INTRODUCTION

Brain metastases (BMs) are the most common malignant intracranial tumors in adults, and the most common primary tumors of BMs are lung cancers.1 With the development of targeted therapies, the overall survival of cancer patients is improving, and the incidence of BMs is also increasing.2 Identifying molecular features of BMs has become essential for the effective management and treatment of BMs.3,4 Because of the limited availability of BM samples, determining the molecular features for BMs is largely based on molecular testing of primary tumors or fluid biopsy. However, the molecular features are different between primary lung cancers and BMs of lung cancer.5,6 Therefore, evaluating molecular events in BM samples is critical for effective treatment.

In this study, we examined the molecular characteristics of pathological samples of BMs by determining the DNA and RNA mutational profiles of 43 cases of BM using the Oncomine™ Focus Assay.7


2 | MATERIALS AND METHODS

2.1 | Cases and clinical samples

This study included 43 cases with BMs and 1 case with glioblastoma that underwent surgical resection at Beijing Tiantan Hospital. Freshly prepared (within 2 weeks after surgery) formalin-fixed, paraffin-embedded (FFPE) samples were analyzed using the Oncomine™ Focus Assay (Thermo Fisher), as previously reported.7 The average amplicon coverage was above 5000x. The study protocol was approved by the Institutional Review Board and Ethics Committee of Beijing Tiantan Hospital.

2.2 | PCR and Sanger sequencing

PTPRZ1-MET (ZM) was detected by PCR and Sanger sequencing. The primer sequences are as follows: forward 5′-TGCCG CCTGGATAAACCTC-3′ and reverse 5′-CGTGAAGTTGGGAA GCTGA-3′. The PCR reaction was run for 50 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s, followed by final extension at 72°C for 5 min. PCR products (166 bp) were extracted from an agarose gel (1.5%) after electrophoresis and verified by Sanger sequencing.

2.3 | Immunohistochemistry

Immunohistochemistry (IHC) was performed as previously described.8,9 The primary antibodies included anti-c-MET antibody (1:500; Abcam); anti-p-MET antibody (1:200; CST); anti-Ki-67 antibody (1:100; ZSGB-Bio), anti-TTF-1 antibody, (MAB Technology), anti-CK antibody (kit-0009; MAB Technology), anti-GFAP antibody (ZSGB-Bio), and anti-Syn antibody (ZSGB-Bio).

3 | RESULTS AND DISCUSSION

We determined the DNA and RNA mutational profiles of one glioblastoma and 43 BM cases, including three BMs of breast cancer, one BM of colon cancer, one BM of rectal carcinoma, one BM of maxillary sinus, and 37 BMs of lung cancer. The case characteristics are summarized in Table 1.

We detected single-nucleotide mutations, gene copy number variations, and gene fusions in glioblastoma and BMs (Figure 1 and Table 2). Common mutational events, including EGFR amplification, EGFRvIII, and FGFR1-TACC1 fusion, were detected in the glioblastoma case. ERBB2 amplification, MYC amplification, and EGFRvIII were detected in the BMs of breast cancers. MYC amplification was observed in the BM of maxillary sinus. KRAS mutation and FGFR1 amplification were detected in the BM of colon cancer. We also detected common mutations in the BMs of lung cancer, including EGFR P.L858R, EGFR exon 19 fragment deletion, EGFR p.T790M, KRAS p.G12D, EGFR amplification, EML4_ALK (E13A20), and others (Figure 1A and Table 2).

Remarkably, we detected ZM in two of 37 BMs of lung cancer (Figure 1A and Table 2). The breakpoints of ZM in both cases are exon 1 of PTPRZ1 to exon 2 of MET (hg19, chromosome 7:121513611 to chromosome 7:116339124) (Figure 1B). We validated the presence of ZM in FFPE samples of the two cases by gel-based PCR and Sanger sequencing (Figure 1C).

The first case (Lung_13), a 56-year-old woman, received pneumonectomy of the left upper lung, gamma knife therapy, and craniotomy of the right front lobe 4 years previously. The previous BM harbored EGFR exon 19 deletion and was treated with Tarceva. A lesion was detected in the left front lobe 2 months before surgery, and the patient was treated with Atezolizumab (Figure 2A). The surgical specimen was diagnosed as BM from lung adenocarcinoma with acinar and papillary growth pattern and was positive for pan-Ck and TTF-1 in IHC.

Molecular testing of the surgical specimen showed the coexistence of EGFR p.E746_A750 (exon 19) deletion (mutant allele frequency [MAF]: 73.29%), p.T790M (MAF: 39.81%), EGFR amplification, EGFRvIII, ZM, and FGFR1-TACC1 (Table 2). The MAF of EGFR mutations suggests EGFR pT790M was a later event than EGFR pE746_A750 (exon 19) deletion. Previous studies showed that MET amplification and METex14 could be secondarily activated as a resistance mechanism for EGFR-targeted therapy.10-12 Here, ZM might be secondarily activated after EGFR-targeted therapy.

The second case (Lung_15), a 50-year-old man, was admitted to the hospital with headache and dizziness for 1 month. Preoperative MRI showed a space-occupying lesion in the left cerebellar hemisphere of the brain (Figure 2B). CT revealed a mass in the lower lobe of the left lung. Postoperative pathological examination of the brain revealed a metastatic tumor from lung, with active proliferation, positive immunostaining for pan-Ck, and diffuse nuclear TTF-1 expression. These findings suggest that ZM can also occur in untreated BMs of lung cancer.

ZM is an oncogenic variant of the MET gene that we previously identified in adult gliomas.13 The breakpoints identified in this study are consistent with our findings in glioma. ZM was proven to be a therapeutic target for secondary glioblastoma and infantile glioma.14,15 To the best of our knowledge, ZM has never been reported in other tumor types. ZM was only detected in glioma in the cancer genome atlas (TCGA) pan-cancer gene fusion dataset (Figure S1).

METex14 was detected in 16.2% (95% confidence interval [CI]: 6.2% to 32.0%) of the 37 BMs of lung cancer. This is higher than the rates reported in lung adenocarcinoma (approximately 3%) and other lung neoplasms (approximately 1%-2%).16 METex14 was proven to be a valuable therapeutic target in clinical trials of non–small cell lung cancer (including BMs).7,12 We found that METex14 occurs at a higher proportion (14%) in secondary glioblastomas than in pan-glioma (0.4%).14,16 METex14 was also detected at a higher proportion in BMs of lung cancer in our study compared with primary lung cancer in previous reports. One possible reason is the molecular divergence between BMs and primary tumors,5 and METex14 tends
TABLE 1 Clinical characteristics of cases in this study

| Sample ID | Sex  | Age | Lobe          | Lateral | Primary tumor  |
|-----------|------|-----|---------------|---------|---------------|
| Breast_1  | Female | 29  | Cerebellum    | Right   | Breast cancer |
| Breast_2  | Female | 44  | Parietal      | Right   | Breast cancer |
| Breast_3  | Female | 47  | Cerebellum    | Right   | Breast cancer |
| Colon_1   | Female | 48  | Cerebellum    | Right   | Colon cancer  |
| GBM       | Male  | 45  | Frontal/temporal | Right   | Glioma        |
| Lung_1    | Female | 49  | Frontal       | Right   | Lung cancer   |
| Lung_2    | Male  | 62  | Occipital     | Left    | Lung cancer   |
| Lung_3    | Male  | 72  | Frontal       | Left    | Lung cancer   |
| Lung_4    | Male  | 57  | Frontal (multiple lobes) | Right   | Lung cancer   |
| Lung_5    | Male  | 55  | Frontal       | Left    | Lung cancer   |
| Lung_6    | Male  | 56  | Frontal       | Right   | Lung cancer   |
| Lung_7    | Male  | 51  | Occipital     | Right   | Lung cancer   |
| Lung_8    | Male  | 52  | Frontal/parietal | Left    | Lung cancer   |
| Lung_9    | Female | 65  | Frontal       | Left    | Lung cancer   |
| Lung_10   | Male  | 76  | Occipital     | Left    | Lung cancer   |
| Lung_11   | Male  | 58  | Temporal/occipital | Right   | Lung cancer   |
| Lung_12   | Female | 53  | Frontal       | Left    | Lung cancer   |
| Lung_13   | Female | 56  | Frontal/parietal | Left    | Lung cancer   |
| Lung_14   | Female | 49  | Frontal/temporal | Left    | Lung cancer   |
| Lung_15   | Male  | 50  | Cerebellum    | Left    | Lung cancer   |
| Lung_16   | Male  | 56  | Cerebellum    | Right   | Lung cancer   |
| Lung_17   | Male  | 59  | Parietal/occipital | Right   | Lung cancer   |
| Lung_18   | Male  | 58  | Cerebellum    | Left    | Lung cancer   |
| Lung_19   | Male  | 57  | Temporal/parietal | Right   | Lung cancer   |
| Lung_20   | Male  | 57  | Occipital     | Right   | Lung cancer   |
| Maxillary_1 | Male  | 47  | Frontal       | Right   | Maxillary sinus |
| Rectal_1  | Female | 55  | Frontal       | Bilateral | Rectal carcinoma |

FIGURE 1 Molecular characteristics of brain metastases. A. The DNA and RNA mutational profile in formalin-fixed, paraffin-embedded (FFPE) samples of 43 brain metastases and a glioblastoma. B. Schematic fusion configuration of case Lung_15 showing PTPRZ1-MET fusion. C. PCR products of cases by PTPRZ1-MET (exon1-exon2) specific primers and Sanger sequencing results.
| Case ID | Molecular characteristic (copy number/fusion format/mutant allele frequency) |
|--------|--------------------------------------------------------------------------------|
| GBM    | EGFR amplification (29.54); EGFR_EGFR (E1:E8); FGFR3-TACC3 (F17T7); FGFR3-TACC3 (F17T8); FGFR3-TACC3 (F17T9) |
| Rectal_1| No alterations                                                                   |
| Colon_1| KRAS p.G12V (36.19%); FGFR1 amplification (5.74)                                   |
| Maxillary_1| MYC amplification (5.84); EGFR_EGFR (E1:E8)                                      |
| Breast_1| No alterations                                                                   |
| Breast_2| MYC amplification (8.94); EGFR_EGFR (E1:E8)                                      |
| Breast_3| ERBB2 amplification (32.33)                                                     |
| Lung_1  | EGFR 658R (50.33%)                                                               |
| Lung_2  | ERBB2 amplification (9.57)                                                       |
| Lung_3  | BRAF p.D594E (61.59%); MET_MET (M13:M15)                                         |
| Lung_4  | EGFR p.L858R (70.67%); ERBB2 amplification (6.31)                                 |
| Lung_5  | EGFR p.E746_A750 (exon 19) del (38.47%)                                          |
| Lung_6  | EGFR_EGFR (E1:E8)                                                                |
| Lung_7  | EGFR_EGFR (E1:E8)                                                                |
| Lung_8  | MET_MET (M13:M15)                                                                |
| Lung_9  | KRAS p.G12D (13.94%); EGFR_EGFR (E1:E8)                                          |
| Lung_10 | No alterations                                                                   |
| Lung_11 | MET_MET (M13:M15)                                                                |
| Lung_12 | EML4_ALK (E13A20); JAK3 p.S493C (5.01%)                                           |
| Lung_13 | EGFR p.T790M (39.81%); EGFR p.E746_A750 (exon 19) del (73.72%); EGFR amplification (8.03); EGFR_EGFR (E1:E8); PTPRZ1_MET (P1M2); FGFR1_TACC1 (F17T7) |
| Lung_14 | EGFR p.L858R (15.64%); EGFR_EGFR (E1:E8)                                         |
| Lung_15 | EGFR_EGFR (E1:E8); PTPRZ1_MET (P1M2)                                              |
| Lung_16 | EGFR p.L858R (94.80%); EGFR amplification (12.39); EGFR_EGFR (E1:E8)             |
| Lung_17 | No alterations                                                                   |
| Lung_18 | KRAS p.G12C (53.1%); MET_MET (M13:M15)                                           |
| Lung_19 | RET p.M918T (52.36%)                                                             |
| Lung_20 | MET-MET (M13:M15); KIF5B-RET (K15R12)                                             |
| Lung_21 | EGFR p.E746_A750 (exon 19) del (67.25%); CTNNB1 p.S33F (12.53%)                  |
| Lung_22 | KRAS p.G12V (44.54%)                                                             |
| Lung_23 | EML4_ALK (E6A20); CTNNB1 p.S45F (35.39%)                                         |
| Lung_24 | FGFR1_TACC1 (F17T7); EGFR_EGFR (E1:E8)                                           |
| Lung_25 | EGFR p.L858R (61.71%); EGFR amplification (6.56)                                  |
| Lung_26 | EGFRp.E746_P753 (exon 19) delinsV5 (51.29%); FAM131B_BRAF (F2B9)                 |
| Lung_27 | EML4_ALK (E6A20); ALK p.I1171N (20.1%)                                           |
| Lung_28 | EML4_ALK (E6A20)                                                                 |
| Lung_29 | EGFR p.L858R (44.31%); FGFR3_TACC3 (F17T11); MYC amplification (5.93); CDK4 amplification (13.34) |
| Lung_30 | MET_MET (M13:M15)                                                                |
| Lung_31 | IDH1 p.R132H (41.55%); FGFR3_TACC3 (F17T11); MYC amplification (7.86)            |
| Lung_32 | EGFR p.E746_A750 (exon 19) del (94.66%); AR amplification (9.43); EGFR amplification (15.29) |
| Lung_33 | EGFR p.L747_P753 (exon 19) delinsS (52.16%); CTNNB1 p.S37C (40.1%)               |
| Lung_34 | EGFR p.L747_T751 (exon 19) del (51.78%)                                          |
| Lung_35 | No alterations                                                                   |
| Lung_36 | EGFR p.L858R (12.56%); PIK3CA p.E545K (16.3); MYC amplification (7.91)            |
| Lung_37 | EGFR p.L747_P753 (exon 19) delinsS (15.87%); EGFR amplification (15.20)           |
to occur in BMs of advanced lung cancer. Another possible reason is that the occurrence rate of METex14 is underestimated in the DNA-based molecular testing, and RNA-based testing is challenged in fluid biopsy. The incidence of METex14 in lung cancer BMs needs validation in a larger cohort.

ZM enhances MET signaling activation through autophosphorylation of MET. METex14 also causes sustained activation of MET by blocking the ubiquitin-mediated degradation of MET. We performed IHC in the two cases harboring ZM and the four cases (with sufficient materials) harboring METex14; a case with wild-type MET was used as a comparison. Strong positive c-MET and positive p-MET signals were observed in cases harboring ZM or METex14, but not in the case with wild-type MET (Figure 2C). These results suggest that ZM and METex14 are critical molecular events that may represent therapeutic targets for BMs.

Notably, we observed that METex14 and EGFR mutations were mutually exclusive in the 37 BMs of lung cancer. These findings suggested MET RNA variants were another important RTK signaling-activated driver event, other than EGFR mutations, in BMs of lung cancer. Other fusions, including EML4-ALK,
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