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ORIGINAL ARTICLE – CLINICAL ONCOLOGY

Aggregate analysis based on TCGA: *TTN* missense mutation correlates with favorable prognosis in lung squamous cell carcinoma

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Abstract

Purpose Lung cancer prevalence with its high mortality rate is a trending topic globally. Non-small cell lung cancer (NSCLC) is the most common type of lung cancer. The human gene *TTN* encoding for TITIN protein is known as major mutation gene in many types of tumor including NSCLC. However, it is still controversial that *TTN* is a cancer-associated candidate considering tumor heterogeneity and complex genetic structure. In-depth researches on correlation between *TTN* mutation and NSCLC are still limited and discussable.

Methods Related somatic mutation profiles and attached clinical data were from The Cancer Genome Atlas (TCGA) lung project. Clinical relevance analysis of *TTN* mutation was evaluated using univariate analysis and a binary logistic regressive model. Survival analysis and screening of independent prognostic factors in mutation types were conducted by Cox proportional hazards models and Kaplan–Meier methods.

Results Available data covering lung adenocarcinoma ($n=517$) and lung squamous cell carcinoma ($n=492$) were analyzed. *TTN* genetic mutations exhibited significant association with lung squamous cell carcinoma. Patients with lung squamous cell carcinoma possessed favorable overall survival benefits from *TTN* mutant type and both favorable overall survival and disease-free survival benefits from *TTN*/*TP53* double mutation. For patients with lung squamous cell carcinoma, about 85% of subjects with *TTN* mutation harbored missense variations, which was an independent indicator of good prognosis.

Conclusions Missense mutation of *TTN* may act as a beneficial role in lung squamous cell carcinoma, but not in lung adenocarcinoma.

Keywords TCGA · Lung cancer · Mutation · *TTN* · *TP53* · Prognosis

Abbreviations

TCGA	The Cancer Genome Atlas
NSCLC	Non-small cell lung cancer
LUAD	Lung adenocarcinoma
LUSC	Lung squamous cell carcinoma
HR	Hazard ratio
CI	Confidence interval

Introduction

The longest-known coding gene *TTN*, which was named *CMD1G* (cardiomyopathy, dilated 1G), has been reported mutated frequently in many tumor types including lung squamous cell carcinoma, lung adenocarcinoma and colon adenocarcinoma (Kim et al. 2013). However, it is still controversial for *TTN* as a significant cancer-associated gene only through several bioinformatics analyses. The points of this controversy focus on the false-positive results brought from its giant complicated structure and heterogeneity in the mutational processes (Hofree et al. 2013; Kim et al. 2017; Lawrence et al. 2013). Meanwhile, *TTN* truncating mutations are identified strongly relating to cardiac and skeletal muscle

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diseases, but not in cancer, leaving us great conjecture on its roles in cancer (Ceyhan-Birsoy et al. 2013; Herman et al. 2012; Schafer and de Marvao 2017; Watanabe et al. 2016). However, limited studies have been investigated deeply on the relevance among *TTN* and cancer. One of the limited researches on *TTN* and cancer has stated that *TTN* or *TP53* mutation in breast cancer does not show strong discriminative power for patient survival. But patients with mutations of certain cancer-associated genes including *AKT1, NCOA3, ARID1A* or *MAP3K1* enjoy a favorable prognosis, and these mutations are exactly mutually exclusive with *TTN*/*TP53* co-mutation (Kim et al. 2017). It suggests that *TTN* and *TP53* double mutation may participate in breast cancer by means of a common downstream pathway with the participation of the other four mentioned genes on the signaling network. Based on these reported studies, the prognostic effects of *TTN* and *TP53* double mutation are explored in this article as well.

NSCLC occupies about 92% of whole-lung cancer cases based on the database utilized in the eighth edition of the TNM classification of lung cancer and takes adenocarcinoma and squamous carcinoma as the majority (Rami-Porta et al. 2014). In addition, *TTN* and *TP53* are the two most common mutant genes in NSCLC including both lung adenocarcinoma and lung squamous cell carcinoma programs queried from The Cancer Genome Atlas (TCGA) lung project (online only). *TP53* remains a very well-known high priority in kinds of tumor. On the other hand, combination analyses of *TP53* and cancer-associated genes have been commonly conducted to observe clinical outcomes and provide well-founded molecular typing for targeted therapy (Labbe et al. 2017; VanderLaan et al. 2017). However, compared to *TP53*, controversies around *TTN* mentioned before, to a certain extent, have discouraged researchers from further analysis. Rare relevance researches of *TTN* mutation in cancer are reported up to now.

TP53, the well known tumor-suppressor gene, has been detected mutations in the exon region in 30–50% of patients with lung adenocarcinoma and 80% of lung squamous cell carcinoma approximately (Maki-Nevala et al. 2016; Seo et al. 2012). *TP53* mutation had been mentioned as a significant independent poor prognostic indicator in NSCLC (Deben et al. 2017; Mitsudomi et al. 1993). Certain research revealed significantly independent poor prognostic effect from "non-disruptive mutation" (HR 6.11 [95% CI 1.43–26.09], $P=0.01$), which was defined as lower predicted degree of disturbance to the structure and function of *TP53*-coding protein, for advanced NSCLC patients (Molina-Vila et al. 2014). This result is incompatible with the reported negative influence of protein changes from *TP53* mutation. Another study elucidated a neutral effect of *TP53* mutation (exons 5–8) for prognosis of NSCLC patients (HR 0.99 [95% CI 0.77–1.28], $P=0.95$) (Ma et al. 2016). As a consequence, the prognostic effect of *TP53* in NSCLC remains controversial as well.

This study focuses on the correlation between clinical outcomes of *TTN* mutations and NSCLC. Attached analyses are explorations of relationships among *TTN* and *TP53* double mutation in NSCLC. This research aims to provide the fundamental basis for further researches on *TTN* and NSCLC.

Methods

Data collection

Standardized high-quality data including lung adenocarcinoma and lung squamous cell carcinoma programs were collected from The Cancer Genome Atlas (TCGA) online database (http://cancergenome.nih.gov). Both somatic mutation profiles and clinical data were required. Immunostaining image of slides were analyzed for pathological grading of NSCLC samples by two professional investigators who were blinded to the sample information. We artificially defined genetic status as wild-type (WT) or mutant-type (MT) without further subtyping. Lung adenocarcinoma and lung squamous cell carcinoma were, respectively, named LUAD and LUSC in this paper following conventions of TCGA.

Clinical relevance analysis

Clinical relevance of *TTN* mutation in NSCLC was evaluated with *P* value, hazard ratio (HR), 95% confidence interval (CI) by univariate analysis and binary logistic regressive model including the following variables: race (Asian, Black or African American, White), histology (squamous, adenocarcinoma), daily smoking amounts, smoking time, gender (male, female), age at diagnosis(<55, 55–65, 65–75, >75 years), pathological stage (I, II, III, IV), histological grading [high (well), moderate (moderately), low (poorly)] and history of chemotherapy/radiation/ molecular targeted/ neoadjuvant treatment.

Survival analysis

Overall survival (OS) and disease-free survival (DFS) analysis of patients with *TTN* mutation or combined mutation were estimated for the assessment of prognostic effect and predictive effect after initial treatment severally. OS, which was the primary end point, was defined as time from random assignment to death from any cause or last follow-up in surviving patients. DFS was defined as time from random assignment to new tumor-associated events after initial treatment or death from any cause or last follow-up in surviving patients. Cox proportional hazards models and

Kaplan–Meier methods (Log-rank test) were utilized to conduct survival analysis and draw survival curves. Screening of independent prognostic and predictive factors in mutation types was conducted by a Cox proportional hazards model including frame mutation, stop gain mutation, and missense mutation variates.

Statistical tools

In this study, IBM SPSS software (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp) was applied for univariate and multivariate analysis and Stata Statistical Software (StataCorp.2011.Stata Statistical Software: Release 12.College Station, TX: StataCorp LP) was used for survival analysis and survival curves performing. We set all statistical significance at $P < 0.05$ and all P values in this study were two sided.

Results

Available patients' data

1009 cases of NSCLC patients diagnosed with lung adenocarcinoma ($n = 517$) and lung squamous cell carcinoma ($n = 492$) from TCGA lung project were available for further analysis. About 989 patients (LUAD $n = 504$, LUSC $n = 485$) were eligible for survival analysis. Details of the quantity of available patients relating to different variates are shown, respectively, as LUAD (Online Resource 1) and LUSC (Online Resource 2).

Clinical relevance of *TTN* in NSCLC

Genetic mutant-type of *TTN* was detected in 62.0% (626/1009, 95% CI [0.590–0.650]) of total NSCLC samples. About 79.7% (95% CI 0.761–0.832) of lung squamous cell carcinoma and 54.7% (95% CI 0.504–0.590) of lung adenocarcinoma patients harbored *TTN* mutation. Histology type (multivariable Cox model $P < 0.001$, HR 3.924 [95% CI 2.164–7.116]) and molecular targeted treatment history (multivariable Cox model $P = 0.001$, HR 3.102 [95% CI 1.607–5.987]) were proved to be independent influence factors of *TTN* mutation by a binary logistic regressive model (Table 1). Furthermore, scores on histological grading approached significance (multivariable Cox model $P = 0.040$). Factors including race, gender, age, pathological stage, daily smoking amounts/time and history of radiation/chemotherapy/neoadjuvant treatment exhibited no independent influence on *TTN* mutation in NSCLC (Online Resource 4).

Survival analysis of *TTN* in LUSC and LUAD

210 out of 485 patients with lung squamous cell carcinoma died and 238 patients had new events after initial treatment including death. Median follow-up times of OS and DFS were 22.2 months (range 0–176 months, mean 32.4 months) and 19.7 months (range 0–176 months, mean 29.9 months) respectively. According to the Cox proportional hazards model (Online Resource 5), *TTN* mutation was an independent significant favorable prognostic indicator (multivariable Cox model $P = 0.013$, HR 0.390 [95% CI 0.186–0.817]) (Fig. 1a) but not a predictive indicator (multivariable Cox model $P = 0.096$) (Fig. 1b) in LUSC. On the other hand, 181 out of 504 LUAD patients died and 237 patients had new events after initial treatment including death. Median follow-up time of OS and DFS was 21.9 months (range 0.1–241.6 months, mean 30.4 months) and 20.2 months (range 0.1–241.6 months, mean 28.5 months) respectively. However, *TTN* mutation did not manifest significant prognostic (multivariable Cox model $P = 0.534$) (Fig. 1c) and predictive value (multivariable Cox model $P = 0.104$) (Fig. 1d) for lung adenocarcinoma.

Prognostic and predictive effect of *TTN* mutation combined with *TP53* mutation

TP53 mutation (including copy number variations) was detected in 86.2% (424/492, 95% CI 0.831–0.892) of LUSC subjects. Around 72.2% of LUSC patients harbored concurrent *TTN* mutation. Patients with *TTN*/*TP53* double mutation had significantly better OS (multivariable Cox model

Table 1 Binary logistic regression analysis in NSCLC

Characteristics	No. of *TTN* mutation/no. of patients (%)	*P* value	HR [95% CI]
Histology			
Squamous vs adenocarcinoma	392/492 (79.7%) vs 283/517 (54.7%)	< 0.001	3.924 [2.164–7.116]
Molecular targeted treatment history			
Yes vs no	198/271 (73.1%) vs 359/554 (64.8%)	0.001	3.102 [1.607–5.987]
Histological grading		0.040	
High vs low	60/99 (60.6%) vs 284/392 (72.4%)	0.041	0.334 [0.117–0.955]
Moderate vs low	329/515 (63.9%) vs 284/392 (72.4%)	0.032	0.503 [0.269–0.942]

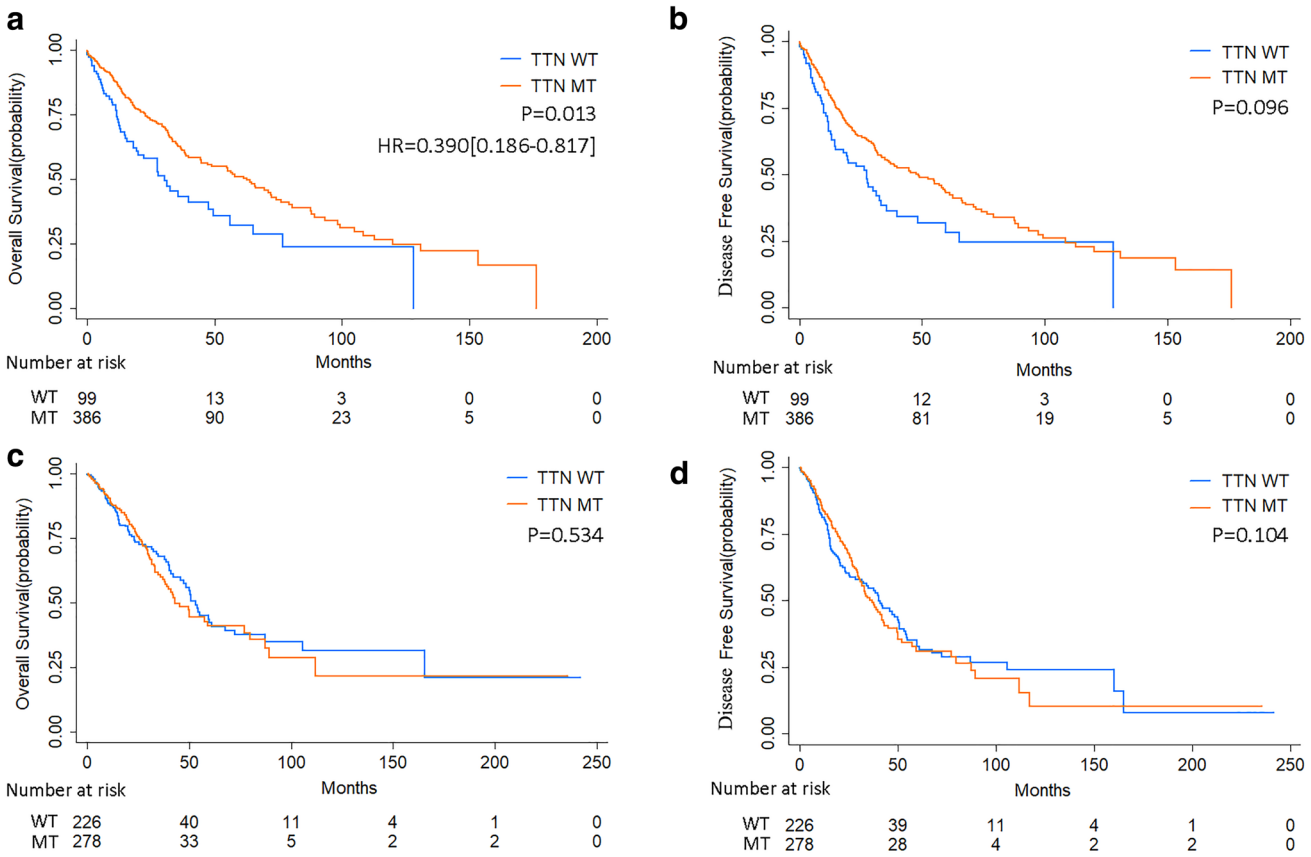

Fig. 1 Unadjusted Kaplan–Meier curves of OS and DFS effect of according to *TTN* mutation status. **a** OS analysis in LUSC. **b** DFS analysis in LUSC. **c** OS analysis in LUAD. **d** DFS analysis in LUAD. *P* value and HR [95% CI] were given by Cox proportional hazards models

$P=0.001$, HR 1.923 [95% CI 1.330–2.779]) (Fig. 2a) and better DFS after initial treatment (multivariable Cox model $P=0.013$, HR 1.565 [95% CI 1.099–2.230]) (Fig. 2b) compared with patients with *TP53* single mutation (multivariable Cox model result is shown in Online Resource 6).

TP53 mutation was found in 51.1% (264/517, 95% CI 0.467–0.554) of LUAD patients meanwhile 35.6% of a total 517 patients had *TTN*/*TP53* double mutation. However, there was no significant difference in OS (multivariable Cox model $P=0.801$) (Fig. 2c) and DFS (multivariable Cox model $P=0.666$) (Fig. 2d) on the basis of mutation status (multivariable Cox model result is shown in Online Resource 7).

Mutation types of *TTN* in lung squamous cell carcinoma

In 392 mutation subjects out of 492 lung squamous cell carcinoma cases, one subject could have a different number of *TTN* single-nucleotide variations from the other (range 1–12 loci, individual case 17 or 30 loci) and mutation types of *TTN* in one patient could be various. Among the 392 cases, 104 cases had only one nucleotide-site variation of *TTN* while 288 of subjects which constituted a majority of mutation cases observed *TTN* nucleotide variations in more than 1 locus (Fig. 3a). Kaplan–Meier survival analysis showed that patients with variations of two or more loci obtained only a better overall survival benefit than patients with *TTN* wild-type and only one site alteration [Log-rank OS $P=0.0160$ (Fig. 3b), DFS $P=0.1777$ (Fig. 3c)].

A total of 1232 single-nucleotide variations were found in above 392 patients. According to the transcription consequence, missense mutations accounted for the top most of mutation types ($n=761$). The second ranked type was synonymous alterations ($n=265$), followed by intron alterations ($n=105$), stop gain mutations ($n=53$), and frameshift mutations ($n=32$). Besides, a small proportion of patients were found as in-frame mutations ($n=4$), 3′ Prime UTR alterations ($n=2$) and splice site mutations ($n=10$) (Fig. 3d). For patients with mutations of about moderate-to-high degree of disturbance to the structure and function of *TTN*-coding protein predicted by TCGA (online only), 314 patients out of 392 harbored missense mutations while 43 with stop gain mutations and 25 with frame mutations. Moreover, for patients with mutations of low degree of disturbance, 159 patients bore synonymous alterations and 92 harbored intron

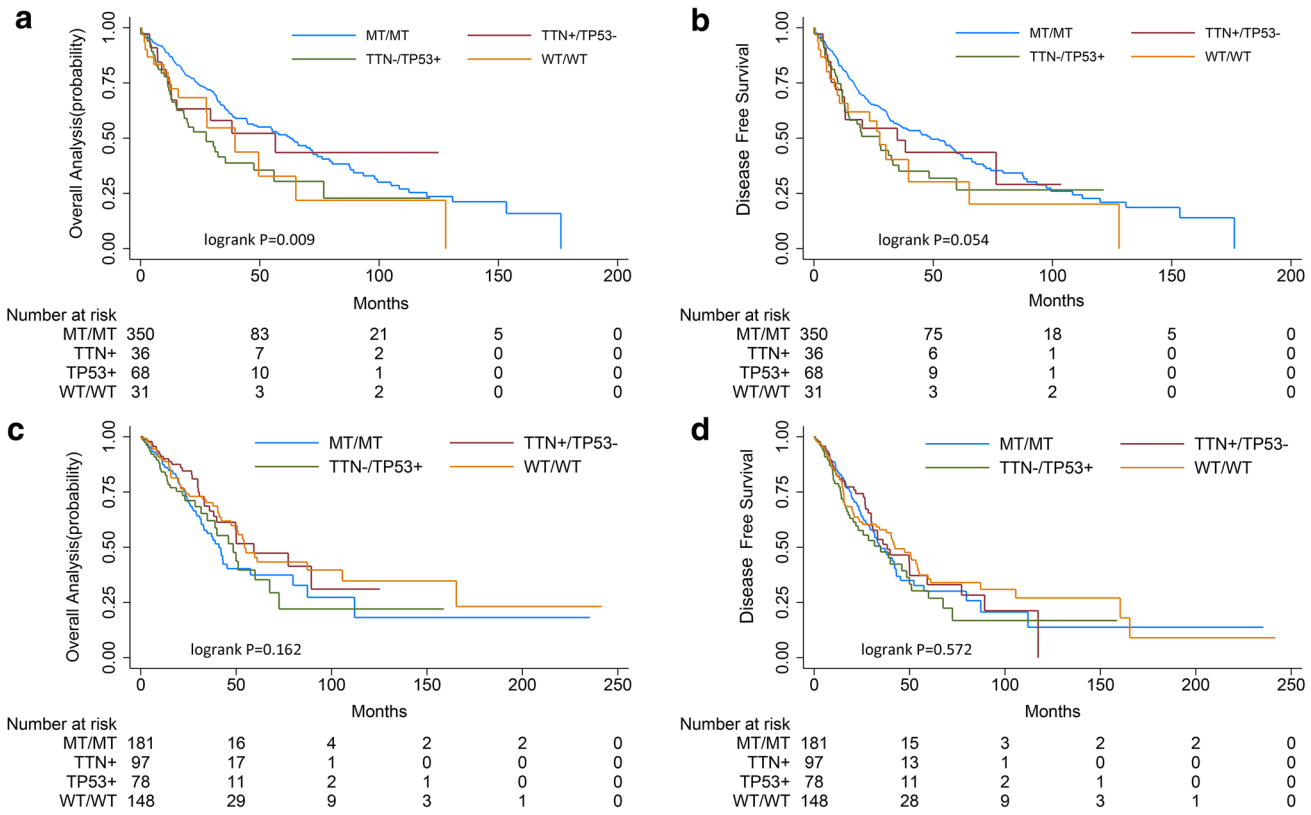

Fig. 2 Unadjusted Kaplan–Meier curves of OS and DFS effect according to *TTN* and *TP53* mutation status (*TTN*/*TP53* combination) (MT, +; WT, −). **a** OS analysis in LUSC. **b** DFS analysis in LUSC. **c** OS analysis in LUAD. **d** DFS analysis in LUAD

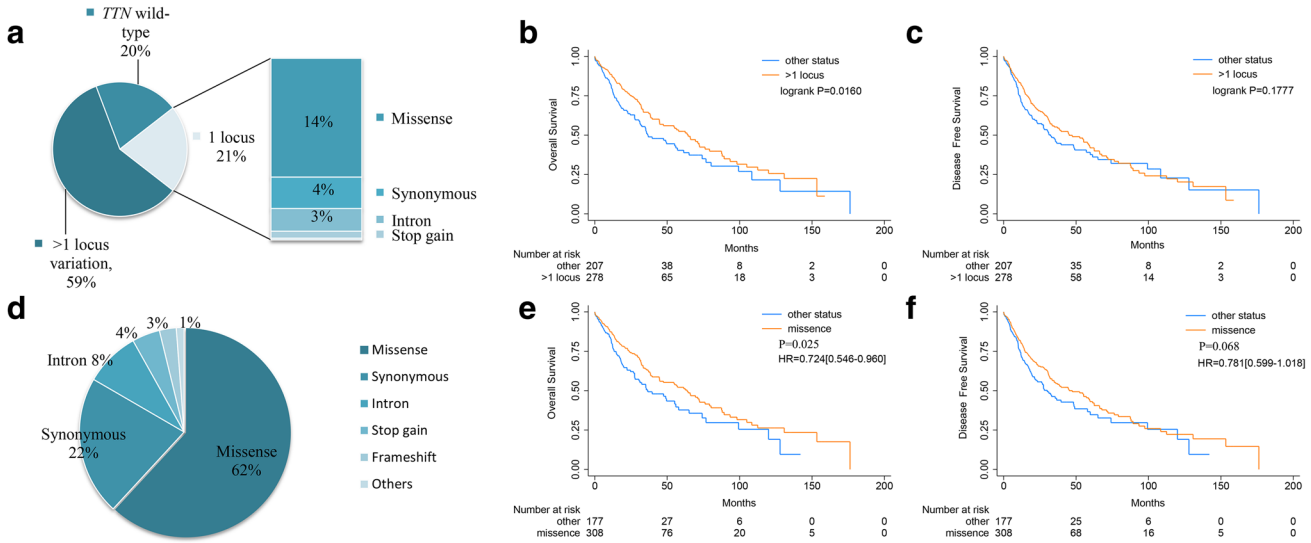

Fig. 3 Aggregate analysis of *TTN* mutation types in lung squamous cell carcinoma. **a** Pie chart ($n=492$) of patients with more than one *TTN* nucleotide variation locus and other status. **b, c** Unadjusted Kaplan–Meier curves of OS/DFS effect according to amounts of *TTN* nucleotide variations. **d** Pie chart ($n=1232$) of *TTN* mutation types. **e, f** Unadjusted Kaplan–Meier curves of OS/DFS effect according to mutation types

alterations. The other mutations including splice site mutations and 3′ Prime UTR alterations were not discussed alone because of low incidence (Table 2). Multivariate Cox regression model identified that missense mutation was an independent favorable prognostic indicator for lung squamous cell carcinoma ($P=0.025$, HR 0.724 [95% CI 0.546–0.960]) (Fig. 3e) but not a significant independent predictive indicator ($P=0.068$, HR 0.781 [95% CI 0.599–1.018]) (Fig. 3f).

Prognostic and predictive effect of *TTN*/*EGFR*, *TTN*/*ALK*, *TTN*/*ROS1*, *TTN*/*RET* combination in LUSC

EGFR, ALK, ROS1, and *RET* mutations (including Copy number variations) were detected, respectively, in 18.5% (91/492, 95% CI 0.151–0.219), 12.6% (62/492, 95% CI 0.097–0.155), 15.4% (76/492, 95% CI 0.122–0.187) and 14.0% (69/492, 95% CI 0.109–0.171) of LUSC subjects (Online Resource 8). As shown in Fig. 4 and Online Resource 9–10, patients with *TTN*/*EGFR* ($P=0.011$, HR 0.526 [95% CI 0.320–0.863]), *TTN*/*ROS1* ($P=0.017$, HR 0.551 [95% CI 0.338–0.900]), *TTN*/*RET* ($P=0.035$, HR 0.587 [95% CI 0.358–0.962]) double-mutant tumors had significantly better OS compared with patients with double WT tumors. Meanwhile, patients with *TTN*/*RET* double-mutant tumors had significantly better DFS ($P=0.022$, HR 0.572 [95% CI 0.354–0.923]) compared with patients with double WT tumors. Furthermore, patients with *TTN* single mutation obtained significantly better OS and DFS benefit compared with patients with double WT tumors throughout the whole TTN/oncogenes combination analysis (most $P<0.005$). The Cox model adjusted by the four oncogenes suggested that *TTN* remained an independent prognostic factor for lung squamous cell carcinoma ($P=0.022$, HR 0.572 [95% CI 0.354–0.923]) (Online Resource 11).

Table 2 COX analysis of *TTN* mutation types in lung squamous cell carcinoma

Events	No.of patients with events	*P* value	HR [95% CI]
Missense mutations	314	0.025	0.72 [0.546–0.960]
Stop gain mutations	43	0.170	0.68 [0.391–1.180]
Frame mutations	25	0.967	1.01 [0.499–2.060]
Synonymous alterations	159	0.150[a]	–
Intron alterations	92	0.224[a]	–
Splice site mutations[b]	10	–	–
3′ Prime UTR alterations[b]	2	–	–

No.of total deaths: $n=210$; No. of total patients: $n=485$

[a]*P* value of synonymous and intron alterations are given by logrank test

[b]Splice site and 3′ prime UTR altertions are not analyzed because of low incidents

Discussion

In this study, subsequent analyses on lung squamous cell carcinoma and lung adenocarcinoma are conducted separately, since *TTN* mutation shows significant difference between lung squamous cell carcinoma and lung adenocarcinoma. Although *TTN* mutation also shows significant relevance with gender, smoking situations through univariate analysis (Online Resource 3), the binary logistic regressive model does not identify them as independent influence factors for *TTN* mutation. Meanwhile, patients with *TTN* mutation are prone to possessing a history of molecular targeted treatment which suggests that *TTN* mutation may tend to co-mutate with oncogenes. However, the details about molecular targeted drugs are unknown and we further discuss the combination analysis of *TTN* and common oncogenes later. Besides, *TTN* mutation is significantly different between poorly differentiated tumor and moderately/well differentiated tumor ($P=0.040$) which cannot explain why *TTN* plays a role in better survival. More messages are required to explain the relationship between *TTN* and significant clinical findings.

Based on these results, we further focus on the survival analysis of *TTN* in the two histological subtypes of NSCLC, respectively. As far as our information goes, it is the first time to assess prognostic and predictive value of *TTN* single and dual mutation in NSCLC. *TTN* manifests no prognostic and predictive effect for lung adenocarcinoma. However, this situation is completely opposite in lung squamous cell carcinoma. We firstly report that *TTN* mutation displays favorable prognostic value in lung squamous cell carcinoma ($P=0.013$), but does not reach the conventional significance level of predictive value ($P=0.096$). Meanwhile, patients with *TTN*/*TP53* double mutation would have both better OS and DFS benefit compared with patients with $TTN_{WT}/TP53_{MT}$ status which means that *TTN* and *TP53* mutation may have a synergistic effect in lung squamous cell carcinoma. Our work suggests that *TTN* mutation influences the prognosis of patients with lung squamous cell carcinoma and mutation means beneficial.

It is not the first time for researchers to find gene mutation indicating good prognosis. Previous study has mentioned that patients with *PIK3CA* mutation enjoy a significantly better overall survival benefit ($P=0.042$) than non-mutated cases in lung squamous cell carcinoma (McGowan et al. 2017). Another research has showed that patients with colorectal cancers would obtain a better prognosis when they harbored POLE proofreading domain mutation (HR 0.34, $P=0.006$) which was possibly caused by increasing

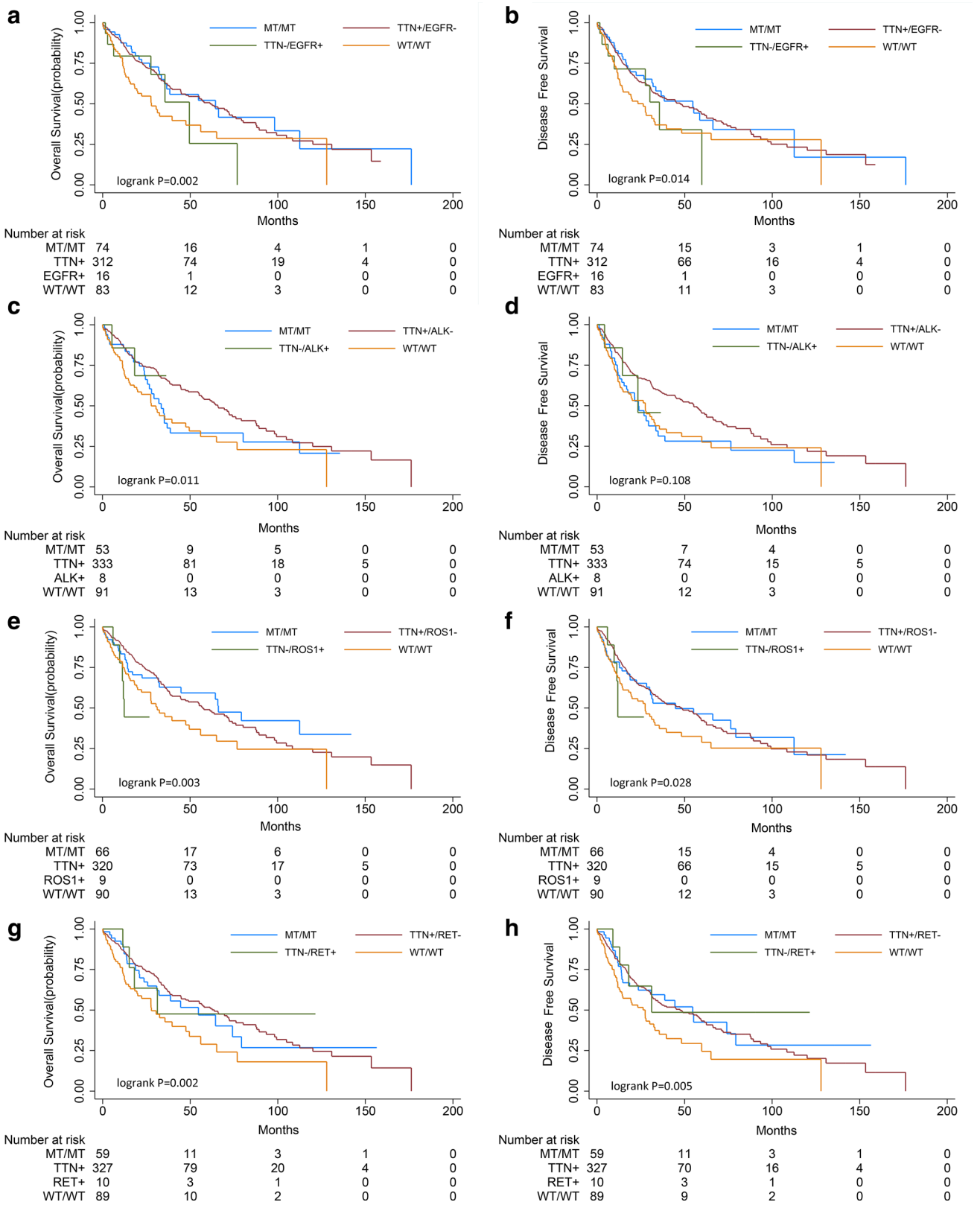

Fig. 4 Unadjusted Kaplan–Meier curves of OS and DFS effect of according to *TTN* and oncogenes mutation status in lung squamous cell carcinoma (MT, +; WT, −). **a** OS analysis of *TTN*/*EGFR* combination. **b** DFS analysis of *TTN*/*EGFR* combination. **c** OS analysis of *TTN*/*ALK* combination. **d** DFS analysis of *TTN*/*ALK* combination. **e** OS analysis of *TTN*/*ROS1* combination. **f** DFS analysis of *TTN*/*ROS1* combination. **g** OS analysis of *TTN*/*RET* combination. **h** DFS analysis of *TTN*/*RET* combination

expression of cytotoxic T-cell markers and effector cytokines or enhancing CD8+ lymphocyte infiltrating (Domingo et al. 2016). Besides, NSCLC patients with lymphatic spread ($P=0.014$) or advanced NSCLC patients ($P=0.011$) would possess favorable prognosis when they harbor A type 3 expression (Schmidt et al. 2016).

Then we discuss the mutation types of *TTN* in lung squamous cell carcinoma to explain this phenomenon. It is difficult to point out which site variation happened most since the same nucleotide locus variation could happen in two samples at most. Single sample could have various mutation types at the same time. Meanwhile, according to corresponding transcription ending, missense mutations and synonymous variations are common. It is interesting to find that patients with two or more *TTN* nucleotide variations possess better prognosis compared to those patients with only one site variation and wild-type ($P=0.016$). Besides, missense mutation is an independent prognostic indicator for good prognosis of lung squamous cell carcinoma ($P=0.025$, HR 0.72) rather than a predictive indicator ($P=0.068$, HR 0.781) which is exactly the same as the previous conclusion. Considering missense mutations occurring in 85% of mutated cases, it strongly indicates that two or more *TTN* nucleotide variations indicating better prognosis may owe to missense mutations. It is well known that a single-nucleotide missense change commonly results in a codon which codes for a different amino acid (Minde et al. 2011). Thus, whether mutant peptide segments somehow triggering certain immune reaction or improving some response to drugs to help patients obtain better survival remains unknown. Further researches are required to confirm this conjecture.

Finally, we talk about the co-mutation of *TTN* with well known oncogenes including *EGFR, ALK, ROS1*, and *RET*. The results show that *TTN* appears to play a central role in these co-mutation effects. The Cox model confirms that *TTN* mutation is still an independent prognostic factor for lung squamous cell carcinoma after adjustment by above oncogenes. We also list the co-mutation results (single-nucleotide variation only) of the comparison of these four oncogenes. Coexistence of possible pathogenic single-nucleotide variations rarely occurs between *EGFR, ALK, ROS1*, and *RET*.

In summary, *TTN* missense mutation acts a beneficial prognostic role in lung squamous cell carcinoma. This phenomenon is not suitable for lung adenocarcinoma. *TTN*/*TP53* double mutation has favorable prognostic and predictive value for lung squamous cell carcinoma as well. Through the popularization and development of sequencing technology and other technology, further clinical trials and basic researches will be required to explain the relevance and mechanism of *TTN* and lung squamous cell carcinoma.

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Data availability The data sets generated during and/or analyzed during the current study are available in the Genomic Data Commons (GDC) Data Portal of TCGA repository that does not issue data sets with DOIs (https://portal.gdc.cancer.gov/).

Compliance with ethical standards

Conflict of interest We declare that we have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Ceyhan-Birsoy O et al (2013) Recessive truncating titin gene, TTN, mutations presenting as centronuclear myopathy. Neurology 81:1205–1214. https://doi.org/10.1212/WNL.0b013e3182a6ca62

Deben C et al (2017) Deep sequencing of the TP53 gene reveals a potential risk allele for non-small cell lung cancer and supports the negative prognostic value of TP53 variants. Tumour Biol J Int Soc Oncodev Biol Med. https://doi.org/10.1177/1010428317694327

Domingo E et al (2016) Somatic POLE proofreading domain mutation, immune response, and prognosis in colorectal cancer: a retrospective, pooled biomarker study. Lancet Gastroenterol Hepatol 1:207–216. https://doi.org/10.1016/s2468-1253(16)30014-0

Herman DS et al (2012) Truncations of titin causing dilated cardiomyopathy. N Engl J Med 366:619–628. https://doi.org/10.1056/NEJMoa1110186

Hofree M, Shen JP, Carter H, Gross A, Ideker T (2013) Network-based stratification of tumor mutations. Nat Methods 10:1108–1115. https://doi.org/10.1038/nmeth.2651

Kim N, Hong Y, Kwon D, Yoon S (2013) Somatic mutaome profile in human cancer tissues. Genom Inform 11:239–244. https://doi.org/10.5808/gi.2013.11.4.239

Kim YA, Madan S, Przytycka TM (2017) WeSME: uncovering mutual exclusivity of cancer drivers and beyond. Bioinformatics (Oxf Engl) 33:814–821. https://doi.org/10.1093/bioinformatics/btw242

Labbe C et al (2017) Prognostic and predictive effects of TP53 co-mutation in patients with EGFR-mutated non-small cell lung cancer (NSCLC). Lung Cancer (Amst Neth) 111:23–29. https://doi.org/10.1016/j.lungcan.2017.06.014

Lawrence MS et al (2013) Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature 499:214–218. https://doi.org/10.1038/nature12213

Ma X et al (2016) Prognostic and predictive effect of TP53 mutations in patients with non-small cell lung cancer from adjuvant cisplatin-based therapy randomized trials: a LACE-bio pooled analysis. J Thorac Oncol Off Publ Int Assoc Study of Lung Cancer 11:850–861. https://doi.org/10.1016/j.jtho.2016.02.002

Maki-Nevala S et al (2016) Hot spot mutations in Finnish non-small cell lung cancers. Lung Cancer (Amst Neth) 99:102–110. https://doi.org/10.1016/j.lungcan.2016.06.024

McGowan M, Hoven AS, Lund-Iversen M, Solberg S, Helland A, Hirsch FR, Brustugun OT (2017) PIK3CA mutations as prognostic factor in squamous cell lung carcinoma. Lung Cancer (Amst Neth) 103:52–57. https://doi.org/10.1016/j.lungcan.2016.11.018

Minde DP, Anvarian Z, Rudiger SG, Maurice MM (2011) Messing up disorder: how do missense mutations in the tumor suppressor protein APC lead to cancer? Mol Cancer 10:101. https://doi.org/10.1186/1476-4598-10-101

Mitsudomi T, Oyama T, Kusano T, Osaki T, Nakanishi R, Shirakusa T (1993) Mutations of the p53 gene as a predictor of poor prognosis in patients with non-small-cell lung cancer. J Natl Cancer Inst 85:2018–2023

Molina-Vila MA et al (2014) Nondisruptive p53 mutations are associated with shorter survival in patients with advanced non-small cell lung cancer. Clin Cancer Res Off J Am Assoc Cancer Res 20:4647–4659. https://doi.org/10.1158/1078-0432.ccr-13-2391

Rami-Porta R, Bolejack V, Giroux DJ, Chansky K, Crowley J, Asamura H, Goldstraw P (2014) The IASLC lung cancer staging project: the new database to inform the eighth edition of the TNM classification of lung cancer. J Thorac Oncol Off Publ Int Assoc Study Lung Cancer 9:1618–1624. https://doi.org/10.1097/jto.0000000000000334

Schafer S, de Marvao A (2017) Titin-truncating variants affect heart function in disease cohorts and the general population. Nat Genet 49:46–53. https://doi.org/10.1038/ng.3719

Schmidt LH et al (2016) Blood group antigen A type 3 expression is a favorable prognostic factor in advanced NSCLC. Lung Cancer (Amst Neth) 92:8–14. https://doi.org/10.1016/j.lungcan.2015.11.019

Seo JS et al (2012) The transcriptional landscape and mutational profile of lung adenocarcinoma. Genome Res 22:2109–2119. https://doi.org/10.1101/gr.145144.112

VanderLaan PA et al (2017) Mutations in TP53, PIK3CA, PTEN and other genes in EGFR mutated lung cancers: correlation with clinical outcomes. Lung Cancer (Amst Neth) 106:17–21. https://doi.org/10.1016/j.lungcan.2017.01.011

Watanabe H et al (2016) A rapid functional decline type of amyotrophic lateral sclerosis is linked to low expression of TTN. J Neurol Neurosurg Psychiatry 87:851–858. https://doi.org/10.1136/jnnp-2015-311541