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ORIGINAL ARTICLE

Histologic transformation of non-small-cell lung cancer in brain metastases

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Abstract

Background Treatment strategies differ substantially for small-cell lung cancer (SCLC), adenocarcinoma and squamous-cell cancer (SCC). Therefore, it is of important significance to identify histologic transformation. There are no reports on histologic transformation in brain metastases (BM) to date. The aim of this study was to analyze the histologic transformation in BM for the first time.

Methods Medical records were reviewed and patients with both resected BM and primary tumors were examined retrospectively. The histologic diagnosis was confirmed by H&E staining, and additional diagnostic immunohistochemical stains were performed at the discretion of the pathologists. Characteristics of histologic transformation in BM were analyzed.

Results 3 of 24 patients (12.5%) with both resected BM and primary non-small-cell lung cancers (NSCLCs) had evidence of histologic transformation in BM. One case with SCC transformed to adenocarcinoma in brain, one case with adenocarcinoma transformed to SCLC, and another case with adenocarcinoma transformed to SCC. The three cases of histologic transformation were all spontaneous and had not tested gene status.

Conclusions We disclosed the histologic transformation of NSCLC in BM at a frequency not as low as expected, and speculated it as an evolution promoted by intratumor heterogeneity, though it warrants further prospective multi-institution investigations with comprehensive molecular analysis. Our findings provided further impetus for surgery when the metastatic or recurrent lesion is resectable, and repeated pathologic evaluation to help tailor individualized treatment.

Keywords Histologic transformation · Non-small-cell lung cancer · Brain metastases · Small-cell lung cancer · Surgery

Introduction

Lung cancer is the leading cause of cancer deaths worldwide. Its two broad histologic subtypes are small-cell lung cancer (SCLC) which accounts for 15% of cases, and non-small-cell lung cancer (NSCLC) which mainly includes adenocarcinoma, squamous-cell cancer (SCC) and large-cell cancer. Treatment strategies differ substantially for SCLC, adenocarcinoma and SCC, which suggests inherent differences in tumor biology. Therefore, it is of important significance to identify histologic transformation to help tailor individualized treatment. Histologic transformation from treated mutated lung adenocarcinoma to SCLC is described as one of the major mechanisms for acquired resistance to epidermal growth factor receptor (EGFR) [1–13] or anaplastic lymphoma kinase (ALK) [14–17] tyrosine kinase inhibitors (TKIs). Recent case reports have also illustrated that transformation from treated mutated lung adenocarcinoma to SCC is another possible mechanism for acquired

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resistance to EGFR TKIs,[18–21] so as transformation to sarcomatoid carcinoma is to ALK TKIs [22]. Although the majority of the published transformed cases have been identified from TKI-resistant lung adenocarcinoma cases, histologic transformation could also occur spontaneously at progress after surgery or chemotherapy [2, 23–25]. In spite of all the published transformed cases, to our knowledge, there are no reports on histologic transformation in brain metastases (BM) in the literature to date. BM is the most common tumor in the brain, and associated with a dismal prognosis. We analyzed the histologic transformation in BM in patients with both surgically resected BM and primary tumors for the first time.

Materials and methods

Patient eligibility

A total of 47 patients who underwent both surgical resections for BM and primary tumors at our institution from 2004 to 2015 were examined retrospectively. One patient who presented with synchronous primary tumors was excluded from this study. Eight patients were also excluded because their histologic diagnoses could not be confirmed as their formalin-fixed and paraffin-embedded samples were not adequate. Finally, 38 patients were eligible. Medical records were reviewed to obtain clinical information. Stage was classified according to the seventh edition of the TNM classification for NSCLC. The research was carried out according to the principles set out in the Declaration of Helsinki 1964 and all subsequent revisions. The ethics committee of our institution approved the protocol (2017015). Informed consent was obtained from every subject. All patients with lung cancer had brain magnetic resonance imaging as part of their initial staging preoperatively. The patients with BM had maximal safe tumor resection. The patients with lung cancer had lobectomy or ipsilateral pneumonectomy. Complete mediastinal LN dissection or systematic mediastinal LN sampling was performed during surgery.

Histologic analysis

All surgical samples underwent histologic review by two experienced pathologists. The histologic diagnosis was confirmed by H&E staining, and additional diagnostic immunohistochemical stains were performed at the discretion of the pathologists. All immunohistochemical staining was performed on representative tissue sections from formalin-fixed and paraffin-embedded tissue blocks. The histologic diagnosis was based on the standard criteria defined by the World Health Organization classification. An immunohistochemical analysis was performed with microwave antigen retrieval and with the HRP/DAB Kit (PV-9000, ZSGB-BIO, Beijing, China).

Results

Medical records were reviewed to identify 38 patients who underwent both surgical resections for BM and primary tumors at our institution from 2004 to 2015. Among them there were 27 cases of lung cancer, including 19 cases of adenocarcinoma, 5 cases of SCC, 2 cases of SCLC, and 1 case of combined SCLC with adenocarcinoma.

NSCLC (63.2%) was the predominant primary tumor. Baseline characteristics of the 24 patients with NSCLC are shown in Table 1. The median age when BM diagnosed was 56 years (range 40–72 years). There were more men (62.5%) than women (37.5%). Almost half of the patients had smoking history. Adenocarcinoma took account for 79.2%, while SCC 20.8%. Upon the initial diagnosis, six patients (25.0%) had Stage I, four patients (16.7%) had Stage II, seven patients (29.2%) had Stage III, and seven patients

Table 1 Baseline characteristics of patients with both surgically resected brain metastases and their primary non-small-cell lung cancers

Patient characteristics	$n=24$
Age when brain metastases diagnosed (years)	
Median	56
Range	40–72
Sex—*n* (%)	
Female	9 (37.5)
Male	15 (62.5)
Smoking history—*n* (%)	
Yes	13 (54.2)
No	11 (45.8)
Histologic subtype of lung lesions—*n* (%)	
Adenocarcinoma	19 (79.2)
Squamous-cell cancer	5 (20.8)
Stage upon the initial diagnosis—*n* (%)	
I	6 (25.0)
II	4 (16.7)
III	7 (29.2)
IV (oligometastasis in brain)	7 (29.2)
The first surgery—*n* (%)	
Lung resection	17 (70.8)
Brain resection	7 (29.2)
Time to brain metastases after thoracic surgery, months	
Median	25
Range	1–66
Maximal diameter of brain metastases (cm)	
Median	3
Range	1–5.2

(29.2%) had Stage IV NSCLC with three or fewer metastatic lesions (oligometastasis) in brain. The seven Stage IV patients underwent brain resection before lung resection. The median time to BM after thoracic surgery was 25 months (range 1–66 months) for the 17 patients without BM upon the initial diagnosis. The median maximal diameter of the oligometastasis in brain was 3 cm. There was no recurrent cancer in lung at the time of BM resection.

Unexpectedly, 3 of 24 patients (12.5%) with NSCLC had evidence of histologic transformation in BM (Table 2). One case with moderately differentiated SCC with negative immunostaining for thyroid transcription factor (TTF)-1 and NapsinA transformed to poorly differentiated adenocarcinoma with focal squamous metaplasia with positive immunostaining for TTF-1 and NapsinA, focally positive for P63, and negative for cytokeratin (CK)5/6, CK8/18 and glial fibrillary acidic protein (GFAP) (Fig. 1); one case with moderately differentiated adenocarcinoma with negative immunostaining for CD56 and neuron-specific enolase (NSE) transformed to combined SCLC with peripherally a shade of adenocarcinoma with positive immunostaining for CD56, NSE, Syn and CgA, partially positive for TTF-1, and negative for CK and GFAP (Fig. 2); and one case with moderate-poorly differentiated adenocarcinoma with positive immunostaining for CK8/18, P63 and TTF-1, and negative for CK5/6, Syn, CgA and CD56 transformed to poorly differentiated SCC with positive immunostaining for P63 and TTF-1, partially positive for CK8/18, and focally positive for CK5/6 (Fig. 3). There was no evidence of tumors with combined histology in any of the three thoracic surgical specimens. The three patients were all female without smoking history. Upon the initial diagnosis, one patient had Stage I, one patient had Stage II, and one patient had Stage IV NSCLC with oligometastasis in brain. The time to BM after thoracic surgery was 25 and 66 months for the Stage I and Stage II patient, respectively. The maximal diameter of the oligometastasis in brain was 2.6, 5 and 1.3 cm for the three patients, respectively (Table 2). Case 1 underwent lung resection and was diagnosed with pathologic Stage T1N0M0 SCC when she was 55 years old in October 2012, and then 2 years later she underwent brain resection because she experienced slurred speech and seizures and her brain MRI revealed a 2.6 × 2.0 × 1.8-cm enhancing mass with surrounding edema in right frontal lobe (Fig. 4a-c), was diagnosed with metastatic lung adenocarcinoma, received stereotactic radiosurgery, and has been living free of tumor since then. Case 2 underwent lung resection and was diagnosed with pathologic Stage T1N1M0 adenocarcinoma when she was 43 years old in June 2007, and received four cycles of adjuvant chemotherapy, and then 5 years later she underwent brain resection because she experienced headache, dizziness and vomiting and her brain MRI revealed a 5 × 4 × 4-cm enhancing mass with surrounding edema in left frontal lobe (Fig. 4d–f), was diagnosed with metastatic SCLC, received whole brain radiation therapy and four cycles of chemotherapy with etoposide and cisplatin, and then had abdominal metastases and malignant pleural effusion and died of cancer 4 months later. Case 3 underwent brain resection because she experienced headache and right hemiparesis and her brain MRI revealed a 1.3 × 1 × 1-cm enhancing mass with surrounding edema in left frontal lobe and was diagnosed with metastatic lung SCC when she was 52 years old in August 2010, then 4 months later she underwent lung resection, was diagnosed with pathologic Stage T2N2M1b adenocarcinoma, received 4 cycles of chemotherapy, and was lost to follow-up thereafter. None had been treated with TKIs before the two resections. The three cases of histologic transformation were all spontaneous, and had not tested gene status. Moreover, the BM histologic subtype of the case whose primary lung tumor's histologic subtype is combined SCLC with adenocarcinoma, is SCLC alone.

Discussion

Recently, cases of histologic transformation of NSCLC have been increasingly published. Among them the most frequently reported is the histologic transformation from

Table 2 Characteristics of patients with histologic transformation in brain metastases

Patient characteristics	Case 1	Case 2	Case 3
Age when brain metastases diagnosed (years)	56	48	52
Sex	Female	Female	Female
Smoking history	No	No	No
Histologic subtype of lung lesions	Squamous-cell cancer	Adenocarcinoma	Adenocarcinoma
Histologic subtype of brain metastases	Adenocarcinoma	Small-cell cancer	Squamous-cell cancer
Stage upon the initial diagnosis	I	II	IV
The first surgery	Lung	Lung	Brain
Time to brain metastases after thoracic surgery (months)	25	66	-
Maximal diameter of brain metastases (cm)	2.6	5	1.3

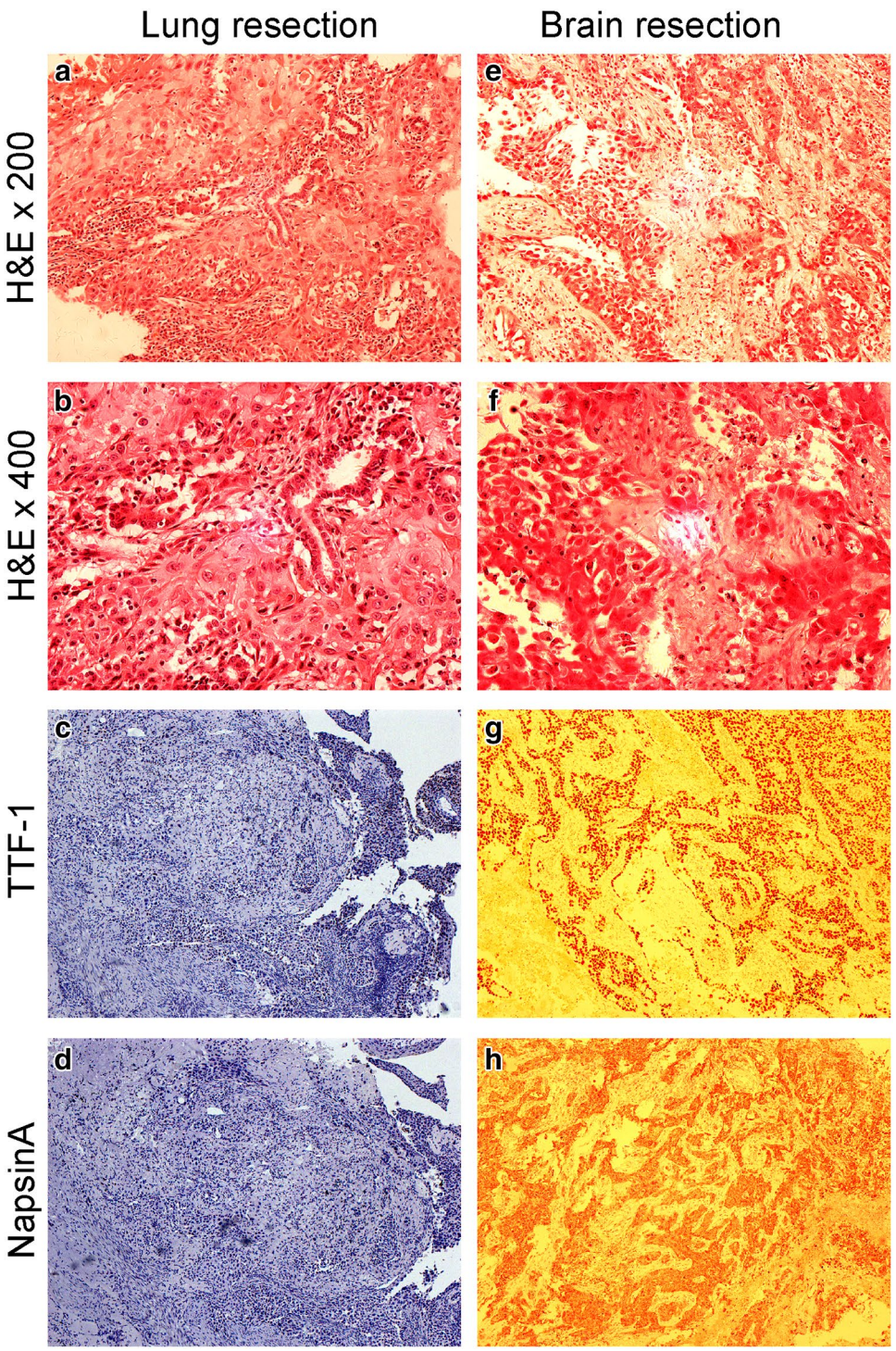

Fig. 1 Case 1's tumor tissue at lung resection (**a**–**d**) and brain resection (**e**–**h**) with histologic transformation from moderately differentiated squamous cell cancer to poorly differentiated adenocarcinoma with focal squamous metaplasia. H&E stained material demonstrating histologic changes from lung resection (**a, b**) to brain resection (**e, f**) with immunohistochemical staining for NapsinA and TTF-1 on lung resection (**c, d**) and brain resection (**g, h**). H&E, hematoxylin and eosin

treated mutated lung adenocarcinoma to SCLC, which is found as one of the major mechanisms for acquired resistance to EGFR [1–13] or ALK [14–17] TKIs. In a case series in which repeat biopsies were done when resistance developed in lung adenocarcinoma patients given EGFR TKIs, transformation to SCLC was reported in 14% of cases, [3] while less than 5% in another case series [4]. The different frequencies of this unusual histologic phenomenon observed, may have been partly due to the different frequencies of pathologic evaluation of drug-resistant specimens in clinical care. Another possible mechanism for acquired resistance to EGFR TKIs is histologic transformation from treated mutated lung adenocarcinoma to SCC [18–21]. Moreover, histologic transformation to sarcomatoid

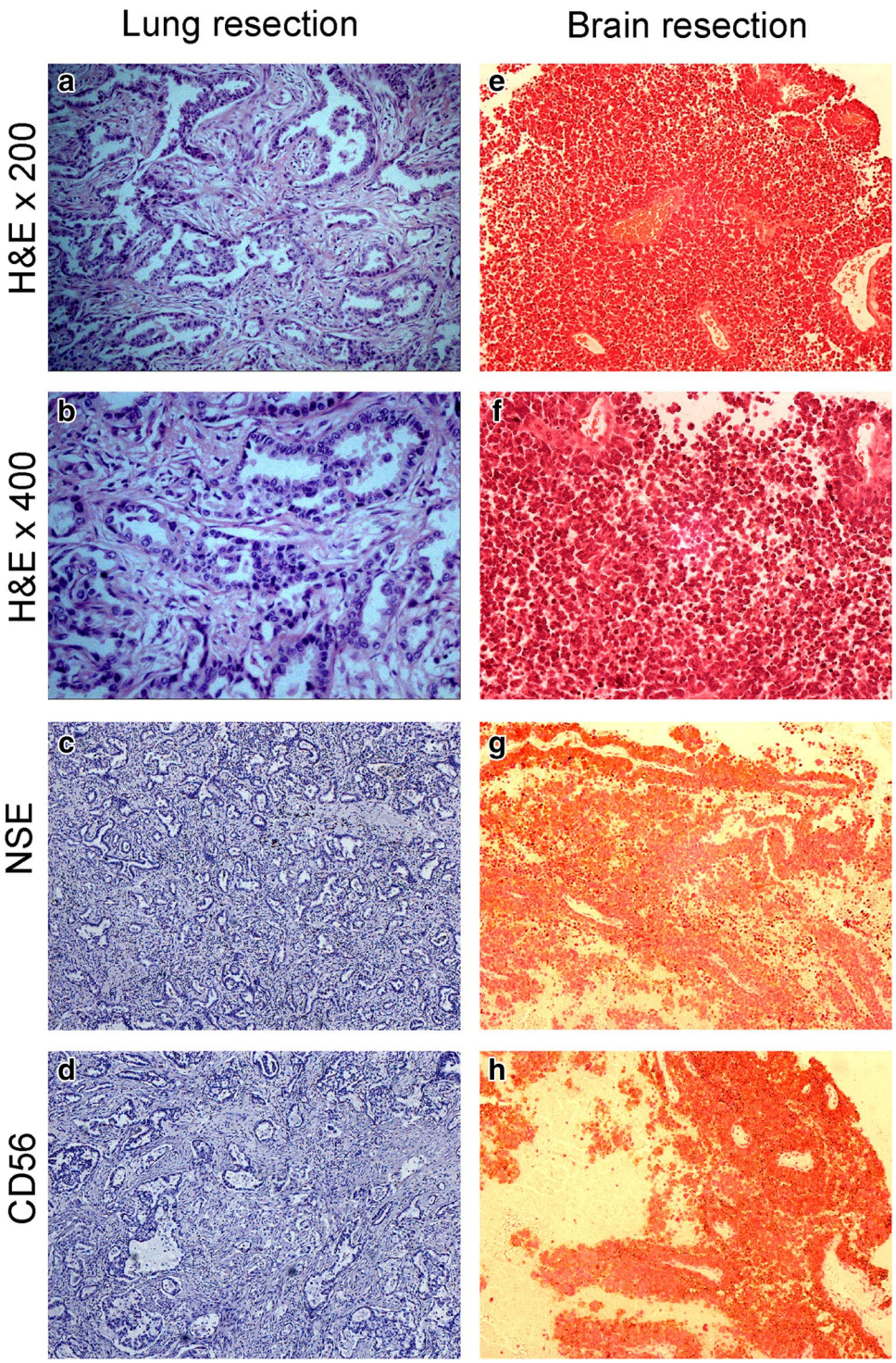

Fig. 2 Case 2's tumor tissue at lung resection (**a**–**d**) and brain resection (**e**–**h**) with histologic transformation from moderately differentiated adenocarcinoma to combined small cell cancer with peripherally a shade of adenocarcinoma. H&E stained material demonstrating histologic changes from lung resection (**a, b**) to brain resection (**e, f**) with immunohistochemical staining for CD56 and NSE on lung resection (**c, d**) and brain resection (**g, h**). H&E, hematoxylin and eosin

carcinoma from ALK-rearranged lung adenocarcinoma through epithelial–mesenchymal transition, is indicated as another possible mechanism for acquired resistance to ALK TKIs [22]. Nonetheless, this phenomenon is not restricted to TKI-resistant tumor specimens, histologic transformation of EGFR mutant lung adenocarcinoma to SCC could also occur without prior exposure to EGFR TKIs [23] so could histologic transformation of non-EGFR-mutant [2] or driver-unknown [24] adenocarcinoma to SCLC. Furthermore, Hsu et al. showed a case with histologic evolution from lung adenocarcinoma harboring neither EGFR mutations nor ALK translocation, to adenocarcinoma with sarcomatoid changes, squamous cell carcinoma with sarcomatoid changes, and squamous cell carcinoma after chemotherapy

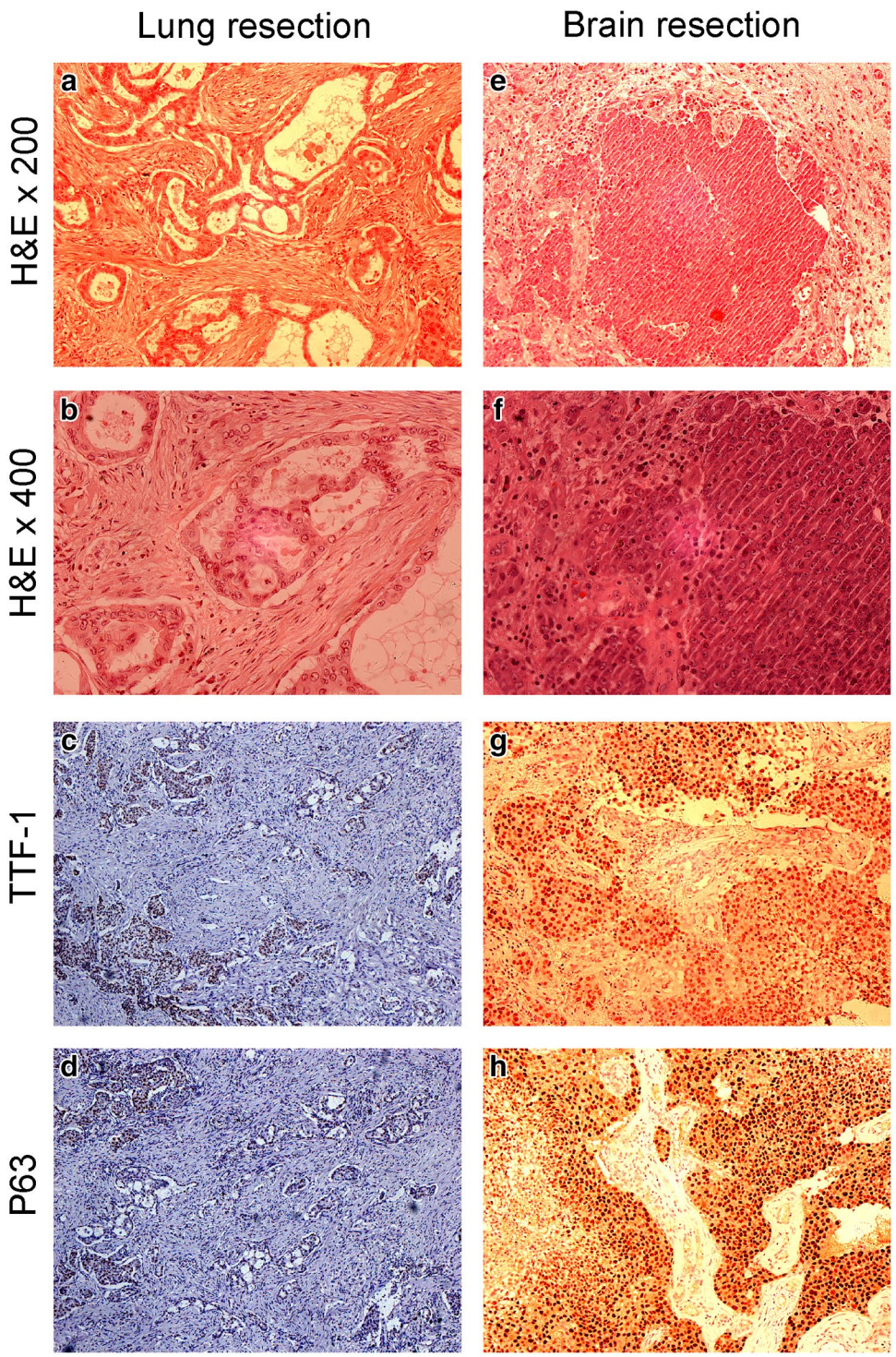

Fig. 3 Case 3's tumor tissue at lung resection (**a**–**d**) and brain resection (**e**–**h**) with histologic transformation from moderate-poorly differentiated adenocarcinoma to poorly differentiated squamous cell cancer. H&E stained material demonstrating histologic changes from lung resection (**a, b**) to brain resection (**e, f**) with immunohistochemical staining for P63 and TTF-1 on lung resection (**c, d**) and brain resection (**g, h**). H&E, hematoxylin and eosin

and immunotherapy [25]. It is suggested that histologic transformation can occur independently of EGFR or ALK mutational status. The reason for the much less reports of histologic transformation in patients who are treated and progress on surgery or chemotherapy, may be the limited data of repeated histologic evaluation on such patients. Despite all the published cases of histologic transformation, there are no reports on histologic transformation in BM or histologic transformation from SCC to adenocarcinoma so far. Unexpectedly, we observed that 3 of 24 patients (12.5%) with both surgically resected BM and primary NSCLCs, had evidence of histologic transformation in BM for the first time. One case with SCC transformed to adenocarcinoma in brain, one case with adenocarcinoma transformed to

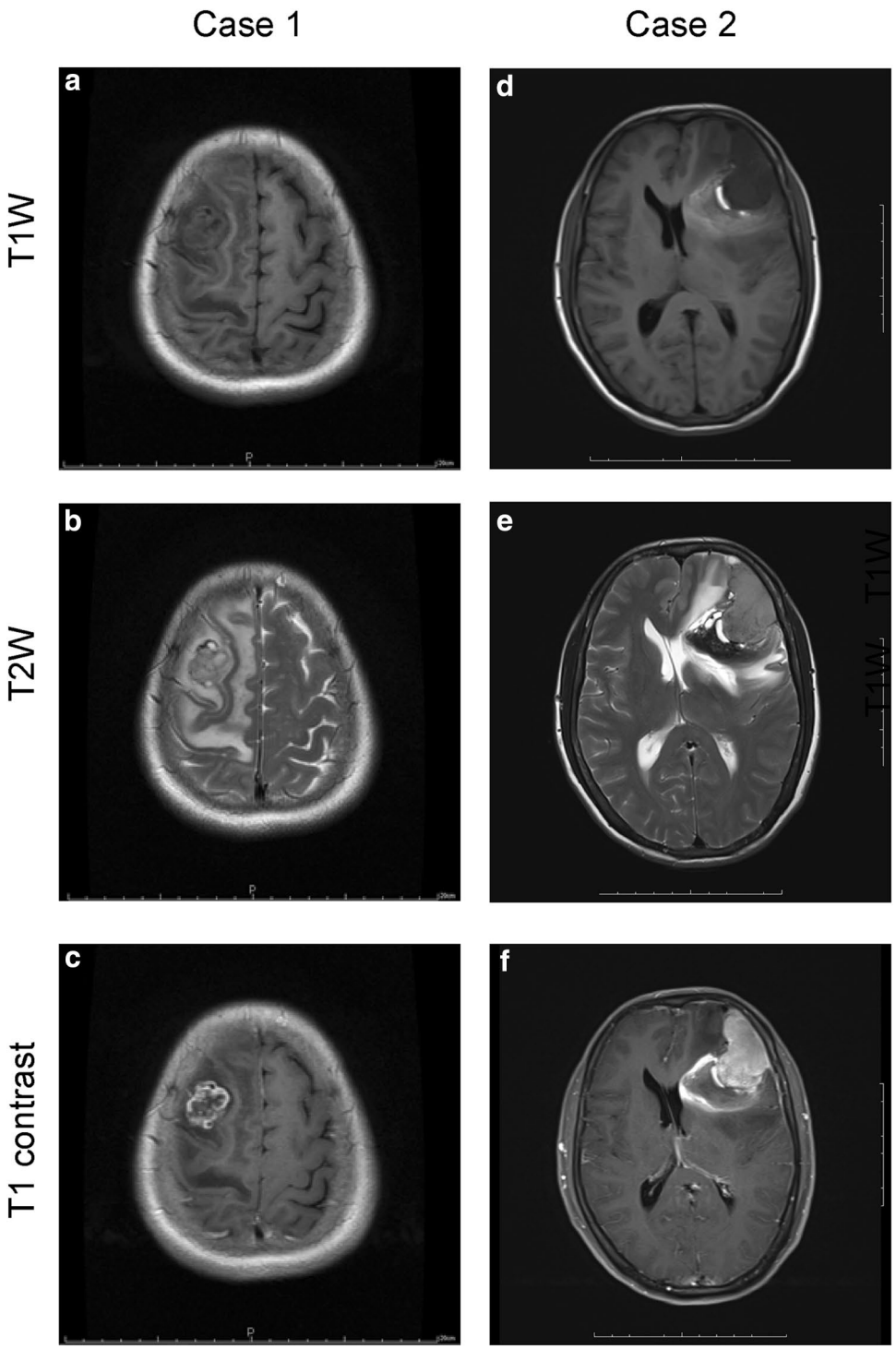

Fig. 4 The brain MRI of Case 1 (**a**–**c**) and Case 2 (**d**–**f**) before brain resection. Case 1's T1W (**a**), T2W (**b**) and T1 contrast (**c**) MRI revealing an enhancing mass with surrounding edema in right frontal lobe. Case 2's T1W (**d**), T2W (**e**) and T1 contrast (**f**) MRI revealing an enhancing mass with surrounding edema in left frontal lobe

SCLC, and another case with adenocarcinoma transformed to SCC. The three cases of histologic transformation were all spontaneous. The frequency of histologic transformation of NSCLC in BM is not as low as expected in our case series.

It is essential to identify histologic transformation when it happens, because SCLC, adenocarcinoma and SCC are treated differently in clinic owing to their distinct biology and genomic abnormalities. It is believed that patients with transformed SCLC should be given standard therapies for SCLC. The patient with transformed SCLC in brain should receive whole brain radiation therapy, not stereotactic radiosurgery, and 4–6 cycles of chemotherapy with etoposide and cisplatin. The patient with transformed adenocarcinoma in brain should be encouraged to have molecular testing

because of more chance to benefit from TKIs. There is supposed superior efficacy and reduced toxicity for cisplatin and pemetrexed in the patient with transformed adenocarcinoma, while superior efficacy for cisplatin/gemcitabine in the patient with transformed SCC. BM represents an important public health burden that is ten times more common than malignant primary brain tumors, whose incidence is rising, as the improved systemic therapies well control extracranial disease to prolong survival, but poorly control intracranial disease due to their poor penetration of blood–brain barrier. Primary lung cancers are the most common source, accounting for half of BM. Advances in surgical technique have rendered upfront resection in the standard of care for solitary BM. Surgery is a key treatment modality to consider, especially for BM over 3 cm in size or otherwise bulky lesions causing neurologic symptoms, and when tissue is necessary to establish a diagnosis. Meanwhile, evidence from retrospective series suggested survival benefits from tumor resection for selected patients of good prognosis with up to three metastatic sites. It is becoming increasingly clear that precision medicine will demand continual assessment of each cancer's evolution over the course of development, to identify the optimal treatment strategies. It is promising that technologies to assess cancers via noninvasive measures such as circulating tumor cell analyses, plasma DNA analyses, or molecular radiology may eventually obviate the need for invasive procedures. But histologic pathology is not replaceable, at least for now. Precise histologic evaluation is crucial for appropriate management. Recently, stereotactic radiosurgery alone is increasingly preferred for limited BM over surgical resection followed by stereotactic radiosurgery when the two treatments are both considered suitable for patients with known history of NSCLC. Nevertheless, our findings demonstrate that concern still exists regarding histologic diagnosis of BM even with confirmed history of resected primary NSCLC. Our investigation provided new insights into our understanding of histologic transformation and emphasized that when the metastatic or recurrent lesion is resectable, surgical intervention is an effective treatment option with better histologic evaluation.

Differentiation into a subtype cancer was thought to occur early in tumorigenesis driven by fundamental genetic differences. The underlying mechanisms of histologic transformation are not completely understood. One mechanism may have been pluripotent tumor cells with divergent differentiation. Mutation or loss of Retinoblastoma1 (RB1) has been found in 100% of the human SCLC tumors sequenced [24]. A recent study demonstrated that EGFR TKI–resistant lung adenocarcinoma and SCLC share a common clonal origin, and that EGFR TKI–resistant SCLCs are branched out early from the lung adenocarcinoma clones that harbor completely inactivated RB1 and TP53 [10]. Another study also revealed RB loss in resistant EGFR mutant lung adenocarcinomas that transform to SCLCs, as well as increased neuroendocrine marker, decreased EGFR expression and greater sensitivity to BCL2 family inhibition compared with resistant NSCLCs, suggesting that this subset of resistant cancers ultimately adopt many of the molecular and phenotypic characteristics of classical SCLC [11]. Genomic characterizations of matched BM and primary tumors revealed branched evolution [26, 27]. Progress of NSCLC is likely a dynamic evolution both spatially and temporally promoted by intratumor heterogeneity revealed through multiregion sequencing [28–30]. A very recent prospective investigation found widespread intratumor heterogeneity for both somatic copy-number alterations and mutations, through multiregion whole-exome sequencing in early-stage NSCLC, which was associated with an increased risk of recurrence or death, and heterogeneous driver alterations that occurred later in evolution were found in more than 75% of the tumors and were common in PIK3CA and NF1 and in genes that are involved in chromatin modification and DNA damage response and repair [28]. Another possible mechanism of histologic transformation is tumors with combined histology owing to the limited amount of specimen obtained by core biopsy or fine-needle aspirates. Most of the previous reported cases of histologic transformation are recognized with biopsy specimens. Combined SCLC was noticed in only 1–3.2% of all SCLCs, whereas as high as 9–26% in surgical specimens. Interestingly, we did happen to find that the BM histologic subtype of the case with combined SCLC with adenocarcinoma is SCLC alone in our study. Nevertheless, our transformed cases could rule out the possibility of tumors with combined histology, because thoracic surgery provided sufficient tissue and adequate sampling. Our results implied that histologic transformation is more likely a cancer evolution both spatially and temporally activated by intratumor heterogeneity. Cancer cells may transform before or after they locate in brain. It is likely that the brain microenvironment does not play an indispensable role in histologic transformation as cases of extracranial histologic transformation of NSCLC have been repeatedly reported. We assume that there is intratumor heterogeneity in metastatic potential and preferential metastatic sites as well as transformation potential, and histologic transformation of BM is likely to happen when the vigorous metastatic "seeds" with immense transformation potential have a specific affinity for the brain "soil".

Our study has some limitations. First, it is a single-institution retrospective analysis. The study may have selection bias and the results should be interpreted cautiously. Second, it lacks additional tumor genomic analysis. Third, our sample size is relatively small. However, our eligible matched surgical specimens of BM and primary tumors are superior

in terms of both quality and quantity. Histologic transformation in BM has not so far been depicted in the literature. Slowly and staggeringly, but we have taken the first step.

Conclusions

In summary, we disclosed the histologic transformation of NSCLC in BM at a frequency not as low as expected, and speculated it as an evolution stimulated by intratumor heterogeneity, though it warrants further prospective multi-institution investigations with comprehensive molecular analysis. Our findings provided further impetus for surgery when the metastatic or recurrent lesion is resectable, and repeated pathologic evaluation to help tailor individualized treatment.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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