Clinicopathologic and Molecular Characteristics of and Diagnostic Dilemmas in Invasive Breast Carcinoma with Choriocarcinomatous Pattern apropos a New Case: A Literature Review with New Findings

Sun-Young Jun\textsuperscript{a} Nara Yoon\textsuperscript{a} Soyeon An\textsuperscript{a} Young-Joon Kang\textsuperscript{b} Chang Suk Park\textsuperscript{c}

\textsuperscript{a}Department of Pathology, Incheon St. Mary’s Hospital, College of Medicine, The Catholic University of Korea, Incheon, Republic of Korea; \textsuperscript{b}Department of Surgery, Incheon St. Mary’s Hospital, College of Medicine, The Catholic University of Korea, Incheon, Republic of Korea; \textsuperscript{c}Department of Radiology, Incheon St. Mary’s Hospital, College of Medicine, The Catholic University of Korea, Incheon, Republic of Korea

\textbf{Keywords}
Breast cancer · Invasive breast cancer with choriocarcinomatous pattern · Human chorionic gonadotropin · Sequencing · TP53 · PTEN

\textbf{Abstract}

\textbf{Background}: Invasive breast carcinoma with a choriocarcinomatous pattern (IBC-CP) is extremely rare, and its molecular basis is yet unclear. The choriocarcinomatous pattern is characterized by the biphasic arrangement of multinucleated syncytiotrophoblast-like cells around clusters of monotypic tumor cells in a hemorrhagic background, along with \(\beta\)-human chorionic gonadotropin (\(\beta\)-hCG) expression. The differentiation of IBC-CP from metastatic choriocarcinoma of the breast (MC-B) is difficult due to the histologic similarity.

\textbf{Methods}: Based on a literature review and our own case, the clinicopathologic differences between IBC-CP patients \((n = 17)\) and MC-B patients \((n = 8)\) were analyzed. Moreover, in our case of IBC-CP, next-generation sequencing (NGS) comparative analysis was conducted for both choriocarcinomatous and invasive breast carcinoma (IBC) components. \textbf{Results}: Compared to the MC-B patients, the IBC-CP patients were older \((p < 0.001)\) and less frequently had past histories of gestational trophoblastic disease/pregnancy-abortion \((p = 0.001)\) and distant metastases \((p = 0.005)\). Our case, a 49-year-old female patient, presented with masses in the right breast and axilla. Following neoadjuvant chemotherapy, a radical mastectomy found an 8.5-cm-sized tumor. Microscopically, multinucleated syncytiotrophoblast-like cells were observed around mononuclear tumor cells with hemorrhage and necrosis. Some tumor cells showed \(\beta\)-hCG immunopositivity, which was compatible with IBC-CP. NGS results showed a missense mutation in exon 5 of the \(TP53\) gene in both the choriocarcinomatous and IBC components. Meanwhile, copy number loss in the \(PTEN\) gene was only identified in the choriocarcinomatous components. \textbf{Conclusion}: The present IBC-CP case is triple-negative breast cancer with \(TP53\) mutation. The \(PTEN\) gene may be associated with choriocarcinomatous differentiation. Obtaining a medical history is mandatory to exclude metastatic lesions.

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Sun-Young Jun and Nara Yoon contributed equally to this work as first authors.
Introduction

Choriocarcinoma, a highly aggressive neoplasm with trophoblastic differentiation, is typically gestational in origin [1]. Nongestational choriocarcinoma of germ cell origin rarely develops at a young age [2]. Choriocarcinoma characteristically shows a biphasic pattern of multinucleated syncytiotrophoblasts around a sheet-like arrangement of mononuclear trophoblasts in an extensive hemorrhagic and necrotic background. In addition, multinucleated and mononuclear trophoblasts usually express β-human chorionic gonadotropin (β-hCG). This choriocarcinomatous feature has been found alone or in association with carcinomas of the stomach, colon, and urinary bladder [1]. The choriocarcinomatous pattern has rarely been reported in breast tumors.

There are two distinct terms in the literature used to refer to a choriocarcinomatous pattern in the breast: primary invasive breast carcinoma with a choriocarcinomatous pattern (IBC-CP) [3–13] and metastatic choriocarcinoma to the breast (MC-B) (Table 1) [14–21]. All MC-B in the literature were of gestational choriocarcinoma origin (Table 1). The recommended treatment for patients with IBC-CP is surgical resection, and the surgical management is the same as that used for other primary breast cancers. The chemotherapy regimen for IBC-CP patients remains uncertain; previous reports have demonstrated poor responses to chemotherapy [22]. In contrast, choriocarcinoma is aggressive and metastasizes widely, but responds well to chemotherapy [22]. Therefore, it is important to properly distinguish between the two diseases so that the appropriate treatment protocols can be initiated immediately, since surgery can be avoided in MC-B patients if it is correctly recognized. Because metastatic choriocarcinomas rarely occur in patients after the local management of gestational trophoblastic disease (GTD; hydatidiform moles or choriocarcinoma), term pregnancies, or abortions, obtaining a patient’s history can help in diagnosing a metastasis [1]. Meanwhile, the presence of ductal carcinoma in situ (DCIS) or invasive breast carcinoma (IBC) is more likely to suggest primary breast carcinoma [15]. Since Saigo and Rosen [13] described the first IBC-CP case in 1981, 16 cases of IBC-CP have been reported [3–12]. Several theories have been proposed in attempts to explain the unusual choriocarcinomatous differentiation in breast tumors, one of which partially or completely refers to choriocarcinoma resulting from a metaplastic process within the primary tumor [13]. However, the origin and molecular basis of IBC-CP remain unclear. Xing et al. [1] recently revealed that TP53 was the only mutated gene in nongestational choriocarcinomas, including 1 case of endometrial adenocarcinoma with a choriocarcinomatous pattern and 1 case of pure choriocarcinoma of pararectal soft tissue. Specifically, in endometrial adenocarcinoma with a choriocarcinomatous pattern, the TP53 mutation was detected in the choriocarcinomatous component but not in the adenocarcinoma component [1]. It is therefore necessary to study whether there is a genetic difference between the choriocarcinomatous and IBC components in IBC-CP.

In this study, we report an IBC-CP case in which next-generation sequencing (NGS) comparative analysis was performed for both the choriocarcinomatous and IBC components. We also analyze previous reports presenting choriocarcinomatous components in the breast to differentiate between IBC-CP and MC-B.

Materials and Methods

Search Strategy and Data Extraction
Several databases (PubMed, Google Scholar, and Embase) were searched through May 2021 for reports on the subjects of “breast carcinoma with choriocarcinomatous,” “choriocarcinoma of breast,” “breast cancer and choriocarcinoma,” and “metastatic choriocarcinoma and breast.” The eligible studies were limited to full-text publications in English. The reference lists of the included articles were also reviewed. Reports published prior to 1981 were excluded because IBC-CP was first described in 1981.

The relevant clinicopathologic data were extracted from each manuscript. The clinical data included the patient’s age and sex, presenting symptoms, previous medical history, serum β-hCG level, treatment including adjuvant chemotherapy and radiotherapy, recurrence or metastasis, follow-up, and survival. The pathologic data included the location and size of the tumor, presence of IBC and/or DCIS, histological type of IBC, Ki-67 labeling index, estrogen receptor (ER) and progesterone receptor (PR) status, human epidermal growth factor receptor 2 (HER2) status, and β-hCG immunoreactivity.

Statistical Analysis
All statistical analyses were performed using SPSS software (version 17.0; SPSS Inc., Chicago, IL, USA). The means of the two groups were compared using the Mann-Whitney test. The categorical variables were compared using the χ² and Fisher exact tests. The survival rate was investigated based on the descriptions in the reports using the Kaplan-Meier method. p values of <0.05 were considered to represent statistically significant differences.

Immunohistochemical and Molecular Analyses
Immunohistochemical staining was performed using a Ventana BenchMark XT automated slide stainer (Ventana Medical Systems, Tucson, AZ, USA) and a DAKO Omnis automated stainer (Agilent Technologies, Glostrup, Denmark) according to the
### Table 1. Previous case reports presenting CCs in the breast

| Reference          | Age, yr | Location  | Size, cm | LN metastasis | Serum β-hCG level | Distant metastasis at presentation | Treatment | IBC or DCIS | Immunohistochemistry | Survival, mo | Past Hx of associated tumors |
|--------------------|---------|-----------|----------|---------------|-------------------|-------------------------------------|-----------|-------------|---------------------|--------------|--------------------------|
| **Primary IBC-CP** |         |           |          |               |                   |                                     |           |             |                     |              |                          |
| Present case       | 49      | Rt        | 8.5      | 2/28          | NS                | Lung later                          | NeoCTx + MRM | IBC         | All (−)             | 96.9         | + in CC                  | AWD (12) No  |
| Zhu et al. [3]     | 32      | Lt        | 3.2      | NS            | ↑                 | Kidney\(^{g}\) and lung\(^{h}\)  | Ex + CTx\(^{e}\) | No          | All (−)             | 75.0         | + in CC                  | NED (37) NS  |
| Sung et al. [4]    | 56      | ROUQ      | 2.4      | 0/4           | No                | MRM                                 | MRM        | IBC         | All (−)             | 50.0         | + in CC                  | NED (NS) No  |
| Canbay et al. [5]  | 31      | Lt        | NS       | Yes           | 0                 | NeoCTx\(^{g}\) + MRM               | No         | IBC         | All (−)             | NS           | +                        | NS No         |
| Akbulut et al. [6] | 53      | Lt        | 3.5      | 0/10          | NS                | No                                   | PM         | IBC         | ER−/PR−/HER2+        | 80.0         | + in CC                  | NED (72) NS  |
| Akbulut et al. [6] | 50      | Rt        | 4.0      | 0/19          | NS                | No                                   | MRM        | IBC         | All (−)             | 80.0         | + in CC                  | NED (48) NS  |
| Siddiquei et al. [7]| 56        | Rt        | 3.5      | No            | No                | No                                   | PM         | IBC/DCIS    | ER+/PR+             | High         | + in CC                  | NS Hx of osteosarcoma |
| Resetkova et al. [8]| 38        | Rt        | 1.0      | No            | ↑                 | No                                   | Ex + CTx   | Medullary   | All (−)             | NS           | + in CC/IHC              | NED (12) In pregnancy |
| Resetkova et al. [8]| 54        | Rt        | 10.0     | No            | ↑                 | Lungs, neck, and pelvis later        | NeoCTx + MRM + CTx + Metaplastic/DCIS | All (−)    | NS         | + in CC/IHC           | DOD (6) Hx of endometrial cancer |
| Erhan et al. [9]   | 59      | Rt        | 2.5      | 4/19          | NS                | No                                   | PM + CTx + RTx | No          | ER−/PR−/HER2+        | 17.0         | + in CC                  | NS NS         |
| Erhan et al. [9]   | 48      | Rt        | 2.5      | 0/16          | NS                | No                                   | MRM + CTx + RTx | IBC         | ER−/PR−/HER2+        | 40.0         | + in CC                  | NED (24) NS  |
| Erhan et al. [9]   | 58      | Rt        | 4.0      | 12/19         | NS                | No                                   | MRM + CTx + RTx | DCIS        | ER−/PR−/HER2+        | 10.0         | + in CC                  | NED (48) NS  |
| Erhan et al. [9]   | 49      | Rt        | 1.6      | 0/20          | NS                | No                                   | Ex         | DCIS        | All (−)             | 2.0          | + in CC                  | NS NS         |
| Giannotti Filho et al. [10] | 50 | ROUQ | 7.0 | 0/20 | NS | No | PM | IBC | ER+/PR− | NS | + in CC | NS NS |
| Murata et al. [11] | 38      | ROUQ      | 5.0      | 44/44         | NS                | Lungs, chest wall, and liver later   | M + CTx\(^{e}\) + RTx + H | IBC         | ER−      | NS | + in CC/IHC | DOD (7) Hx of ovarian teratoma |
| Green [12]         | 71      | Rt        | 2.5      | 20/21         | NS                | NS                                   | M + H      | Mucinous    | NS     | NS | + in CC | NED (4) NS |
| Saigo and Rosen [13]| 55        | Lt        | 2.5      | No            | NS                | Lung\(^{h}\) and multiple LN      | MRM        | IBC/DCIS    | NS    | NS | + in CC | DOD (7) Hx of medullary carcinoma of breast\(^{h}\) |
| **MC-B**           |         |           |          |               |                   |                                     |           |             |                     |              |                          |
| Khanna and Singh [14]| 27     | ROUQ | NS | NS | ↑ | Lungs | CTx\(^{d}\) | NS | NS | NS | NS | AWD (NS) With G-Choriocarcinoma and Hx of spontaneous abortion |
| Hemati et al. [15] | 41      | Both, multiple | 3.0 | NS | ↑ | Lungs, liver, kidneys | Ex + CTx\(^{e}\) | No | NS | NS | + | DOD (1) Hx of H-mole |
| Kalra et al. [16]  | 27      | RILQ      | 6.5      | NS            | ↑                 | NS                                   | Bx         | NS          | NS          | NS | +                        | NS Hx of H-mole |
| Choi and Park [17] | 48      | LOUQ      | 1.0      | NS            | ↑                 | Lungs and nasal cavity               | CTx\(^{f}\) | NS          | NS          | NS | NS | AWD (7) Hx of H-mole |
| Fowler et al. [18] | 32      | ROLQ      | NS | NS | ↑ | Lungs, brain later | Bx + CTx\(^{f}\) + RTx | NS | NS | NS | NS | DOD (12) 6 weeks postpartum |
manufacturer’s protocols. Immunoreactivity was interpreted through light microscopic examination and evaluated by a pathologist (S.-Y.J.). All antibodies used in the present case are listed in Table 2.

To compare the molecular bases of the choriocarcinomatous lesion and IBC, NGS was conducted while utilizing the Oncomine Comprehensive Assay v1 Panel (OCP; Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer’s instructions [23]. The OCP included 143 genes in total, of which 73 oncogenes were interrogated for mutational hotspots and 26 tumor suppressor genes were interrogated for all exons. The OCP also provides the capability to detect copy number variations in 49 genes and fusion drivers in 22 genes (available at https://www.thermofisher.com/kr/ko/home/clinical/preclinical-companion-diagnostic-development/oncomine-oncology/oncomine-cancer-research-panel-workflow.html.)

Briefly, both IBC (tumor #1, partly containing pleomorphic discohesive cells) and choriocarcinomatous (tumor #2) components of formalin-fixed, paraffin-embedded tumor sections were manually micro-dissected. Normal control tissue in each case was also dissected from the adjacent nonmalignant tissue. DNA and RNA were extracted and quantified using the RecoverAll™ Multi-Sample RNA/DNA Isolation Workflow (Ambion, Austin, TX, USA) and Qubit 2.0 fluorometer (Thermo Fisher Scientific). DNA and RNA libraries were generated from 20 ng of each of DNA and RNA per sample using the Ion AmpliSeq Library Kit 2.0 (Thermo Fisher Scientific). DNA and RNA libraries were used to prepare templated ion sphere particles with the Ion 540 Chef kit and the Ion S5™ Chef system (both Thermo Fisher Scientific). Sequencing was performed using the Ion S5 Sequencer and Ion 540 Chips, and the sequencing data were analyzed using Torrent Suite version 5.2.2 and Ion Reporter version 5.2 (both Thermo Fisher Scientific). Variants were identified using the Ion Torrent Variant Caller plug-in (v5.2.2.41) and Ion Reporter software v5.2 (both Thermo Fisher Scientific). ANNOVAR software (http://www.openbioinformatics.org/annovar/) was also used for the functional annotation of identified single-nucleotide polymorphisms to investigate their genomic location and any variations in information [24].

### Table 2. Antibodies used in this study

| Antibody | Clone | Dilution | Supplier |
|----------|-------|----------|----------|
| ER       | SP1   | 1:150    | Leica    |
| PR       | SP42  | 1:400    | Leica    |
| HER2     | A0485 | Prediluted | DAKO    |
| Ki-67    | MIB-1 | 1:400    | DAKO    |
| β-hCG    | 234A-15 | 1:1,000 | Cell Marque |
| Cytokeratin | AE1/AE3 | 1:600 | DAKO |
| PS3      | DO-7  | 1:300    | DAKO    |
| GATA3    | L50-823 | 1:200  | Cell Marque |
| PTEN     | Y184  | 1:100    | Abcam   |
| GCDFP-15 | 23A3  | 1:50     | Neomarkers |
| Mammoglobin | 31A5   | Prediluted | Ventana |
| SOX10    | 383A-76 | 1:25    | Cell Marque |

### Table 1 (continued)

| Reference | Age (yr) | Sex | Location | LN metastasis | Size (cm) | LN, lymph node; NS, not stated; β-hCG, beta-human chorionic gonadotropin | Treatment | Survival method |
|-----------|----------|-----|----------|---------------|-----------|---------------------------------|-----------|-----------------|
| Alvarez et al. [19] | 29 | LOUQ | No | 1 | Lungs, skin and brain later | MRM + CX + PTX + RNX | DOD (25) | NS |
| Kumar et al. [20] | 22 | Lt | ROLQ | No | 4.0 | Lungs, brain later | Ex | NS |
| Tsukamoto et al. [21] | 29 | RILQ | No | 3.0 | Liver, brain later | Ex | NS |

Distant metastasis at presentation: LungLt; brain later; MRM + CTxh + RTx; DCIS; NS; NS; NS; NS; DOD (1); Hx of elective abortion; AC (cyclophosphamide and doxorubicin); 5-FU (5-fluorouracil); EMA-CO (etoposide, methotrexate, actinomycin D, and cyclophosphamide and vincristine); MACIII (methotrexate, actinomycin D, and cyclophosphamide); Etoposide, methotrexate, doxorubicin, vincristine, and cyclophosphamide; MAC (methotrexate, actinomycin D, and cyclophosphamide) → EMA and 5-FU and cisplatin → ifosfamide with mesna.
Clinicalpathologic Features of IBC-CP and MC-B

Ultimately, 19 articles including IBC-CP (n = 16) and MC-B (n = 8) patients were identified as eligible for this study. The clinicopathologic features of the IBC-CP (including our case) and MC-B patients are summarized in Tables 1 and 3. All patients were female. In IBC-CP, the patient ages ranged from 31 years old to 71 years old (mean, 49.8 years; SD, 10.3 years). The mean size of the tumor was 4.0 cm (range, 1.0–10.0 cm), and most tumors developed in the right breast (13/17, 76.5%). Serum β-hCG levels were typically not checked at presentation but elevated levels were found in 3 of the 17 patients (13/17, 76.5%), and this tumor was accompanied by extensive central necrosis and skin involvement (Fig. 2a). Enlargement of the axillary and supraclavicular lymph nodes was also observed, and the largest of these was 4.5 cm in the greatest dimension. IBC with axillary involvement was pathologically confirmed by core needle biopsy. The patient presented with cT4cN3 disease in the initial evaluation, so neoadjuvant chemotherapy – which consisted of four cycles of doxorubicin 60 mg/m² and cyclophosphamide 600 mg/m² every 3 weeks followed by four cycles of docetaxel 75 mg/m² every 3 weeks – was administrated. Then, a radical mastectomy was performed, and a residual tumor invading the skin and nipple was found with nodal metastases in 2 out of 28 axillary lymph nodes. Ac-

Clinical Characteristics of the Present Case

This study was approved by the Institutional Review Board (approval number, OC18TESI0052) with a waiver of the requirement to obtain patient consent. A 49-year-old female patient with no previous medical history or underlying disease visited the hospital due to masses in the right breast and axillary region. Magnetic resonance imaging revealed an irregular tumor of 9.2 cm in size, and this tumor was accompanied by extensive central necrosis and skin involvement (Fig. 2a). Enlargement of the axillary and supraclavicular lymph nodes was also observed, and the largest of these was 4.5 cm in the greatest dimension. IBC with axillary involvement was pathologically confirmed by core needle biopsy. The patient presented with cT4cN3 disease in the initial evaluation, so neoadjuvant chemotherapy – which consisted of four cycles of doxorubicin 60 mg/m² and cyclophosphamide 600 mg/m² every 3 weeks followed by four cycles of docetaxel 75 mg/m² every 3 weeks – was administrated. Then, a radical mastectomy was performed, and a residual tumor invading the skin and nipple was found with nodal metastases in 2 out of 28 axillary lymph nodes. Ac-

Table 3. Comparison between IBC-CP and MC-B patients

|          | IBC-CP  | MC-B   | p value |
|----------|---------|--------|---------|
| Age, yr; mean ± SD | 49.8±10.3 | 31.9±8.5 | <0.001* |
| Tumor size, cm; mean ± SD | 4.0±2.5 | 3.5±2.0 | 0.920   |
| Localization, n (%) |          |        |         |
| Lt | 4 (23.5) | 3 (37.5) |          |
| Rt | 13 (76.5) | 4 (50.0) |          |
| Both | 0 | 1 (12.5) |          |
| Serum β-hCG, n (%) |          |        |         |
| Elevated level | 1 (25.0) | 0 |          |
| Normal level | 3 (75.0) | 8 (100) | 0.333   |
| Metastasis to the axillary node, n (%) |          |        |         |
| No | 8 (57.1) | – |          |
| Yes | 6 (42.9) | – |          |
| Distant metastasis, n (%) |          |        |         |
| No | 11 (68.8) | 0 |          |
| Yes | 5 (31.2) | 7 (100) | 0.005*  |
| Associated IBC/DCIS, n (%) |          |        |         |
| Absent | 2 (11.8) | 1 (50.0) | 0.298   |
| Present | 15 (88.2) | 1 (50.0) |          |
| Past history of GTD, abortion, and/or pregnancy, n (%) |          |        |         |
| No | 7 (87.5) | 0 |          |
| Yes | 1 (22.5) | 8 (100) | 0.001*  |

*Statistically significant at p < 0.05. 
Only cases with available data were analyzed.
According to the European Guideline, there was no response to chemotherapy in either the tumor or the nodes [25]. The patient received postoperative adjuvant chemotherapy with capecitabine and radiation therapy. After being transferred to another hospital, lung metastases were discovered during a clinical follow-up. The patient is still alive while receiving chemotherapy one year after surgery.

**Pathological and Molecular Characteristics of the Present Case**

Grossly, an ill-defined firm tumor (8.5 × 7.0 × 5.5 cm) showing a whitish tan-colored firm cut surface with hemorrhage and necrosis had invaded the skin and nipple (Fig. 2b, c). Microscopically, the tumor was predominantly composed of irregular solid nests of oval-shaped mononuclear epithelial cells with fibrotic stroma and necrosis (Fig. 3a), which were multifocally surrounded by numerous multinucleated syncytiotrophoblast-like giant cells with hemorrhage (Fig. 3b). In addition, pleomorphic discohesive invasive carcinomatous cells were focally observed in about 1% of the entire tumor volume (Fig. 3c). There were no definite DCIS components. Immunohistochemically, less than 5% of the tumor cells were positive for GATA-binding protein 3 (GATA3), and all of these were IBC components (Fig. 3d). By contrast, the multinucleated syncytiotrophoblast-like giant cells and pleomorphic discohesive cells were negative (Fig. 3e, f). In addition, mammaglobin was only expressed in a few tumor cells of IBC components (online suppl. Fig. 1A; see www.karger.com/doi/10.1159/000522621 for all online suppl. material). Further, SOX10 was extensively expressed regardless of tumor components (online suppl. Fig. 1B), while GCDFP-15 was completely negative (online suppl. Fig. 1C). All tumor cells were heterogeneously positive for cytokeratin (Fig. 3g, h), and a few oval-shaped mononuclear cells displayed β-hCG immunopositivity (Fig. 3i). Neither previous medical history nor imaging studies identified other malignant tumors, so this tumor was diagnosed as IBC-CP.

![Fig. 1. Survival analysis. The survival time of IBC-CP patients tended to be longer than that of MC-B patients.](image)

![Fig. 2. Representative radiologic and gross images. Magnetic resonance imaging showed an irregular tumor accompanied by extensive central necrosis and skin involvement (a). On gross examination, an ill-defined firm tumor (8.5 × 7.0 × 5.5 cm) invading the skin and nipple (b), and a whitish tan-colored firm cut surface with hemorrhage and necrosis (c) were observed.](image)
In NGS, nucleotide sequencing only revealed a missense mutation (NM_000546: c.524G>A; p.R175H) in exon 5 of the TP53 gene. This mutation was identically observed in both the choriocarcinomatous lesion (allelic frequency, 82.9%) and IBC (allelic frequency, 78.1%). However, copy number loss in the PTEN gene was only identified in the choriocarcinomatous components. Immunohistochemically, all tumor cells exhibited strong p53 positivity, i.e., the mutant phenotype (Fig. 4a, b). Interestingly, immunoreactivity for PTEN was lost in the syncytiotrophoblast-like cells of the choriocarcinomatous lesion, whereas it was intact in IBC and pleomorphic discohesive cells (Fig. 5a–c). All tumor cells were completely negative for the ER, PR, and HER2. The tumor cells exhibited a high Ki-67 labeling index of 96.9%, which was calculated digitally.

Fig. 3. Representative microscopic images. Irregular solid nests of oval-shaped mononuclear cells with fibrotic stroma (a), surrounding multinucleated syncytiotrophoblast-like giant cells with hemorrhage (b), and pleomorphic discohesive invasive carcinomatous cells (e). GATA3-positive oval-shaped mononuclear cells (d), but GATA3-negative multinucleated syncytiotrophoblast-like giant cells (e) and pleomorphic discohesive cells (f). Heterogeneous cytokeratin immunoreactivity in multinucleated syncytiotrophoblast-like giant cells, mononuclear cells, (g) and pleomorphic discohesive invasive carcinomatous cells (h). A few mononuclear cells displaying β-hCG immunopositivity (i) (a–c, H&E; d–f, GATA immunostaining; g, h, cytokeratin immunostaining; i, β-hCG immunostaining) (a–h, ×100 magnification; i, ×200 magnification).
Discussion/Conclusion

We report a rare case of IBC-CP with a NGS comparative analysis of the choriocarcinomatous and the IBC component. Moreover, based on a literature review, we analyze the clinicopathological characteristics of IBC-CP and compare them to those of MC-B.

The optimal chemotherapy regimen for IBC-CP is still unclear. IBC-CP patients received neoadjuvant chemotherapy or postoperative chemotherapy using fluorouracil, epirubicin, doxorubicin, actinomycin D, cyclophosphamide, and other agents as monotherapy or in combination therapy, but the efficacies of these treatments were not reported (Table 1). Meanwhile, Zhu et al. [3] reported that capecitabine was effective for the treatment of IBC-CP. In general, 5-fluorouracil is recognized as an effective drug for the treatment of choriocarcinoma, and it is speculated that capecitabine, an oral prodrug of 5-fluorouracil, may exert superior therapeutic efficacy toward IBC-CP [3]. Our patient received neoadjuvant chemotherapy consisting of doxorubicin and cyclophosphamide plus docetaxel and postoperative adjuvant chemotherapy with capecitabine, and this treatment showed poor therapeutic efficacy. Further studies on chemotherapy for patients with IBC-CP are needed.

TP53 is the second most common gene with a somatic mutation in breast cancer, as it has a listed frequency of 27% in the Catalogue of Somatic Mutations in Cancer database [26]. By contrast, somatic mutations in the PTEN gene were identified in 6% of the primary IBC cases [26]. The mutation spectra of TP53 and PTEN as well as PIK3CA and AKT1 reflected subgroup specificity with clini-
Breast Carcinoma with Choriocarcinomatous Pattern

PTEN is the third most frequently mutated gene in TNBC after TP53 and PIK3CA [26]. In chemotaxis, the temporal and spatial distribution of the phosphatidylinositol 3-kinase (PI3K) and the tumor suppressor gene PTEN regulates cytokinesis [29]. Polarity is established by the local accumulation of phosphatidylinositol 3,4,5-triphosphate (PIP3) at the cell’s leading edge, but the loss of PTEN fails to modulate the PIP3 levels, leading to defects in cytokinesis [29]. As a consequence, the nuclei of the cells are duplicated, resulting in large multinucleated cells [29]. In the present case, copy number loss in the PTEN gene was only identified in the choriocarcinomatous components, and immunoreactivity for PTEN was only lost in the syncytiotrophoblast-like cells of the choriocarcinomatous lesion, which was consistent with the role known to be played by the PTEN gene in cytokinesis. In our case, multinucleated giant cells were dispersed in the pleomorphic discohesive invasive carcinoma components. Interestingly, these pleomorphic discohesive invasive cells had TP53 mutations without PTEN mutations in NGS, and they also showed weakly positive PTEN immunostaining. We therefore speculated that this area may be a transitional zone between IBC and the choriocarcinomatous lesion. However, we failed to define the genetic changes via NGS because the pleomorphic discohesive invasive carcinoma components were too scarce for tumor cells to be collected by manual dissection. Therefore, further studies are needed to elucidate the relationship between choriocarcinomatous differentiation and PTEN gene mutations, such as multi-institutional research considering multiple cases.

Cytokeratin immunohistochemically showed heterogeneity depending upon the tumor component. Multinucleated syncytiotrophoblast-like giant cells were weakly and focally positive for cytokeratin, whereas IBC and the polymorphic discohesive cells were strongly and extensively positive. In a metaplastic carcinoma case containing sarcomatoid and squamous elements reported by Resetkova et al. [8], syncytiotrophoblast-like cells were demonstrated only within the sarcomatoid component, and they were cytokeratin negative. Accordingly, the PTEN gene status and immunohistochemical finding of PTEN and cytokeratin may support choriocarcinomatous differentiation or metaplasia of the tumor cells.

Placental β-hCG mediates antitumor effects during pregnancy by imprinting a permanent genomic signature of the mammary gland refractory to malignant transformation through cell differentiation, apoptosis, and the inhibition of growth [30]. In contrast, ectopically expressed β-hCG in breast cancer paradoxically exerts a tumor-promoting function, which is associated with poor prognosis [30]. Immunoreactivity for β-hCG has been reported in 12–60% of primary breast carcinoma cases, and elevated serum β-hCG levels have been reported in 14% of primary breast carcinoma patients [31]. The tumorigenic effect of β-hCG has been suggested to block the apoptotic effect of transforming growth factor β1 in cancer cells through TGFβ-receptor binding [30]. In addition, β-hCG appears to promote invasion by downregulating E-cadherin, an invasive suppressor that plays a significant role in epithelial cell-to-cell adhesion [30]. In an experimental study of transgenic mice overexpressing β-hCG, multiple neoplasms developed, such as breast cancer accompanied by impaired Wnt signaling in the mammary gland [30].

Previous studies of IBC-CP cases showed that not only were choriocarcinomatous cells β-hCG positive but so were IBC cells, as in our case [3–13]. As was expected, 2 cases of MC-B that underwent β-hCG immunohistochemical analysis showed immunopositivity [15, 16]. Altogether, the β-hCG-positive findings did not help differentiate between IBC-CP and MC-B, and they appear to be related to the adverse parameters of the tumor.

The cancer-promoting function of ectopically expressed β-hCG in tumor cells has come to serve as the basis for the development of possible therapeutic strategies through immunological approaches. A β-hCG DNA vaccine effectively inhibited the growth of breast cancer cells in a mouse model [30], while a monoclonal antibody against β-hCG conjugated with cytotoxic drugs enhanced the killing rates of β-hCG-expressing cancer cells [30]. Therefore, the fact that these immunological strategies target ectopically β-hCG-expressing cancer cells suggests that β-hCG may be a valuable target in breast cancer therapy [30].

GATA3 is sensitive to breast and urothelial carcinomas, and it is used as a marker to differentiate breast and urothelial carcinomas from other carcinomas. However, GATA3 is also important for trophoblast differentiation [28, 32]. Mirkovic et al. [32] stated that all trophoblast lineages of hydatidiform moles as well as all GTDs – including...
choriocarcinomas, placental site trophoblastic tumors, and epithelioid trophoblastic tumors – were positive for GATA3. In our case, a few IBC components were positive for GATA3, while the multinucleated syncytiotrophoblast-like cells and pleomorphic discohesive cells were negative. GCDFP-15 is one of the proteins detected in breast cyst fluid, and it is also known as a specific marker of the breast [28]. GCDFP-15 is expressed in approximately 60% of primary breast carcinomas [28]. In previous reports presenting choriocarcinomatous components in the breast, GCDFP-15 immunostaining was performed in 4 IBC-CP cases; of these, two were positive [3, 7] and the other two were negative [8]. Mammaglobin and SOX10 have also been used as sensitive breast markers. In TNBC specifically, the expressions of GATA3, GCDFP-15, and mammaglobin are lower than they are in other types of breast cancer [33]. Meanwhile, it has been reported that SOX10 is positive in 40–70% of TNBCs, even in the case of GATA3 negativity [34, 35]. Similarly, our IBC-CP case, a TNBC, was focally positive for GATA3 and mammaglobin and completely negative for GCDFP-15, but it was extensively positive for SOX10. The immunopositivity of GATA3, GCDFP-15, and mammaglobin in a tumor favors primary breast carcinoma in the clinical setting, but negative staining in a tumor does not exclude a breast origin. It is also recommended to determine whether the breast lesion is primary or metastatic by combining clinical information and imaging findings while keeping in mind that GATA3 may be positive in cells of a trophoblast lineage.

Our study had a few limitations. First, the number of published MC-B cases is very small, and there is a lack of pathologic data on primary gestational tumors. Therefore, selection bias cannot be excluded. Although a history of GTD, abortion, and pregnancy is important in the diagnosis of MC-B, a patient reported by Resetkova et al. [8] was diagnosed with IBC-CP despite the fact that the breast tumor was detected during pregnancy and found to be GCDFP-15 negative. When IBC and/or DCIS was present in the periphery of a tumor, that tumor tended to be diagnosed as IBC-CP. However, in a case report by Alvarez et al. [19], the tumor was diagnosed as MC-B due to a history of abortion despite accompanying DCIS. Second, the histologic and immunohistochemical findings of breast tumors in MC-B were also incomplete. It was impossible to compare the presence or absence of IBC or DCIS, the immunophenotype of the breast tumor, and the β-hCG immunostaining pattern. Third, in IBC-CP patients, serum β-hCG levels were not typically measured at presentation, so the difference from MC-B patients could not be determined. Therefore, further multi-institutional studies with a larger number of IBC-CP and MC-B cases are needed for more accurate comparative studies.

In conclusion, the present IBC-CP case is TNBC with TP53 mutation, and the copy number loss of the PTEN gene may be associated with choriocarcinomatous differentiation. The survival time of IBC-CP patients tended to be longer than that of MC-B patients. When an older patient with no history of GTD, abortion, or pregnancy presents a breast lesion with a choriocarcinomatous pattern without distant metastasis, IBC-CP may be suspected first. In other words, it is crucial to take a medical history to rule out metastatic lesions.

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Statement of Ethics

This study was approved by the Institutional Review Board (approval number, OC18TESI0052) with a waiver of the requirement to obtain patient consent.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Concepts, design, and statistical analysis: S.-Y.J.; definition of intellectual contents: S.-Y.J. and N.Y.; literature search and data acquisition: S.A.; clinical studies: Y.-J.K. and C.S.P.; data analysis: N.Y.; manuscript preparation: S.-J.Y. and N.Y.; manuscript editing and review: S.-Y.J. All authors critically read and approved the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material. Further inquiries can be directed to the corresponding author.
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