症　例

若年男性に発生した肺原発高分化胎児型腺癌の1切除例

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要　旨

症例は21歳男性．健診の胸部X線写真にて右下肺野に異常陰影が指摘されたため，当院を紹介された．右肺S^{10}に45×30 mmの内部に空洞を伴う腫瘤影を認め，気管支鏡下生検にて，腺癌と診断され，高分化胎児型腺癌が疑われた．胸腔鏡下右肺下葉切除術を施行し，永久病理組織診断の結果，高分化胎児型腺癌40×30 mm pT2aN0M0，pStageIBと診断された．術後補助化学療法としてUFTを2年間内服し，現在，術後2年6ヵ月で無再発生存中である．

索引用語：高分化胎児型腺癌，低悪性度胎児型腺癌，桑実胚
well-differentiated fetal adenocarcinoma (WDFA), low-grade adenocarcinoma of the fetal lung type (L-FLAC), morule

はじめに

胎児型腺癌は，胎児期の気道上皮に類似した腺管構造を特長とする稀な腫瘍であり，全肺癌の約0.1％と報告されている[1]．今回，高分化胎児型腺癌の切除例を経験したので，文献的考察を加えて報告する．

症　例

症　例：21歳，男性．

主　訴：特記事項なし．

既往歴・家族歴：特記事項なし．

喫煙歴：なし．

現病歴：大学の健康診断での胸部X線写真で右下肺野に異常陰影が指摘され，精査目的に当院を紹介された．

入院時現症：身長162 cm，体重47 kg．血圧122/76 mmHg，脈拍70/分・整．心・肺音に異常なく，表在リンパ節は触知しなかった．貧血・黄疸・浮腫なども認めなかった．

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入院時血液検査所見：血液生化学検査では，T-Bilが1.54 mg/dl (D-Bil 0.07)と軽度上昇を認める他は，明らかな異常を認めなかった．腫瘍マーカーは，CEA 2.2 ng/ml，CYFRA＜0.5 ng/ml，pro-GRP 29.3 pg/ml，AFP 1.7 ng/ml，SCC 1.0 ng/ml，NSE 9.6 ng/ml，HCG＜1.0 mIU/mlと，いずれも正常範囲内であった．

胸部X線所見：右下肺野に，内部に空洞を伴う47×42 mmの腫瘤影を認めた（Fig. 1-a）．

胸腹部CT所見：右肺S^{10}に，内部に空洞を伴う45×30 mmの腫瘤影を認めた（Fig. 1-b）．縦隔リンパ節腫大は認めなかった．

PET所見：上記腫瘤にのみFDGの異常集積を認めた（SUVmax 7.3)(Fig. 1-c)．

気管支鏡検査：可視範囲内に明らかな病変は認めなかった．右$B^{10}b$より擦過検体及び生検検体を採取し，細胞診，組織診にて腺癌と診断，桑実胚（morule）を散在性に認めたため高分化胎児型腺癌が疑われた．

手術所見：腫瘍は右肺下葉S^{10}に認めた．明らかな胸膜浸潤，胸膜播種，胸水等は認めず，胸腔内洗浄細胞診も陰性であった．胸腔鏡下右肺下葉切除術，縦隔リンパ節郭清術（ND2a-2）を施行した．

病理学的所見：肉眼的には，中心に空洞を有する，40

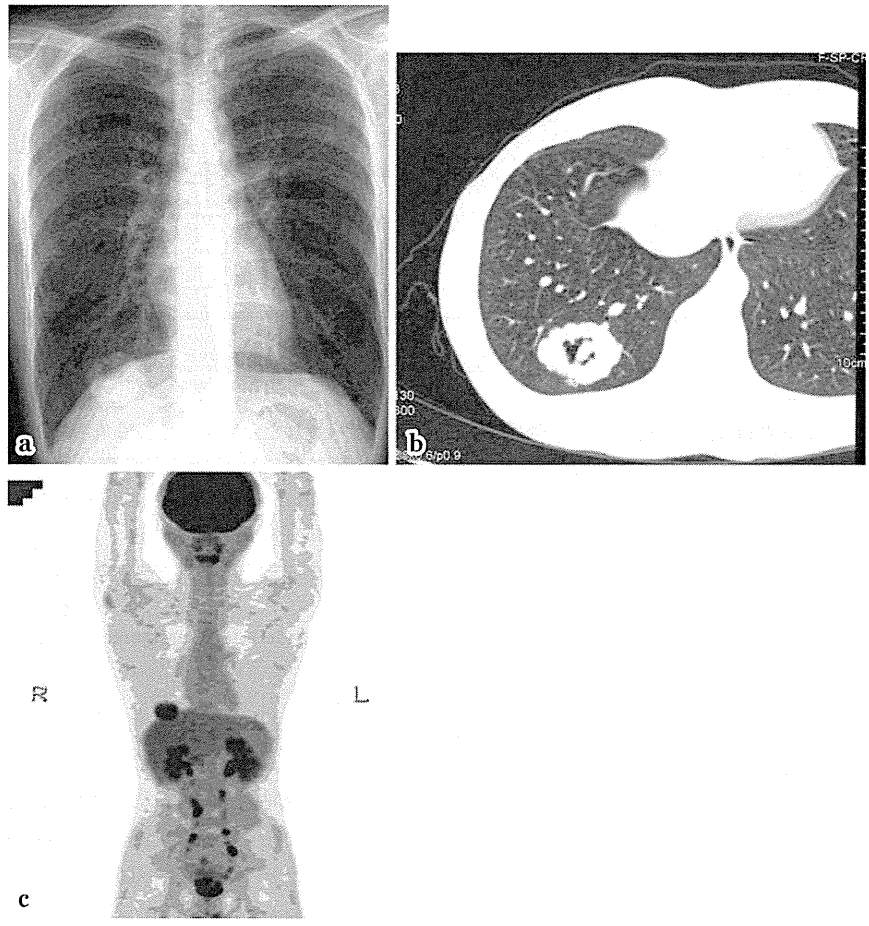

Fig. 1 Chest radiograph showing a mass in the right lower field (**a**). Chest computed tomography showing a 45×30-mm cavity-forming mass in the right lower lobe (**b**). PET scan revealed a marked accumulation of FDG in the tumor with a maximum standardized uptake value of 7.3 (**c**).

×30 mmの境界明瞭で白色調の腫瘤状病変を認めた(Fig. 2-a)．組織学的には，細長い核が柵状に並び，内腔面に胞体を有する管腔構造を示し，その腺管から連続して豊富な好酸性細胞質をもつ多形細胞からなる桑実胚を認めた．桑実胚の核はスリガラス状（optically clear nucleus）であった(Fig. 2-b, c)．間質は全体的に乏しく，腫瘍は上皮性成分からなり，間葉成分は認めなかった．免疫染色では，腫瘍細胞は，核および細胞質優位に，β-catenin染色陽性を示した(Fig. 2-d)．以上の所見から，高分化胎児型腺癌と診断した．胸膜浸潤は認めず，リンパ節転移も陰性であり，pT2aN0M0, pStageIBと診断した．

術後経過：術後補助化学療法としてUFTを2年間内服し，現在，術後2年6ヵ月で無再発生存中である．

考　察

胎児型腺癌は，胎児期の気道上皮に類似した腺管構造を特長とする稀な腫瘍であり，その頻度は，Zaidiら[1]の報告では，肺癌と病理学的に診断された2720例中3例(0.1%）が本腫瘍であった．

1961年にSpencer[2]が，胎児肺に類似した，上皮・間葉成分から成る腫瘍を，この組織像が腎芽腫（nephroblastoma）に類似することから，肺芽腫（pulmonary blastoma；PB)として報告した．1982年にはKradinら[3]が，肉腫成分を欠く肺芽腫（pulmonary endodermal tumor resembling fetal lung；PET)を報告したが，これが胎児

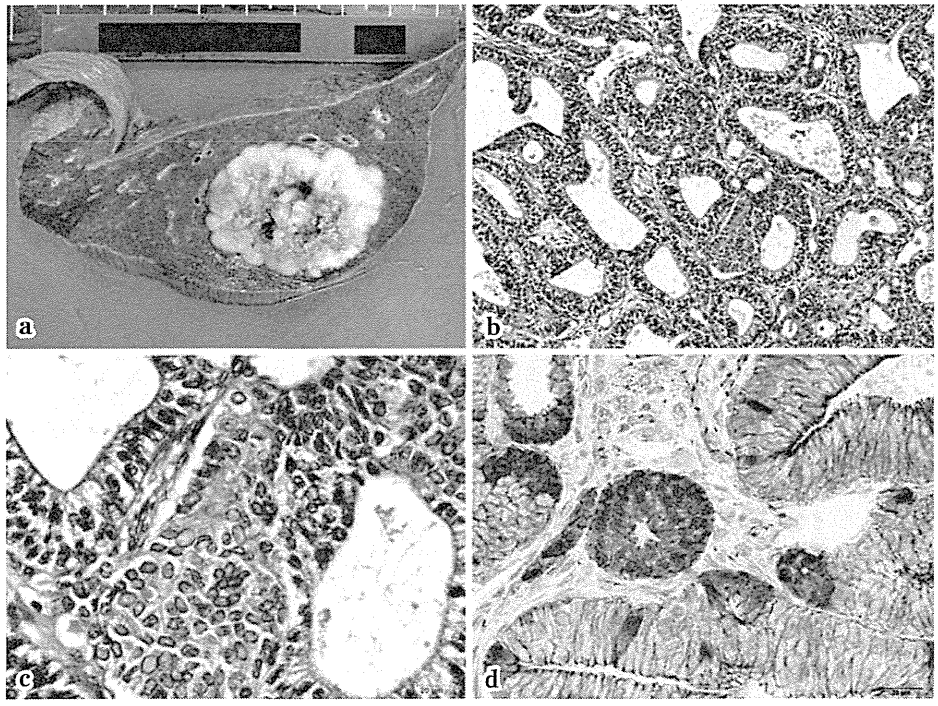

Fig. 2 Resected specimen showed a well-defined cavity-forming solid tumor in the right lower lobe **(a)**. Histological findings showed complex neoplastic glandular structures resembling the fetal lung, and the nodules consisted of small solid nests of tumor cells (morules) **(b, c**; HE stain), and, immunohistochemically, the epithelial cells were positive for anti-β-catenin antibody in the nucleus and cytoplasm **(d)**.

型腺癌の初めての報告である．1984年にKodamaら[4]はwell-differentiated adenocarcinoma stimulating fetal lung tubules/adenocarcinoma of fetal lung type，1991年にKossら[5]はwell-differentiated fetal adenocarcinoma（WDFA）という名称を用いているが，1999年版WHO分類において，同腫瘍が初めて，高分化胎児型腺癌（WDFA）として記載され，腺癌の亜型に分類された．

一方，症例が蓄積されるに従い，過去にPET/WDFAとして報告されてきた腫瘍のなかに，臨床病理学的に大きく異なる腫瘍が存在することが明らかとなり，1998年にNakataniら[6]は，従来のPET/WDFAに相当する，悪性度の低いものを低悪性度胎児型腺癌(low grade adenocarcinoma of the fetal lung type；L-FLAC)とし，悪性度の高いものを高悪性度胎児型腺癌（high grade adenocarcinoma of the fetal lung type；H-FLAC）と呼称することを提案した．

以上のような歴史的変遷を経て，2004年版WHO分類では，L-FLAC，H-FLACを包括する胎児型腺癌（fetal adenocarcinoma）として，腺癌の特殊型に分類された．2011年に国際肺癌学会などから提案された肺腺癌の分類においても，2004年版WHO分類と同様に記載されている[7]．

L-FLAC/WDFAの発生に関しては，β-cateninという諸臓器で増殖・分化に重要な役割を果たしている細胞内分子があり，そのβ-catenin遺伝子変異を含むWntシグナル伝達系の活性化が関与していることが示唆されている．β-catenin遺伝子変異により，本来主として細胞膜に局在する同タンパクの細胞質内貯留・核内移行が進み，癌化に関係する標的遺伝子の転写を促進すると考えられている．肺癌全体でのβ-catenin遺伝子変異は2-4%程度とされ，L-FLAC/WDFAに特異的とされる[8]．一方，L-FLAC/WDFAに特徴的な腺様構造は，甲状腺乳頭癌，子宮や卵巣の類内膜腫瘍，胆嚢の幽門腺型腺腫，膵芽腫，大腸腺腫/腺癌などの組織型と類似するが，これらの腫瘍においても，β-catenin遺伝子変異がしばしば認められるとされる[8]．

Table 1 Summary of the 31 Reported Cases of Well-Differentiated Fetal Adenocarcinoma Resected in Japan

No.	reported year	author	age (years)	sex	smoking	symptoms	preoperative diagnosis	location	extent of resection	lymph node dissection (LND)	size (mm)	pT	pN	M	pStage	recurrence	outcome (months)
1	1985	Mitsuoka	39	M	ND	none	none	RUL (S2)	lobectomy	ND	70	2b	0	0	IIA		alive (72)
2	1986	Ogawa	33	F	ND	none	none	LUL (S1+2)	lobectomy	LND	60	2b	0	0	IIA	+	alive (55)
3	1987	Tanimura	27	F	−	none	none	LUL (S3)	lobectomy	mediastinal	90	3	0	0	IIB		alive (52)
4	1990	Nakamura	46	F	−	cough, sputum	none	LUL (S5)	lobectomy	mediastinal	30	1b	0	0	IA		alive (7)
5	1991	Fukase	35	F	ND	none	none	RUL (S1)	lobectomy	ND	30	1b	0	0	IA		alive (60)
6	1992	Sato	32	F	−	cough	none	LLL (S6)	lobectomy	mediastinal	80	3	0	0	IIB		alive (5)
7	1992	Higashiyama	52	M	+	chest discomfort, weight loss	none	RLL (S6)	pneumonectomy	hilar	60	2b	0	0	IIA	+	dead (28)
8	1992	Higashiyama	36	M	+	none	SCC	RLL (S10)	lobectomy	mediastinal	24	1b	0	0	IA		alive (88)
9	1992	Higashiyama	62	M	+	none	Sq	RLL (S9)	lobectomy	mediastinal	20	1a	0	0	IA		alive (42)
10	1993	Shimada	31	F	ND	none	none	RUL (S2)	lobectomy	mediastinal	45	2a	ND	0	ND	ND	ND
11	1995	Fujino	33	F	−	none	PB	RLL (S6)	pneumonectomy	ND	90	3	1	0	IIIA		alive (12)
12	1996	Okano	33	F	ND	none	none	RUL (S3)	bilobectomy	mediastinal	50	2a	0	0	IB		alive (11)
13	1997	Izumi	32	F	+	none	Ad	RUL (S2)	lobectomy	mediastinal	30	1b	0	0	IA	+	alive (33)
14	1997	Matsumoto	30	F	ND	none	Ad	LUL (S3)	lobectomy	ND	32	2a	0	0	IB		alive (48)
15	1998	Nakatani	35	M	+	none	ND	RUL (S3)	ND	ND	14	1a	0	0	IA		alive (24)
16	1998	Nakatani	35	F	−	none	ND	RUL	ND	ND	22	1b	0	0	IA		alive (24)
17	1998	Nakatani	39	M	+	none	ND	LUL (S1+2)	ND	ND	25	1b	0	0	IA		alive (10)
18	1998	Nakatani	40	F	+	cough, hemosputum	ND	RML	ND	ND	30	1b	0	0	IA		alive (120)
19	1998	Nakatani	35	F	+	none	ND	RUL	ND	ND	30	1b	0	0	IA		alive (48)
20	1998	Nakatani	33	F	+	none	ND	LUL	ND	ND	35	2a	0	0	IB		alive (108)
21	1998	Nakatani	45	M	+	none	ND	LUL (S4)	ND	ND	45	2a	0	0	IB		alive (72)
22	1998	Nakatani	55	F	+	eye pain	ND	RLL	ND	ND	50	2a	1	1	IV	(advanced)	dead (24)
23	1998	Nakatani	19	M	ND	ND	ND	LUL (S1+2)	ND	ND	15	1a	ND	0	ND	ND	ND
24	2001	Tatebayashi	27	F	−	none	none	LUL (S3/4)	lobectomy	mediastinal	26	1b	0	0	IA		alive (8)
25	2001	Sawamoto	24	F	ND	cough, fever	Ad	RUL	bilobectomy	mediastinal	120	3	0	0	IIB	ND	ND
26	2003	Kawai	58	M	ND	none	none	LLL (S10)	lobectomy	mediastinal	32	2a	0	0	IB		alive (36)
27	2003	Mori	38	M	+	none	Ad	LUL (S4)	lobectomy	mediastinal	12	1a	0	0	IA	ND	ND
28	2006	Sato	36	M	ND	none	Ad	RLL	bilobectomy	mediastinal	41	2a	0	0	IB		alive (38)
29	2011	Takeshita	32	F	+	cough	none	LLL (S8)	lobectomy	LND	17	1a	0	0	IA		alive (36)
30	2011	Yamaguchi	15	F	−	none	Ad	RLL (S9/10)	bilobectomy	mediastinal	55	2b	0	0	IIA		alive (8)
31	2012	Present case	21	M	−	none	Ad	RLL (S10)	lobectomy	mediastinal	40	2a	0	0	IB		alive (30)

ND: not described, SCC: small cell carcinoma, Sq: squamous cell carcinoma, PB: pulmonary blastoma, Ad: adenocarcinoma, RUL: right upper lobe, RML: right middle lobe, RLL: right lower lobe, LUL: left upper lobe, LLL: left lower lobe.

L-FLAC/WDFAは，肉眼的には境界明瞭でしばしば分葉状，白色から黄白色の充実性腫瘤である．組織学的には，胎児期の気道上皮に類似した未熟で複雑に分枝する腺管構造を認め，その腺管から連続して多形細胞からなる桑実胚を認める．この桑実胚は，moruleと呼ばれ，本腫瘍の特徴であり，moruleの核はスリガラス状で，optically clear nucleusと表現される．β-catenin免疫染色にて，moruleや分枝中の腺管の先端で，核および細胞質優位に発現を認めるのも本腫瘍の特徴である．さらにL-FLAC/WDFAの診断においては，H-FLACとの鑑別が必要になる．H-FLACは，L-FLAC/WDFAに類似した複雑な乳頭腺管状構造を形成するが，臨床像が大きく異なる．L-FLAC/WDFAが若年から中年のやや女性優位に発生し，予後が比較的良好なのに比べ，H-FLACは高齢の男性優位に発生し，予後は不良とされる．H-FLACはmorule形成は認めず，β-catenin免疫染色においても，細胞膜有意の発現を示す点などでL-FLAC/WDFAと鑑別が可能である[8]．本症例では，moruleを認め，免疫染色で腫瘍細胞の核および細胞質優位に，β-catenin染色陽性を示したことなどよりL-FLAC/WDFAと診断した．

1998年のNakataniらの報告以前は，胎児型腺癌は，その多くが，「肉腫成分を欠く肺芽腫」として報告され，肺芽腫との比較で述べられている．そのため，moruleやβ-catenin免疫染色の記載がなく，L-FLAC/WDFAとH-FLACの鑑別については，臨床的に判断せざるをえないものもあるが，我々が検索しうる限り，本邦では本症例を含めて31例の手術例が報告されている（Table 1）[6,9-27]．

発症時の年齢は中央値で35歳（15–62歳）と若く，男女比は1：1.5（男性12例，女性19例）で女性が多かった．約80%（30人中23人）が無症状で，健康診断の胸部X線写真等が診断契機であった．術前の確定診断は困難なことが多く，病理学的に悪性と診断されたものは半数以下（22人中10人）で，そのうち，本症例も含めた7人が腺癌，1人が肺芽腫と診断されていた．原発肺葉には特に差は認められなかった．手術術式では，全摘が2例，2葉切が4例，葉切が16例，記載なしが9例であった．腫瘍径は中央値で32 mm（12–120 mm），リンパ節転移は10%以下（29人中2人），約70%はI期で発見されていた（29人中22人，肺癌取扱い規約　第7版）．予後については，低悪性度で比較的良好であり，腫瘍死は約10%（28人中2人）であった．

治療としては，手術が第一選択と考えられている．術後補助療法については，本邦での報告に関しては，放射線療法の報告はなく，化学療法については，本症例を含めて3例のみに施行されていた．本症例では，患者の希望もあり，IB期の腺癌の術後補助化学療法に準じて，2年間のUFT内服を選択した．

稀な腫瘍であるため，切除範囲，リンパ節郭清についての検討の報告はなく，また手術方法と同様に，術後補助療法や再発時の治療についても一定の見解はない．今後の検討課題と思われる．

ま と め

今回，非常に稀な高分化胎児型腺癌の切除例を経験したので報告した．術後補助化学療法としてUFTを2年間内服し，現在，術後2年6ヵ月で無再発生存中である．

文　献

1. Zaidi A, Zamvar V, Macbeth F, Gibbs AR, Kulatilake N, Butchart EG. Pulmonary blastoma: medium-term results from a regional center. Ann Thorac Surg 2002; **73**: 1572-5.
2. Spencer H. Pulmonary blastomas. J Pathol Bacteriol 1961; **82**: 161-5.
3. Kradin RL, Young RH, Dickersin GR, Kirkham SE, Mark EJ. Pulmonary blastoma with argyrophil cells and lacking sarcomatous features (pulmonary endodermal tumor resembling fetal lung). Am J Surg Pathol 1982; **6**: 165-72.
4. Kodama T, Shimosato Y, Watanabe S, Koide T, Naruke T, Shimase J. Six cases of well-differentiated adenocarcinoma simulating fetal lung tubules in pseudoglandular stage. Comparison with pulmonary blastoma. Am J Surg Pathol 1984; **8**: 735-44.
5. Koss MN, Hochholzer L, O'Leary T. Pulmonary blastomas. Cancer 1991; **67**: 2368-81.
6. Nakatani Y, Kitamura H, Inayama Y, Kamijo S, Nagashima Y, Shimoyama K, et al. Pulmonary adenocarcinomas of the fetal lung type: a clinicopathologic study indicating differences in histology, epidemiology, and natural history of low-grade and high-grade forms. Am J Surg Pathol 1998; **22**: 399-411.
7. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, et al. International association for the study of lung cancer/American thoracic society/

European respiratory society international multidisciplinary classification of lung adenocarcinoma. J Thorac Oncol 2011; **6**: 244-85.

8. 中谷行雄，宮城洋平，廣島健三．胎児型肺腺癌と肺芽腫．加藤治文，西條長宏，福岡正博，小林紘一，海老原善郎，井内康輝，他編．MOOK 肺癌の臨床 2008～2009．東京：篠原出版新社；2008: 27-38.
9. 光岡明夫，和田洋巳，伊藤元彦．肉腫成分を欠いた Pulmonary Blastoma（Pulmonary Endodermal Tumor Resembling Fetal Lung）の1症例．肺癌 1985; **25**: 555-8.
10. 小川純一，井上宏司，小出司郎策，川田志明，正津　晃，長村義之．胎児肺細気管支に類似した管腔を形成する高分化型腺癌の1例．日胸 1986; **45**: 68-72.
11. 谷村繁雄，友安　浩，伴場次郎，正木幹雄，松下　央．胎児肺に類似する組織像を示した肺高分化型腺癌の1手術例．肺癌 1987; **27**: 307-11.
12. 中村聡人，小阪真二，福瀬達郎，玉田二郎，能登原憲司．胎児肺型肺腺癌の1例．日呼外会誌 1990; **4**: 478-84.
13. 深瀬真之，斎藤清子，石原　良，由岐義広，河本廣志，奥村　浩，他．肉腫成分を欠いた pulmonary blastoma（pulmonary adenocarcinoma of fetal type：胎児肺型腺癌）の1手術例．日胸 1991; **50**: 217-21.
14. 佐藤邦彦，前田和信，一瀬幸人，矢野篤次郎，原　信之，大田満夫．高分化胎児型肺腺癌の1手術例．日胸外会誌 1992; **40**: 1792-6.
15. 東山聖彦，横内秀起，土井　修，児玉　憲，建石竜平．「胎児肺型肺腺癌」外科切除6症例の臨床病理学的検討．日呼外会誌 1992; **6**: 760-6.
16. 島田友幸，阿保七三郎，北村道彦，橋本正治，四釜俊夫，南谷佳弘．Pulmonary blastoma の1亜型　胎児肺類似肺高分化型腺癌の1手術例．日胸外会誌 1993; **41**: 1026-9.
17. Fjino S, Asada Y, Konishi T, Asakura S, Kato H, Mori A. Well-differentiated fetal adenocarcinoma of lung. Lung Cancer 1995; **13**: 311-6.
18. 岡野高久，井岡二朗，田部志郎，中根佳宏，西山勝彦．Pulmonary blastoma（well differentiated fetal adenocarcinoma）の1例．日胸外会誌 1996; **44**: 69-73.
19. 泉　浩，見上光平，二川俊郎，細田泰之，益田貞彦，植草利公．Well-differentiated fetal adenocarcinoma（WDFA）の1切除例．肺癌 1997; **37**: 231-5.
20. 松本武敏，児玉哲郎，松本武夫，金子　司，岩崎聖二，川上喜久，他．肉腫成分を欠いた pulmonary blastoma（pulmonary endodermal tumor resembling fetal lung）の1例．日臨細胞会誌 1997; **36**: 44-8.
21. 舘林孝幸，菅野　恵，鈴木利光，新田澄郎．Well differentiated fetal adenocarcinoma の1手術例．日呼外会誌 2001; **15**: 131-5.
22. 澤本博史，村田繁利，真武邦茂，角南俊也，牛島泰宏，亀田祐子．Pulmonary blastoma の1例．臨床放射線 2001; **46**: 597-600.
23. 河合俊典，三宅　隆，原　享子，原　史人，中嶋健博．胎児型肺腺癌の1例．胸部外科 2003; **56**: 340-3.
24. 森　正一，関戸好孝，重光希久生，吉岡　洋，今泉宗久，下方　薫．βカテニン遺伝子に変異が認められた well differentiated fetal adenocarcinoma の1例．肺癌 2003; **43**: 351-5.
25. Sato S, Koike T, Yamato Y, Yoshiya K, Honma K, Tsukada H. Resected well-differentiated fetal pulmonary adenocarcinoma and summary of 25 cases reported in Japan. Jpn J Thorac Cardiovasc Surg 2006; **54**: 539-42.
26. 竹下伸二，村松　高，四万村三恵，古市基彦，大森一光，塩野元美．肺高分化胎児型腺癌の1症例．日呼外会誌 2011; **25**: 639-42.
27. 山口　央，大崎敏弘，大庭ひろみ，杉本幸弘，海老規之，山本英彦．15歳で発見された高分化胎児型腺癌の1例．肺癌 2011; **51**: 742-6.

A resected case of well-differentiated fetal adenocarcinoma of the lung in a young male adult

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Fetal adenocarcinoma is a rare type of malignant lung tumor resembling fetal lung tissue, and is estimated to account for only 0.1% of all pulmonary malignant neoplasms. We report a resected case of well-differentiated fetal adenocarcinoma. A 21-year old man was found to have a tumor shadow in the right lower field of a chest radiograph as part of a medical examination. Chest computed tomography showed a 45×30-mm cavity-forming mass in the right lower lobe, and transbronchial biopsy revealed adenocarcinoma. A right lower lobectomy was performed via video-assisted thoracoscopy. The post-operative pathological diagnosis was well-differentiated fetal adenocarcinoma, Stage IB (pT2aN0M0). The patient was treated with postoperative adjuvant chemotherapy, and there had been no evidence of recurrence as of 30 months postoperatively.

Immunohistochemical studies of pulmonary large cell neuroendocrine carcinoma: A possible association between staining patterns with neuroendocrine markers and tumor response to chemotherapy

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Objective: Pulmonary large cell neuroendocrine carcinoma is a rare high-grade malignant tumor. Because large cell neuroendocrine carcinoma is rare, the optimal treatment, including perioperative chemotherapy, has not been defined. We retrospectively analyzed the correlation among the effectiveness of perioperative chemotherapy in treating large cell neuroendocrine carcinoma, pathologic stage, and immunoreactivity to neuroendocrine markers.

Methods: A total of 63 patients with pulmonary large cell neuroendocrine carcinoma undergoing surgical resection from 2001 to 2009 were included. The resected tumors were immunohistochemically stained with the 3 neuroendocrine markers synaptophysin, chromogranin A, and neural cell adhesion molecule. We categorized patients who were positive for all 3 markers as the triple-positive group and those who were negative for 1 or 2 markers as the non–triple-positive group.

Results: Perioperative chemotherapy resulted in better overall survival than surgery alone ($P = .042$). Multivariate analysis of survival revealed that perioperative chemotherapy was a significant independent prognostic factor (hazard ratio, 0.323; 95% confidence interval, 0.112-0.934; $P = .0371$). Among the patients who received perioperative chemotherapy, the non–triple-positive group had a significantly greater 5-year survival rate than the triple-positive group ($P = .0216$). Moreover, among the non–triple-positive group, a significantly greater 5-year survival rate was observed for the patients who underwent surgery with chemotherapy than for those who underwent surgery without chemotherapy ($P = .0081$). In contrast, no difference was found in 5-year survival between patients with chemotherapy and those without chemotherapy when the tumors were triple positive.

Conclusions: Our results suggest that perioperative chemotherapy might benefit the survival of patients with pulmonary large cell neuroendocrine carcinoma, in particular when the tumors are not immunoreactive to all 3 neuroendocrine markers. (J Thorac Cardiovasc Surg 2012;■:1-8)

GTS

Pulmonary large cell neuroendocrine carcinoma (LCNEC), proposed as a separate tumor category by Travis and colleagues[1] in 1991, is distinguished from typical carcinoid, atypical carcinoid, and small-cell lung carcinoma (SCLC) by its morphologic and biologic features. In 1999, the World Health Organization classified LCNEC as a variant of large cell carcinoma.[2] Pulmonary LCNEC represents about 2% to 3% of all lung malignancies and is associated with a worse prognosis than other non–SCLC (NSCLC), even in the early stage.[3-6] However, in a recent Japanese study with a large sample size, Asamura and colleagues[7] reported that no prognostic difference was found between pulmonary LCNEC and SCLC.

Several small-scale retrospective studies have demonstrated that perioperative chemotherapy could improve the survival of patients with pulmonary LCNEC. Perioperative chemotherapy is recommended even for patients with resectable stage I LCNEC because of its aggressive course, remarkably dismal prognosis, and high potential for metastasis.[8-11] However, owing to the rarity of this tumor, the incidence, prognosis, and optimal treatment remain to be determined.

In the present study, we retrospectively analyzed the efficacy of perioperative chemotherapy in treating pulmonary LCNEC. Furthermore, we examined the correlation between the sensitivity of LCNEC and perioperative chemotherapy and the immunohistochemical staining patterns of

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Abbreviations and Acronyms
CPT-11 = irinotecan
LCNEC = large cell neuroendocrine carcinoma
NCAM = neural cell adhesion molecule
NSCLC = non–small-cell lung carcinoma
SCLC = small-cell lung carcinoma
VP-16 = etoposide

the tumors with 3 immunohistochemical neuroendocrine markers, synaptophysin, chromogranin A, and neural cell adhesion molecule (NCAM). Although our experiences in 2 institutions do not allow us to reach a definite conclusion owing to the small number of subjects, the present preliminary study may be useful in generating a hypothesis to determine the immunohistochemical biomarkers to predict LCNEC's response to perioperative chemotherapy in future prospective multi-institutional trials.

METHODS

We retrospectively examined the clinical data of 63 patients with pulmonary LCNEC who underwent complete surgical resection from 2001 to 2009. All follow-up data were current as of December 31, 2011. All patients who underwent surgery in 2009 were included in the present study, because more than 2 years have passed since their surgery. The median follow-up period was 32.3 months (range, 2.8-95.3 months). The Hyogo Cancer Center and Kobe University Hospital institutional review boards approved the study, and each participant provided informed consent. LCNEC was diagnosed using the following histopathologic criteria: (1) neuroendocrine morphology such as an organoid, palisading, rosette-like, or trabecular growth pattern; (2) high mitotic count (≥11/10 high-power fields [HPF]); (3) tumor necrosis (often large zone); (4) large cell size with low nuclear/cytoplasmic ratio, vesicular or fine chromatin, and/or frequent nucleoli; and (5) positive immunostaining for 1 or more of the neuroendocrine markers, synaptophysin, chromogranin A, and NCAM.[2]

Immunohistochemical stains were performed on 4-mm-thick, formalin-fixed, paraffin-embedded sections. The deparaffinized sections underwent CC1 buffer pretreatment (pH 8.5, ethylenediaminetetraacetic acid; Roche, Mannheim, Germany) and were immunostained for the markers with the streptavidin-biotin technique with an automated immunostainer (Benchimark; Ventana, Tucson, Ariz) according to the manufacturer's instructions. Antibodies against chromogranin A (polyclonal, 1:500 dilution; Dako, Glostrup, Denmark), synaptophysin (monoclonal, clone 27G12, 1:2 dilution; Nichirei, Tokyo, Japan), CD56 (NCAM; monoclonal, 1:100 dilution; Novocastra, Newcastle, UK), and Ki-67 (monoclonal 1:100 dilution; Dako) were used.

All samples were evaluated by an expert pathologist (C.O.) without knowledge of the patient's outcome. Plural sections, more than 10 sections in most cases, were prepared in each case, and 1 representative specimen involving tumor was selected for immunohistochemistry. The final results were reported as negative (no positive cells) or positive (immunoreactive). Proliferative activity was expressed as the MIB-1 index, which was calculated as the proportion of Ki-67–positive cells by counting 500 to 1000 cancer cells. The mitotic counts were performed using an Olympus BX53 microscope at a magnification of ×400, counting 3 sets of 10 HPF for each tumor. The area with the greatest numbers of mitoses was counted. In the present study, we included pure LCNEC and combined LCNEC, in which at least 1 portion of neuroendocrine differentiation or morphology in NSCLC was LCNEC. The medical records provided information on the patient age, gender, smoking status, pathologic stage, perioperative chemotherapy, and operative procedure. The determination of disease stage was based on the TNM classification using the International Union Against Cancer staging system.[12]

We classified patients into 2 groups to investigate the correlation between the sensitivity of LCNEC to perioperative chemotherapy and the results of immunohistochemical staining of neuroendocrine markers. We categorized the patients who were positive for all 3 neuroendocrine markers (ie, synaptophysin, chromogranin A, and NCAM) as the triple-positive group and those who were negative for 1 or 2 of the markers as the non–triple-positive group. Statistical analyses were performed using JMP, version 8, software (SAS Institute, Cary, NC). Student's *t*-test and the chi-square test were performed to assess the significance of the differences in age, gender, smoking status, surgical procedure, and pathologic stage between the triple-positive and non–triple-positive groups. Survival was calculated using the Kaplan-Meier method, and differences in the distributions were evaluated using the log-rank test. The Cox proportional hazards model was used to evaluate the association between the prognostic factors and survival rate after pulmonary resection, with the hazards ratio and 95% confidence intervals. Significance was set at $P < .05$.

RESULTS

The clinicopathologic characteristics of the 63 patients with pulmonary LCNEC who underwent surgical resection are listed in Table 1. The patient age ranged from 30 to 84 years (mean age, 67.0 years). Of the 63 patients, 54 (87%) were men, and 58 (92%) were former or current smokers. The surgical procedures included 55 lobectomies, 2 segmentectomies, and 6 wedge resections with complete resection (R0). Of the 55 lobectomies, 8 bronchoplastic procedures were performed and 6 extended resections were required because of tumor invasion into the adjacent tissue, including muscle and rib (n = 3), parietal pleura (n = 1), and vagal nerve (n = 2). Of the 6 patients who underwent extended resection, 5 were treated with chemotherapy. Because these patients had advanced-stage disease and the number of the subjects was small, no correlation was found between the extent of resection and the outcome.

The distribution of pathologic stage was stage IA in 19 patients (30%), stage IB in 16 (25%), stage IIA in 5 (8%), stage IIB in 11 (18%), stage IIIA in 9 (14%), and stage IIIB in 3 patients (5%). The mean MIB-1 index for all patients was 62.7% (range, 5.2%–90.5%), and the mean mitotic count was 71.2/10 HPF (range, 14–153/10 HPF). All 63 patients had tumor necrosis.

Perioperative platinum-based chemotherapy was administered to 23 (37%) of the 63 patients. We have used the criterion of tumor size more than 3 cm in offering chemotherapy for patients with stage I disease since 2004. Thus, 8 of 35 patients with stage I received chemotherapy. Also, 9 of 16 with stage II and 6 of 12 with stage III received chemotherapy. Induction chemotherapy was administered to 3 patients at clinical stage III and adjuvant chemotherapy was administered to 20 patients at clinical stage I/II. Three patients received preoperative mediastinal radiotherapy (40 Gy) combined with induction chemotherapy. No patient

TABLE 1. Patient characteristics (n = 63)

Factor	Total	Triple positive	Non–triple positive	*P* value
Patients (n)	63	31	32	
Age (y)				.0473
Mean	67.0	64.4	69.5	
Range	30–84	30–78	41–84	
Gender				.2578
Male	54 (87)	25 (81)	29 (91)	
Female	9 (13)	6 (19)	3 (9)	
Smoking status				.1512
Former or current	58 (92)	27 (87)	31 (97)	
Never smoked	5 (8)	4 (13)	1 (3)	
Surgical procedure				.3416
Lobectomy	55 (87)	26 (84)	29 (90)	
Segmentectomy	2 (3)	3 (10)	0 (0)	
Wedge resection	6 (10)	2 (6)	3 (10)	
Pathologic stage				.6044
IA	19 (30)	11 (35)	8 (25)	
IB	16 (25)	7 (23)	9 (28)	
IIA	5 (8)	3 (10)	2 (6)	
IIB	11 (18)	3 (10)	8 (25)	
IIIA	9 (14)	5 (16)	4 (13)	
IIIB	3 (5)	2 (6)	1 (3)	
MIB-1 index (%)				.5029
Mean	62.7	61.2	64.4	
Range	5.2–90.5	5.2–90.0	5.8–90.5	
Mitotic counts (/10 HPF)				.3538
Mean	71.2	64.7	77.9	
Range	14–153	14–122	20–153	

Data in parentheses are percentages. *Triple positive,* Positive for synaptophysin, chromogranin A, and neural cell adhesion molecule; *Non–triple positive,* negative for 1 or 2 neuroendocrine markers (synaptophysin, chromogranin A, and neural cell adhesion molecule); *HPF,* High-powered fields.

underwent postoperative radiotherapy. The chemotherapy regimens are listed in Table 2.

The results of immunohistochemical staining for the 3 neuroendocrine markers are summarized in Table 3. Although the percentage of reactive cells ranged very much, the intensity of immunostaining was not so variegated for all 3 neuroendocrine markers. Of the 63 tumors, 40 (63%) were positive for synaptophysin, 36 (57%) for chromogranin A, and 59 (94%) for NCAM. Finally, 31 tumors (49%) were positive for all 3 neuroendocrine markers and 32 (51%) were negative for 1 or 2 markers. The clinicopathologic characteristics and chemotherapy regimens of patients in the triple-positive group and non–triple-positive group are listed in Tables 1and 2, respectively. Although the triple-positive group was significantly younger than the non–triple-positive group, no significant differences were seen in the distribution of other characteristics between the 2 groups. Also, no morphologic differences were found between the 2 groups in the neuroendocrine structures such as rosettes and ribbon-like arrangements, necrosis, mitotic counts, and MIB-1 index.

TABLE 2. Regimens of perioperative platinum-based chemotherapy (n = 23)

Regimen	Triple positive (n = 12)	Non–triple positive (n = 11)
Induction chemotherapy		
CDDP + VP-16	1	0
CDDP + VNR	0	1
CBDCA + DOC	1	0
Adjuvant chemotherapy		
CDDP + CPT-11	5	2
CBDCA + PTX	2	4
CDDP + VNR	2	3
CBDCA + VP-16	1	1

Triple positive, Positive for synaptophysin, chromogranin A, and neural cell adhesion molecule; *Non–triple positive,* negative for 1 or 2 neuroendocrine markers (synaptophysin, chromogranin A, and neural cell adhesion molecule); *CDDP,* cisplatin; *VP-16,* etoposide; *VNR,* vinorelbine; *CBDCA,* carboplatin; *DOC,* docetaxel; *CPT-11,* irinotecan; *PTX,* paclitaxel.

The overall 5-year survival rate among the 63 patients was 44.9%. Significantly longer survival was observed for the patients who underwent surgery with chemotherapy than for those who underwent surgery without chemotherapy (74.4% and 32.3%, respectively; $P = .042$; Figure 1, *A*).

Next, we evaluated whether the effects of perioperative chemotherapy were seen in patients with different stages. Although there was a tendency for longer survival for the patients with stage I disease who underwent surgery and chemotherapy compared with those who underwent surgery without chemotherapy, the small number of subjects did not allow us to obtain a statistically significant difference (85.7% and 35.2%, respectively; $P = .1129$; Figure 1, *B*). Similarly, no statistically significant difference in survival between the patients with and without chemotherapy at stage II/III (68.8% and 25.6%, respectively; $P = .1243$; Figure 1, *B*). Multivariate analysis of survival was

TABLE 3. Immunohistochemical staining of 3 neuroendocrine markers (n = 63)

Neuroendocrine marker	Patients (n)
Synaptophysin	
Positive	40 (63)
Negative	23 (37)
Chromogranin A	
Positive	36 (57)
Negative	27 (43)
NCAM	
Positive	59 (94)
Negative	4 (6)
Triple positive	31 (49)
Non–triple positive	32 (51)

Data in parentheses are percentages. *NCAM,* Neural cell adhesion molecule; *Triple positive,* Positive for synaptophysin, chromogranin A, and neural cell adhesion molecule; *Non–triple positive,* negative for 1 or 2 neuroendocrine markers (synaptophysin, chromogranin A, and neural cell adhesion molecule).

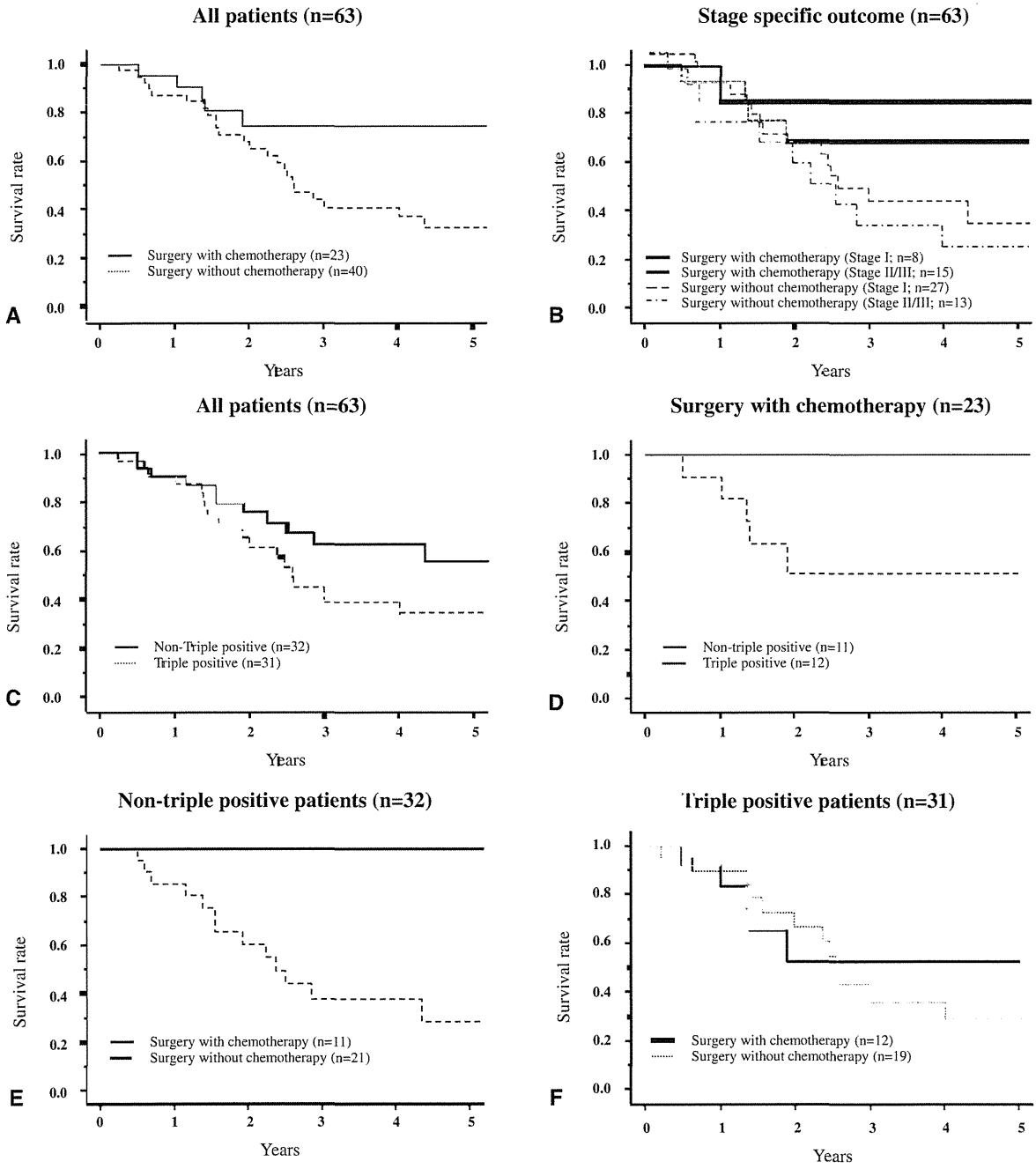

FIGURE 1. A, Comparison of survival of patients with large cell neuroendocrine carcinoma who underwent surgery with perioperative chemotherapy and those who underwent surgery alone. B, Comparison of stage-specific survival of patients with large cell neuroendocrine carcinoma who underwent surgery with perioperative chemotherapy and those who underwent surgery alone (stage I vs stage II/III). C, Comparison of survival of the non–triple-positive group and triple-positive group. D, Comparison of survival of the non–triple-positive group and triple-positive group among patients who received perioperative chemotherapy. E, Comparison of survival of non–triple-positive patients who underwent surgery with perioperative chemotherapy and those who underwent surgery without perioperative chemotherapy. F, Comparison of survival of triple-positive patients who underwent surgery with perioperative chemotherapy and those who underwent surgery without perioperative chemotherapy. *Non–triple positive,* Negative for 1 or 2 neuroendocrine markers (synaptophysin, chromogranin A, and neural cell adhesion molecule); *Triple positive,* positive for synaptophysin, chromogranin A, and neural cell adhesion molecule.

TABLE 4. Multivariate analysis of prognostic factors and survival (Cox proportional hazards model)

Variable	HR	95% CI	*P* value
Age (<75 vs ≥75 y)	1.030	0.466–2.279	.9409
Gender (male vs female)	1.091	0.400–2.967	.8659
Pathologic stage (I vs II/III)	0.645	0.286–1.455	.2904
Surgical procedure (lobectomy vs sublobar resection)	1.048	0.287–3.824	.9431
Treatment (surgery with chemotherapy vs surgery alone)	0.323	0.112–0.934	.0371

HR, Hazard ratio; *CI*, confidence interval.

performed using 5 clinical prognostic factors (age, gender, pathologic stage, surgical procedure, and surgery with or without chemotherapy; Table 4). Patients who underwent surgery with chemotherapy had a significantly better prognosis than those who underwent surgery without chemotherapy (hazards ratio, 0.323; 95% confidence interval, 0.112–0.934; $P = .0371$).

Next, we examined whether the clinical outcome of patients with LCNEC correlated with the immunohistochemical characteristics determined by immunoreactivity for 3 neuroendocrine markers. No significant difference was found in 5-year survival between the triple-positive and non–triple-positive patients (34.0% and 55.3%, respectively; $P = .1312$; Figure 1, *C*). No statistically significant difference was found in survival among the single-positive, double-positive, and triple-positive patients (data not shown). Among the patients who received perioperative chemotherapy, a significantly greater 5-year survival rate was observed in the non–triple-positive group than in the triple-positive group (100% and 51.9%, respectively; $P = .0216$; Figure 1, *D*). Moreover, in the non–triple-positive group, a significantly greater survival rate at 5 years was observed in patients who underwent surgery with adjuvant chemotherapy than in those who underwent surgery without chemotherapy (100% and 34.5%, respectively; $P = .0081$; Figure 1, *E*). In contrast, in the triple-positive group, no difference was found in 5-year survival between the patients who underwent surgery with adjuvant chemotherapy and those who underwent surgery without chemotherapy (51.8% and 28.1%, respectively; $P = .7682$; Figure 1, *F*).

We further analyzed the correlation of chemotherapy benefits and immunoreactivity patterns of neuroendocrine markers in patients with different stages. The patients with stage I and stage II/III did not differ in overall survival in the non–triple-positive group (53.2% and 56.3%, respectively; $P = .8910$; Figure 2, *A*). Survival differences were also not found in the triple-positive group between stage I and stage II/III (36.8% and 28.2%, respectively; $P = .6460$; Figure 2, *B*).

Because a limited number of patients with stage I disease received perioperative chemotherapy, we failed to show a statistically significant survival difference between the patients with and without chemotherapy in the non–triple-positive patients (100% and 40.6%, respectively; $P = .2002$; Figure 2, *C*) and the triple-positive patients (80% and 25.2%, respectively; $P = .2606$; Figure 2, *D*). However, perioperative chemotherapy resulted in a significantly greater 5-year survival rate in the non–triple-positive group patients with stage II/III than in the triple-positive group (100% and 17.9%, respectively; $P = .0074$; Figure 2, *E*). No correlation was found between the use of perioperative chemotherapy and the survival of patients with stage II/III disease in the triple-positive group. In the group of patients with triple-positive tumors, the 5-year survival rate of the patients with chemotherapy and without chemotherapy was 34.3% and 33.3%, respectively ($P = .6108$; Figure 2, *F*).

DISCUSSION

Neuroendocrine lung tumors comprise a spectrum of epithelial neoplasms ranging from low-grade carcinoid tumor to SCLC. Although most SCLCs show neuroendocrine differentiation on immunohistochemistry or electron microscopy,[13] a significant minority of NSCLCs (approximately 10%–30%) show neuroendocrine differentiation. NSCLCs with neuroendocrine differentiation are considered to result in an especially poor prognosis. Several reports have indicated that NSCLCs with neuroendocrine differentiation were clinically aggressive with greater chemosensitivity; however, other studies have not shown any correlation.[8,14] A 5-year survival rate of 15% to 57% has been reported for all stages of LCNEC.[10,11] Sarkaria and colleagues[11] reported a 5-year survival rate of 37% for patients with stage IB-IIIA LCNEC who did not receive perioperative platinum-based chemotherapy compared with 51% in those patients who received it. Saji and colleagues[10] reported that the 5-year survival rate for patients undergoing perioperative chemotherapy was 87.5% and that of patients without perioperative chemotherapy was 58.5%.[10] Our results were similar.

Thus, we assumed that pulmonary LCNEC might have several features that make it sensitive to chemotherapy and tried to evaluate the association between the 3 neuroendocrine markers that are essential for the diagnosis of LCNEC and the responsiveness to chemotherapy. Positive immunostaining for 1 or more neuroendocrine markers among synaptophysin, chromogranin A, and NCAM is necessary to diagnose pulmonary LCNEC. Synaptophysin is a synaptic vesicle glycoprotein with 4 transmembrane domains; however, its exact function is unknown.[15] Chromogranin A is the major member of the granin family of acidic secretory glycoproteins and plays multiple roles in the secretory process.[16] NCAM, a glycoprotein, is a member of the immunoglobulin superfamily and contributes to the function of cell–cell adhesion.[17] Although all 3 markers are present in neuroendocrine cells, it remains possible

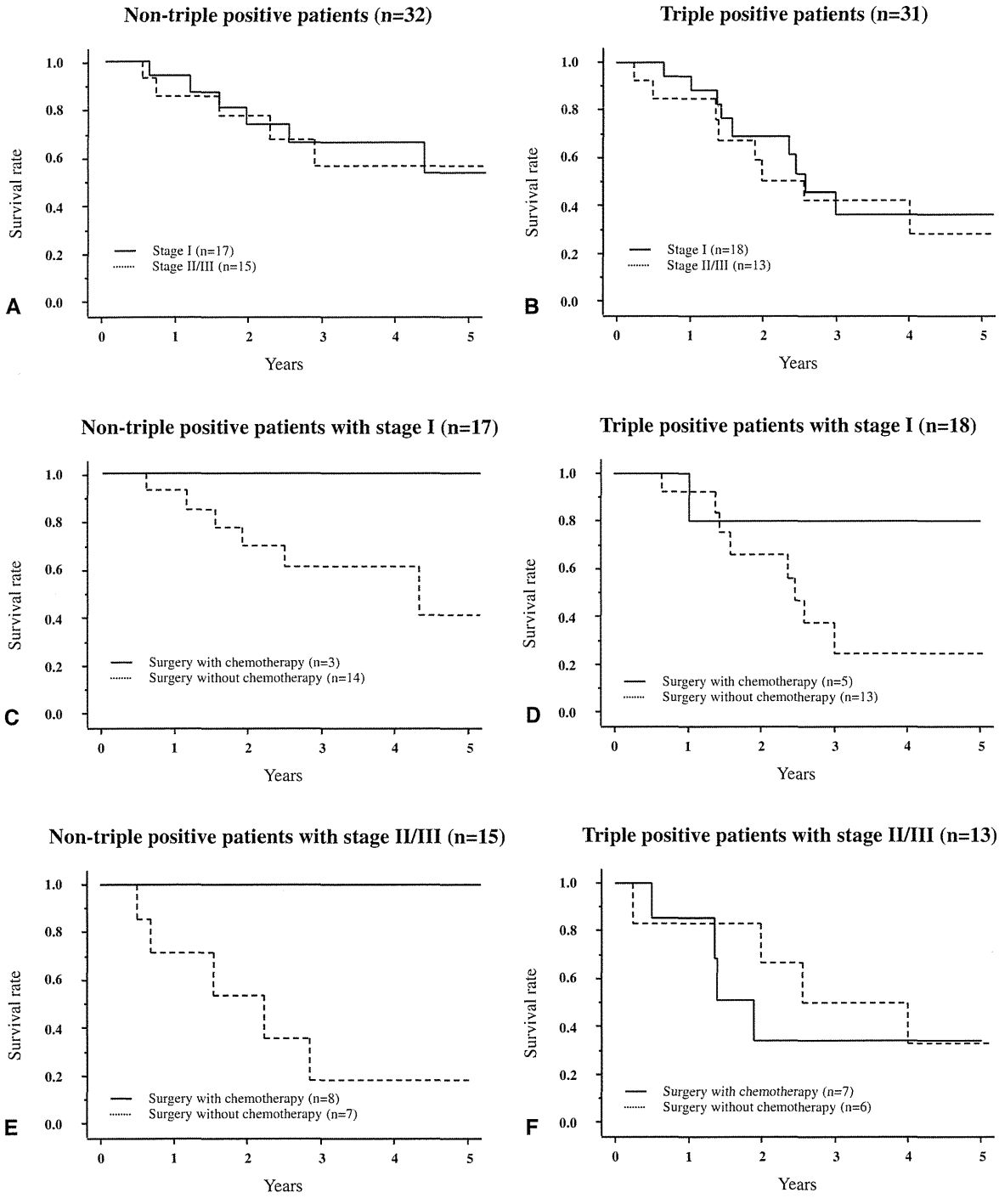

FIGURE 2. A, Comparison of stage-specific survival of patients with non–triple-positive large cell neuroendocrine carcinoma. B, Comparison of stage-specific survival of patients with triple-positive large cell neuroendocrine carcinoma. C, Comparison of survival of patients with stage I nontriple-positive disease who underwent surgery with perioperative chemotherapy and those who underwent surgery without perioperative chemotherapy. D, Comparison of survival of patients with stage I triple-positive who underwent surgery with perioperative chemotherapy and those who underwent surgery without perioperative chemotherapy. E, Comparison of survival of patients with stage II/III non–triple-positive disease who underwent surgery with perioperative chemotherapy and those who underwent surgery without perioperative chemotherapy. F, Comparison of survival of patients with stage II/III triple-positive disease who underwent surgery with perioperative chemotherapy and those who underwent surgery without perioperative chemotherapy. *Non–triple positive,* Negative for 1 or 2 neuroendocrine markers (synaptophysin, chromogranin A, and neural cell adhesion molecule); *Triple positive,* positive for synaptophysin, chromogranin A, and neural cell adhesion molecule.

that LCNEC does not have all 3 proteins. It was reported that neuroendocrine markers are often negative in poorly differentiated neuroendocrine cancers,[18] and it is estimated that LCNEC in the non–triple-positive group tended to be poorly differentiated and associated with a poor prognosis. Our results have demonstrated that the 5-year survival rate of patients who did not undergo perioperative chemotherapy in the non–triple-positive group was 34.5%. The addition of perioperative chemotherapy improved the prognosis of LCNEC in the non–triple-positive group but did not improve the prognosis of LCNEC in the triple-positive group. We considered that LCNEC might become resistant to chemotherapy through coexistence and mutual interaction of synaptophysin, chromogranin A, and NCAM and might lose the ability to resist because of the deficiency of the mutual interaction, owing to a lack of any of the 3 proteins.

Various studies have analyzed LCNEC's prognostic factors using other immunohistochemical staining and gene expression profiles.[19-21] However, no report has more clearly demonstrated the sensitivity of pulmonary LCNEC to perioperative chemotherapy than has our study. Moreover, it is also considered valuable that we can describe this result with 3 well-known biomarkers that are necessary for the diagnosis of pulmonary LCNEC. Furthermore, it was reported that carcinoids that exhibit good prognosis have a low response rate to chemotherapy and SCLCs that show a poor prognosis have a high initial response rate to chemotherapy.[18,22] Considered with our results, it is likely that triple-positive LCNEC was rich in neuroendocrine character, similar to carcinoids, and the non–triple-positive LCNECs were poor in neuroendocrine character, similar to SCLCs.

In the present study, antibody staining was designated as negative when none of the tumor cells were stained and as positive when any degree of immunoreactivity was found. We determined the cutoff value using the following scoring system: 0, no positive cells; 1+, less than 10% of cells positive; 2+, 10% to 50% of cells positive; and 3+, more than 50% of cells positive. From this analysis, the optimal cutoff, defined as the value that best separated a poor prognostic group from a better prognostic group, was nonimmunoreactive (negative) vs immunoreactive (positive) for the neuroendocrine markers. The evaluation separating "positive" from "negative," without any counting of cells, was easily and quickly performed with high reproducibility, which could be an advantage in possible future use in the clinical setting.

At present, most LCNECs are diagnosed using surgically resected specimens and rarely using biopsy or cytology specimens. Almost all publications concerning resected LCNEC have been based on the retrospective analyses of surgical specimens.[23] We used postoperative specimens to diagnose pulmonary LCNEC and to categorize them as either triple positive or non–triple positive. However, it would be difficult to categorize LCNEC according to our criteria using small biopsy specimens or cytologic specimens because heterogeneity and focal and scattered positivity of immunostaining against the neuroendocrine markers are not unusual. Therefore, this method might not be applicable for neoadjuvant chemotherapy.

Regarding the perioperative chemotherapy regimens for pulmonary LCNEC, platinum-based regimens that include etoposide (VP-16) or irinotecan (CPT-11), which are standard for SCLC, are more effective than other platinum-based regimens for NSCLCs, because pulmonary LCNEC is genetically and immunohistochemically more similar to SCLC than to NSCLC.[10,24,25] In our study, 10 (43%) of the 23 patients underwent a platinum-based regimen that included VP-16 or CPT-11. In addition, 3 (27%) of 11 patients in the non–triple-positive group received a platinum-based regimen that included VP-16 or CPT-11, in contrast to 7 (58%) of 12 patients in the triple-positive group. We considered that our result (ie, the sensitivity of LCNEC to perioperative chemotherapy in the non–triple-positive group), was not affected by the regimen of chemotherapy that included VP-16 or CPT-11.

Evidence is increasing that surgical resection alone is insufficient as treatment of LCNEC, even for stage I disease, and perioperative platinum-based chemotherapy might provide a survival advantage for patients with stage I LCNEC.[9,10] Our results have demonstrated that patients with stage I LCNEC tended to benefit from perioperative chemotherapy, although we failed to demonstrate a significant difference because only a small number of patients with stage I received perioperative chemotherapy. In the patients with stage I, perioperative chemotherapy tended to be associated with longer survival in the non–triple-positive group, as well as in the triple-positive group.

Although we acknowledge our study's limitations (a small number of subjects and short-term follow-up), our results have demonstrated that perioperative chemotherapy can enhance survival for the patients in the non–triple-positive group, although no correlation was seen between chemotherapy and survival in the triple-positive group. We believe these preliminary results are a reasonable rationale for a larger study to determine the correlation between chemotherapy response and neuroendocrine immunoreactivity in patients with LCNEC.

CONCLUSIONS

Our results have suggested that perioperative chemotherapy can be an important therapeutic option in the treatment of pulmonary LCNEC, particularly in the non–triple-positive patients. In the future, prospective multi-institutional studies with larger sample sizes should be conducted to verify the validity of our findings. Continued studies, including molecular studies, are also important to further improve the treatment stratification of patients with LCNEC.

References

1. Travis WD, Linnoila RI, Tsokos MG, Hitchcock CL, Cutler GB Jr, Nieman L, et al. Neuroendocrine tumors of the lung with proposed criteria for large-cell neuroendocrine carcinoma: an ultrastructural, immunohistochemical, and flow cytometric study of 35 cases. *Am J Surg Pathol.* 1991;15:529-53.
2. Travis WD, Corrin B, Shimosato Y. Histological typing of lung and pleural tumors. In: World Health Organization International Histological Classification of Tumors. 3rd ed. Berlin: Springer-Verlag; 1999.
3. Cooper WA, Thourani VH, Gal AA, Lee RB, Mansour KA, Miller JI. The surgical spectrum of pulmonary neuroendocrine neoplasms. *Chest.* 2001;119:14-8.
4. Iyoda A, Hiroshima K, Toyozaki T, Haga Y, Fujisawa T, Ohwada H. Clinical characterization of pulmonary large cell neuroendocrine carcinoma and large cell carcinoma with neuroendocrine morphology. *Cancer.* 2001;91: 1992-2000.
5. Takei H, Asamura H, Maeshima A, Suzuki K, Kondo H, Niki T, et al. Large cell neuroendocrine carcinoma of the lung: a clinicopathologic study of eighty-seven cases. *J Thorac Cardiovasc Surg.* 2002;124:285-92.
6. Battafarano RJ, Fernandez FG, Ritter J, Meyers BF, Guthrie TJ, Cooper JD, et al. Large cell neuroendocrine carcinoma: an aggressive form of non-small cell lung cancer. *J Thorac Cardiovasc Surg.* 2005;130:166-72.
7. Asamura H, Kameya T, Matsuno Y, Noguchi M, Tada H, Ishikawa Y, et al. Neuroendocrine neoplasms of the lung: a prognostic spectrum. *J Clin Oncol.* 2006; 24:70-6.
8. Iyoda A, Hiroshima K, Toyozaki T, Haga Y, Baba M, Fujisawa T, et al. Adjuvant chemotherapy for large cell carcinoma with neuroendocrine features. *Cancer.* 2001;92:1108-12.
9. Iyoda A, Hiroshima K, Moriya Y, Takiguchi Y, Sekine Y, Shibuya K, et al. Prospective study of adjuvant chemotherapy for pulmonary large cell neuroendocrine carcinoma. *Ann Thorac Surg.* 2006;82:1802-7.
10. Saji H, Tsuboi M, Matsubayashi J, Miyajima K, Shimada Y, Imai K, et al. Clinical response of large cell neuroendocrine carcinoma of the lung to perioperative adjuvant chemotherapy. *Anticancer Drugs.* 2010;21:89-93.
11. Sarkaria IS, Iyoda A, Roh MS, Sica G, Kuk D, Sima CS, et al. Neoadjuvant and adjuvant chemotherapy in resected pulmonary large cell neuroendocrine carcinomas: a single institution experience. *Ann Thorac Surg.* 2011;92:1180-6.
12. Sobin L, Wittekind CH, eds. TNM classification of malignant tumours. 6th ed. New York: Wiley-Liss; 2002. p. 99-103.
13. Guinee DG Jr, Fishback NF, Koss MN, Abbondanzo SL, Travis WD. The spectrum of immunohistochemical staining of small-cell lung carcinoma in specimens from transbronchial and open-lung biopsies. *Am J Clin Pathol.* 1994;102:406-14.
14. González-Aragoneses F, Moreno-Mata N, Cebollero-Presmanes M, García-Yuste M, Cañizares-Carretero MA, Molins-López-Rodó L, et al. Prognostic significance of synaptophysin in stage I of squamous carcinoma and adenocarcinoma of the lung. *Cancer.* 2007;110:1776-81.
15. Leung ASY, Cooper K, Leung FJWM. Manual of Diagnostic Antibodies for Immunohistology. London; Greenwich Medical Media: 1999:307.
16. Gosney JR, Gosney MA, Lye M, Butt SA. Reliability of commercially available immsunocytochemical markers for identification of neuroendocrine differentiation in bronchoscopic biopsies of bronchial carcinoma. *Thorax.* 1995;50:116-20.
17. Ionescu DN, Treaba D, Gilks CB, Leung S, Renouf D, Laskin J, et al. Nonsmall cell lung carcinoma with neuroendocrine differentiation—an entity of no clinical or prognostic significance. *Am J Surg Pathol.* 2007;31:26-32.
18. Gustafsson BI, Kidd M, Chan A, Malfertheiner MV, Modlin IM. Bronchopulmonary neuroendocrine tumors. *Cancer.* 2008;113:5-21.
19. Faggiano A, Sabourin JC, Ducreux M, Lumbroso J, Duvillard P, Leboulleux S, et al. Pulmonary and extrapulmonary poorly differentiated large cell neuroendocrine carcinomas: diagnostic and prognostic features. *Cancer.* 2007;110:265-74.
20. Harada M, Yokose T, Yoshida J, Nishiwaki Y, Nagai K. Immunohistochemical neuroendocrine differentiation is an independent prognostic factor in surgically resected large cell carcinoma of the lung. *Lung Cancer.* 2002;38:177-84.
21. Beasley MB, Lantuejoul S, Abbondanzo S, Chu WS, Hasleton PS, Travis WD, et al. The P16/cyclin D1/Rb pathway in neuroendocrine tumors of the lung. *Hum Pathol.* 2003;34:136-42.
22. Turrisi AT III, Kim K, Blum R, Sause WT, Livingston RB, Komaki R, et al. Twice-daily compared with once-daily thoracic radiotherapy in limited small-cell lung cancer treated concurrently with cisplatin and etoposide. *N Engl J Med.* 1999;340:265-71.
23. Travis WD, Rush W, Flieder DB, Falk R, Fleming MV, Gal AA, et al. Survival analysis of 200 pulmonary neuroendocrine tumors with clarification of criteria for atypical carcinoid and its separation from typical carcinoid. *Am J Surg Pathol.* 1998;22:934-44.
24. Rossi G, Cavazza A, Marchioni A, Longo L, Migaldi M, Sartori G, et al. Role of chemotherapy and the receptor tyrosine kinases KIT, PDGFRalpha, PDGFRbeta, and Met in large-cell neuroendocrine carcinoma of the lung. *J Clin Oncol.* 2005; 23:8774-85.
25. Rusch VW, Klimstra DS, Venkatraman ES. Molecular markers help characterize neuroendocrine lung tumors. *Ann Thorac Surg.* 1996;62:798-809. 710.

000 Immunohistochemical studies of pulmonary large cell neuroendocrine carcinoma: A possible association between staining patterns with neuroendocrine markers and tumor response to chemotherapy

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Perioperative chemotherapy might play an important role in pulmonary LCNEC treatment. Immunohistochemical analyses revealed that patients with tumors that were negative for at least 1 of the markers, synaptophysin, chromogranin A, and neural cell adhesion molecule, might benefit more from chemotherapy than those with immunoreactivity for all 3 neuroendocrine markers.

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Psf3 is a prognostic biomarker in lung adenocarcinoma

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ABSTRACT

Psf3 is a member of the evolutionarily conserved heterotetrameric complex GINS (Go-Ichi-Ni-San), which consists of Sld5, Psf1, Psf2, and Psf3. Previous studies have suggested that some GINS complex members are upregulated in cancer, but the status of Psf3 expression in lung adenocarcinoma has not been investigated. The objective of the current study was to determine whether Psf3 plays a role in lung adenocarcinoma by investigating clinical samples. We investigated the status of Psf3 expression in cancer cells of 125 consecutive resected lung adenocarcinomas by immunohistochemistry. Increased Psf3 expression was observed in 27 (21.6%) of the 125 cases. Further, univariate analysis and log-rank test indicated a significant association between Psf3 expression and lower overall survival rate ($P = 0.0001$ and $P < 0.0001$, respectively). Multivariate analysis also indicated a statistically significant association between increased Psf3 expression and lower overall survival rate (hazard ratio, 5.2; $P = 0.0027$). In a subgroup analysis of only stage I patients, increased Psf3 expression was also significantly associated with a lower overall survival rate ($P = 0.0008$, log-rank test). Moreover, the Ki67 index level was higher in the Psf3-positive group than in the Psf3-low positive group ($P < 0.0001$, Mann–Whitney U-test). Our results indicated that Psf3 can serve as a prognostic biomarker in lung adenocarcinoma.

1. Introduction

Psf3 is a member of the evolutionarily conserved heterotetrameric complex GINS comprising Sld5, Psf1, Psf2, and Psf3. GINS was originally identified in *Saccharomyces cerevisiae*, and its *Xenopus laevis* homolog has been characterized in egg extracts [1–3]. In *Eukarya*, the GINS complex associates with the mini-chromosome maintenance (MCM) proteins Mcm2–7 and with Cdc45 to form the Cdc45, Mcm2–7, GINS (CMG) complex, which in turn regulates both the initiation and the progression of DNA replication [4–7]. The CMG complex constitutes the eukaryotic replicative DNA helicase and contributes to the recruitment of the replicative polymerases essential for the synthesis of leading and lagging strands [7–10]. While the GINS components that play a part in the initiation of DNA replication seem to have an important role in the accelerated DNA replication of cancer cells, the oncological significance of them is not yet clear.

Several recent reports have suggested that Psf1 is required for the acute proliferation of cells, particularly immature cells such as stem cells and progenitor cells and that this protein is useful in the successful detection of cancer stem cells [11–14]. Moreover, previous studies have suggested that some GINS complex members are upregulated in cancer, and some GINS components may be useful in the detection of cancer stem cells.

Although several studies have suggested that GINS components play a role in cancer [15–18], the expression status of these components in lung adenocarcinoma has not yet been examined. Therefore, we sought to evaluate the expression status of Psf3 by immunohistochemical examination of surgically resected samples of human primary lung adenocarcinoma tissue. We also investigated whether Psf3 expression in tumor tissues influenced the outcome of these patients.

2. Materials and methods

2.1. Patients

The study population comprised 125 consecutive patients (71 males, 54 females) who were examined and treated at Kobe

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Table 1
Association between increased expression of Psf3 and clinicopathological characteristics in 125 patients with lung adenocarcinoma.

Variable	Total	Psf3		*P*-value
		Low positive	Positive	
No. patients (%)	125	98	27	NA
Age in years, mean ± SD (range)	67.4 ± 8.8 (42–84)	67.6 ± 8.6 (42–84)	66.5 ± 9.6 (42–81)	0.573
Gender				
Male/female	71/54	55/43	16/11	0.770
T factor				
T1/T2/T3/T4	69/42/4/10	60/29/1/8	9/13/3/2	0.0077*
N factor				
N0/N1/N2/N3	87/13/24/1	76/8/14/0	11/5/10/1	0.0013*
M factor				
M0/M1	122/3	97/1	25/2	0.226
Stage				
I/II/III/IV	82/12/28/3	73/7/17/1	9/5/11/2	0.0004*
P factor				
0/1/2/3	86/19/12/8	72/17/7/2	14/2/5/6	0.0003*
PA invasion				
Negative/positive	101/24	82/16	19/8	0.120
PV invasion				
Negative/positive	74/51	64/34	10/17	0.008*
LY invasion				
Negative/positive	75/50	64/34	11/16	0.021*

LY, lymphatic duct; NA, not applicable; PA, pulmonary artery; PV, pulmonary vein.
* Significant *P*-value.

University Hospital between 2001 and 2004 for lung adenocarcinoma. All cases underwent complete resection in this study. Of the 125 patients, 55, 27, eight, four, 23, five and three had stage IA, IB, IIA, IIB, IIIA, IIIB and IV tumors, respectively (Table 1). Of the N2/N3 patients, three, one and eight patients received induction chemotherapy, radiation and chemoradiotherapy, respectively. Four patients were administered postoperative adjuvant chemotherapy. The study protocol was approved by the Regional Ethics Committee for Clinical Research of Kobe University, and the study was conducted according to the principles of the Declaration of Helsinki. All patients provided written informed consent. Details of the clinical and demographic information, prognostic factors, and disease progression were collected retrospectively.

2.2. Immunohistochemistry

Formalin-fixed, paraffin-embedded specimens were cut at the maximal area of tumor mass into 5-μm-thick slices, and the sections were deparaffinized with xylene and rehydrated with ethanol. For antigen retrieval, the specimens were placed in Dako REAL-Target Retrieval Solution (Dako, Glostrup, Denmark) at 98 °C for 20 min. Mouse anti-human Psf3 monoclonal antibodies (1:500; GeneStem Co., Ltd., Osaka, Japan) were used as the primary antibodies for the detection of Psf3. The Dako LSAB2 Universal (DAB) kit (Dako) was used for endogenous peroxidase blocking, treatment with a secondary antibody against anti-mouse and anti-rat immunoglobulin antibody, and the visualization of HRP. Hematoxylin staining was used as the counterstain. Photographs of the stained sections were obtained using a camera mounted on a Keyence BZ-8000 digital microscope (Keyence, Osaka, Japan).

2.3. Classification of immunohistochemical staining patterns

Immunochemically stained sections were classified by light microscopy. Because Psf3 is a nuclear protein, only sharply defined areas of HRP staining in the nuclei were judged as Psf3 staining. If HRP staining was observed in other structures, such as the cytoplasm, it was judged as background staining. Psf3 localized and functioned only in the nuclei in the previous reports and any specific staining of Psf3 in the cytoplasm was not detected in this study. This assessment method ensured objective and reproducible measurement. The ratio of the cells positive for nuclear staining in a given microscopic field (×200) was determined for each tissue sample, and the expression status was assessed on the basis of this ratio. The status of Psf3 expression as follows: if more than 50% of cancer cells in any microscopic field (×200) of tumor tissues showed nuclear staining, the tissues were considered Psf3 positive; if the ratio of positive nuclear staining was lower than 50% for all the examined microscopic fields, the tissue was deemed Psf3 low positive.

2.4. Statistical analysis

Associations between Psf3 expression on cancer cells and clinicopathological features were determined using the χ^2-test. Survival was examined using the Kaplan–Meier method, and the significance of the difference was evaluated by a log-rank test. Variable effects on survival time were investigated using Cox's regression model. Statistical analysis was performed using the software JMP version 8 (SAS Institute, Cary, NC, USA). A threshold level of 0.05 was set for statistical evaluation.

3. Results

3.1. Psf3 expression in cancer cells of human lung adenocarcinoma

The expression status of Psf3 was determined in 125 lung adenocarcinomas and the adjacent normal lung tissues by immunohistochemistry (IHC), with the use of anti-human Psf3 monoclonal antibodies. In normal lung tissues, Psf3 expression was not detected (Fig. 1A). In some tumor tissues, the nuclei of cancer cells were stained in a scattered pattern, and the ratio of the Psf3-positive cells was less than 10% (Fig. 1B). In contrast, some tissues showed stained nuclei clustered in some areas of tumor tissues, and the ratio of stained cells in such tissue samples was more than 80% (Fig. 1C). These tissue samples showing clustered nuclear staining were classified as Psf3 positive. Thus, we determined the status of Psf3 expression as follows: if more than 50% of cancer cells in any microscopic field (×200) of tumor tissues showed nuclear staining, the tissues were considered Psf3 positive; if the ratio of positive nuclear staining was lower than 50% for all the examined

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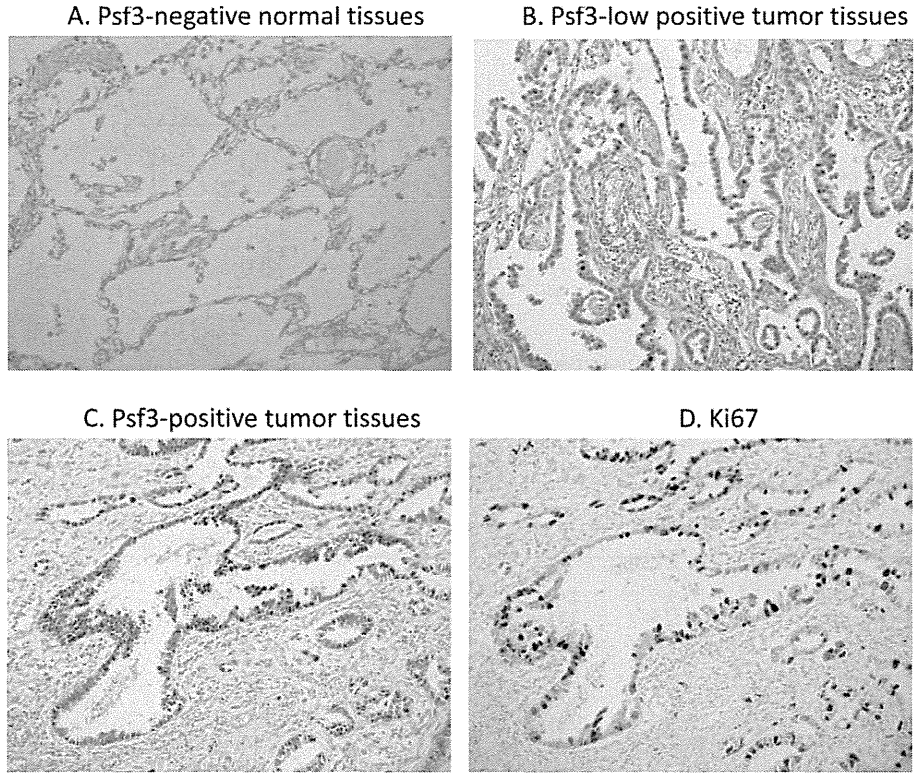

Fig. 1. An immunohistochemical analysis of the Psf3 expression status in cancer cells of human primary lung adenocarcinoma is illustrated. (A) Psf3-negative normal lung tissues. The staining of nuclei was not detected in epithelial or interstitial tissue. (B) Psf3-low positive tumor tissues. The nuclei of cancer cells were stained in a scattered pattern. The ratio of the positive cells was less than 10% (C) Psf3-positive tumor tissues. The stained nuclei were clustered at some regions of the tumor tissues. The ratio of the stained cells in these areas was more than 80%. (D) Immunohistochemical analysis of the expressions of Psf3 and Ki67 in serial sections. While almost all nuclei of the cancer cells were stained with the Psf3 antibody, Ki67 staining was observed in a scattered pattern in the same areas. Ki67 index; 40%.

microscopic fields, the tissue was deemed Psf3 low positive. Of the specimens examined, 98 (78.4%) were low positive for Psf3, while 27 (21.6%) were positive for Psf3 expression.

3.2. Relationship between Psf3 expression and clinicopathological characteristics of patients

In order to evaluate the role of Psf3 in lung adenocarcinoma, we investigated whether Psf3 expression was associated with any of clinicopathological variables in the 125 enrolled cases of primary lung adenocarcinoma (Table 1). The results of the analysis revealed that Psf3 expression was significantly associated with T factor ($P=0.0077$), TNM stage ($P=0.0004$), P factor ($P=0.0003$), lymph node metastasis ($P=0.02$), invasion of the pulmonary vein ($P=0.008$), and cancer cell invasion of the lymphatic ducts ($P=0.02$). No significant relationship was noted between Psf3 expression and age ($P=0.57$), gender ($P=0.77$), distant metastasis ($P=0.22$), and cancer spread to the pulmonary artery ($P=0.12$). These results suggest that increased Psf3 expression may enhance cancer cell proliferation and tumor progression, thereby resulting in the spread of cancer cells into the tumor vessels.

3.3. Increased expression of Psf3 was related to poor patient prognosis

Using the data collected from 125 study patients, we evaluated their prognosis and its relationship to the expression of Psf3. Follow-up data of all the 125 cases were available, for at least 5 years after surgery. We examined the overall survival (OS) of Psf3-low positive and Psf3-positive groups and found a statistically significant difference between the 2 groups by using the log-rank test ($P<0.0001$). The survival of Psf3-low positive patients was greater than that of the Psf3-positive patients (Fig. 2A). A univariate analysis indicated that among the clinicopathological factors, gender (male), tumor classification, lymph node metastasis, invasion of the pulmonary vein, and increased Psf3 expression correlated with the outcome (Table 2). Further assessment using the Cox multivariate analysis indicated that gender (male), lymph node metastasis, and increased Psf3 expression were statistically significant predictors for poor OS (Table 2).

3.4. Increased expression of Psf3 was also related to poor patient prognosis in stage I lung adenocarcinoma

In the current study, we analyzed the association of clustered Psf3 expression in stage I lung adenocarcinoma. Among the stage I cases, 9 (11.0%) and 73 (89.0%) patients were classified as Psf3 positive and Psf3 low positive, respectively (Table 1). A survival analysis that included only stage I patients revealed that the OS curve for the Psf3-positive group was lower than that for the Psf3-low positive group. The log-rank test showed that the intergroup difference was statistically significant ($P=0.0008$; Fig. 2B).

3.5. Relationship between Psf3 expression and Ki67 index

We examined the relationship between increased Psf3 expression and cancer cell proliferation. We used the Ki67 (MIB-1) expression index as an indicator of cell proliferation. In this study, the Ki67 index was calculated using the maximal section of the tumor mass. Using the Mann–Whitney *U*-test, the Ki67 index level

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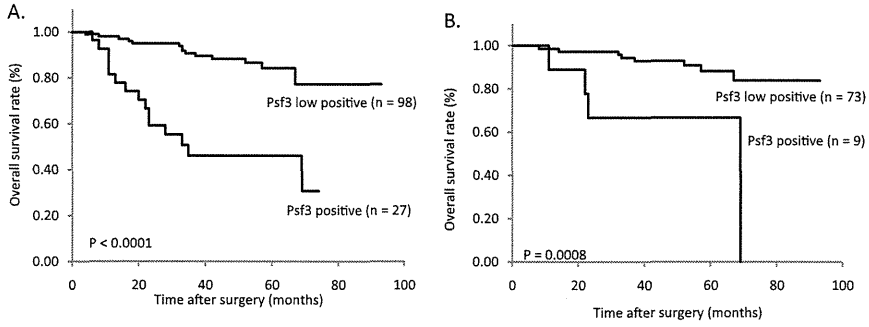

Fig. 2. (A) Kaplan–Meier plot of the overall survival rate in 125 patients with lung adenocarcinoma and its relationship to the Psf3 expression status. *P*-value was determined using the log-rank test. (B) Kaplan–Meier plot of the overall survival rate in 82 patients with lung adenocarcinoma and its relationship to the Psf3 expression status in stage I patients. *P*-value was determined using the log-rank test.

Table 2
Univariate and multivariate analyses of the association between the overall survival of 125 patients with lung adenocarcinoma and prognostic factors by Cox proportional hazard models.

Variable	Hazard ratio	95% confidence interval	*P*-value
Univariate			
Age	1.01	0.96–1.06	0.621
Gender (male versus female)	3.21	1.26–8.20	0.014*
T factor (T1<)	3.27	1.38–7.78	0.0071*
LN (negative versus positive)	5.62	2.32–13.5	0.0001*
PV invasion (negative versus positive)	3.40	1.44–8.00	0.0051*
Psf3 (low positive versus positive)	6.91	2.70–17.6	0.0001*
Multivariate			
Age	1.03	0.97–1.09	0.260
Gender(male versus female)	4.27	1.36–13.4	0.012*
T factor (T1<)	2.30	0.78–6.75	0.129
LN (negative versus positive)	4.08	1.32–12.5	0.014*
PV invasion (negative versus positive)	0.92	0.29–2.88	0.886
Psf3 (low positive versus positive)	5.22	1.77–15.3	0.0027*

LN, lymph node metastasis; PV, invasion of the pulmonary vein.
* Significant *P*-value.

was found to be higher in the Psf3-positive group than in the Psf3-low positive group. The median Ki67 index was 5% and 17% in the Psf3-low positive and Psf3-positive tumors, respectively (Fig. 3).

Additionally, immunochemical staining of Ki67 was also performed on serial sections that were used for Psf3 staining. The ratio of Ki67-positive cancer cells was found to be higher in areas where excessive staining of the nuclei of cancer cells was observed when tested with the Psf3 antibody (Fig. 1C and D). However, different staining patterns were observed for Ki67 and Psf3. While almost all nuclei of cancer cells were stained with the Psf3 antibody in the clustered area (Fig. 1C), Ki67 staining was observed in a scattered pattern, and the ratio of Ki67-positive nuclei was less than 50% (Fig. 1D).

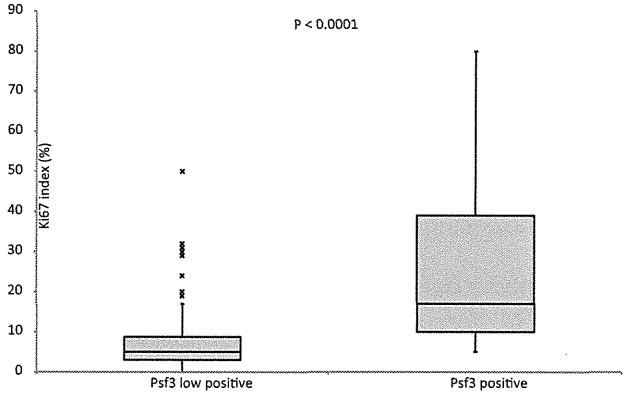

Fig. 3. Ki67 index (%) in lung adenocarcinoma samples and its relationship to the Psf3 expression status. *P*-value was determined using the Mann–Whitney *U*-test.

3.6. *Psf3: most powerful predictor of poor prognosis in lung adenocarcinoma patients*

We have previously performed IHC for 10 cancer-related proteins (CDC45, HIF1, sirt1, E-cadhelin, Nectin3, and the proteins listed in Table 3 other than Psf3) with the same paraffin-embedded specimens of the 125 cases investigated in this study [19,20] and examined the relationship between their expression in cancer cells and the prognosis of the patients. Univariate analysis revealed the significant association of 5 of the proteins with poor prognosis. To clarify the prognostic value of Psf3 expression in cancer cells, we statistically compared the expression levels of these 5 proteins and Psf3. Multivariate analysis revealed that Psf3 was the strongest predictor of poor prognosis (Table 3).

4. Discussion

Psf3 is a member of the GINS complex, along with Sld5, Psf1, and Psf2. Psf1 is tightly regulated at the transcriptional level in stem cells and enables the successful detection of cancer stem cells [11–14]. Therefore, it seems reasonable that other GINS components may also facilitate the detection of cancer stem cells in tumors. Cancer stem cells, which are resistant to anti-cancer drugs and irradiation, appear to be responsible for tumor growth in hematological and solid cancers. The detection of these cells is critical for identifying molecular targets to inhibit their growth. We conducted

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