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ORIGINAL ARTICLE – CLINICAL ONCOLOGY

Comparative analysis of co-occurring mutations of specific tumor suppressor genes in lung adenocarcinoma between Asian and Caucasian populations

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Abstract

Introduction Mutated tumor suppressor genes (TSG) such as *TP53, STK11*, and *MGA* are widely-reported. We hypothesized the presence of single mutation or co-occurring mutations in these specific genes may represent a significant therapeutic target for lung adenocarcinoma.

Methods We sequenced lung adenocarcinoma samples from 677 East-Asian patients, combined them with those from cBioPortal public database (including TCGA) and performed a comparative analysis between Asian and Caucasian populations.

Results East-Asian lung adenocarcinomas presented distinct driver-mutational distribution compared to that of Caucasians (79% vs 56%, $p<0.001$). Similar results were observed in TSG mutations of *TP53* (35% vs 46%, $p=0.150$), *STK11* (4% vs 17%, $p=0.006$) and *MGA* (10% vs 4%, $p=0.166$). Compared with none-mutational cases, the patients harboring TSG mutations are more likely to be male ($p=0.009$), smokers ($p<0.001$), and more advanced disease ($p=0.004$). In addition, the TSG-mutated tumors had poorer differentiation ($p<0.001$), and more likely to be solid or micropapillary-predominant adenocarcinomas ($p<0.001$). Survival analysis showed that both overall survival (OS, $p<0.001$) and post-recurrence survival (PRS, $p<0.001$) became worse with the accumulation of TSG mutations. However, the prognostic variety was not found in Caucasian patients. Moreover, multivariate analysis proved the accumulation of TSG mutations independently predicts both unfavorable OS (HR = 0.435, 95% CI 0.245–0.774, $p=0.005$) and PRS (HR = 0.491, 95% CI 0.269–0.894, $p=0.020$) in East-Asian patients, adjusting all other survival-associated factors.

Conclusions Co-occurring mutations of specific TSGs define unfavorable subgroups of lung adenocarcinoma, implying that the tumor promotion mechanisms contribute to the heterogeneity in tumor evolution. However, the Caucasian population did not show the same results, providing insights into the molecular basis underlying the striking racial disparities of this disease and evidence for different gene-panel designs for different population in the purpose of targeted therapy.

Keywords Lung adenocarcinoma · Tumor suppressor gene (TSG) · Survival

Introduction

Lung cancer is the leading cause of cancer-related mortality, with more than one million deaths occurring annually worldwide (Siegel et al. 2017). Despite therapeutic advances over

Yiliang Zhang and Yuan Ma contributed equally to this study.

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the last several decades, the overall 5-year survival remains only 16% (Siegel et al. 2017). One of the reasons is that a great part of the patients still cannot benefit from targeted therapy (Sequist et al. 2013; Rosell et al. 2012; Mitsudomi et al. 2010), because lung cancer is a molecularly heterogeneous disease that is markedly distinct in tumor gene mutation profiles (Campbell et al. 2016; Cancer Genome Atlas Research N 2014).

Cancer has been traditionally typified by a stepwise accumulation of mutations in driver oncogenes and tumor suppressors (Kleppe and Levine 2014). The proteins coded by tumor suppressor genes (TSG), either have a damping or repressive effect on the regulation of the cell cycle or promote apoptosis, and sometimes do both (Sherr 2004). Mutated *TP53, STK11*, and *MGA*, which belong to the family of TSG, are prevalence in lung adenocarcinoma and function in different tumor-promoting mechanisms (Cancer Genome Atlas Research N 2014). Mutation in *TP53* occurs frequently in non-small cell lung cancer (NSCLC), especially in squamous cell carcinoma (~ 86.4%) though less common in adenocarcinoma (~ 54.1%) (Campbell et al. 2016; Weir et al. 2007; Ding et al. 2008). *STK11* encodes a serine/threonine protein kinase known as liver kinase β1 (Shackelford and Shaw 2009). The most common *STK11* mutations are deletion or inactivating mutations (Gao et al. 2010, 2011; O'Neill et al. 2007; Ji et al. 2007; Matsumoto et al. 2007; Shah et al. 2008; Sanchez-Cespedes et al. 2002), and the murine studies provided strong evidence for its tumor-suppressing function (Ji et al. 2007). Heterodimerization of MGA with MYC activates and mediates transcription activities (Dang 2012; Hurlin et al. 1999). Protein MGA binds to MAX and inhibits MYC-dependent cell transformation (Hurlin et al. 1999). Loss-of-function (frameshift and nonsense) mutations of *MGA* in lung adenocarcinoma may suggest a hitherto unappreciated potential mechanism of MYC pathway activation for mutation, and were mutually exclusive with focal MYC amplification (Cancer Genome Atlas Research N 2014). Another study regarded *MGA* as a TSG, whose inactivating mutations may contribute to solid tumor development (Jo et al. 2016). In addition, an inactivating mutation of *MGA* was found in small-cell lung cancers (Romero et al. 2014). Recent studies have illuminated the presence of commonly co-occurring mutations in *KRAS* with *TP53* and *STK11* (Schabath et al. 2016). However, the impact of *STK11* and *MGA* somatic mutation on disease progression and survival of patients with lung adenocarcinoma are rarely reported, and moreover, it is largely unknown about co-occurring mutations of *TP53, STK11*, and *MGA* in lung adenocarcinoma.

This study focuses on comparing specific TSG-mutational spectrums in East-Asian and Caucasian lung adenocarcinoma, and the association between accumulation of these gene mutations and clinicopathological characteristics of the patients. These results allowed us to figure out the impact of co-occurring mutations of specific TSGs on the heterogeneity in tumor evolution.

Results

Clinicopathological characteristics of Caucasians and East-Asians with lung adenocarcinoma

A total of 677 East-Asians with resected lung adenocarcinoma were included in this study. There were 327 (48.3%) males and 350 (51.7%) females with an average age of 60.3 years (range 27 to 84). Among these patients, 464 (68.5%) were never smokers and 213 (31.5%) were former or current smokers. The detail characteristics and gene mutational statue of the East-Asian patents are summarized in Table 1. We employed four lung adenocarcinoma studies with gene mutation data that are available cBioPortal

Table 1 Clinicopathological features of Caucasians and East-Asians with lung adenocarcinoma

Variants	Caucasians ($n = 588$)	East-Asians ($n = 677$)
Age		
< 60	103 (17.5%)	315 (46.6%)
≥ 60	284 (48.3%)	362 (53.4%)
NA	201 (34.2%)	– (0)
Gender		
Male	199 (33.8%)	327 (48.2%)
Female	218 (37.1%)	350 (51.8%)
NA	171 (29.1%)	– (0)
Smoke		
Never	26 (4.4%)	464 (68.6%)
Current	149 (25.3%)	213 (31.4%)
NA	413 (70.2%)	– (0)
Pathologic stage		
I	83 (14.1%)	386 (57.1%)
II	31 (5.3%)	73 (10.8%)
III	22 (3.7%)	218 (32.1%)
NA	452 (76.9%)	– (0)
Driver mutation		
EGFR	92 (15.6%)	416 (62.4%)
KRAS	185 (31.5%)	53 (8.0%)
HER2	12 (2.0%)	14 (2.1%)
BRAF	39 (6.6%)	11 (1.7%)
ALK	32 (5.4%)	43 (6.5%)
TSG mutation		
TP53	270 (45.9%)	234 (34.5%)
STK11	101 (17.2%)	29 (4.1%)
MGA	24 (4.1%)	67 (9.8%)

NA not available, *TSG* tumor suppressor gene

website. 588 Caucasian patients were enrolled in the study who seemed to consist of more elderly patients (48.3% vs 17.5%, $p < 0.001$) and more smokers (25.3% vs 4.4%, $p < 0.001$) (Table 1).

Distinct mutation profile of lung adenocarcinomas between East-Asian and Caucasian patients

The prevalence of common oncogenic driver mutations in East-Asian patients was much higher than that of Caucasian patients (78% vs 56%, $p = 0.002$). We found that driver mutational frequency was 78% and the top two drivers were *EGFR* (61.4%) and *KRAS* (7.8%) in East-Asian patients. Contrarily, the most common driver was *KRAS* (31.7%), and the second was *EGFR* (15.5%) in Caucasian patients. In addition, Caucasian lung adenocarcinoma carried much more *KRAS* (31.5% vs 8.0%) and *BRAF* (6.6% vs 1.7%) mutation than East-Asians. As to the TSG mutations, the prevalence of *MGA* mutation was higher in East-Asians compared to that of Caucasian patients (9.8% vs 4.1%, $p < 0.001$). However, *TP53* (35.4% vs 45.9%, $p < 0.001$) and *STK11* (4.1% vs 17.2%, $p < 0.001$) mutations were on opposite set (Table 1; Fig. 1).

Co-occurring TSG mutations in East-Asian patients

There were 389 (57.5%) patients with none mutational TSG, 247 (36.5%) patients harboring one mutation, 40 (6.0%) patients harboring two mutations, and only one patient with simultaneous mutations in all three TSGs. Clinicopathologic parameters analyzed included age, sex, smoking history, tumor cell differentiation, pathologic stage, and predominant subtypes. Compared with the none-mutational patients, the TSG-mutated cases were more likely to be male ($p = 0.009$) and smokers ($p < 0.001$). As the TSG-mutational accumulation, the tumor became poorer differentiation ($p < 0.001$) and harbored more advanced disease ($p = 0.004$). As to predominant subtypes, the good-prognostic lepidic component decreased, but the poor solid cases increased. In addition, the mutation statue of TSG was not significantly associated with age ($p = 0.708$) and driver gene mutation ($p = 0.675$) (Table 2).

Survival analysis

In East-Asian cohort, 61 (6.0%) patients who lost contact were excluded from the survival analysis. There was no death during the perioperative period. Finally, a total of 636 East-Asians was studied in detail for survival outcomes, including 369 (58.0%) patients with no TSG mutation, 230 (36.2%) with one TSG mutation and 37 (5.8%) with two TSG mutations. The median follow-up time were 38.6 months. To investigate the TSG mutational accumulation impacts in Caucasian population, we also employed TCGA patients with follow-up data to analysis survival condition. After excluding African, Asian and stage IV cases, 218 Caucasian patients enrolled in survival analysis (Supplemental Table 1).

In univariate analysis, the East-Asian patients harboring any single mutation of *TP53* (5-year OS: 75.1% vs 81.6%, $p = 0.039$), *STK11* (5-year OS: 59.3% vs 80.2%, $p = 0.004$) or *MGA* (5-year OS: 67.3% vs 80.9%, $p = 0.037$) showered a worse OS than the respective wild-type cases (Fig. 2a–c). The RFS difference between the TSG mutational patients and wild type was significant regarding *TP53* and *STK11* ($p = 0.026$; $p = 0.012$) but not *MGA* ($p = 0.373$) (Fig. 2d–f). Interestingly, when investigated the prognostic impact of co-occurring TSG mutations, we found that the patients with no TSG mutation shared best survival outcomes, while those with two TSG mutations got the worst, which meant that both RFS ($p = 0.007$) and OS ($p < 0.001$) got worse with the accumulation of the TSG mutations (Fig. 3a, b). However,

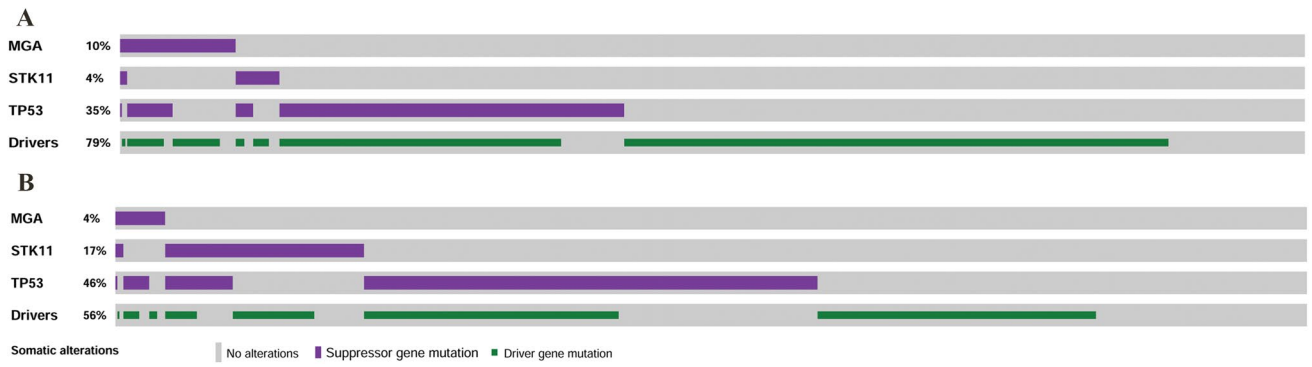

Fig. 1 Co-occurring mutation profiles in specific tumor suppressor genes (*TP53, STK11*, and *MGA*) and common driver mutation in oncogenic genes of East Asian and Caucasian lung adenocarcinoma samples. The prevalence of *MGA* mutation (9.8% vs 4.1%, $p < 0.001$) was higher in East Asian lung adenocarcinoma samples, but *STK11* (4.1% vs 17.2%, $p < 0.001$) and *TP53* (35.4% vs 45.9%, $p < 0.001$) were much higher in Caucasian patients

Table 2 Features of lung adenocarcinomas harboring specific tumor suppressor gene mutations (*TP53, STK11* and *MGA*)

Variable	None mutated gene ($n = 389$)	One mutated gene ($n = 247$)	Two mutated gene ($n = 40$)	*P*
Age				0.708
< 60	176 (45.2%)	120 (48.6%)	19 (47.5%)	
≥ 60	213 (54.8%)	127 (51.4%)	21 (52.5%)	
Gender				0.009
Male	168 (43.2%)	135 (54.7%)	23 (57.5%)	
Female	221 (56.8%)	112 (45.3%)	17 (42.5%)	
Smoke				< 0.001
Never (%)	289 (74.3%)	155 (62.8%)	20 (50.0%)	
Current (%)	100 (25.7%)	92 (37.2%)	20 (50.0%)	
Differentiation				< 0.001
Well	34 (8.9%)	4 (1.7%)	– (0)	
Moderate	242 (63.5%)	139 (57.7%)	14 (35.9%)	
Poor	105 (27.6%)	98 (40.7%)	25 (64.1%)	
Pathologic stage				0.004
I	240 (61.7%)	133 (53.8%)	13 (32.5%)	
II	41 (10.5%)	26 (10.5%)	6 (15.0%)	
III	108 (27.8%)	88 (35.6%)	21 (52.5%)	
Subtypes				< 0.001
Acinar	189 (48.6%)	123 (49.8%)	18 (45%)	
Lepidic	72 (18.5%)	20 (8.1%)	1 (2.5%)	
Papillary	39 (10.0%)	31 (12.6%)	5 (12.5%)	
Micropapillary	3 (0.7%)	7 (2.8%)	– (0)	
Solid	42 (10.8%)	52 (21.1%)	14 (35.0%)	
IMA	29 (7.5%)	13 (5.3%)	2 (5.0%)	
Others	15 (3.9%)	1 (0.4%)	– (0)	
Driver mutational status				0.675
Pan positive	311 (81.8%)	196 (80.3%)	29 (76.3%)	
Pan negative	69 (18.2%)	48 (19.7%)	9 (23.7%)	

IMA invasive mucinous adenocarcinoma

in the Caucasian cohort, Kaplan–Meier analysis revealed no predicting role of TSG mutations for either RFS ($p = 0.669$) or OS ($p = 0.109$) (Fig. 3c, d).

In multivariate survival analysis after adjusted for age, gender, smoking history, predominant subtyping, pathologic stage, and driver gene mutational status, accumulating TSG mutations proved to be an independent predictor for poor OS in East-Asian patients compared with no mutation (HR = 3.503, 95% CI 2.009–6.107, $p = 0.004$), but not for RFS (Table 3). However, in the Caucasian cohort, multivariate analysis revealed no predicting role of TSG mutations for either OS or RFS (Table 4).

Discussion

In recent years, with the advance of next-generation sequencing (NGS) technology, driver-mutated oncogenes have been systematically identified for all major cancer types, especially through consortium projects such as TCGA or ICGC. However, a great part of lung cancer patients cannot benefit from the targeted therapies for lack of diver mutations (Kwak et al. 2010; Bergethon et al. 2012; Drilon et al. 2013; Stephens et al. 2004). Our study explored the status of tumor suppressor mutations in East Asian with lung adenocarcinomas and found that co-mutations of the gene *TP53, STK11*, and *MGA* resulted in poor prognosis. However, similar results were not observed in Caucasians population, which providing insights into the molecular basis underlying the striking racial disparities of this disease and evidence for different gene-panel designs for different population in the purpose of targeted therapy.

Public NGS consortium projects usually characterized the mutations from patients with mixed racial backgrounds or only Caucasian population. East-Asian patients have distinct driver mutational spectrum in from Caucasian population. Sun et al. and Kawaguchi et al. have reported that EGFR mutation rate was nearly 60%, KRAS mutation

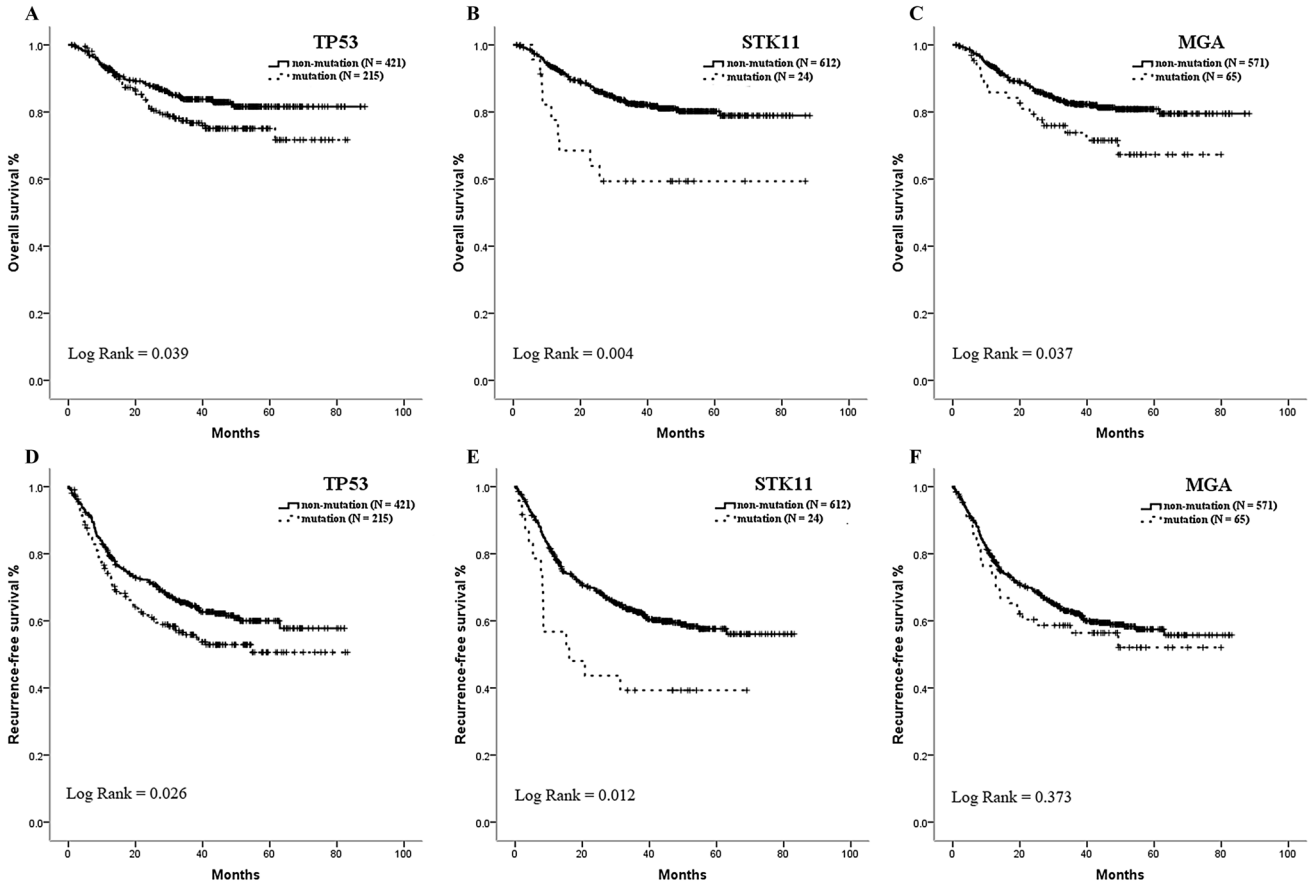

Fig. 2 Kaplan–Meier survival curve for overall survival (OS) and recurrence-free survival (RFS) according to tumor suppressor genes' status shows three of which have significant difference. **a** OS in *TP53* mutation and non-mutation, **b** OS in *STK11* mutation and non-mutation, **c** OS in *MGA* mutation and non-mutation, **d** RFS in *TP53* mutation and non-mutation, **e** RFS in *STK11* mutation and non-mutation, **f** RFS in *MGA* mutation and non-mutation

rate was nearly 8%, and BRAF mutation rate only was 1–2% in Chinese and Japanese with lung adenocarcinoma (Kawaguchi et al. 2016; Sun et al. 2010), while Caucasians who EGFR mutation rate was nearly 14% and KRAS mutation rate was nearly 33%, BRAF mutation rate was nearly 10% (Cancer Genome Atlas Research N 2014; Ding et al. 2008). Therefore, identifying the heterogeneity in tumor evolution and different patient populations became a critical foundation for developing novel therapeutic strategies. In this research, we compared the driver and specific TSG mutations in East-Asian with lung adenocarcinoma from FUSCC and Caucasian patients from public researches. We found that common oncogenic driver mutation frequency was similar to the previous research. However, *TP53* (46% vs 35%) and *STK11* (17% vs 4%) of TSGs are more mutation frequency in Caucasian patients than East-Asian. Another point to note is that *MGA* (10% vs 4%) seldomly-reported in East-Asians has twice higher mutation rate than that of Caucasian patients. These results suggested that lung adenocarcinoma is a disease with striking racial disparities, providing more information to fulfill the needs of personalized medicine.

Co-mutations indicate certain important cancer-related genes simultaneously in the same lung cancer sample. Co-occurring mutations of cancer-related genes may contribute to heterogeneity in tumor biology. Previous studies reported that the increased mutation burden will lead to a worse prognosis for patients with cancer, but which genes play a major role is still unclear. Schabath et al. reported that 442 lung adenocarcinoma patients commonly co-occurring mutations in STK11 and TP53 tumor suppressors with KRAS-mutant tumors (Schabath et al. 2016). However, there are few studies on co-mutations of cancer-related genes and prognosis in Asian populations. In this study, we compared the effects of co-mutation of tumor suppressor genes between Asian lung adenocarcinoma population and Caucasian population. After Kaplan–Meier analysis, we found that any one of TSGs (*TP53, STK11* and *MGA*) could bring worse prognosis. These TSGs belong to different tumor promotion mechanisms which is fatal to cell growth and cell-cycle-related

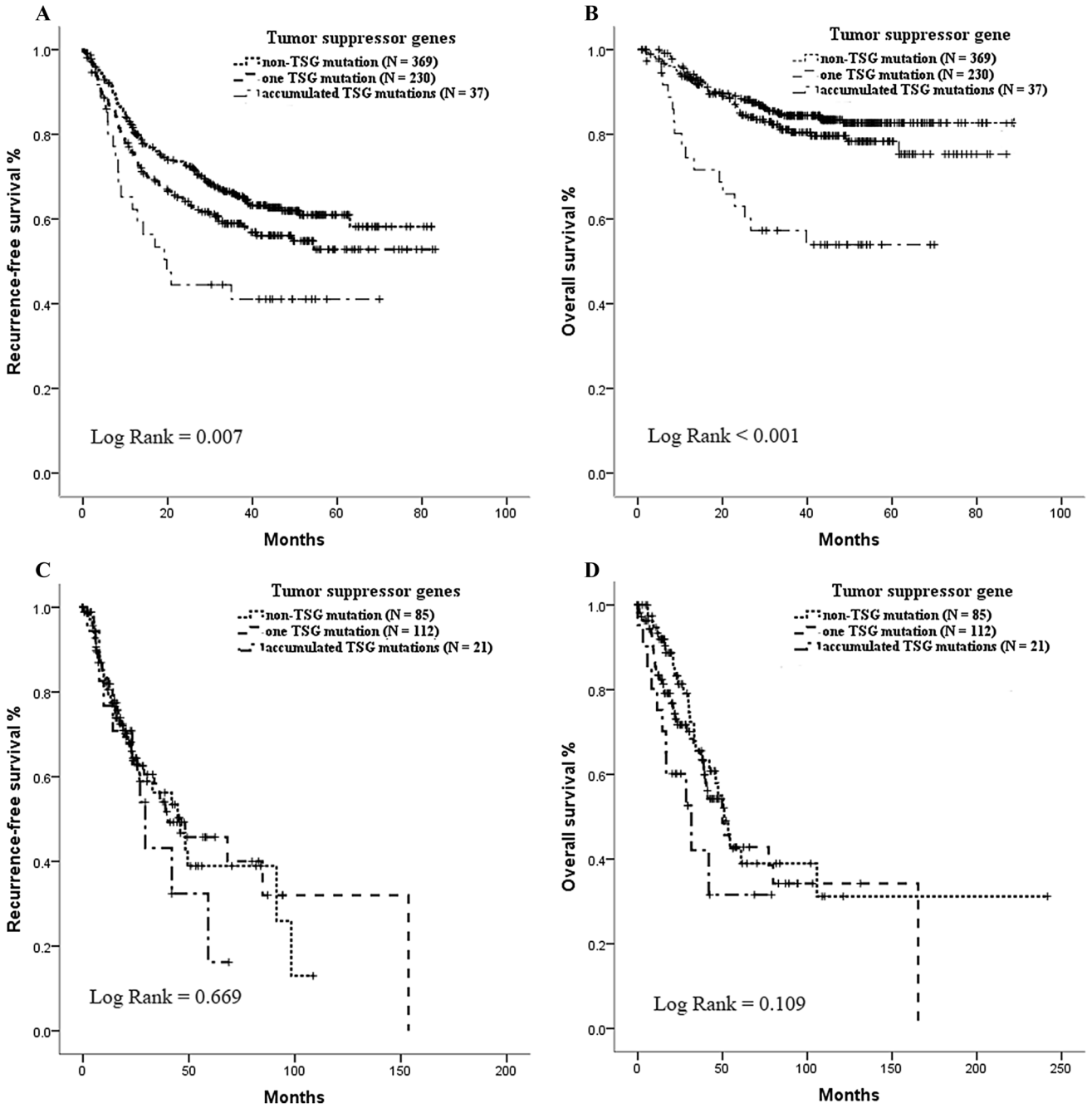

Fig. 3 There was significant difference in recurrence-free survival (RFS; $p = 0.007$) or overall survival (OS; $p < 0.001$) analyzed by accumulation of TSG mutations in East Asian lung adenocarcinoma samples (**a, b**) but not in Caucasian (RFS; $p = 0.669$, OS; $p = 0.109$) (**c, d**)

genes and promotes oncogenesis. The burden of specific-mutated TSGs is a huge impact factor for clinical features of patients with lung cancer. Compared with none-mutational TSG patients, TSG-mutated patients significant enriched in male, smokers, poor tumor differentiation, and advanced disease. In addition, TSG-mutated patients significant enriched in micropapillary or solid predominant subtype which resulted in worse outcomes. More attention should be paid to this aggressive lung adenocarcinoma subtype to identify new molecular targets. TSGs' mutation has few related to common oncogenic driver mutation panel including single-driver gene mutational statue (data no shown). Therefore, we believe that the most common EGFR and KRAS mutation not directly related to the co-mutation of the three tumor suppressor genes. The mutation rate of other driver genes is too low to have statistical significance. These

Table 3 Univariate and multivariate overall and relapse-free analysis of East-Asian with lung adenocarcinoma

Variable	OS								RFS							
	Univariate				Multivariate				Univariate				Multivariate			
	HR	95% CI	95% CI	*p*	HR	95% CI	95% CI	*p*	HR	95% CI	95% CI	*p*	HR	95% CI	95% CI	*p*
Age, years																
≥ 60/< 60	1.188	0.822	1.717	0.358	NA	NA	NA	NA	0.928	0.722	1.193	0.559	NA	NA	NA	NA
Gender																
Male/female	1.640	1.134	2.373	0.009[a]	1.278	0.696	2.347	0.429	1.517	1.179	1.952	0.001[a]	1.271	0.853	1.895	0.239
Smoking history																
Yes/no	2.304	1.596	3.326	0.000[a]	1.716	0.932	3.161	0.083	2.113	1.638	2.727	0.000[a]	1.769	1.178	2.657	0.006[a]
Stage																
II and III/I	9.062	5.538	14.828	0.000[a]	8.013	4.864	13.200	0.000[a]	5.662	4.286	7.479	0.000[a]	5.209	3.920	6.921	0.000[a]
Driver gene																
Mut/WT	1.751	1.144	2.682	0.010[a]	0.642	0.412	1.000	0.050[a]	1.293	0.940	1.779	0.114	NA	NA	NA	NA
TSG																
One Mut/WT	1.261	0.847	1.876	0.253	1.021	0.682	1.529	0.921	1.279	0.982	1.667	0.068	1.075	0.820	1.409	0.599
Two Mut/WT	3.503	2.009	6.107	0.000[a]	2.287	1.303	4.016	0.004[a]	1.990	1.242	3.188	0.004[a]	1.306	0.810	2.104	0.273

CI confidence interval, *OS* overall survival, *RFS* relapse-free survival, *HR* hazard ratio, *NA* not applicable, *TSG* tumor suppressor gene, *Mut* mutated gene, *WT* wild type

[a]Statistical significance

Table 4 Univariate and multivariate overall and relapse-free survival analysis of TCGA patients with lung adenocarcinoma

Variable	OS								RFS							
	Univariate				Multivariate				Univariate				Multivariate			
	HR	95% CI	95% CI	*p*	HR	95% CI	95% CI	*p*	HR	95% CI	95% CI	*p*	HR	95% CI	95% CI	*p*
Age, years																
≥ 60/< 60	1.282	0.774	2.123	0.334	NA	NA	NA	NA	1.069	0.656	1.740	0.789	NA	NA	NA	NA
Gender																
Male/female	0.927	0.602	1.428	0.731	NA	NA	NA	NA	0.757	0.485	1.181	0.220	NA	NA	NA	NA
Smoking history																
Yes/no	0.772	0.497	1.197	0.248	NA	NA	NA	NA	0.915	0.576	1.455	0.707	NA	NA	NA	NA
Stage																
II and III/I	3.328	2.121	5.224	0.000[a]	3.232	2.048	5.099	0.000[a]	2.380	1.517	3.733	0.000[a]	2.405	1.523	3.796	0.000[a]
Driver gene																
Mut/WT	0.970	0.630	1.494	0.891	NA	NA	NA	NA	1.146	0.730	1.799	0.554	NA	NA	NA	NA
TSG																
One Mut/WT	1.251	0.782	2.002	0.351	1.061	0.660	1.705	0.806	0.947	0.589	1.521	0.821	0.846	0.524	1.365	0.493
Two Mut/WT	2.095	1.041	4.215	0.038[a]	1.668	0.826	3.369	0.154	1.302	0.638	2.659	0.468	1.078	0.524	2.214	0.839

CI confidence interval, *OS* overall survival, *RFS* relapse-free survival, *HR* hazard ratio, *NA* not applicable, *TSG* tumor suppressor gene, *Mut* mutated gene, *WT* wild type

[a]Statistical significance

results suggested that as the burden of three specific TSGs mutations increased, the proliferation of tumor cells and the influence of metastatic signal pathways are gradually increased, resulting in tumor invasion and dissemination. These specific TSGs were closely associated to poorer cell differentiation and more advanced disease.

The association of some specific TSGs (*TP53* and *STK11*) mutation and survival in lung cancer patients has been investigated in numerous studies, most of which have reported the poor prognostic role of mutated TSGs in lung cancer (Shah et al. 2008; Sanchez-Cespedes et al. 2002; Gibbons et al. 2014; Facchinetti et al. 2017). Only several researches investigated *MGA* function in colorectal cancer and Natural killer/T-cell lymphoma (Jo et al. 2016; Jiang et al. 2015). Here, we further revealed that accumulation of TSG mutations subsets was an independent poor prognostic marker for OS validated by multivariate survival analysis incorporating clinicopathologic variables and status of well-identified driver mutations in East-Asian patients. However, when investigating into Caucasian patients, no RFS or OS difference was observed between mutation and none-mutation TSG groups. These results suggested the molecular mechanism of co-occurring TSG mutations might be associated with poor prognosis of lung adenocarcinoma. It also revealed the mutational burden of specific TSGs is a prognostic marker for East-Asian but not for Caucasian populations. In addition, we found that the recurrence of multiple metastases was more likely to occur in patients with two co-occurring TSG lung adenocarcinomas ($p = 0.031$). This aggressive pattern of recurrence provides clinical evidence for the findings in the mouse model research and may explain the reason why mutated TSGs patients shared similar RFS but ended in much poor OS with none mutated patients in Asian populations.

In interpreting these findings, several limitations inherent to this study must be considered. First, we did not get adjacent non-tumor tissues served as a control which may eliminate the interference caused by polymorphic mutations. In addition, when comparing the mutational rates between the lung adenocarcinoma populations, we first assessed the sequencing depth of exons and found that samples from Chinese patients had a considerably greater depth than those from Caucasian, and this could have some bias. Nonetheless, somatic mutations were evaluated by the most critical screening criteria to eliminate false positives and germline mutations which have been generally accepted by the published sequencing studies may reduce errors.

In conclusion, co-occurring mutations of specific TSGs define unfavorable subgroups of East-Asian lung adenocarcinoma, implying that the tumor promotion mechanisms contribute to the heterogeneity in tumor evolution. However, the Caucasian population did not get the same results, providing insights into the molecular basis underlying the striking racial disparities of this disease and evidence for different gene-panel designs for different population in the purpose of targeted therapy.

Patients and methods

Patient population

Between 2008 and 2013, consecutive patients with newly diagnosed primary lung cancer were retrospectively selected. After pre-operative work-up (enhanced thoracic computed tomography, abdominal ultrasonography, brain magnetic resonance imaging, and bone scan for all patients and positron emission tomography/computed tomography for some) to exclude regional and systemic disease, patients underwent surgery with curative intent at Fudan University Shanghai Cancer Center (FUSCC). Lymphadenectomy was routinely done for all patients, and adjuvant chemotherapy was suggested for those who had nodal metastases. Inclusion criteria for this study included: (1) patients with pulmonary tumors underwent complete resection with curative intent; (2) tumors were pathologically confirmed as lung adenocarcinoma through immunohistochemistry (IHC) methods, according to the new WHO classification of lung tumors (Travis 2015), by two independent pathologists; and (3) sufficient tissue for comprehensive mutational analyses. Tumor tissues sampled just after the surgical resection, and were immediately stored in liquid nitrogen. Written informed consent was obtained from the patients and the study was approved by the Ethics of Human Research Committee of FUSCC.

Molecular analysis

DNA was extracted from the samples, and quality-control assessments were performed as described previously (Cancer Genome Atlas Research N 2012). *EGFR, KRAS, HER2*, and *BRAF* hot region exon were amplified by polymerase chain reaction (PCR) using KOD-plus DNA polymerase and cDNAs (Sun et al. 2010; Pan et al. 2014a, b). Direct dideoxynucleotide method sequencing was performed to analyze the gene alterations. A combined strategy of reverse transcriptase PCR and quantitative real-time PCR was performed to detect *ALK* fusions. Fluorescent in situ hybridization was used as a validation for these fusion genes (Wang et al. 2012a, b, 2014; Pan et al. 2014). Multiplexed, targeted deep sequencing was performed on Ion Torrent (Life Technologies, Shanghai, China), using an Ion Ampliseq custom panel. *TP53, STK11*, and *MGA* were selected on the basis of the previous report (Cancer Genome Atlas Research N 2014) and were evaluated to cover whole critical mutations (Data Supplement Fig. 1). According to TSG mutational accumulation of *TP53, STK11*, and *MGA*, we divided the

population into three groups: no mutation, only one mutation of any gene, and co-occurring mutations in any two of TSGs.

We employed four lung adenocarcinoma studies with gene mutation data that are available cBioPortal website (http://www.cbioportal.org/data_sets.jsp). They come from four different resource including Imielinksi et al. (2012), Rizvi et al. (2015), TCGA (2014) and Ding et al. (2008). After excluded 12 Africa and Asian patients, 588 Caucasian patients were enrolled in this study. The prognostic survival data of Caucasians who diagnosis with lung adenocarcinoma has been selected from Xena Public Data Hubs https://xenabrowser.net/datapages/?cohort=TCGA%20Lung%20Adenocarcinoma%20(LUAD). These patients are the same as the TCGA database cases.

Statistical analysis

Pearson's Chi-squared test or Fisher's exact test (when the count in any cell of a contingency table was less than required) was performed to detect the association between gene alteration and clinicopathological characteristics. The Kaplan–Meier method was used to estimate the survival curve, and the log-rank test was used to compare the survival data. Cox regression was used for multivariate analysis to assess the effect of covariates on OS, RFS, and PRS. SPSS for Windows version 19.0 (IBM Corporation, Armonk, NY, USA) was used to process data; 0.05 was chosen as the type I error; and therefore, a p value < 0.05 indicated statistical significance.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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