly in carcinoma tissue showing a mean of $36 \pm 33\%$ (s.d.; $P = 0.01$; two-sided Wilcoxon rank test). Analysing staining intensity, non-neoplastic lung tissue showed an intensity score of 0.73 ± 0.84 (s.d.) as compared to 0.82 ± 0.85 (s.d.) in NSCLC ($P = 0.08$; two-sided Wilcoxon rank test). The combined H-score also differed significantly between lung cancers (60 ± 71 (s.d.)) and non-neoplastic lung tissue (39 ± 47 (s.d.); $P = 0.001$, two-sided Wilcoxon rank test).

MTSS1 is differentially expressed in diverse subtypes of NSCLC. The metastasis suppressor 1 expression levels were significantly increased in NSCLC compared with non-neoplastic lung tissues. In addition, also the percentage of MTSS1-expressing

tumour cells revealed significant differences between histological NSCLC subtypes. The highest MTSS1 expression was seen in LAC compared with LCC or SCC (Table 2; $P < 0.001$ Kruskal–Wallis).

Decrease in MTSS1 expression was almost paralleled by loss of differentiation of NSCLC (Table 2; $P = 0.001$).

Decrease of MTSS1 expression is associated with advanced tumour stages and metastatic disease. Additional statistical analyses revealed that MTSS1 expression continuously decreased with increasing pT and pN stage. This proved to be of statistical significance concerning local tumour spread (percentage: $P = 0.016$; intensity: $P = 0.021$; H-score: $P = 0.014$; Wilcoxon rank test; Table 3). In addition, MTSS1 expression was almost statistically significantly lower in patients presenting with lymph node metastases as compared with those staged pN0 (percentage: $P = 0.086$; intensity: $P = 0.036$; H-score: $P = 0.062$; Mann–Whitney *U*-test; Table 3).

We also analysed MTSS1 expression in correlation with the presence of metastatic disease. As hilar lymph nodes are not infrequently infiltrated by continuous cancer growth and then

Table 2. Summary of the statistical analysis of MTSS1 expression in correlation with histologic type and grading

	Percentage of positive tumour cells		Intensity score		H-score	
	Mean ± s.d.	P-value	Mean ± s.d.	P-value	Mean ± s.d.	P-value
Histology						
LAC	49.0 ± 35.3	<0.001	1.1 ± 0.9	<0.001	92.3 ± 84.4	<0.001
SCC	26.5 ± 27.9		0.6 ± 0.7		37.1 ± 49.6	
LCC	34.7 ± 34.5		0.8 ± 0.8		60.6 ± 67.8	
Non-squamous						
Squamous	44.2 ± 35.6	<0.001	1.0 ± 0.9	<0.001	81.5 ± 80.4	<0.001
	26.5 ± 27.9		0.6 ± 0.7		37.1 ± 49.6	
Grading						
G1 (n = 20)	68.2 ± 30.0	0.001	1.7 ± 0.9	0.001	141.4 ± 81.4	<0.001
G2 (n = 88)	33.9 ± 31.9		0.8 ± 0.9		59.4 ± 77.6	
G3 (n = 141)	33.7 ± 32.9		0.7 ± 0.7		52.1 ± 60.0	
G4 (n = 14)	27.2 ± 28.2		0.8 ± 0.8		45.9 ± 54.8	

Abbreviations: LAC = lung adenocarcinoma; LCC = large-cell carcinoma; MTSS1 = metastasis suppressor 1; SCC = squamous cell carcinoma. Kruskal–Wallis test for histological type and grading; Mann–Whitney U-test for squamous vs non-squamous histology.

Table 3. Summary of the statistical analysis regarding the correlation of MTSS1 and tumour stage and lymph nodal metastasis status

	Percentage of positive tumour cells		Intensity score		H-score	
	Mean ± s.d.	P-value	Mean ± s.d.	P-value	Mean ± s.d.	P-value
pT						
pT1	43.9 ± 35.1	0.016	1.0 ± 1.0	0.021	82.5 ± 85.9	0.014
pT2	37.5 ± 33.4		0.8 ± 0.8		61.2 ± 67.1	
pT3	28.3 ± 30.5		0.6 ± 0.7		43.6 ± 59.7	
pT4	19.8 ± 25.4		0.5 ± 0.8		30.0 ± 43.4	
pN						
pN0	38.5 ± 32.9	0.262	0.9 ± 0.8	0.127	65.1 ± 72.1	0.198
pN1	32.0 ± 32.9		0.7 ± 0.8		55.1 ± 72.2	
pN2	31.9 ± 33.4		0.7 ± 0.8		49.6 ± 61.3	
pN0 vs pN+						
pN0	38.5 ± 32.9	0.086	0.9 ± 0.8	0.036	65.1 ± 72.1	0.062
pN+	31.6 ± 33.0		0.7 ± 0.8		51.1 ± 65.1	
UICC stage						
Early (I and II)	38.4 ± 33.5	0.072	0.9 ± 0.8	0.096	65.6 ± 73.8	0.067
Advanced (III and IV)	30.8 ± 32.1		0.7 ± 0.8		48.5 ± 60.5	
UICC stage vs MTSS1 downregulation						
	No MTSS1 downregulation		MTSS1 downregulation			
	No. of patients	Per cent (%)	No. of patients	Per cent (%)		
UICC stage						
Early (I and II)	100	62.5	60	44.4		
Advanced (III and IV)	51	51.5	48	48.5		
$P = 0.081 (\chi^2)$						

Abbreviation: MTSS1 = metastasis suppressor 1. Kruskal–Wallis test for pT and pN; Mann–Whitney U-test for pN0 vs pN+.

classified as nodal positive, we divided our cohort according to the UICC stages. We dichotomized the cohort into ‘early cancer’ including the UICC stages I and II as these do not show metastatic spread to mediastinal lymph nodes and ‘advanced cancer’, which included UICC stages III and IV. In the latter group, metastatic spread can present either as mediastinal lymph node metastasis and/or as separate tumour nodules. Thus, advanced cancer stages III and IV can be assumed as metastatic NSCLC. In early UICC stages I and II, MTSS1 expression was 38.4 ± 33.5% (s.d.) at mean and decreased to 30.8 ± 32.1 (s.d.) in advanced UICC stages III and IV ($P = 0.072$; Mann–Whitney U-test; Table 3). In analogy, staining intensity and H-score were decreased in advanced stages, too (intensity: $P = 0.096$; H-score: $P = 0.067$; Mann–Whitney U-test; Table 3).

MTSS1 is an independent prognostic factor in SCCs. Overall survival analysis of our cohort proved to be of representative

measures concerning tumour stage, lymph node metastases, and age-related survival as described previously (Kayser *et al*, 2010, 2011, 2012, 2013).

With regard to the mean fraction of positive cells in normal lung tissue in which in 20% of cells MTSS1 expression could be detected immunohistochemically, we chose this cutoff for MTSS1 downregulation in cancer tissue, as this event seems to occur later in the time course of biological tumour transformations. NSCLC patients without MTSS1 downregulated tumours had a statistically significant better prognosis compared with those with a fraction of less than 20% MTSS1-positive tumour cells ($P = 0.007$; log rank). As MTSS1 expression was tightly correlated with histological tumour type, this also became evident in subgroup analyses: analysing MTSS1 downregulation with regard to the presence or absence of histologically predominant squamous differentiation, MTSS1 downregulation was of prognostic significance in SCC, only (Figure 2A and B).

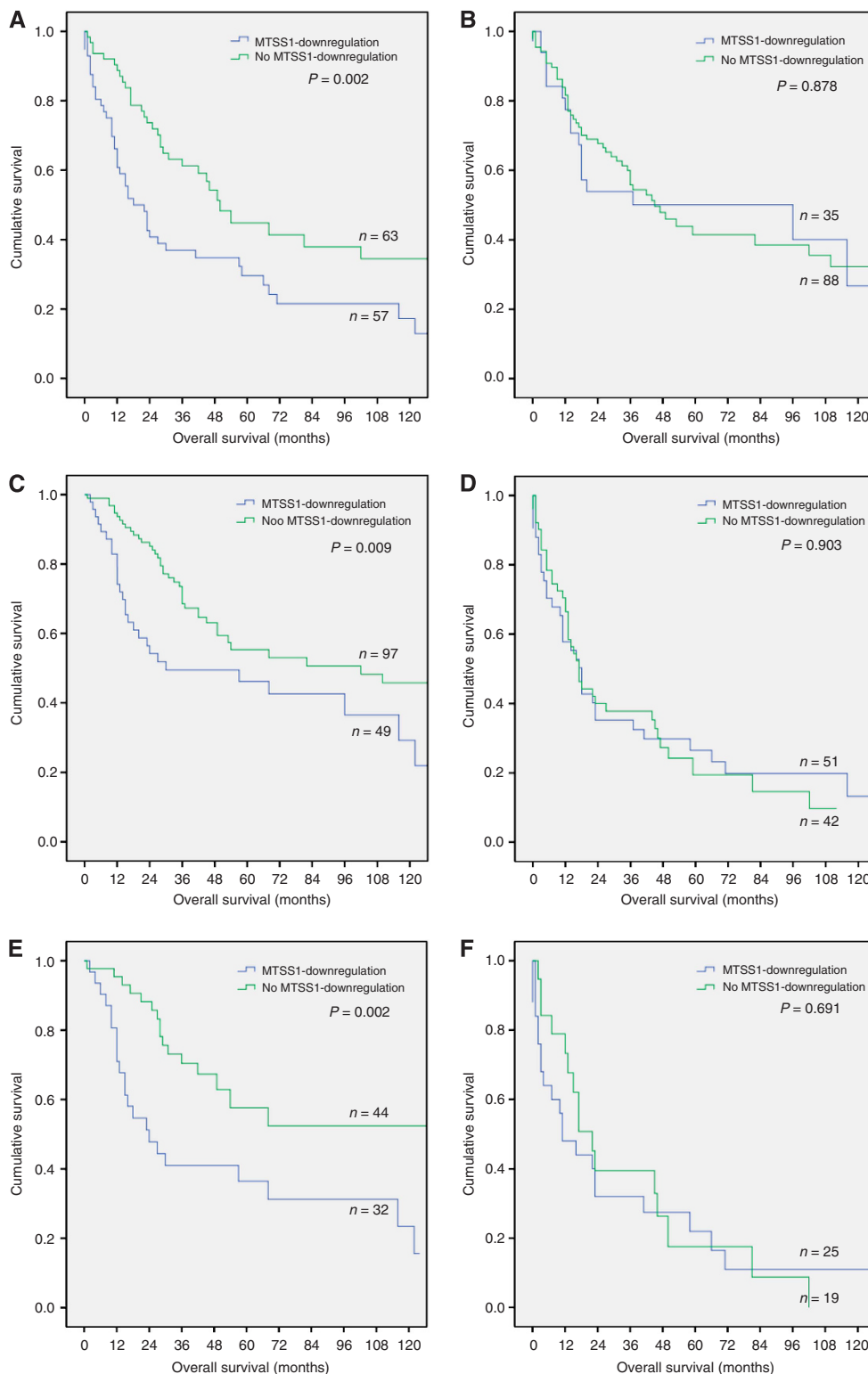


Figure 2. Kaplan–Meier survival plots regarding histological tumour entity (A and B) as well as early (C and E) and advanced cancer (D and F) and MTSS1 downregulation. MTSS1 downregulation is a prognostic factor in SCC, the early cancer UICC stages I and II, and especially in early-stage SCC. (A) Squamous cell carcinoma ($P=0.002$; log rank). (B) Non-squamous NSCLC (LAC and LCC; $P=0.878$; log rank); (C) early UICC stages I and II including the whole cohort ($P=0.009$; log rank); (D) advanced UICC stages III and IV including the whole cohort ($P=0.903$; log rank); (E) early UICC stages I and II in SCC ($P=0.002$; log rank); (F) advanced UICC stages in SCC ($P=0.691$; log rank).

In a multivariate Cox-regression analysis which included all of the prognostically relevant parameters (UICC stage, patient’s age (cutoff 65 years (cohort median) and histological grading) beside MTSS1 downregulation, the latter proved to be an independent prognostic factor in SCC ($P=0.019$), but not in the whole cohort

of NSCLC ($P=0.115$; Table 4) or in LAC ($P=0.250$) or LCC ($P=0.456$).

MTSS1 downregulation may serve as a predictor for metastasising events. The metastasis suppressor 1 expression was significantly

Table 4. Multivariate analysis using the Cox-regression algorithm to elucidate an independent impact of MTSS1 downregulation on patient's survival in the whole cohort as well as in early and advanced cancer stages

	Hazard ratio SCC	P-value SCC	Hazard ratio Whole cohort	P-value Whole cohort
Number of patients	120		238	
UICC stage	1.92	<0.001	1.79	<0.001
Patient's age (cutoff 65 years)	1.79	0.013	1.53	0.014
MTSS1 downregulation	1.75	0.019	1.31	0.115
Histological grading	1.63	0.038	1.50	0.002
Early cancer				
Number of patients	75		142	
UICC stage	2.01	0.045	2.29	0.001
Patient's age (cutoff 65 years)	2.37	0.012	1.59	0.061
MTSS1 downregulation	2.43	0.008	1.80	0.020
Histological grading	1.00	1.000	1.48	0.026
Advanced cancer				
Number of patients	44		93	
UICC stage	1.83	0.577	1.24	0.588
Patient's age (cutoff 65 years)	1.50	0.241	1.41	0.165
MTSS1 downregulation	1.52	0.237	1.04	0.883
Histological grading	2.46	0.020	1.47	0.058

Abbreviations: MTSS1 = metastasis suppressor 1; SCC = squamous cell carcinoma.

lower in advanced stages not only concerning absolute figures (Table 3) but also comparing the fraction of cases with respect to MTSS1 downregulation (Table 3).

Furthermore, subgroup analysis of early cancer as well as advanced cancer revealed that the prognostic effect of MTSS1 downregulation is evident in the group of UICC stages I and II, only. Within these early cancer group, MTSS1 downregulation is a predictor of poor patient survival in SCC (Figure 2C–F), contrasting advanced cancer stages III and IV. In these, significant prognostic results could not be observed (Figure 2, Table 4). In the multivariate Cox-regression analysis again, MTSS1 downregulation in early cancer stages proved to be an independent prognostic factor in SCC ($P=0.008$; Table 4).

DISCUSSION

Lung cancer is still the leading cause of death in patients suffering from malignant disease (Jemal *et al*, 2011, 2013). Owing to metastatic tumour disease and despite advances in modern chemotherapy regimens, cure from lung cancer is a relatively rare event (Pao, 2012). We aimed to determine the impact of MTSS1 as a new prognostic marker in NSCLC, focussing on a possible association with metastasising events and survival.

Low levels of MTSS1 expression have been reported previously in diverse tissues including lung (Mattila *et al*, 2003; Bompard *et al*, 2005). Accordingly, we were able to demonstrate MTSS1 expression in approximately 20% of non-neoplastic parenchymal cells in lung tissue. MTSS1 upregulation was shown to correlate with malignant transformation, for example, in melanomas and head and neck SCCs (Dawson *et al*, 2012; Mertz *et al*, 2014). Ma *et al* (2007) have shown that an increase of MTSS1 expression parallels the progression of hepatocellular carcinogenesis. However, once hepatocellular carcinoma is established, MTSS1 expression was downregulated.

Here we show that MTSS1 is highly expressed in NSCLC compared with non-neoplastic lung tissues. However, there is a significant diversity of MTSS1 expression between different histological subtypes of NSCLC, which indeed is of clinico-pathological significance: In subgroup analyses, MTSS1

downregulation was particularly important in SCC. Within these, we identified MTSS1 downregulation as a strong and independent prognostic parameter. This supports the observation that SCCs have a different biological profile compared with LACs and LCCs. Recent studies have shown that expression levels of thymidylate synthase are higher in SCCs (Pao and Girard, 2011) and this histological subtype of NSCLC does show a better response to the chemotherapeutic agent gemcitabine as compared with pemetrexed (Scagliotti *et al*, 2008). Furthermore, Jin *et al* (2009) detected distinct DNA-methylation and different folate levels in different NSCLC subtypes. In agreement with these studies, our data further support the importance of histological typing with the aim of patient centralised therapy regimes in NSCLC.

In normal lung tissue, highest staining intensity was observed in alveolar lining cells, especially type II pneumocytes and only to a lesser extend in bronchial epithelium. Basal cells of the bronchi did not show a distinct positive staining for MTSS1. These observations along with the current stem cell theory on the origin of the different histological subtypes of NSCLC (Kotton and Fine, 2008; Eramo *et al*, 2010) closely correlate with the fact that LACs did show a higher immunohistochemical expression of MTSS1 than SCCs.

In this context, MTSS1 expression is negatively correlated with prognostic clinico-pathological features such as pT, nodal metastasis, or histological grading (Tables 2 and 3). We could observe downregulation of MTSS1 in some NSCLC cases, but mean expression levels even in highly malignant tumours still remained higher than in non-neoplastic lung tissues. This might point out that MTSS1 expression has an important role in the initiation of cancer development and sustaining malignancy. A fact that has been investigated in several studies (Xie *et al*, 2011b; Du *et al*, 2012; Mertz *et al*, 2014) but its functional ways of action have yet not been fully understood (Zhong *et al*, 2013). Xie *et al* (2011a) could reproduce the interrelation between downregulation and invasiveness in oesophageal cancer cell lines and Wang *et al* (2013) reported similar results in cholangiocarcinomas.

Several functions have been attributed to MTSS1, which at least in part might explain the findings of our study: As a multi-functional scaffolding protein MTSS1 is involved in cytoskeleton interaction, especially with actin (Mattila *et al*, 2003; Woodings *et al*, 2003; Lin *et al*, 2005; Lee *et al*, 2007). This interaction is

associated with higher motility and invasiveness of tumour cell lines, which is suggestive of faster infiltrative tumour growth and metastatic behaviour (Mattila *et al*, 2003, 2007; Loberg *et al*, 2005). Furthermore, a link between the sonic hedgehog signalling pathway and MTSS1 was revealed by Callahan *et al* (2004): MTSS1 binding to Gli1, Gli2, or the Gli-complex-associated protein Sufu enhances gene expression responsive to sonic hedgehog signalling and leads to promotion of tumour cell growth (Gonzalez-Quevedo *et al*, 2005). Overexpression of MTSS1 in the carcinogenesis of NSCLC can therefore be explained as MTSS1 is known to promote cell proliferation. This is reflected by our data of MTSS1 overexpression in NSCLC in comparison with non-neoplastic lung tissue. Fujita *et al* (1997) could show that dysregulation of the sonic hedgehog pathway is more pronounced in squamous cell cancer cell lines as compared with adenocarcinoma cell lines. A finding that was partly reproduced by Huang *et al* (2010) who revealed different expression patterns of molecules involved in the hedgehog signalling pathway in tumour samples of different histological cell types. These findings in correlation with our data that MTSS1 downregulation is of prognostic value especially in SCC of the lung, again support the importance of accurate histological and immunohistochemical subtyping of NSCLC for prognostic and therapeutic reasons.

Lee *et al* (2002) first reported MTSS1 in 2002 as a potential metastasis suppressor gene in bladder cancer and named it 'missing in metastasis' as it was not expressed in metastatic cell lines. In our study, we could confirm that MTSS1 is significantly downregulated in metastatic NSCLC with positive regional lymph nodes and in advanced tumour stages. MTSS1 therefore seems to be first overexpressed to promote carcinogenesis and with ongoing tumour progression downregulation of MTSS1 expression – according to our data – is associated with a more aggressive biological behaviour. Comparing true metastatic tumour stages III and IV with the early cancer stages I and II in which lymph node involvement is often due to direct invasion of these, our data can reproduce these findings. According to our subgroup analysis, early NSCLC stages not only showed higher expression levels of MTSS1 but also the amount of downregulated NSCLC cases was significantly lower in this group compared with advanced cancer stages (Table 3). In addition, this is reflected by our results of the survival analysis, in which MTSS1 downregulation proved to be of prognostic significance. In this respect, MTSS1 expression also has been shown to be a predictor for poor patient survival in breast, liver, upper GI, and bladder cancer patients (Hicks *et al*, 2006; Ma *et al*, 2007; Wang *et al*, 2007, 2013; Parr and Jiang, 2009; Liu *et al*, 2010; Xie *et al*, 2011a).

As MTSS1 downregulation was associated with lymph nodal metastases and with the setting of true metastatic events, this protein may also serve as a predictor of metastasising NSCLC. This hypothesis is further supported as MTSS1 downregulation is especially in early cancer stages I and II of significant prognostic value and indicates aggressive biological behaviour of NSCLC, particularly of SCC. Keeping in mind that prognosis in NSCLC is in the vast majority of cases determined by metastatic disease, MTSS1 downregulation can be supposed as an event before the occurrence of metastases. As MTSS1 downregulation proves to be an independent prognostic factor in SCC and this is especially significant in early cancer, it could be clinically useful to stratify these patients into a high- and a low-risk group with the aim to individualise cancer treatment especially in the adjuvant setting. As newly developed small molecules for personalised chemotherapeutic regime are almost exclusively available for NSCLC showing a predominant non-squamous cell differentiation (Pao and Girard, 2011; Pao *et al*, 2011), investigation of MTSS1 expression could offer new possibilities in the treatment for patients suffering from SCC of the lung.

In conclusion, we demonstrate that MTSS1 is significantly overexpressed in NSCLC compared with non-neoplastic lung tissue. Therefore, it can be assumed that MTSS1 is involved in the carcinogenesis of NSCLC. Furthermore, we show that MTSS1 downregulation is associated with tumour progression and is an independent prognostic parameter in SCC of the lung, particularly in early UICC stages I and II. To the best of our knowledge, this is the first study on MTSS1 expression in NSCLC and includes one of the largest patient cohorts in the study of MTSS1. Based on our results MTSS1 may serve not only as a new prognostic biomarker for NSCLC but also as a putative predictor for metastasising events to occur in early cancer patients. We therefore propose that investigation of MTSS1 downregulation is useful to stratify patients suffering from early-stage NSCLC into different risk groups. Thus, it may also serve as a helpful marker in the decision-making process of additional adjuvant therapy application. Furthermore, owing to its functional activities in actin filament organisation and as a co-transcription factor in the sonic hedgehog signalling pathway (Mattila *et al*, 2003; Callahan *et al*, 2004; Mattila *et al*, 2007), it holds strong potential to open new targeted therapeutic possibilities in personalised cancer treatment regimes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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