Pleomorphic Lobular Carcinoma with Lipid-producing Activity: A Report of 2 Cases

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This case report presented 2 cases of pleomorphic lobular carcinoma (PLC) of the breast with lipid-producing activity, a type of cancer that has not been previously reported in the literature. The patients were 2 Japanese women aged 67 and 57 years, respectively, who presented with the chief complaint of an indolent tumor mass of the left breast. Both patients underwent breast conserving surgery and axillary sentinel node biopsy. Pathological analysis of breast tissue specimens revealed carcinoma cells, most of which contained abundant granular or foamy cytoplasm, enlarged round nuclei with prominent nucleoli, and intracytoplasmic lumens (ICLs). Digested periodic acid-Schiff (PAS) staining revealed diastase-resistant PAS staining in the ICLs, while Sudan III and oil -red -O staining revealed lipid granules in the cytoplasm. Immunohistochemically, the carcinoma cells from both cases tested negative for E-cadherin but positive for gross cystic disease fluid protein-15, cytokeratin, and mucin 1. Consideration of these findings led to a diagnosis of PLC with lipid-producing activity.

Key words: Breast cancer, Lipid-producing activity, Pleomorphic lobular carcinoma, Pleomorphic lobular carcinoma in situ

INTRODUCTION

Pleomorphic lobular carcinoma (PLC), a rare cancer of the breast affecting less than 1% of the female breast cancer population, is described as a variant of classic invasive lobular carcinoma (ILC) [1, 2]. Acceptance of PLC as a clinically important tumor subtype with aggressive behavior and relatively poor prognosis is increasing [3]. In this case report, we describe our recent diagnosis of 2 cases that demonstrated such heterogeneity. Although the detection of lipid granules in the cytoplasm by Sudan III staining initially led us to suspect lipid-rich carcinoma of the breast, further testing led to both cases as PLC with lipid -producing activity, which may be a new variant of PLC.

CASE REPORT

Case 1

Clinical presentation

A 67-year-old Japanese woman presented with the chief complaint of a lump in her left breast. On physical examination, the hard lump was 2.5cm in size with unclear margins. The results of all laboratory tests, including tumor markers, were normal. However, mammography (MMG) revealed segmental and pleomorphic microcalcification (Fig. 1A), and ultrasonography (US) detected a hypoechoic lesion measuring 3.9 cm in the upper outer quadrant of the left breast (Fig. 1B). The results of core needle biopsy led to the diagnosis of ductal components of carcinoma and suspicion of lipid-rich carcinoma or apocrine carcinoma. The patient underwent breast-conserving surgery (BCS) and axillary sentinel node biopsy (SLNB). After surgery, she received a total dose of 60-Gy radiation therapy for her left breast. She elected not to receive adjuvant therapy, and remained free from local recurrence or distant metastasis.

Pathological findings

Gross examination revealed a mass lesion without a clear border on the sections of the breast tissue specimen. Microscopically, the tumor was composed of clusters of dilated ducts filled with carcinoma cells, some of which exhibit comedo necrosis and calcification (Fig. 2A). Lumen structural formation was not observed, and loss of cohesion was prominent. Many of the carcinoma cells contained abundant and granular cytoplasm and enlarged round nuclei, prominent nucleoli, and focally intracytoplasmic lumens (ICL; Fig. 2B). Digested periodic acid-Schiff (PAS) staining revealed diastase-resistant PAS staining in the ICL (Fig. 2B). Sudan III and oil -red -O staining revealed the presence of lipid -secretory granules in the cytoplasm (Fig. 2C), a finding subsequently confirmed by electron microscopy (Fig. 2H). No invasive growth could be identified. Immunohistochemically, the carcinoma cells tested negative for E-cadherin (Fig. 2D) and positive for cytokeratin and gross cystic disease fluid protein-15 (GCDFP-15) (Fig. 2E) (Table 1). Consideration of these findings led to a final diagnosis of PLC in situ. Table 2 contains a summary of the histochemical and

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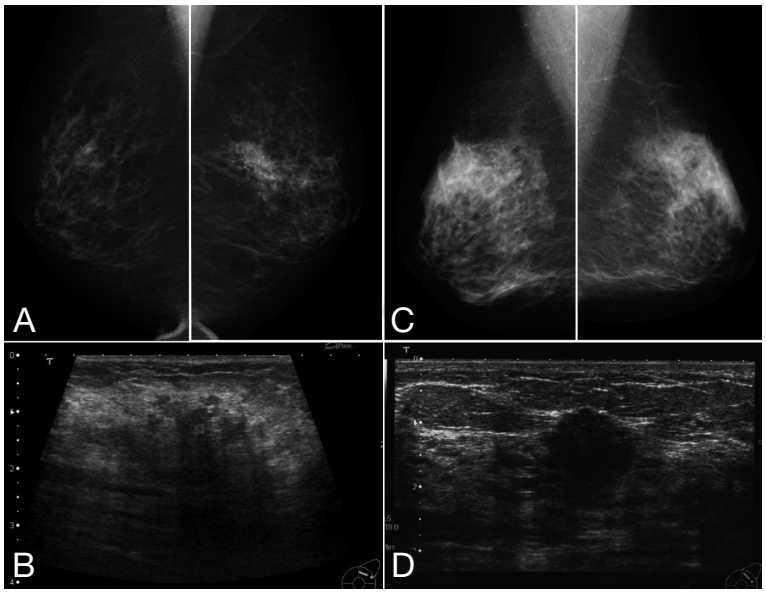

Fig. 1 Mammography (MMG) and ultrasonography (US) features of the 2 cases
A. MMG of case 1. In the mediolateral oblique (MLO) position, segmental and pleomorphic microcalcification is detected in the upper -outer area of the left breast. B. US of case 1 revealing a hypoechoic lesion, measuring 3.9 cm, in the upper -outer quadrant of the left breast. C. MMG of case 2. In the MLO position, an ill-defined mass with an irregular margin is detected in the upper -outer area of the left breast. D. US of case 2 revealing a hypoechoic lesion, measuring 2.0 × 1.3 cm, with an irregular shape and a heterogeneous internal echoic level.

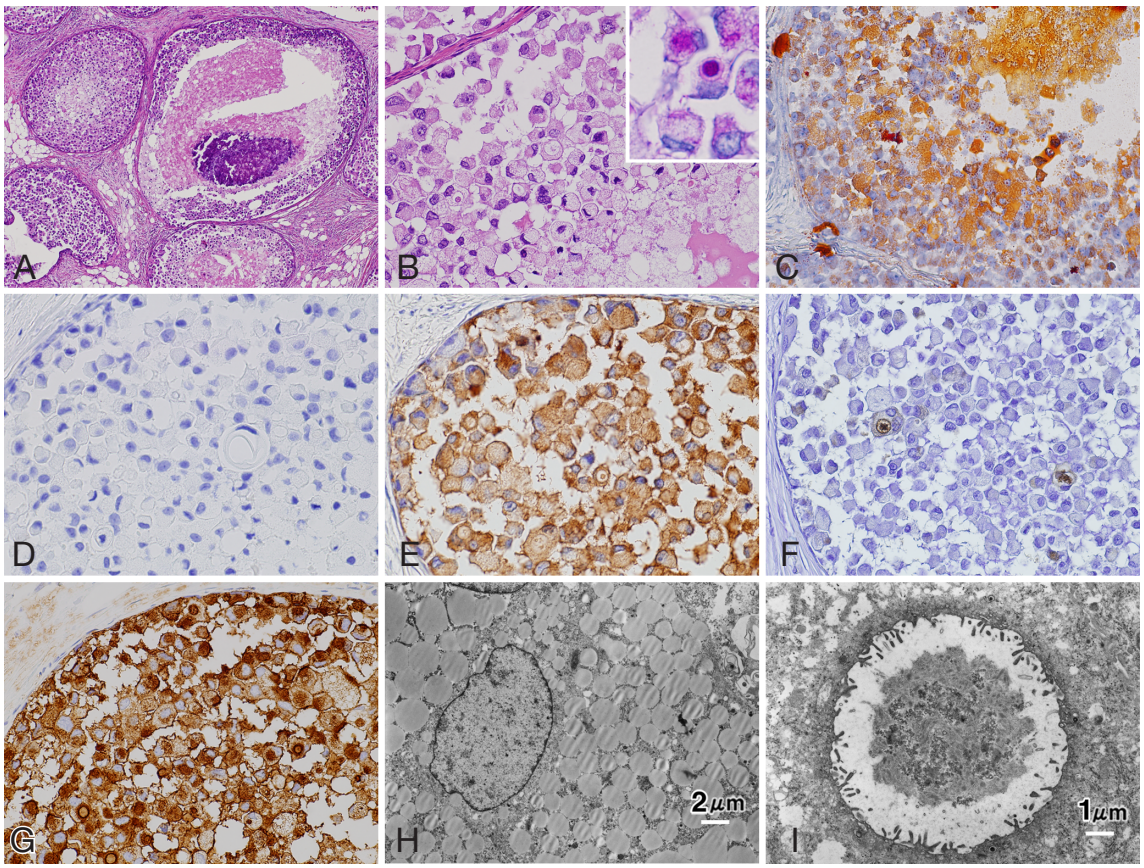

Fig. 2 Microscopic and electroscopic features of case 1
A. The tumor is composed of clusters of dilated ducts filled with carcinoma cells, some of which exhibit comedo necrosis and calcification. B. Under high-power view, lumen structural formation is not observed and the loss of cohesion is prominent. Many carcinoma cells contain abundant and granular cytoplasm with enlarged round nuclei and prominent nucleoli. Insert: Intracytoplasmic lumen (ICL). C. Sudan III staining indicates the presence of lipid granules in the cytoplasm. D. Immunohistochemical analysis indicates carcinoma cells negative for E-cadherin. E. Immunohistochemical analysis indicates carcinoma cells positive for gross cystic disease fluid protein-15 (GCDFP-15). F. Several ICLs test positive for lactoferrin. G. Carcinoma cells test strongly positive for mucin 1 (MUC1). H. Electron microscopy features of lipid-secretory granules. I. Electron microscopy displaying ICLs with micro-villous structures on the lumen side.

Table 1 Summary of antibodies used in immunohistochemical analysis

Antibodies	Clone	Method	Manufacturer	Dilution
ER	6F11	Ventana BenchMark	Roche Tissue Diagnostics	Undiluted
PgR	16	Ventana BenchMark	Roche Tissue Diagnostics	Undiluted
AR	2F12	Bond-maX	Leica	1: 20
HER2	Polyclonal	Dako Autostainer	Dako	Undiluted
E-Cadherin	36B5	Bond-maX	Leica	1: 40
GCDFP-15	D6	Bond-maX	COVANCE	1: 100
α-LA	Polyclonal	Dako Autostainer	Dako	1: 100
Lactoferrin	Polyclonal	Dako Autostainer	Dako	1: 100
MUC1	Ma695	Bond-maX	Leica	1: 50

ER: estrogen receptor; PgR: progesterone receptor; AR: androgen receptor; HER2: human epidermal growth factor receptor type 2; GCDFP-15: gross cystic disease fluid protein-15; α-LA: alpha-lactalbumin; MUC1: mucin 1
Dako Autostainer: Dako, Glostrup, Denmark;
Ventana BenchMark: Roche Tissue Diagnostics, Basel, Switzerland;
Bond-maX: Leica Biosystems Newcastle Ltd., Newcastle, United Kingdom

immunohistochemical study results.

Case 2

Clinical presentation

A 57-year-old Japanese woman consulted us after detecting an elastic, firm mass in the upper left lateral quadrant of her left breast approximately 2 cm in diameter. All laboratory test results were normal. However, MMG revealed an ill-defined mass with an irregular margin (Fig. 1C), while US revealed a hypoechoic lesion measuring 2.0 × 1.3 cm with an irregular shape and heterogeneous internal echoic level (Fig. 1D). The results of fine-needle aspiration cytology suggested a malignant tumor. The patient underwent BCS and SLNB, after which her left breast was treated with a total dose of 60-Gy radiation therapy. She began hormonal therapy with an aromatase inhibitor after surgery, and subsequently remained free from local recurrence or distant metastasis.

Pathological findings

Gross examination of the breast tissue specimen revealed an irregularly shaped, white tumor measuring approximately 1.8 × 1.5 cm. Microscopically, the tumor contained a diffuse proliferation of carcinoma cells, most of which contained round nuclei with prominent nucleoli and foamy and eosinophilic cytoplasm, accompanied by stromal fibrosis and invasion of carcinoma cells into the surrounding fat tissues (Fig. 3A). Lobular carcinoma in situ (LCIS) components were detected around the tumor. PAS staining indicated the presence of many diastase-resistant ICLs (Fig. 3C-2), while Sudan III staining indicated the presence of lipid -secretory granules in cells containing vacuolated cytoplasm (Fig. 3B). Immunohistochemical analysis (Table 2) indicated the absence of E-cadherin (Fig. 3D) and the presence of GCDFP-15 and cytokeratin (Fig. 3G) in the carcinoma cells. Consideration of these findings led to the final diagnosis of invasive pleomorphic carcinoma of the breast.

DISCUSSION

In 1982, PLC was initially described by Dixon *et al.* as a histologically distinct variant of ILC with apocrine differentiation [1]. Pleomorphic lobular carcinoma in situ (PLCIS) is an uncommon variant of LCIS first described by Frost *et al.* in 1996 [4]. The morphology of PLC and PLCIS has been described as that of large, discohesive cells containing eccentric nuclei and moderate to marked pleomorphism that exhibit either (1) abundant granular and eosinophilic cytoplasm or (2) foamy cytoplasm with a histiocytoid feature or ICL [5]. Immunohistochemically, PLC and PLCIS share the ILC characteristics of cell-membrane immune reactivity to E-cadherin, and are typically reactive to GCDFP-15 [2, 5]. The clinical presentation and mammographic abnormalities of PLCIS, including microcalcification, architectural distortion, and/or high density, are similar to those of ductal carcinoma in situ (DCIS) but differ from those of classic LCIS [6, 7].

The 2 cases described here shared the characteristics of PLC, i.e., loss of cell -membrane immune reactivity to E-cadherin and reactivity to GCDFP-15. Morphologically, the carcinoma cells of our 2 patients shared characteristics with the carcinoma cells of PLC cases, including enlarged, round nuclei with prominent nucleoli; abundant, eosinophilic, and foamy cytoplasm; and in some cells, ICLs that were detected by PAS staining. Furthermore, Sudan III and oil -red -O staining and electron microscopy revealed that the carcinoma cells possessed a lipid -secretory tendency. Immunohistochemically, our cases tested positive for alpha-lactalbumin or lactoferrin [8], which is also a characteristic of lipid-rich carcinoma. Although lipid-rich carcinoma cells also contain abundant and foamy cytoplasm, and most contain pleomorphic nuclei, they typically test positive for E-cadherin [9] and negative for GCDFP-15. Whereas Fisher *et al.* reported that cytoplasmic lipids may be observed in almost one-third of the well-recognized histologic types of breast cancers [10], the literature contains no descriptions of breast cancers exhibiting lipid -producing activity.

Differential breast cancer diagnoses other than PLC include apocrine carcinoma and glycogen-rich carcinoma [2, 3], which present with cells characterized by abundant foamy to granular cytoplasm. However, no breast carcinoma has been reported as exhibiting lipid -producing activity to date. Apocrine carcinoma, which also tests positive for GCDFP-15, is defined as a carcinoma demonstrating apocrine differentiation in

Table 2 Summary of histochemical and immunohistochemical analysis results

	Classical LC	Pleomorphic LC	Lipid-rich carcinoma	Case 1 (PLCIS)	Case 2 (PLC)
PAS	+ / −	+ / −	−	+	+
d-PAS	+ / −	+ / −	−	+	+
Oil- red- O	***	***	+	+	+
ER	+	+ / −	−	−	−
PgR	+	+ / −	−	−	−
AR		+		+	+
HER2	***	***	***	2+ (FISH −)	−
E-Cadherin	−	−	+	−	−
GCDFP-15	−	+	−	+	+
α-LA	***	***	+	−	+
Lactoferrin	***	***	+	+	−
MUC1	+ / −	+ / −	***	+	+

***: Unknown; LC: lobular carcinoma; PLCIS: pleomorphic lobular carcinoma in situ; PLC: pleomorphic lobular carcinoma; PAS: periodic acid-Schiff testing; ER: estrogen receptor; PgR: progesterone receptor; AR: androgen receptor; HER2: human epidermal growth factor receptor type 2; FISH: fluorescent in situ hybridization; GCDFP-15: gross cystic disease fluid protein-15; α -LA: alpha-lactalbumin; MUC1: mucin 1

more than 90% of tumor cells. Although glycogen-rich carcinomas contain PAS-positive granular cytoplasm, the PAS-positive granules are digested by diastase. Histologically, PLCIS has been misdiagnosed as high nuclear-grade DCIS associated with pleomorphic nuclei, comedo necrosis, and calcification [3]. Detection of loss of E-cadherin cell-membrane immune reactivity assists in differentiating LCIS from DCIS.

Regarding genomic subtype, classic ILC and pure PLC are generally categorized into the "luminal" tumor subgroup [2, 11], but PLC cases tend to exhibit fewer estrogen/progesterone receptors (ER/PgR) and a greater number of human epidermal growth factor type 2 receptors (HER2/neu) [12, 13]. When compared to classic cases of LCIS, cases of PLCIS exhibit lower levels of ER/PgR expression [14, 15]. A recent gene- expression profiling study categorized 3 of 4 cases of PLC as the "molecular apocrine type" [16]. Our cases tested negative for ER/PgR and HER2 but, as do most cases of PLC [3] and apocrine carcinoma [17], tested positive for androgen receptors (AR).

Based on the results of their studies, Eusebi *et al.* [5] and Weidner and Semple [18] described PLC as an aggressive subtype of ILC. However, we found that there was less than 10% Ki67 in our 2 cases, indicating that they are not aggressive subtypes of ILC. Interestingly, the carcinoma cells in our cases tested strongly positive for mucin 1 (MUC1), for which the signet ring cells of ILC cases also test positive [3]. A gene encoding a membrane-bound protein that is a member of the mucin family, MUC1, is associated with better prognosis, especially in early-stage breast cancer [19].

An extensive review of the literature indicates that our description of the 2 cases of PLC with lipid -producing activity is the first description of such a diagnosis. We attribute this to the rarity of PLC as a type of breast cancer and to the difficulty of detecting a lipid -producing activity, which requires performing the histochemical method of intracellular -lipid identification on non-paraffin embedded frozen tissue sections. As demonstrated by our exhaustive testing and investigation, the application of extra effort in testing may yield surprising results.

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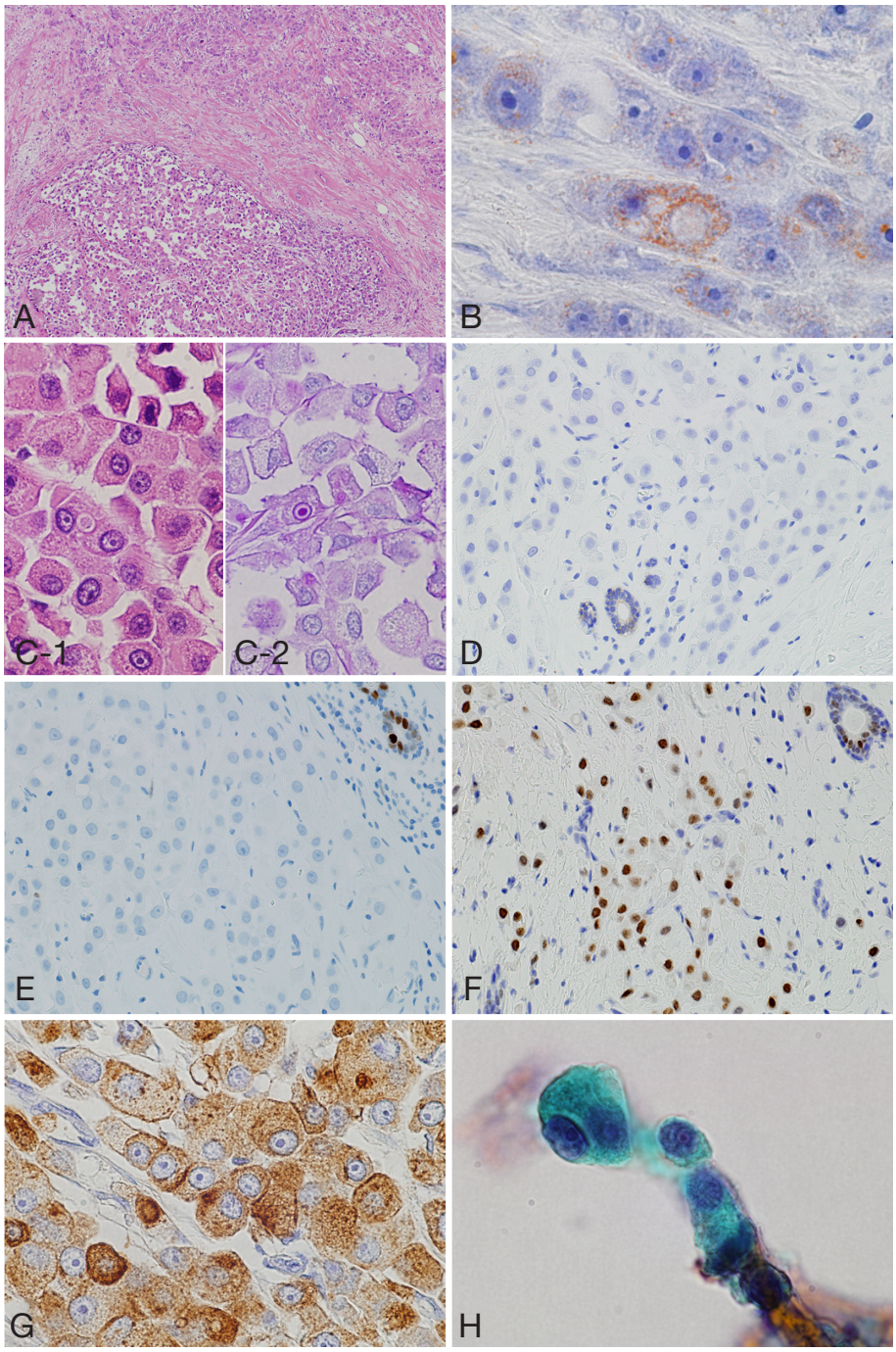

Fig. 3 Histological and cytological features of case 2
A. The tumor exhibiting diffuse proliferation of carcinoma cells accompanied by stromal fibrosis in the upper right section and lobular carcinoma in-situ components in the lower left section. B. Sudan III staining indicates the presence of lipid granules in cells containing vacuolated cytoplasm. C-1. Most carcinoma cells contain round nuclei with prominent nucleoli and granular and eosinophilic cytoplasm. C-2. Periodic acid-Schiff (PAS) staining indicates the presence of ICLs. D. Immunohistochemical analysis indicates absence of E-cadherin reactivity in carcinoma cells while normal ductal epithelial cells are present. E. Carcinoma cells testing negative for estrogen receptors. F. Carcinoma cell nuclei testing positive for androgen receptors. G. Carcinoma cells testing positive for GCDFP-15. H. The cytological features of carcinoma cells include round nuclei with prominent nucleoli. An ICL is identified by Papanicolaou staining.

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