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ORIGINAL ARTICLE – CLINICAL ONCOLOGY

Synchronicity of genetic variants between primary sites and metastatic lymph nodes, and prognostic impact in nodal metastatic lung adenocarcinoma

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Abstract

Purpose Nodal positive lung adenocarcinoma includes wide range of survival. Several methods for the classification of nodal-positive lung cancer have been proposed. However, classification considering the impact of targetable genetic variants are lacking. The possibility of genetic variants for the better stratification of nodal positive lung adenocarcinoma was estimated.

Methods Mutations of 36 genes between primary sites and metastatic lymph nodes (LNs) were compared using next-generation sequencing. Subsequently, mutations in *EGFR* and *BRAF*, rearrangements in ALK and *ROS1* were evaluated in 69 resected pN1–2M0 adenocarcinoma cases. Recurrence-free survival (RFS), post-recurrence survival (PRS), and overall survival (OS) were evaluated with respect to targetable variants and tyrosine kinase inhibitor (TKI) therapy after recurrence.

Results About 90% of variants were shared and allele frequencies were similar between primary and metastatic sites. In 69 pN1–2M0 cases, *EGFR*/*ALK* were positive in primary sites of 39 cases and same *EGFR*/*ALK* variants were confirmed in metastatic LNs of 96.7% tissue-available cases. Multivariate analyses indicated positive *EGFR*/*ALK* status was associated with worse RFS (HR 2.366; 95% CI 1.244–4.500; $P=0.009$), and PRS was prolonged in cases receiving TKI therapy (no post-recurrence TKI therapies, HR 3.740; 95% CI 1.449–9.650; $P=0.006$). OS did not differ with respect to targetable variants or TKI therapy.

Conclusions Cases harbouring targetable genetic variants had a higher risk of recurrence, but PRS was prolonged by TKI therapy. Classification according to the targetable genetic status provides a basis for predicting recurrence and determining treatment strategies after recurrence.

Keywords Lung adenocarcinoma · Lymph node metastasis · NGS · Recurrence · EGFR · ALK

Introduction

Lung cancer with metastatic lymph nodes (LNs) is an advanced status with variation in prognosis. Nodal-positive cases have a high risk of recurrence, even after complete resection. Recurrence is a crucial issue for prognosis after resection, and unresectable recurrent cases harbouring targetable genetic variants are good candidates for targeted therapies. Although several further classification methods have been proposed for nodal-positive cases by analysing the metastatic lymph node (LN) status, methods based on targetable genetic variants are lacking. Additionally, the prognostic impact of genetic aberrations in metastatic LNs is unclear. This study aimed to compare the status of targetable genetic variants at primary sites and metastatic LN of lung adenocarcinoma and to explore classification methodology

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for nodal-positive lung adenocarcinoma based on genetic status and prognostic value.

Materials and methods

Study design

Nodal-positive lung adenocarcinoma cases resected between 2005 and 2016 at Hiroshima University Hospital (Hiroshima, Japan) were retrospectively reviewed. To estimate the spread of genetic variation in nodal metastatic cases, genetic heterogeneity between primary sites and metastatic LNs was first evaluated by next-generation sequencing (NGS). Two pairs of fresh frozen tissues from primary sites and corresponding metastatic LNs were used. Subsequently, mutations in *EGFR* and *BRAF* and rearrangements in *ALK* and *ROS1*, which are current targetable variants, were detected in primary sites of pN1–2 cases by clinically available methods. In *EGFR*/*ALK*/*BRAF*/*ROS1*-positive cases, the same targetable variants were evaluated in metastatic LNs. The prognostic impact of targetable variants on pN1–2 patients was evaluated based on recurrence-free survival (RFS), post-recurrence survival (PRS), and overall survival (OS).

pN1–2 cases after neoadjuvant chemotherapy/chemoradiotherapy, proven to be incomplete resection (R1–2), pN3, or distant metastasis were excluded. All included cases were pathologically diagnosed according to the classification system of the International Association for the Study of Lung Cancer (IASLC)/American Thoracic Society (ATS)/European Respiratory Society (ERS) (Travis et al. 2011) or 2015 WHO classification (Travis et al. 2015) by two pathologists (Y. T. and K. K.). TNM stage was determined according to the IASLC TNM classification system (eighth edition) (Rami-Porta et al. 2018). This study, including the utilisation of surgical specimens and collection and analysis of clinicopathological data, was approved by the institutional review board at Hiroshima University Hospital (E-1231).

Next-generation sequencing for comprehensive genetic analysis

For NGS, fresh frozen tissues obtained by surgical resection were used. DNA was extracted using the QIAamp DNA Micro Kit (56403; QIAGEN GmbH, Hilden, Germany). Extracted DNA was prepared for NGS using HaloPlex HS (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. MiSeq (Illumina Inc., San Diego, CA, USA) was utilized for NGS as previously described (Ito et al. 2017). Sequence reads were processed and mapped to a human genome reference sequence (hg19) using SureCall 3.5.1.46 (Agilent Technologies). The evaluated genes and coverage for each targeted region are shown in Supplemental Table 1. Our custom panel was designed to cover 36 genes with a median coverage of 99.57% (80.89–100%) (Ito et al. 2017). If a mutation met at least one of the following criteria, it was excluded from the analysis as false positive detection: allele frequency < 0.05, number of variant alleles < 10, filtered read depth < 100, or variants defined as benign or likely benign using the default settings of SureCall.

Detection of *EGFR*/*ALK*/*BRAF*/*ROS1* variants at primary sites and metastatic LNs

The *EGFR* mutation status in Exon 18, 19, and 21 was evaluated by the peptide nucleic acid-locked nucleic acid (PNA–LNA) polymerase chain reaction (PCR) clamp method by an institutional laboratory or external estimation body as described previously (Nagai et al. 2005; Ito et al. 2018). *BRAF* mutation (V600E) was evaluated by LNA PCR in the institutional laboratory. The conditions for LNA PCR and sequence of primers and probes are described in Supplemental Table 2. DNA was extracted from frozen sections as described above or from formalin-fixed paraffin-embedded (FFPE) tissues using a QIAamp DNA FFPE Tissue Kit (56404; QIAGEN GmbH).

Rearrangements in *ALK* and *ROS1* were evaluated according to the College of American Pathologists (CAP)/IASLC/Association for Molecular Pathology (AMP) guideline (Kalemkerian et al. 2018). Immunohistochemistry (IHC) was regarded as an equivalent alternative to fluorescence in situ hybridization (FISH) for *ALK* rearrangement testing (Kalemkerian et al. 2018). IHC was conducted at the Pathological Department of Hiroshima University Hospital using commercial antibody (727071; NICHIREI BIOSCIENCES INC., Tokyo, Japan) and FISH was performed using the Vysis ALK Break Apart FISH Probe Kit (6N38-21; Abbott JAPAN, Tokyo, Japan) by an external examining body. *ROS1* rearrangements were screened by IHC using a commercial antibody (3287; Cell Signaling Technology, Danvers, MA, USA) at an institutional laboratory. Positive IHC results for *ROS1* were confirmed by cytomolecular methods using a commercial kit (A163; RIKEN GENESIS CO., Ltd., Tokyo, Japan) by an external examination body. For the detection of *EGFR*/*ALK*/*ROS1* variants, the same external examination body (LSI Medience Corp., Tokyo, Japan) was utilized.

Statistical analyses

RFS was calculated from the day of operation to the day when recurrence was detected radiologically. PRS was calculated from the day of recurrence to the day of death from any cause. OS was calculated from the day of operation to the day of death from any cause. To compare continuous numerical variables, the Mann–Whitney *U* test was used.

For frequencies, significance was evaluated using the Chi squared, Yates, or Fisher's exact probability test. RFS, PRS, and OS were estimated using the Kaplan–Meier method and compared using log-rank tests. Univariate and multivariate analyses were conducted by a Cox proportional hazards model with a backward stepwise procedure. Probability values were derived from two-tailed tests and $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA) and StatMate V (ATMS Co., Ltd., Tokyo, Japan).

Results

For NGS analyses of primary sites and metastatic LNs, the median read depth and percentage of target regions coverage were 416 (106–1238) and 92.39% (92.13–92.73%), respectively. The genetic variants and allele frequencies were similar between primary sites and metastatic LNs. A heat map of genetic variants showed a symmetric pattern between primary sites and corresponding metastatic LNs in the two cases (Fig. 1a). Moreover, as shown in a Venn diagram, 92.8% (64/69) and 87.0% (67/77) of variants were shared between primary and metastatic sites in case 1 and case 2, respectively (Fig. 1b).

A total of 69 pN1–2 adenocarcinoma cases were enrolled and patient characteristics are shown in Table 1. All 69 cases were resected with curative intensity after clinical staging using computed tomography (CT), positron-emission tomography (PET)-CT, and brain magnetic resonance imaging (MRI). As targetable variants, *EGFR* mutations were confirmed in 35 out of 69 pN1–2 cases. *ALK* rearrangements were positive in 4 cases (2 cases were positive by IHC and 2 cases were positive by FISH). *BRAF* mutations and *ROS1* rearrangements were not detected (3 cases were checked by the cytomolecular method for *ROS1* rearrangement after IHC screening). The median follow-up term was 43.5 (3.75–128.2) months. Forty-seven cases relapsed and 27 cases received tyrosine kinase inhibitor (TKI) therapy after recurrence (gefitinib in 15 patients, erlotinib in 9 patients, afatinib in 8 patients, and alectinib in 1 patient).

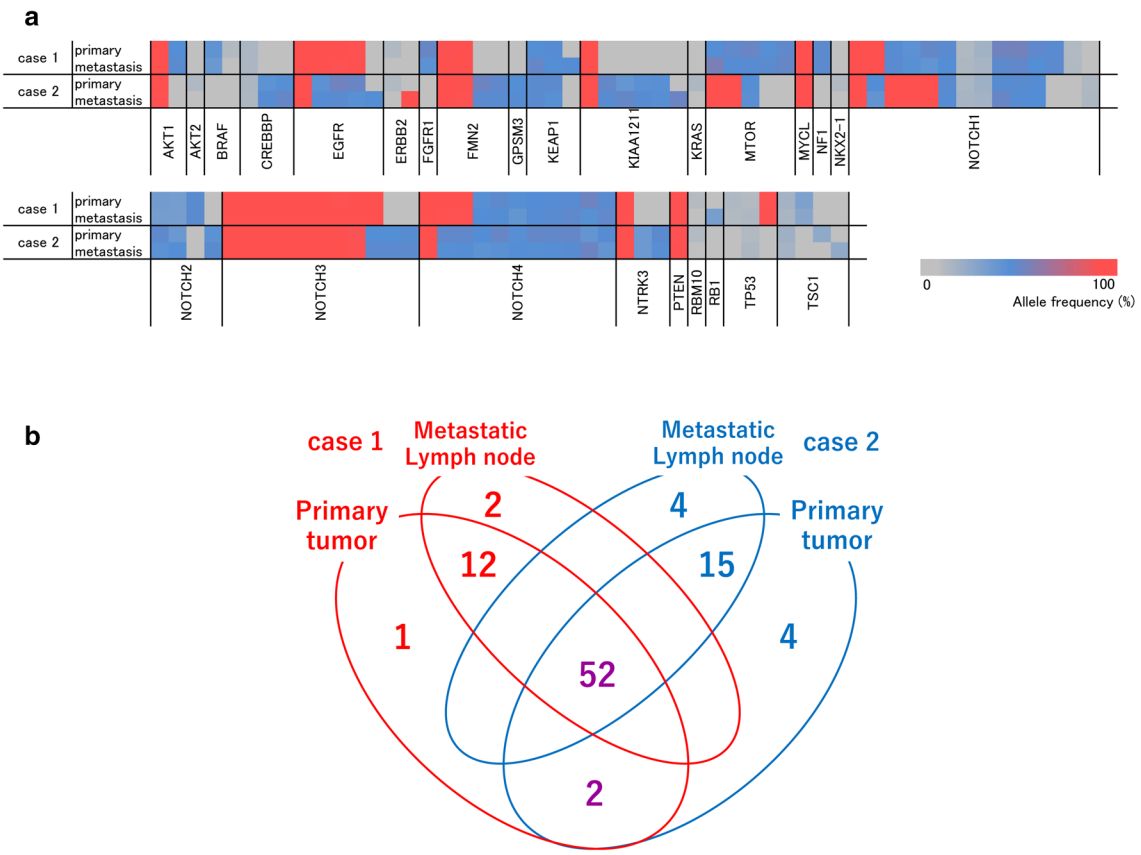

Fig. 1 NGS-based comparative analysis of mutation type and allele frequency between primary sites and metastatic LNs in two cases. **a** Heat map showing the allele frequency of each detected variant. **b** Venn diagram indicating the number of detected genetic variants in primary and/or metastatic LN

Table 1 Clinicopathological characteristics of pN1–2 adenocarcinoma cases ($N=69$)

Clinicopathological characteristic	Cases ($N=69$)
Age, years	
Median (range)	68 (45–89)
Interquartile range	13.75
Sex, *N* (%)	
Male	44 (63.8)
Female	25 (36.2)
Smoking status, *N* (%)	
Current or ex-smoker	41 (59.4)
Never smoker	28 (40.6)
Surgical procedure, *N* (%)	
Lobectomy	60 (87.0)
Segmentectomy	8 (11.6)
Wedge resection	1 (1.5)
Predominant subtype, *N* (%)	
Lepidic	4 (5.8)
Acinar	6 (8.7)
Papillary	42 (60.9)
Micropapillary	3 (4.3)
Solid	12 (17.4)
IMA	2 (2.9)
Pathological node descriptor, *N* (%)	
N1a	18 (26.1)
N1b	7 (10.1)
N2a1	14 (20.3)
N2a2	7 (10.1)
N2b	23 (33.3)
Pathological stage, *N* (%)	
IIB	22 (31.9)
IIIA	43 (62.3)
IIIB	4 (5.8)
Number of metastatic LN	
Median (range)	2 (1–35)
Interquartile range	6
Ratio of metastatic LN among resected LN, %	
Median (range)	20 (3.3–100)
Interquartile range	32
Classification by nodal zone, *N* (%)	
N1a	18 (26.1)
N1b	7 (10.1)
N2a	30 (43.5)
N2b	14 (20.3)
Adjuvant platinum doublet chemotherapy, *N* (%)	
Y	41 (59.4)
N	28 (40.6)
Targetable variant status, *N* (%)	
EGFR mutation	35 (50.7)
ALK rearrangement	4 (5.8)
EGFR/*ALK*/*BRAF*/*ROS1* negative	30 (43.5)
Pleural invasion, *N* (%)	
Y	25 (36.2)
N	44 (63.8)
Lymphatic invasion, *N* (%)	
Y	47 (68.1)
N	22 (31.9)
Vascular invasion, *N* (%)	
Y	41 (59.4)
N	28 (40.6)
Recurrence, *N* (%)	
Y	47 (68.1)
N	22 (31.9)
TKI therapy after recurrence, *N* (%)	
Y	27 (57.4)
N	20 (42.6)

IMA invasive mucinous adenocarcinoma, *LN* lymph node, *N* no, *TKI* tyrosine kinase inhibitor, *Y* yes

Among 39 *EGFR*/*ALK*-positive pN1–2 cases, LN tissues were available for 30 cases and the same *EGFR*/*ALK*-positive status was confirmed in 29 cases (concordance ratio 96.7%).

RFS and OS were evaluated in *EGFR*/*ALK*-positive and *EGFR*/*ALK*-negative cases. PRS and OS were evaluated in patients who received TKI therapy after recurrence. Genetic results and populations for prognostic comparisons are summarised in Fig. 2.

The median RFS were 16.9 and 20.5 months for *EGFR*/*ALK*-positive and -negative cases, respectively [hazard ratio (HR) 1.80, 95% confidence interval (CI) 1.02–3.20; $P=0.043$]. The median PRS were 34.5 months and 18.7 months for cases with and without TKI therapy, respectively (HR 0.44, 95% CI 0.14–0.91; $P=0.032$). There was no significant difference in OS between *EGFR*/*ALK*-positive and -negative cases (median OS: 46.3 months vs. 38.5 months, respectively, HR 0.99, 95% CI 0.45–2.20; $P=0.99$) or with and without TKI therapies cases (median OS: 49.3 months vs. 38.5 months, respectively, HR 0.65, 95% CI 0.27–1.46; $P=0.28$) (Fig. 3).

Univariate analyses revealed that the ratio of metastatic LNs and *EGFR*/*ALK*-positive status were significantly associated with poorer RFS, and no TKI therapy after recurrence was associated with shorter PRS. Multivariate analysis revealed that male sex, no adjuvant platinum doublet chemotherapy, ratio of metastatic LNs, and *EGFR*/*ALK*-positive status were predictive factors for worse RFS (for male sex, no adjuvant platinum doublet chemotherapy, ratio of metastatic LN, and positive *EGFR*/*ALK* status, HR 2.276, 1.912, 1.012, and 2.366; 95% CI 1.141–4.542, 1.042–3.511,

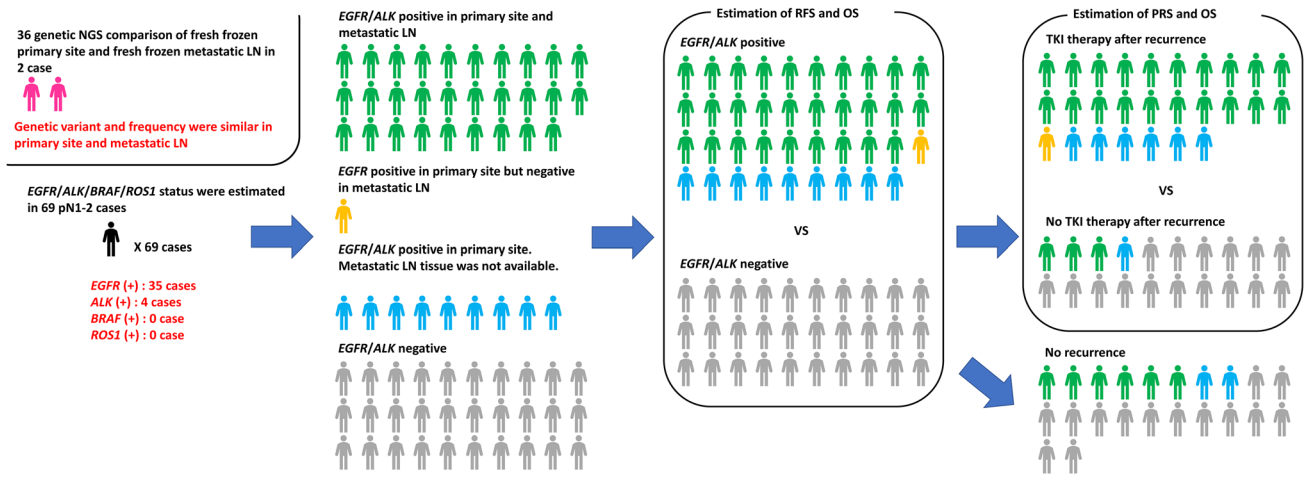

Fig. 2 Summary of genetic variants and populations for prognostic comparison

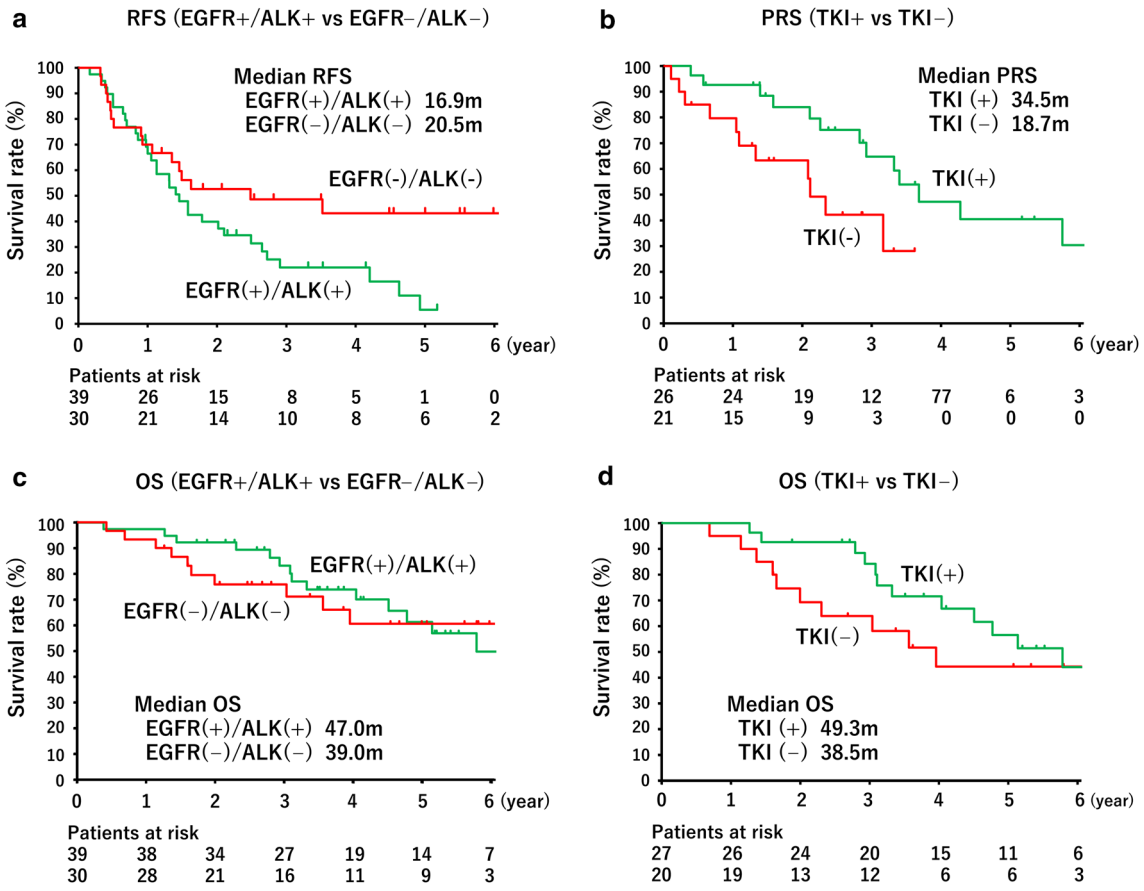

Fig. 3 RFS, PRS, and OS curves based on *EGFR* mutation/*ALK* rearrangement status or TKI treatment. **a** RFS curves according to *EGFR*/*ALK* status. **b** PRS curves according to post-recurrence TKI therapies. **c** OS curves according to *EGFR*/*ALK* status. **d** OS curves according to post-recurrence TKI therapies

1.001–1.024, and 1.244–4.500; $P = 0.020$, 0.037, 0.033, and 0.009, respectively) (Table 2). N2 metastatic status and no TKI therapies after recurrence were predictive factors for shorter PRS (for N2 status and no post-recurrence TKI therapies, HR 3.652 and 3.740; 95% CI 1.022–13.047 and 1.449–9.650; $P = 0.046$ and 0.006, respectively) (Table 3).

Table 2 Univariate and multivariate analyses of RFS

Prognostic factor	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age	1.021 (0.995–1.048)	0.115	1.017 (0.982–1.054)	0.341
Male sex	1.435 (0.786–2.618)	0.239	2.276 (1.141–4.542)	0.020*
Sublobar resection	1.165 (0.519–2.614)	0.711	0.800 (0.344–1.860)	0.604
No adjuvant platinum doublet therapy	1.417 (0.802–2.503)	0.230	1.912 (1.042–3.511)	0.037*
MP/solid/IMA predominance	0.789 (0.391–1.589)	0.506	0.986 (0.401–2.425)	0.975
Pleural invasion	1.408 (0.801–2.476)	0.234	1.167 (0.630–2.162)	0.624
Lymphatic invasion	1.129 (0.620–2.056)	0.691	1.089 (0.515–2.303)	0.824
Vascular invasion	1.284 (0.715–2.306)	0.402	1.180 (0.569–2.450)	0.657
Metastasis on N2 station	1.576 (0.844–2.946)	0.154	1.131 (0.527–2.427)	0.752
Number of metastatic LNs	1.029 (0.985–1.074)	0.199	0.991 (0.930–1.057)	0.783
Ratio of metastatic LNs	1.013 (1.002–1.024)	0.017	1.012 (1.001–1.024)	0.033*
Metastasis on N2b zone	1.837 (0.952–3.542)	0.070	1.449 (0.706–2.976)	0.312
Positive *EGFR*/*ALK*	1.850 (1.009–3.393)	0.047	2.366 (1.244–4.500)	0.009*

IMA invasive mucinous adenocarcinoma, *LNs* lymph nodes, *MP* micropapillary

*$P < 0.05$

Table 3 Univariate and multivariate analyses of PRS

Prognostic factor	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age	1.040 (0.997–1.086)	0.069	1.032 (0.988–1.077)	0.156
Male sex	1.016 (0.440–2.347)	0.971	1.044 (0.994–1.097)	0.084
Sublobar resection	0.649 (0.189–2.230)	0.492	0.646 (0.176–2.367)	0.510
MP/solid/IMA predominance	1.180 (0.338–4.119)	0.796	0.604 (0.161–2.261)	0.454
Pleural invasion	0.848 (0.379–1.898)	0.688	0.549 (0.214–1.411)	0.213
Lymphatic invasion	2.131 (0.840–5.407)	0.111	1.778 (0.686–4.609)	0.237
Vascular invasion	1.676 (0.704–3.991)	0.244	1.315 (0.462–3.744)	0.608
Metastasis on N2 station	2.211 (0.654–7.472)	0.202	3.652 (1.022–13.047)	0.046*
Number of metastatic LNs	1.023 (0.959–1.091)	0.488	1.007 (0.908–1.116)	0.895
Ratio of metastatic LNs	1.006 (0.991–1.021)	0.422	1.004 (0.984–1.025)	0.698
Metastasis on N2b zone	1.303 (0.558–3.042)	0.541	0.708 (0.266–1.883)	0.489
Positive *EGFR*/*ALK*	0.483 (0.201–1.162)	0.104	0.852 (0.149–4.855)	0.857
No post-recurrence TKI therapy	2.551 (1.056–6.162)	0.037	3.740 (1.449–9.650)	0.006*

IMA invasive mucinous adenocarcinoma, *LNs* lymph nodes, *MP* micropapillary, *TKI* tyrosine kinase inhibitor

*$P < 0.05$

Discussion

Lung cancer with metastatic LN is no longer early-stage. Nodal-positive cases are conventionally classified as N1, N2, or N3 according to the location of the metastatic LN. Nodal-positive cases vary with respect to prognosis; new classification methodologies have been proposed (Wei et al. 2011; Jonnalagadda et al. 2011; Saji et al. 2011; Wisnivesky et al. 2011; Rusch et al. 2007, 2009) and their usefulness has been compared (Ito et al. 2013; Lee et al. 2016). Nodal-positive cases have a high risk of recurrence even after complete resection, and PRS is prolonged by EGFR–TKI treatment (Takenaka et al. 2015; Jeon et al. 2015; Shimada et al. 2013). Nevertheless, a classification that considers the post-recurrence course of treatment has not been proposed. We focused on the therapeutic course after recurrence and propose a classification method for nodal-positive cases based on targetable genetic features.

Reported concordances of genetic status between primary and metastatic sites vary according number of invested genes or utilized methodologies. Several studies have compared the genetic status of primary and metastatic sites, focusing on one to three genes, and have reported a wide range of

concordance ratios of 26.7–100% (Kalikaki et al. 2008; Park et al. 2009; Cortot et al. 2010; Schmid et al. 2009; Matsumoto et al. 2006). A well-designed study (Yatabe et al. 2011) and another study using an NGS approach (Vignot et al. 2013) concluded that genetic discordance between primary and metastatic sites is rare. Xie et al. also used NGS and showed that driver gene mutations correspond completely (100%) between primary sites and metastatic LNs when copy number variation is not considered (Xie et al. 2018). The results of these three studies were similar to ours. We utilized resected fresh frozen tissues with sufficient volumes and found that both variant types and allele frequencies were similar between primary sites and metastatic LNs for 36 genes. Although previous studies have not indicated the association between genetic heterogeneity or homogeneity on prognosis, we demonstrated the clinical usefulness of genetic similarity for predicting RFS and PRS. Our results suggested clinical usefulness on the genetic variant-based stratification of nodal-positive cases.

Previous studies have proposed classification methodologies based on characteristics of resected metastatic LNs, for example, the number, ratio, or zone of metastatic LNs (Wei et al. 2011; Jonnalagadda et al. 2011; Saji et al. 2011; Wisnivesky et al. 2011; Rusch et al. 2007, 2009). They assessed status of the whole metastatic LNs. Genetic analyses of all resected LNs are not clinically practical. However, our results suggested that analysis of whole LNs is not necessary, as long as the genetic status of primary site is evaluated. In current study, *EGFR* mutation and *ALK* rearrangement are related to worse RFS. Regarding to proposed methodologies, the ratio of metastatic LNs was significantly related to poorer RFS but not PRS. The number or zone of metastatic LNs was not a significant predictor of worse RFS or PRS. Moreover, these previously proposed methodologies have various limitations. For example, precise counts of LNs are often difficult to obtain due to adhesion among LNs. Intraoperative location of LNs can differ even among expert thoracic surgeons (Watanabe et al. 2002). Systematic LN resection can be omitted if LN metastasis is not proven intraoperatively. The advantages of nodal classification by genetic status are that this approach is qualitative, reproducible, and does not necessarily require precise counts or locations of metastatic LNs. Moreover, the status is directly relevant to the choice of treatment after recurrence. Among 31 recurrent cases harbouring *EGFR* mutation or *ALK* rearrangement, 27 cases (87.1%) received TKI treatment and PRS was prolonged.

EGFR mutation and *ALK* rearrangement are driver genetic variants and target of molecular therapy. We previously suggested *EGFR* mutations are associated to increased risk of recurrence as driver mutations in invasive lung adenocarcinoma (Ito et al. 2018). *ALK* rearrangement is suggested to be associated with shorter disease-free survival in resected lung adenocarcinoma (Gao et al. 2017; Chaft et al. 2018). In the current study, genetic status at the recurrent site was not confirmed. The indication of TKIs were determined based on the genetic status of the primary site and PRS was prolonged by TKI therapies. This suggests that targetable driver variants are conserved not only in metastatic LN but at the site of recurrence. OS was not prolonged by TKI therapy, in part owing to the small number of cases and previous generation TKIs were used. Considering that newer TKIs have been developed with better therapeutic impact compared to the former-generation TKIs (Soria et al. 2018; Camidge et al. 2018), it is possible that a significant difference in OS will be reached in another cohort that includes more cases receiving newer TKIs.

This study had some limitations. This was a single-institution retrospective study with a small number of cases. *EGFR* mutations could not be confirmed in metastatic LN in one case, despite the positive status at the primary site. Probably due to the sampling error; the case included metastasis only on one LN. We have not evaluated whether a targetable variant confirmed at one metastatic site could be confirmed at all other metastatic sites. Further exploration is warranted to evaluate whether nodal classification based on genetic status is possible only from the metastatic site status. Additionally, the validation of mutation-based classification and the impact on OS should be evaluated by multi-centre, prospective studies including a large number of cases.

Conclusions

Similar genetic statuses were confirmed in primary sites and metastatic LNs. Cases harbouring targetable genetic variants had a higher risk of recurrence, but post-recurrence survival was prolonged by TKI therapy. Stratification of nodal-positive cases based on targetable genetic variants can be clinically useful for the prediction of recurrence and choice of treatment.

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Compliance with ethical standards

Conflict of interest All authors declare no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institu-

tional 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.

References

Camidge DR, Kim HR, Ahn MJ, Yang JC, Han JY, Lee JS et al (2018) Brigatinib versus crizotinib in ALK-positive non-small-cell lung cancer. N Engl J Med 379:2027–2039. https://doi.org/10.1056/NEJMoa1810171

Chaft JE, Dagogo-Jack I, Santini FC, Eng J, Yeap BY, Izar B et al (2018) Clinical outcomes of patients with resected, early-stage ALK-positive lung cancer. Lung Cancer 122:67–71. https://doi.org/10.1016/j.lungcan.2018.05.020

Cortot AB, Italiano A, Burel-Vandenbos F, Martel-Planche G, Hainaut P (2010) KRAS mutation status in primary nonsmall cell lung cancer and matched metastases. Cancer 116:2682–2687. https://doi.org/10.1002/cncr.25014

Gao Q, Li P, Jiang X, Zhan Z, Yan Q, Zhang B et al (2017) Worse disease-free, tumor-specific, and overall survival in surgically-resected lung adenocarcinoma patients with *ALK* rearrangement. Oncotarget 8:86066–86081. https://doi.org/10.18632/oncotarget.20973

Ito M, Yamashita Y, Tsutani Y, Misumi K, Harada H, Miyata Y et al (2013) Classifications of n2 non-small-cell lung cancer based on the number and rate of metastatic mediastinal lymph nodes. Clin Lung Cancer 14:651–657. https://doi.org/10.1016/j.cllc.2013.04.012

Ito M, Miyata Y, Hirano S, Kimura S, Irisuna F, Ikeda K et al (2017) Therapeutic strategies and genetic profile comparisons in small cell carcinoma and large cell neuroendocrine carcinoma of the lung using next-generation sequencing. Oncotarget 8:108936–108945. https://doi.org/10.18632/oncotarget.22426

Ito M, Miyata Y, Kushitani K, Yoshiya T, Kai Y, Tsutani Y et al (2018) Increased risk of recurrence in resected EGFR-positive pN0M0 invasive lung adenocarcinoma. Thorac Cancer 9:1594–1602. https://doi.org/10.1111/1759-7714.12866

Jeon JH, Kang CH, Kim HS, Seong YW, Park IK, Kim YT (2015) Prognostic and predictive role of epidermal growth factor receptor mutation in recurrent pulmonary adenocarcinoma after curative resection. Eur J Cardiothorac Surg 47:556–562. https://doi.org/10.1093/ejcts/ezu177

Jonnalagadda S, Smith C, Mhango G, Wisnivesky JP (2011) The number of lymph node metastases as a prognostic factor in patients with N1 non-small cell lung cancer. Chest 140:433–440. https://doi.org/10.1378/chest.10-2885

Kalemkerian GP, Narula N, Kennedy EB, Biermann WA, Donington J, Leighl NB et al (2018) Molecular testing guideline for the selection of patients with lung cancer for treatment with targeted tyrosine kinase inhibitors: American Society of Clinical Oncology endorsement of the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology Clinical Practice Guideline Update. J Clin Oncol 36:911–919. https://doi.org/10.1200/JCO.2017.76.7293

Kalikaki A, Koutsopoulos A, Trypaki M, Souglakos J, Stathopoulos E, Georgoulias V et al (2008) Comparison of EGFR and K-RAS gene status between primary tumours and corresponding metastases in NSCLC. Br J Cancer 99:923–929. https://doi.org/10.1038/sj.bjc.6604629

Lee GD, Kim DK, Moon DH, Joo S, Hwang SK, Choi SH et al (2016) A comparison of the proposed classifications for the revision of N descriptors for non-small-cell lung cancer. Eur J Cardiothorac Surg 49:580–588. https://doi.org/10.1093/ejcts/ezv134

Matsumoto S, Takahashi K, Iwakawa R, Matsuno Y, Nakanishi Y, Kohno T et al (2006) Frequent EGFR mutations in brain metastases of lung adenocarcinoma. Int J Cancer 119:1491–1494. https://doi.org/10.1002/ijc.21940

Nagai Y, Miyazawa H, Huqun Tanaka T, Udagawa K, Kato M et al (2005) Genetic heterogeneity of the epidermal growth factor receptor in non-small cell lung cancer cell lines revealed by a rapid and sensitive detection system, the peptide nucleic acid-locked nucleic acid PCR clamp. Cancer Res 65:7276–7282. https://doi.org/10.1158/0008-5472.CAN-05-0331

Park S, Holmes-Tisch AJ, Cho EY, Shim YM, Kim J, Kim HS et al (2009) Discordance of molecular biomarkers associated with epidermal growth factor receptor pathway between primary tumors and lymph node metastasis in non-small cell lung cancer. J Thorac Oncol 4:809–815. https://doi.org/10.1097/JTO.0b013e3181a94af4

Rami-Porta R, Call S, Dooms C, Obiols C, Sánchez M, Travis WD et al (2018) Lung cancer staging: a concise update. Eur Respir J 17:1800190. https://doi.org/10.1183/13993003.00190-2018

Rusch VW, Crowley J, Giroux DJ, Goldstraw P, Im JG, Tsuboi M et al (2007) The IASLC Lung Cancer Staging Project: proposals for the revision of the N descriptors in the forthcoming seventh edition of the TNM classification for lung cancer. J Thorac Oncol 2:603–612. https://doi.org/10.1097/JTO.0b013e31807ec803

Rusch VW, Asamura H, Watanabe H, Giroux DJ, Rami-Porta R, Goldstraw P et al (2009) The IASLC Lung Cancer Staging Project: a proposal for a new international lymph node map in the forthcoming seventh edition of the TNM classification for lung cancer. J Thorac Oncol 4:568–577. https://doi.org/10.1097/JTO.0b013e3181a0d82e

Saji H, Tsuboi M, Yoshida K, Kato Y, Nomura M, Matsubayashi J et al (2011) Prognostic impact of number of resected and involved lymph nodes at complete resection on survival in non-small cell lung cancer. J Thorac Oncol 6:1865–1871. https://doi.org/10.1097/JTO.0b013e31822a35c3

Schmid K, Oehl N, Wrba F, Pirker R, Pirker C, Filipits M (2009) EGFR/KRAS/BRAF mutations in primary lung adenocarcinomas and corresponding locoregional lymph node metastases. Clin Cancer Res 15:4554–4560. https://doi.org/10.1158/1078-0432.CCR-09-0089

Shimada Y, Saji H, Yoshida K, Kakihana M, Honda H, Nomura M et al (2013) Prognostic factors and the significance of treatment after recurrence in completely resected stage I non-small cell lung cancer. Chest 143:1626–1634. https://doi.org/10.1378/chest.12-1717

Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH et al (2018) Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. N Engl J Med 378:113–125. https://doi.org/10.1056/NEJMoa1713137

Takenaka T, Takenoyama M, Yamaguchi M, Toyozawa R, Inamasu E, Kojo M et al (2015) Impact of the epidermal growth factor receptor mutation status on the post-recurrence survival of patients with surgically resected non-small-cell lung cancer. Eur J Cardiothorac Surg 47:550–555. https://doi.org/10.1093/ejcts/ezu227

Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y et al (2011) International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society international multidisciplinary classification of lung adenocarcinoma. J Thorac Oncol 6:244–285. https://doi.org/10.1097/JTO.0b013e318206a221

Travis WD, Brambilla E, Nicholson A et al (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(9):1243–1260

Vignot S, Frampton GM, Soria JC, Yelensky R, Commo F, Brambilla C et al (2013) Next-generation sequencing reveals high concordance

of recurrent somatic alterations between primary tumor and metastases from patients with non-small-cell lung cancer. J Clin Oncol 31:2167–2172. https://doi.org/10.1200/JCO.2012.47.7737

Watanabe S, Ladas G, Goldstraw P (2002) Inter-observer variability in systematic nodal dissection: comparison of European and Japanese nodal designation. Ann Thorac Surg 73:245–248 **(discussion 248–249)**

Wei S, Asamura H, Kawachi R, Sakurai H, Watanabe S (2011) Which is the better prognostic factor for resected non-small cell lung cancer: the number of metastatic lymph nodes or the currently used nodal stage classification? J Thorac Oncol 6:310–318. https://doi.org/10.1097/JTO.0b013e3181ff9b45

Wisnivesky JP, Arciniega J, Mhango G, Mandeli J, Halm EA (2011) Lymph node ratio as a prognostic factor in elderly patients with pathological N1 non-small cell lung cancer. Thorax 66:287–293. https://doi.org/10.1136/thx.2010.148601

Xie F, Zhang Y, Mao X, Zheng X, Han-Zhang H, Ye J et al (2018) Comparison of genetic profiles among primary lung tumor, metastatic lymph nodes and circulating tumor DNA in treatment-naïve advanced non-squamous non-small cell lung cancer patients. Lung Cancer 121:54–60. https://doi.org/10.1016/j.lungcan.2018.05.002

Yatabe Y, Matsuo K, Mitsudomi T (2011) Heterogeneous distribution of EGFR mutations is extremely rare in lung adenocarcinoma. J Clin Oncol 29:2972–2977. https://doi.org/10.1200/JCO.2010.33.3906