Original article

Immunohistochemical molecular expression profile of metastatic brain tumor for potent personalized medicine.

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Abstract

The recent progress in molecular targeting therapy may a yield the personalized therapeutic strategy to the patients with metastatic brain tumor (MBT), which is the most frequently encountered intracranial tumors. For this purpose, we explored the molecular expression profile of MBT to establish the pathological basis for personalized diagnosis. We employed 166 cases of MBT specimens including 70 cases of lung cancer and 34 cases of breast cancer, and performed immunostaining for multiple molecules such as EGFR, COX-2 and O-6-methylguanine-DNA methyltransferase (MGMT), which could be target molecules for therapeutic agents or predict the drug efficacy. The loss of MGMT expression was observed in about 20 to 40 % of MBT derived from lung, breast and gastrointestinal cancers, indicating the possible treatment with temozolomide to MBT patients. In addition, MBT expressed various receptor tyrosine kinases such as EGFR and HER2, and signal transduction molecules such as phospho-mTOR and COX-2 independently to their tumor origin, offering individualized medication with molecular targeting drugs. Moreover, we identified the alteration of molecular expression profile in 4 MBT cases during the recurrence. Our results not only explore the molecular characteristics of MBT, but also propose potent personalized medicine to MBT patients. (195 words)

Introduction

The metastatic brain tumor (MBT) is the most frequently encountered intracranial tumor, although the reported incidence rates of MBT probably underestimates the true incidence because of underdiagnosis and inaccurate reporting[1]. The most common primary lesions of MBT in adults are the lung and the breast[1], and more than 80 % of MBT are located in the cerebral hemisphere and around 15 % are found in the cerebellum[2]. If MBT is found in the cancer patients after surgery and/or the targeted chemotherapies against the primary tumor, the clinician usually offers the best supportive care. In fact, most of the chemotherapeutic agents are thought to be less effective compared to those for the primary lesion, mainly due to the blood-tumor barrier in the brain[1, 3]; thus the cancer patients' prognosis with brain metastasis is extremely poor even after the multimodal combination therapy of surgical resection, radiotherapy and chemotherapy.

The recent progress in molecular targeting therapy provides promising tumor type-specific and personalized treatment to the cancer patients, especially of lung and breast cancer. Regarding MBT treatment, the dramatic responses of gefitinib and lapatinib to brain metastasis of lung adenocarcinoma and breast cancer were recently reported[4-8], and there are many ongoing clinical trials with molecular targeting drugs

against MBT[1]. To obtain the maximal therapeutic effect, a personalized pathological diagnosis based on the molecular expression profile beyond the types of primary organ is needed.

The extensive analyses for the molecular expression profiles in MBT are limited because of the difficulty to obtain a large number of tumor samples from brain metastasis. Several reports revealed the expression and the alteration of several molecular markers such as EGFR, COX-2, and VEGF-C in MBT from lung cancer, although they failed to identify the clinical benefits from their studies[9-11]. In 234 cases of breast cancer, EGFR expression was identified in 18.4 %, although no detailed analysis for the brain metastasis was performed[12]. Here we performed immunohistochemical analysis to obtain the molecular expression profile of MBT to establish the pathological basis for personalized diagnosis which would be useful to offer a personalized therapeutic strategy to the patients with metastatic brain tumor.

Materials and Methods

Ethical requirements

The study using human samples was performed with the approval of the Internal Review Board on Ethical Issues of Hokkaido University Graduate School of Medicine, Sapporo, Japan.

Patients' demography and tumor specimens

We employed 166 metastatic brain tumor specimens diagnosed between January 2003 and May 2012 in our faculty for histological examination. The patients had been diagnosed with primary brain tumor or MBT without identification of primary lesions, and had undergone radical surgery. Formalin-fixed paraffin-embedded tissue blocks were prepared from surgical specimens, and sections were sliced and stained with hematoxylin and eosin (HE) for routine histopathological examination. The final diagnosis of MBT and identification of primary tumor were performed by routine histological examination and immunohistochemical analysis. Characteristics of the patients are summarized in Table 2. Ninety-nine were male and 67 were female. Median age at surgery was 62.4 years (range 51 - 73).

Immunohistochemical analysis using tissue microarray (TMA)

TMA were constructed using Tissue Micro Arrayer, JF-4 (Sakura Finetek, Tokyo).

Cylindrical cores of 3.0 mm in diameter were taken from each tissue block. Immunohistochemical staining was performed as follows: The TMA sections were incubated with indicated primary antibody and reacted with a dextran polymer reagent combined with secondary antibodies and peroxidase (Envision/HRP; Dako). The primary antibodies with the conditions for antigen retrieval used in this analysis are summarized in Table 2. Each slide was evaluated independently by three pathologists (Y. K., H. M., and H. N.). Immunostaining was evaluated for both the proportion and staining intensity of tumor cells in each case. The proportion was assessed according to the percentage of immunopositive cells as follows: 0, 0 %; +1, less than 10 %; +2, 10 to 50 %; and +3, greater than 50 %. The staining intensity was evaluated as weak (+1), moderate (+2) and strong (+3). The membranous staining of PDGFRβ, EGFR (wild type: WT), EGFR(L858R), EGFR(del), cKit and cMET, or nuclear staining of MGMT were also restrictedly evaluated. The sum of the proportion score and intensity score was evaluated as follows: MGMT and EGFR (WT), 3+≦ positive; the others, 4+ ≦ positive.

Results

Characteristics of patients and primary lesions of MBT

A summary of the patients is shown in Table 2 and Supplemental Fig. 1. Median age of the patients was 62.4 years (ranging from 51 to 73). Ninety-nine patients were men and 67 were women. The most frequent lesion was the lung in males (53.5 %) and the breast in females (50.1 %). The gastrointestinal tract cancer (stomach, colon and rectum) was followed and 13.3 % was primary-unknown at the time of initial pathological diagnosis. These results were almost compatible with a recent report from Europe[1].

MGMT expression in MBT

Previously, MGMT expression in metastatic lung cancer and melanoma was evaluated[13, 14], and we also established the immunohistochemical validation of MGMT expression in surgical specimens[15]; thus, we performed immunostaining to explore the MGMT expression in MBT. MGMT was positive in 55 % of total MBT, and the positive ratio (75.0 – 79.4 %) in MBT of breast and gastrointestinal origin was much higher than that of lung (53.3 %), while MBT derived from renal cancer revealed relatively low expression rate of MGMT (28.6 %) (Table 3). The representative images of MGMT

nuclear expression is indicated in Fig. 2 (T, U).

Expression profile of therapeutic target molecules in MBT

To propose the potent personalized medicine to MBT patients with currently available molecular targeting drugs, we evaluated the expression profile of the multiple target molecules including EGFR (WT), EGFR (L858R), EGFR (del 746-750), PDGFRβ, HER2, cKit, cMET, phospho-mTOR, and COX-2 by immuohistochemistry. As shown in Figure 2, the expression of PDGFRβ, EGFR, HER2, cKit, and cMET was found in the cell membrane, and phospho-mTOR and COX-2 was located in the cytoplasm and partially in the cell membrane (phospho-mTOR). The positive rate of each molecule according to the primary tumor is summarized in Table 2, Supplemental Fig. 2 and Supplemental Fig. 3. EGFR (WT) was highly expressed in MBT derived from breast (35.3 %) and kidney (85.7 %) as well as lung (45.7 %). In addition, the mutant form of EGFR (L858R), which indicates favorable chemosensitivity to gefitinib[4], was identified in 3 cases of MBT from breast cancer. HER2 was also expressed in metastatic gastric cancer (25.0 %) and lung cancer (2.9 %), which were lower than breast cancer (52.9 %). cMET and phospho-mTOR were ubiquitously identified in a higher rate except in MBT from liver and kidney, while a relative high expression of

cKit was observed in MBT of unknown origin (40.9 %). COX-2 expression was also identified in MBT from lung, breast colorectal and kidney in about 20 %.

Alteration of molecular expression profile during the recurrence

Throughout this analysis, we experienced 13 cases in which the patients underwent recurrence of MBT and second radical surgery. Although the alteration of gene expression profile between the primary tumor and brain metastasis was previously reported[9-12, 16, 17], the study of sequential recurrent MBT has not been reported yet. We analyzed 13 cases of recurrent MBT and obtained interesting results in which 4 cases represented dramatic changes in molecular expression profile such as loss and gain of EGFR expression including wild type and also mutant forms, cKit and COX-2 (Table 4 and Fig. 3).

Discussion

Here we explored the molecular expression profile of MBT and found various molecular markers in MBT such as EGFR, HER2 and MGMT, suggesting potent personalized medication to MBT patients.

Temozolomide (TMZ) is an oral alkylating agent used for the treatment of malignant glioma and malignant melanoma[1]. The therapeutic mechanism of TMZ depends on its ability to alkylate/methylate DNA, which usually occurs at the N-7 or O-6 positions of guanine residues, resulting in the death of tumor cells[18]. However, if tumor cells express an enzyme called O-6-methylguanine-DNA methyltransferase (MGMT), they are able to repair this type of DNA damage, and therefore diminish the therapeutic efficacy of temozolomide[19]. Although multiple retrospective and prospective phase II trials with TMZ to MBT were reported, the therapeutic response was not dramatic[5]. This might be because that the expression status of MGMT was not considered in these clinical trials. In fact, as shown in our analysis and also in previous report[14], more than 50 % of MBT expressed MGMT; in particular, MBT of breast and gastrointestinal cancer revealed a high positive rate of MGMT. Therefore, selection of the MGMT-negative MBT patients by immunohistochemistry, unconcerned with tumor origin, might yield a promising therapeutic response of TMZ to MBT

patients.

The promising personalized treatment with the molecular targeting drugs such as gefitinib and lapatinib is expected to be the new therapeutic strategy for MBT patients. In fact, many clinical trials with molecular targeting drugs against MBT are ongoing[1]. The EGFR-expressing MBT might be sensitive for cetuximab treatment, because crossing of the blood-brain barrier and accumulation in brain metastasis of cetuximab was reported[20]. MBT with a mutation form of EGFR (L858R) could be a promising candidate for gefitinib treatment, although only 2 breast cancer cases out of a total of 166 cases were isolated in our analysis. The large number of MBT cases being positive for cMET and/or phospho-mTOR might indicate a possible target for clinical trials with MET inhibitors and mTOR inhibitors[1]. A recent comparative genome-wide expression analysis in breast cancer patients with brain metastasis identified COX-2 as a mediator of cancer cell passage through the blood–brain barrier, and the treatment efficacy of NSAIDs to the mice with brain metastasis of breast cancer was proven [21, 22]. Therefore the overexpression of COX-2 in MBT suggests the anti-cancerous effect of COX-2 inhibitors, such as celecoxib, to MBT. These results encourage us to offer the challenging personalized molecular targeting therapy; however, we must consider the discrepancy between the immunohistochemical expression of target molecules in tumor

cells and the therapeutic efficacy of the molecular targeting drugs.

The alteration of gene expression profile between primary and metastatic tumor was reported in various types of cancer such as lung, colorectal and breast[9-11, 16, 17]. In this analysis, we isolated 13 cases of sequential recurrent brain metastasis including 4 cases in which the molecular expression profile was altered. Especially, lung cancer cases represented a paradoxical alteration of EGFR expression in which one case showed loss of expression but the other case acquired a gain of expression and additional mutation of L858R. The loss of expression could result from therapeutic response by EGFR targeting drugs such as gefitinib, while gain of expression and also acquisition of additional mutation might explain that two sequential MBT were derived from different clones of tumor cells in the same primary lung cancer. The detailed analysis of clinical courses and multiple gene expression analysis between the primary and metastatic tumor will elucidate the interesting phenomena described above. In addition, these results inform us that reevaluation of molecular expression profile by re-brain biopsy might be required to perform the promising personalized medicine to MBT patients.

In conclusion, we explored the immunohistochemical molecular expression profile of MBT which could be target molecules for therapeutic agents or predict the drug

efficacy. Our results could be a pathological basis for personalized diagnosis which would be useful to offer a personalized therapeutic strategy to the patients with metastatic brain tumor.

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References

1 Preusser M, Capper D, Ilhan-Mutlu A, Berghoff AS, Birner P, Bartsch R, *et al.* Brain metastases: pathobiology and emerging targeted therapies. *Acta neuropathologica*. 2012; **123**: 205-22.

2 Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (eds). WHO Classification of Tumours of the Central Nervous System, 4th Edition, IARC Press, Lyons, 2007.

3 Lockman PR, Mittapalli RK, Taskar KS, Rudraraju V, Gril B, Bohn KA, *et al.* Heterogeneous blood-tumor barrier permeability determines drug efficacy in experimental brain metastases of breast cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2010; **16**: 5664-78.

4 Hotta K, Kiura K, Ueoka H, Tabata M, Fujiwara K, Kozuki T, *et al.* Effect of gefitinib ('Iressa', ZD1839) on brain metastases in patients with advanced non-small-cell lung cancer. *Lung Cancer*. 2004; **46**: 255-61.

5 Olson JJ, Paleologos NA, Gaspar LE, Robinson PD, Morris RE, Ammirati M, *et al.* The role of emerging and investigational therapies for metastatic brain tumors: a systematic review and evidence-based clinical practice guideline of selected topics. *Journal of neuro-oncology*. 2010; **96**: 115-42.

6 Lin NU, Carey LA, Liu MC, Younger J, Come SE, Ewend M, *et al.* Phase II trial of lapatinib for brain metastases in patients with human epidermal growth factor receptor 2-positive breast cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2008; **26**: 1993-9.

7 Jones RL, Smith IE. Efficacy and safety of trastuzumab. *Expert opinion on drug safety*. 2004; **3**: 317-27.

8 Sawaki M, Ito Y, Tada K, Mizunuma N, Takahashi S, Horikoshi N, *et al.* Efficacy and safety of trastuzumab as a single agent in heavily pretreated patients with HER-2/neu-overexpressing metastatic breast cancer. *Tumori*. 2004; **90**: 40-3.

9 Milas I, Komaki R, Hachiya T, Bubb RS, Ro JY, Langford L, *et al.* Epidermal growth factor receptor, cyclooxygenase-2, and BAX expression in the primary non-small cell lung cancer and brain metastases. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2003; **9**: 1070-6.

10 Italiano A, Saint-Paul MC, Caroli-Bosc FX, Francois E, Bourgeon A, Benchimol D, *et al.* Epidermal growth factor receptor (EGFR) status in primary colorectal tumors correlates with EGFR expression in related metastatic sites: biological and clinical implications. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO*. 2005; **16**: 1503-7.

11 Saad AG, Yeap BY, Thunnissen FB, Pinkus GS, Pinkus JL, Loda M, *et al.* Immunohistochemical markers associated with brain metastases in patients with nonsmall cell lung carcinoma. *Cancer*. 2008; **113**: 2129-38.

12 Sihto H, Lundin J, Lundin M, Lehtimaki T, Ristimaki A, Holli K, *et al.* Breast cancer biological subtypes and protein expression predict for the preferential distant metastasis sites: a nationwide cohort study. *Breast cancer research : BCR*. 2011; **13**: R87.

13 Wu PF, Kuo KT, Kuo LT, Lin YT, Lee WC, Lu YS, *et al.* O(6)-Methylguanine-DNA methyltransferase expression and prognostic value in brain metastases of lung cancers. *Lung Cancer*. 2010; **68**: 484-90.

14 Ingold B, Schraml P, Heppner FL, Moch H. Homogeneous MGMT immunoreactivity correlates with an unmethylated MGMT promoter status in brain metastases of various solid tumors. *PloS one*. 2009; **4**: e4775.

15 Sasai K, Akagi T, Aoyanagi E, Tabu K, Kaneko S, Tanaka S. O6-methylguanine-DNA methyltransferase is downregulated in transformed astrocyte cells: implications for anti-glioma therapies. *Molecular cancer*. 2007; **6**: 36.

16 Gomez-Roca C, Raynaud CM, Penault-Llorca F, Mercier O, Commo F, Morat L, *et al.* Differential expression of biomarkers in primary non-small cell lung cancer and metastatic sites. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer*. 2009; **4**: 1212-20.

17 Yoshida S, Takahashi H. Expression of extracellular matrix molecules in brain metastasis. *Journal of surgical oncology*. 2009; **100**: 65-8.

18 Brandsma D, van den Bent MJ. Molecular targeted therapies and chemotherapy in malignant gliomas. *Current opinion in oncology*. 2007; **19**: 598-605.

19 Jacinto FV, Esteller M. MGMT hypermethylation: a prognostic foe, a predictive friend. *DNA Repair (Amst)*. 2007; **6**: 1155-60.

20 Rades D, Nadrowitz R, Buchmann I, Hunold P, Noack F, Schild SE, *et al.* Radiolabeled cetuximab plus whole-brain irradiation (WBI) for the treatment of brain metastases from non-small cell lung cancer (NSCLC). *Strahlentherapie und Onkologie : Organ der Deutschen Rontgengesellschaft [et al]*. 2010; **186**: 458-62.

21 Rizzo MT. Cyclooxygenase-2 in oncogenesis. *Clinica chimica acta; international journal of clinical chemistry*. 2011; **412**: 671-87.

22 Bos PD, Zhang XH, Nadal C, Shu W, Gomis RR, Nguyen DX, *et al.* Genes that mediate breast cancer metastasis to the brain. *Nature*. 2009; **459**: 1005-9.

Figure Legends

Fig 1. The molecular expression profile of a total of 166 cases of MBT. Each column and number represents the positive rate of each molecule. The detailed positive rate according to tumor origins is summarized in Table 2.

Fig 2. Representative pictures of positive staining of the following molecules: (A, B) PDGFRβ, (C, D) EGFR (WT), (E, F) EGFR (L858R), (G, H) EGFR (E746-A750del), (I, J) HER2, (K, L) c-kit, (M, N) c-MET, (O, P) p-mTOR, (R, S) COX2, (T, U) MGMT. The primary lesion of each case is as follows: (A, C, G, M, T) lung, (B, L) primary unknown, (D, E, F, H, I) breast, (J) stomach, (K) uterus, (N) gallbladder, (Q) pancreas, (R) colon, (U) esophagus. All pictures are in x400. Scale bars: 200 μm.

Fig 3. The 4 cases of MBT with alteration in molecular expression profile during their sequential recurrence. In Case 1, loss of EGFR (WT) was observed. Case 2 harbored the gain of expression with EGFR (WT) and EGFR (L858R) after the recurrence. In Case 3, gain of EGFR (del) and COX2 expression was shown. In Case 4, c-kit expression was lost after recurrence. The primary lesions of Cases 1, 2, 3 were lung, and Case 4 was breast. All pictures are in x400. Scale bars: 200 μm.

Table1

Table 1 The primary antibodies and the conditions for antigen retrieval used in this analysis.

Antibody	Clone	Type	Dilution	Antigen retrieval	Company
PDGFRβ	C82A3	rabbit	1:200	Water bath (EDTA buffer pH9.0)	Cell Signaling Technology Inc, Danvers, MA, USA
EGFR (Wild type)	31G7	mouse	1:50	Trypsin	NICHIREI Bioscience Inc, Tokyo, Japan
EGFR (L858R)	43B2	rabbit	1:100	Water bath (EDTA buffer pH9.0)	Cell Signaling Technology Inc
EGFR (E746-A750del)	6B6	rabbit	1:50	Water bath (EDTA buffer pH9.0)	Cell Signaling Technology Inc,
HER2	poly	rabbit	1:200	Water bath (citric acid buffer pH 6.0)	DakoCytomation, Glostrup, Denmark
c-kit	poly	rabbit	1:150	Water bath (EDTA buffer pH9.0)	DakoCytomation, Glostrup, Denmark
c-MET	EP1454Y	rabbit	1:150	Water bath (EDTA buffer pH9.0)	EPITOMICS Inc, Burlingam, USA.
p-mTOR	49F9	rabbit	1:100	Water bath (EDTA buffer pH9.0)	Cell Signaling Technology Inc
COX2	poly	rabbit	1:100	Water bath (EDTA buffer pH9.0)	Cayman Chemical, Michigan, USA
MGMT	MT3.1	mouse	1:200	Pressure cook (citric acid buffer pH 6.0)	CHEMICON International Inc, Temecula, USA

Table2

Table 2. Patient Characteristics and primary lesions of MBT

Primary lesion	No.	%	Age, years (Median and range)	Male		Female	
				No.	%	No.	%
Total	166		62.4±11.3	99		67	
Lung	70	42.2%	63.9±9.9	53	53.5%	17	25.4%
Breast	34	20.5%	56.2±11.5	0	0.0%	34	50.7%
Colon and rectum	9	5.4%	73.8±5.3	6	6.1%	3	4.5%
Stomach	8	4.8%	65.4±6.0	8	8.1%	0	0.0%
Pancreas, biliary duct, liver	8	4.8%	55.9±7.2	5	5.1%	3	4.5%
Kidney	7	4.2%	65.3±11.7	6	6.1%	1	1.5%
Esophagus	3	1.8%	66.7±2.9	3	3.0%	0	0.0%
Ovary	2	1.2%	50.0±14.0	0	0.0%	2	3.0%
Uterus	2	1.2%	55.0±1.0	0	0.0%	2	3.0%
Thyroid	1	0.6%	62	1	1.0%	0	0.0%
Unknown	22	13.3%	64.5±13.0	17	17.2%	5	7.5%

Table3

Table 3. Molecular expression profile of MBT according to the primary lesions

Primary lesion	Total No.	%	PDGFRβ Positive % (No.)	EGFR (WT) Positive % (No.)	EGFR (L858R) Positive % (No.)	EGFR (del) Positive % (No.)	HER2 Positive % (No.)	c-kit Positive % (No.)	c-MET Positive % (No.)	p-mTOR Positive % (No.)	COX2 Positive % (No.)	MGMT Positive % (No.)
Lung	70	42.2%	1.4% (1)	45.7% (32)	0.0% (0)	2.9% (2)	2.9% (2)	12.9% (9)	81.4% (57)	31.4% (22)	20.0% (14)	53.3% (32)
Breast	34	20.5%	0.0% (0)	35.3% (12)	8.8% (3)	0.0% (0)	52.9% (18)	20.6% (7)	70.6% (24)	52.9% (18)	17.6% (6)	79.4% (27)
Colorectal	9	5.4%	0.0% (0)	11.1% (1)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	55.6% (5)	66.7% (6)	22.2% (2)	77.8% (7)
Stomach	8	4.8%	0.0% (0)	12.5% (1)	0.0% (0)	0.0% (0)	25.0% (2)	0.0% (0)	62.5% (5)	12.5% (1)	0.0% (0)	75.0% (6)
Pancreas	2	1.2%	0.0% (0)	50.0% (1)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	50.0% (1)	0.0% (0)	50.0% (1)
Bile duct	2	1.2%	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	50.0% (1)	100.0% (2)	0.0% (0)	0.0% (0)
Liver	4	2.4%	0.0% (0)	25.0% (1)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	25.0% (1)
Kidney	7	4.2%	0.0% (0)	85.7% (6)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	14.3% (1)	28.6% (2)	14.3% (1)	28.6% (2)
Esophagus	3	1.8%	0.0% (0)	66.7% (2)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	66.7% (2)	0.0% (0)	0.0% (0)	66.7% (2)
Ovary	2	1.2%	0.0% (0)	100.0% (2)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	100.0% (2)	50.0% (1)	0.0% (0)	100.0% (2)
Uterus	2	1.2%	0.0% (0)	100.0% (2)	0.0% (0)	0.0% (0)	0.0% (0)	50.0% (1)	50.0% (1)	50.0% (1)	0.0% (0)	50.0% (1)
Thyroid	1	0.6%	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	100.0% (1)	100.0% (1)	0.0% (0)	0.0% (0)
Unknown	22	13.3%	4.5% (1)	22.7% (5)	0.0% (0)	0.0% (0)	13.6% (3)	40.9% (9)	63.6% (14)	27.3% (6)	9.1% (2)	50.0% (11)

≧50%　20-50%

Table4

Table 4. Number of patients with recurrent MBT

Primary lesion	Total number	Recurrence (+) No. (%)	Alteration in profile (+) No. (%)
Lung	70	7 (10 .0%)	3 (4.3 %)
Breast	34	3 (11.3 %)	1 (2.9 %)
Colorectal	9	0 (0 %)	—
Stomach	8	2 (25.0 %)	0 (0 %)
Pancreas	4	0 (0 %)	—
Bile duct	2	0 (0 %)	—
Liver	2	0 (0 %)	—
Kidney	2	0 (0 %)	—
Esophagus	3	0 (0 %)	—
Ovary	2	1 (50.0 %)	0 (0 %)
Uterus	2	0 (0 %)	—
Thyroid	1	0 (0 %)	—
Unknown origin	22	0 (0 %)	—

Figure1

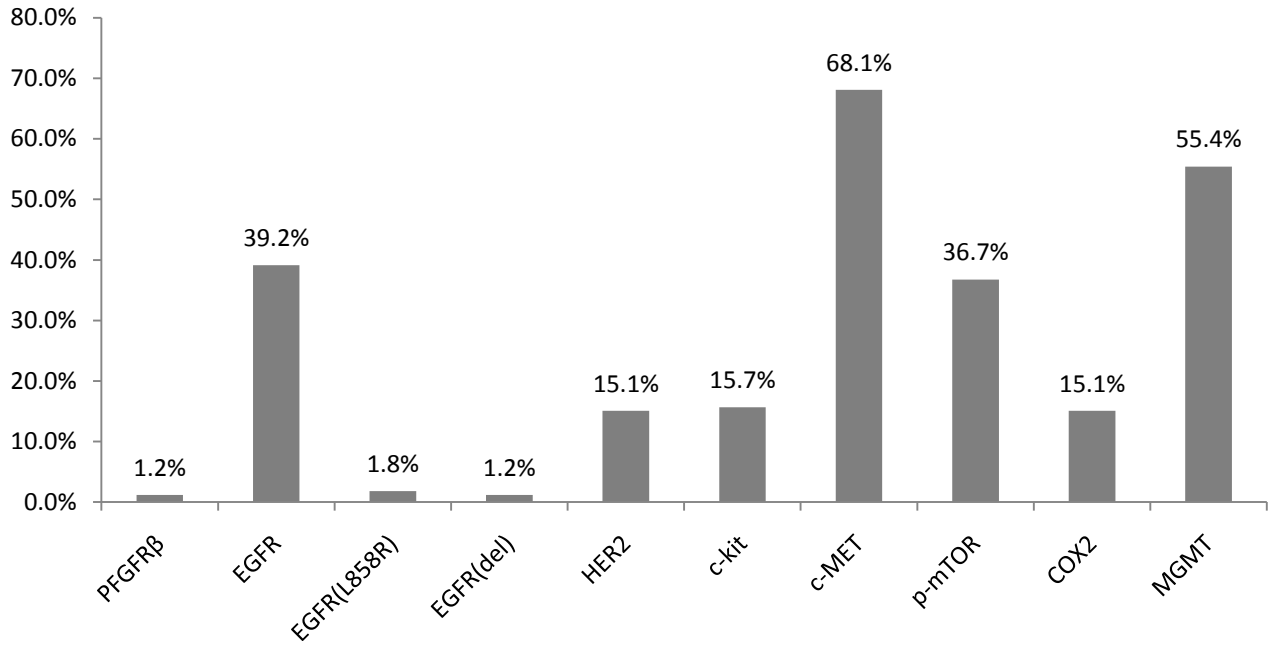

80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
1.2%
39.2%
1.8%
1.2%
15.1%
15.7%
68.1%
36.7%
15.1%
55.4%
PFGFRβ
EGFR
EGFR(L858R)
EGFR(del)
HER2
c-kit
c-MET
p-mTOR
COX2
MGMT

Figure2

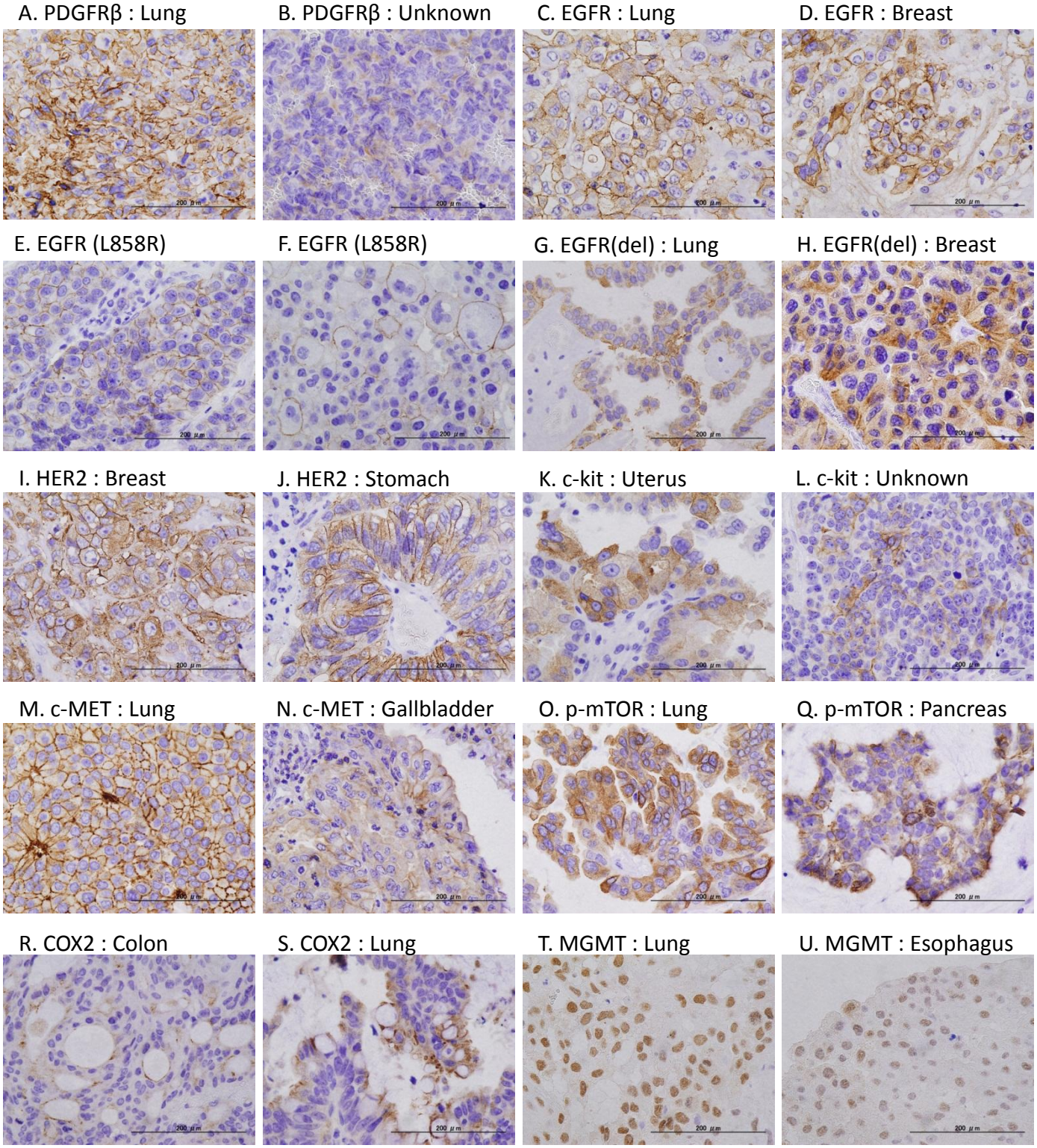
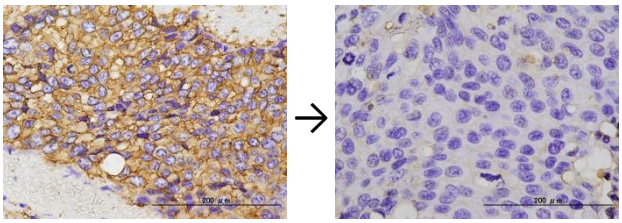

A. PDGFRβ : Lung
B. PDGFRβ : Unknown
C. EGFR : Lung
D. EGFR : Breast
E. EGFR (L858R)
F. EGFR (L858R)
G. EGFR(del) : Lung
H. EGFR(del) : Breast
I. HER2 : Breast
J. HER2 : Stomach
K. c-kit : Uterus
L. c-kit : Unknown
M. c-MET : Lung
N. c-MET : Gallbladder
O. p-mTOR : Lung
Q. p-mTOR : Pancreas
R. COX2 : Colon
S. COX2 : Lung
T. MGMT : Lung
U. MGMT : Esophagus

Figure3

Case 1. Lung

EGFR

(+) (-)



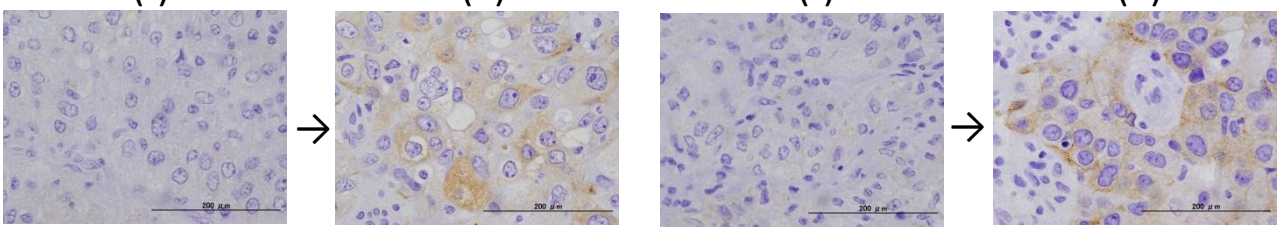
Case 2. Lung

EGFR

(-) (+)

EGFR (L858R)

(-) (+)



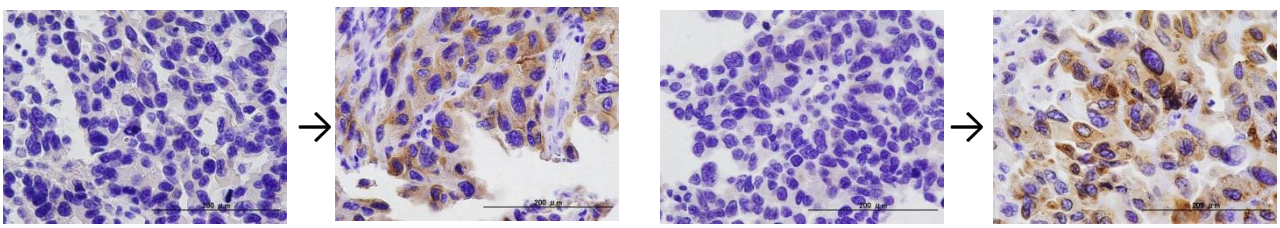
Case 3. Lung

EGFR (del)

(-) (+)

COX2

(-) (+)



Case 4. Breast

c-Kit

(+) (-)