Kosin Medical Journal 2015;30:131-139.
http://dx.doi.org/10.7180/kmj.2015.30.2.131

KMJ
Original Article

Mucinous precursor lesions of mucinous carcinoma in breast: Incidence and histopathologic features

Min Jung Jung, Young Ok Kim

Department of Pathology, College of Medicine, Kosin University, Busan, Korea

유방 점액암종의 점액성 전구병변: 빈도 및 조직병리학적인 특성

정민정, 김영옥

고신대학교 의과대학 병리학교실

Objectives: Columnar cell lesion (CCL), atypical ductal hyperplasia (ADH) and ductal carcinoma in situ (DCIS) may be premalignant lesion of mammary invasive carcinoma. A few recent investigators reported that the precursor lesions exhibited mucin production and they might be potential precursor lesion for mucinous carcinoma (mCA). This study aims to investigate the incidence and histopathologic characteristics of mucinous precursor lesions, including mucinous DCIS (mDCIS) and mucinous CCL (mCCL).
Methods: We retrospectively analyzed invasive carcinomas with mucin. Cases were grouped into three subgroups: pure mCA, mixed mCA, and invasive carcinoma of no special type with mucin production (IC of NST-m). Precursor lesions were evaluated with PAS and alcian blue staining.
Results: Total 27 cases of invasive carcinoma with mucin were analysed and classified as 18 pure mCA, 7 mixed mCA, and 2 IC of NST-m. mDCISs were found in 12 pure mCA, 4 mixed mCA and 2 IC of NST-m. mCCLs were found in 7 pure mCA and 2 mixed mCA. Majority of mucin was identified in both cytoplasm and ductal lumen, while some tumors exhibited only cytoplasmic mucin. We also observed three patterns of mDCIS classifiable by location of mucin and architecture of tumor cells.
Conclusions: Cytoplasmic mucin suggested that mucinous feature of precursor lesions in the vicinity of mCA might not be a passive morphologic finding but be involved in development of mCA.

Key Words: Breast, Mucin, Mucinous carcinoma, Precursor

Traditionally, intraductal proliferative lesions, such as usual ductal hyperplasia (UDH), atypical ductal hyperplasia (ADH), ductal carcinoma in situ (DCIS) were considered as pre-malignant lesion with different level of risk for progression to carcinoma. Recently several new insights about more complex relationship between intraductal proliferative lesions and invasive carcinoma were made, and as one of them, "flat

Corresponding Author: Young Ok Kim, Department of Pathology, College of Medicine, Kosin University, 262, Gamcheon-ro, Seo-gu, Busan 49267, Korea
Tel: 82-51-990-6325 Fax: 82-51-990-3080 E-mail: suajoon@ns.kosinmed.or.kr

Received: Oct. 1, 2014
Revised: Dec. 18, 2014
Accepted: Jan. 4, 2015

epithelial atypia" has been considered as precursor lesion with overlapping morphologic, immunohistochemical and molecular features of ADH and low grade DCIS.[1,2]

Columnar cell lesions (CCL), including columnar cell change (CCC), columnar cell hyperplasia (CCH), flat epithelial atypia (FEA) has roughly similar morphology and genetic alterations.[3] Immunohistochemically, CCLs have strong and diffuse positive reaction for estrogen receptor /progesterone receptor, and negative reaction for Her2/neu, and these findings are similar with those of ADH and low grade DCIS. Furthermore, it has been reported that they exhibit monoclonality by human androgen receptor gene and loss of heterozygosity similar to those of ADH/low grade DCIS, and these genetic alteration has step-wise increase in the number and complexity according to degree of proliferation.[4,5] These morphologic and genetic findings may support a hypothesis that CCL may be an intermediary step progression of some ADH, low grade DCIS and low grade invasive carcinoma, even though their risk for progression into invasive carcinoma is very low.[6,7]

Mucinous carcinomas (mCA) of breast are pathologically classified into pure mCA, mixed mCA, and invasive carcinoma of no special type (IC of NST) by extent of mucin production, and their prognosis is differently predicted.[3] Recent a few investigators reported identification of mucin in the ductal lumen or cytoplasm of precursor lesions, such as DCIS, ADH, CCL or UDH and called them as mucinous ductal carcinoma in situ (mDCIS), mucinous atypical ductal hyperplasia (mADH) and mucinous columnar cell lesion (mCCL). They suggest that these may play role as precursor lesion for mCA.[8-11]

The aim of this study was to investigate the incidence and histopathologic characteristics of mucinous precursor lesions in the vicinity of mCA.

Materials and Methods

With approval from the Institutional Review Board of Gospel Hospital, excised or resected invasive carcinomas with mucin, regardless of the extent of the mucinous component, were retrieved from the files of the Department of Pathology, Gospel Hospital, Kosin University, Busan, Korea, between 2008 and 2013 by computer-assisted search program. After review of preceding or subsequent breast excisions or resections, cases were grouped into three subgroups by proportion of invasive carcinoma with mucin to overall invasive carcinoma based on WHO classification: 1) pure mCA (case with more than 90% mucin), 2) mixed mCA (case with 50-90% mucin), and 3) invasive carcinoma of no special type with mucin production (IC of NST-m) (case with less than 50% mucin)[3]. We noted histologic type of non-invasive carcinoma components, and presence or absence of lymph node metastasis. Near the invasive carcinoma with mucin production, we noted presence and histologic features of precursor lesions, including DCIS and CCL. When any mucinous material was identified in the ductal lumen or cytoplasm by histochemistry using periodic acid-Schiff (PAS) and alcian blue (pH 2.5), samples were considered as mDCIS or mCCL according to morphologic features by H-E stain. The location of the mucinous contents was classified into ductal lumen, cytoplasm, or both. The method of histochemistry was as follows. For PAS staining, formalin-fixed paraffin-embedded tissues were cut into 4 μm sections, oxidized in 0.5% periodic acid solution for 10 minutes, rinsed in deionized (DI) water, placed in Schiff reagent for 10 minutes, washed

in tap water for 1 minute, counterstained in Harris' Hematoxylin for 30 seconds, washed in DI water for 1 minute, placed in hydrochloric acid for 1 second, washed in DI water for 1 minute, dehydrated, cleared and mounted. For alcian blue (pH 2.5) staining, formalin-fixed paraffin-embedded tissues were cut into 4 μm sections, treated in 3% glacial acetic acid for 30 minutes, stained in alcian blue solution for 30 minutes, washed in tap water for 1 minute, counterstained in nuclear fast red for 5 minutes, and washed in tap water for 1 minute, dehydrated, cleared and mounted.

Results

We collected 27 cases of invasive carcinoma with mucin for 6 years, and they were grouped into 18 pure mCA, 7 mixed mCA, and 2 IC of NST-m. Their clinicopathologic characteristics were summarized in Table 1. Only two of them, nonmucinous component of invasive carcinoma ($p=0.002$) and lymph node metastasis ($p<0.001$) were significant, however it was limited value due to low number of corresponding cases.

DCIS, CCL, mDCIS and mCCL were present in 14/9/12/7 cases in 18 pure mCA, 7/3/4/2 cases in 7 mixed mCA, and 2/0/2/0 cases in 2 IC of NST-m, respectively (Fig. 1). The mucinous feature was identified in 86%/57%/100% and 78%/67%/0% of DCIS and CCL associated with pure CA, mixed CA, IC of NST-m, respectively. Majority of mucin in mDCIS and mCCL was located in both the cytoplasm and ductal lumen, however, 4 mDCIS (3/12 mDCIS associated with pure mCA and 1/4 mDCIS associated with mixed mCA) and 2 mCCL (1/7 mCCL associated with pure mCA and 1/2 mCCL associated with mixed mCA) exhibited mucinous contents only in the cytoplasm without intraluminal location (Table 2).

The morphology of mDCIS was variable, and it was grouped into three patterns by location of mucin and

Table 1. Clinicopathologic characteristics of invasive carcinoma with mucin

Characteristic \ Group	Pure mCA (n=18)	Mixed mCA (n=7)	IC of NST-m (n=2)
Gender	Female (18)	Female (7)	Female (2)
Age (average) (Min-Max)	49.9 years (29-73)	57.7 years (40-77)	45 years (41-49)
Operation type	M (9) E (1) E+M (8)	M (5) E+M (2)	M (1) E+M (1)
Tumor size (average) (Min-Max)	25.72mm (3-58)	22.4mm (10-50)	23.5mm (22-25)
Lymph node metastasis	-	-	+ (1)
Non-mucinous component	- (16) IC with SRC diff (1) IC of NST (1)	IC of NST (7)	IC of NST (2)
Microcalcification	+ (13)	+ (5)	+ (2)

mCA, mucinous carcinoma; IC of NST-m, invasive carcinoma of no special type with mucin production; Min, minimal; Max, maximal; M, mastectomy; E, excision; E+M, excision with subsequent mastectomy; +, present; -, absent; IC with SRC diff, invasive carcinoma with signet-ring cell differentiation; IC of NST, invasive carcinoma of no special type

architecture of proliferative cells: pattern 1) DCIS with overdistended duct by luminal mucin and partially lined by attenuated or minimally-to-mildly proliferative intraductal tumor cells; pattern 2) DCIS with luminal mucin, without feature of pattern 1; and pattern 3) mucin in minute intercellular spaces or intracytoplasmic vacuoles, without features of pattern 1 or pattern 2 (Fig. 2). Some cases exhibited only one pattern of mDCIS, however others exhibited mixed patterns of mDCIS. Although variable patterns were identified in all groups, most common pattern was pattern 3 (Fig. 3).

Discussion

In our study, mucin contents were identified in 76% (12 mDCIS/14 DCIS associated with pure mCA and 4 mDCIS/7 DCIS associated with mixed mCA) of DCIS associated with pure mCA and mixed mCA. It was lower than 95% (84 mDCIS/88 DCIS associated with pure mCA and mixed mCA) by Kryvenko et al.,[10] even though they defined the cases with only luminal expansion of ducts with mucin as mDCIS different to ours. In our study, cytoplasmic and intraluminal mucin were identified in 100% (12/12 mDCIS associated with

Table 2. Histopathologic characteristics of mucinous precursor lesions

Group \ Precursor	Case	DCIS	mDCIS (location / pattern)	CCL	mCCL (location)
Pure mCA (n=18)	1	+	+ (L,C / pattern 1,2)	+	+ (L,C)
	2	+	-	-	NA
	3	+	+ (C/ pattern 3)	+	-
	4	+	-	+	+ (C)
	5	+	+ (L,C / pattern 1,2,3)	+	+ (L)
	6	+	+ (L,C / pattern 1,2,3)	-	NA
	7	+	+ (L,C / pattern 1,2,3)	-	NA
	8	-	NA	-	NA
	9	+	+ (C / pattern 3)	+	+ (L,C)
	10	+	+ (L,C / pattern 1,2,3)	-	NA
	11	-	NA	-	NA
	12	-	NA	-	NA
	13	+	+ (L,C / pattern 1,3)	+	+ (L,C)
	14	-	NA	+	+ (L,C)
	15	+	+ (L,C / pattern 2,3)	+	+ (L,C)
	16	+	+ (L,C / pattern 2)	-	NA
	17	+	+ (L,C / pattern 3)	+	-
	18	+	+ (C / pattern 3)	-	NA
Mixed mCA (n=7)	19	+	+ (L,C / pattern 1,2,3)	-	NA
	20	+	-	+	+ (L,C)
	21	+	+ (C / pattern 3)	+	-
	22	+	-	-	NA
	23	+	+ (L,C / pattern 3)	+	+ (C)
	24	+	+ (L,C / pattern 1,2,3)	-	NA
	25	+	-	-	NA
IC of NST-m (n=2)	26	+	+ (L,C / pattern 2,3)	-	NA
	27	+	+ (L,C / pattern 3)	-	NA

mCA, mucinous carcinoma; IC of NST-m, invasive carcinoma of no special type with mucin production; DCIS, ductal carcinoma in situ; mDCIS, mucinous ductal carcinoma in situ; CCL, columnar cell lesion; mCCL, mucinous columnar cell lesion; +, present; -, absent; NA, not available; L, ductal lumen; C, cytoplasm

pure mCA and 4/4 mDCIS associated with mixed mCA) and 75% (9/12 mDCIS associated with pure mCA and 3/4 mDCIS associated with mixed mCA) of mDCIS associated with pure mCA and mixed mCA. Although many cases simultaneously exhibited cytoplasmic and intraluminal mucin, 4 cases exhibited mucin in only cytoplasmic location.

We also found mCCL in 78% (7 mCCL/9 CCL associated with pure mCA) of CCL associated with pure mCA and 75% (7 mCCL/9 CCL associated with pure mCA and 2 mCCL/3 CCL associated with mixed mCA) of CCL associated with pure mCA and mixed mCA, in either ductal luminal or cytoplasmic location. These results were higher than the 68% of mCCL (13 mCCL/19 CCL associated with 46 pure mCA) by Verschuur-Maes et al..[8] They reported that the

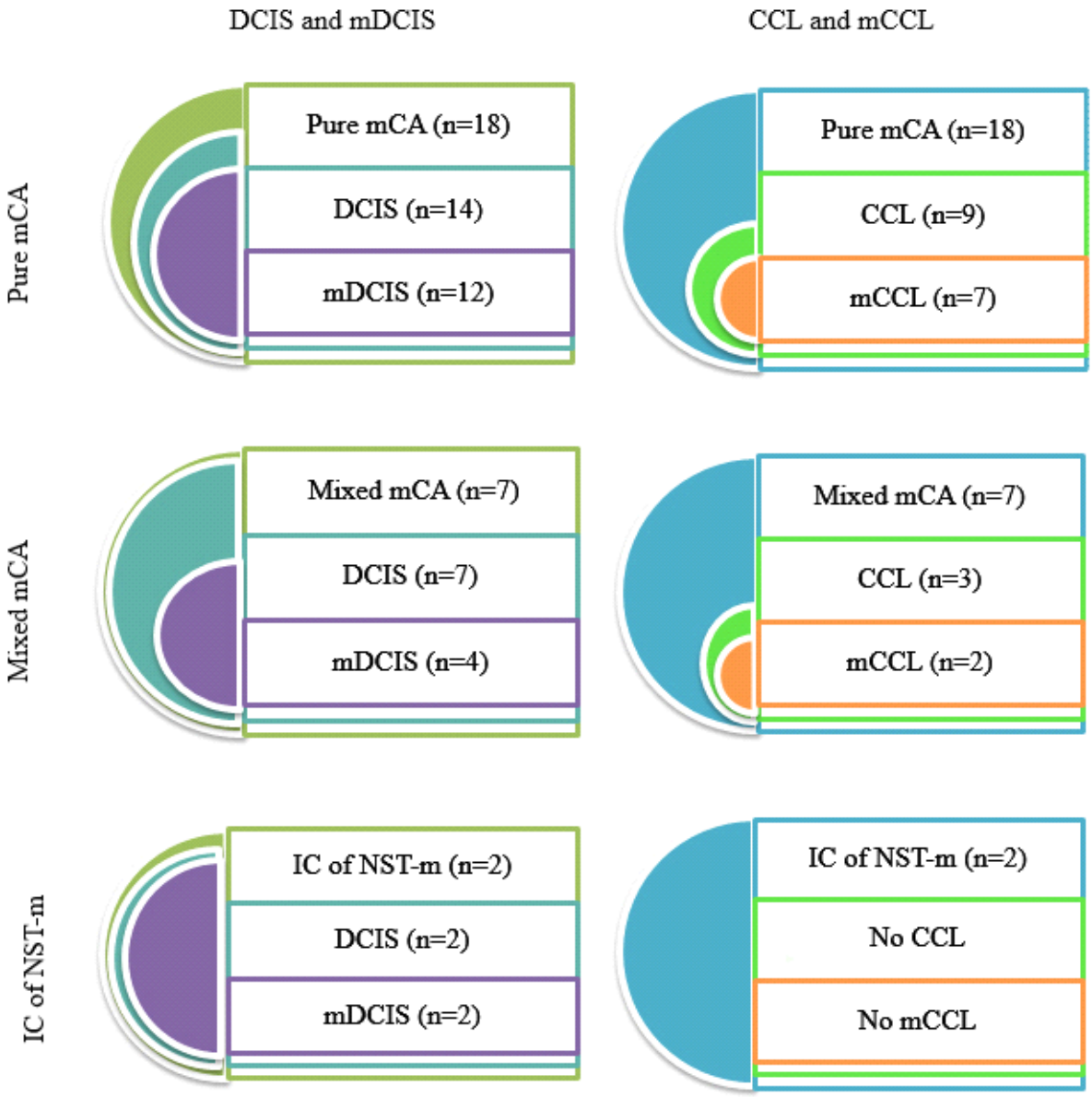

Figure 1. Distribution of ductal carcinoma in situ, mucinous ductal carcinoma in situ, columnar cell lesion and mucinous columnar cell lesion is variable in each groups of invasive carcinoma with mucin. (DCIS, ductal carcinoma in situ; mDCIS, mucinous ductal carcinoma in situ; CCL, columnar cell lesion; mCCL, mucinous columnar cell lesion; mCA, mucinous carcinoma; IC of NST-m, invasive carcinoma of no special type with mucin production)

intraluminal mucin (80%) was more frequent than cytoplasmic mucin (33%) in mCCL associated with pure mCA. However in our study, cytoplasmic and intraluminal mucin in mCCL had same frequency in 85% (6 mCCL/7 CCL associated with pure mCA) of CCL associated with pure mCA and 88% (7 mCCL/8 CCL associated with pure mCA and 1 mCCL/1 CCL associated with mixed CA) of CCL associated with pure mCA and mixed mCA. Almost of them had both cytoplasmic and intraluminal mucin, but each one case exhibited mucin in only cytoplasmic or intraluminal location.

In overall, cytoplasmic mucin was often coexisted with intraluminal mucin, however 22% of mDCIS (4/18 total mDCIS) and 11% of mCCL (1/9 total mCCL) exhibited mucinous contents only in cytoplasm without

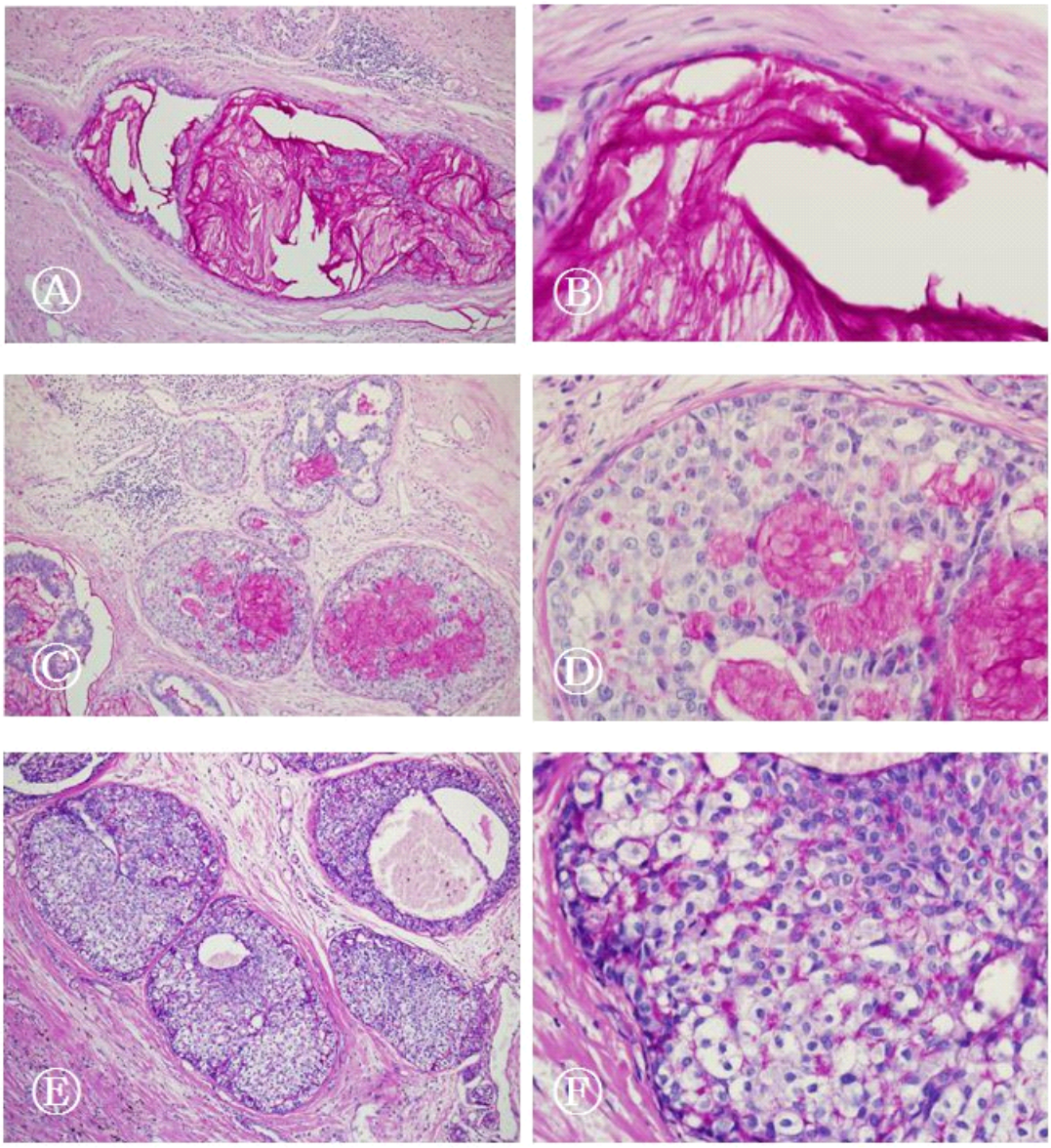

Figure 2. Mucinous ductal carcinoma in situ is histologically classified into three patterns. (A) In pattern 1, ductal carcinoma in situ exhibits ducts overextended by intraluminal mucin. (B) They are partially lined by attenuated ductal epithelium. (C, D) In pattern 2, ductal carcinoma in situ exhibits intraluminal mucin without ductal overextension. (E, F) In pattern 3, ductal carcinoma in situ exhibits minute intercellular spaces or intracytoplasmic vacuoles filled with mucin without distinct ductal mucin. (Hematoxylin-Eosin stain, A, C, E: x40, B, D, F: x400)

intraluminal mucinous content. This result can be interpreted that mucin in DCIS and CCL is result of their own mucin production, not a passive spread of mucin through the mammary duct-lobular system. And cytoplasmic mucin should be included of investigation of mucinous precursor lesions of mCA.

Although no hypothesis for progression of mCCL into mDCIS or mCA has been confirmed, a significantly higher incidence of mCCL in mCA compared to IC of NST has been reported.8 We also found that the incidence of mDCIS and mCCL tended to be higher in pure mCA than mixed mCA. This tendency could be interpreted as evidence supporting the potential role of mucinous precursor lesions in carcinogenesis of mCA. Although the high incidence (100%) of mDCIS in IC of NST-m is discordant with this interpretation, the interpretation of this group is limited due to small number of cases in this study.

We observed three patterns of mDCIS classifiable by location of mucin and architecture of tumor cells. Pattern 1 and pattern 3 were similar histopathologic morphology with mDCIS described by Kryvenko et al..[10] Pattern 1 was observed in samples of pure mCA (42%) and mixed mCA (29%), but no IC of NST-m. Pattern 2 and pattern 3 were observed in all three groups, with the lowest frequency in the mixed mCA group (pattern 2: 29%; pattern 3: 57%) compared to pure mCA (pattern 2: 50%; pattern 3: 71%) and IC of NST-m (pattern 2: 50%; pattern 3: 100%). The interpretation for these results has limitation due to small number of cases and additional validation is needed with more cases in each groups. Nonetheless, noteworthy was that pattern 3 mDCIS, which may be subtle in H-E stain, was most frequent.

mCA has been classified as favorable group among the breast cancer, and uncommon lymph node metastasis has been suggested as the reason for its more favorable prognosis, compared to prognosis in IC of

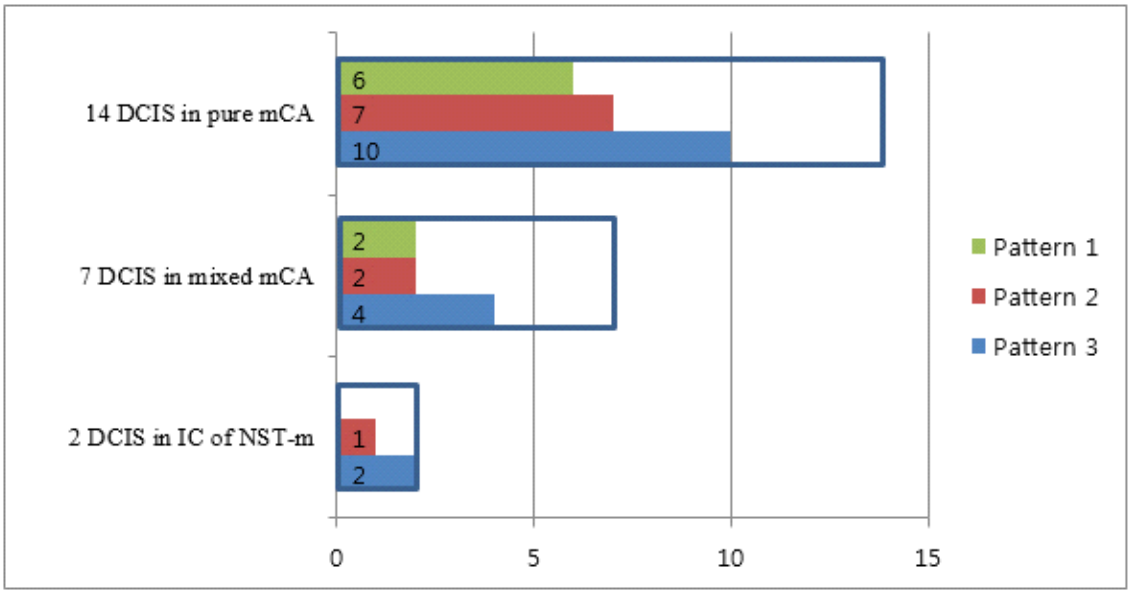

Figure 3. Three patterns of mucinous ductal carcinoma in situ are identified in three groups of invasive carcinoma with mucin. Some cases exhibit more than one pattern of mucinous ductal carcinoma in situ, and most common pattern is pattern 3. (DCIS, ductal carcinoma in situ, mCA, mucinous carcinoma; IC of NST-m, invasive carcinoma of no special type with mucin production)

NST.[12-14] In our study, no patients with pure mCA or mixed mCA exhibited lymph node metastasis, but one patient with IC of NST-m had lymph node metastasis. Its metastatic lymph node exhibited mucinous carcinoma component in part, even though the main mammary lesion exhibited relatively low proportion (10%) of mucinous carcinoma component to whole invasive carcinoma.

In conclusion, CCL and DCIS in vicinity of mCA often had mucinous component and the frequency of mucinous precursor lesions was different among the each groups of invasive carcinoma with mucin. Mucin was often identified in both the ductal lumen and cytoplasm, while some samples exhibited mucinous contents in the cytoplasm without intraluminal mucin. These findings suggested that a mucinous feature of precursor lesion in the vicinity of mCA might not be a passive morphologic finding but be involved in mCA development. We also observed three histopathologic patterns of mDCIS. Variable patterns were identified in the each groups of invasive carcinoma with mucin production, and most common pattern was pattern 3, which might be subtle in H-E stain. Further study of mucinous precursor lesions will be helpful in understanding the carcinogenesis of mammary mucinous carcinoma.

Acknowledgements

A major part of this study was presented at the poster session of the Third International Breast Cancer Symposium, 25-26 April 2014, Jeju, Korea.

References

1. Abdel-Fatah TM, Powe DG, Hodi Z, Reis-Filho JS, Lee AH, Ellis IO. Morphologic and molecular evolutionary pathways of low nuclear grade invasive breast cancers and their putative precursor lesions: further evidence to support the concept of low nuclear grade breast neoplasia family. Am J Surg Pathol 2008;32:513-23.
2. Simpson PT, Gale T, Reis-Filho JS, Jones C, Parry S, Sloane JP, et al. Columnar cell lesions of the breast: the missing link in breast cancer progression? A morphological and molecular analysis. Am J Surg Pathol 2005;29:734-46.
3. Sunil R. Lakhani IOE, Stuart J Schinitt, Puay Hoon Tan, Marc J. van de Vijver. WHO classification of tumours of the breast. 4th Edition ed. Fred T. Bosman ESJ, Sunil R. Lakhani, Hiroko Ohgaki, editor. Geneva: International Agency for Research on Cancer; 2012.
4. Go EM, Tsang JY, Ni YB, Yu AM, Mendoza P, Chan SK, et al. Relationship between columnar cell changes and low-grade carcinoma in situ of the breast--a cytogenetic study. Hum Pathol 2012;43:1924-31.
5. Moinfar F, Man YG, Bratthauer GL, Ratschek M, Tavassoli FA. Genetic abnormalities in mammary ductal intraepithelial neoplasia-flat type ("clinging ductal carcinoma in situ"): a simulator of normal mammary epithelium. Cancer 2000;88:2072-81.
6. Schnitt SJ. The diagnosis and management of pre-invasive breast disease: flat epithelial atypia--classification, pathologic features and clinical significance. Breast Cancer Res 2003;5:263-8.
7. Abdel-Fatah TM, Powe DG, Hodi Z, Lee AH, Reis-Filho JS, Ellis IO. High frequency of coexistence of columnar cell lesions, lobular neoplasia, and low grade ductal carcinoma in situ with invasive tubular carcinoma and invasive lobular carcinoma. Am J Surg Pathol 2007;31:417-26.
8. Verschuur-Maes AH, Van Diest PJ. The mucinous variant of columnar cell lesions. Histopathology 2011;58:847-53.
9. Gadre SA, Perkins GH, Sahin AA, Sneige N, Deavers MT, Middleton LP. Neovascularization in mucinous ductal carcinoma in situ suggests an alternative pathway for invasion. Histopathology 2008;53:545-53.
10. Kryvenko ON, Chitale DA, Yoon J, Arias-Stella J, 3rd, Meier FA, Lee MW. Precursor lesions of mucinous carcinoma of the breast: analysis of 130 cases. Am J Surg

Pathol 2013;37:1076-84.
11. O'Connell JT, Shao ZM, Drori E, Basbaum CB, Barsky SH. Altered mucin expression is a field change that accompanies mucinous (colloid) breast carcinoma histogenesis. Hum Pathol 1998;29:1517-23.
12. Di Saverio S, Gutierrez J, Avisar E. A retrospective review with long term follow up of 11,400 cases of pure mucinous breast carcinoma. Breast Cancer Res Treat 2008;111:541-7.
13. Matsukita S, Nomoto M, Kitajima S, Tanaka S, Goto M, Irimura T, et al. Expression of mucins (MUC1, MUC2, MUC5AC and MUC6) in mucinous carcinoma of the breast: comparison with invasive ductal carcinoma. Histopathology 2003;42:26-36.
14. Komaki K, Sakamoto G, Sugano H, Morimoto T, Monden Y. Mucinous carcinoma of the breast in Japan. A prognostic analysis based on morphologic features. Cancer 1988;61:989-96.