Histology and Histopathology

From Cell Biology to Tissue Engineering

Diagnostic utility of CD205 in breast cancer: Simultaneous detection of myoepithelial cells and dendritic cells in breast tissue by CD205

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Summary. Background. CD205 can be used to detect myoepithelial cells (MECs) and dendritic cells (DCs) in breast tissue. However, the usefulness of CD205 immunostaining in the pathological diagnosis of breast tumors is not fully understood. The objective of this study was to re-evaluate CD205 co-expression with other MEC markers, such as p63 and CD10, in nonneoplastic and neoplastic breast tissue and to evaluate its pathological diagnostic utility in these types of breast cancer.

Material and methods. Nonneoplastic breast tissue samples with a terminal duct lobular unit and duct were obtained from fibroadenoma and mastopathy patients. Neoplastic breast tissue samples included ductal carcinoma *in situ* (DCIS) (n=43) and invasive ductal carcinoma (IDC) (n=60), including the tubule-forming type (n=20). These specimens were investigated by CD205, p63, and CD10 immunostaining.

Results. In addition to p63 and CD10, CD205 was expressed on MECs in nonneoplastic breast and DCIS tissue samples; CD205 was simultaneously detected on DCs that had infiltrated DCIS and IDC tumor nests. CD205 was expressed on cancer cells themselves in only 7.3% of the breast cancer samples. The number of intratumoral CD205$^+$ DCs in tubular IDC was significantly higher than that in DCIS (P<0.01).

Conclusion. Because CD205 was simultaneously detected on MECs and DCs in the same breast tissue sections, it may be useful for distinguishing tubular IDC from DCIS.

Key words: CD205, Myoepithelial cell, Dendritic cell, Breast tissue, Pathological diagnosis

Introduction

CD205 (also known as DEC205, LY-75, CLEC13B, and gp200-MR6) is an endocytic receptor homologous to the macrophage mannose receptor. CD205 is expressed on dendritic cells (DCs), activated plasmacytoid DCs, alveolar macrophages and thymic epithelial cells (Ohtani, 2013). Some previous studies have indicated that CD205 is expressed in some tumors (Nonaka et al., 2007; Faddaoui et al., 2016; Merlino et al., 2019).

Myoepithelial cell (MEC) markers, such as p63, CD10, α-smooth muscle actin, smooth muscle myosin heavy chain, calponin, and maspin are useful for identifying MECs in breast tissue (Moriya et al., 2009; Umekita et al., 2018). Schnitt et al. reported that high-grade ductal carcinoma *in situ* (DCIS) could reduce the frequency of MECs (Schnitt et al., 2012). Conversely, maspin expression by tumor cells themselves could be a poor prognostic factor for invasive breast cancer patients (Berardi et al., 2013).

Furthermore, al-Tubuly et al. (1996) demonstrated that CD205 expression is associated with benign and

most *in situ* tumors, while it is absent in the majority of invasive carcinomas, thus raising the possibility that the loss of CD205 expression may play a role in tumorigenesis. They also noted that cells of the outer layer, i.e., MECs, which are positive for CD10 and/or smooth muscle actin, homogeneously expressed CD205 (al-Tubuly et al., 1996). Conversely, it has been reported that CD205 expression is elevated in triple negative breast cancer compared to other breast cancer types (Merlino et al., 2019). Thus far, the usefulness of CD205 immunostaining in the pathological diagnosis of breast tumors is not fully understood.

The objective of this study was to re-evaluate CD205 expression along with that of other MEC markers, such as p63 and CD10, in nonneoplastic and neoplastic breast tissue samples, including DCIS, invasive ductal carcinoma (IDC), and invasive lobular carcinoma (ILC), and to evaluate its pathological diagnostic utility in these types of breast cancer. In this study, among MEC markers, p63 and CD10 were used because they are useful for identifying MECs, but not myofibroblasts or vascular smooth myocytes, on routine pathological diagnosis (Moriya et al., 2009). DC infiltration into the tumor nest may closely reflect cancer invasion into the adjacent stroma, which may be compatible with invasive breast carcinoma (Tsuge et al., 2000). Therefore, we compared the frequency of intratumoral CD205$^+$ DCs, especially between tubular IDC and DCIS.

Materials and methods

Tissue specimens

A total of 128 female patients were included. The specimens included 123 neoplastic samples from breast cancer patients with DCIS [n=43; low nuclear grade (n=14, mean age 59.1 years, range 37-78), intermediate nuclear grade (n=11, mean age 57.2 years, range 40-78), and high nuclear grade (n=18, mean age 63.1 years, range 36-83)] (Schnitt et al., 2012), conventional IDC of no special type [n=60; tubule forming type (n=20, mean age 56.3 years, range 30-74), solid type (n=20, mean age 58.1 years, range 40-81), and scirrhous type (n=20, mean age 57.3 years, range 39-79)], and ILC (n=20, mean age 68.8 years, range 46-86); in addition, 5 nonneoplastic breast samples were examined, including the terminal duct lobular unit (TDLU) and duct (n=5, mean age 38.0 years, range 25-49) 5 mm away from fibroadenoma and mastopathy. Pathological diagnoses were made at Yamagata University Hospital, Yamagata Prefectural Shinjo Hospital, Sanyudo Hospital, and Yamagata Saisei Hospital between 2003 and 2013. All samples were reviewed and evaluated by two pathologists (O.R. and Y.M.), and the type of IDC was classified according to the Japanese Classification of Breast Cancer, 18th edition (2018) (Japanese Breast Cancer Society, 2018). Tissues were fixed in 10% neutral buffered formalin for 6-12 hours at room temperature, embedded in paraffin,

and used for immunohistochemical and immunofluorescence double staining.

This study was approved by the Research Ethics Committee of Yamagata University Faculty of Medicine (2018-249) and was performed in accordance with the Declaration of Helsinki.

Single and double immunohistochemical (IHC) and double immunofluorescence staining

Immunohistochemistry was performed as previously described (Ohe et al., 2018) using antibodies against CD205 (11A10; mouse IgG1, Novocastra, Leica Biosystems, Nussloch, Germany), CD205 (LY-75; EPR5233; rabbit IgG, Abcam, Cambridge, BK), p63 (4A4; mouse IgG2a, ϰ, Nichirei Biosciences, Tokyo, Japan), and CD10 (56C6; mouse IgG1, Nichirei Biosciences). Phosphate-buffered saline (PBS; 0.01 M, pH 7.4), Universal Negative Control-Mouse (N1698; DAKO, Agilent Technologies, Santa Clara, CA), and Universal Negative Control-Rabbit (N1699; DAKO, Agilent Technologies) were used as negative controls.

Three-micron-thick sections were deparaffinized, and endogenous peroxidase activity was blocked with methanol containing 0.3% hydrogen peroxide. Antigen retrieval was performed using citrate (pH 6; Nichirei Biosciences) or EDTA (Antigen Retrieval Solution, pH 9; Nichirei Biosciences) in an autoclave (2 atmospheres, 121°C, 20 min). The sections were incubated with primary antibodies at room temperature overnight. The streptavidin-biotin peroxidase labelling method was used (UltraTech HRP Streptavidin-Biotin Detection system, PN IM2391; Immunotech, Marseille, France). Positive reactions were detected by a brown color using 3,3'-diaminobenzidine tetrahydrochloride. The sections were then counterstained with hematoxylin. The streptavidin (Streptavidin/AP; DAKO, Agilent Technologies)-biotin alkaline phosphatase labelling method and Anti-Mouse EnVision$^+$ System-HRP-Labeled Polymer (DAKO, Agilent Technologies) were used for double staining. Positive reactions were detected by a blue and red color produced by BCIP/NBT (BCIP/NBT Substrate System, DAKO, Agilent Technologies) and AEC (Simple Stain AEC Solution, Nichirei Biosciences), respectively. Double immunofluorescence staining of formalin-fixed paraffin-embedded tissue sections was performed as previously described (Meng et al., 2015).

The number of positive MECs and tumor cells per objective field (x200) was calculated in the 10 areas with the most positive cells per case by single staining (Table 1). Positive MECs for CD205, p63, and CD10 were counted in a total of ≥6,500 cells from DCIS cases. Similarly, the number of CD205/p63$^+$ or CD205/CD10$^+$ MECs per objective field (x200) was calculated in the 10 areas with the most positive cells per case by double staining. We evaluated the correlations based on these data. The number of CD205$^+$ DCs and tumor cells per objective field (x200) was calculated in the 10 areas only between tubular IDC and DCIS because it is not difficult

to differentiate DCIS from other types of IDC, e.g., solid and scirrhous types.

Statistical analysis

The Spearman test was used to examine the number of CD205^{+} and p63^{+} or CD10^{+} cells double stained in nonneoplastic and neoplastic breast tissue samples. The Mann-Whitney test was used to compare the number of intratumoral CD205^{+} DCs between tubular IDC and DCIS. Statistical analyses in this study were performed using JMP, version 14 (SAS Institute, Tokyo, Japan). Differences with a P value <0.05 were considered significant in each analysis.

Results

Expression of CD205 and MEC markers in the TDLU and duct of nonneoplastic breast tissue samples

Double IHC staining of MECs in the TDLU and duct of nonneoplastic breast tissue samples showed CD205 on the cell membrane, p63 in the nucleus, and CD10 in the cytoplasm of MECs (Fig. 1A,B). Double IHC staining for CD205 and p63/CD10 showed a good correlation in both the TDLU (CD205-p63, r_s=0.68, P<0.01; CD205-CD10, r_s=0.60, P<0.01) and duct (CD205-p63, r_s=0.98, P<0.01; CD205-CD10, r_s=0.96, P<0.01) in nonneoplastic breast tissue samples. Double immunofluorescence staining also confirmed that CD205 and p63 or CD10 were simultaneously expressed on MECs (Fig. 1C-F). Although CD205 was expressed on some luminal cells of the duct, as previously described (al-Tubuly et al., 1996), it was not difficult to evaluate CD205^{+} MECs because MECs were morphologically distinct from luminal cells.

Expression of CD205 and MEC markers in neoplastic breast tissue samples

Single IHC staining for CD205, p63 and CD10 revealed frequently identifiable MECs in all grades of DCIS (Table 1) (Fig. 2A). Conversely, MECs were not identifiable in any neoplastic breast tissue samples of any type of IDC or ILC. The sensitivity of CD205^{+} MEC to differentiate DCIS from IDC was 81.4% (35 of 43). The specificity of that was 100% (0 of 60). The sensitivity and specificity of p63 or CD10 was as follows; 88.4% (38 of 43) and 100% (0 of 60), 90.7% (39 of 43) and 100% (0 of 60). The proportion of CD205, p63, and CD10 expression on MECs was prone to reduce in high nuclear grade DCIS compared with non-high nuclear grade DCIS [CD205, 61.1% (11/18) vs. 96% (24/25); p63, 72.2% (13/18) vs. 100% (25/25); CD10, 77.8% (14/18) vs. 100% (25/25)]. Double IHC staining showed the simultaneous expression of CD205/p63 and CD205/CD10 on MECs in all grades of DCIS, with good correlations (CD205-p63, r_s=0.62, P<0.01; CD205-CD10, r_s=0.46, P<0.01) (Fig. 2B). CD205 was expressed on cancer cells themselves in only 9 cases (Table 1) (Fig. 2C). Neither p63 nor CD10 was expressed on cancer cells themselves in our cases.

Comparison of intratumoral CD205^{+} DCs between tubular IDC and DCIS

This study compared the frequency of CD205^{+} DCs between tubular IDC and DCIS. More intratumoral CD205^{+} DCs were present in tubular IDC [1.48±1.77 cells/objective field (x200)] than in DCIS [0.05±0.26 cells/objective field (x200)] (P<0.01) (Fig. 2A,D,E).

Discussion

Our study yielded two major findings. First, we confirmed that the number of CD205^{+} MECs was well correlated with that of p63^{+} or CD10^{+} MECs in nonneoplastic and neoplastic breast tissue samples. Moreover, in most DCIS samples, MECs tended to remain in the tumor nests. Second, more intratumoral CD205^{+} DCs were found in tubular IDC than in DCIS. These results could provide the first indication that CD205 immunostaining may be useful for distinguishing tubular IDC from DCIS.

Table 1. Expression of CD205, p63, and CD10 in neoplastic breast tissue, as detected by immunohistochemistry.

Neoplastic tissue	n	Myoepithelial cells			CD205+ tumor cells
		CD205	p63	CD10	
Ductal carcinoma *in situ*					
Low nuclear grade	14	100% (14/14)	100% (14/14)	100% (14/14)	14.3% (2/14)
Intermediate nuclear grade	11	90.9% (10/11)	100% (11/11)	100% (11/11)	0% (0/11)
High nuclear grade	18	61.1% (11/18)	72.2% (13/18)	77.8% (14/18)	11.1% (2/18)
Invasive carcinoma					
Invasive ductal carcinoma					
Tubule-forming type	20	0% (0/20)	0% (0/20)	0% (0/20)	5% (1/20)
Solid type	20	0% (0/20)	0% (0/20)	0% (0/20)	5% (1/20)
Scirrhous type	20	0% (0/20)	0% (0/20)	0% (0/20)	5% (1/20)
Invasive lobular carcinoma	20	0% (0/20)	0% (0/20)	0% (0/20)	10% (2/20)

In this study, CD205 was expressed on MECs in nonneoplastic breast tissue samples and DCIS samples (Table 1). Furthermore, the sensitivity for MEC of CD205 was at the same degree as that of p63 or CD10, and the number of CD205+ cells showed a good correlation with that of p63+/CD10+ cells. These results
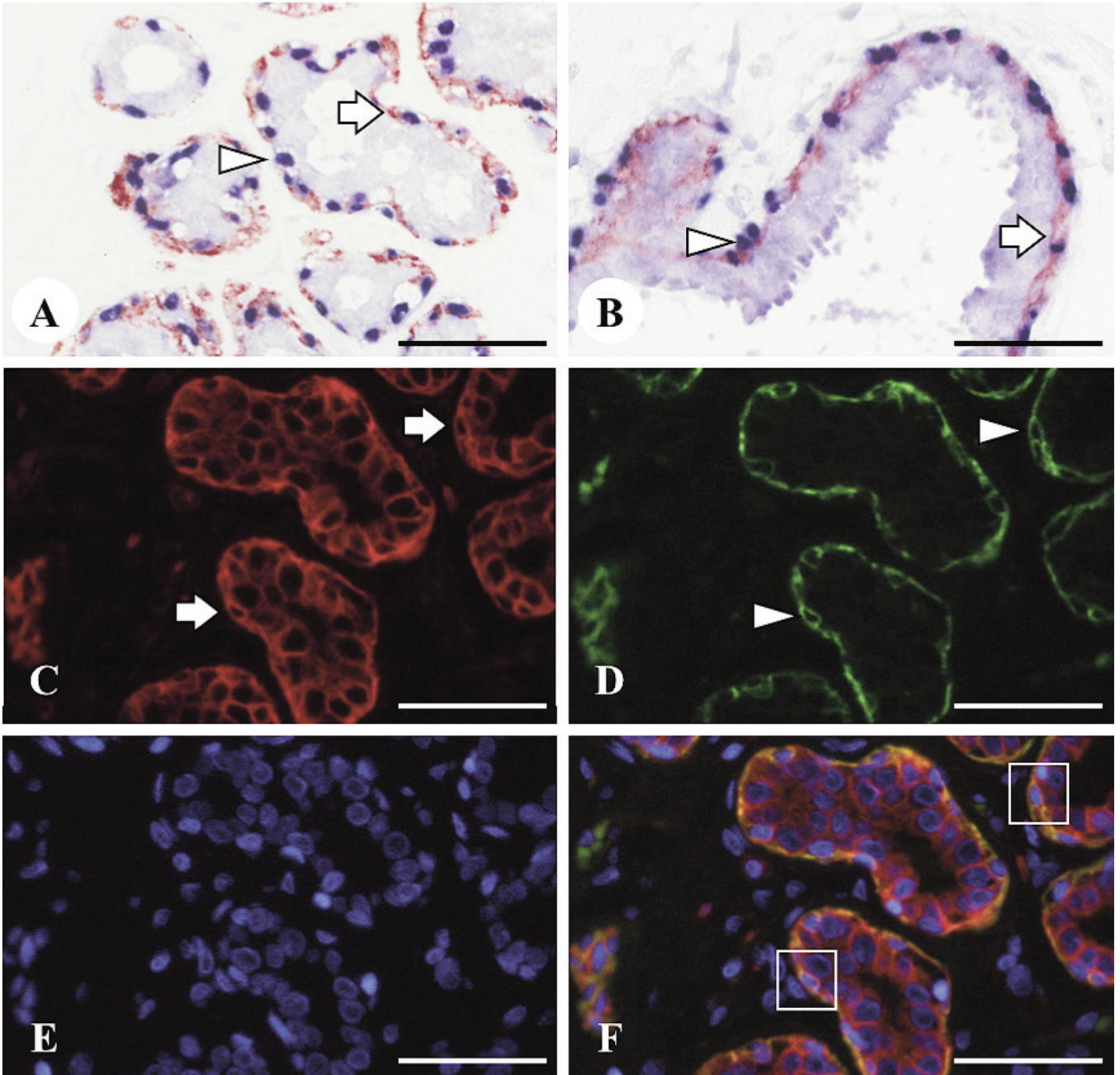

Fig 1. Double immunohistochemical staining for CD205 and p63 **(A, B)** and double immunofluorescence staining for CD205 and CD10 **(C-F)** in nonneoplastic breast tissue samples. With double immunohistochemical staining, CD205 is observed on the cell membrane **(A, B**; AEC, red) and p63 is observed in the nucleus **(A, B**; BCIP/NBT, blue). CD205 and p63 are expressed on myoepithelial cells (MECs) in the terminal duct lobular units (TDLUs) **(A)** and ducts **(B)** of nonneoplastic breast tissue samples. Arrows indicate CD205+ MECs and arrowheads indicate p63+ MECs **(A, B)**. CD205 **(C**; rhodamine, red) indicates epithelial cells and MECs (arrows) in the TDLU. CD10 **(D**; FITC, green) indicates MECs (arrowheads) in the TDLUs. Nuclear staining was performed with DAPI **(E)**. Cells positive for both CD205 and CD10 are also found among MECs in a similar localization pattern **(F**; merged, yellow, squares). Scale bars: 50 μm.

suggested that CD205 may be a candidate marker for identifying MECs, in addition to p63 and CD10. A previous study reported that the proportion of smooth muscle myosin heavy chain expression on MECs was significantly reduced in high nuclear grade DCIS compared with non-high nuclear grade DCIS although that of p63 and CD10 had no significant difference (Hilson et al., 2009). Our results were slightly different
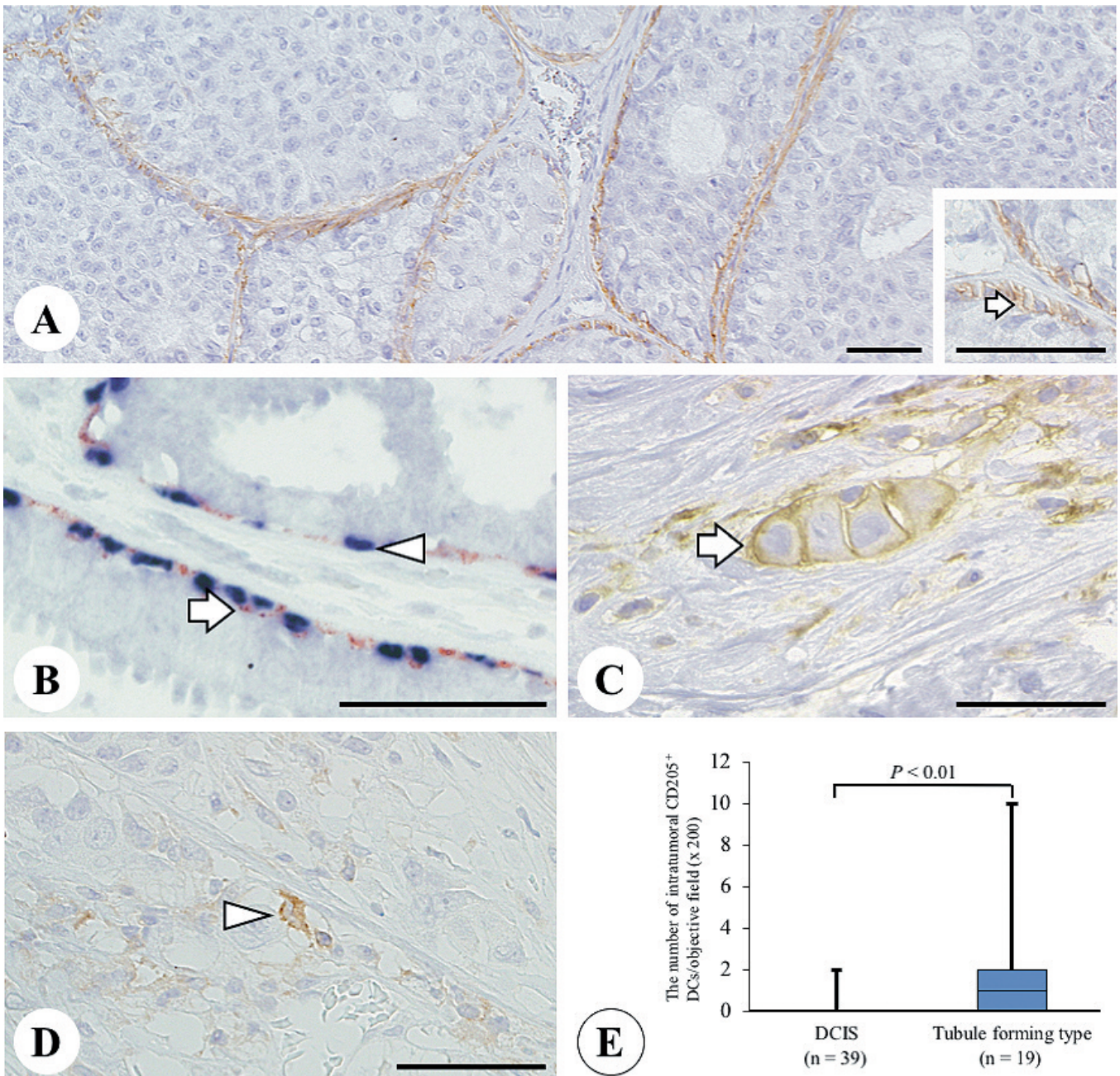

Fig. 2. Immunohistochemical staining for CD205 and p63 in ductal carcinoma *in situ* (DCIS) samples **(A, B, E)** and staining for CD205 in invasive ductal carcinoma (IDC) samples **(C-E)**. With single immunohistochemical staining, myoepithelial cells (MECs) are positive for CD205 **(A)**. CD205 expression is present on the cell membrane of MECs (**A**: Inset, arrow). MECs double positive for CD205 (**B**; AEC, red) and p63 (**B**; BCIP/NBT, blue) are observed in DCIS. Arrows indicate CD205^{+} MECs, and arrowheads indicate p63^{+} MECs. CD205 is infrequently expressed on the breast cancer cells themselves (**C**; arrow). CD205^{+} dendritic cells (DCs) (arrowhead) infiltrated tumor nests in IDC **(D)** but not DCIS **(A)**. The number of intratumoral CD205^{+} DCs in tubular IDC [1.48±1.77 cells/objective field (x200)] is greater than that in DCIS [0.05±0.26 cells/objective field (x200)] (P<0.01) **(E)**. Scale bars: 50 µm.
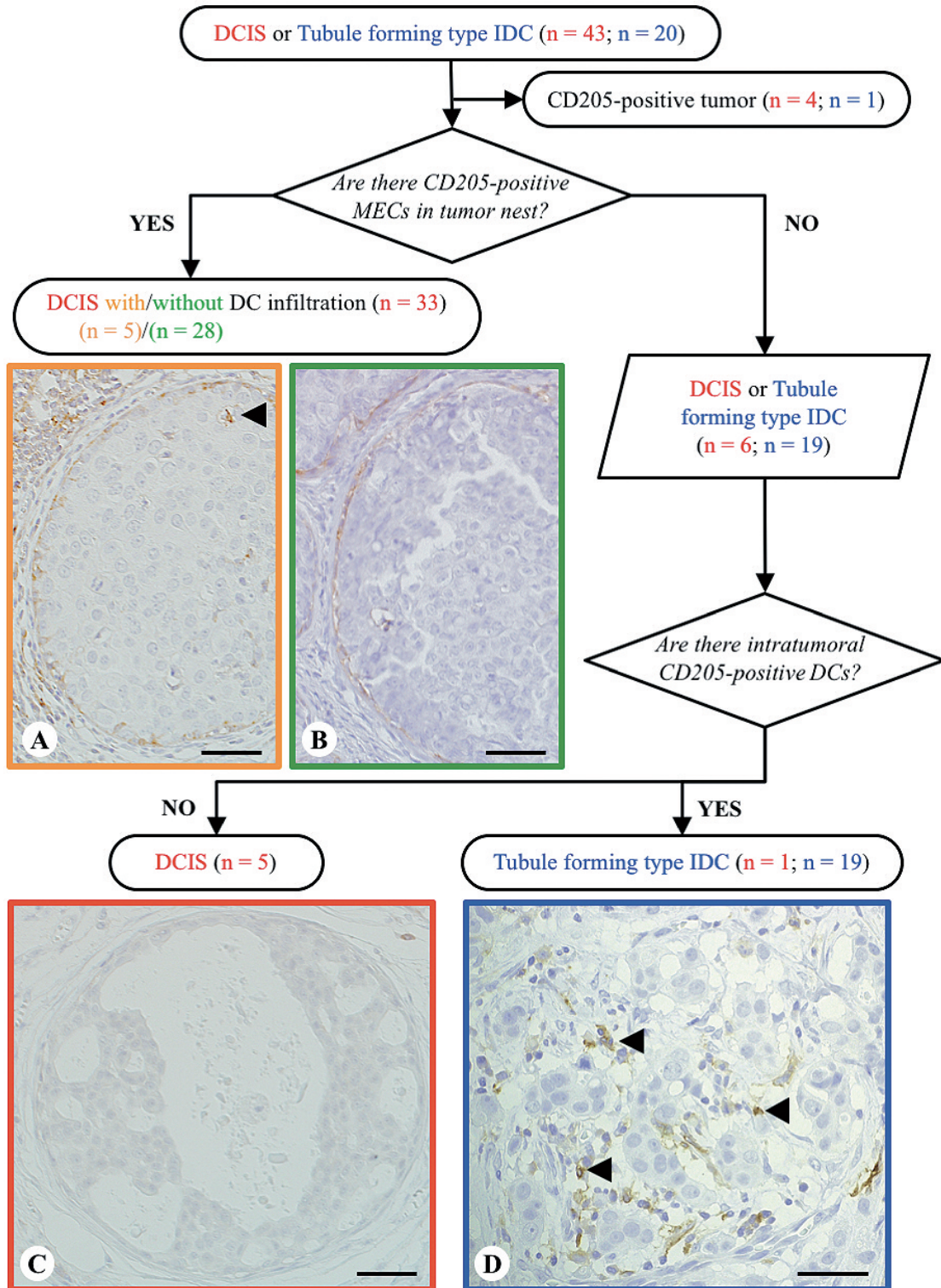
Fig. 3. Differential diagnostic flow chart for ductal carcinoma *in situ* (DCIS) **(A-C)** and tubular invasive ductal carcinoma (IDC) **(D)** by CD205 immunostaining. The specimens included 63 neoplastic samples from breast cancer patients with DCIS (n=43) and tubular IDC (n=20) are re-evaluated by this algorithm. The presence of CD205+ myoepithelial cells (MECs) in tumor nests with/without intratumoral CD205+ dendritic cell (DC) infiltration suggests DCIS (**A, B**; low nuclear grade DCIS, n=12; intermediate nuclear grade DCIS, n=10, high nuclear grade DCIS, n=11). The absence of CD205+ MECs in tumor nests without intratumoral CD205+ DCs suggests DCIS (**C**; high nuclear grade DCIS, n=5). The absence of CD205+ MECs in the tumor nest with intratumoral CD205+ DCs suggests tubular IDC (**D**; intermediate nuclear grade DCIS, n=1; tubular IDC, n=19). The sensitivity of CD205 to differentiate DCIS from IDC is 97.4% (38 of 39) by applying this algorithm. The specificity of that is 95% (19 of 20). There are five cases in which CD205 was expressed by tumor cells themselves (low nuclear grade DCIS, n=2; high nuclear grade DCIS, n=2; tubular IDC, n=1). Arrowheads indicate CD205+ DCs. Scale bars: 50 µm.

in that the proportion of CD205, p63, and CD10 expression on MECs was prone to reduce in high nuclear grade from non-high nuclear grade DCIS. Therefore, some MEC markers may be sometimes useless for identifying high nuclear grade DCIS-associated MEC.

Previous studies have indicated that CD205 is expressed in high-grade serous carcinoma of the ovary, thymoma, pancreatic cancer, and diffuse large B-cell lymphoma (Nonaka et al., 2007; Faddaoui et al., 2016; Merlino et al., 2019). Though CD205 was downregulated in breast cancer (al-Tubuly et al., 1996), this expression was elevated in triple negative breast cancer compared to other breast cancer types, recently (Merlino et al., 2019). Faddaoui et al. reported that the CD205 (LY-75) gene corresponds to metastatic potential (Faddaoui et al., 2016). Moreover, Merlino et al. demonstrated that a novel and selective antibody-drug conjugate (ADC), MEN1309/OBT076, acted against CD205 positive tumors (Merlino et al., 2019). Our results showed that CD205 was expressed by tumor cells themselves in only 7.3% (9/123) of breast cancer cases, although it could not be investigated whether these cases were triple negative. Therefore, clinicopathological features of CD205^{+} breast cancer cases should be investigated for future studies.

Until now, the frequency of DCs in breast cancer tissue has not been fully understood. Heys SD, et al. reported that the number of infiltrating CD205^{+} cells in large breast cancers (>3 cm) or locally advanced breast cancers is not associated with the effect of chemotherapy (Heys et al., 2012). Tsuge et al. indicated that DC infiltration into the tumor nest suggests tumor invasion into the adjacent stroma (Tsuge et al., 2000). Similarly, in this study, CD205 could also be used to identify intratumoral DCs (Fig. 2D), and more intratumoral CD205^{+} DCs were found in the tubular IDC samples than in the DCIS samples (P<0.01) (Fig. 2E), suggesting that an increased number of tumor-infiltrating CD205^{+} DCs [probably more than one CD205^{+} DC per microscopic field (x200)] may be useful for distinguishing tubular IDC from DCIS.

Our CD205 immunostaining results demonstrate the simultaneous detection of MECs and DCs in the same tissue sections, which could provide an algorithm for the pathological differential diagnosis between tubular IDC and DCIS (Fig. 3). The presence of CD205^{+} MECs in the tumor nest with the absence of intratumoral CD205^{+} DCs favors DCIS, while the presence of intratumoral CD205^{+} DCs favors tubular IDC. Indeed, by applying this algorithm to this study data, the sensitivity of CD205 to differentiate DCIS from IDC raised to 97.4% (38 of 39) from 81.4% (35 of 43), which was the sensitivity of the ordinary method confirming only MECs. Furthermore, this algorithm was useful to distinguish DCIS from tubular IDC when the proportion of MEC markers reduced in high nuclear grade DCIS (Fig. 3C).

In conclusion, because CD205 can simultaneously reveal MECs and DCs in breast tissue, it may be useful for distinguishing between tubular IDC and DCIS.

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