Supporting Information

Figure S1 5′-RACE products using FFPE tissues. Our modified 5′-RACE faithfully isolated cDNA fragments for *EML4-ALK* (A) or *KIF5B-ALK* (B) from known ALK- positive tumors.
(TIF)

Figure S2 Putative cDNA sequence of KLC1-ALK. The putative full-length cDNA of *KLC1-ALK* was synthesized from the frozen tissue with KIF5B-ALK fusion expression.
(PDF)

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Author Contributions

Conceived and designed the experiments: KT HM. Performed the experiments: YT MS SS ES SH RA. Analyzed the data: YT MS HM KT. Contributed reagents/materials/analysis tools: RA TN. Wrote the paper: KT HM.

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Case Report

Favorable response to crizotinib in three patients with echinoderm microtubule-associated protein-like 4–anaplastic lymphoma kinase fusion-type oncogene-positive non-small cell lung cancer

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The echinoderm microtubule-associated protein-like 4 (EML4)–anaplastic lymphoma kinase (ALK) is a recently identified fusion-type oncoprotein that exists in approximately 5% of non-small cell lung cancer (NSCLC). It has been demonstrated that NSCLC driven by EML4-ALK is strongly addicted to this fusion-type oncokinase. A clinical trial of crizotinib (PF-02341066) sponsored by Pfizer has proven this oncogene addiction in humans by demonstrating a high response rate to inhibition of ALK kinase activity. In the present study, we report on three cases harboring *EML4-ALK* rearrangement who were enrolled in the trial (A8081001, NCT00585195). All three patients showed favorable responses to the ALK-specific tyrosine kinase inhibitor. (*Cancer Sci* 2011; 102: 1602–1604)

Case report

Clinical findings in three patients treated with crizotinib are described below.

Case 1. A 30-year-old man who had never smoked presented with smoldering pneumonia in October 2008. A diagnosis of Stage IIIB (cT2N3M0) lung adenocarcinoma was made and the patient subsequently received two cycles of systemic chemotherapy with cisplatin and vindesine, with concurrent thoracic irradiation, followed by two cycles of consolidation with carboplatin and paclitaxel. This treatment regimen resulted in stable disease. The primary tumor did not have epidermal growth factor receptor (*EGFR*) mutations, but immunohistochemistry (IHC) was positive for anaplastic lymphoma kinase (ALK) protein using the intercalated antibody-enhanced polymer (iAEP) method with 5A4 (Abcam, Cambridge, UK) as the primary antibody (Fig. 1a).[1] Fluorescence *in situ* hybridization (FISH), using probes described elsewhere,[2] confirmed echinoderm microtubule-associated protein-like 4 (*EML4*)–*ALK* rearrangement (Fig. 1b). Multiplex RT-PCR[1,3] further showed that this patient's fusion type was variant 3 (Fig. 1c). The patient was enrolled in the trial in April 2009.[4] The patient's persistent cough disappeared within 2 weeks of initiation of oral crizotinib therapy (250 mg twice daily) and the primary tumor shrank. Although there was no change in the size of the mediastinal lymph nodes, PET CT demonstrated marked reductions in the accumulation of fluorodeoxyglucose (^{18}F) after 12 months (Fig. 1d). Transient mild nausea and diarrhea were observed for the first 4 weeks of treatment, but were well controlled over the subsequent 19 months without any other toxicity. Follow-up MRI of the brain in November 2010 showed the appearance of asymptomatic but multiple new lesions, despite favorable control outside the central nervous system (CNS). The patient received whole-brain radiotherapy in December 2010; thereafter, crizotinib was resumed after a 4-week break. The patient is currently continuing crizotinib treatment for 24 months in total.

Case 2. A 29-year-old man who was a heavy smoker presented with progressive lumbago in May 2009. Shortly thereafter, sudden onset symmetrical leg paralysis developed. Because MRI revealed tumors in multiple segments of the thoracic and lumbar spine, emergent radiotherapy to the 10th–12th thoracic vertebrae was performed. This patient's disease was diagnosed as Stage IV (cT2N3M1) lung adenocarcinoma with single brain and multiple bone metastases. Tests for *EGFR* mutations were negative and the patient was given two cycles of systemic chemotherapy with cisplatin and vinorelbine. After insertion of a cyber knife into the brain lesion, the patient was given a cycle of carboplatin and paclitaxel, resulting in stable disease. On the evidence of ALK-positive iAEP IHC (Fig. 2a) with confirmatory FISH-positive *EML4-ALK* rearrangement, oral crizotinib therapy (250 mg twice daily) was started in September 2009. All tumors except for the brain lesion shrank significantly and the patient was able to cease opioid within 1 month. Mild nausea, alternating diarrhea and constipation, and the persistence of light spectrum image bothered the patient for a few months and dysgeusia lasted throughout the duration of treatment, but these adverse events were not so severe as to diminish the patient's activities of daily living. After 6 months, a chest CT showed complete regression of the thoracic lesions and bone scintigraphy showed a marked reduction in the accumulation of ^{88m}Tc (Fig. 2b). The brain lesion remained stable and other pre-existing tumors disappeared for 9 months. However, multiple new lesions appeared in the liver and crizotinib therapy was therefore discontinued. Thereafter, the patient received four cycles of carboplatin, pemetrexed, and bevacizumab and is now being followed-up with the latter two agents being used as maintenance therapy.

Case 3. A 31-year-old man who was a light smoker presented with an asymptomatic abnormal shadow on chest X-ray found as part of the annual medical checkup in August

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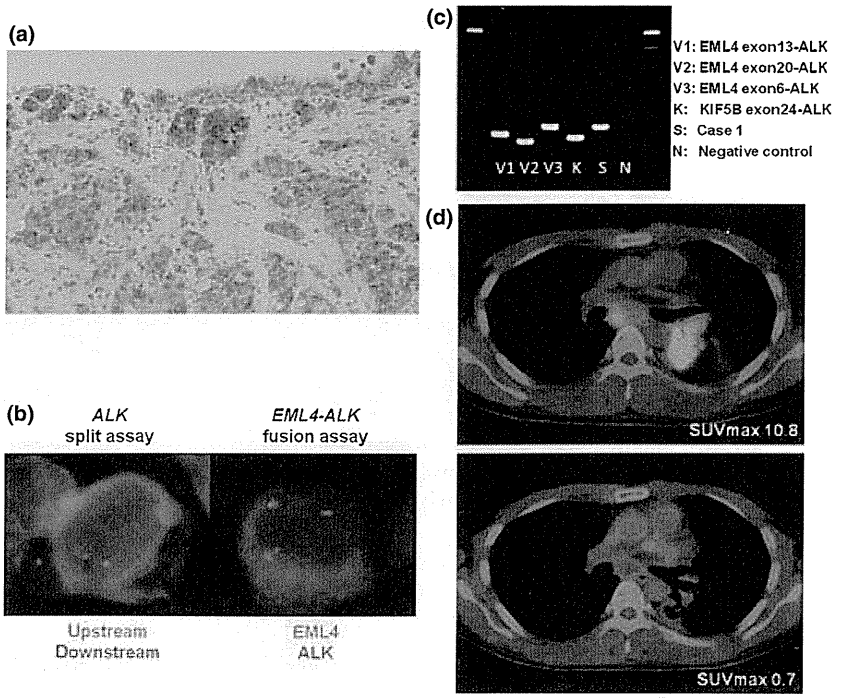

Fig. 1. (a) Intercalated antibody-enhanced polymer immunohistochemistry showing anaplastic lymphoma kinase (ALK) protein expression in acinar-type adenocarcinoma in Case 1. (b) Fluorescence *in situ* hybridization confirmed the *EML4-ALK* fusion gene. The ALK split assay was performed by labeling with probes for the upstream (green) and downstream (red) region of the *ALK* locus. The *EML4-ALK* fusion assay was performed by labeling with probes for *EML4* (green) and *ALK* (red). (c) Multiplex RT-PCR demonstrated the presence of *EML4-ALK* variant (V) 3. (d) Scans (PET CT) before (upper) and 12 months after (lower) crizotinib treatment. Accumulation of fluorodeoxyglucose (^{18}F) decreased markedly with maximum standardized uptake value (SUVmax) decreasing from 10.8 to 0.7. EML4, echinoderm microtubule-associated protein-like 4.

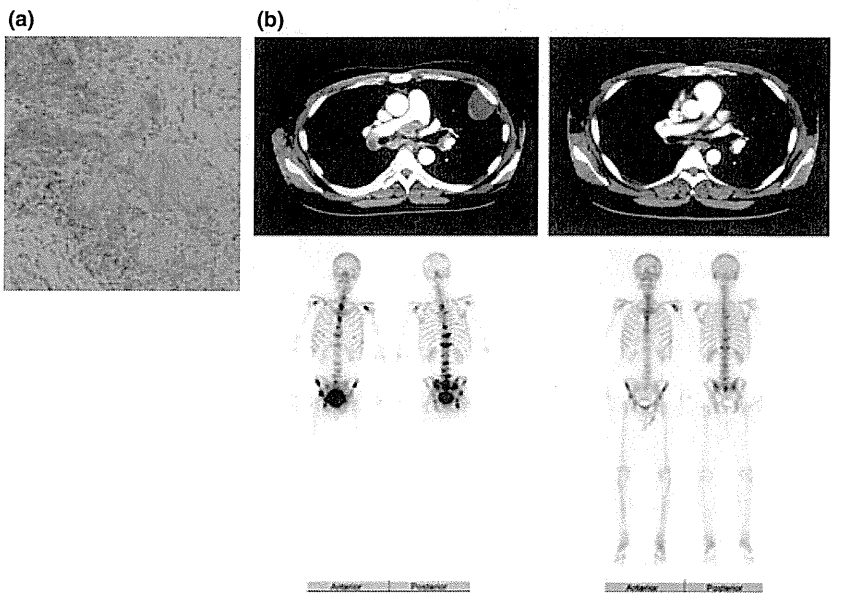

Fig. 2. (a) Intercalated antibody-enhanced polymer immunohistochemistry showing anaplastic lymphoma kinase protein expression in acinar-type adenocarcinoma in Case 2. (b) Chest CT and bone scintigraphy before (left) and 6 months after (right) crizotinib treatment. The tumors in the thorax almost disappeared and accumulation of ^{88m}Tc in multiple bones also decreased markedly.

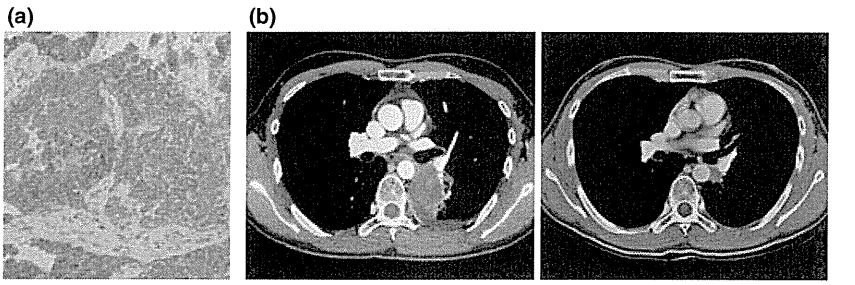

Fig. 3. (a) Intercalated antibody-enhanced polymer immunohistochemistry showing anaplastic lymphoma kinase protein expression in acinar-type adenocarcinoma in Case 3. (b) Chest CT before (left) and 12 months after (right) crizotinib treatment. Left pleural effusion disappeared and the primary tumor in the S6 segment shrank significantly.

2009. This patient's disease was diagnosed as Stage IIIB (cT4N0M0) lung adenocarcinoma with pleural dissemination. Chemoradiotherapy with cisplatin and vinorelbine failed after one cycle. Because tests for *EGFR* mutations were negative, but iAEP IHC (Fig. 3a) and FISH analyses revealed his tumor to be positive for *EML4-ALK*, the patient was started on oral crizotinib (250 mg twice daily) in December 2009. He achieved partial response shortly thereafter and the favorable

effect has lasted thus far for 17 months (Fig. 3b). The patient is currently continuing on crizotinib treatment. As for adverse effects, mild nausea and diarrhea were noted temporarily for the first 4 weeks of treatment, but persistence of vision spectrum and dysgeusia have lasted for the duration of the treatment period.

Discussion

The malignant transformation of approximately 5% of non-small cell lung cancer (NSCLC), especially adenocarcinoma, is caused by EML4-ALK and it is mutually exclusive for *EGFR* and *KRAS* mutations.[3,5–7] Basic research has shown that *EML4-ALK*-driven NSCLC is strongly addicted to this fusion-type oncogene.[5,8–10] Crizotinib is an experimental agent that targets ALK and mesenchymal-epithelial transition (MET) kinases and it is not currently approved by any regulatory agency. Pfizer's trial of crizotinib has demonstrated a high response rate (57%) in the *ALK* fusion-positive NSCLC population, indicating that these tumors have strong addiction exclusive to this oncokinase.[4] The three patients reported herein were all treated as part of the Pfizer trial after they had met the eligibility criteria and provided informed consent. All three patients showed a substantial responses to crizotinib. Although a minority of NSCLC arises from the *ALK* fusion-type oncokinase, most of ALK-positive NSCLC occur in younger people.[7] Thus, it is encouraging that ALK inhibitors will become available for use in the clinical setting. Moreover, crizotinib is also attractive as a potent ''pain killer'' because it was so effective against the pain produced by the bone metastases in Case 2 as to completely relieve the patient's cancer-related pain.

As for safety, temporary Grade 1 nausea and diarrhea were observed in all three patients and Grade 1 constipation coexisted in Case 2. Grade 1 dysgeusia of hot taste and visual disturbances (transient persistence of vision spectrum noted on moving from the dark to the light) occurred in Cases 2 and 3. These adverse events, except for dysgeusia, were reported in the trial[4] and all were tolerable. Crizotinib did not affect liver function, renal function, or blood cell counts in the three cases reported here.

Some responders (including two cases in the present study) develop resistance to crizotinib after a certain period, similar to most responders to EGFR tyrosine kinase inhibitors. This is a serious issue not to be overlooked. Based on the occurrence (Case 1) and unresponsiveness (Case 2) of brain metastases, crizotinib may not fully penetrate from the bloodstream into the CNS at the dose given in this trial. Costa *et al.*[11] recently reported a similar case to Case 1 in the present study, in whom the concentration of crizotinib was much lower in the cerebrospinal fluid (CSF) than in the plasma (0.0014 *vs.* 0.53 mol/L, respectively). Costa *et al.*[11] suggested that the low CSF:plasma ratio (0.0026) implied poor blood–brain barrier penetration of crizotinib, which may explain the persistent systemic disease control with crizotinib but the concurrent appearance of brain metastases.

Furthermore, two *de novo* mutations in the tyrosine kinase domain of *ALK* cDNA (G4374A and C4493A) have been identified recently from a patient who showed resistance to crizotinib.[12] These mutations result in substitutions of cysteine to tyrosine (C → Y) and leucine to methionine (L → M) at positions corresponding to amino acids 1156 and 1196, respectively. The L1196 of ALK corresponds to threonine (T) at position T315 in ABL and T790 in EGFR, the most frequently substituted position conferring resistance to inhibitors for these kinases. Conversely, C1156Y located adjacent to the αC helix on the N-terminal side has been proposed to allosterically hinder the binding of ALK inhibitors.[12] Such resistant mutations may provide another explanation for the lack of a response of brain metastases to crizotinib. Regarding the hepatic metastases in Case 2, it is unlikely that the hepatocyte growth factor/MET pathway or *MET* amplification (known resistant mechanisms for EGFR inhibitors) participated in this setting because crizotinib is a dual inhibitor for both ALK and MET kinases. We must investigate the mechanisms underlying the resistance to crizotinib to develop new treatment strategies, as well as second-generation ALK inhibitors, to overcome resistance.

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Disclosure Statement

KT has acted in an advisory role for Chugai Pharmaceutical Co., Ltd (Tokyo, Japan). The other authors report no potential conflicts of interest. None of the authors was an investigator in the trial conducted by Pfizer.

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Clinical Cancer Research

Pulmonary Inflammatory Myofibroblastic Tumor Expressing a Novel Fusion, PPFIBP1–ALK: Reappraisal of Anti-ALK Immunohistochemistry as a Tool for Novel ALK Fusion Identification

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Abstract

Purpose: The anaplastic lymphoma kinase (ALK) inhibitor crizotinib has been used in patients with lung cancer or inflammatory myofibroblastic tumor (IMT), both types harboring ALK fusions. However, detection of some ALK fusions is problematic with conventional anti-ALK immunohistochemistry because of their low expression. By using sensitive immunohistochemistry, therefore, we reassessed "ALK-negative" IMT cases defined with conventional immunohistochemistry (approximately 50% of all examined cases).

Experimental Design: Two cases of ALK-negative IMT defined with conventional anti-ALK immunohistochemistry were further analyzed with sensitive immunohistochemistry [the intercalated antibody-enhanced polymer (iAEP) method].

Results: The two "ALK-negative" IMTs were found positive for anti-ALK immunohistochemistry with the iAEP method. 5′-rapid amplification of cDNA ends identified a novel partner of ALK fusion, protein-tyrosine phosphatase, receptor-type, F polypeptide-interacting protein-binding protein 1 (PPFIBP1) in one case. The presence of PPFIBP1–ALK fusion was confirmed with reverse transcriptase PCR, genomic PCR, and FISH. We confirmed the transforming activities of PPFIBP1–ALK with a focus formation assay and an *in vivo* tumorigenicity assay by using 3T3 fibroblasts infected with a recombinant retrovirus encoding PPFIBP1–ALK. Surprisingly, the fusion was also detected by FISH in the other case.

Conclusions: Sensitive immunohistochemical methods such as iAEP will broaden the potential value of immunohistochemistry. The current ALK positivity rate in IMT should be reassessed with a more highly sensitive method such as iAEP to accurately identify those patients who might benefit from ALK-inhibitor therapies. Novel ALK fusions are being identified in various tumors in addition to IMT, and thus a reassessment of other "ALK-negative" cancers may be required in the forthcoming era of ALK-inhibitor therapy. *Clin Cancer Res; 17(10); 3341–8. ©2011 AACR.*

Introduction

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase that was discovered in anaplastic large cell lymphoma (ALCL) in the form of a fusion protein, NPM–ALK. (1, 2). In addition to ALCL (fused to NPM, TPM3, TPM4, ATIC, TFG, CLTC, MSN, MYH9, or ALO17; refs. 1–10), ALK has further been found to generate fusions in inflammatory myofibroblastic tumor (IMT; TPM3, TPM4, CLTC, CARS, RANBP2, ATIC, or SEC31L1; refs. 10–15), ALK-positive large B-cell lymphoma (CLTC, NPM, SEC31L1, or SQSTM1; 16–19), lung cancer (EML4 or KIF5B; refs. 20, 21), and ALK-positive histiocytosis (TPM3; ref. 22) Besides, some ALK fusions have been reported without showing histopathologic evidence: TPM4–ALK in esophageal squamous cell carcinoma (23, 24), TFG–ALK in lung adenocarcinoma (25), and EML4–ALK in colon and breast carcinomas (26). The wild-type ALK is mainly expressed in the developing nervous system, and is usually not expressed in other normal tissues (27). A fusion protein formation with a partner through chromosomal translocations is the most common mechanism of ALK overexpression and ALK kinase domain activation. These features render ALK fusion oncokinase an ideal molecular target.

Recently, the ALK inhibitor crizotinib has been used in patients with lung cancer or IMT, both types harboring ALK fusions (28, 29). The compound showed a 57% response rate in lung cancers (28), and a strong response for several months in IMT (29). Crizotinib and other ALK inhibitors

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Translational Relevance

Anaplastic lymphoma kinase (ALK) inhibitors have become one of the most promising groups of molecularly targeted drugs. Therefore, ALK is no longer a mere research target or simply a diagnostic marker, but is directly linked to the therapeutic benefit of patients harboring the fusions.

Pathologic diagnoses for ALK fusion-positive tumors have been made reliably with anti-ALK immunohistochemistry. Since the discovery of EML4–ALK, however, an unexpected problem in anti-ALK immunohistochemistry has become apparent, that is, the inability to detect a low level of EML4–ALK expression. To overcome this, we developed the intercalated antibody-enhanced polymer immunohistochemistry, which successfully detected EML4–ALK.

In other words, this indicates that unknown ALK fusions, particularly those expressed at a low level, may wait to be discovered in "ALK-negative" tumors defined with conventional immunohistochemistry. In the forthcoming era of ALK-inhibitor therapy, "ALK-negative" tumors should be reassessed with a high sensitive immunohistochemistry and, if positive, be further examined with appropriate molecular method(s).

have thus become one of the most promising groups of molecularly targeted drugs. Therefore, the sensitive and accurate identification of ALK fusion in tumors has also become clinically relevant, because it is no longer a mere research target or simply a diagnostic marker, but is directly linked to the therapeutic benefit of patients harboring the fusions.

Identification of such ALK fusions, especially within ALCL, has been prompted by the immunohistochemical staining pattern with antibodies to ALK. In ALCL, the most common ALK fusion is NPM–ALK (comprising approximately 80% of all cases), and its immunohistochemical staining pattern is both nuclear and cytoplasmic. NPM has a nuclear localization signal in the C-terminal region, and therefore the heterodimers of wild-type NPM with NPM–ALK fusion protein are transported to the nucleus whereas NPM–ALK homodimers remain within the cytoplasm (30). In contrast, other fusions do not localize in the nucleus and do not show a nuclear staining pattern in anti-ALK immunohistochemistry. Interestingly, each ALK fusion usually has its own characteristic anti-ALK immunohistochemical staining pattern, because the subcellular localization of ALK fusions is dependent on the corresponding fusion partners. Anti-ALK immunohistochemistry has thus become a highly useful tool for both research and diagnostic purposes.

Since the discovery of EML4–ALK fusion in lung cancer (20), however, an unexpected problem in anti-ALK immunohistochemistry has become apparent, that is, the inability to detect a low level of fusion expression. To overcome this, we developed the intercalated antibody-enhanced polymer (iAEP) method, which moderately raises sensitivity in the immunohistochemical detection system (21). With this very simple method, anti-ALK immunohistochemistry has become a potent weapon in the diagnosis of EML4–ALK-positive lung cancer (21, 31–33). Other researchers used an anti-ALK rabbit monoclonal antibody, which is usually more sensitive than mouse monoclonal antibody, which can stain EML4–ALK (34). However, most EML4–ALK-positive lung cancer tissues do not stain well with conventional anti-ALK immunohistochemical methods because of the low message/protein level of EML4–ALK (21, 35). The expression level of a fusion gene depends on the promoter activity of the 5′-side gene, and that of EML4 is likely to be lower than that of the other ALK fusion partner genes, which may explain why EML4–ALK had not been discovered until 12 years after the development of the first anti-ALK antibody became available for immunohistochemistry (36). In other words, a tumor that immunostains for ALK only by a sensitive immunohistochemistry method may harbor a novel ALK fusion. Interestingly, in this study, we detected 2 IMT cases positive for ALK immunohistochemistry only when stained by iAEP method (21), and successfully identified a novel fusion gene, protein-tyrosine phosphatase, receptor-type, F polypeptide-interacting protein-binding protein 1 (PPFIBP1)–ALK.

Materials and Methods

Materials

Pathologic specimens from 2 pulmonary IMT cases, originally diagnosed as fibrous histiocytoma (1988: case 1, 45-year-old male; 1998: case 2, 34-year-old female), were reassessed morphologically and immunohistochemically. Surgically removed tumor specimens were routinely fixed in 20% neutralized formalin and embedded in paraffin for conventional histopathologic examination. For case 2, total RNA was extracted from the corresponding snap-frozen specimen and purified with the use of an RNeasy Mini kit (Qiagen). The study was approved by the institutional review board of the Japanese Foundation for Cancer Research.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue was sliced at a thickness of 4 μm, and the sections were placed on silane-coated slides. For antigen retrieval, the slides were heated for 40 min at 97°C in Target Retrieval Solution (pH 9.0; Dako). For the conventional staining procedure, the slides were incubated at room temperature with Protein Block Serum-free Ready-to-Use solution (Dako) for 10 minutes and then with primary antibodies against ALK (5A4), smooth muscle actin, muscle-specific actin (HHF35), CD34, cytokeratins (AE1/AE3), S100, or desmin for 30 minutes. The immune complexes were then detected with dextran polymer reagent (EnVision + DAB system; Dako) and an AutoStainer instrument (Dako). The iAEP method was also used for the sensitive detection of ALK, as described previously (21).

Isolation of PPFIBP1–ALK fusion

To obtain cDNA fragments corresponding to a novel *ALK* fusion gene, we used a 5′-RACE method with the SMART RACE cDNA Amplification Kit (Clontech) according to the manufacturer's instructions, with a minor modification: the ALK2458R primer (5′-GTAGTTGGGGTTGTAGTCGGT-CATGATGGT-3′) was used as the gene-specific reverse primer.

From the oligo(dT)-primed cDNA obtained from case 2 RNA, a 471bp cDNA fragment containing the fusion point was specifically amplified with the primers PPFIBP1-592F (5′-AGAGACACAGAGGGGCTGATT-3′) and ALK3078RR (5′-ATCCAGTTCGTCCTGTTCAGAGC-3′).

PCR analysis of genomic DNA for *PPFIBP1–ALK* in case 2 was carried out with a pair of primers flanking the putative fusion point, PPFIBP1-607F (5′-CTGATTCAGGAGATCA-ATGATTTGAGGT-3′) and Fusion-RT-AS (5′-TCTTGCCAG-CAAAGCAGTAGTTGG-3′).

From the cDNA, a full-length cDNA for *PPFIBP1–ALK* was amplified by PCR with the PA-w-cDNA-in-S primer (5′-TATCTGGGTTGGAATTTGCCCCTG-3′) and the KA-w-cDNA-in-AS primer (5′-TGAGTGTGCGACCGAGCTCAGG-3′) and PrimeSTAR HS DNA polymerase (TakaraBio).

FISH

FISH analysis of gene fusion was carried out with bacterial artificial chromosome (BAC) clone-derived DNA probes for *ALK* and *PPFIBP1*. Unstained sections (4 μm thick) were subjected to hybridization with an *ALK*-split probe set (Abbott) or BAC clone-derived probes for *ALK* (RP11-984I21, RP11-62B19) and *PPFIBP1* (RP11-1060J15). Hybridized slides were then stained with DAPI and examined with the fluorescence microscope BX51 (Olympus).

Transformation assay for ALK fusion proteins

Analysis of the transforming activity of PPFIBP1–ALK was carried out as described previously (20, 37, 38). Briefly, the pMXS-based expression plasmid for PPFIBP1–ALK, EML4–ALK variant 1, or NPM–ALK was used to generate recombinant ecotropic retrovirus, followed by individual infection of mouse 3T3 fibroblasts (39). Formation of the transformed foci was evaluated after culturing the cells for 14 days. The same set of 3T3 cells was subcutaneously injected into nu/nu mice, and tumor formation was examined after 20 days. The animal experiments were approved by the animal ethics committee of Jichi Medical University.

Results

Morphology and immunophenotype of PPFIBP1–ALK-positive IMT

Histopathologic analysis of the 2 IMT cases revealed a marked proliferation of cells composed of somewhat histiocytoid spindle cells showing a fascicular or storiform pattern. The tumor cells were uniform and had pale eosinophilic cytoplasm and an oval vesicular nucleus, within which a small nucleolus was centrally located. Mild inflammatory infiltrate containing lymphocytes, plasma cells, foamy histiocytes, and multinucleated giant cells was observed (Fig. 1A and 1D). The immunophenotype of the 2 cases was negative for smooth muscle actin,

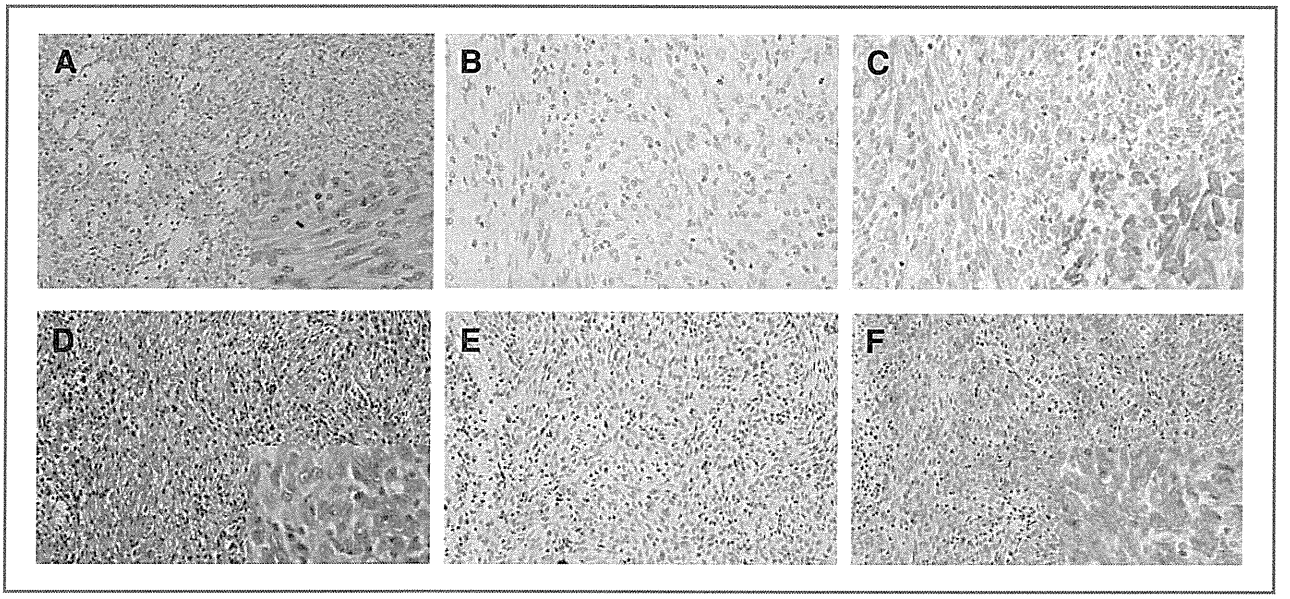

Figure 1. Histopathology of PPFIBP1–ALK-positive IMT. Diffuse proliferation of histiocytoid spindle cells showing a fascicular or storiform pattern. The tumor cells were uniform and had pale eosinophilic cytoplasm and an oval vesicular nucleus, within which a small nucleolus was centrally located. Mild inflammatory infiltrate containing lymphocytes, plasma cells, and foamy histiocytes is observed (A and D). The tumor cells were negative for ALK with conventional anti-ALK immunohistochemistry (B and E), but were clearly positive for ALK when the iAEP method was used. The staining pattern is diffuse cytoplasmic (C and F). Case 1 (A–C), Case 2 (D–F).

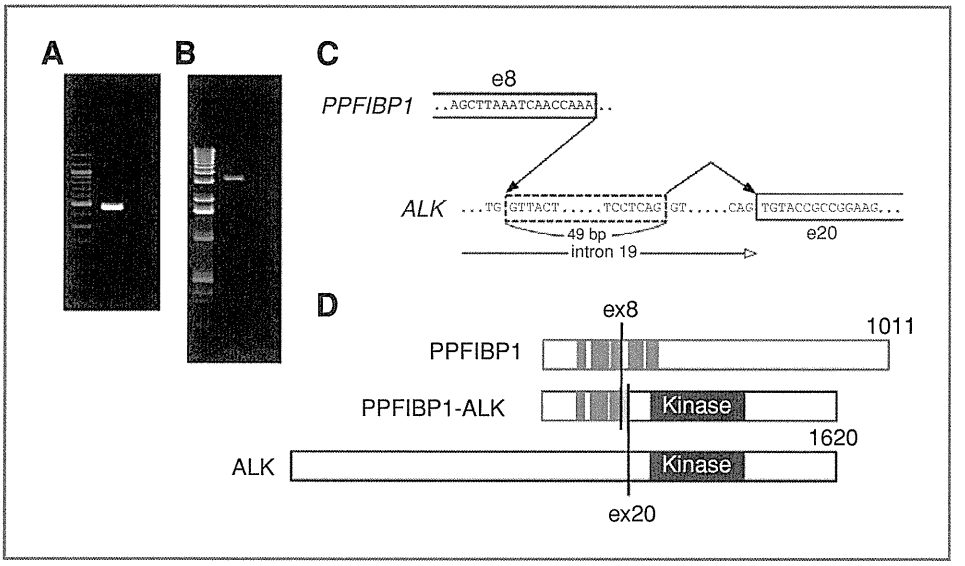

Figure 2. Identification of PPFIBP1–ALK: a PCR product of 471 bp covering the fusion point of *PPFIBP1–ALK* cDNA was specifically amplified from the tumor cells of case 2. The left lane contains DNA size standards (100 bp ladder). The right lane represents no template control (A). A PCR product of approximately 3 kbp covering the genomic fusion point of *PPFIBP1–ALK* was specifically amplified from the tumor cells of case 2. The left lane contains DNA size standards (1 kbp ladder). The right lane represents no template control (B). In our 5′-RACE products, exon 8 of *PPFIBP1* cDNA was fused to a 49 bp sequence in intron 19 of *ALK*, followed by exon 20 of *ALK* (C). PPFIBP1 contains 5 coiled-coil domains. A chromosome translocation, t(2;10)(p23;p11), generates a fusion protein in which the top 3 coiled-coil domains of PPFIBP1 and the intracellular region of ALK (containing the tyrosine kinase domain) are conserved. Numbers indicate amino acid positions of each protein (D).

HHF35, CD34, AE1/AE3, and S100. Desmin was focally positive in case 1, but was negative in case 2.

Identification of PPFIBP1–ALK as a novel ALK fusion gene

We conducted anti-ALK immunohistochemistry on 2 morphologically typical pulmonary IMT cases, originally diagnosed as fibrous histiocytoma. Immunostaining for ALK with the conventional polymer method led to the revised diagnosis of "ALK-negative" IMT (Fig. 1B and E). In the present study, anti-ALK immunohistochemistry with the iAEP method, however, showed a diffuse positive cytoplasmic staining (Fig. 1C and F), indicating the possibility of ALK fusion to a novel partner gene, the expression level of which is modest. To address this issue, in case 2 we conducted 5′-RACE assay for the isolation of an upstream cDNA to the ALK kinase domain cDNA, for which snap-frozen material was available.

Interestingly, we isolated a cDNA fragment containing exon 8 of *PPFIBP1* followed by a 49 bp-sequence within intron 19 of *ALK* and coupled to exon 20 of *ALK* (Fig. 2), suggesting the presence of a novel fusion between *PPFIBP1* and *ALK* genes. Because insertion of the intronic 49 bp allows an in-frame fusion between the 2 genes, this rearrangement likely produces a novel fusion-type tyrosine kinase. To confirm the genomic rearrangement responsible for the *PPFIBP1–ALK* fusion, a genomic PCR assay (Fig. 2B) and both *ALK* split and *PPFIBP1–ALK* fusion FISH assays (Fig. 3) were carried out. All results were consistent with the presence of t(2;12)(p23;p11) leading to the generation of *PPFIBP1–ALK*. Owing to the limited material available in case 1, only the FISH analyses were carried out. Surprisingly, these results also indicate the presence of *PPFIBP1–ALK* (Fig. 3, Supplementary Fig. 2A–C).

Transforming activities of PPFIBP1–ALK

To prove that the t(2;12)(p23;p11) rearrangement leads to the production of PPFIBP1–ALK kinase, in case 2 we attempted to amplify from the cDNA a full-length cDNA encoding the protein. By using a sense primer at the 5′-untranslated region of *PPFIBP1* mRNA (GenBank accession no. NM_003622) and an antisense primer at

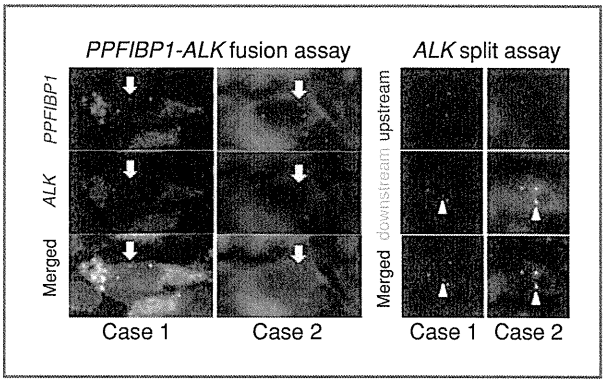

Figure 3. FISH analyses for *PPFIBP1–ALK*: sections of tumors positive for PPFIBP1–ALK were subjected to FISH analyses. In *PPFIBP1–ALK* fusion assays (left) the fusion genes are indicated by arrows. In *ALK* split assays (right) the 3′-sides of *ALK* are indicated by arrowheads. The color of fluorescence for the BAC clones and the case numbers in each hybridization are indicated. Nuclei are stained blue with DAPI.

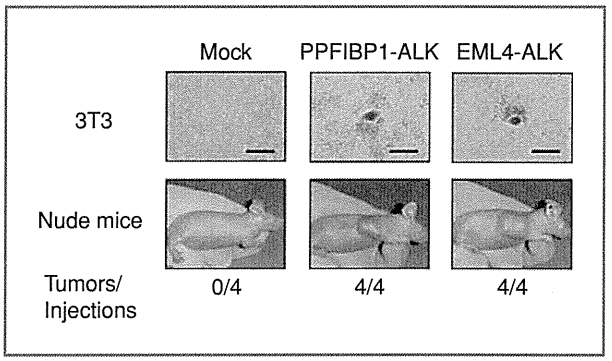

Figure 4. Transforming potential of PPFIBP1–ALK. Top, mouse 3T3 fibroblasts were infected with retroviruses encoding PPFIBP1–ALK or EML4–ALK or with the corresponding empty virus (Mock). The cells were photographed after 14 days of culture. Scale bars, 400 μm. Bottom, Nude mice were injected subcutaneously with the corresponding 3T3 cells, and tumor formation was examined after 14 days. The number of tumors formed per 4 injections is indicated at the bottom.

the 3′-untranslated region of *ALK* mRNA (GenBank accession no. NM_004304), a full-length *PPFIBP1–ALK* cDNA of 2488 bp was successfully amplified, which should have produced a fusion kinase of 811 amino acids with a predicted molecular weight of 90,740 Da (Supplementary Fig. 1).

To examine the transforming potential of PPFIBP1–ALK, a recombinant ecotropic retrovirus was generated to express PPFIBP1–ALK, which was used to infect mouse 3T3 fibroblasts. As shown in Figure 4, PPFIBP1–ALK produced hundreds of transformed foci over 14 days of culture, which was comparable with the observation with EML4–ALK. Furthermore, subcutaneous injection of the infected 3T3 cells into the shoulder of nude mice revealed that those expressing either PPFIBP1–ALK or EML4–ALK formed large tumors *in vivo*.

Discussion

Since their discovery in 1994, appropriate diagnosis of ALK fusion-positive tumors with conventional anti-ALK immunohistochemistry methods has been accepted. However, EML4–ALK in lung adenocarcinoma, identified in 2007, did not stain positive for ALK with conventional immunohistochemistry methods (21, 35). We developed a sensitive immunohistochemistry method, the iAEP method, and successfully stained EML4–ALK with ordinary anti-ALK mouse monoclonal antibodies (21, 31–33). Such observation further indicates a possibility that staining cancer specimens with sensitive immunohistochemical methods (such as iAEP) may detect novel ALK fusions in the "ALK-negative" tumors defined by conventional anti-ALK immunohistochemistry methods. On the basis of this hypothesis, we have identified a novel ALK fusion in "ALK-negative" IMT.

Caution is needed in practical settings. For example, rhabdomyosarcoma, especially of the alveolar type, often expresses wild-type ALK at a detectable level with conventional anti-ALK immunohistochemistry (40). Moreover, in our experience, a small portion of small cell carcinoma and large cell endocrine carcinoma of the lung, and some sarcomas, may be positive for ALK by iAEP immunohistochemistry, expressing wild-type ALK. Therefore, in order to specifically detect ALK fusions with sensitive anti-ALK immunohistochemistry, a confirmatory test by using FISH, RT-PCR, or similar is usually required. If a tumor is positive for a confirmatory test and the suspected partner gene is not a reported one, 5′-RACE or inverse reverse transcriptase PCR methods can be used for the identification of the suspected partner. Even if overexpressed, wild-type ALK may not be oncogenic (20, 21, 37, 38), although some investigators have suggested that wild-type ALK overexpression above a certain threshold level drives the growth of neuroblastoma (41). Further investigation will be required to clarify if wild-type ALK overexpression is a target for ALK inhibitor therapy.

IMT is a rare mesenchymal tumor that has been referred to as inflammatory pseudotumor, plasma cell granuloma, fibroxanthoma, fibrous histiocytoma, pseudosarcomatous myofibroblastic tumor, and invasive fibrous tumor of the tracheobronchial tree (42). It occurs in the soft tissues as well as in the viscera and the lung, and is more likely to occur in children and young adults. Histologically, IMT is composed of a variable admixture of bland, spindle-shaped myofibroblast-like cells and an inflammatory component of lymphocytes, eosinophils, plasma cells, and macrophages. Recent genetic studies have elucidated clonal chromosomal abnormality involving 2p23, at which ALK is located, in a subset of IMT. The expression of ALK fusion proteins is detected by anti-ALK immunohistochemistry in approximately 50% of IMT cases (42), in which various ALK fusion genes have been reported (Table 1). Collectively, these lines of evidence support ALK-positive IMT being a distinct neoplastic entity. However, the other 50% of IMT cases are negative for anti-ALK immunohistochemistry, and thus in terms of pathogenesis it remains unknown whether these ALK-negative IMTs should be included in the same entity or not. In fact, 1 ALK-negative IMT case did not respond to crizotinib therapy (29). However, we have detected a novel ALK-fusion in "ALK-negative" IMT that subsequently proved positive for ALK with the iAEP immunohistochemistry method. Therefore, unexpectedly lowly expressed ALK fusions may explain the pathogenesis of a portion of "ALK-negative" IMT cases. PPFIBP1–ALK represents such an ALK fusion, although we do not yet know what proportion of "ALK-negative" IMTs can be attributed to this novel subtype. "ALK-negative" IMT warrants screening with the iAEP method to detect this fusion or other, unrecognized, ALK fusions.

PPFIBP1 codes liprin beta 1 (also called PTPRF-interacting protein-binding protein 1). This 114 kDa protein is a member of the leukocyte common antigen–related (LAR) transmembrane tyrosine phosphatase-interacting protein family that may regulate LAR protein properties via interaction with another member of the family, liprin alpha1 (43). Liprin beta 1 expresses in intestinal lymphatic endothelial cells *in vitro* and lymphatic vasculature *in vivo*,

Table 1. ALK fusion partners in well-documented IMT cases

Partner	Locus	Age	Sex	Site	Year, First author
TPM3	1p23	30	F	Lung	2000, Lawrence
		23	F	Abdomen	2000, Lawrence
		4	M	Lung	2006, Yamamoto
		29	F	Ileum	2006, Milne
		4	M	Lung	2007, Kinoshita
TPM4	19p13	1	M	Abdomen	2000, Lawrence
		6	M	Mesentery	2003, Hisaoka
		25	M	Prostate	2003, Hisaoka
		5	M	Mesentery	2006, Yamamoto
		5	F	Urinary bladder	2006, Yamamoto
CLTC	17q23	3	F	Neck	2001, Bridge
		37	M	Pelvis	2001, Bridge
		2	M	Thoracic cavity	2006, Yamamoto
		6	M	Mesentery	2006, Yamamoto
		0	F	Mediastinum	2007, Patel
CARS	11p15	0	M	Abdomen	2002, Cools
		10	M	Neck	2003, Debelenko
RANBP2	2q13	7	M	Abdomen	2003, Ma
		0	M	Abdomen	2003, Ma
		2	M	Abdomen	2007, Patel
		34	M	Liver	2008, Chen
		44	M	Abdomen	2010, Butrynski
ATIC	2q35	46	M	Urinary bladder	2003, Debiec-Rychter
SEC31L1	4q21	23	M	Abdomen	2006, Panagopoulos
PPFIBP1	12p11	45	M	Lung	Present case 1
		34	F	Lung	Present case 2

and plays an important role in the maintenance of lymphatic vessel integrity in *Xenopus* tadpoles (44). PPFIBP1 has 5 coiled-coil domains in exons 5 through 12, and the upper 3 domains are conserved in fusion form with ALK (Fig. 2D). The coiled-coil domain is shared in all ALK fusion partners (except for NPM, MSN, and SQSTM1), with which the ALK fusion proteins homodimerize leading to constitutive activation of ALK kinase domains (8, 19). As expected, in the present study, the oncogenicity of PPFIBP1–ALK was clearly confirmed with an *in vitro* focus formation assay and an *in vivo* tumorigenicity assay.

The difference in subcellular localization has contributed to the discovery/identification of various ALK fusions. Likewise, the difference in the expression level found is here proved important in the accurate detection of fusion proteins. Sensitive immunohistochemical methods such as iAEP will broaden the potential value of immunohistochemistry, which is a simple and long-established histopathologic technique in the fields of research and diagnosis. The ALK positivity rate (approximately 50%) in IMT should be reassessed with these more sensitive methods, possibly leading to the identification of novel ALK fusions and more candidates for ALK inhibitor therapy. A novel ALK fusion, VCL–ALK, has recently been identified in renal cancers (45, 46). In addition to IMT, therefore, a reassessment of diverse "ALK-negative" human cancers may be required in the forthcoming era of ALK inhibitor therapy.

Disclosure of Potential Conflicts of Interest

K. Takeuchi, scientific advisor for developing an anti-ALK iAEP immunohistochemistry kit (ALK Detection Kit, Nichirei Bioscience, Japan) and in charge of pathology screening for ALK fusions using the immunohistochemistry kit and an original probe set for ALK split FISH assay in a clinical trial of an ALK inhibitor (AF802, Chugai, Japan). The other authors disclosed no potential conflicts of interest.

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症　例

肺野に孤立性陰影を認めた悪性胸膜中皮腫の1例

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背景：悪性胸膜中皮腫の細胞診は，ほとんどが胸水が対象になっている．胸部CTで肺野に腫瘤陰影を認め肺腫瘍が疑われたが，経気管支的穿刺吸引細胞診を行い腫瘍細胞が得られた悪性胸膜中皮腫を経験した．経気管支的穿刺吸引細胞診で腫瘍細胞が得られる悪性胸膜中皮腫はまれである．

症例：50歳代，女性．検診での胸部X線写真で異常陰影を指摘され，胸部CTで右中葉領域に腫瘤が認められた．経気管支的穿刺吸引細胞診では，小型の異型細胞で構成される細胞集塊が認められたが，核の大きさが小さく，異型性に乏しいため悪性細胞と判定できなかった．確定診断のための胸腔鏡下肺部分切除による組織診および免疫染色にて，上皮型悪性胸膜中皮腫と診断された．

結論：肺に腫瘤を形成した悪性胸膜中皮腫症例において，経気管支的穿刺吸引細胞診で悪性細胞が採取された．細胞診に際して臨床情報を十分に把握し診断をする必要がある．

Key words：Malignant pleural mesothelioma，Transbronchial aspiration cytology，Case report

I. はじめに

悪性胸膜中皮腫は，アスベストとの関連で注目されている疾患で，治療抵抗性であり予後不良である．わが国では2008年の中皮腫の死亡数が1170人[1)]であり，その数は年々増加し社会的問題となっている．悪性胸膜中皮腫は胸水貯留で発見されることが多く，胸水細胞診で診断される機会が多い．また，胸膜腫瘤として認められる場合には，経皮的穿刺や胸腔鏡下の生検による診断が行われる．

今回，胸部CTで肺腫瘍が疑われ，胸腔鏡下肺部分切除術を行い，悪性胸膜中皮腫と診断された症例の経気管支的穿刺吸引細胞診で認められた異型細胞について報告する．

II. 症　　例

患　者：50歳代，女性．
既往歴：特記すべき事項なし．
喫煙歴：20本×30年．
職　業：清掃業でアスベスト曝露歴がある．
現病歴：住民検診の胸部X線写真で右下肺野に胸部異常陰影を指摘された（Photo. 1）．胸部CTでは右中葉領域に腫瘤陰影を認め，肺腫瘍の疑いで精査目的に当院を紹介され受診した．自覚症状はなく，胸部CT上は右中葉から右中下葉間の葉間胸膜にかけて4.5×3.5 cm大の孤立性の辺縁円滑な類円形腫瘤が認められた．肺内または葉間胸膜に存在する腫瘤と考え，原発性肺癌や孤立性線維性腫瘍が疑われた（Photo. 2）．両側胸膜には明らかなプラークや石灰化は認められなかった．診断目的の経気管支的穿刺吸引細胞診では，異型細胞が出現したが悪性と判定できず疑陽

Malignant pleural mesothelioma showing a solitary shadow in the lung field——A case report——

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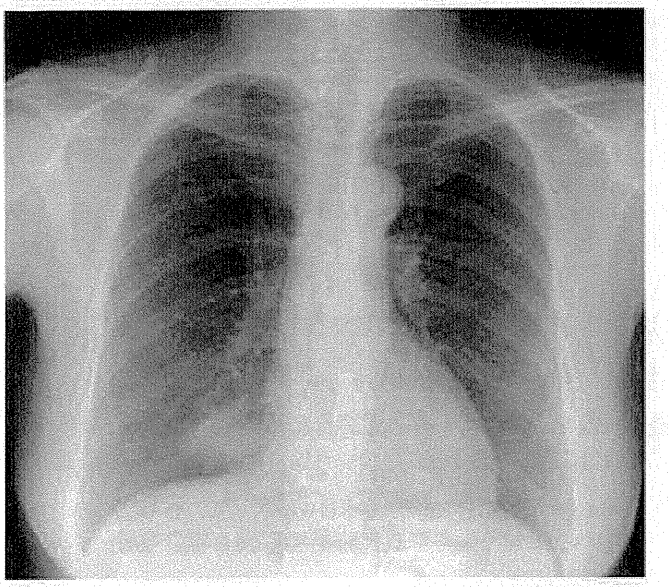

Photo. 1 Chest X-ray showing an abnormal round shadow in the lower right lung field.

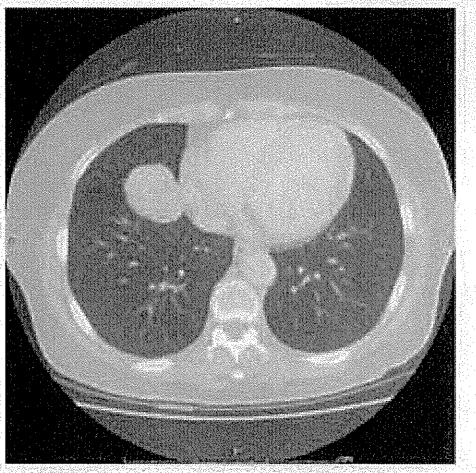

Photo. 2 Chest CT showing a well-demarcated mass 4.5 cm in diameter.

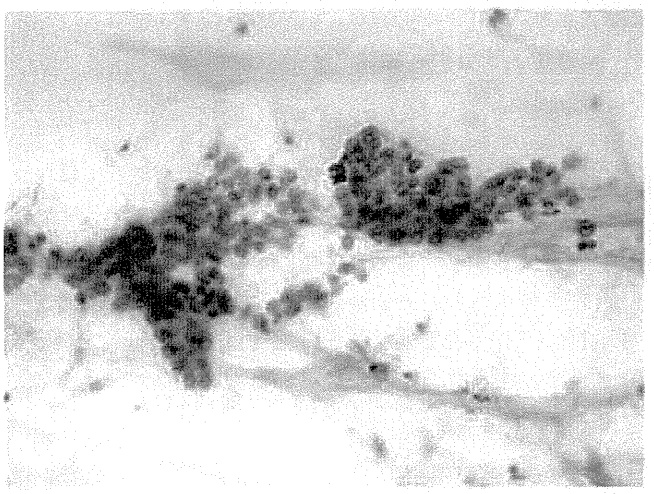

Photo. 3 Cytological transbronchial needle aspiration cytology (TNAC) findings. Note sheet-like cell clusters (Papanicolau (Pap.) staining, ×40).

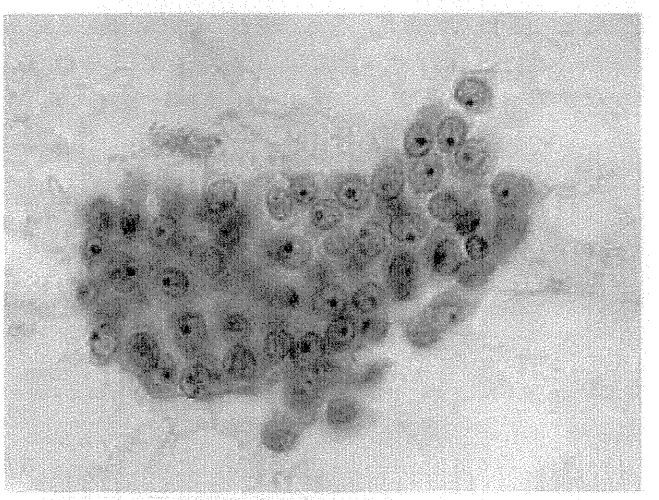

Photo. 4 Cytological TNAC findings. Note cells with predominant nucleoli and a fine granular chromatin pattern (Pap. staining, ×100).

性と判定された．経気管支鏡下の生検でも悪性細胞が採取されず，組織診も陰性であった．確定診断目的に胸腔鏡下右中葉部分切除術を施行された．術中洗浄胸水細胞は陰性であった．手術時に胸膜から発生する腫瘍であることが判明した．腫瘍の捺印細胞診には，経気管支的穿刺吸引細胞診とほぼ同様の細胞が出現したが，腫瘍細胞，悪性と判定した．切除標本の組織診，免疫組織染色により上皮型悪性胸膜中皮腫と診断された．根治的な胸膜肺全摘術は希望せず再発時に考慮することで経過観察となった．患者は術後2年以上経過しているが，現在も再発はなく経過観察中である．

III. 細胞所見

気管支鏡下の穿刺吸引細胞診では，きれいな背景の中に小型の異型細胞が集塊として出現し，集塊の多くはシート状で配列は規則正しかった（Photo. 3）．2核細胞や多核細胞の出現は認めなかった．核は小型類円形で円滑，核縁は菲薄でクロマチンは細顆粒状で均等分布を示していた．細胞質はライトグリーン好性で，核小体は明瞭であるが，個々の細胞は多形性に乏しかった（Photo. 4）．悪性とする所見は乏しく，増殖した気管支上皮細胞との鑑別は困難であった．しかし，一部に細胞密度が高く，重積性のある集塊がみられ（Photo. 5），また腫大した核小体から，腺癌も否定できないため疑陽性と判定した．なお，紡錘型の異型細胞

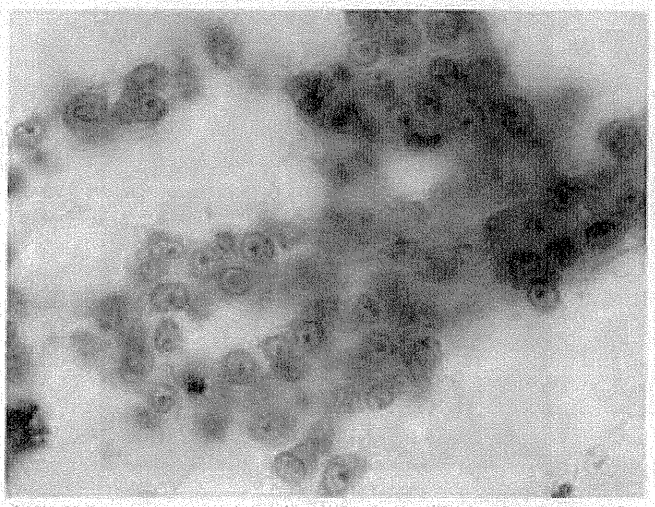

Photo. 5 Cytological TNAC findings. Cell cluster showing partial overlapping (Pap. staining, ×100).

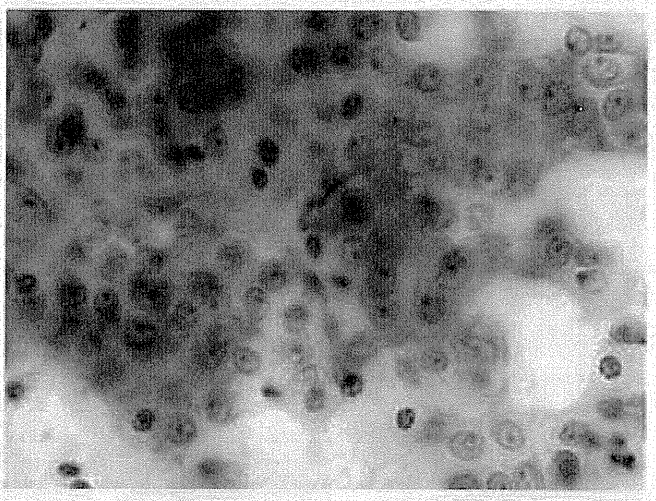

Photo. 6 Cytological imprinted cytology findings. Large cell cluster showing overlapping (Pap. staining, ×100).

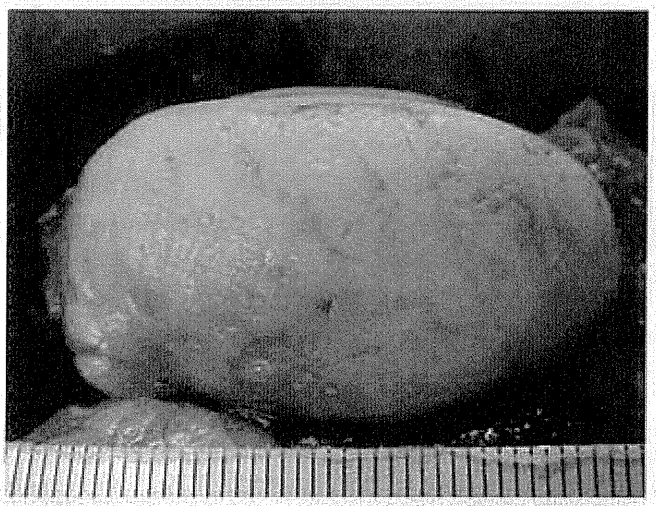

Photo. 7 Cut surface of hard solid yellow elastic tumor

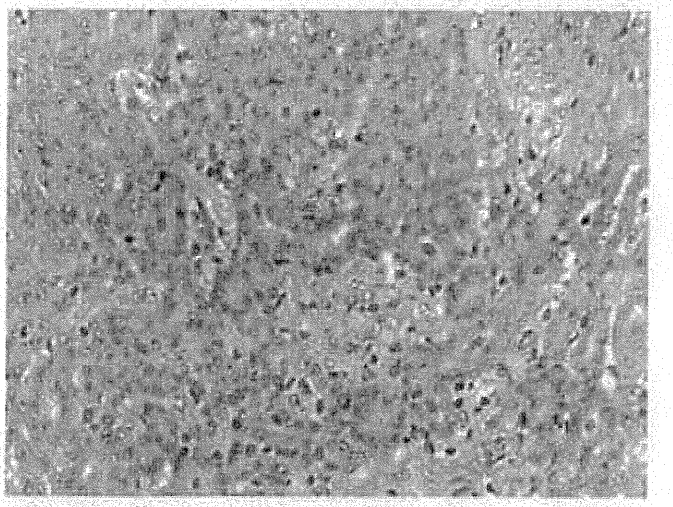

Photo. 8 Histological findings of malignant pleural mesothelioma (HE staining, ×40).

はみられなかった．捺印細胞診では，経気管支的穿刺吸引細胞と同様の小型多辺形～立方状細胞が大型集塊として出現した（Photo. 6）．胸膜腫瘍であることが判明していたが，孤立性繊維性腫瘍は紡錘形細胞を認めないので否定的であった．細胞は異型に乏しいが集塊の一部は重積性を示し，著明な核小体が認められたことから，悪性と判定し，悪性胸膜中皮腫を疑った．

IV. 病理組織所見

肉眼所見：中葉の胸膜に発生し，右肺S5領域に突出する4 cm大の腫瘍があり，心嚢と横隔膜には播種と判断される被膜を有する黄色腫瘍を認めた．腫瘍は充実性で炭粉沈着や壊死は認めなかった（Photo. 7）．

組織所見：HE染色では，明瞭な核小体を持つ円形～楕円形核を有し比較的豊富な胞体をもつ腫瘍細胞が線維性間質を伴って索状胞巣状，リボン状に増生していた（Photo. 8）．腫瘍細胞は胸膜表面に露出し，中葉実質に浸潤していた．免疫染色ではCalretinin（+），D2-40（+），HBME-1（+），WT-1（+）と中皮マーカーがすべて陽性で（Photo. 9），肺胞上皮マーカーはTTF-1（−），Napsin A（−）と陰性，また腺癌マーカーはCEA（−），Ber-EP4（−）といずれも陰性であった．また，心嚢と横隔膜の腫瘍も同じ所見であった．以上の結果から，びまん性悪性胸膜中皮腫（上皮型）の診断となった．アスベスト小体の組織学的検索を行ったが検索範囲内には確認できなかった．

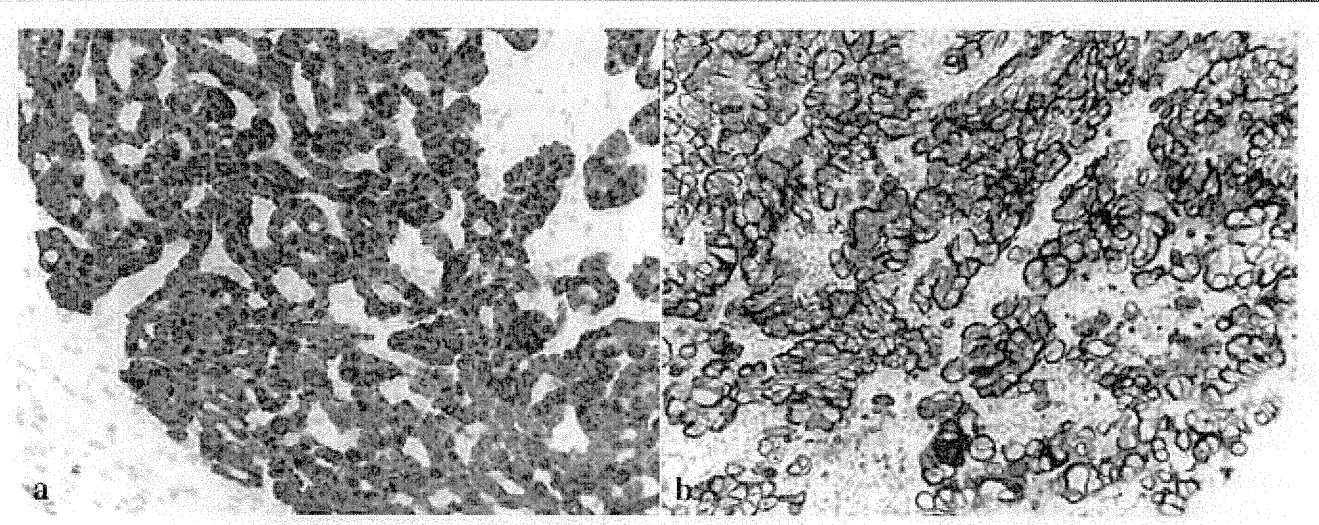

Photo. 9 Tumor cells positive for (a) calretinin and (b) D2-40 (Immunostaining, ×40).

V. 考　　察

悪性胸膜中皮腫はしばしば胸水貯留を認め，胸水細胞診で診断されることが多い．胸水細胞診での中皮腫の診断率は32～76%であるが[2]，25%という報告もある[3]．胸水貯留を認めない胸膜中皮腫は約20%であり[1]，これらの診断はX線透視下あるいはCTガイド下の経皮的穿刺や胸腔鏡下での腫瘤生検や細胞診が行われている．今回の症例は初診時の画像診断で肺腫瘍が疑われたため，経気管支的穿刺吸引細胞診が行われた．細胞診では，明瞭な核小体をもつ小型多辺形の異型細胞が集塊として出現したが，シート状で規則的な配列で，細胞の異型性が乏しかったため，悪性の診断までにはいたらなかった．また，経気管支的に異型細胞が採取されたため，肺内病変と考え悪性中皮腫の可能性は考慮されなかった．

悪性胸膜中皮腫は胸膜表面を覆う中皮細胞から発生する腫瘍で，胸膜の表面に沿って浸潤するように進展する[3]．悪性胸膜中皮腫の画像所見の特徴は，通常胸水貯留がみられ，胸膜肥厚像や胸膜沿いに広がる結節や腫瘤であり[6～8]，びまん性胸膜中皮腫といわれる．本症例では，胸部CT上，右中葉領域に約4 cm大の辺縁円滑の類円形腫瘤を呈していた．一方，いわゆる限局型胸膜中皮腫も少なく，報告例では50例に満たない[9]．しかもその多くは胸壁に接して存在するもので，肺野に孤立性陰影を呈するものはきわめてまれであると推測される．本症例はびまん性胸膜中皮腫の範疇に入るが画像上肺野に孤立腫瘤陰影を呈した．腫瘍が中下葉間胸膜に発生し，中葉領域に浸潤して腫瘤を形成したため，肺腫瘍のような形態をとったと考えられる．

悪性胸膜中皮腫の細胞所見は主に体腔液の細胞診として解説されている．胸水中の胸膜中皮腫細胞は，腺癌細胞のみならず，反応性中皮細胞との鑑別も困難とされている[10～12]．胸水細胞診の中皮腫細胞は，孤立性ならびに散在性に，あるいは大型集塊（乳頭状，球状，まりも状）として出現する[10]．また，比較的平面的なシート状配列を示す．経気管支的擦過細胞診で悪性胸膜中皮腫細胞を認めた症例報告[13]では，その細胞像は胸水細胞診と同様で，乳頭状の細胞集塊を呈し，核は類円形で核形不整に乏しく，核の位置は中心性で，細胞質はライトグリーン好性であったと報告されている．本症例では大型の球状，まりも状の集塊はみられず，主に平面的な配列であった．胸水細胞診で中皮腫細胞の特徴とされる細胞相接像や相互封入体，またいわゆるhump様細胞突起などの所見や2核細胞や多核細胞は認められなかった．また，画像上も肺野の孤立性陰影であったため肺癌を第一に考え，経気管支的に採取された異型細胞を気管支上皮由来の細胞と考え，異型が乏しかったことや核中心性であったことから悪性の判定ができず，中皮腫の診断ができなかった．

今回，われわれが経験した悪性胸膜中皮腫の症例は，細胞診で確定診断までにはいたらなかったが，経気管支的穿刺吸引細胞診でも，悪性胸膜中皮腫が診断対象となる可能性がある．また，職業歴や画像所見などの臨床情報を十分に把握することが必要である．

VI. ま　と　め

経気管支的穿刺吸引細胞診にて悪性胸膜中皮腫細胞が得られた症例を経験した．本症例の細胞は，小型細胞からなる単調な結合性の強いシート状の細胞集塊として認められた．CT画像上，肺野の孤立性陰影であり，経気管支的に

採取された異型細胞を肺由来のものと判断した．

細胞診断においても，臨床情報を十分に把握して診断する必要がある．

Abstract

Background：Malignant pleural mesothelioma is usually diagnosed by pleural effusion cytology, but finding atypical cells by transbronchial aspiration cytology in malignant pleural mesothelioma is rare.

Case：A woman in her 50s seen for an abnormal chest X-ray shadow was found in chest computed tomography (CT) to have a right middle lung lobe mass. Atypical cells in sheets observed in transbronchial aspiration cytology smears could not be diagnosed as malignant cells because nuclei were small and less atypical. The tumor was finally diagnosed as malignant pleural mesothelioma by histological and immunohistochemical staining of specimens obtained through video-assisted thoracic surgery (VATS) lung resection.

Conclusion：In this case of malignant pleural mesothelioma, the tumor was located in the lung and atypical cells were obtained by transbronchial aspiration cytology, underscoring the importance of cytological diagnosis made together with clinical information.

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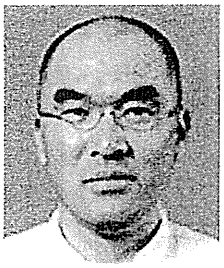
mTOR阻害剤：everolimus

Mammalian target of rapamycin：everolimus



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◎経口 mTOR 阻害剤 everolimus は 2010 年に，転移性腎細胞癌患者に対する第 3 の分子標的治療薬として厚生労働省に承認された．本稿では，第Ⅲ相臨床試験により抗腫瘍効果が明らかにされている腎細胞癌を中心に，mTOR シグナルの基礎から乳癌や胃癌，悪性リンパ腫など現在進行中の他の癌腫での現況など，私見を交えて解説する．

Key word：腎細胞癌，mTOR阻害剤，分子標的治療，エベロリムス，臨床試験

Rapamycin 誘導体のエベロリムス(everolimus)(RAD001，ノバルティスファーマ，図 1)は経口 mTOR(mammalian target of rapamycin)阻害剤として知られ，移植患者の免疫抑制剤としては，サーティカン®(Certican®：0.25 mg, 0.5 mg, 0.75 mg)の商品名でわが国でも臨床使用されてきた．mTOR 阻害剤は抗腫瘍作用ももち，2010 年に転移性腎細胞癌患者に対する 3 番目の分子標的治療薬，アフィニトール®(5 mg)として，わが国でも 2010 年 4 月から臨床投与が開始された．

本稿では mTOR シグナルの概論から，腎細胞癌と mTOR シグナル，everolimus の臨床効果，気をつけたい副作用，および他癌腫での現況について，私見を交えて解説していく．

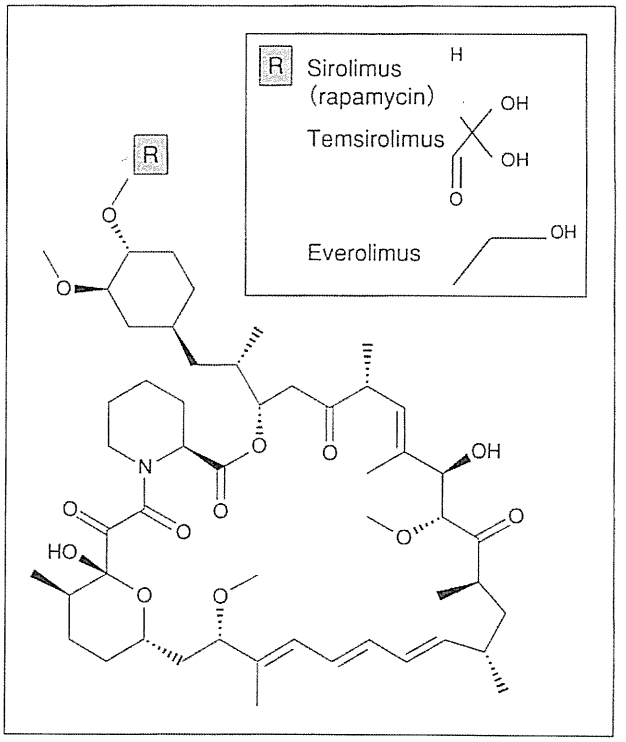

図 1　mTOR阻害剤の構造式
Rapamycin，temsirolimus，everolimus の構造式．

mTORシグナル

mTOR は serine/threonine protein kinase のひとつで，転写や蛋白質合成，細胞の成長や増殖などの機能に重要な働きをしているとされる．マクロライド系抗生物質 rapamycin は sirolimus ともよばれ，細菌からの自然抽出物である[1]．Rapamycin は，細胞内に取り込まれたとき，その細胞内受容体とされる FK506 binding protein 12(FKBP12)と結合し，この結合体 FKBP12-rapamycin complex が mTOR に結合し，そのシグナルを阻害す

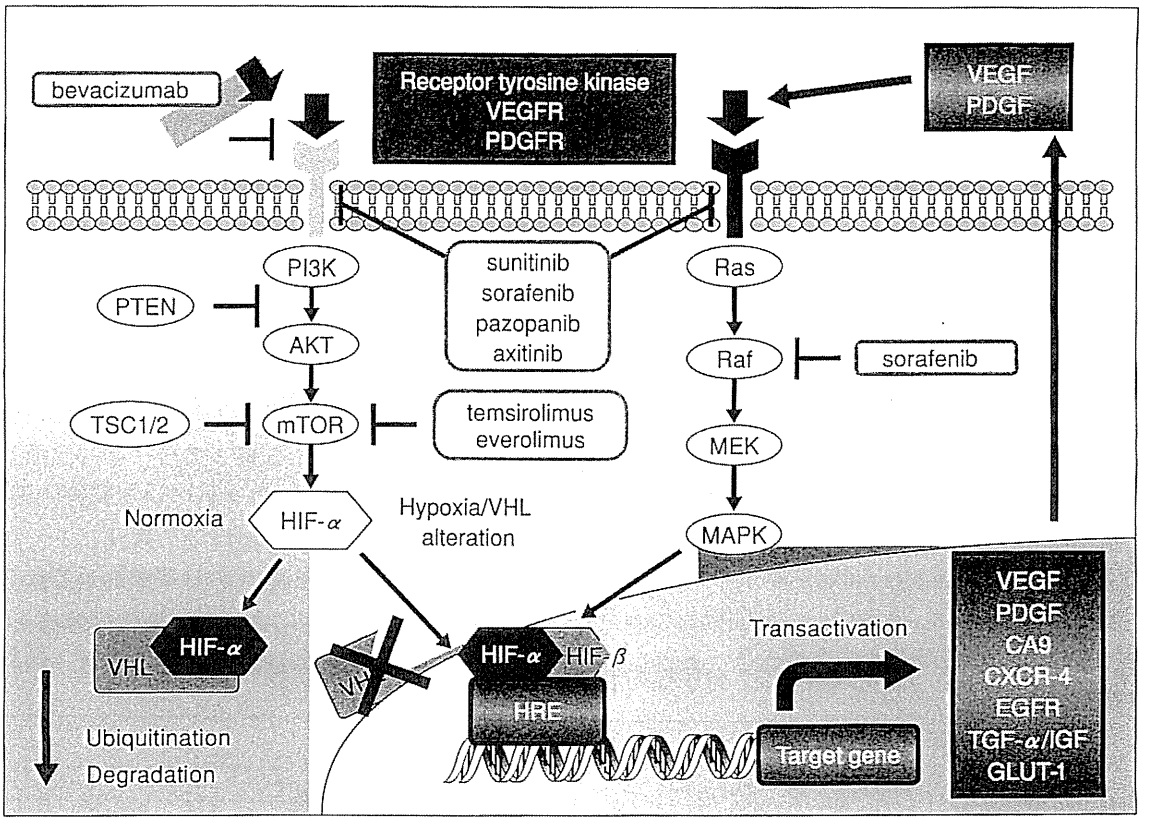

図 2　mTORシグナルと分子標的治療薬

る[2]．そのため，mTOR は FKBP12-rapamycin associated protein 1(FRAP1)ともよばれ，FRAP1 遺伝子にコードされている．mTOR はシグナルを受け取ると，regulatory associated protein of mTOR (Raptor)と結合し，rapamycin 感受性である mTOR complex 1(mTORC1)，あるいは rapamycin-insensitive companion of mTOR(Rictor)と結合し，rapamycin 抵抗性である mTOR complex 2(mTORC2)という 2 種類の機能的結合体を形成する．everolimus の標的は前者の mTORC1 である[1,2]．

mTORC1 の代表的な標的は p70-S6 Kinase1 (S6K1)と，eukaryotic initiation factor 4E(eIF4E) binding protein 1(4E-BP1)である．mTOR シグナルの活性化によってさまざまな成長因子の合成が促進され，これらの成長因子によって誘導されたシグナルが活性化し，増殖カスケードが築かれる仕組みである(図 2)．

腎細胞癌とmTORシグナル

腎細胞癌の 80%を占める淡明細胞腎細胞癌では，癌抑制遺伝子である VHL 遺伝子の変異や欠失により VHL 蛋白質の機能異常が起こり，低酸素状態ばかりでなく正常酸素状態においても hypoxia inducible factor-1(HIF-1α)が恒常的に発現を認める HIF1-β と結合し，常時活性状態になるとされている[3]．さらに，乳頭状腎細胞癌の原因遺伝子の c-Met や fumarate hydratase(FH)の異常によっても HIF が活性化されるとされる[4]．HIF は本来，低酸素状態で賦活される遺伝子群の活性化を促進するが，その代表的な標的のひとつは血管内皮増殖因子受容体(vascular endothelial growth factor receptor：VEGFR)や血小板由来成長因子受容体(platelet derived growth factor receptor：PDGFR)を介した増殖シグナルであり，その結果，mTOR シグナルが活性化される(図 2)．

腎細胞癌に対するeverolimusの臨床試験

Everolimus は rapamycin と同様に FKBP12 に結合し，mTOR シグナルを直接阻害し，腫瘍増殖および血管新生をダイレクトに阻害する効果をもっている[5]．主要な代謝酵素は CYP3A4 で，トランスポーターとして P 糖蛋白質の働きが指摘されている[6]．Memorial Sloan-Kettering Cancer Center(MSKCC)の Motzer らを中心として行われ