ONLINE FIRST

This is a provisional PDF only. Copyedited and fully formatted versión will be made available at final publication

HISTOLOGY AND HISTOPATHOLOGY

ISSN: 0213-3911
e-ISSN: 1699-5848

TRIM11 expression in non-small cell lung cancer is associated with poor prognosis

Authors: Christiane Kuempers, Tobias Jagomast, Finn-Ole Paulsen, Carsten Heidel, Sabine Bohnet, Stefanie Schierholz, Markus Reischl, Eva Dreyer, Till Olchers, Martin Reck, Jutta Kirfel and Sven Perner

DOI: 10.14670/HH-18-647
Article type: ORIGINAL ARTICLE
Accepted: 2023-06-26
Epub ahead of print: 2023-06-26

TRIM11 expression in non-small cell lung cancer is associated with poor prognosis

Christiane Kuempers[1*], Tobias Jagomast[2], Finn-Ole Paulsen[1,3], Carsten Heidel[1,4], Sabine Bohnet[2], Stefanie Schierholz[5], Markus Reischl [6], Eva Dreyer[1], Till Olchers[7,9], Martin Reck[7,9], Jutta Kirfel[1], Sven Perner[8]

[1]Institute of Pathology, University Hospital Schleswig-Holstein, Campus Luebeck, Ratzeburger Allee 160, 23538 Luebeck, Germany

[2]Medical Clinic III, Pulmonology, University Hospital Schleswig-Holstein, Campus Luebeck, Ratzeburger Allee 160, 23538 Luebeck, Germany

[3]Department of Oncology, Hematology and Bone Marrow Transplantation with Division of Pneumology, University Medical Center Hamburg-Eppendorf, Martinistr. 52, 20246 Hamburg, Germany

[4]Department of Haematology and Oncology, Sana Hospitals, Kronsforder Allee 71-73, 23560 Lübeck, Germany

[5]Department of Surgery, Medical University of Schleswig-Holstein, Campus Luebeck, Ratzeburger Allee 160, 23538 Luebeck, Germany

[6]Institute for Automation and Applied Informatics, Karlsruhe Institute of Technology, Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Karlsruhe, Germany

[7]Department of Thoracic Oncology, LungenClinic Grosshansdorf, Wöhrendamm 80, 22927 Großhansdorf, Germany

[8]Institute for Hematopathology Hamburg, Fangdieckstraße 75A, 22547 Hamburg, Germany.

[9] Airway Research Center North (ARCN), Member of the German Center for Lung Research (DZL), Großhansdorf, Germany

***Corresponding author**

Christiane Kuempers, MD

Institute of Pathology, University Hospital Schleswig-Holstein, Campus Luebeck, Ratzeburger Allee 160 (Building V50)

23538 Luebeck, Germany

Phone: +49 451 500-15826

Fax : +49 451 500-15804

E-Mail: christiane.kuempers@uksh.de

Keywords: TRIM11, NSCLC, prognostic, biomarker, immunohistochemistry

Short title: TRIM11 expression in NSCLC

Abstract

Background: Despite promising results of targeted therapy approaches, non-small cell lung cancer (NSCLC) remains the leading cause of cancer-related death. Tripartite motif containing 11 (TRIM11) is part of the TRIM family of proteins, playing crucial roles in tumor progression. TRIM11 serves as an oncogene in various cancer types and has been reported to be associated with a poor prognosis. In this study, we aimed to investigate the protein expression of TRIM11 in a large NSCLC cohort and to correlate its expression with comprehensive clinico-pathological data.

Methods: Immunohistochemical staining of TRIM11 was performed on a European cohort of NSCLC patients (n = 275) including 224 adenocarcinomas and 51 squamous cell carcinomas. Protein expression was categorized according to staining intensity as absent, low, moderate and high. To dichotomize samples, absent and low expression was defined as weak and moderate and high expression was defined as high. Results were correlated with clinico-pathological data.

Results: TRIM11 was significantly more highly expressed in NSCLC than in normal lung tissue and significantly more highly expressed in squamous cell carcinomas than in adenocarcinomas. We found a significantly worse 5-year overall survival for patients who highly expressed TRIM11 in NSCLC.

Conclusions: High TRIM11 expression is linked with a poor prognosis and might serve as a promising novel prognostic biomarker. Its assessment could be implemented in future routine diagnostic workup.

Abbreviations

adNSCLC - pulmonary adenocarcinoma

IHC - Immunohistochemistry

NSCLC – non-small cell lung cancer

OS – overall survival

PQC - protein quality control

RING - really interesting new gene

sqNSCLC - pulmonary squamous cell carcinoma

TMA - tissue microarray

TRIM11 - Tripartite motif containing 11

Introduction

Despite promising results of recent approaches in personalized therapies, non-small cell lung cancer (NSCLC) still harbors the highest mortality rate among cancers (Siegel et al., 2020). This illustrates the importance of identifying new biomarkers and novel therapeutic targets for lung cancer (Mandell et al., 2020). Lung adenocarcinoma (adNSCLC) is the major subtype of non-small cell lung cancer, followed by lung squamous cell carcinoma (sqNSCLC) (Travis et al., 2015). Molecular targeted therapy has significantly improved the survival of adNSCLC patients, while fewer advances have been made in the treatment of sqNSCLC (Hur et al., 2019).

Tripartite motif (TRIM) containing proteins were identified as relevant biomarkers of cancer, where they may show decreased or increased levels of expression and may also have prognostic value (Mandell et al., 2020). They are a subfamily of the Really Interesting New Gene (RING) type E3 ubiquitin ligase family, containing more than 70 subtypes, one of which is TRIM11. TRIM proteins are composed of an evolutionarily conserved RING domain, 1 or 2 B-box motifs and a coiled-coil region (RBCC) (Hatakeyama, 2011).

Recently, increasing evidence has suggested that TRIMs are key players in regulation of protein quality control (PQC), which is essential in the elimination of misfolded proteins and maintaining of cellular homeostasis (Zhang et al., 2020). With this, TRIM family proteins play significant roles in various conditions, such as cell proliferation and development or DNA damage-repair. Dysregulated PQC is associated with various diseases, such as neurodegenerative disorders and cancer (Zhang et al., 2020). There is evidence in the literature that TRIM proteins, especially TRIM11, promote tumorigenesis by removing misfolded proteins and reducing oxidative stress during oncogenic growth. It is assumed that the capacity to degrade misfolded proteins is augmented during oncogenic transformation, and that the higher degradation power is attributable to the upregulation of the TRIM system. However, the link between misfolded proteins and tumorigenesis is still poorly understood (Chen et al., 2017a).

Noteworthy, TRIMs may provide insight into the development of novel TRIM targeted cancer therapies (Mandell et al., 2020). A potential approach in drug-design might be targeting the RING domain, known to be crucial for TRIM functionality. However, inhibitors specific to TRIM RING domains have not yet been reported so far, but because small molecule inhibitors of the RING domains from other protein families exist (Bulatov et al., 2018), one might suggest that TRIM RING inhibition may be feasible. Other possibilities for TRIM-

directed cancer therapy would be to target the activity of specific domains critical to TRIM function in cancer and/or to interfere with the interactions between a TRIM subtype and its cancer-relevant binding partners (Mandell et al., 2020).

TRIM11 is known to be overexpressed in cell lines and tissues of malignant tumors, e.g., high-grade gliomas (Di et al., 2013), breast cancer (Song et al., 2019), ovarian cancer (Chen et al., 2017b), hepatocellular cancer (Zhang et al., 2017) and gastric cancer (Lan et al., 2021) besides lung cancer (Huang et al., 2019; Wang et al., 2021) and its overexpression was found to be correlated with poor prognosis.

With regard to NSCLC, there are promising *in vitro* studies on adenocarcinoma cell lines demonstrating that knockdown of TRIM11 suppresses and TRIM11 overexpression favors tumorigenesis (Huang et al., 2019; Wang et al., 2021) implicating TRIM11 as potential prognostic biomarker for treatment of lung adenocarcinomas.

Despite these promising findings from the literature there is, however, hardly any data on TRIM11 protein expression on NSCLC. In this study, we aimed to investigate the protein expression of TRIM11 in a large NSCLC cohort and to correlate its expression with comprehensive clinico-pathological data.

Material and Methods

Cohort

European patients (n=275) with NSCLC (224 adNSCLC, 51 sqNSCLC) undergoing surgical resection were enrolled in this retrospective study. With this distribution (81.5% adNSCLC, 18.5% sqNSCLC), there is a clearer predominance of adNSCLC over sqNSCLC than known from the literature (Alduais et al., 2023). The mean age of the patients (153 female, 122 male) at initial diagnosis was 66.1 years (64.3 for female, 67.5 for male). 197 (71.6%) were

smokers, 11 (4%) were non-smokers and for 67 (24.4%) smoking status was unknown. At the time of last follow up, 185 patients were alive and 36 were deceased. For 54 patients, follow-up data were missing. Chemotherapy-naive primary lung cancer tissues were available from 242 patients, while tissue from lymph node metastases and distant metastases tissues without corresponding primary tumor were available from 1 and 32 patients, respectively. From the 242 primary tumor cases, 9 patients had corresponding lymph node metastases, 2 had lymph node and distant metastases and 1 had distant metastases so that we investigated 230 primary tumors without metastases. From 4 patients, multiple (up to 2 metastases) were collected. Adjacent noncancerous lung tissue was obtained from 89 patients. Tumors were graded according to the 2015 World Health Organization Classification of Lung Tumors. 5 of the primary tumors were graded as G1 (2.1%), 128 as G2 (52.9%) and 109 as G3 (45%). For determination of tumor state, 8th Edition of UICC/ TNM staging system was used. From primary tumors, 13 (5.4%), 51 (21.1%), 54 (22.3%), 33(13.6%), 13 (5.8%), 44 (18.2%) and 34 (14%) were classified as pT1a, pT1b, pT1c, pT2a, pT2b, pT3 and pT4, respectively. The mutation status was known for part (69/224=30.8%) of the adNSCLC samples. In the subcohort of adNSCLC, 14 out of 69 (20.3%) showed *EGFR* mutations and 55 (79.7%) showed *EGFR* wildtype.

All data were anonymized before being included in the study cohort. Archived tissue blocks were collected from 2005 to 2017. This study was approved by the Internal Review Board of University of Luebeck (file number 16-277). The basic clinic-pathological data of our study cohort is summarized in Table 1.

Immunohistochemistry (IHC)

IHC staining was performed according to the manufacturer's instructions, using the Ventana Discovery (Ventana Medical System) automated staining system. In brief, slides were

incubated at 37 degrees Celsius with the primary antibody anti-TRIM11 1:100, clone ab 111694, Abcam.

Tissue microarrays (TMA) were constructed from Formalin-fixed paraffin-embedded tissue blocks from tumors and corresponding normal lung tissue. Each sample was represented in triplicates of 0.6 mm diameter cores. A tumor sample was incorporated in further analysis if at least one core was evaluable. Staining was considered positive if staining was nuclear.

Stained slides were scanned (Panoramic Desk, 3DHistech) and evaluation of the staining intensity was performed by the semiautomated Image Software Definiens Tissue Studio 2.1 (Definiens Inc), as reported before (Offermann et al., 2019). Here, tumor cell areas were manually annotated for each TMA core as ROI by a pathologist in order to exclude stromal cells, immune cells or necrosis. In samples of benign lung tissue, only pneumocytes were annotated. Within the annotated areas, a continuous spectrum of tumor cell nuclei brown staining intensity (mean brown-maximum range of readout from 0.003 to 0.73) was obtained. Based on re-evaluation of IHC staining by two independent pathologists (CK and SP), samples were categorized into 4 groups: absent expression (< 0.08), low expression (0.08 to <0.23), moderate expression (0.23 to <0.38) and high expression (≥ 0.38).

To dichotomize samples into weak and strong staining, absent and low expression was defined as weak and moderate and high expression was defined as strong expression. For patients with multiple metastases, an average TRIM11 expression was calculated.

Statistical analyses

For statistical analyses and data visualization, R software (version 4.0.2, R Foundation, Vienna, Austria; http://www.R-project.org) was used. Wilcoxon rank-sum test was used to associate TRIM11 expression with tissue type, and to analyze for correlation of TRIM11

expression with clinic-pathological characteristics chi square tests were used. Kaplan-Meier curves were used to illustrate overall survival (OS) in dependency of TRIM11 expression of primary tumors and statistically proved by log-rank tests. All tests were two-tailed and a p-value of < 0.05 was considered significant.

Results

TRIM11 expression pattern

TRIM11 showed nuclear staining. Expression pattern of TRIM11 between the cores originating from one tumor sample was homogenous, implicating neglectable intratumoral heterogeneity. Overall expression between tumor samples varied with a range of expression intensities from absent to strong immunoreactivity (Nuclear mean brown intensity values 0.003-0.73). Figure 1 provides exemplary pictures of immunohistochemical stainings.

Intensity of TRIM11 expression was compared between benign lung tissue and primary NSCLC as well as lymph node metastases and distant metastases.

In a first step, TRIM11 expression was compared between benign lung tissue and primary NSCLC including both adNSCLC und sqNSCLC. Here, TRIM11 was significantly shown to be more highly expressed in primary NSCLC compared to benign lung tissue ($p < 0.0001$; Figure 2a). This difference remained significant, after the cohort of primary NSCLC was separated in adNSCLC and sqNSCLC ($p<0.001$ each; Figure 2b). Comparing the two NSCLC subtypes to each other, mean TRIM11 expression was significantly higher in sqNSCLC than in adNSCLC ($p=0.011$; Figure 2b). In lymph node ($n = 12$) and distant metastases ($n=35$) mean TRIM11 expression was found to be significantly lower than in primary tumors ($p<0.001$ for distant metastases, $p = 0.031$ for lymph node metastases; Figure 3) whereas there was no significant

difference between the two metastatic tissues (p=0.058). The metastases were predominantly unpaired, i.e. the corresponding primary tumor was not available. Only in 3 cases were we able to compare the primary tumor with corresponding metastases as matched pairs. Here, we saw an increase in expression levels of TRIM11 in the metastatic tissue, although the difference was not considered significant (p=0.069, not shown).

In a next step, expression of TRIM11 was assessed in primary tumors that had metastasized and those that had not metastasized. For this purpose, we could only study the cases with lymph node metastases, because the number of cases with distant metastases was too small. Considering the whole cohort, primary tumors without lymph node metastases showed a higher TRIM11 expression than primary tumors with lymph node metastases. The difference was not significant (p=0.54). The results were similar for the subcohort of adNSCLC (p=0.24). For the subcohort of sqNSCLC, we found a higher TRIM11 expression in cases with lymph node metastases than in cases without lymph node metastases, equally without significance (p=0.18; not shown).

Correlation of TRIM11 with overall survival

TRIM11 expressions of the primary tumors were used for survival analysis. They were stratified into weak and strong expression of TRIM11. Considering the entire cohort, 239 cases were included in survival analysis (survival data missing in 3 of 241 cases). Kaplan-Meier analysis indicated a significantly longer OS for patients with TRIM11 weakly expressing NSCLC than for patients with strong TRIM11 expression (log-rank test, p = 0.046; Figure 4a). The group with strong TRIM11 expression showed many endpoints early in follow up. However, for both groups 5-year survival rates of 75% were estimated. On the basis of the

4-level system (absent, low, moderate and high expression) no significant differences between the groups could be ascertained (p=0.25; not shown). To assess if the prognostic value of TRIM11 expression was independent of other prognostic factors, univariable and multivariable cox regression was performed. It was found that TRIM11 expression was not an independent prognostic factor for OS (HR = 0.51 (95% CI 0.08-3.07, p=0.459).

Because our cohort consisted mainly of adNSCLC, we additionally performed the survival analyses on the subcohort of adNSCLC only (188 cases). Here, the positive influence of weak TRIM11 expression on OS was even more evident (log-rank test, p=0.0059; Figure 4b).

No sufficient follow up data were available for the assessment of DFS, so that this analysis had to be omitted.

Correlation of TRIM11 expression with other clinico-pathological characteristics

No significant correlation of TRIM11 expression was found with regard to sex, age, smoking-status, N-status, M-status, and UICC-status for the entire cohort and adNSCLC subcohort. One significant result was observed with regard to T-status meaning that the proportion of smaller T stages (T1/2) was higher (77.9%) in the TRIM11 high expressing group than in the TRIM11 low (63.7%) expressing group (p=0.034; Table 2). The latter was also observed in the subcohort of adNSCLC (p=0.008; Table 2).

For the subcohort of adNSCLC, we additionally correlated *EGFR*-status with TRIM11 expression, and found no significance here (p=0.325).

Discussion

Despite promising advances in the therapy of NSCLC, the identification of new prognostic and therapeutically targetable biomarkers is urgently needed. Comparing the two most common types of NSCLC, molecular targeted therapy has improved the survival of adNSCLC patients in a remarkable manner, while fewer advances have been made in the treatment of sqNSCLC (Hur et al., 2019).

The role of TRIM11 in NSCLC has not yet been investigated as thoroughly as in other malignancies, like breast (Song et al., 2019), ovarian (Chen et al., 2017b), hepatocellular (Zhang et al., 2017) or gastric cancer (Lan et al., 2021). Concerning lung cancer, it was found that knockdown of TRIM11 inhibited proliferation of lung cancer cells, significantly suppressed colony formation, enhanced cell apoptosis and reduced glucose uptake (Wang et al., 2021). A study by Huang et al. (2019) indicated that TRIM11 stimulated promoted tumor growth and in further angiogenesis via activation of STAT3/VEGFA signaling in nude mice models. These results indicate TRIM11-mediated mechanism in lung cancer progression.

There are only few investigations dealing with protein expression of TRIM11 on NSCLC. To the best of our knowledge, this is the first study to investigate protein expression of TRIM11 in a large cohort of NSCLC containing both primary adNSCLC and sqNSCLC, which represent the two major subtypes of NSCLC, as well as their metastases.

TRIM11 expression pattern:

In this study, we found TRIM11 to be significantly more highly expressed in primary NSCLC compared to benign lung tissue. The difference remained significant when the cohort was

separated into adNSCLC and sqNSCLC (Figure 2). These data suggest the participation of TRIM11 in the development of NSCLC. These results are in line with studies from the literature. Huang et al. (2019) found a significantly increased intensity of TRIM11 staining in 46 lung adNSCLC tissues than in paired adjacent normal lung tissues. Wang et al. stated an up-regulation of TRIM11 in 10 cases of NSCLC compared to corresponding precancerous lung tissue without specifying the entities in more detail (Wang et al., 2021). .

By comparing the two NSCLC subtypes we found that TRIM11 expression was significantly higher in sqNSCLC than in adNSCLC. To the best of our knowledge, there are no comparative studies which investigated TRIM11 protein expression on sqNSCLC and adNSCLC for which reason our data are not comparable with the literature.

In a next step, we analyzed TRIM11 protein expression in lymph node and distant metastases. In comparison with primary tumors we found mean TRIM11 expression to be significantly lower in metastases whereas there was no significant difference between lymph node and distant metastases (Figure 3). In general, it is not further unusual that protein expression can differ between primary tumors and metastases (Marinova et al., 2019), so that this result is not particularly surprising. This may be an indication that the tumor biology of metastases differs from that of accompanying primary tumors. However, there is a restriction due to different sizes of the comparison groups (242 primary tumors vs 12 lymph node metastases and 35 distant metastases). Furthermore, here it should be taken into account that the metastases were predominantly unpaired, i.e. the corresponding primary tumor was not investigated. Therefore, a direct comparison can only be made to a limited extent here. A general deduction that TRIM11 expression decreases in metastases, e.g. in the sense of a role as tumor suppressor gene (its loss is related to malignant progression) is thus rather not to be drawn. We were able to compare the primary tumor with

corresponding metastases in only 3 cases. However, here we actually saw an increase in expression levels in the metastatic tissue, although the difference was not considered significant (p= 0.069, not shown).

Since metastatic tissue is often not biopsied and therefore not available for examination, we further analyzed whether TRIM11 expression assessed on primary tumors differed between primary tumors with and without lymph node metastases, respectively. The results were not significant.

Prognostic Significance:

By stratifying samples into TRIM11 weak and strong expressing cases, we found a strong TRIM11 protein expression to be associated with worse 5-year OS for the entire cohort and subcohort of adNSCLC (log-rank test p=0.046 and p=0.0059, respectively; Figure 4). However, we did not identify TRIM11 as being an independent prognostic factor. The observation that a high TRIM11 expression correlates with unfavorable survival has also been shown in other studies. Huang et al. also figured out that TRIM11 protein expression negatively correlated with OS (p = 0.044), indicating that TRIM11 is associated with faster progression and a poor prognosis of adNSCLC (Huang et al., 2019). However, the authors did not specify how they defined high and low expression (n=31 and 15, respectively). The authors solely investigated adNSCLC for IHC and it can be stated that their and the present results, which we assessed on the adNSCLC subcohort, are in line (Figure 4b). The inference of prognostic significance of TRIM11 is further supported by the fact that our adNSCLC subcohort is larger (n=188) than the one in the published study from Huang et al. (n=46). They additionally quote the TCGA database with similar results (p=0.027; 92 high and 113 low TRIM11 expressing tumors).

Correlation of TRIM11 expression with clinicopathological characteristics other than survival

We found no significant correlation of TRIM11 expression with regard to sex, age, smoking-status, N-status, M-status, and UICC-status, as well as for the adNSCLC subcohort, no significant correlation with EGFR-mutation status. A significant result was stated concerning T-status meaning that the proportion of smaller T-stages was higher in the TRIM11 high expressing group than in the TRIM11 low expressing group (p=0.034 for the entire cohort, p=0.008 for adNSCLC). Corresponding data in the literature are partially different for this. TRIM11 expression was found to be significantly correlated with clinical TNM stages of adNSCLC (n=46), meaning more highly expressed in stages III and IV than in stages I and II ($p<0.05$) (Huang et al., 2019). Our observation that the proportion of smaller T-stages was higher in the TRIM11 high expressing group than in the TRIM11 low expressing group is in a way in contrast to our survival data demonstrating that a strong TRIM11 protein expression associates with a poor OS. The most plausible explanation for this should be the uncommon stage distribution in our cohort with predominance of small tumor stages (pT1: 48.8%, pT2: 19%, pT3: 18.2%, pT4: 14%). This is primarily due to the fact that we only used tumor tissue from resections and not biopsies for TMA production. Patients who undergo primary surgery tend to have a lower T-stage, whereas patients with high T-stages do not undergo surgery or possibly only after neoadjuvant therapy. However, we did not use tissue after neoadjuvant therapy for the study. This is at the same time a limitation of our study because it does not reflect the real distribution. However, it must also be taken into account that the prognosis depends more on the UICC-stage than on the T-stage, which is only one aspect of the UICC-stage. Therefore, correlation of TRIM11 expression with UICC-stage might be more meaningful than with T-stage. On the other hand, the data regarding the T-stages (Table 2)

also demonstrate that in higher stages (T3/4), which might be more relevant for prognosis than the lower stages (T1/2), proportionally more tumors show a weak TRIM11 expression (n=62/80.5%) than a strong TRIM11 expression (n=15/19.5%). The ratio is stronger than in the group with smaller T-stages (strong expression in n=53/32.7%, weak expression in n=109/67.3%). Since we observed better OS with weak TRIM11 expression, the data are less contradictory when considered in this way.

One further limitation of the current study is that our results are not validated on an independent cohort. Our results are novel and only weakly comparable with preexisting data, since studies in the literature examined TRIM11 expression either only at the mRNA level, or the cohorts were significantly smaller or differently composed than in our study. In the literature there are no data on TRIM11 expression in association with smoking and mutation status of the *EGFR* gene, therefore our data on this are not comparable, but should be investigated in independent studies.

In summary, little is known about TRIM11 expression in NSCLC and its impact on survival. *In vitro* and *in vivo* studies were able to demonstrate TRIM11-mediated mechanisms in tumor progression and there is strong support for targeting TRIMs in an effort to develop effective therapies for different cancer entities. Our findings on a large NSCLC cohort incorporating adNSCLC and sqNSCLC suggest that the TRIM11 protein expression status could offer valuable information about prognosis of NSCLC patients and might serve as an indicator for a meaningful follow-up management. Due to its clear nuclear immunoreactivity and easy to handle classification as strong or weak, its expression is effortless to evaluate. Involvement of TRIM11 immunohistochemistry in future routine diagnostic workup of NSCLC samples

appears therefore meaningful. If a therapeutic approach to target TRIM11 is developed in the future, it would be necessary to investigate whether TRIM11 protein expression could also predict a therapeutic response. Finally, prospective studies from other researchers are necessary to validate our findings on independent cohorts.

Acknowledgments: We thank Eva Dreyer for the excellent technical support.

Funding: Supported with funds from the Section of Medicine at the University of Luebeck J18-2020.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Compliance with Ethical Standards: The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the local ethics council at the University of Lübeck (file number 16-277, 16-278). For the present study, the retrospective part (16-277) of the ethics application applies. It stipulates that statement on consent of the patients for research purposes is required for samples from 2018 onwards. The samples examined in this study originated from earlier years (2005-2017).

Author Contributions: SP and CK planned the research project; ED performed the immunohistochemical stainings; SP and CK evaluated the samples; TJ and MR performed the statistical analysis; CH, SB, SS, FOP, TO and MR provided patients` data; CK, TJ, FOP, CH, SB, SS, MR, ED, TO, MR, JK and SP: wrote and/or revised the manuscript.

All authors have read and agreed to the published version of the manuscript.

Literature

Alduais Y., Zhang H., Fan F., Chen J. and Chen B. (2023). Non-small cell lung cancer (NSCLC): A review of risk factors, diagnosis, and treatment. Medicine (Baltimore) 102, e32899.

Bulatov E., Zagidullin A., Valiullina A., Sayarova R. and Rizvanov A. (2018). Small molecule modulators of RING-Type E3 ligases: MDM and cullin families as targets. Front. Pharmacol. 9, 450.

Chen L., Brewer M.D., Guo L., Wang R., Jiang P. and Yang X. (2017a). Enhanced degradation of misfolded proteins promotes tumorigenesis. Cell Rep. 18, 3143-3154.

Chen Y., Sun J. and Ma J. (2017b). Proliferation and invasion of ovarian cancer cells are suppressed by knockdown of TRIM11. Oncol. Lett. 14, 2125-2130.

Di K., Linskey M.E. and Bota D.A. (2013). TRIM11 is overexpressed in high-grade gliomas and promotes proliferation, invasion, migration and glial tumor growth. Oncogene 32, 5038-5047.

Hatakeyama S. (2011). TRIM proteins and cancer. Nat. Rev. Cancer 11, 792-804.

Huang J., Tang L., Zhao Y. and Ding W. (2019). TRIM11 promotes tumor angiogenesis via activation of STAT3/VEGFA signaling in lung adenocarcinoma. Am. J. Cancer Res. 9, 2019-2027.

Hur J.Y., Kim H.R., Lee J.Y., Park S., Hwang J.A., Kim W.S., Yoon S., Choi C.-M., Rho J.K. and Lee J.C. (2019). CDK7 inhibition as a promising therapeutic strategy for lung squamous cell carcinomas with a SOX2 amplification. Cell. Oncol. Dordr. 42, 449-458.

Lan Q., Tan X., He P., Li W., Tian S. and Dong W. (2021). TRIM11 promotes proliferation, migration, invasion and EMT of gastric cancer by activating β-Catenin signaling. OncoTargets Ther. 14, 1429-1440.

Mandell M.A., Saha B. and Thompson T.A. (2020). The tripartite nexus: Autophagy, cancer, and tripartite motif-containing protein family members. Front. Pharmacol. 11, 308.

Marinova D.M., Mekov E.V., Dimitrova, D.G., Titorenkov, P.T., Mihailov, M.M., Nachev, S.S., Youroukova, V.M., Kostadinov, D.T. and Slavova, Y.G. (2019). Immunohistochemical characteristics of brain metastases and corresponding primary lung cancer. J. BUON Off. J. Balk. Union Oncol. 24, 1626-1637.

Offermann A., Roth D., Hupe M.C., Hohensteiner S., Becker F., Joerg V., Carlsson J., Kuempers C., Ribbat-Idel J., Tharun L., Sailer V., Kirfel J., Svensson M., Andren O., Lubczyk V., Kuefer R., Merseburger A.S. and Perner S. (2019). TRIM24 as an independent prognostic biomarker for prostate cancer. Urol. Oncol. 37, 576.e1-576.e10.

Siegel R.L., Miller K.D. and Jemal A. (2020). Cancer statistics, 2020. CA. Cancer J. Clin. 70, 7-30.

Song W., Wang Z., Gu X., Wang A., Chen X., Miao H., Chu J. and Tian Y. (2019). TRIM11 promotes proliferation and glycolysis of breast cancer cells via targeting AKT/GLUT1 pathway. OncoTargets Ther. 12, 4975-4984.

Travis W.D., Brambilla E., Nicholson A.G., Yatabe Y., Austin J.H.M., Beasley M.B., Chirieac L.R., Dacic S., Duhig E., Flieder D.B., Geisinger K., Hirsch F.R., Ishikawa Y., Kerr K.M., Noguchi M., Pelosi G., Powell C.A., Tsao M.S., Wistuba I. and WHO Panel (2015). The 2015 World Health Organization Classification of lung tumors: Impact of genetic, clinical and radiologic advances since the 2004 classification. J. Thorac. Oncol. 10, 1243-1260.

Wang X.-L., Lu S.-C., Sun C., Jin W.-G., Fan Y.-W., Shu Y.-S., Shi H.-C., Min L.-F. (2021). Tripartite motif protein 11 (TRIM11), an oncogene for human lung cancer via the DUSP6-mediated ERK1/2 signaling pathway. Cancer Biol. Ther. 22, 324-332.

Zhang L., Afolabi L.O., Wan X., Li Y. and Chen L. (2020). Emerging Roles of Tripartite Motif-Containing Family Proteins (TRIMs) in Eliminating Misfolded Proteins. Front. Cell Dev. Biol. 8, 802.

Zhang Z., Xu C., Zhang X., Huang L., Zheng C., Chen H., Wang Y., Ju H. and Yao Q. (2017). TRIM11 Upregulation Contributes to Proliferation, Invasion, and EMT of Hepatocellular Carcinoma Cells. Oncol. Res. 25, 691-699.

Table 1. patients´ baseline characteristics

Fig 1. Exemplary pictures of TRIM11 expression patterns in NSCLC.

adNSCLC with (a): absent (0.04), (b): low (0.15), (c): moderate (0.23) and (d): high (0.38) TRIM11 expression and sqNSCLC with (e): absent (0.07), (f): low (0.14), (g): moderate (0.26) and (h): high (0.51) TRIM11 expression. The figures demonstrate a specific nuclear staining which is homogenous within the cores. (I): Normal lung tissue with absent to low expression in pneumocytes (objective magnification × 100 and x 400, respectively). Nuclear mean brown intensity values indicated in brackets.

Fig 2. TRIM11 expression in dependency of tissue type and tumor histology.

(a) TRIM11 is significantly more highly expressed in primary NSCLC compared to normal lung tissue (p <0.0001). (b) TRIM11 is significantly more highly expressed in sqNSCLC compared to adNSCLC (p <0.011).

Fig 3. TRIM11 expression in primary tumor and metastatic tissue

TRIM11 is significantly more highly expressed in primary NSCLC (n=242) compared to lymph node (n=12) and distant metastases (n=35) (p= 0.031 and <0.001, respectively). There is no significant difference in TRIM11 expression between lymph node and distant metastases (p= 0.058).

Fig 4. Kaplan Meier graphs with a p-value of Log-rank test of 5-year overall survival stratified by dichotomized TRIM11 expression.

TRIM11 expression assessed on (a) primary NSCLC (n=239) and (b) adNSCLC subcohort (n=188) was used to stratify the cohort in two groups with absent and low expression defined as weak and with moderate and high expression defined as strong expression. Upregulation of TRIM11 correlated significantly with a shorter 5-year OS (p = 0.046 for all NSCLC and p=0.0059 for adNSCLC).

Table 2. Overview of clinic-pathological characteristics of the entire cohort and adNSCLC subcohort with TRIM11 strong and weak expressing primary NSCLC

Table 1. patients´ baseline characteristics

	total n=275
patients	
female	153
male	122
survival status	
alive	185
deceased	36
unknown	54
age at surgery (years)	
mean	66.1
median	66.5
range	36-83
smoking status	
smoker	197 (71.6%)
non-smoker	11 (4%)
unknown	67 (24.4%)
composition of the cohort	
adNSCLC	224 (81.5%)
sqNSCLC	51 (18.5%)
solely primary tumors	230
primary tumors with lymphnode metastases	9
primary tumors with distant metastases	1
primary tumors with both lymphnode and distant metastases	2
solely lymphnode metastases	1
solely distant metastases	32
pT-Stage n (%)	
pT1	118 (48.8%)
pT2	46 (19%)
pT3	44 (18.2%)
pT4	34 (14%)
grading	
G1	5 (2.1%)
G2	128 (52.9%)
G3	109 (45%)

Table 2. Overview of clinic-pathological characteristics of the entire cohort and adNSCLC subcohort with TRIM11 strong and weak expressing primary NSCLC

entire cohort

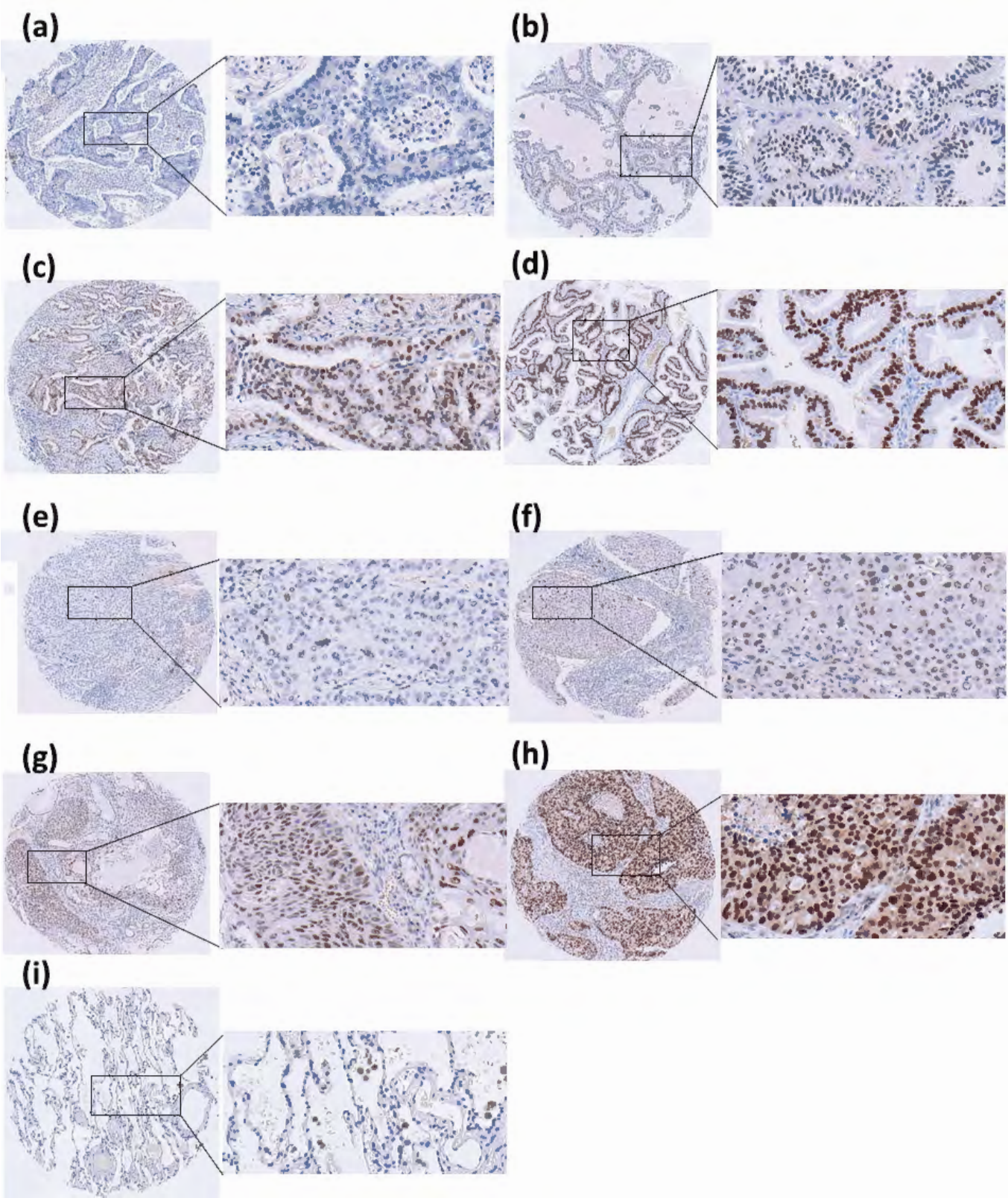
Variable	strong (n=68)	weak (n=171)	total (n=239)	p value
Sex				0.486
male	36 (52.9%)	99 (57.9%)	135 (56.5%)	
female	32 (47.1%)	72 (42.1%)	104 (43.5%)	
Age				0.676
young	31 (45.6%)	83 (48.5%)	114 (47.7%)	
old	37 (54.4%)	88 (51.5%)	125 (52.3%)	
T				0.034
1,2	53 (77.9%)	109 (63.7%)	162 (67.8%)	
3,4	15 (22.1%)	62 (36.3%)	77 (32.2%)	
N				0.703
missing	12	8	20	
0	44 (78.6%)	124 (76.1%)	168 (76.7%)	
+	12 (21.4%)	39 (23.9%)	51 (23.3%)	
M				0.379
missing	53	90	143	
0	15 (100.0%)	77 (95.1%)	92 (95.8%)	
+	0 (0.0%)	4 (4.9%)	4 (4.2%)	
UICC				0.219
1,2	57 (83.8%)	131 (76.6%)	188 (78.7%)	
3,4	11 (16.2%)	40 (23.4%)	51 (21.3%)	
smoking				0.308
missing	8	44	52	
no	5 (8.3%)	6 (4.6%)	11 (5.8%)	
yes	55 (91.7%)	124 (95.4%)	179 (94.2%)	

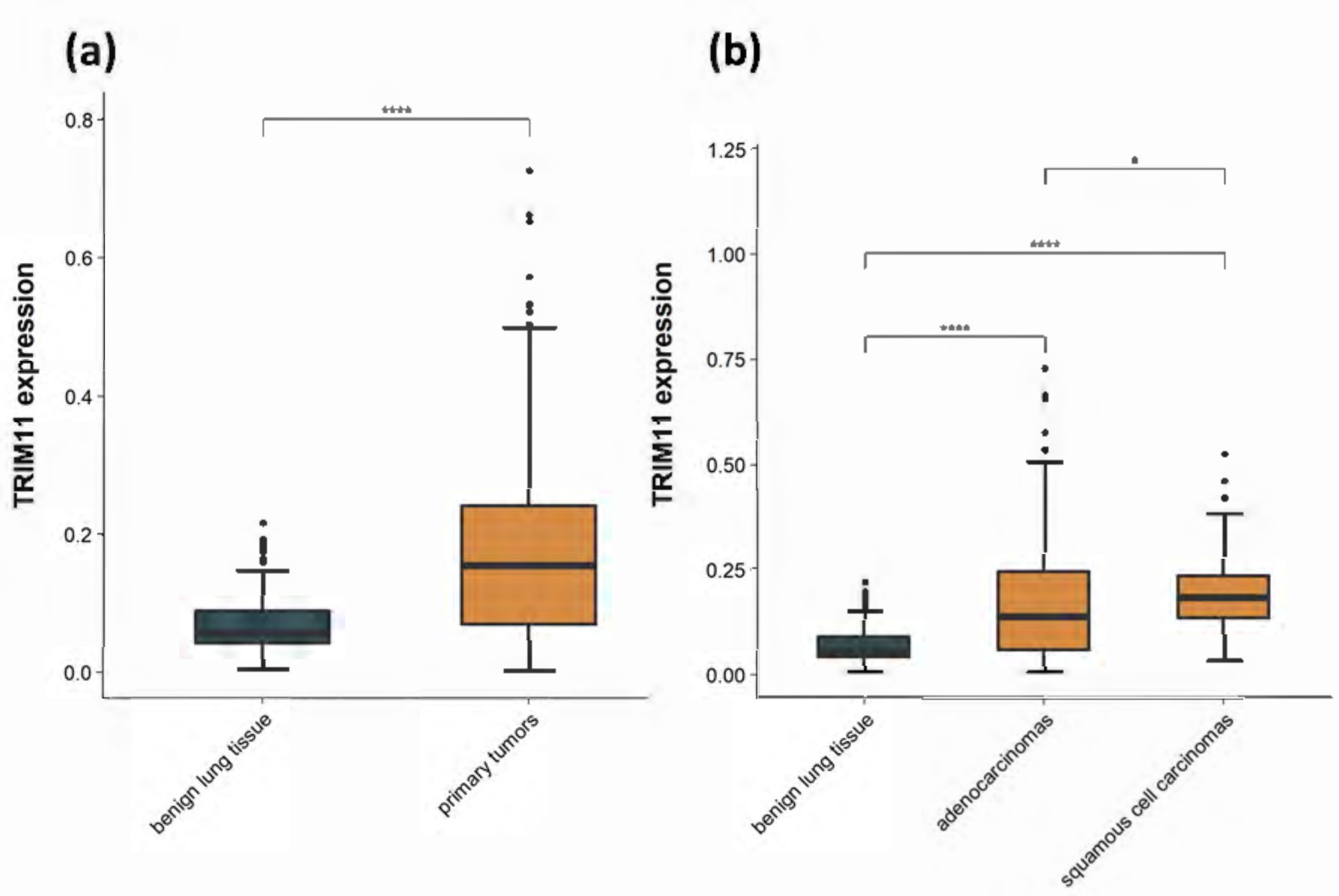
adNSCLC subcohort

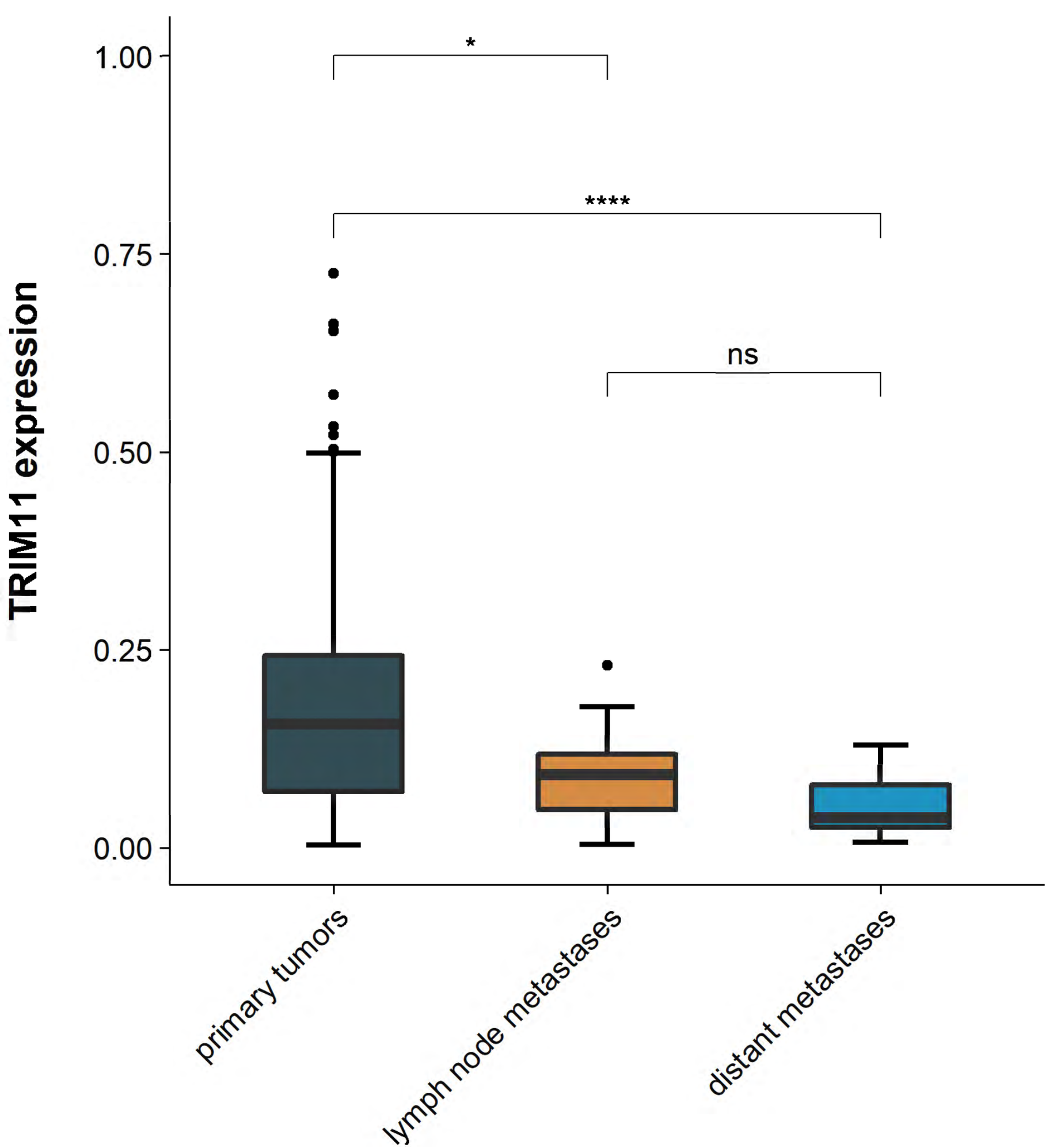
Variable	strong (n=53)	weak (n=138)	total (n=191)	p value
Sex				0.748
male	26 (49.1%)	72 (52.2%)	98 (51.3%)	
female	27 (50.9%)	66 (47.8%)	93 (48.7%)	
Age				0.646
young	23 (43.4%)	65 (47.1%)	88 (46.1%)	
old	30 (56.6%)	73 (52.9%)	103 (53.9%)	
T				0.008
1,2	45 (84.9%)	89 (64.5%)	134 (70.2%)	
3,4	8 (15.1%)	49 (35.5%)	57 (29.8%)	
N				0.247

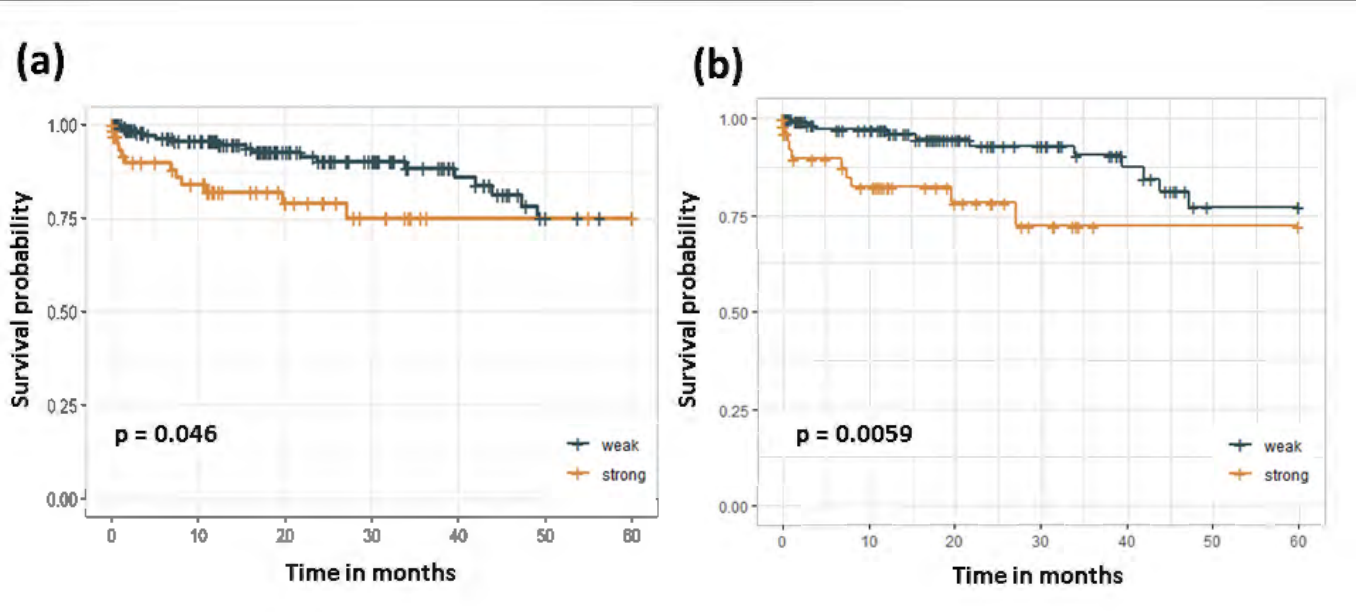
missing	12	7	19	
0	34 (82.9%)	97 (74.0%)	131 (76.2%)	
+	7 (17.1%)	34 (26.0%)	41 (23.8%)	
M				1
missing	52	90	142	
0	1 (100.0%)	47 (97.9%)	48 (98.0%)	
+	0 (0.0%)	1 (2.1%)	1 (2.0%)	
UICC				0.071
1,2	47 (88.7%)	106 (76.8%)	153 (80.1%)	
3,4	6 (11.3%)	32 (23.2%)	38 (19.9%)	
smoking				0.341
missing	2	32	34	
no	5 (9.8%)	6 (5.7%)	11 (7.0%)	
yes	46 (90.2%)	100 (94.3%)	146 (93.0%)	

age: young < median, old > median (66.5 years)


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)


(a)
TRIM11 expression
0.8
0.6
0.4
0.2
0.0
****
benign lung tissue
primary tumors
(b)
TRIM11 expression
1.25
1.00
0.75
0.50
0.25
0.00
*
****
****
benign lung tissue
adenocarcinomas
squamous cell carcinomas


TRIM11 expression
1.00
0.75
0.50
0.25
0.00
*
****
ns
primary tumors
lymph node metastases
distant metastases


(a)
Survival probability
1.00
0.75
0.50
0.25
0.00
p = 0.046
weak
strong
0
10
20
30
40
50
60
Time in months
(b)
Survival probability
1.00
0.75
0.50
0.25
0.00
p = 0.0059
weak
strong
0
10
20
30
40
50
60
Time in months