福岡大医紀（*Med. Bull. Fukuoka Univ.*）：34(1), 15–21, 2007

The Patterns of Pitfalls in the Application of Smooth Muscle Actin (SMA) Immunostaining for Breast Cytological Analyses

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Abstract：The identification of myoepithelial cells (MECs) is useful for breast cytology. Since MECs are not always easily identified morphologically, the immunostaining of smooth muscle actin (SMA) which is a common and a reliable marker for MECs is a useful tool. Our purpose was to investigate the patterns of pitfalls regarding the application of SMA immunostaining for breast cytological analyses. Sixty-five cases of invasive breast carcinoma were available for both immunocytochemical and immunohistochemical examinations. There were 3 patterns of pitfalls as follows. 1) Many SMA positive myofibroblasts mimicking MECs were scattered in the background (18.5%). 2) The clusters with SMA negative tumor cells and SMA positive MECs or myofibroblasts were recognized and found to mimic benign dimorphic clusters (10.8%). 3) The clusters composed of SMA positive tumor cells suggested the possibility of myoepithelial differentiation (9.2%). In conclusion, it is necessary to keep in mind the 3 patterns of pitfalls when performing SMA immunostaining for breast cytological analyses.

Key words：**Breast cytology, Breast carcinoma, Immunostaining, Smooth muscle actin (SMA), Myoepithelial cell, Pitfalls**

Introduction

The identification of myoepithelial cells (MECs) located between ductal epithelial cells and the basal lamina is useful in breast pathology for differentiating benign breast lesions from invasive breast carcinoma. It is also an important point for breast cytology to identify MECs. The problem is that MECs are not always easily identified cytomorphologically. Therefore immunocytochemical staining has been used to demonstrate MECs. The various antibodies that have been studied for this purpose include S100 protein, muscle specific actin (HHF35), and smooth muscle actin (SMA). Previous studies have reported that SMA appeared to be the most reliable marker for the recognition of MECs in three putative markers of MECs(antibodies to S100 protein, HHF-35, and SMA).[1)2)] However, previous studies also reported that the immumostaining for SMA demonstrated not only smooth muscle and MECs, but also myofibroblasts and tumor cells.[1)3)] In our previous study, we investigated the reliability of SMA immunostaining for breast cytology in both benign and malignant cases.

In the present study we used SMA on formalin-fixed, paraffin-embedded tissue sections and their imprint cytologic smears of invasive breast carcinomas. Our purpose was to investigate the patterns of pitfalls regarding the application of SMA immunostaining for breast cytological analyses.

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Materials and Methods

Sixty-five cases of invasive breast carcinomas were available for both immunohistochemical and immunocytochemical examinations. Table 1 shows the histologic subtypes of the breast carcinoma. All the cases were derived from the surgical pathology files of Fukuoka University Hospital ; they were diagnosed as invasive breast carcinoma and classified based on various subtypes. In addition, the cases of invasive ductal carcinomas (IDCs) were classified into three subtypes ; papillotubular type dominantly consisting of cribriform structure, solid-tubular type and scirrhous type with productive fibrosis (Table 1).

At first, the resected specimens were used to prepare imprint cytologic smears and then were embedded in paraffin after formalin fixation. The imprint cytologic smears were fixed with 95% alcohol and then were stained with Papanicolaou stain.

For immunostaining, both imprint cytologic smears and 5-μm-thick tissue sections were processed by alkaline phosphatase labeled streptavidin biotin techniques using the antibody of smooth muscle actin (alpha-SMA; 1 A 4, DAKO, Glostrup, Denmark) with both positive and negative controls.

When immunopositivity was present, the location and the distribution of positivity was carefully recorded for myoepithelial, ductal, and stromal cells.

Results

Table 2 summarizes the results of immunocytochemical staining in the 65 breast carcinomas arranged based on a histologic diagnosis. Table 3 is a summary of the results of immunohistochemical staining in both stroma and tumor cells.

Twenty-five cases (38.5%) showed positivity for

Table 1. Histopathologic Subtypes in Breast Carcinomas

Invasive ductal carcinoma	Papillotubular type	6
	Solid-tubular type	16
	Scirrhous type	28
Mucinous carcinoma		6
Medullary carcinoma		3
Invasive lobular carcinoma		1
Adenoid cystic carcinoma		1
Tubular carcinoma		1
Papillary carcinoma		3
Total		65

Table 2. The results of Immunocytochemical Staining for Imprint Smears

		Group 1 (%)	Group 2 (%)	Group 3 (%)
Invasive ductal carcinoma	papillotubular type	0/6 (0)	3/6 (50)	0/6 (0)
	solid-tubular type	2/16 (12.5)	0/16 (0)	1/16 (6.3)
	scirrhous type	5/28 (17.9)	2/28 (7.1)	2/28 (7.1)
Mucinous carcinoma		3/6 (50)	0/6 (0)	1/6 (16.7)
Medullary carcinoma		0/3 (0)	0/3 (0)	1/3 (33.3)
Invasive lobular carcinoma		0/1 (0)	0/1 (0)	0/1 (0)
Adenoid cystic carcinoma		0/1 (0)	0/1 (0)	1/1 (100)
Tubular carcinoma		0/1 (0)	0/1 (0)	0/1 (0)
Papillary carcinoma		2/3 (66.7)	2/3 (66.7)	0/3 (0)
Total		12/65 (18.5)	7/65 (10.8)	6/65 (9.2)

*Group 1 : Isolated SMA positive fibroblasts are recognized in the background.
*Group 2 : Dimorphic clusters with SMA negative tumor cells and positive fibroblasts are recognized.
*Group 3 : SMA positive tumor cells are recognized.

SMA on immunocytochemical stained smears. They were divided into 3 groups according to the observed patterns of SMA positive cells. Group 1 comprised SMA positive cells which appeared to be myofibroblasts isolated and diffusely scattered in the background (Fig. 1). Group 1 consisted of 2 cases of IDC (solid-tubular type), 5 cases of IDC (scirrhous type), 3 cases of mucinous carcinoma and 2 cases of papillary carcinoma. Group 2 showed SMA positive cells which formed clusters with SMA negative tumor cells and mimicked a benign dimorphic pattern (Fig. 2). Group 2 consisted of 7 (10.8%) cases including 3 cases of IDC (papillotubular type), 2 cases of IDC (scirrhous type) and 2 cases of papillary carcinoma. In the group 3, SMA positive cells appeared to be tumor cells. Group 3 consisted of 6 (9.2%) cases comprising one case of IDC (solid-tubular type), 2 cases of IDC (scirrhous type), one case of mucinous carcinoma, medullary carcinoma and adenoid cystic carcinoma (Table 2, Fig. 3, Fig. 4).

Immunohistochemical staining of all cases showed a moderate to strong and focal positivity in the stroma of 54 (83.1%) cases and a partial and global positivity in the tumor nests of 6 (9.2%) cases (Table 3).

Table 3. The Results of Immunohistochemical Staining for Tissue Sections

		SMA positive stroma (%)	SMA positive tumor cells (%)
Invasive ductal carcinoma	papillotubular type	3/6 (50)	0/6 (0)
	solid-tubular type	15/16 (93.8)	1/16 (6.3)
	scirrhous type	27/28 (96.4)	3/28 (10.7)
Mucinous carcinoma		4/6 (66.7)	1/6 (16.7)
Medullary carcinoma		2/3 (66.7)	2/3 (66.7)
Invasive lobular carcinoma		0/1 (0)	0/1 (0)
Adenoid cystic carcinoma		0/1 (0)	1/1 (100)
Tubular carcinoma		0/1 (0)	0/1 (0)
Papillary carcinoma		3/3 (100)	0/3 (0)
Total		54/65 (83.1)	6/65 (9.2)

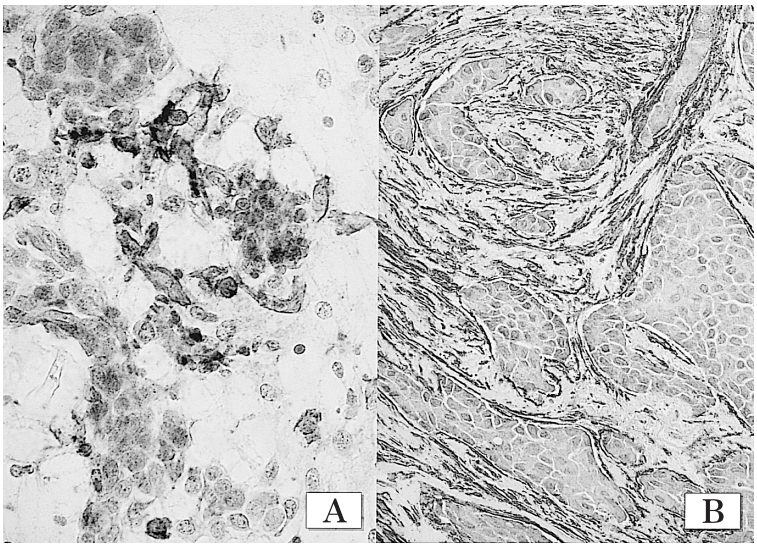

Figure. 1. Microscopic findings of a case of IDC (scirrhous type) in group 1. SMA positive myofibroblasts are scattered and observed to mimic MECs. (Immunocytochemical stain for SMA, ×200, left) Immunohistochemical staining shows SMA positive rich stroma. (Immunohistochemical stain for SMA, ×200, right)

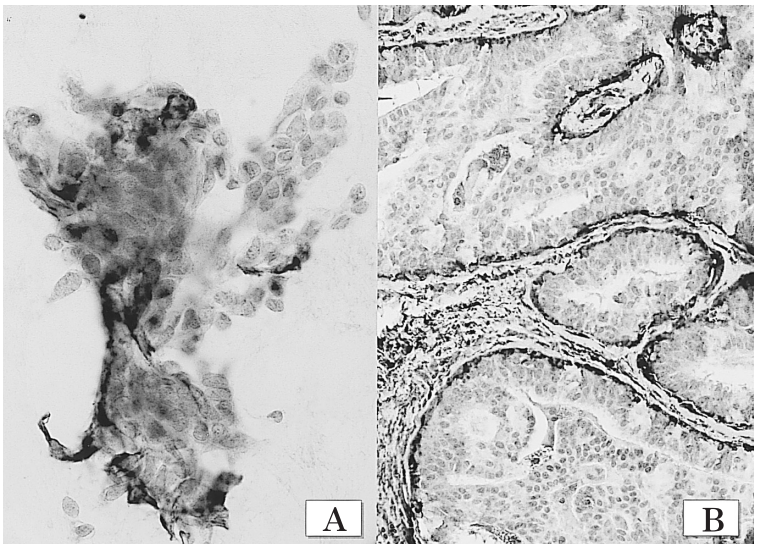

Figure. 2. Microscopic findings of a case of papillary carcinoma in group 2.
SMA positive myofibroblasts are forming a cluster with SMA negative tumor cells and mimicking a benign dimorphic pattern.(Immunocytochemical stain for SMA,×200, left)
The vascular core adjacent to tumor cells shows positivity for SMA. (Immunohistochemical stain for SMA,×200, right)

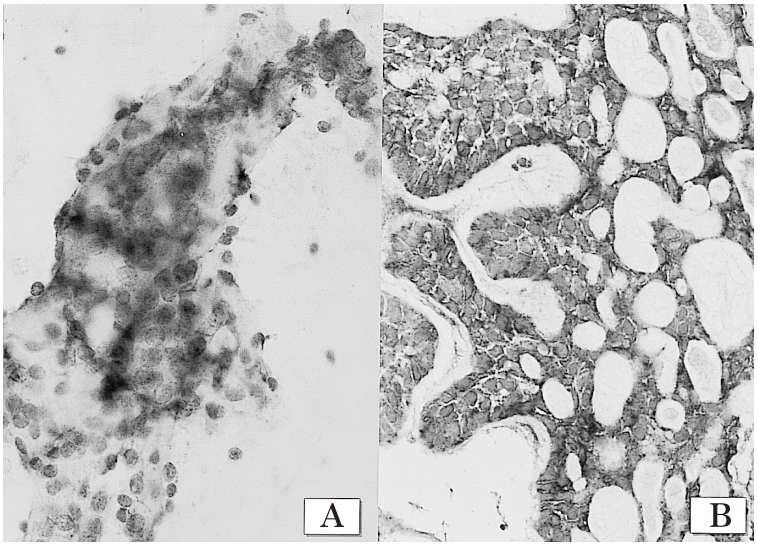

Figure. 3. Microscopic findings of a case of adenoid cystic carcinoma in group 3.
Some tumor cells in the cluster are positive for SMA. (Immunocytochemical stain for SMA,×200, left)
SMA positive tumor cells showing a cribriform pattern. (Immunohistochemical stain for SMA,×200, right)

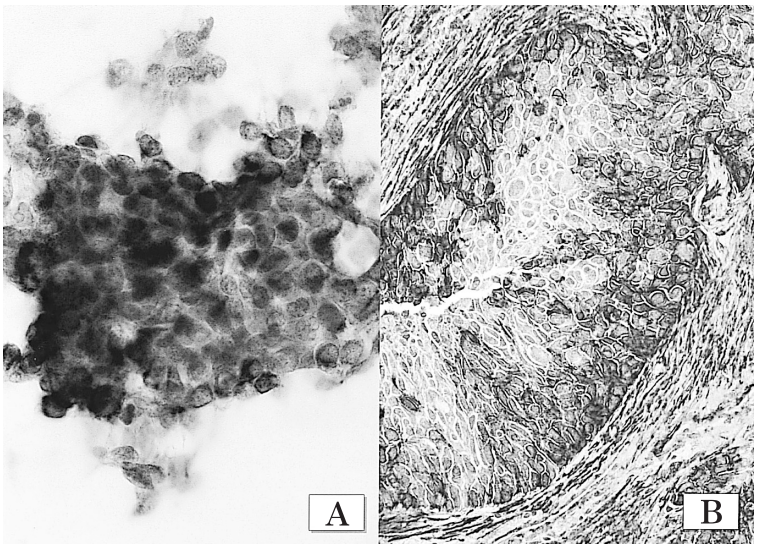

Figure. 4. Microscopic findings of a case of IDC (solid type) in group 3.
Most tumor cells forming a medullary cluster are positive for SMA.
(Immunocytochemical stain for SMA, ×200, left)
SMA positive tumor cells are seen in the periphery of the solid nest.
(Immunohistochemical stain for SMA, ×200, right)

Discussion

MECs are defined by their localization and cytomorphologic appearance as the spindle-cell elements located between the epithelial cells and the basal lamina of the acini and ducts of the breast. In the breast, MECs are universally observed in benign conditions and in some proliferative and neoplastic lesions. They are prominent in proliferative lesions such as intraductal papilloma,[4] proliferative epitheliosis and sclerosing adenosis. The recognition of MECs is a useful tool for making a cytological diagnosis. However, MECs are not always easily identified by cytological analyses. In histology, since MECs are identified based on both cytomorphologic features and structural location. On the other hand, in cytology, since exfoliative cells do not demonstrate a lot of structural information, MECs are almost always identified only by cytomorphologic features. Therefore immunocytochemical staining is thus considered to be a reliable ancillary study to identify MECs.

MECs are characteristically positive for a variety of antibodies that recognize actin (SMA[4)-7)] and HHF-35[8)9)]), smooth muscle myosin heavy chain, certain keratins,[10)] calponin/caldesmon,[11)19)20)] CD10,[20)] glial fibrillary acidic protein[12)] and p63.[19)20)] In these antibodies SMA was a reliable and widespread marker for the recognition of MECs[1)] and it is also valuable for making a cytological diagnosis.

In the group 1, SMA positive cells were isolated and diffusely distributed in the background. They appeared to not be MECs but to be myofibroblasts. The cases of IDC (scirrhous type) occupied a half of group 1 that possessed abundant stroma. Immunohistochemically the stroma in 54 (83.1%) cases showed a strong positivity within the myofibroblastic elements. It is possible for these cases that SMA postitive myofibroblasts exfoliate on cytologic smears.

In group 2, SMA positive cells were found in the clusters with SMA negative tumor cells. A previous study reported that SMA positive MECs were recognized in the benign epithelial cell clusters, but not in the malignant epithelial cell clusters.[2)] However, in our study, in 7 cases (10.8%), SMA positive cells were recognized in the clusters and mimicking benign dimorphic cluster. Group 2 consisted of 3 cases of IDC (papillotubular type), 2 cases of IDC (scirrhous type) and 2 cases of papillary carcinoma. In the cases of IDC (scirrhous type), the tumor cells were suggested to be exfoli-

ated with SMA positive myofibroblasts derived from rich stroma surrounding tumor nests. In the 3 cases of IDC (papillotubular type) and 2 cases of papillary carcinoma that occupied 71.4% of group 2, thus demonstrating two dimorphic patterns. One was a dimorphic pattern with SMA positive MECs and SMA negative tumor cells derived from non-invasive lesions of ductal spredding. The other was a dimorphic pattern with SMA positive myofibroblasts and SMA negative tumor cells. The tumor cells appear to be exfoliated with SMA positive myofibroblasts of a vascular core on the ground of that the stroma of vascular core in papillary structure was immunohistochemically positive for SMA.

In group 3, SMA positive cells appeared to be tumor cells. This "cross-reactivity" suggests the possibility of myoepithelial differentiation and/or a high actin content of breast tumor cells. Several studies have demonstrated myoepithelial differentiation in breast cancer[13)-15)] and myoepitheliomas of the breast, albeit rare, also have been described to show differentiation.[16)17)] One case in group 3 was adenoid cystic carcinoma in which most tumor cells were positive for SMA and thus showed myoepithelial differentiation. In a previous study the definite differentiation towards MECs has been demonstrated in adenoid cystic carcinoma, adenomyoepithelioma, low-grade adenosquamous (syringomatous) carcinoma, pure malignant myoepithelioma and poorly differenntiated myoepithelial-rich breast carcinoma.[18)] The group 3 contained 2 cases of IDC (scirrhous type) and a case of IDC (solid-tubular type) that showed productive fibrosis. A previous study reported that invasive ductal carcinomas with diffuse fibrosis were associated with a myoepithelial immunophenotype of carcinoma cells.[18)]

In conclusion, we herein identified 3 patterns of pitfalls regarding the application of SMA immunostaining to breast cytology.

1) Many SMA positive fibroblasts mimic MECs which are scattered in the background of some cases of invasive breast carcinomas.

2) The dimorphic clusters with SMA negative tumor cells and SMA positive MECs or myofibroblasts mimicking benign dimorphic cluster are recognized in some cases of invasive breast carcinomas that mainly consist of IDC (papillotubular type) and papillary carcinoma.

3) The clusters composed of tumor cells with "cross-reactivity" to SMA are recognized in some invasive breast carcinomas, thus suggesting the possibility of myoepithelial differentiation.

These pitfalls should be kept in mind when assessing of immunocytochemical staining for SMA when making a cytodiagnosis of breast carcinoma.

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(Received on November 10, 2006,
Accepted on January 5, 2007)