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Cancer Research

Correction: Intrinsic Cooperation between $p16^{INK4a}$ and $p21^{Waf1/Cip1}$ in the Onset of Cellular Senescence and Tumor Suppression *In vivo*

In this article (Cancer Res 2010;70:9381–90), which was published in the November 15, 2010 issue of *Cancer Research* (1), an incorrect version of Figure 4A was published. The correct version of the figure is provided below. The maximum number of papillomas per mouse shown in Table 1 was calculated from this figure. Therefore, the conclusions of the data in the article are unaltered by this correction.

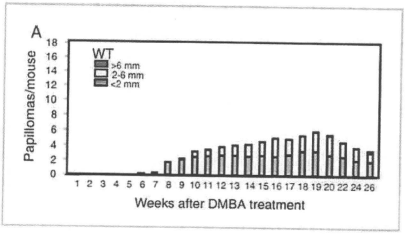

Figure 4A.

Reference

1. Takeuchi S, Takahashi A, Motoi N, Yoshimoto S, Tajima T, Yamakoshi K, et al. Intrinsic cooperation between $p16^{INK4a}$ and $p21^{Waf1/Cip1}$ in the onset of cellular senescence and tumor suppression *in vivo*. Cancer Res 2010;70:9381–90.

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症　例

ラブドイド細胞が目立った肺大細胞神経内分泌癌の１例

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宮内　栄作[3)]　石川　雄一[4)]　平井　康夫[1)]　宝来　威[3)]
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癌研究会有明病院呼吸器内科[3)]，癌研究会癌研究所病理部[4)]

背景：ラブドイド細胞を伴う原発性肺癌はまれであり，その存在は予後不良因子であることが知られている．今回，針生検捺印細胞診においてラブドイド細胞が目立った肺大細胞神経内分泌癌を経験したので報告する．

症例：37歳，男性．右胸部および背部痛と血痰を主訴に当院を受診した．胸部CTにて右上葉に最大径14 cmの腫瘤を認め，CTガイド下針生検を施行した．その捺印細胞診にて，中型類円形で独特の厚みのある細胞質および偏在核が特徴的なラブドイド細胞が多数認められ，肉腫様成分を含む癌や横紋筋肉腫を疑った．生検組織診では腫瘍細胞がrosette様の配列を示し，免疫染色にて神経内分泌への分化を示したことにより肺大細胞神経内分泌癌と診断した．発症から約２ヵ月で癌死した．

結論：細胞診上ラブドイド細胞の存在を指摘することは，その特徴所見より比較的容易であり，臨床的な予後予測に有益であると考えられる．

Key words：Lung carcinoma，Imprint cytology，Rhabdoid cells，Large cell neuroendocrine carcinoma，Case report

I. はじめに

ラブドイド細胞を伴う原発性肺癌はまれであるが，ラブドイド細胞の存在は肺癌の予後不良因子であることが知られており[1)]，現行の肺癌取扱い規約[2)]には，大細胞癌の特殊型であるラブドイド形質を伴う大細胞癌として分類されている．ラブドイド細胞を伴う肺癌についてはいくつかの報告がなされており，主体となる組織型は大細胞癌のほか，腺癌，低分化癌，肉腫様癌があるが，われわれの経験した大細胞神経内分泌癌は非常にまれである[1,3～11)]．ラブドイド細胞の細胞像は，豊富で独特の厚みのある細胞質と，明瞭な核小体を有し，偏在核が特徴的であると報告されている[4～6,9)]．今回，われわれは針生検捺印細胞診においてラブドイド細胞を伴い予後不良であった肺大細胞神経内分泌癌の１例を経験したので報告する．

Large cell neuroendocrine carcinoma of the lung with frequent rhabdoid cells——A case report——
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[1)]Department of Cytology, Cancer Institute Hospital
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論文別刷請求先　〒135-8550 東京都江東区有明３の８の31　癌研究会有明病院細胞診断部　鈴木奈緒子
平成21年２月５日受付
平成21年９月９日受理

II. 症　例

症　例：37歳，男性．
主　訴：右胸部および背部痛，血痰．
喫煙歴：17歳から20年間，20本/日（喫煙指数は400）．
既往歴・家族歴：特記事項なし．

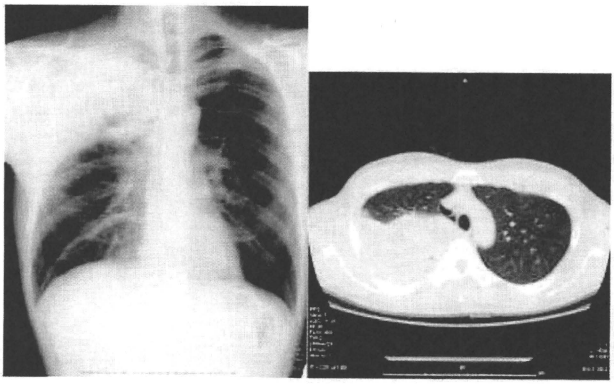

Photo. 1 Chest X-ray and CT showing a well-demarcated nodule 14 cm in diameter in the upper right hepatic field.

現病歴：当院受診2週間前より上記主訴にて近医を受診した．胸部単純X線写真にて，右肺異常陰影を指摘され当院を紹介され初診となった．胸部CTにて右上葉に最大径14 cmの，境界明瞭で内部不均一な腫瘤が認められた（Photo. 1）．CTガイド下針生検組織診，およびその捺印細胞診が施行された．捺印細胞診では肉腫様成分を含む癌や，横紋筋肉腫を疑ったが，生検組織診では肺大細胞神経内分泌癌と診断した．胸水貯留と副腎転移を認め，臨床病期Ⅳ期と診断され，シスプラチン（CDDP）と塩酸イリノテカン（CPT-11）併用化学療法を施行したが，効果が得られなかった．1クール終了後，腫瘍増大と大量胸水による呼吸状態の悪化にて，発症から約2ヵ月で死亡した．剖検の承諾は得られなかった．

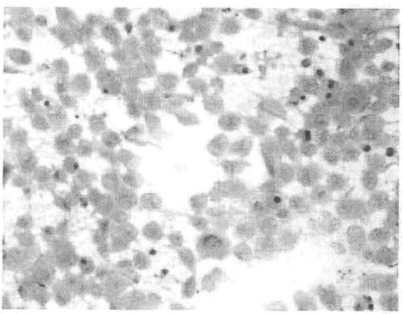

Photo. 2 Imprint cytology showing round, isolated tumor cells (Papanicolaou staining, ×40).

III. 細 胞 像

捺印細胞診では，少量の壊死物質を背景に，腫瘍細胞が孤立散在性ないし一部緩い結合をもって出現していた（Photo. 2）．腫瘍細胞は中型類円形細胞が主体を占めており，単調な印象であった（Photo. 3）．そのほかに大型類円形～短紡錘形の異型の強い細胞の混在を少数認めた（Photo. 4）．主体を占める中型類円形細胞は比較的豊富でライトグリーン好染の独特な厚みのある細胞質と偏在核を有し，ラブドイド細胞と判断した．これらの細胞には腫大した核小体が単個みられ，くびれや切れ込み等の核形不整が目立った．核クロマチンは淡く細顆粒状で，不均等分布を示し，核縁の肥厚は目立たなかった．また，ごく少数の腫瘍細胞の細胞質内に周囲よりも淡染性の小球体がみられた（Photo. 3矢印）．後日，synaptophysin（DAKO）を施行し，陽性であった（Photo. 5）．

重積集塊は認められず，rosette様配列，鋳型様配列，pair cells等の出現も認められなかった．

胸水細胞診では捺印細胞診に出現していた細胞と類似した，腫瘍細胞を孤立散在性に認めた．

IV. 組 織 像

生検組織診では，明瞭な核小体と淡いクロマチンを有す

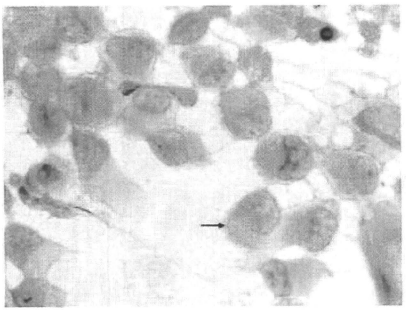

Photo. 3　Higher magnification of imprint cytology. Note rhabdoid cells with globules (arrow) (Papanicolaou staining, ×100).

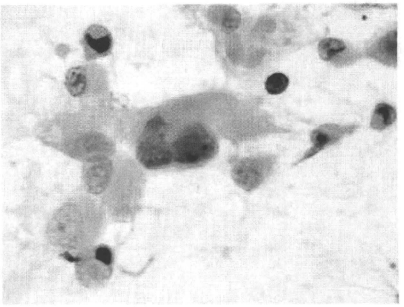

Photo. 4　Higher magnification of imprint cytology. Occasional large, polygonal tumor cells occur among many rhabdoid cells (Papanicolaou staining, ×100).

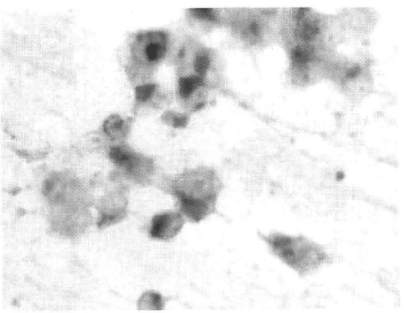

Photo. 5　Immunocytochemical tumor findings. Note synaptophysin positivity in cytoplasm (Immunostaining, ×100).

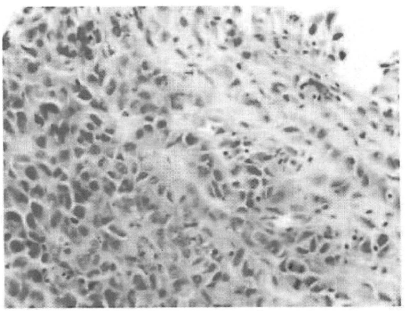

Photo. 6　Histologically, the tumor shows solid growth. Tumor cells show molding and rosette structures (HE staining, ×40).

る核をもつ異型細胞が索状・充実性に増殖し，一部にrosette形成やmoldingを思わせる構造がみられた（Photo. 6）．腫瘍細胞の細胞質は比較的豊富で，一部に好酸性の強い細胞もみられたが，ラブドイド形質を指摘するほどの所見は得られなかった（Photo. 7）．免疫染色ではsynaptophysin（DAKO）が陽性（Photo. 8），vimentin（DAKO）が強陽性，AE1/3（DAKO）とTTF-1（DAKO）が弱陽性を示した．NCAM（Novocastra），chromogranin A（DAKO），Napsin A（IBL）は陰性であった．以上の所見より，大細胞神経内分泌癌と診断した．

V. 考　　察

ラブドイド細胞を伴う癌は小児の腎腫瘍の亜型としてはじめて報告され[12]，その後腎以外の臓器の原発性腫瘍の一部にみられることが報告されている[3,13]．ラブドイド細胞を伴う原発性肺癌については，1996年Cavazzaらによりはじめて報告され[2]，現在では大細胞癌の特殊型にラブドイド形質を伴う大細胞癌として分類されている[2]．ラブドイド形質を伴う肺癌についての既報告では，主体となる組織型として，大細胞癌のほか，腺癌，低分化癌，肉腫様癌がある．本症例の主体となった大細胞神経内分泌癌については，Chettyらが1997年に報告した1例[10]，2000年に報告した

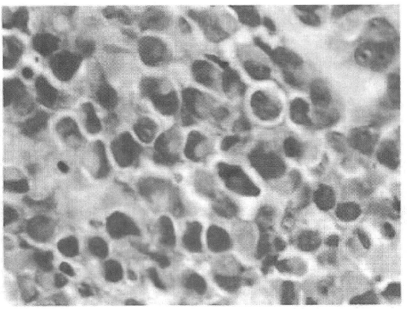

Photo. 7 At higher magnification, some tumor cells showing abundant eosinophilic cytoplasm (HE staining, ×100).

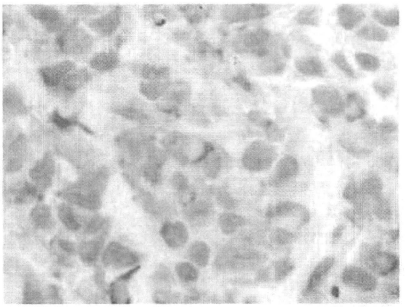

Photo. 8 Immunohistochemical tumor findings. Note strong synaptophysin positivity in cytoplasm (Immunostaining, ×100).

混合癌の一部に大細胞神経内分泌癌成分がみられた2例[11]のみで，非常にまれである．主体となる組織型は上記のとおり多彩であるが，いずれも進行が早いことが報告されている[1,3~11]．すなわち，他臓器の癌においてラブドイド細胞の存在が予後不良因子とされているのと同様に，原発性肺癌においても予後不良因子になると指摘されている[7]．

本症例は生検組織診にて，部分的にrosette形成を思わせる構造がみられ，免疫染色を行ったところ，神経内分泌への分化を示すsynaptophysinが陽性であり，大細胞神経内分泌癌と診断した．組織標本を再検討したところごく少数であるが，ラブドイド細胞の小球体様の物質を確認できる細胞が認められた．その細胞のsynaptophysinの染色性が，小球体部分はsynaptophysin陰性，それ以外の細胞質が陽性であったため，同一の細胞が神経内分泌の性質とラブドイド形質をもっていると考えられる（Photo. 8）．大細胞神経内分泌癌は，一般に予後不良とされており，Asamuraらは，大細胞神経内分泌癌切除後の全病理病期の5年生存率は40.3%，病理病期Ⅰ期に限っても57.8%と，小細胞癌に匹敵する予後不良な腫瘍であると報告している[14]．今回われわれが経験した症例は発見時にすでに病期Ⅳ期で，約2ヵ月で死亡した．またChettyらの報告した大細胞神経内分泌癌にラブドイド細胞が伴った症例も3～12ヵ月で死亡した[10,11]．これは大細胞神経内分泌癌にラブドイド形質が伴ったことにより，早い臨床経過をたどったと考えられ，ラブドイド形質という因子が悪性度をより高めたと推測される．

本症例の細胞診では，大細胞神経内分泌癌と診断しえる，結合性の強い重積集塊の出現や，rosette様配列，鋳型様配列，pair cells等の所見[15]は確認されず，大細胞神経内分泌癌との診断にはいたらなかった．標本上多数のラブドイド細胞が孤立散在性ないし一部緩い結合をもつ小集団として認められた．それらは腎やその他の臓器の腫瘍の一部にみられるラブドイド細胞の形態学的な既報告と同様に，豊富で独特の厚みのある細胞質，偏在核，明瞭な核小体などの特徴的所見により[4~6,9,12]，その存在を指摘することは容易であった．また，細胞質の小球体については，Papanicolaou染色において本例と同様に周囲の細胞質よりも淡染性であった例[4]のほか，ライトグリーン好染の例[6]，または不明瞭の例[9]とさまざまであった．

組織学的には，全腫瘍細胞のうちラブドイド細胞の含有率10%未満の症例では，ラブドイド細胞を含まない症例とほぼ同等の予後であるのと比べ，含有率が10%以上の症例では有意に予後不良との報告があり[7]，現在ではラブドイド細胞の含有率が10%以上のものをラブドイド形質と診断するのが一般的である[1,3,7]．本症例の生検組織診では，すでに述べたように大細胞神経内分泌癌との診断にいたった．組織診再検討の結果，腫瘍細胞の細胞質は比較的豊富で，一部に好酸性の強い細胞もみられた．さらに細胞診標本にてsynaptophysinを施行し，組織診と同様の染色結果が得られたことにより，細胞診と組織診に出現している細胞は同一のものと判断した．しかしながら，組織診ではラブドイド形質を指摘するほどの所見には欠け，生検組織診ではラブドイド形質を伴う肺癌との診断にはいたらなかった．生検材料という限られた検体では，ラブドイド形質を示唆する所見やその含有率を指摘することは困難であったと考えられる．本症例の細胞診では，ラブドイド細胞が多数認められたが，全腫瘍のうちラブドイド細胞の占める割合についての評価は困難である．

細胞診上，ラブドイド細胞の存在を指摘することは，そ

の特徴的所見により容易である．さらに，ラブドイド細胞の存在は予後に深く関係すると推測されるため，たとえ少量であっても形態学上ラブドイド細胞を指摘することは臨床上重要であると考えられる．

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Abstract

Background：Pulmonary carcinoma with rhabdoid cells is a rare tumor with a dismal prognosis. We report a case of large cell neuroendocrine carcinoma with frequent rhabdoid cells found in imprint cytology.

Case：A 37-year-old man reporting right chest and back pain and bloody sputum was found in chest computed tomography (CT) to have a well-demarcated mass 14 cm in diameter in the upper right hepatic lobe. Imprint cytology from the biopsy specimen obtained by CT-guided needle aspiration showed many rhabdoid cells against a necrotic background. Rhabdoid cells were round, with thick cytoplasm and occasional globules. Nuclei were unevenly distributed in tumor cells. Although carcinoma with sarcomatous elements or rhabdomyosarcoma was highly suspected on cytology, the definitive diagnosis of large cell neuroendocrine carcinoma was made based on histological analysis based on immunohistochemical staining. The patient died of this disease two months after onset.

Conclusion：Rhabdoid cells are easily found in cytology due to their typical features, making a cytological approach useful in definitively diagnosing pulmonary carcinoma having rhabdoid cells.

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Successful Treatment with Pemetrexed in a Patient with Mucinous Bronchioloalveolar Carcinoma

Long-Term Response Duration with Mild Toxicity

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A 36-year-old female former smoker presented with a productive cough. One year after visiting our hospital in February 2008, chest computed tomography (CT) revealed diffuse bilateral pulmonary nodules combined with a consolidation shadow (Figure 1*A*). A definite diagnosis could not be made by bronchofiberscopic examination; however, CT-guided needle biopsy specimens revealed bronchioloalveolar carcinoma (BAC) of mucinous subtype (Figure 2). Her clinical stage was T4N2M1a according to the seventh edition of the tumor, node, metastasis classification. Activating epidermal growth factor receptor (EGFR) gene mutations were not detected in her biopsy specimens. She received chemotherapy with gemcitabine and carboplatin as her first-line chemotherapy; however, her disease progressed after four cycles of chemotherapy. She then received further chemotherapy with docetaxel, erlotinib, paclitaxel, and irinotecan; however, neither regimen was effective, and her symptoms worsened. In May 2009, pemetrexed was approved for non-small cell lung cancer in Japan and was chosen as her sixth-line regimen and started in June 2009. The initial dose of pemetrexed was 500 mg/m^2 with vitamin B_{12} and folic acid supplementation. Chest CT after the two cycles of chemotherapy showed a radiographic response, and her symptoms also improved. The dose of pemetrexed was reduced to 400 mg/m^2 from the fourth cycle because of grade 3 liver dysfunction (Common Terminology Criteria for Adverse Events, version 3). Other adverse events were urticaria, skin hyperpigmentation, and general fatigue; however, they were all generally mild. Chest CT showed continuous improvement (Figure 1*B*, *C*), and her liver function has been stable since the dose reduction. Pemetrexed is currently being administered for its 20th cycle, and she is doing very well.

DISCUSSION

BAC is a distinctive form of lung adenocarcinoma and is further divided into two subtypes: mucinous and nonmucinous.[1] Although approximately 20% of adenocarcinomas have BAC features, "pure" BAC represents less than 5% of adenocarcinomas.[2] Historically, BAC was believed to be rather refractory to cytotoxic chemotherapy, and it is still debatable whether cytotoxic chemotherapy is equally effective in BAC and other types of adenocarcinoma.[2] Recently, it became widely known that the frequency of activating mutations of EGFR, the strongest predictive factor of a response to EGFR tyrosine kinase inhibitors, such as gefitinib and erlotinib, is significantly higher in BAC[3]; however, the frequency of EGFR mutations is significantly lower in the mucinous subtype than in the nonmucinous subtype.[4]

Our patient presented with BAC of the mucinous subtype, and her tumor did not express activating EGFR mutations. She received multiple lines of chemotherapy, including platinum based, docetaxel, and erlotinib; however, only pemetrexed was effective.

Pemetrexed is a multitargeted antifolate agent and has been approved as standard first-line (combination with platinum) and second-line chemotherapy for non-small cell lung cancer, and more recently, maintenance chemotherapy with pemetrexed has been under debate. Interestingly, pemetrexed is significantly more effective for nonsquamous than squamous histology.[5] One possible explanation is that the expression of thymidylate synthase, one of the molecular targets of pemetrexed, is generally higher in squamous than nonsquamous histology; however, it needs further confirmation.

We performed immunohistochemical examination to detect the echinoderm microtubule-associated protein-like 4 gene and the anaplastic lymphoma kinase gene, fusion gene, using the intercalating antibody-enhanced polymer

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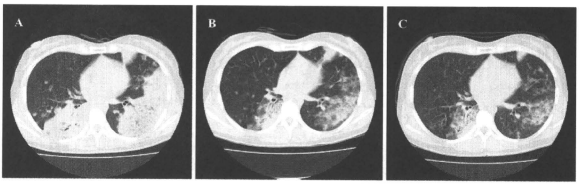

FIGURE 1. Computed tomography (CT) of the chest showed diffuse bilateral pulmonary nodules combined with consolidation shadow before pemetrexed treatment (*A*). CT after 12 cycles (*B*) and 18 cycles (*C*) showed continuous improvement of the shadow.

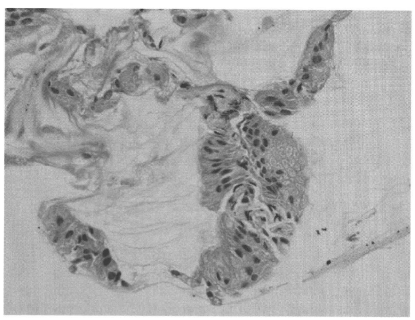

FIGURE 2. Biopsy specimen. There are a few fragments of columnar tumor cells containing mucin in the cytoplasm (hematoxylin and eosin stained).

method[6]; however, her tumor did not harbor the echinoderm microtubule-associated protein-like 4 gene and the anaplastic lymphoma kinase gene, fusion gene.

Finally, pemetrexed was safely administered for more than 1 year to our patient without deterioration of the performance status. This may also indicate the usefulness of pemetrexed as maintenance chemotherapy. Further investigations of pemetrexed are needed in patients with BAC.

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Early Release Paper

Identification of a novel fusion, SQSTM1-ALK, in ALK-positive large B-cell lymphoma

by Kengo Takeuchi, Manabu Soda, Yuki Togashi, Yasunori Ota, Yasunobu Sekiguchi, Satoko Hatano, Reimi Asaka, Masaaki Noguchi, and Hiroyuki Mano

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Identification of a novel fusion, SQSTM1-ALK, in ALK-positive large B-cell lymphoma

Running title: SQSTM1-ALK-positive large B-cell lymphoma

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Abstract

ALK-positive large B-cell lymphoma is a rare subtype of lymphoma, and most cases follow an aggressive clinical course with a poor prognosis. We examined an ALK-positive large B-cell lymphoma case showing an anti-ALK immunohistochemistry pattern distinct from those of 2 known ALK fusions, CLTC-ALK and NPM-ALK, for the presence of a novel ALK fusion; this lead to the identification of SQSTM1-ALK. SQSTM1 is an ubiquitin binding protein that is associated with oxidative stress, cell signaling, and autophagy. We showed transforming activities of SQSTM1-ALK with a focus formation assay and an in vivo tumorigenicity assay using 3T3 fibroblasts infected with a recombinant retrovirus encoding SQSTM1-ALK. ALK-inhibitor therapies are promising for treating ALK-positive large B-cell lymphoma, especially for refractory cases. SQSTM1-ALK may be a rare fusion, but our data provide novel biological insights and serve as a key for the accurate diagnosis of this rare lymphoma.

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Introduction

Anaplastic lymphoma kinase-positive large B-cell lymphoma (ALK+LBCL) is a rare subtype of lymphoma that was first described in 1997.(1) Approximately 50 cases have been reported to date,(2) with most cases (60%) following an aggressive clinical course.(3) In well-characterized cases, 3 genes have been reported as a fusion partner of *ALK*: *clathrin* (*CLTC-ALK*),(4-6) *nucleophosmin* (*NPM-ALK*),(7-8) and *SEC31A* (*SEC31A-ALK*).(9) In this paper, we report a case of ALK+LBCL that harbored a novel ALK fusion partner, sequestosome1 (SQSTM1).

Design and Methods

Materials

Biopsied specimens were fixed in 20% neutralized formalin and embedded in paraffin for conventional histopathological examination. We extracted DNA and total RNA from the snap-frozen specimens and subsequently purified the samples. Written informed consent was obtained from the patient. The study was approved by the Institutional Review Board of the Japanese Foundation for Cancer Research.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue was used. For antigen retrieval, we heated the slides for 40 min at 97°C in Target Retrieval Solution (pH 9.0; Dako), and subsequently detected the immune complexes with a dextran polymer reagent (EnVision+DAB system, Dako) and an AutoStainer instrument (Dako).

Isolation of *ALK* fusion cDNA

To obtain cDNA fragments corresponding to novel ALK fusion genes, we used an inverse reverse transcription-polymerase chain reaction (RT-PCR) method slightly modified from one previously reported.(10) Double-stranded cDNA was synthesized from 2 μg of total RNA with 1 pM of the primer ALKREVex22-23 (5′-TGGTTGAATTTGCTGATGATC-3′) and a cDNA Synthesis System (Roche), and was self-ligated by incubation overnight with T4 DNA ligase (TaKaRa Bio). We subjected the resulting circular cDNA to PCR (35 cycles of 94°C for 15 sec, 62°C for 30 sec, and 72°C for 1 min) with primers ALKREV3T (5′-CTGATGGAGGAGGTCTTGCC-3′) and ALKFWDex20-21

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(5′-ATTCGGGGTCTGGGCCAT-3′) in a final volume of 20 μl. We subjected 1 μl of the 1:100 diluted reaction products to a second PCR step (the same settings as above), with primers ALKREV4T (5′-GGTTGTAGTCGGTCATGATGGTC-3′) and ALKFWDex21-22 (5′-AGTGGCTGTGAAGACGCTGC-3′) in a final volume of 20 μl. The resulting products were purified by gel extraction and directly sequenced in both directions with primers ALKFWDex20-21 and ALKREV4T.

The fusion point of *SQSTM1-ALK* cDNA was amplified by RT-PCR with primers SQSTM1 565F (5′-AAACACGGACACTTCGGGT-3′) and ALK3078RR (5′-ATCCAGTTCGTCCTGTTCAGAGC-3′).

Full-length *SQSTM1-ALK* cDNA was obtained from the specimen by RT-PCR with primers SQSTM1v1-F90 (5′-CTCGCTATGGCGTCGCTCACCGTGAA-3′) and KA-W-cDNA-out-AS (5′-CCACGGTCTTAGGGATCCCAAGG-3′).

Fluorescence in situ hybridization (FISH)

We performed FISH analysis of the gene fusion for unstained slides (4 μm thick) with bacterial artificial chromosome (BAC) clone-derived DNA probes for *ALK* (RP11-984I21, RP11-62B19) and *SQSTM1* (RP11-55M16).

Transformation assay for ALK fusion protein

We performed analysis of the transforming activity of SQSTM1-ALK as described previously.(11-13) Briefly, cDNA for SQSTM1-ALK was inserted into the retroviral expression plasmid pMXS.(14) The resulting plasmid and similar pMXS-based expression plasmids for EML4-ALK variant 1 or NPM-ALK were used to generate recombinant ecotropic retroviruses, which were then used to infect mouse 3T3 fibroblasts. We evaluated formation of transformed foci after culturing the cells for 14 days. We subcutaneously injected the same set of 3T3 cells into nu/nu mice and examined tumor formation after 20 days.

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PCR for *IGH* gene rearrangement

Genomic PCR was used for amplification of the rearranged *IGH* gene using the primers FR2A 5′-TGG(A/G)TCCG(A/C)CAG(C/G)C(C/T)(C/T)CNGG-3′ and LJH 5′-ACCTGAGGAGACGGTGACC-3′. Several clones were sequenced after subcloning the PCR product into pGEM-T-Easy Vector (Promega).

Results and Discussion

Case presentation

A 67-year-old man was admitted with a tumor in the left side of his neck. A systemic workup revealed swelling of cervical, mediastinal, and hilar lymph nodes. Blood counts were within normal ranges. Lactose dehydrogenase was slightly elevated (223 IU/L) in peripheral blood with high IgG (2,425 mg/dL), normal IgA (157 mg/dL) and low IgM (32 mg/dL) levels.

Histopathological examination of the biopsied specimen from the cervical lymph node showed a diffuse infiltrate of tumor cells with a round, vesicular nucleus containing a centrally located large nucleolus. The cytoplasm was abundant (Figure 1A). These features may be consistent with immunoblasts or plasmablasts, but the size of tumor cells was large compared with typical immunoblasts and plasmablasts. Immunophenotypically, the tumor cells were negative for CD3, CD4, CD5, CD10, CD20, CD57, CD79a, and most cytokeratins (CK5/6, CK8, CK19, CK20); focally positive for CD30 and cytokeratins (AE1/AE3, CAM5.2, CK7, CK18) (Figure 1B); weakly positive for PAX5; and positive for CD138 (Figure 1C), EMA, and ALK (Figure 1D). The positivity of focal cytokeratin, which has been reported in a small proportion of ALK+LBCL cases,(15) and the cytomorphology of this case may have led to a misdiagnosis of undifferentiated metastatic carcinoma. The presence of *ALK* translocation was demonstrated by an ALK split FISH assay, which was performed at a commercial laboratory (data not shown). The tumor cells were positive for PAX5, which is suggestive of ALK+LBCL. However, we carefully excluded a possibility of metastasis of ALK-positive lung cancer(10) because the tumor cell were positive for some cytokeratins and immunohistochemistry for immunoglobulins was not evaluable due to background staining. Immunohistochemistry for TTF1 was negative, which is usually positive in ALK-positive lung cancers.(16) In addition, PCR and sequencing analyses revealed that *IGH* was monoclonally rearranged and somatically hypermutated (data not shown).

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The patient was diagnosed as having ALK+LBCL and achieved complete remission after 6 cycles of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) treatment. Four months later, however, he relapsed.

Identification of SQSTM1-ALK

The 2 major ALK fusions in ALK+LBCL are CLTC-ALK and NPM-ALK, and they show a coarse granular cytoplasmic pattern and a nuclear and cytoplasmic pattern in anti-ALK immunohistochemistry, respectively. In the present case, anti-ALK immunohistochemistry showed a diffuse cytoplasmic staining pattern with ill-demarcated spots (Figure 1D), which was different from either of the former 2 patterns. Therefore, we carried out inverse RT-PCR to examine the presence of a novel fusion of *ALK*. We indeed isolated a cDNA containing the exon 5 of *SQSTM1* in-frame fused to the exon 20 of *ALK* (Figure 2A). A separate RT-PCR assay amplified the fusion point of *SQSTM1-ALK* cDNA (data not shown). To confirm the chromosome rearrangement, we performed *SQSTM1-ALK* fusion FISH. This result was consistent with the presence of a t(2;5)(p23.1;q35.3) leading to the generation of *SQSTM1-ALK* (Figure 2B). The complete sequences of SQSTM1-ALK are shown in Supplementary Figure 1.

SQSTM1 is a ubiquitin binding protein that is associated with oxidative stress, cell signaling, and autophagy.(17-20) Autophagosomal membrane protein LC3/Atg8 binds SQSTM1 and makes SQSTM1-containing protein aggregate to the autophagosome.(21) Mutations within *SQSTM1* are identified in patients with Paget disease of bone.(22)

SQSTM1 is located very near *NPM*, which is on 5q35.1. Therefore, the cytogenetic findings of the NPM-ALK-positive and the SOSTM1-ALK-positive lymphomas may be similar, because of which SQSTM1-ALK occurrence in lymphoma may be underestimated. As mentioned, however, NPM-ALK and SQSTM1-ALK differ in terms of the anti-ALK immunostaining pattern. NPM has a nuclear transport signal, while SQSTM1 does not. Therefore, NPM-ALK shows a nuclear and cytoplasmic staining pattern while SQSTM1-ALK shows only a cytoplasmic staining pattern. ALK is a representative "promiscuous" molecule because of its various fusion partners. The subcellular localization of ALK fusions depends on the fusion partners. The anti-ALK immunohistochemical staining pattern is, therefore, a simple and useful means to identify the possible partner in a tested case, and in fact, has prompted the identification of many ALK fusion partners, including the present case.

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Transforming activities of SQSTM1-ALK

We generated a recombinant retrovirus encoding SQSTM1-ALK and used it to infect cultured 3T3 fibroblasts. Infection with the virus, but not with an empty virus, resulted in the formation of multiple transformed foci in vitro (Figure 2C). As control experiments for formation, EML4-ALK (variant 1) and NPM-ALK similarly produced transformed foci (data not shown). The same 3T3 cells were injected into nude mice for an in vivo tumorigenicity assay. As expected, 3T3 cells expressing SQSTM1-ALK developed subcutaneous tumors at all injection sites within an observation period of 20 days (Figure 2D), confirming the transforming potential of the novel fusion kinase, SQSTM1-ALK.

All ALK fusion partners identified so far except moesin (MSN) have a coiled-coil domain(s) in their sequences, and the domain is conserved in its fusion form. The coiled-coil domain allows the protein to homodimerize. The tyrosine kinase domain of the ALK fusions is constitutively phosphorylated and activated through homodimerization via the coiled-coil domain. It has been speculated that the binding properties of MSN to cell membrane proteins lead to the dimerization of MSN-ALK proteins, enabling the constitutive phosphorylation of the chimeric MSN-ALK protein. (23) SQSTM1 does not harbor a coiled-coil domain and does not bind to membrane proteins. Instead, it has the Phox and Bem1p (PB1) domain in its N-terminus and forms heteromeric and homomeric complexes mediated by this domain. (24) Therefore, SQSTM1-ALK probably homodimerizes through the PB1 domain, leading to constitutive activation of the ALK kinase domain.

In conclusion, we reported a novel ALK fusion, SQSTM1-ALK, and its oncogenicity. ALK+LBCL is an aggressive lymphoma with poor prognosis;(3) ALK inhibitors are promising therapeutic agents for this condition. SQSTM1-ALK may be a rare fusion, but our data provide novel biological insights and may serve as a key to the accurate diagnosis of this rare lymphoma.

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Authorship and Disclosures

KT, MN, and HM conceived the study, collected and analyzed the data, and drafted the paper; KT and YO contributed to the pathology diagnosis; YS and MN contributed patient care; and MS, YT, YO, SH, and RA performed special studies and analyzed the data.

The authors reported no potential conflicts of interest.

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