Mutational characteristics of bone metastasis of lung cancer

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Background: Roughly 30–40% of lung cancer (LC) patients develop bone metastasis during the course of disease. The genetic differences between primary LC and matched bone metastasis are not yet fully understood.

Methods: A total of 40 LC patients with bone metastasis were collected and 450 targeted cancer-related genes were sequenced for genomic-alteration (GA) identification.

Results: Among the 40 LC patients, 33 had adenocarcinomas and 7 had squamous cell carcinomas. The metastatic sites of the 33 lung adenocarcinomas (LUADs) were the pelvis (6 patients), spine (16 patients), and limbs (11 patients). A total of 425 and 422 GAs were detected in the primary and metastatic lesions, respectively. The most common GAs were epidermal growth factor receptor (EGFR) mutations, which had mutation rates of 85.0% and 72.5% in the primary and metastatic lesions, respectively, and *tumor protein 53 (TP53)* mutations, which had mutation rates of 52.5% and 67.5% in the primary and metastatic lesions, respectively. Metastases to the pelvis and spine were most commonly accompanied by *factor receptor substrate 2 (FRS2), cyclin-dependent kinase 4 (CDK4),* and *murine double minute 2 (MDM2)* amplification, and *cyclin-dependent kinase inhibitor 2A (CDKN2A)* deletion. The concordance between primary lung squamous cell carcinoma (LUSC) and corresponding metastasis was significantly higher than that of primary LUAD and corresponding metastasis (P=0.033). Compared to limb and pelvis metastases, the shared mutation in spine metastasis was significantly lower (P=0.016 and P=0.023, respectively). In matched primary LUSCs and bone metastasis lesions, there was no significant difference in the distribution of the tumor mutational burden (TMB) (P=0.9). Conversely, a significant difference of the TMB distribution was detected in pairs of primary LUAD and corresponding bone metastasis lesions (P=0.021).

Conclusions: The consistency of mutation patterns between primary LC lesions and matched bone metastases may vary in terms of metastatic sites, but is very high in general. There was a significant difference in the TMB between primary LUAD and matched bone metastatic lesions. Our findings contribute to molecular understandings of primary LC and matched bone metastatic lesions.

Keywords: Bone metastasis; lung cancer (LC); next generation sequencing (NGS); genomic alterations; tumor mutational burden

Introduction

Lung cancer (LC) is one of the most common cancers in the world (1). LC patients are prone to bone metastasis, which may be accompanied by bone pain, fracture, hypercalcemia, nerve compression and other bone-related events that seriously affect patients' quality of life (2,3). Once a tumor metastasizes to the bone, it is almost incurable and has a high mortality rate (4). However, the early detection of LC is still challenging, and most patients have bone metastasis at first diagnosis (5).

The incidence of bone metastasis in LC is about 22.3%, which is related to the location and pathological type of the primary cancer (6,7). Routine diagnostic methods include biopsy, imaging, and laboratory examination. The most common sites of bone metastasis of LC is the spine, which accounts for nearly 50% of bone metastasis (8). The application of next-generation sequencing (NGS) technology is widely used in the auxiliary diagnosis and target therapy of cancers (9,10). In recent years, with the rapid development of precision medicine in LC, the survival of patients with advanced LC has improved significantly (11). *Epidermal growth factor receptor* (*EGFR*) is the most widely used driving gene for the targeted treatment of LC, and responds well to EGFR tyrosine kinase inhibitors (12). Kondraciuk *et al.* reported the genomic characters and examined risk factors for prognosis of thyroid cancer bone metastasis (13). Previous studies have shown that micro RNA, Dickkopf1, and insulin-like growth factor binding protein 3 are potential therapeutic targets for LC bone metastasis (14).

There is heterogeneity between primary lung and bone metastases. Previous studies have shown that bone metastasis of LC may be related to mutations in primary LC. Taverna *et al.* reported that interactions between the EGFR gene pathway and bone microenvironment affect the bone metastasis of LC (15). The mutation of the *Kirsten rat sarcoma viral oncogene homolog* (*KRAS*) gene may be associated with the postoperative recurrence and metastasis of LC (16). *Echinoderm microtubule-associated protein-like 4 anaplastic lymphoma kinase* (*EML4-ALK*) fusion is common in LC patients with distant organ metastasis, which suggests that the *ALK* gene fusion is involved in the bone metastasis of LC (17-19). Mukai *et al.* reported the high expression of *mesenchyma-to-epithelial transition* (*MET*) in both primary and bone metastasis of LC patients (20), and suggested that drugs targeted at *MET* amplification, such as crizotinib and cabotinib, would have a certain effect on LC patients with bone metastasis.

Many reports have focused on the molecular mediators of non-small-cell lung carcinoma bone metastasis; however, its underlying mechanism remains unclear. In this study, we aimed to analyze the molecular differences between the primary LC and the matched bone metastases and provide evidence for the precise treatment of bone metastasis of LC. We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/apm-21-1595).

Methods

Patient enrollment and sample collection

A total of 40 LC patients with bone metastasis at The Second Affiliated Hospital of Zhejiang University School of Medicine were enrolled in this study. Formalin-fixed paraffin-embedded (FFPE) tumor tissues from both primary and bone metastatic lesions were collected and transferred to OrigiMed, Shanghai for genetic variation detection. Matched blood samples were also collected as a control. Genomic deoxyribonucleic acid (DNA) was prepared using the QIAamp DNA FFPE Tissue Kit and the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. The concentration of DNA was measured by Qubit and normalized to 20–50 ng/μL. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethical Committee of The Second Affiliated Hospital of Zhejiang University School of Medicine (No. 2020143), and informed consent was provided by all patients.

Identification of GAs and the TMB

The genomic mutations were identified using the NGS-based YuanSu450 gene panel (OrigiMed, Shanghai, China), which covers the coding exons of 450 cancer-related genes and 64 selected introns in 39 genes that are frequently rearranged in solid tumors. The genes were captured and sequenced with a mean depth of 800× using the Illumina NextSeq 500. Genomic alterations (GAs), including single nucleotide variants (SNVs), insertion-deletion polymorphisms (Indels), copy number variation, were identified using MuTect (v.1.17), PINDEL (v.2.04), and Control-FREEC (v9.4), respectively. Gene fusions were detected through an in-house pipeline, and gene rearrangements were assessed by the Integrative Genomics

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Table 1 Clinicopathologic features of the cohort

Variable	n (%)
Total	40
Gender	
Male	25 (62.5)
Female	15 (37.5)
Age, median [range]	63 [45–83]
Smoking status	
Smoking	18 (45.0)
Nonsmoking	22 (55.0)
TMB, median [range]	
Primary	3.7 [0.6–41.4]
Metastases	3.1 [0–0.7]
Primary subtype	
Lung adenocarcinoma	33 (82.5)
Lung squamous cell carcinoma	7 (17.5)
Bone metastases loci	
Pelvis	7 (17.5)
Spine	17 (42.5)
Limb	16 (40.0)

TMB, tumor mutational burden.

Viewer (IGV). The tumor mutational burden (TMB) was estimated by counting the somatic mutations, including SNVs and Indels, per mega base of the sequence examined in each patient. Driver mutations and known germline alterations were not counted.

Statistical analysis

The statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). Fisher's exact test was used for the significant differences analyses. A $P<0.05$ was considered statistically significant.

Results

Patient characteristics

Forty LC patients with bone metastases were enrolled in this study. Among them, there were 25 males and 15 females, 18 (45.0%) patients had a history of smoking for

>20 years and 22 (55.0%) never smoked. Patients had a median age of 63 years (range, 45–83 years). According to the primary subtype, 33 (82.5%) patients had lung adenocarcinoma (LUAD) and 7 (17.5%) had lung squamous cell carcinoma (LUSC). The bone metastasis sites of this cohort included 7 (17.5%) pelvis, 17 (42.5%) spine, and 16 (40.0%) limb sites (see *Table 1*).

Different characterization of GAs between primary LC and bone metastasis

A total of 604 clinically relevant GAs were evaluated in this cohort (see *Figure 1*). The mutational characteristics of LUSC and LUAD were different. For example, the amplifications of *mitogen-activated protein kinase 13 (MAP3K13)*, *phosphatidylinositol 3-kinase catalytic alpha polypeptide (PIK3CA)*, *B-cell lymphoma 6 (BCL6)*, *E26 transformation-specific variant transcription factor 5 (ETV5)*, and *Kelch-like protein 6 (KLHL6)* were more frequent in LUSC than LUAD (see *Figure 1*). Only 2 patients had the *KRAS* G12V mutation for which the corresponding primary tumor was LUAD.

In primary tissues, the most common mutated genes included *EGFR* (62.5%, 25/40), *tumor protein 53 (TP53)* (50.0%, 20/40), *cyclin-dependent kinase inhibitor 2A (CDKN2A)*, *high mobility group protein 2 (HMGA2)*, and *murine double minute 2 (MDM2)* (15.0%, 6/40, for each), and *mucin 16 (MUC16)*, *cyclin-dependent kinase 4 (CDK4)*, *v-myc avian myelocytomatosis viral oncogene homolog (MYC)*, *protein kinase C iota (PRKCI)*, and *Tetraspanin 31 (TSPAN31)* (12.5%, 5/40, for each). The bone metastasis samples from 39 patients were successfully sequenced, and the most common mutated genes include *EGFR* and *TP53* (64.1%, 25/39, for both), *PRKCI* (17.9%, 7/39), *factor receptor substrate 2 (FRS2)*, *MAP3K13*, and *PIK3CA* (15.4%, 6/39, for each), and *BCL6*, *CDKN2A*, *CDKN2B*, *ETV5*, *KLHL6*, *MUC16*, *SRY-Box Transcription Factor 2 (SOX2)*, and *telomerase ribonucleic acid component (TERC)* (12.8%, 5/39, for each). In this cohort, 235 (40.2%, 235/585) GAs were shared in paired primary lesions and bone metastases, 165 (28.2%, 165/585) GAs were specifically detected in primary lesions, and 185 (31.6%, 185/585) GAs were specifically detected in bone metastases. However, no mutation was associated with bone metastases and primary tumors.

Association between mutated genes and metastatic locations in LUAD
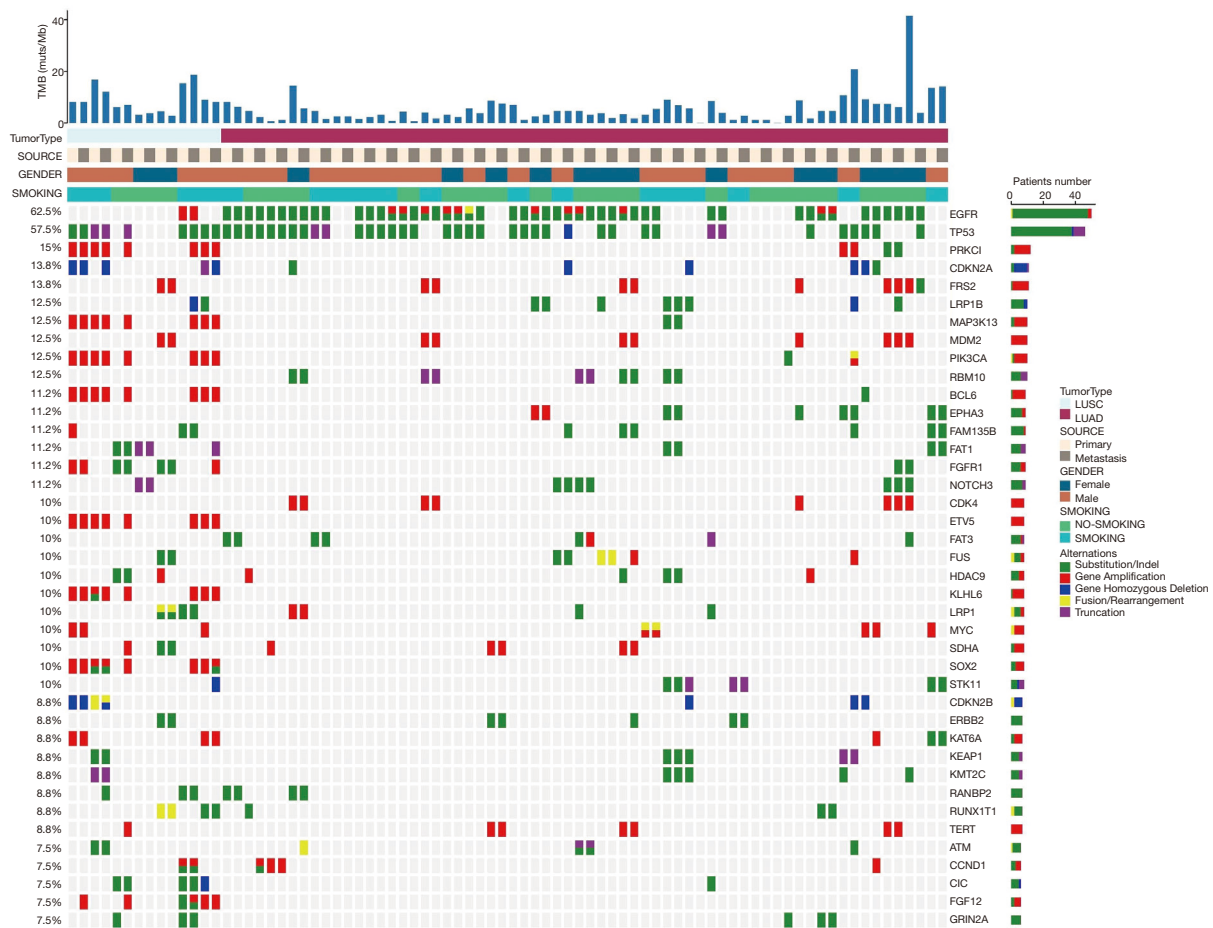
Given the heterogeneity between LUSC and LUAD

Figure 1 Mutational landscape of paired primary tumors and bone metastases of 40 LC patients. The X-axis shows each case sample, and the Y-axis shows each mutated gene. The upper bar graph shows the TMB values of the patients. The bar graph on the right shows the number of each mutated gene. Tumor type: blue represents LUSC and red represents LUAD; Source: light yellow represents primary tumors and grey represents bone metastases; Gender, blue represents female and brown represents male; Smoking: green represents no-smoking and blue represents smoking; Alterations: green represents substitution/indel mutations, red represents gene amplification mutations, blue represents gene homozygous deletion mutations, yellow represents fusion/rearrangement mutations, and purple represents truncation mutations. LC, lung cancer; TMB, tumor mutational burden; LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma.

and the small number of samples, we selected 33 patients with primary LUAD tumors to further investigate the molecular characterizations of different metastatic loci. The mutational profiling of LUAD showed a higher frequency of *FRS2*, *CDK4*, and *MDM2* amplification, and the homozygous deletion of *CDKN2A* in patients with pelvis and spine metastases, but not in those with the limb metastases (see *Figure 2*). Based on the primary and metastatic mutational profile of each patient, the mean frequency of shared mutations of patients with limb, pelvic, and spine metastases were 63.1% (range, 0.0–91.7%),

58.4% (range, 44.4–80.0%), and 38.7% (range, 5.26–80.0%), respectively. A statistical analysis showed that the frequency of shared mutations in the spine metastases was significantly lower than the frequency in the limbs and the pelvis (P=0.016 and P=0.023, respectively; see *Figure 3*).

Decreased TMB in LUAD with bone metastasis

To explore the relationship between the TMB and metastatic loci, available TMB values were measured from both primary and metastatic samples. The median TMB
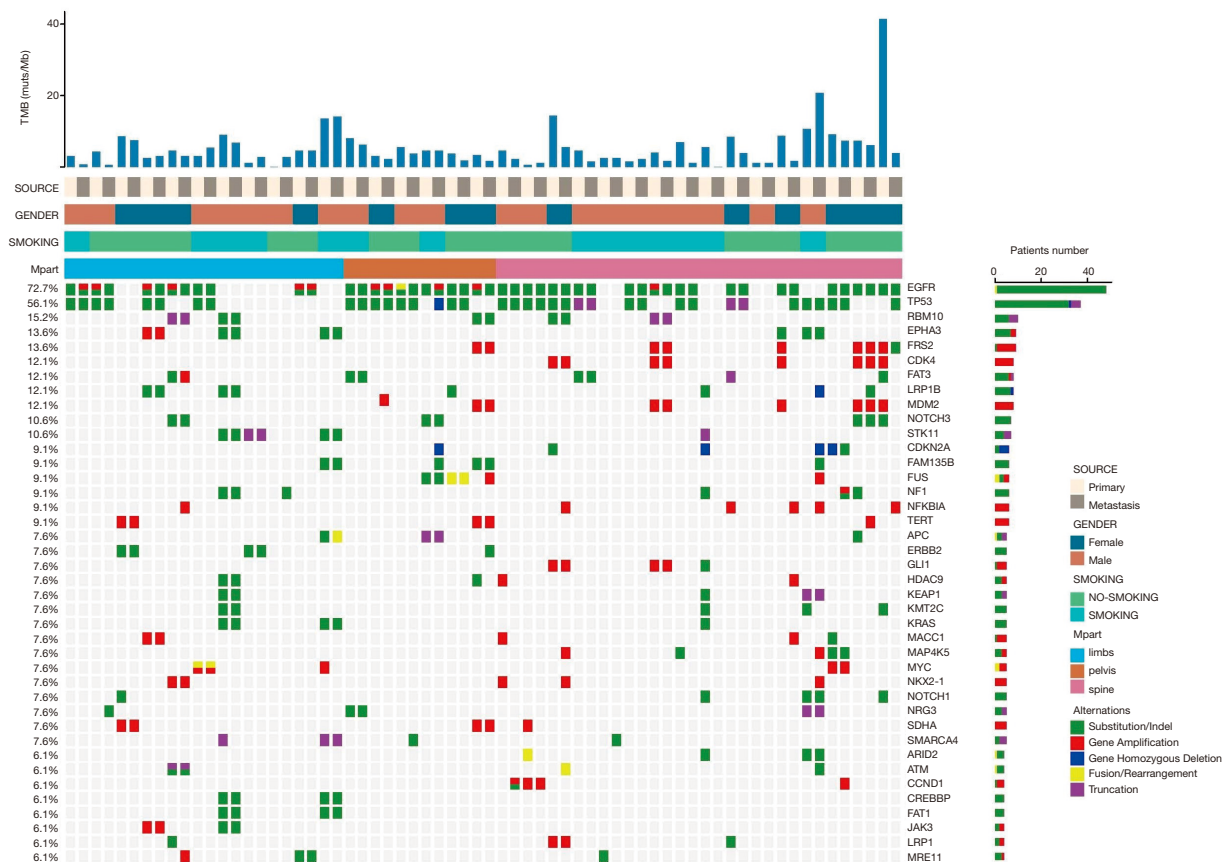
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Figure 2 Mutational landscape of paired primary tumor and bone metastases of 33 LUAD patients. The X-axis shows each case sample, and the Y-axis shows each mutated gene. The upper bar graph shows the TMB value of the patients. The bar graph on the right shows the number of each mutated gene. Source: light yellow represents primary tumors and grey represents bone metastases. Gender, blue represents female and brown represents male. Smoking: green represents no-smoking and blue represents smoking. Mpart: blue represents limb metastasis, red represents pelvis metastasis, and pink represents spine metastasis. Alterations: green represents substitution/indel mutations, red represents gene amplification mutations, blue represents gene homozygous deletion mutations, yellow represents fusion/rearrangement mutations, and purple represents truncation mutations. LUAD, lung adenocarcinoma; TMB, tumor mutational burden.

values of primary and metastatic tumors were 3.7 and 3.1 muts/Mb, respectively. According to the subtypes of primary lesions, our results showed that the TMB value of bone metastasis was significantly lower than that of primary LUAD (P=0.021), but there was no significant difference in TMB values between bone metastasis and primary LUSC (P=0.9; see *Figure 4A*). Additionally, the TMB value of the bone metastasis of LUAD was also significantly lower than that of bone metastasis of LUSC (P=0.0098; see *Figure 4A*). We also compared the TMB value in different metastatic loci of LUAD, but no significant difference was found (see *Figure 4B*).

Discussion

Different subtypes of LC have preferential metastatic sites, such as liver metastasis of small cell LC, and brain metastasis of small cell LC and adenocarcinoma (21,22). The percentage of bone metastases in LC patients ranges from 20% to 40% (23). Research on the epidemiological and prognostic factors of LC bone metastasis has progressed (24,25); however, the molecular characterization of LC bone metastasis remains unclear. To evaluate the difference in molecular characteristics between primary LC and bone metastasis, we performed NGS detections on the primary

and bone metastasis samples of 40 patients, which included 33 LUAD and 7 LUSC samples.

Heterogeneity widely exists in primary tumors and bone metastases. Krawczyk *et al.* reported that the mutation rate of *EGFR* in bone metastases was 75%, but only 12.8% in primary tumors (26). *EGFR* mutations are common in bone
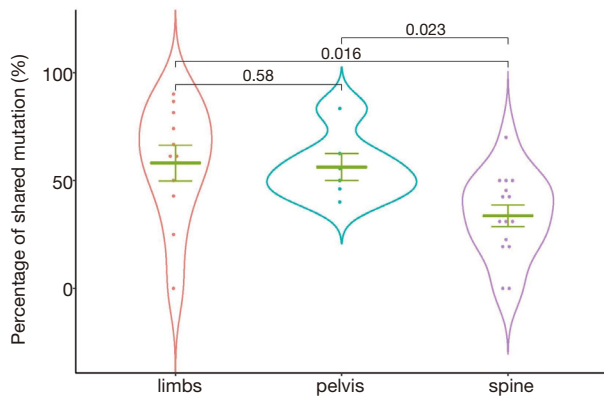

Figure 3 Comparison of the consistency of mutations between different metastasis sites and primary LUAD. The X-axis represents the different metastasis sites, and the Y-axis represents the consistency of mutations for each patient. LUAD, lung adenocarcinoma.

metastases of LUAD (27,28). Guan *et al.* showed that the incidence of bone metastasis in patients with the *EGFR* mutation was higher than those with *EGFR* wild-type mutation (29). In the present study, our results supported the wide heterogeneity of primary and metastatic lesions, and found only 40% of coexisting mutations. Among the mutations that were only detected in the primary tumor or corresponding metastasis of each patient, the *EGFR* mutation was still the most common mutation. The statistical analysis did not reveal any mutations associated with primary tumor or bone metastasis. This may be due to the small sample size of this cohort; future studies should be conducted with larger cohorts.

The *KRAS* gene is a factor of the mitogen-activated protein kinase (MAPK) pathway, which is downstream of the EGFR, and its mutation can promote cancer cell development (30). *KRAS* mutations commonly occur in about 30% of LUADs and 5% of LUSCs, and mutations in exon 12 are the most frequent (31). Different subtypes of *KRAS* mutations are associated with different types of metastasis. Previous studies have shown that the *KRAS* G12C mutation occurs more frequently in western patients with developed bone metastasis, while the *KRAS* G12V mutation occurs more frequently in western patients with developed pleuro-pericardial metastasis (32,33).
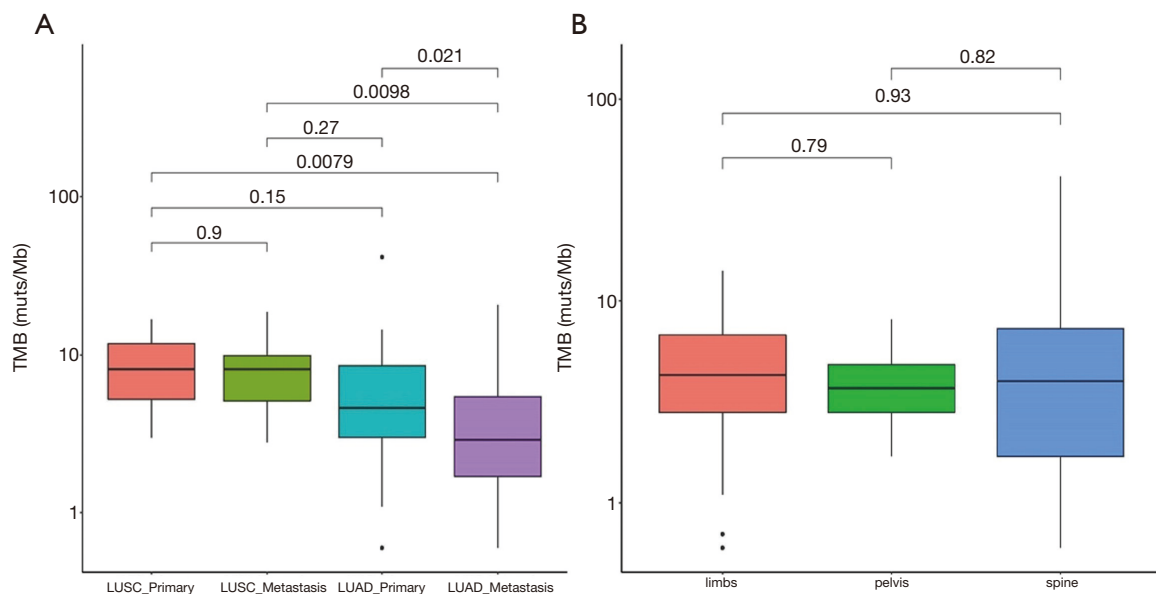

Figure 4 Distribution of TMB in this cohort. (A) Comparison of TMB value between the primary tumor and metastasis sites. (B) The distribution of TMB in different metastatic sites of LUAD. The X-axis represents the different primary sites or bone metastatic sites, and the Y-axis represents the TMB value of each sample. TMB, tumor mutational burden; LUAD, lung adenocarcinoma.

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Interestingly, while all the samples in this cohort came from LC patients with bone metastasis, only 3 patients harbored *KRAS* G12V mutation, including a patient who failed to detect genomic detection of bone metastasis sample. This suggests that the *KRAS* mutation is not characteristic in Chinese LC patients with bone metastasis, and that there are different mechanisms between the Chinese and Western patient groups.

The TMB represents the number of somatic mutations per mega base of tumor DNA and may further guide the selection of checkpoint inhibitors for patients (34). A high TMB has been reported to be associated with the generation of neoantigens and potential clinical responses to immunotherapies (35). In a clinical LC trial, patients with a high TMB benefited significantly from immunotherapy, which improved progression-free survival and response rates (36). Given the heterogeneity of primary and metastatic tumors, we also compared the TMB value between primary and metastatic tumors. Interestingly, the TMB decreased significantly after bone metastasis in LUAD, but no significant TMB difference was found between primary LUSC and its bone metastasis. These results suggest that the benefits of immunotherapy may be affected if LUAD has metastasized to the bone.

Bone metastasis of LC commonly occurred in the spine and pelvis, followed by the limbs. Tani *et al.* reported that mesometastasis, which is defined as bone metastasis of the forearm or lower leg, might be a poor prognostic factor (37). A recent systematic meta-analysis identified several prognostic factors, including age, gender, disease factors, and increasing the time from diagnosis to surgery (38). In the present study, our results revealed that the metastatic spine had a higher heterogeneity than metastatic pelvis or limbs. The heterogeneity of a tumor may affect the outcome of a targeted therapy (39). We deduced that this heterogeneity may be a potential factor for the poor prognosis of metastatic spine. However, neither significant GAs nor significant different TMB were detected among the metastatic spine, pelvis, and limbs.

Recently, great progress has been made in targeted therapy such as EGFR tyrosine kinase inhibitors (TKIs) in lung cancer (40). The treatment of the bone metastasis of lung cancer is challenging. When the metastatic bone lesion was identified, the treatment of primary lung lesion and metastatic bone lesion should both be considered. However, the question of genetic heterogeneity between the primary tumor and the corresponding bone metastases is pivotal for the treatment. In this study, our results showed the high consistency of mutation patterns between primary LC lesions and matched bone metastases. This indicate that the effective treatment of primary lung cancer may be also suitable for matched bone metastasis of lung cancer, such as the EGFR-TKI treatment for the bone metastasis of lung cancer with sensitive EGFR mutations (41,42).

In conclusion, this study identified the GAs of 40 pairs of primary LC and bone metastases, revealed the decreased TMB value of bone metastasis of LUAD, and the high heterogeneity between primary LUAD and the metastatic spine. Our results suggest that heterogeneity may be a potential factor for the poor prognosis of LC patients with bone metastasis, and immunotherapy should be used with caution among LUAD patients with bone metastasis. This study had a number of limitations, including that only a small number of patients participated in this study. In the future, a large cohort study needs to be conducted to examine the precise treatment of bone metastasis of LC.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://dx.doi.org/10.21037/apm-21-1595

Data Sharing Statement: Available at https://dx.doi.org/10.21037/apm-21-1595

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/apm-21-1595). XS and QH report they are an employee of OrigiMed Co. Ltd. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work, including ensuring that questions related to the accuracy or integrity of any part of the work have been appropriately investigated and resolved. The study was conducted in accordance with the Declaration of

Helsinki (as revised in 2013). The study was approved by the Ethical Committee of The Second Affiliated Hospital of Zhejiang University School of Medicine (No. 2020143), and informed consent was provided by all patients.

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