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Case Report

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Clonal evolution and molecular profiling of recurrent maxillary ameloblastoma with lung metastasis
harboring *SMO* and *PIK3CA* mutations

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Abstract

Purpose This study investigated genetic alterations in recurrent maxillary ameloblastoma with pulmonary metastases.

Methods We analyzed tissue from a patient with a fifth recurrence of maxillary ameloblastoma and bilateral pulmonary metastases who underwent resection of the maxillary and lung lesions. Immunohistochemistry (IHC) for CK19, p53, and MIB1 was performed on the recurrent maxillary tumor and metastatic lung lesions. Genetic alterations were evaluated using a 225-gene cancer panel.

Results Histologically, the recurrent maxillary tumor showed moderate atypia, whereas the metastatic lung tumor showed mild atypia. IHC demonstrated focal p53 positivity in the recurrent maxillary tumor and

diffuse p53 positivity in the metastatic lung tumor. Genomic analysis identified identical *SMO* (L412F) and *PIK3CA* (E545K) mutations in the recurrent maxillary tumor and metastatic lung lesion.

Conclusion These findings support the involvement of Hedgehog and phosphoinositide 3-kinase (PI3K) signaling in the pathogenesis of metastatic maxillary ameloblastoma and demonstrate the potential value of genetic profiling for identifying therapeutic targets in aggressive or metastatic odontogenic tumors.

Keywords: Ameloblastoma, *SMO* (L412F); *PIK3CA* (E545K), lung metastasis, IHC

Introduction

Ameloblastoma is the most common benign odontogenic tumor. It may arise from remnants of the dental lamina or enamel organ, the epithelial lining of an odontogenic cyst, or basal cells of the oral mucosa [1,2]. The reported ratio of women to men is 1:1.1, and the tumor occurs most frequently in adults aged 30–50 years [3]. Approximately 80% of ameloblastomas arise in the mandible and 20% in the maxilla, although these tumors may also be associated with other odontogenic tumors [4]. Painless swelling is the most common presenting symptom, but ameloblastoma can also cause progressive bone destruction. In the 2005 World Health Organization (WHO) classification, ameloblastomas that metastasized were classified as odontogenic carcinomas. In 2017, the WHO reclassified metastasizing ameloblastoma as a benign epithelial odontogenic tumor and distinguished it from ameloblastic carcinoma [5]. The lungs are the most common metastatic site, followed by the cervical lymph nodes [3]. Reported recurrence rates

after resection range from 5.0% to 30% [6-8]. Radical resection is the standard treatment for recurrent disease, but it can cause facial deformity and does not eliminate the risk of further recurrence. Consequently, conservative approaches, including chemotherapy and radiotherapy, have been investigated [9-12]. Next-generation sequencing has recently identified clinically relevant genetic alterations and enabled comparisons between primary tumors and their metastases [13-15]. Heikinheimo et al. reported the B-Raf proto-oncogene, serine/threonine kinase (*BRAF*) V600E mutation in 62.5% of ameloblastomas [16]. Sweeney et al. found smoothened, frizzled class receptor (*SMO*) mutations, including L412F and W535L, in 39% of ameloblastomas. *SMO* and *BRAF* mutations are associated with tumor location: 69% of tumors with *BRAF* mutations arose in the mandible, whereas 82% of tumors with *SMO* mutations arose in the maxilla [17].

At Shizuoka Cancer Center, a DNA panel targeting 225 cancer-related genes enables comparison of genetic alterations between recurrent and metastatic lesions [18]. In this study, we used this panel to compare, for the first time, the genetic alterations in recurrent maxillary ameloblastoma and its pulmonary metastasis.

Materials and Methods

Ethical statement

The Institutional Review Board of Shizuoka Cancer Center approved all aspects of this study (permit

number T2025-11-20251-5).

Clinical samples

Tissue samples from the fifth recurrent maxillary ameloblastoma and the pulmonary metastases were obtained during surgical resections at Shizuoka Cancer Center. The recurrent maxillary tumor and the left pulmonary metastasis were resected on separate dates in 2022, and the right pulmonary metastasis was resected in 2023. The primary tumor and the first four recurrences were resected at an outside hospital; therefore, tissue samples from those lesions were unavailable.

Analysis using a 225-gene cancer panel

Formalin-fixed, paraffin-embedded (FFPE) tissue blocks from the recurrent maxillary ameloblastoma and pulmonary metastasis were cut into 10- μ m-thick sections. DNA was extracted from the FFPE tissue and purified as previously described [18]. Tumor DNA from the maxillary and pulmonary lesions was analyzed using a panel of 225 cancer-related genes listed by Hatakeyama et al. [18]. Target capture was performed by hybridization, followed by sequencing using the clinical 225S Cancer Panel platform at SRL & Shizuoka Cancer Center Collaborative Laboratories, Inc. (Shizuoka, Japan), a laboratory accredited by the College of American Pathologists (CAP) and certified under the Clinical Laboratory Improvement Amendments (CLIA), as previously described [18].

Immunohistochemistry

Immunohistochemistry (IHC) was performed using a Leica Bond-III automated stainer (Leica, Tokyo,

Japan). Heat-induced antigen retrieval was performed with Bond Epitope Retrieval Solution 2 for 20 min at 100 °C in accordance with the manufacturer's protocol and as previously described [18, 19]. The following primary antibodies were used: mouse monoclonal anti-CK19 antibody (61010; 1:800; PROGEN, Heidelberg, Germany), mouse monoclonal anti-p53 antibody (clone DO-7; PA0057; ready to use; Leica Biosystems, Newcastle, UK), and mouse monoclonal anti-Ki-67 antibody (clone MIB1; M7240; 1:100; Agilent Technologies, Santa Clara, CA, USA).

Results

The clinical course is summarized in Fig. 1. The patient presented with a fifth recurrence of maxillary ameloblastoma accompanied by bilateral pulmonary metastases. The recurrent maxillary tumor and both pulmonary metastases were surgically resected, and the resected tissues were analyzed using the 225-gene cancer panel. Gadolinium-enhanced T1-weighted imaging showed that the fifth recurrent maxillary tumor occupied the maxillary sinus (Fig. 2a). Macroscopically, the poorly circumscribed solid mass almost completely occupied the maxillary sinus (Fig. 2b). Histologically, the tumor filled the maxillary sinus and showed solid, nested, sheet-like, and palisading growth patterns with proliferation of osteoclast-like giant cells (Fig. 3a–d). Tumor cells showed moderate nuclear atypia, frequent mitotic figures, and squamous metaplasia (Fig. 3e and f). The stroma was highly cellular and contained cells with moderate atypia and frequent mitotic figures (Fig. 3g). IHC showed cytoplasmic CK19 positivity and focal p53 positivity in the

tumor cells. The MIB1 labeling index was approximately 30% in hot spots (Fig. 4a–c). In contrast, stromal cells were negative for CK19, showed limited p53 positivity, and had an MIB1 labeling index of approximately 10% in hot spots (Fig. 4d–f). Radiologic findings suggested bilateral pulmonary metastases; therefore, left and right lung segmentectomies were performed. Nodules were identified in segment 1 + 2 of the left lung (Fig. 5a) and segment 9 of the right lung (Fig. 5b). The left and right pulmonary nodules measured 1.5 × 1.2 cm and 1.0 × 1.0 cm, respectively. Histologic examination confirmed tumor involvement in both nodules. In the left lung, tumor cells were distributed along the periphery of the nodule (Fig. 5c and d). Their morphology resembled that of the maxillary tumor, with solid and nested growth, peripheral palisading, squamous metaplasia, and moderate atypia (Fig. 5e and f). The stroma showed moderate cellularity and mild atypia, with few mitotic figures (Fig. 5g). Tumor cells showed diffuse p53 positivity (Fig. 6a), and the MIB1 labeling index was approximately 20% in hot spots (Fig. 6b). The right pulmonary lesion had the same pathologic features as the left pulmonary lesion (data not shown).

Genetic alterations in the recurrent maxillary tumor and pulmonary metastasis were analyzed using the 225-gene cancer panel. DNA purity, assessed by the absorbance ratio at 260 nm/280 nm, was 1.93 for the recurrent maxillary tumor and 1.94 for the pulmonary metastasis (Table 1). The DNA integrity number (DIN) was 3.0 and 3.7, respectively, and the estimated tumor content was 80% and 20%, respectively. The recurrent maxillary tumor harbored missense mutations in *AXL* (W448L), *CDH1* (V832M), *FGFR2* (N549K), *MAP3K1* (R162C), *MAP3K4* (R90Q), *PIK3CA* (E545K), *SMO* (L412F), and *TACC3* (R594C)

(Table 2). A *BAP1* frameshift variant (D458Gfs*11) was detected in both the recurrent maxillary tumor and the pulmonary metastasis (Tables 2 and 3). Splice variants in *KDM6A* and *PBRM1* were detected in the recurrent maxillary tumor. The pulmonary metastasis harbored missense mutations in *AXL* (W448L), *CDHI* (V832M), *FGFR2* (N549K), *MAP3K4* (R90Q), *PIK3CA* (E545K), *SMO* (L412F and V494M), *TACC3* (R594C), and *TP53* (R273C) (Table 3). In the recurrent maxillary tumor, the variant allele frequencies (VAFs) of *AXL* (W448L), *BAP1* (D458Gfs*11), *CDHI* (V832M), *FGFR2* (N549K), *KDM6A*, *MAP3K1* (R162C), *MAP3K4* (R90Q), *PBRM1*, *PIK3CA* (E545K), *SMO* (L412F), and *TACC3* (R594C) were 44.76%, 81.7%, 42.37%, 96.96%, 37.16%, 2.25%, 49%, 95.74%, 37.54%, 63.84%, and 50%, respectively. According to ClinVar, the clinical significance of *CDHI* (V832M), *FGFR2* (N549K), *PIK3CA* (E545K), and *SMO* (L412F) in the recurrent maxillary tumor was benign, likely pathogenic, pathogenic/likely pathogenic, and pathogenic, respectively. In the pulmonary metastasis, the VAFs of *AXL* (W448L), *BAP1* (D458Gfs*11), *CDHI* (V832M), *FGFR2* (N549K), *MAP3K4* (R90Q), *PIK3CA* (E545K), *SMO* (L412F), *SMO* (V494M), *TACC3* (R594C), and *TP53* (R273C) were 46.4%, 20.23%, 48.43%, 41.64%, 47.88%, 12.47%, 18.89%, 19.15%, 47.94%, and 12.14%, respectively. According to ClinVar, the clinical significance of *CDHI* (V832M), *FGFR2* (N549K), *PIK3CA* (E545K), *SMO* (L412F), and *TP53* (R273C) in the pulmonary metastasis was benign, likely pathogenic, pathogenic/likely pathogenic, pathogenic, and conflicting interpretations of pathogenicity, respectively.

Discussion

Ameloblastoma frequently recurs, and recurrent tumors may invade locally and metastasize to the lungs [20, 21]. Metastases to multiple organs occur in 1%–4% of ameloblastomas and are associated with disease that is difficult to treat [20]. Characterizing the molecular alterations in paired recurrent and metastatic lesions is therefore important for understanding tumor progression.

We identified the same *SMO* (L412F) mutation in the recurrent maxillary ameloblastoma and multiple bilateral pulmonary metastases. To our knowledge, an identical activating *SMO* mutation has not previously been molecularly confirmed in paired recurrent and metastatic lesions. This finding supports a common clonal origin and suggests that *SMO* activation was maintained during metastatic progression. *SMO* encodes a key transmembrane receptor in the Hedgehog signaling pathway, and recurrent activating mutations such as L412F cause ligand-independent pathway activation [22, 23]. *SMO* mutations occur in approximately one-third of ameloblastomas and are generally mutually exclusive with *BRAF* V600E mutations [23, 24]. Accordingly, this tumor belongs to a molecular subgroup driven by Hedgehog signaling rather than mitogen-activated protein kinase (MAPK) pathway activation. The presence of this variant in anatomically distinct lesions suggests that *SMO* (L412F) was an early truncal event in tumor evolution rather than a genetic alteration acquired during metastatic progression.

The lower VAF in the pulmonary metastasis more likely reflects differences in tumor purity and clonal composition than loss of the driver mutation. Metastatic ameloblastoma most commonly involves

the lungs and often presents as multiple bilateral pulmonary nodules that retain the benign histologic appearance of the primary tumor [20, 25]. However, molecular characterization of paired maxillary and metastatic lesions remains extremely limited. To our knowledge, this study provides the first molecular evidence of clonal continuity between recurrent maxillary ameloblastoma and its pulmonary metastases. Identification of an activating *SMO* mutation also raises the possibility of therapy targeting the Hedgehog pathway. Nevertheless, the therapeutic sensitivity of tumors harboring *SMO* (L412F) remains incompletely understood, and additional clinical evidence is required before molecularly targeted therapy can be recommended for metastatic ameloblastoma.

Molecular analysis identified the same *SMO* (L412F) and *PIK3CA* (E545K) mutations in the recurrent maxillary ameloblastoma and pulmonary metastasis. *PIK3CA* (E545K) is a recurrent activating alteration in the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway and has previously been identified in ameloblastoma [26, 27]. The concordant presence of both mutations provides molecular evidence of a clonal relationship between the recurrent and metastatic tumors and indicates that alterations in the Hedgehog and PI3K/AKT pathways were maintained during metastatic dissemination. Although the contributions of these co-occurring mutations to metastatic behavior remain unclear, their persistence in the pulmonary metastasis suggests that activation of these pathways may have contributed to tumor progression and maintenance of the metastatic phenotype. The absence of *BRAF* V600E indicates a *BRAF* wild-type molecular subtype in which *SMO*-associated signaling may predominate. To our knowledge, this case is a

unique example of the same combination of *SMO* and *PIK3CA* mutations occurring in recurrent maxillary ameloblastoma and multiple pulmonary metastases. Despite the lower VAFs in the metastatic lesion, persistence of *SMO* (L412F) and *PIK3CA* (E545K) supports the presence of a shared ancestral clone. This genetic concordance further supports the metastatic origin of the pulmonary tumors and provides insight into the molecular evolution of metastasizing ameloblastoma.

We detected *TP53* (R273C) in the pulmonary metastasis but not in the recurrent maxillary ameloblastoma. In contrast, *SMO* (L412F) and *PIK3CA* (E545K) were present in both tumors. This distinct distribution may provide insight into tumor evolution. The shared *SMO* and *PIK3CA* mutations may represent early ancestral alterations in the founding clone, whereas *TP53* (R273C) may have arisen later in a subclone that gave rise to the pulmonary metastasis. Thus, the genomic profile of the metastatic lesion may reflect both preservation of ancestral driver alterations and acquisition of additional alterations during metastatic progression. The restriction of *TP53* (R273C) to the metastatic lesion raises the possibility that acquisition or selection of this alteration contributed to the aggressive phenotype of the metastatic clone. However, a causal relationship cannot be established from a single case.

Histologic distinction between ameloblastoma and ameloblastic carcinoma (AC) remains challenging because both entities share features such as peripheral palisading of columnar cells and central stellate reticulum-like areas [28-30]. However, AC is diagnosed on the basis of overt cytologic malignancy. In this case, focal areas showed nuclear atypia and increased proliferative activity, with an MIB1 labeling

index of 30% in hot spots. Recurrent ameloblastomas may exhibit cellular atypia that mimics malignancy [31]. Such atypia may result from chronic inflammation and altered vascularity after multiple surgical procedures rather than true malignant transformation [29, 30]. AC arising in the maxilla or mandible often shows necrosis and a *TP53* mutation, whereas the recurrent maxillary tumor in this case showed neither feature. Moreover, the pulmonary metastasis had less nuclear atypia and a lower MIB1 labeling index than the recurrent maxillary tumor. We therefore diagnosed recurrent metastasizing maxillary ameloblastoma rather than AC. Nevertheless, the five recurrences and the pronounced nuclear atypia and high MIB1 labeling index in the recurrent maxillary tumor warrant close monitoring for possible future malignant transformation.

Declarations

Funding: This work was supported by the Japan Agency for Medical Research and Development (AMED; project number 2021-12).

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

Ethical approval: The Institutional Review Board of Shizuoka Cancer Center approved all aspects of this study (permit number T2025-11-20251-5). All procedures performed in this study were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Consent to Participate: Informed consent was obtained in accordance with the requirements approved by

the Institutional Review Board of Shizuoka Cancer Center.

Consent for publication: Consent for publication was obtained for every individual person's data included in the study.

Availability of data and materials: No datasets were generated or analyzed during the current study.

Code Availability This study did not use any custom code or software that is not publicly available.

Author contributions

Y.Y. performed the experiments and prepared the figures. K.O., Y.T., and R.M. contributed to the study conception and data collection. Y.O., T.M., and K.U. supervised the study and edited the manuscript. F.S. performed the histopathologic diagnosis, conceived the study, and wrote the manuscript. All authors reviewed and approved the manuscript.

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Figure legends

Fig. 1 Clinical course of the patient. The patient underwent treatment for four recurrences of maxillary ameloblastoma at an outside hospital between 48 and 59 years of age. At Shizuoka Cancer Center, the fifth recurrent maxillary tumor and bilateral pulmonary lesions were surgically resected. Tissue samples from the maxillary tumor and pulmonary metastases were then analyzed using the 225-gene cancer panel.

Fig. 2 Radiologic and macroscopic findings of the fifth recurrent maxillary tumor. (a) Gadolinium-enhanced T1-weighted image showing the maxillary tumor (arrow). (b) Cut surface showing a solid tumor that almost completely occupies the maxillary sinus (arrows).

Fig. 3 Histologic findings of the fifth recurrent ameloblastoma in the maxillary sinus. (a) Low-power view showing a solid ameloblastoma occupying the maxillary sinus. Original magnification, $\times 0.3$. (b) Tumor cells arranged in solid nests and sheets (asterisks), with intervening stromal cells (triangles). (c) Osteoclast-like giant cells within the tumor. Original magnification, $\times 10$ in (b) and (c). Tumor cells show (d) peripheral palisading (arrows), (e) moderate atypia with focal mitotic figures (arrows), and (f) squamous metaplasia (arrows). (g) The highly cellular stroma shows moderate atypia and frequent mitotic figures (arrows). Original magnification, $\times 20$ in (d) and $\times 40$ in (e)–(g).

Fig. 4 Immunoreactivity for CK19, p53, and MIB1 in tumor and stromal cells of the maxillary lesion. (a) Cytoplasmic CK19 positivity in tumor cells. (b) Focal p53 positivity in tumor cells. (c) MIB1 labeling index of approximately 30% in a hot spot. (d) Negative CK19 staining in stromal cells. (e) Focal p53 positivity in stromal cells. (f) MIB1 positivity in stromal cells. Original magnification, $\times 40$ in (a)–(f).

Fig. 5 Radiologic and histologic findings of pulmonary metastatic ameloblastoma. Computed tomography (CT) shows metastatic nodules in (a) segment 1 + 2 of the left lung and (b) segment 9 of the right lung (white arrows). Histologic findings of the left pulmonary metastasis are shown at original magnifications of (c) $\times 0.3$ and (d) $\times 10$. (e, f) Tumor cells show solid nests, peripheral palisading (arrows in f), and squamous metaplasia (asterisks in f), resembling the maxillary tumor. (g) The stroma is focally myxoid and shows moderate atypia. Original magnification, $\times 40$ in (e)–(g).

Fig. 6 Immunoreactivity for p53 and MIB1 in tumor cells of the pulmonary metastasis. (a) Diffuse p53 positivity in tumor cells. (b) MIB1 labeling index of approximately 20% in a hot spot. Original magnification, $\times 40$ in (a) and (b).

Tables

Table 1. DNA purity, DNA integrity number (DIN), and estimated tumor content in the recurrent maxillary tumor and pulmonary metastasis.

Table 2. Genetic alterations identified in the recurrent maxillary tumor using the 225-gene cancer panel.

Table 3. Genetic alterations identified in the pulmonary metastasis using the 225-gene cancer panel.

Fig.1

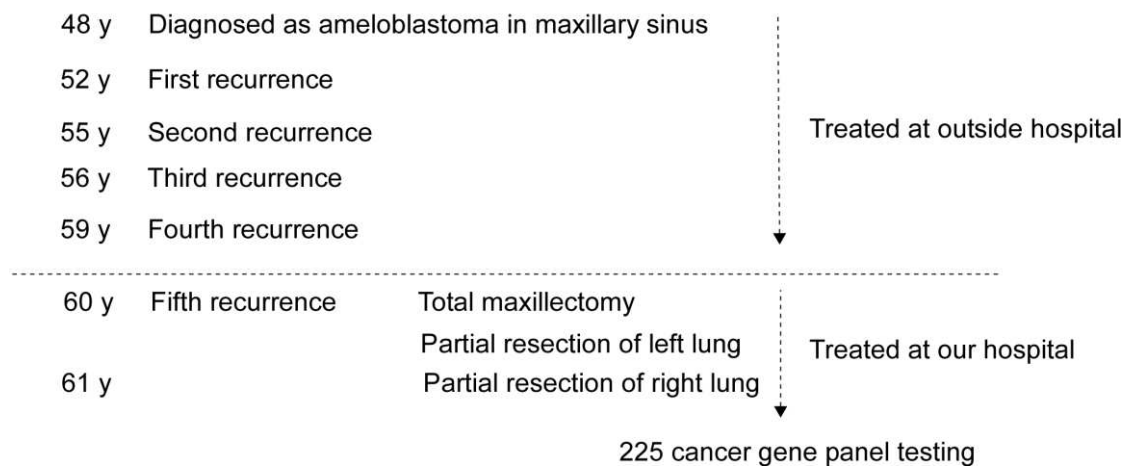
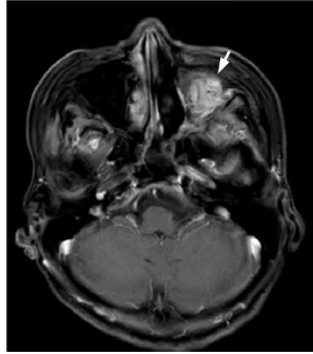


Fig.2

a



b



Fig.3

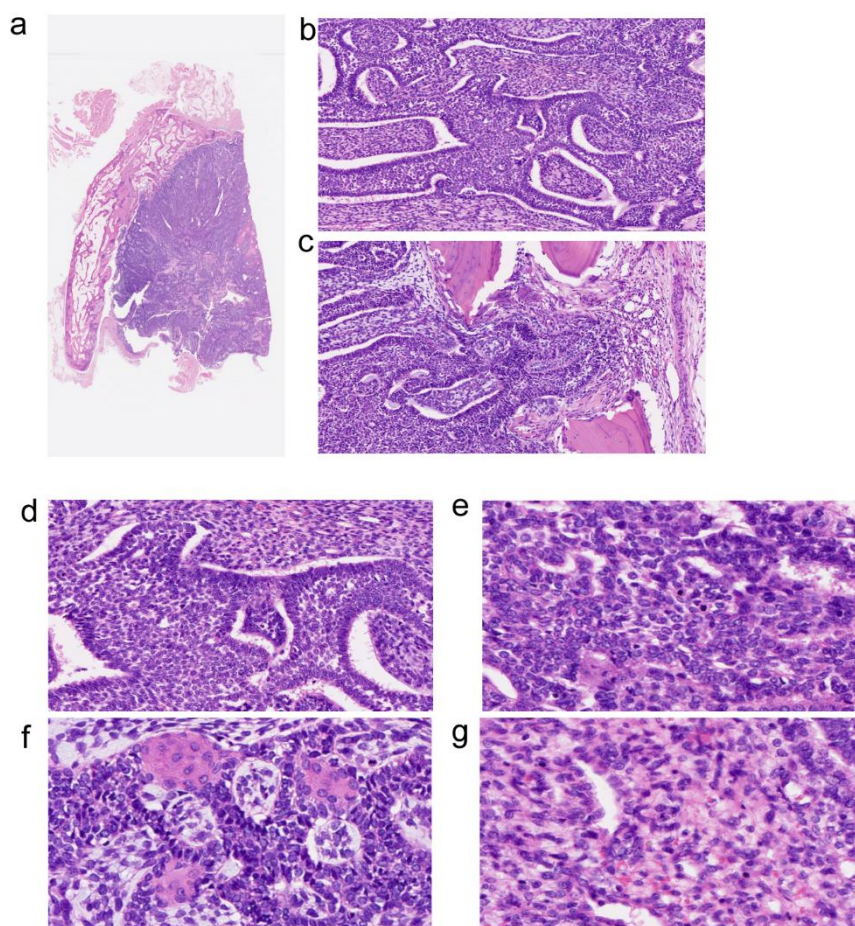


Fig.4

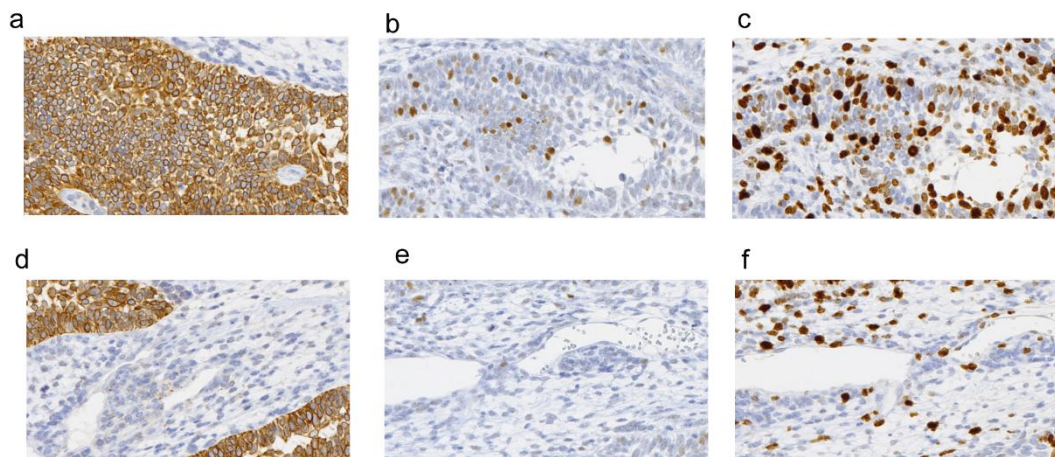


Fig.5

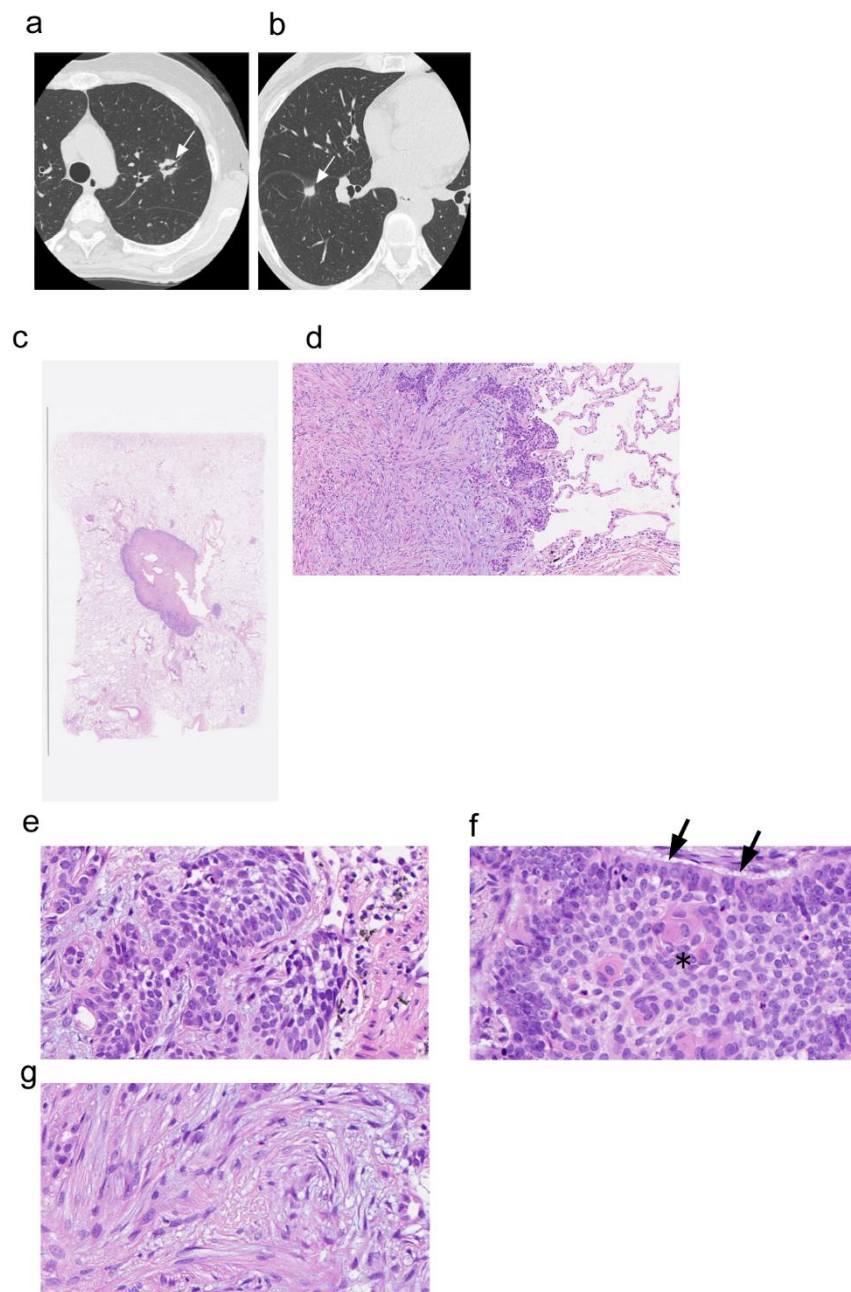


Fig.6

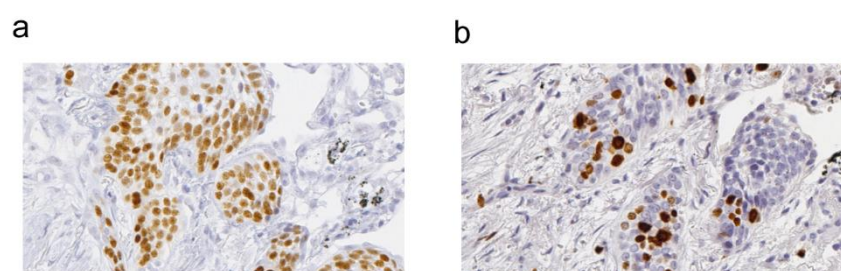


Table. 1

Specimen	DNA purity (260nm/280nm)	DNA Integrity Number (DIN)	tumor cell content (%)
Maxilla	1.93	3	80
Lung	1.94	3.7	20

Table. 2

Gene name	Variant	AF (%)	Transcript Name	Sequence Ontology	ClinVar.Clinical_Significance
AXL	W448L	44.76	NM_021913.4	missense_variant	-
BAP1	D458Gfs*11	81.7	NM_004656.3	frameshift_variant	-
CDH1	V832M	42.37	NM_004360.4	missense_variant	Benign
FGFR2	N549K	96.96	NM_000141.4	missense_variant	Likely Pathogenic
KDM6A	-	37.16	NM_021140.3	splice_acceptor_variant	-
MAP3K1	R162C	2.25	NM_005921.1	missense_variant	-
MAP3K4	R90Q	49	NM_005922.3	missense_variant	-
PBRM1	-	95.74	NM_018313.4	splice_acceptor_variant	-
PIK3CA	E545K	37.54	NM_006218.3	missense_variant	Pathogenic/Likely Pathogenic
SMO	L412F	63.84	NM_005631.4	missense_variant	Pathogenic
TACC3	R594C	50	NM_006342.2	missense_variant	-

Table. 3

Gene name	Variant	AF(%)	Transcript Name	Sequence Ontology	ClinVar.Clinical_Significance
AXL	W448L	46.4	NM_021913.4	missence_variant	-
BAP1	D458Gfs*11	20.23	NM_004656.3	frameshift_variant	-
CDH1	V832M	48.43	NM_004360.4	missence_variant	Benign
FGFR2	N549K	41.64	NM_000141.4	missence_variant	Likely Pathogenic
MAP3K4	R90Q	47.88	NM_005922.3	missence_variant	-
PIK3CA	E545K	12.47	NM_006218.3	missence_variant	Pathogenic/Likely Pathogenic
SMO	L412F	18.89	NM_005631.4	missence_variant	Pathogenic
SMO	V494M	19.15	NM_005631.4	missence_variant	-
TACC3	R594C	47.94	NM_006342.2	missence_variant	-
TP53	R273C	12.14	NM_000546.5	missence_variant	Conflicting Interpretations Of Pathogenicity