Apocrine Carcinoma as Triple-negative Breast Cancer: Novel Definition of Apocrine-type Carcinoma as Estrogen/Progesterone Receptor-negative and Androgen Receptor-positive Invasive Ductal Carcinoma

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Objective: Apocrine carcinoma, a subtype of invasive ductal carcinoma of the breast, expresses androgen receptor (AR), but often lacks estrogen receptor (ER) and progesterone receptor (PgR). In the present study, the author immunohistochemically defined apocrine-type carcinoma as ER−/PgR−/AR+ invasive ductal carcinoma and analyzed the significance of apocrine-type carcinoma as triple-negative breast cancer.

Methods: Four hundred and forty breast cancers from 429 cases were immunostained for estrogen receptor, progesterone receptor, androgen receptor, human epidermal growth factor receptor type 2 (HER2), p53, Ki-67 and epidermal growth factor receptor. The lesions included 58 in situ malignancies (including 13 apocrine-type lesions) and 325 invasive ductal carcinomas (including 44 apocrine type).

Results: Of 91 estrogen receptor-negative invasive ductal carcinomas, 44 (48%) belonged to apocrine-type carcinoma, and overexpression of human epidermal growth factor receptor type 2 (HER2), p53, Ki-67 and epidermal growth factor receptor type 2 overexpressed. Invasive ductal carcinomas in the triple-negative breast cancer category (n = 51) were divided into triple-negative, androgen receptor-positive (apocrine, n = 21) and quadruple-negative (non-apocrine, n = 30). p53 overexpression was more often seen in the apocrine-type triple-negative breast cancer (18/21 = 86%) than in the non-apocrine type (14/30 = 46%) (P < 0.05). Ki-67 labeling was significantly higher in the non-apocrine type (58%) than in the apocrine type (37%) (P < 0.01). Epidermal growth factor receptor is consistently expressed in triple-negative breast cancers (16/16 = 100% in apocrine and 18/20 = 90% in non-apocrine).

Conclusions: Androgen receptor should be added to immunohistochemical panels, since apocrine-type invasive ductal carcinoma, resembling basal-like phenotypes, may show clinical behaviors different from the basal-like triple-negative breast cancer.

Key words: apocrine carcinoma — androgen receptor — triple-negative breast cancer — immunohistochemistry — pathology
INTRODUCTION

Apocrine carcinoma is a microscopically defined type of invasive ductal carcinoma (IDC) of the breast (1). The apocrine cancer cells possess large round nuclei and plump, eosinophilic, granular and sharp-bordered cytoplasm, occasionally associated with an apical decapitation secretion pattern (apical snouting). Apocrine carcinoma shows growth patterns seen in common IDCs, and ductal carcinoma in situ (DCIS) occasionally accompanies the apocrine phenotype (2). It is known that this type of breast cancer commonly expresses androgen receptor (AR) but is often devoid of estrogen receptor (ER) and progesterone receptor (PgR) (2–5). Overexpression of human epidermal growth factor receptor type 2 (HER2) is frequently seen in apocrine carcinoma (6), while HER2-negative apocrine carcinoma can be phenotyped as triple-negative breast cancer (TNBC) (7).

TNBC commonly shows high-grade nuclear atypia and aggressive clinical behavior with poor prognosis (8–12). Because of negativity of hormone receptors (HRs) and HER2, chemotherapy should be the choice of therapy. Genotyping using DNA microarray analysis reveals that TNBC often belongs to the basal-like type (13).

Apocrine metaplasia in benign breast lesions invariably shows the ER−/PgR−/AR+ phenotype, and this expression pattern is retained in classical apocrine carcinoma. In the present study, the author defined apocrine-type carcinoma as ER−/PgR−/AR+ IDC. A total of 440 breast cancer lesions sampled mainly by needles from 429 cases were evaluated immunohistochemically for ER, PgR, AR, HER2, p53 oncoprotein, Ki-67, epidermal growth factor receptor (EGFR), cytokeratin (CK) 5/6, CK14 and gross cystic disease fluid protein 15 (GCDFP15). It is expected that appropriate pathological evaluations of HER2-negative apocrine-type carcinoma, which may be distinctive from the basal-like TNBC, should assist at considering effective treatment strategy in apocrine-type TNBC cases.

PATIENTS AND METHODS

MATERIALS

A total of 440 breast cancer lesions were sampled from 429 cases by needle biopsy (369 cases), excisional biopsy (11 cases) and surgical removal (50 cases), in the period from January 2008 to December 2011. Most of the samples were sent from open practitioners Matsuo Clinic, Toyohashi, Aichi, Yamada Clinic, Ichinomiya, Aichi, and Hayashi Clinic, Nagoya, Aichi, and the other cases were obtained at Saishukan Hospital, Kitanagoya, Aichi, and Keiyu Hospital, Yokohama, Kanagawa. Synchronous double cancers were seen in five cases, while two different histological patterns were separately evaluated in six, including three cases: apocrine-type DCIS plus IDC and two cases of apocrine-type plus non-apocrine IDC. Patients’ ages ranged from 25 to 93 years with the mean of 55.8 (20s: 5 cases, 30s: 41 cases, 40s: 119 cases, 50s: 102 cases, 60s: 88 cases, 70s: 53 cases, 80s: 20 cases and 90s: 2 cases). Three male cases were aged 78, 81 and 93.

Histological types are summarized in Table 1, including 54 ductal carcinomas in situ and 325 IDCs. DCIS lesions were subclassified as follows: apocrine 13 (24%) and non-apocrine 41 (comedo 9, cribriform 25, papillary 3 and neuroendocrine 4). The Scarff–Bloom–Richardson histological grading system (14) was applied to the IDCs.

Since apocrine cellular features are empirically seen in the form of IDCs (2), apocrine carcinoma was included in the IDC category. In the present study, the author dared to define apocrine-type carcinoma immunohistochemically as ER−/PgR−/AR+ IDC, since apocrine metaplastic cells invariably showed the ER−/PgR−/AR+ phenotype. Forty-four (14%) of 325 IDCs were categorized as apocrine-type carcinoma. Histological evaluation revealed that 22 (50%) were categorized as classical apocrine carcinoma. The patients’ ages ranged from 25 to 93 years (mean: 56.4) for IDCs, from 37 to 85 (mean: 59.9) for apocrine-type carcinoma and from 41 to 85 (mean: 61.7) for classical apocrine carcinoma.

The author also evaluated 50 needle biopsy specimens of benign breast lesions, including 14 intraductal papillomas, 13 fibroadenomas, 7 benign phyllodes tumors, 7 mastopathy lesions, 6 tubular/ductal adenomas, 1 hamartoma and 1 mucocle-like tumor (mean age: 42.9 years). Apocrine metaplasia was observed in 11 lesions, including 5 intraductal papillomas, 3 mastopathy lesions, 2 fibroadenomas and 1 ductal adenoma (mean age: 47.8).

<table>
<thead>
<tr>
<th>Typing</th>
<th>Case no.</th>
<th>Age</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-invasive ca.</td>
<td>58</td>
<td>53.2</td>
<td>(27–85)</td>
</tr>
<tr>
<td>DCIS</td>
<td>54</td>
<td>53.6</td>
<td>(27–85)</td>
</tr>
<tr>
<td>Apocrine</td>
<td>13</td>
<td>57.8</td>
<td>(35–77)</td>
</tr>
<tr>
<td>Non-apocrine</td>
<td>41</td>
<td>52.2</td>
<td>(27–85)</td>
</tr>
<tr>
<td>Others</td>
<td>4</td>
<td>48.0</td>
<td>(39–64)</td>
</tr>
<tr>
<td>Apocrine</td>
<td>50</td>
<td>59.9</td>
<td>(37–85)</td>
</tr>
<tr>
<td>Special 1</td>
<td>51</td>
<td>57.0</td>
<td>(36–89)</td>
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<tr>
<td>Special 2</td>
<td>6</td>
<td>40.5</td>
<td>(33–58)</td>
</tr>
<tr>
<td>Total</td>
<td>440</td>
<td>55.8</td>
<td>(25–93)</td>
</tr>
</tbody>
</table>

G1, Grade 1; G2, Grade 2; G3, Grade 3; DCIS, ductal carcinoma in situ; ICPC, intracystic papillary carcinoma; LCIS, lobular carcinoma in situ; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; MUC, mucinous carcinoma; MPC, micropapillary carcinoma; NEC, neuroendocrine carcinoma; MED, medullary carcinoma; CCC, clear cell carcinoma; ACC, adenosid cystic carcinoma.
METHODS

All the specimens were fixed in 10% formalin and embedded in paraffin wax. Paraffin sections of 4 μm thickness were mounted onto the aminosilane-coated glass slides. Hematoxylin and eosin (H&E) staining was performed for evaluating histological features. The amino acid polymer technique (Simple Stain Max-PO, Nichirei Bioscience, Tokyo, Japan) was utilized for immunoperoxidase staining for ER, PgR, AR, HER2, p53 oncoprotein, Ki-67, EGFR, CK5/6, CK14 and GCDFP15. EGFR, CK5/6, CK14 and GCDFP15 were evaluated in 304 lesions, including 223 IDCs. When necessary, immunostaining for chromogranin A and CD56 (neural cell adhesion molecule) was added, in order to evaluate neuroendocrine nature of the tumor cells. The antibodies and the soaking solutions for heat-induced epitope retrieval are listed in Table 2. For hydrated heating of deparaffinized sections, pressure pan heating at 121°C for 10 min was employed (15). After releaving the pressure, sections were left in the solution for more than 30 min for gradual cooling. The nuclei were counterstained with Mayer’s hematoxylin.

The judging criteria of the immunostaining were as follows.

(i) HRs (ER, PgR and AR): the 1% criterion was adopted: when the nuclei were stained in not less than 1% of the cancer cells, it was judged as positive.

(ii) HER2 and EGFR: 3+, strongly expressed along the plasma membrane; 2+, moderately expressed; 1+, weakly expressed; and –, no staining. In the present study, only 3+ positivity was regarded as ‘overexpressed’. In the case of HER2, the international 30% criterion was utilized for the judgment, although in most cases, tumor cells showed uniform reactivity.

(iii) p53 oncoprotein: 3+, more than two-thirds stained; 2+, more than one-third but no more than two-thirds; +, no more than one-third stained; –, negative. Here, 2+ or 3+ were judged as overexpressed.

(iv) Ki-67: the mean percentage of nuclear positivity was evaluated in a stepwise way, such as 1, 2, 3, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80 and 90%.

(v) CK5/6, CK14 and GCDFP15: when more than 10% of the tumor cells showed cytoplasmic reactivity, the judgment was positive.

STATISTICAL ANALYSIS

Statistical analysis was performed with the χ² test. For comparing Ki-67 labeling, the Wilcoxon rank-sum test (for comparison between two groups) or the Kruskal–Wallis test (for comparison among three or more groups) was utilized. A value of P < 0.05 was regarded as statistically significant.

ETHICAL ISSUE

Informed consent was orally obtained from each patient, and all the immunostained data in an addendum report of the signed histopathological diagnosis were accessible to patients when the treatment plan was presented by attending physicians. Written informed consent was prepared in Keiyu Hospital. The present clinical study was approved by the Ethics Committees for Epidemiological and Clinical Studies of Fujita Health University (#11-047), Saishukan Hospital and Keiyu Hospital. The study outline was open to the public in the homepage of the Department of Pathology, Fujita Health University School of Medicine.

RESULTS

BENIGN BREAST LESIONS

The duct-lining cells in the non-cancerous mammary tissue invariably served as internal positive controls for ER and PgR. Ductal cells in benign neoplasm occasionally showed AR expression in the nuclei, though fundamentally negative. Non-neoplastic mammary tissue never showed overexpression of HER2 and p53. Weak membrane reactivity of HER2 was often noted in the normal duct-lining cells. Ki-67 labeling was infrequent in the non-neoplastic ductal cells.

Normal myoepithelial cells consistently expressed EGFR, CK5/6 and CK14. These three markers were inconsistently

<table>
<thead>
<tr>
<th>Antibody against</th>
<th>Clone</th>
<th>Source</th>
<th>Dilution</th>
<th>Solution for heat-induced antigen retrieval</th>
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<tbody>
<tr>
<td>ER</td>
<td>6F11</td>
<td>Novocastra</td>
<td>1:50</td>
<td>1 mM EDTA, pH 8</td>
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<tr>
<td>PgR</td>
<td>16</td>
<td>Novocastra</td>
<td>1:100</td>
<td>1 mM EDTA, pH 8</td>
</tr>
<tr>
<td>AR</td>
<td>AR441</td>
<td>Dako</td>
<td>1:100</td>
<td>1 mM EDTA, pH 8</td>
</tr>
<tr>
<td>HER2</td>
<td>rabbit serum</td>
<td>Nichirei</td>
<td>1:400</td>
<td>10 mM citrate buffer, pH 6</td>
</tr>
<tr>
<td>p53</td>
<td>D07</td>
<td>Dako</td>
<td>1:100</td>
<td>1 mM EDTA, pH 8</td>
</tr>
<tr>
<td>Ki-67</td>
<td>MIB-1</td>
<td>Dako</td>
<td>1:100</td>
<td>1 mM EDTA, pH 8</td>
</tr>
<tr>
<td>EGFR</td>
<td>EGFR25</td>
<td>Novocastra</td>
<td>1:100</td>
<td>1 mM EDTA, pH 8</td>
</tr>
<tr>
<td>CK5/6</td>
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<td>Dako</td>
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<td>1 mM EDTA, pH 8</td>
</tr>
<tr>
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<td>LL002</td>
<td>YLEM</td>
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<tr>
<td>GCDFP15</td>
<td>D6</td>
<td>Calbiochem</td>
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<tr>
<td>CD56</td>
<td>1B6</td>
<td>Novocastra</td>
<td>1:100</td>
<td>10 mM citrate buffer, pH 6</td>
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</table>

ER, estrogen receptor; PgR, progesterone receptor; AR, androgen receptor; HER2, human epidermal growth factor receptor type 2; EGFR, epidermal growth factor receptor; CK5/6, cytokeratin 5/6; CK14, cytokeratin 14; GCDFP15, gross cystic disease fluid protein 15; CD56, cluster of differentiation 56; EDTA, ethylenediamine tetraacetic acid.
expressed in proliferative duct-lining cells in benign lesions such as intraductal papilloma, fibroadenoma and mastopathic breast.

Apocrine metaplasia was seen in 11 benign breast lesions and consistently showed such intranuclear HR expression as ER_2/PgR_2/AR+. EGFR was frequently positive along the basolateral membrane of apocrine metaplastic cells. Representative features of apocrine metaplasia in intraductal papilloma are illustrated in Fig. 1. CK5/6 and CK14 were fundamentally negative in the metaplastic cells. Ki-67 labeling was lower in the metaplastic cells than in the surrounding non-metaplastic ductal cells. GCDFP15 was consistently expressed in apocrine metaplasia, while non-metaplastic duct-lining cells occasionally revealed positivity.

**BREAST CANCER LESIONS**

Tables 3 and 4 summarize the results of immunostaining for ER, PgR, AR, HER2, p53 and Ki-67 in breast cancer types and IDCs, respectively.

**HORMONE RECEPTOR EXPRESSION**

ER, PgR and AR were expressed in 330 (75%), 276 (63%) and 242 (55%) of 440 breast cancers, respectively. Among the 325 IDCs, 234 (72%), 194 (60%) and 173 (53%) expressed ER, PgR and AR, respectively. The PgR-positive cases consistently expressed ER, and the PgR-positive cancer cells were never more than the ER-positive cancer cells, as has been commented in the 11th St Gallen consensus meeting 2009 (16). Of 173 AR-positive IDCs, 44 (25%) were ER_2/PgR_2 and immunohistochemically categorized in apocrine-type carcinoma (Figs 2 and 3).

Regarding DCIS, 13 lesions were judged as apocrine type (ER_2/PgR_2/AR+). Non-apocrine-type DCIS lesions (n = 41), intracystic papillary carcinoma (ICPC, n = 3) and lobular carcinoma in situ (LCIS, n = 1) consistently expressed ER, and most of them (41/45 = 91%) expressed PgR. AR was less often positive in 21 (47%) lesions.

The other types of invasive malignancy, all cases of invasive lobular carcinoma (ILC, n = 19), mucinous carcinoma (MUC, n = 18), micropapillary carcinoma (MPC, n = 7) and neuroendocrine carcinoma (NEC, n = 7) were positive for ER, and most of them were also positive for PgR (41/51 = 80%). The expression rate of AR was also high in ILC and MUC (31/37 = 84%), but was less frequent in MPC and NEC (4/14 = 29%). Medullary carcinoma (MED, n = 4), clear cell carcinoma (CCC, solid and papillary variant, n = 1) and adenoid cystic carcinoma (ACC, n = 1) were negative for all the three HRs.

When 281 non-apocrine-type IDCs were graded into G1 through G3, all the G1 lesions (n = 120) expressed ER and most of the G2 lesions (83/95 = 87%) expressed ER, but ER was positive only in 31 (47%) of 66 G3 lesions (P < 0.001). PgR was expressed in 104 of 120 (87%) in G1 and 68 of 95 (72%) in G2, but 22 of 66 (34%) in G3 (P < 0.001). AR was positive in 63 of 120 (53%) in G1, 50 of 95 (53%) in G2 and 16 of 66 (24%) in G3 (P < 0.001).
Overexpression of HER2 and p53

Overexpression of HER2 and p53 was seen in 78 (18%) and 100 (23%), respectively, among all the 440 lesions examined. Of 281 non-apocrine IDCs, HER2 and p53 were overexpressed in 40 (14%) and 49 (17%) lesions, respectively. In contrast, apocrine-type IDCs (n = 44) significantly (P < 0.001) overexpressed HER2 and p53: 23 of 44 (52%) for HER2 and 33 of 44 (75%) for p53 (Fig. 2). HER2-negative apocrine-type IDCs (n = 21) should be regarded as a form of TNBC (Fig. 3).

The histological grade of IDCs significantly affected the overexpression (Table 4). Among non-apocrine-type IDCs, HER2 overexpression was seen in 3% (4/120) for G1, 20% (26% (17/66) for G2, and 42% (28/66) for G3 (P < 0.001). When the apocrine-type IDCs were graded, no significant difference was recognized in HER2 overexpression (4/8 (50%) for G1, 9/20 (45%) for G2 and 10/16 (63%) for G3), while p53 overexpression was dependent upon the histological grade (4/8 (50%) for G1, 15/20 (75%) for G2 and 14/16 (88%) for G3) (P < 0.05).

High rate of overexpression of HER2 and p53 (P < 0.001) was also seen in apocrine-type DCIS: 8 of 13 (62%) for HER2 and 5 of 13 (38%) for p53. In 45 non-apocrine-type non-invasive lesions (DCIS, ICPC and LCIS), overexpression was identified in 5 (11%) for HER2 and 2 (4%) for p53 (mainly seen in comedo-type: 4/9 for HER2 and 2/9 for p53).

Overexpression of HER2 and p53 was infrequent in non-IDC types of invasive malignancy (ILC, MUC, MPC, NEC MED, CCC and ACC): 2 of 51 (4%) and 6 of 51 (12%), respectively. Of note is that MED lacked the overexpression (only one of four showed 2+ positivity for p53).

**Ki-67 Labeling Index**

Ki-67 labeling indices (mean and range) are shown in Table 3. It was very high (mean: 74%) in the MED and CCC groups, and low in the ILC, MUC, MPC and NEC groups (mean: 16%) and ACC (10%). The labeling indices were low (mean: 13%) in DCIS (n = 54), with apocrine-type DCIS (16%) vs. non-apocrine-type DCIS (12%).

The Ki-67 labeling indices were dependent upon the histological grade of IDCs (Table 4). In 281 non-apocrine IDCs,
the mean labeling indices were 11% for G1 (n = 120), 22% for G2 (n = 95) and 55% for G3 (n = 66) (P < 0.001). The indices of the apocrine-type IDCs (n = 44) were 39% (Figs 2 and 3): 14% for G1 (n = 8), 35% for G2 (n = 20) and 55% for G3 (n = 16) (P < 0.001).

Figure 2. Representative case (44 years old) of human epidermal growth factor receptor type 2 (HER2)-overexpressed apocrine-type carcinoma, non-classical (G2) (Simple Stain-Max method after heat-induced antigen retrieval). Apocrine-type cancer cells with plump, amphophilic cytoplasm in H&E (a) are negative for ER (b), but with nuclear staining for AR (c). HER2 is overexpressed along the plasma membrane (d), and p53 is strongly positive in the nuclei (e). Ki-67 labeling is moderate (25%) in degree (f).

Figure 3. Representative case (62 years old) of HER2-negative classical apocrine carcinoma (G2) (Simple Stain-Max method after heat-induced antigen retrieval). Apocrine-type cancer cells showing plump, eosinophilic cytoplasm in H&E (a) are negative for ER (b), but with nuclear staining for AR (c). HER2 is only weakly (1+) stained on the plasma membrane (d), while p53 is strongly positive in the nuclei (e). Ki-67 labeling is around 10% (f).

Relationship Between HR Expression and Other Parameters

In 325 IDCs, HR expression was evaluated in correlation with the overexpression of HER2, p53 and Ki-67 labeling index (Tables 3 and 4).
ER was expressed in a total of 234 IDCs and negative in 91 lesions (apocrine type 44 and non-apocrine type 47). In ER-positive lesions, HER2 and p53 were overexpressed in 23 (10%) and 27 (12%) lesions, respectively. Overexpression was significantly ($P < 0.001$) frequent in the ER-negative lesions: 40/91 (44%) for HER2 and 55/91 (60%) for p53. The mean Ki-67 labeling indices were much higher in the ER-negative lesions (47%) than in the ER-positive lesions (19%) ($P < 0.001$). Of the ER-negative lesions, the apocrine type more often showed overexpression of HER2 (23/44 = 52%) and p53 (33/44 = 75%) than the non-apocrine type, in which overexpression of HER2 and p53 was seen in 17 of 47 (36%) ($P = 0.099$) and 22 of 47 (47%) ($P < 0.01$), respectively. In contrast, the mean Ki-67 labeling indices were higher in the non-apocrine type (55%) than in the apocrine type (39%) ($P < 0.001$).

AR was expressed in 173 IDCs, including 44 apocrine-type carcinomas. Of 129 ER+/AR+ non-apocrine-type IDCs, HER2 and p53 were overexpressed in 12 (9%) and 22 (17%), respectively. Among 152 AR-negative IDCs, ER was expressed in 105 (69%) and PgR in 90 (59%), HER2 and p53 were overexpressed in 28 (18%) and 27 (18%), respectively. The mean Ki-67 labeling indices in AR-negative non-apocrine-type IDCs (30%) were higher than AR-positive non-apocrine type (19%) ($P < 0.001$) and were comparable with the apocrine type (39%).

**GROUPING APOCRINE-TYPE AND NON-APOCRINE-TYPE IDCs**

According to the expression pattern of HRs and HER2, 325 IDCs were divided into six groups (Table 5). The first and third groups should be classified as TNBC.

(i) HER2-negative apocrine type (ER−/PgR−/AR+/HER2−), $n = 21$.

(ii) HER2-overexpressed apocrine type (ER+/PgR−/AR+/HER2+), $n = 23$.

(iii) HR-negative, HER2-negative = quadruple-negative breast cancer (ER−/PgR−/AR−/HER2−), $n = 30$.

(iv) HR-negative, HER2-overexpressed (ER+/PgR−/AR−/HER2+), $n = 17$.

(v) ER-positive, HER2-negative (ER+/PgR+/AR−/HER2−), $n = 211$.

(vi) ER-positive, HER2-overexpressed (ER+/PgR+/AR+/HER2+), $n = 23$.

p53 overexpression was most frequently seen in HER2-negative (−) apocrine type 18 of 21 (86%), followed by HER2-overexpressed (HER2OE) apocrine type 15 of 23 (65%), HR−/HER2OE type 8 of 17 (47%), HR−/HER2− (quadruple-negative) type 14 of 30 (47%), ER+/HER2OE type 8 of 23 (35%) and ER+/HER2− type 19 of 211 (9%).

The mean Ki-67 labeling indices were highest in HR−/HER2− (quadruple-negative) lesions (58%), followed by HR−/HER2OE type (49%), HER2OE apocrine type (41%), HER2− apocrine type (37%), ER+/HER2OE type (35%) and ER+/HER2− type (18%).

The percentages of G3 lesions were highest in HR−/HER2− (quadruple-negative) lesions 26 of 30 (87%), followed by HR−/HER2OE type 9 of 17 (53%), HER2OE apocrine type 10 of 23 (43%), ER+/HER2OE type 8 of 23 (35%), HER2− apocrine type 6 of 21 (29%) and ER+/HER2− type 23 of 211 (11%).

When HER2− apocrine type was compared with quadruple-negative type, p53 overexpression rate was significantly high ($P < 0.05$), Ki-67 labeling was low ($P < 0.01$) and the percentage of G3 was low ($P < 0.001$).

**EXPRESSION OF EGFR, CK5/6, CK14 AND GCDFP15**

Expression of EGFR, CK5/6, CK14 and GCDFP15 was evaluated in a total of 304 lesions, including 223 IDCs (Table 6). Among IDCs, EGFR was positive in most (94%) of the apocrine type (HER2− 16/16 and HER2OE 17/19, Fig. 3) and in HR−/HER2− (quadruple-negative) type (18/20 = 90%). HR−/HER2OE type expressed EGFR in 6 of 11 (55%). The EGFR-positive rate was lower in ER+/HER2OE type 5 of 15 (33%) and in ER+/HER2− type 13 of 142 (9%) ($P < 0.001$).

CK5/6 expression showed the same tendency to EGFR expression, with the highest expression rate seen in HR−/HER2− type.

**Table 5. Six groups of IDCs, according to the expression of HRs and HER2**

<table>
<thead>
<tr>
<th>Type</th>
<th>Case no.</th>
<th>ER+</th>
<th>PgR+</th>
<th>AR+</th>
<th>HER2OE 3+</th>
<th>p53OE 2+/3+</th>
<th>Ki-67 labeling (range)</th>
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<tr>
<td>Apocrine type, HER2-negative (G1 = 4, G2 = 11, G3 = 6)</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>21</td>
<td>0</td>
<td>18</td>
<td>37% (10–80%)</td>
</tr>
<tr>
<td>Apocrine type, HER2-overexpressed (G1 = 4, G2 = 9, G3 = 10)</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>23</td>
<td>23</td>
<td>15</td>
<td>41% (5–70%)</td>
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<tr>
<td>HR-negative, HER2-negative (G1 = 0, G2 = 4, G3 = 26)</td>
<td>30</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>14</td>
<td>58% (10–80%)</td>
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<td>HR-negative, HER2-overexpressed (G1 = 0, G2 = 8, G3 = 9)</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>8</td>
<td>49% (20–80%)</td>
</tr>
<tr>
<td>ER-positive, HER2-negative (G1 = 116, G2 = 72, G3 = 23)</td>
<td>211</td>
<td>211</td>
<td>176</td>
<td>117</td>
<td>0</td>
<td>19</td>
<td>18% (1–80%)</td>
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<tr>
<td>ER-positive, HER2-overexpressed (G1 = 4, G2 = 11, G3 = 8)</td>
<td>23</td>
<td>23</td>
<td>18</td>
<td>12</td>
<td>23</td>
<td>8</td>
<td>35% (10–70%)</td>
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</table>

HR: hormone receptor.
HER2– (quadruple-negative) lesions 14 of 20 (70%) and the lowest in ER+/HER2– lesions 8 of 142 (6%). Apocrine type commonly expressed CK5/6 in 21 of 5 (60%; Fig. 4) \( (P < 0.05) \). The expression rate of CK14 was relatively low, with the highest expression seen in HR–/HER2– lesions 6 of 20 (30%).

Eight apocrine-type DCIS lesions were examined for EGFR, CK5/6 and CK14. All the lesions were immunoreactive for EGFR, five for CK5/6 and three for CK14. In 30 non-apocrine-type DCIS lesions, six (20%) were positive for EGFR, eight (27%) for CK5/6 and six (20%) for CK14. The data for EGFR were statistically significant \( (P < 0.001) \). Expression of these markers was infrequent in the ILC, MUC, MPC and NEC groups, as shown in Table 6. All MED \( (n = 2) \) and CCC \( (n = 1) \) exhibited immunoreactivity of EGFR, CK5/6 and CK14.

**Table 6.** Expression of EGFR, CK5/6, CK14 and GCDFP15 in breast cancer

<table>
<thead>
<tr>
<th>Type</th>
<th>Examined</th>
<th>EGFR</th>
<th>CK5/6</th>
<th>CK14</th>
<th>GCDFP15</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCIS</td>
<td>38</td>
<td>14 (37%)</td>
<td>13 (34%)</td>
<td>9 (24%)</td>
<td>29 (76%)</td>
</tr>
<tr>
<td>Apocrine</td>
<td>8</td>
<td>8 (100%)</td>
<td>5 (63%)</td>
<td>3 (38%)</td>
<td>6 (75%)</td>
</tr>
<tr>
<td>Non-apocrine</td>
<td>30</td>
<td>6 (20%)</td>
<td>8 (27%)</td>
<td>6 (20%)</td>
<td>23 (77%)</td>
</tr>
<tr>
<td>ICPC + LCIS</td>
<td>4</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>3 (75%)</td>
</tr>
<tr>
<td>IDC</td>
<td>223</td>
<td>75 (34%)</td>
<td>48 (22%)</td>
<td>17 (8%)</td>
<td>120 (54%)</td>
</tr>
<tr>
<td>Apo/HER2–</td>
<td>16</td>
<td>16 (100%)</td>
<td>10 (63%)</td>
<td>3 (19%)</td>
<td>10 (63%)</td>
</tr>
<tr>
<td>Apo/HER2OE</td>
<td>19</td>
<td>17 (89%)</td>
<td>11 (58%)</td>
<td>2 (11%)</td>
<td>15 (79%)</td>
</tr>
<tr>
<td>HR–/HER2–</td>
<td>20</td>
<td>18 (90%)</td>
<td>14 (70%)</td>
<td>6 (30%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>HR–/HER2OE</td>
<td>11</td>
<td>6 (55%)</td>
<td>3 (27%)</td>
<td>0 (0%)</td>
<td>3 (27%)</td>
</tr>
<tr>
<td>ER+/HER2–</td>
<td>142</td>
<td>13 (9%)</td>
<td>8 (6%)</td>
<td>4 (3%)</td>
<td>86 (61%)</td>
</tr>
<tr>
<td>ER+/HER2OE</td>
<td>15</td>
<td>5 (33%)</td>
<td>2 (14%)</td>
<td>2 (14%)</td>
<td>4 (27%)</td>
</tr>
<tr>
<td>Special 1</td>
<td>36</td>
<td>5 (14%)</td>
<td>2 (6%)</td>
<td>1 (3%)</td>
<td>13 (36%)</td>
</tr>
<tr>
<td>MED + CCC</td>
<td>3</td>
<td>3 (100%)</td>
<td>3 (100%)</td>
<td>3 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>304</td>
<td>97 (32%)</td>
<td>66 (22%)</td>
<td>30 (10%)</td>
<td>165 (54%)</td>
</tr>
</tbody>
</table>

Special 1: ILC 13, MUC 11, MPC 6, NEC 6; Apo, apocrine.

**Figure 4.** Expression of EGFR, CK5/6 and CK14 in apocrine-type carcinoma, non-classical (G3) in a 50 years old case (Simple Stain-Max method after heat-induced antigen retrieval). The cancer cells possess plump, amphophilic, granular and sharp-bordered cytoplasm in H&E (a). EGFR is strongly expressed along the plasma membrane (b). CK5/6 (c) and, though less frequently, CK14 (d) are demonstrated in the cytoplasm of the cancer cells.

**HISTOLOGICAL RE-EVALUATION OF APOCRINE-LIKE FEATURES BY H&E STAINING**

The immunohistochemical characteristics were compared with the histological features in H&E preparations. When
44 apocrine-type IDCs with the ER−/PgR−/AR+ phenotype were reviewed and judged by the author’s eyes, 22 lesions belonged to classical apocrine carcinoma accompanying plump, eosinophilic and sharp-bordered cytoplasm. Granular cytoplasm was recognized in 17 lesions, vacuolated (foamy) cytoplasm in 10, widened Golgi area in 16 and apical snouting in 4. The tumor cells in 20 lesions (non-classical type), however, were suggestive of but not regarded as typical apocrine, mainly because of cytoplasmic amphophilia and/or agranular cytoplasm and a high nuclear/cytoplasmic ratio. In the remaining two (poorly differentiated type), the tumor cells showed scanty basophilic cytoplasm and G3 atypia, and thus not suggestive of apocrine type in H&E preparations.

Table 7 summarizes marker expression in apocrine-type DCIS and IDC, classical vs. non-classical vs. poorly differentiated. Representative H&E morphology and GCDFP15 immunostaining are illustrated in Fig. 5. All the lesions of the classical apocrine-type DCIS and IDC expressed both EGFR and GCDFP15. EGFR-negative apocrine-type DCIS (two lesions) and GCDFP15-negative apocrine-type IDC (two lesions) and IDC (10 lesions) belonged to non-classical or poorly differentiated apocrine type. Ki-67 labeling index tended to be lower in the classical apocrine-type IDC (31%) than in the non-classical (44%) or poorly differentiated apocrine-type IDC (65%). Statistical significance (P < 0.05) was seen between the classical and non-classical/poorly differentiated groups.
The author also encountered a total of 10 non-apocrine IDCs (3 G2 and 7 G3 lesions), where the histological features were suggestive of the apocrine type (Fig. 6). ER was expressed in seven and PgR in four. AR was positive in three ER-positive lesions. HER2 and p53 were overexpressed in seven and five lesions, respectively. Ki-67 labeling indices ranged from 15 to 50% (mean: 37%). EGFR and CK5/6 were detected in one of eight lesions examined.

To the author’s eyes, the histological difference was delicate and subtle and seemed to be dependent on H&E coloring sequence. Histological evaluation thus showed certain limitations in making a diagnosis of apocrine carcinoma.

DISCUSSION

Apocrine carcinoma is a histologically defined type of breast cancer (1), and it encompasses a range of growth pattern identical to the common IDCs (2). In apocrine carcinoma, AR is commonly expressed, while the positivity rate of ER and PgR is low (4,5). Reported frequency of apocrine carcinoma in breast cancer reveals a variable range between 0.3 and 4% (17). This actually suggests that the histological criteria of apocrine carcinoma are subjective and pathologist-dependent to certain degree and require reproducible and objective markers. Therefore, in the present study, apocrine-type carcinoma was immunohistochemically categorized as ER-/PgR-/AR+ IDC. In fact, apocrine metaplastic cells in benign conditions reproducibly showed the ER-/PgR-/AR+ phenotype.

The author immunohistochemically evaluated a total of 440 breast cancer lesions, including 325 IDCs (apocrine type 44 and non-apocrine type 281). The analysis was particularly focused on the IDC lesions.

ER was expressed in 234 (72%) of 325 IDC lesions, and the ER-negative IDCs were divided into AR-positive apocrine type (n = 44) and AR-negative non-apocrine type (n = 47). All the ER-negative breast cancers were negative for PgR. A considerable percentage (55%) of breast cancers, including 53% of IDCs, were AR-positive, as has been reported earlier (18). AR was co-expressed with ER in 129 of 281 (46%) of the non-apocrine IDCs.

Based upon the association of HER2 overexpression, both the apocrine type and non-apocrine type were further divided into HER2-overexpressed and HER2-negative categories (6). The HER2-negative apocrine type (n = 21) and HER2-negative non-apocrine type (n = 30) belonged to TNBC. In other words, TNBC can be grouped into two according to the AR expression. The author would like to propose the term ‘quadruple-negative breast cancer’ for the HER2-negative non-apocrine type.

Both AR-positive and -negative TNBCs very frequently expressed EGFR and CK5/6 (6,8–12). Overexpression of p53 was also characteristic in these groups (19–22). In the present study, the rate of CK5/6 expression was higher in the non-apocrine TNBC, and the rate of p53 overexpression was higher in the apocrine-type TNBC. The mean Ki-67 labeling indices were higher in the non-apocrine type (58%) than in the apocrine type (37%) (2,23). Most (26/30 = 87%) of the non-apocrine TNBCs belonged to the G3 category, while...
only 6 (29%) of 21 apocrine-type TNBCs were classified as G3.

The apocrine-type DCIS \( n = 13 \), representing all the ER-negative DCIS lesions, showed the phenotypes very similar to the apocrine-type IDCs: high overexpression rates of HER2 and p53 and positivity of EGFR and CK5/6 were noted \((24,25)\). Ki-67 labeling indices (16%) were not particularly high in the apocrine DCIS when compared with non-apocrine DCIS (12%).

Apocrine carcinoma reportedly shows relatively favorable clinical behavior \((26,27)\). From the clinicopathological viewpoint, including the prognosis, pathogenesis and therapy, it seems reasonable that the apocrine-type TNBC should be distinguished from the non-apocrine-type TNBC (quadruple-negative breast cancer). In fact, in the apocrine-type TNBC, the proliferation rate is lower than the quadruple-negative type, and the p53 oncoprotein alteration is more closely related to the carcinogenesis in the apocrine-type malignancy, as has been suggested \((22)\). Hormone therapy targeted against AR may be applicable to the apocrine type \((28)\). Furthermore, the clinical response of molecular-targeted therapy against EGFR may be different between the apocrine-type TNBC and quadruple-negative breast cancer \((7,29,30)\). Biological differences between triple-negative, AR-positive (apocrine type) and quadruple-negative IDCs should be analyzed in future clinical studies.

Recent topics of breast cancer treatment are centered on TNBC commonly accompanying the basal-like DNA profiling. Classification by gene profiling using DNA microarray analysis opened a new era in histological evaluation of breast cancer \((8–10,31)\) and also in the therapeutic implications and prediction of the prognosis \((32,33)\). In order to detect basal-like TNBC, an immunohistochemical approach to demonstrating EGFR, CK5/6 and CK14 became popular, in order to replace the expensive and complicated DNA microarray analysis. However, such immunohistochemical markers as ER, PgR, HER2, p53, EGFR, CK5/6 and CK14 scarcely distinguish the apocrine-type TNBC from the non-apocrine type \((6,7)\). As shown in the present study, apocrine-type carcinomas in the author’s definition were never uncommon (44 in 325 IDCs, 14%), sharing a half (44/91 = 48%) of ER-negative breast cancer, and a half (21/44 = 48%) of the apocrine-type breast cancers were HER2-negative and thus categorized as TNBC \((6)\). In addition, MED, accompanying high-grade nuclear atypia but with a favorable prognosis, and CCC, a rare high-grade malignancy with solid and papillary structures \((1)\) the author happened to experience, also showed the similar immunophenotypes categorizable in TNBC \((7,16)\). The author positively insists that AR should be added to the panel of immunohistochemistry for evaluating breast cancer.

The author emphasizes again that the diagnosis of apocrine-type IDC should be made not only with the histological features but also with immunohistochemical HR localization and believes that the histological criteria of apocrine carcinoma are not necessarily solid and are pathologist-dependent. In the present study, 22 (50%) of 44 ER-/PgR-/AR+ IDCs showed classical apocrine features, but the remaining 21 were not typically apocrine, principally because of amphophilic and/or agranular cytoplasm and a high nuclear/cytoplasmic ratio of the cancer cells. In fact, all IDC lesions of the classical apocrine type expressed both EGFR and GCDFP15, while the expression rate of GCDFP15 was low in the non-classical and poorly differentiated types \((7/17 = 41\%)\), and two non-classical lesions lacked EGFR expression. The low rate of GCDFP15 expression in the non-classical apocrine-type cancers, probably related to abortive phenotypic differentiation, should further be evaluated. Meanwhile, GCDFP15 was not specific to the apocrine type, being commonly expressed in non-apocrine-type DCIS \((23/30 = 77\%)\) and ER+/HER2− IDC \((86/142 = 61\%)\), as has been reported \((34)\).

The author’s microscopic criteria of apocrine carcinoma should be more strict, because the percentage of classical apocrine carcinoma in total breast cancer \((22/440 = 5\%)\) was still high when compared with the reported frequency 0.3–4% \((17)\). Of special note is the high frequency of apocrine-type DCIS: 13 (24%) of 54 lesions belonged to the apocrine type, with 11 (20%) being categorized in classical apocrine DCIS. Meanwhile, a total of 10 non-apocrine-type IDC lesions exhibited microscopic findings suggestive of non-classical apocrine type to the author’s eyes. It has been described so far that low percentage of apocrine carcinoma, judged under a microscope, expressed ER and/or PgR \((2,26,27)\), and thus became the target of hormone therapy. For establishing reproducible and contemporary criteria for the appropriate treatment, apocrine carcinoma could be defined as ER-/PgR-/AR+ IDCs with apocrine-like microscopic appearance. Benign apocrine metaplasia always demonstrated the ER-/PgR-/AR+ phenotype, and this is the key finding for the author’s proposal.

In the present days where immunohistochemical as well as molecular analyses are so popular particularly in the breast cancer field, we pathologists must not stick the old-fashioned histological criteria for diagnosing apocrine carcinoma: histological distinction between apocrine carcinoma and carcinoma with apocrine-like features may not be important. In the near future when specific treatment strategy targeted to AR and/or EGFR expressed on apocrine-type carcinoma might be developed, the objective definition of ER-/PgR-/AR+ IDC the author proposed would be helpful.

Previous studies on the gene expression profile indicated that the ‘molecular apocrine’-type breast cancer showed a defined gene set distinguishable from the basal-like type \((7,29,30)\). Moreover, ‘molecular apocrine’ carcinomas significantly overlapped with the HER2 group. The St Gallen consensus 2009 stated that apocrine carcinoma, MED, and ACC might be categorized in TNBC, but should be distinguished from the basal-like TNBC because of their favorable prognosis \((16)\). Further analysis is definitely needed to focus on the possibility that the basal-like breast cancer
specifically corresponds to the AR-negative TNBC, namely quadruple-negative type.

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Conflict of interest statement

None declared.

References