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Original Article

Invasive cribriform carcinoma in a Chinese population: comparison with low-grade invasive ductal carcinoma-not otherwise specified

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Abstract: Invasive cribriform carcinoma (ICC) and low-grade invasive ductal carcinoma (IDC) were recently considered to belong to a low-grade breast neoplasia family. However, none of publications has compared ICC and low-grade IDC at present. Meanwhile, in order to evaluate prognostic significance of clinicopathological characteristics of different cribriform contents in ICC and invasive breast cancer with less cribriform structures, a retrospective review of fifty-one cases of ICC and forty cases of invasive breast cancer with less cribriform pattern (less than fifty percent) was conducted in a Chinese population. Forty-nine cases of low-grade IDC without cribriform elements were selected as a control. ICC presented more favorable prognostic factors than those of invasive breast carcinoma with less cribriform pattern and low-grade IDC, such as smaller tumor size, less frequent axillary lymph node involvement, higher positive rate of estrogen receptor and/or progestogen receptor expression, and lower proliferation index. The expression of human epidermal growth factor receptor two in ICC and invasive breast cancer with less cribriform pattern was mostly negative. Pure ICC showed less frequency of axillary lymph node involvement, but not its number. The proliferation index in the pure type was lower, although the tumor size in these two types was not obviously different. Tumors contained cribriform structures had a more favorable prognosis than those with low-grade IDC. Considering the tumor biology, and the benign course of pure ICC studied, chemotherapy may not be indicated in the typical case.

Keywords: Invasive cribriform carcinoma, invasive ductal carcinoma, breast, prognosis, China

Introduction

Invasive cribriform carcinoma (ICC) of the breast is characterized by predominant cribriform growth pattern of its invasive component, according to WHO's new definition and Page's description in 1983 [1, 2]. The incidence of ICC was reported to range from 0.3% to 3.5% [1, 3-5]. The pure ICC was defined as almost entirely (>90%) of an invasive cribriform pattern, and that the lesions show a predominantly cribriform differentiation while the remainder component is limited to tubular carcinoma (TC) are also included in the category of ICC. Cases with a component (<50%) of another carcinoma type, other than TC, should be regarded as mixed type of ICC. ICC manifested a better prognosis than that of invasive ductal carcinoma (IDC), not otherwise specified (NOS). The ten-year overall survival for ICC was 90% to 100%, and the outcome of mixed ICC was reported to be less favourable than that of the pure form, but better than that of common ductal carcinoma [1, 4, 6].

Prognostic significance of clinicopathological characteristics in ICC patients is not well established, because of its low incidence and lack of a standard definition. Treatment guidelines for ICC are mostly extrapolated from data based on IDC without clear validation. The tumor may present as a mass but is frequently clinically occult, including hardness for radiological detecting. For this reason, the lesions are usually larger at presentation although they grow slowly at most time [4]. The axillary lymph node (ALN) is less frequently involved in ICC than in IDC [1]. Venable indicated the maximal number of metastatic lymph node (LN) in ICC was not over than three, although there was not obvious difference of positive LN rate among pure ICC, mixed ICC and IDC control [3]. There was also a case report of pure ICC with internal mammary node metastasis [7]. All ICCs express estrogen receptor (ER), however, human epidermal growth factor receptor 2 (HER2) amplification is rarely found. Intraductal carcinoma, generally of the cribriform type, and mutifocality are often found in ICC cases [1-3]. Distant metastasis was rarely reported in ICC cases based on the publications available now.

Recent studies suggested ICC, grade 1 IDC-NOS, TC, and classic invasive lobular carcinomas (ILCs), as well as their non-obligate in situ precursors, should be included into a family of low-grade breast neoplasia, because all these lesions display remarkably similar phenotypic and genetic aberrations [8-13]. It is well known that ICC shows very close similarity to TC either from the morphological features or from the prognosis [1, 3, 14]. The new World Health Organization (WHO) classification for breast carcinoma has even incorporated ICC and TC into one category [2]. Lopez-Garcia recently indicated TCs were similar at the transcriptomic level to grade- and molecular subtype-matched IDC-NOS (mainly low-grade IDC-NOS) [15]. However, none of publications has compared ICC with low-grade IDC-NOS (LG-IDC) at present. This study aims to evaluate the effect caused by different cribriform contents in ICC and invasive breast cancer (IBC) with less cribriform structures as to clinicopathological and prognostic factors. In addition, the study is the first investigation of ICC in a Chinese population. We intended to compare ICC with IBC with less cribriform structures and LG-IDC without cribriform structures, and provided some evidences for establishing the prognostic significance and guidelines for ICC treatment.

Materials and methods

Case selection and clinical evaluation

The medical records of all primary breast carcinoma (12,647 cases) from January 2004 to October 2011 were available in the Department of Breast Cancer Pathology and Research Laboratory, Cancer Hospital of Tianjin Medical University (Tianjin, China). According to the new World Health Organization (WHO) criteria [2] and the description presented by Page et al in 1983 [1], thirty pure ICC cases and twenty-one mixed ICC cases were identified. Patients with ICC and a second breast primary tumor in either breast were excluded from the study if the latter exhibited a non-cribriform architecture. In addition, forty cases of invasive breast carcinoma (IBC) with minor cribriform components (less than 50%) were also consecutively selected as compared with ICC cases. Meanwhile, forty-nine cases of low-grade (grade 1) IDC-NOS (LG-IDC) which did not contain any cribriform elements were randomly selected as a control. The study protocol was approved by the Hospital Human Ethical Committee. Informed consent had been obtained from all patients before their surgery and the examination of the specimens were conducted.

We retrospectively evaluated some demographic and clinicopathological factors, including age at diagnosis, menopausal status, family history (family history of cancer within first- and second-degree relatives), laterality, tumor size, tumor grade, lymph node status, radiological examinations and treatment modalities (type of surgical procedures, use of radiotherapy, chemotherapy, hormone therapy, and targeted therapy). The pathological tumor stage (TNM stage) was assessed according to the criteria established by the 6th edition of the American Joint Committee on Cancer (AJCC) staging manual. Follow-up was undertaken in 48 ICC cases, 32 IBC cases and 49 LG-IDC cases. The mean follow-up time was 29.5 months in ICC cases (range, 3-88 months), 16.5 months in IBC cases (range, 1-61 months) and 52.6 months in LG-IDC cases (range 11-87 months), respectively.

Immunohistochemical procedures and evaluation

Immunohistochemistry (IHC) was performed on formalin-fixed paraffin-embedded tissue blocks

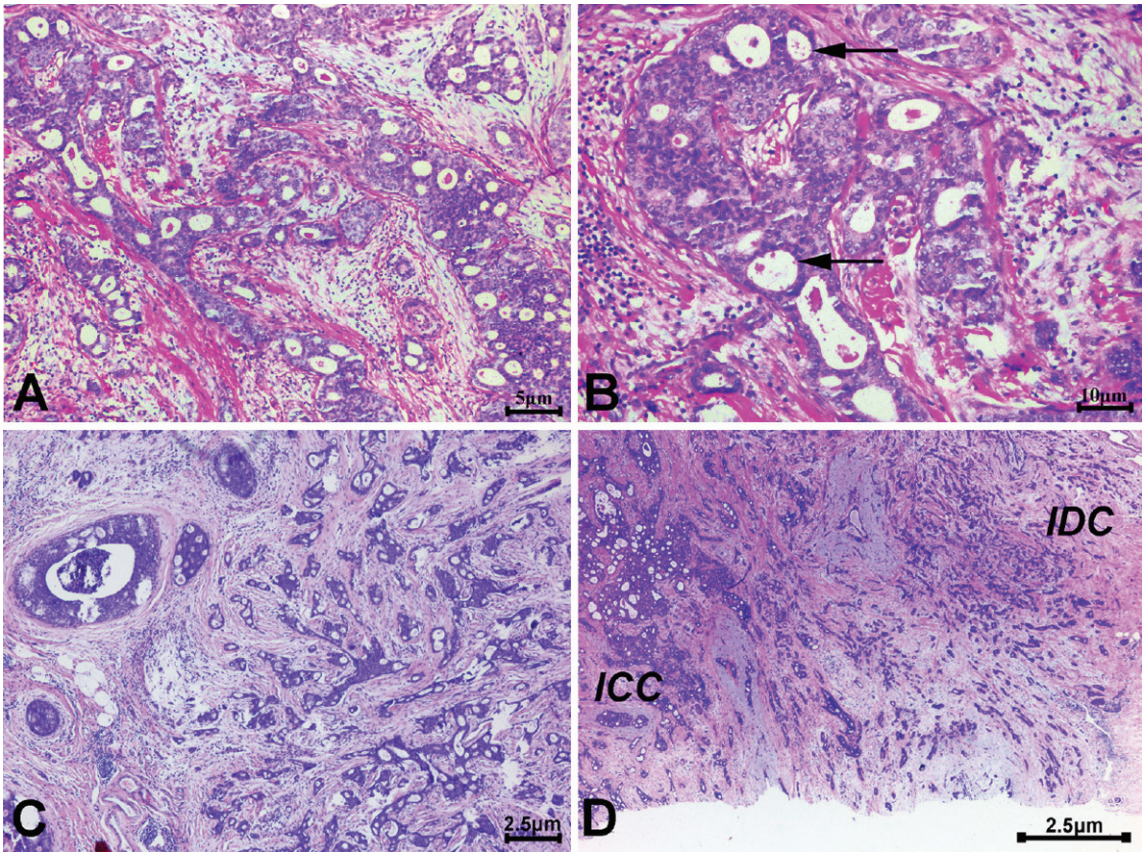

Figure 1. Histology of pure and mixed ICC tumors (HE staining). A: In pure ICC cases, most infiltrating tumor cells are arranged in groups with a cribriform structure, and obvious sclerotic stroma exists between the tumor nests. B: The image of high magnification shows a moderate degree of nuclear pleomorphism in the cells of the cribriform elements in pure ICC. In the sieve pore, mucin-like secretion and apical snouts (arrows indicated) can be found. C: DCIS with a cribriform style is usually found in ICC case. D: In mixed ICC, other tumor elements of minor percentage can be found in the tumor except for cribriform carcinoma, such as IDC. DCIS, ductal carcinoma in situ; ICC, invasive cribriform carcinoma; IDC, invasive ductal carcinoma. Scale bars = 2.5μm in C and D; 5μm in A and 10μm in B.

in all the cases. Briefly, 4 μm sections of tumour tissue were deparaffinized in xylene and hydrated in a graded series of alcohols. Antigen retrieval was performed using a pressure cooker in citrate buffer (pH 6.0) solution. Following incubation in 3% H_2O_2 for 10 min, inactivating the endogenous peroxidase activity, the sections were treated with a blocking solution containing 10% normal goat serum for 20 min at room temperature. Then tissue sections were incubated with primary antibodies at 37°C for 2 h. Primary antibodies used in this study included ER (1:150; Zymed, USA), PR (1:150; Zymed, USA), c-erB-2 (1:100; Invitrogen), Ki-67 (1:75; Zymed, USA), SMA (1:100; Santa Cruz, USA), p63 (1:150; Zymed, USA) and CK5/6 (1:200; Invitrogen). After incubation with antimouse or rabbit biotin-conjugated secondary antibody and streptavidin-horseradish peroxidase (Zymed) for 30 min at 37°C, respectively, color was developed by incubation with 3, 3'-diaminobenzidine tetra-hydrochloride (DAB). The sections were counterstained with hematoxylin. Sections with normal lobules of mammary gland adjacent to tumor were used as an autospecific positive control for ER and PR. And sections of the invasive ductal carcinomas that were positive for HER2, Ki-67, SMA, p63 and CK5/6 were used each time as a positive control. As a negative control, the primary antibody was replaced with normal mouse or rabbit immunoglobulin.

The immunostaining was scored in double blind by two senior pathologists, who were blinded to patients' clinicopathologic characteristics and outcomes. For ER, PR, the location of immunoreactivity, percentage of stained cells, and

intensity were determined. ER+ or PR+ was defined as >1% of the tumor cells presented nuclear staining with different degrees. A positive HER2 result was IHC staining of 3+ (uniform, intense membrane staining of >30% of invasive tumor cells); a negative result is an IHC staining of 0 or 1+; while an equivocal result was an IHC staining of 2+. SMA and CK5/6 stains were considered positive if any cytoplasmic and/or membranous staining was observed. Ki67 and p63 positive stains were nuclear staining and Ki67 status was expressed in terms of percentage of positive cells, with a threshold of 14% of positive cells.

Statistical analysis

The categorical variables were compared among all the groups by using χ^2 and Kruskal-Wallis test. When the null hypothesis was rejected at level of α=0.05, extended *t* test was performed for group comparisons. ER, PR and Ki-67 status were evaluated as the continuous variables according to their scores or percentage of the positive cells. One-way ANOVA was used to test their differences among the 4 groups. The least significant difference test was applied if equal variances were assumed, otherwise Dunnett T3 was used. All statistical tests were two-sided at the 5% level of significance and were performed using the SPSS 16.0 software package (SPSS Inc., Chicago, IL, USA).

Results

We retrospectively reviewed the medical records of 12, 647 women with primary breast cancers diagnosed between January 2004 and October 2011. Of them, 51 patients (0.4%) were diagnosed as ICC. The complete clinical information was available in 50 cases of ICC, as one ICC case had undergone a lumpectomy in outer hospital, while her pathological consultation and radial mastectomy were performed in our hospital. 40 cases of IBC with less than 50% cribriform components and 49 cases of LG-IDC were also selected as controls.

Histopathology and immunoprofile of ICC

Although showing the greatest similarity to TC, ICC is predominantly considered as a distinct clinico-pathological entity. Its diagnosis mainly depends on the histopathological features. Here we found thirty pure ICC cases, since most of invasive elements (exceeding 90%) were predominantly arranged in a sieve-like or cribriform growth pattern (**Figure 1A**). Apical snouts were often found on the surface of the sieve pore and mucin-like secretion could also be found (**Figure 1B**, arrows indicated). The tumor cells were smaller when compared with that of IDC-NOS, and had the low or moderate grade nuclei. Mitoses are rarely seen (**Figure 1B**). Obvious interstitial response was found among the nests (**Figure 1A** and **1B**). The remainder elements in four pure ICC cases (<10%) were TC (**Table 1**). The ductal carcinoma in situ (DCIS) can be seen in 25 cases of pure ICC (83.3%), prevalently in a cribriform style (**Figure 1C**). However, other micropapillary and solid patterns were also found. Twenty-one cases of mixed type were ascertained in our study which was defined as that more than 50% of the invasive component showed a cribriform pattern but other invasive components were not restricted to TC, such as IDC-NOS elements (**Figure 1D**). In some pure ICC cases (four cases), both ICC and TC foci could be found in the same tumor and their reciprocal transition was detected at the boundary of both foci (**Figure 2A** and **2B**). Only in pure ICC group the same well developed cribriform pattern was maintained in the nodal metastases (**Figure 2C** and **2D**). Multifocality was also found in seven ICC cases (13.7%), including six pure cases and one mixed case. Calcification could be found in pure ICC tumors and their metastatic lymph nodes (data not shown). As summarized in **Table 1**, except for the cribriform element, other infiltrating carcinoma components in IBC group were invasive lobular carcinoma, invasive papillary carcinoma and invasive micropapillary carcinoma. It seemed that the remaining carcinoma components were more complicated in this group, because 14 cases (35%) were composed of at least three invasive carcinoma components, while only one (5%) in the mixed ICC.

The tumor cells comprising the cribriform structure in ICC are usually uniform and CK-positive (**Figure 3A** and **3B**), lacking the expression of the markers of myoepithelial cell, such as SMA and p63 (**Figure 3C** and **3D**). Therefore the invasive nature of this tumor could be identified by this mean since DCIS lesion within this tumor was covered by a layer of myoepithelial cells

Table 1. The histologic components in pure, mixed ICC (>50%) and IBC with less cribriform pattern (<50%) of the breast

	exclusive cribriform pattern (%)	associated invasive carcinoma components (%)					≥ 3 invasive carcinoma components (%)
		tubular	IDC-NOS	ILC	IMC	IMPC	
pure n=30	26 (86.7)	4 (13.3)	0	0	0	0	0
Mixed n=21	0	2 (9.5)	19 (90.5)	0	0	0	1 (5)
IBC n=40	0	14 (35)	36 (90)	2 (5)	1 (2.5)	1 (2.5)	14 (35)

IBC, invasive breast carcinoma; ICC, invasive cribriform carcinoma; IDC-NOS, invasive ductal carcinoma-not otherwise specified; ILC, invasive lobular carcinoma; IMC, invasive papillary carcinoma; IMPC, invasive micropapillary carcinoma.

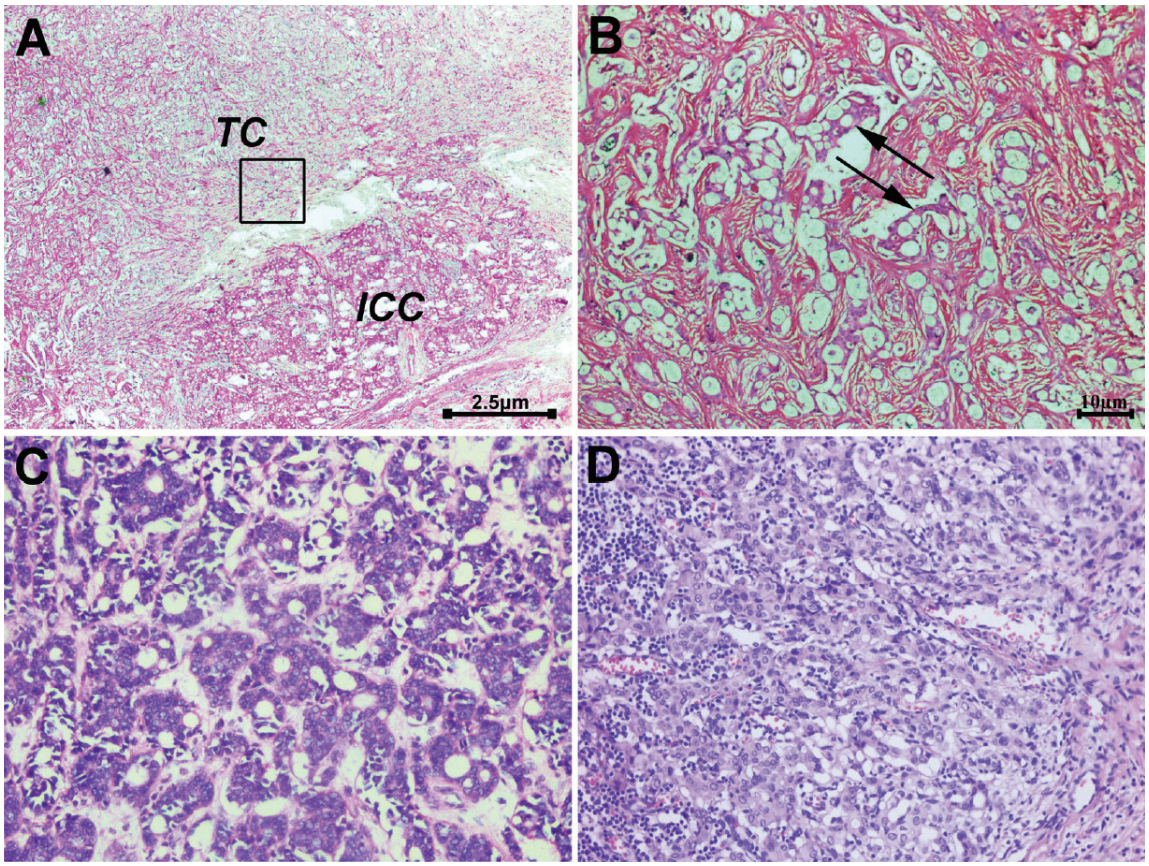

Figure 2. A, B: The association of ICC and TC. A: Both ICC and TC foci are found in the same tumor (pure ICC). B: At the boundary of these two foci, as indicated by the magnified image for the area of box in A, some TC cells seem to divert into ICC (arrows indicated). C, D: The metastatic lymph nodes of pure and mixed ICC. C: Axillary lymph nodal metastasis of pure ICC maintains cribriform pattern, while that of mixed ICC may be IDC phenotype in D: (A-D HE staining). ICC, invasive cribriform carcinoma; IDC, invasive ductal carcinoma; TC, tubular carcinoma. Scale bars = 2.5μm in A; and 10μm in B-D.

(**Figure 3E** and **3F**). Compared with the paraneoplastic normal lobule of mammary gland, tumor cells in ICC showed a strong staining of ER or PR. And the DCIS lesion in ICC appeared a similar staining pattern for ER or PR as the invasive components (**Figure 4A-D**). Moreover, the majority of ICC case displayed positive for ER and PR expression (96.1% and 88.2% VS. 62.5% and 68.8%, respectively); negative for HER2 expression (98.0% VS. 81.3%) and low Ki-67 proliferation index (72.5% VS. 37.5%, Ki67≤14%), as compared with LG-IDC cases (**Figure 5A-D**, **Table 2**).

Clinicopathological characteristics of ICC compared with IBC and LG-IDC

The mean age at diagnosis in ICC and IBC group was 51 years (range 36-71) and 52 years (range

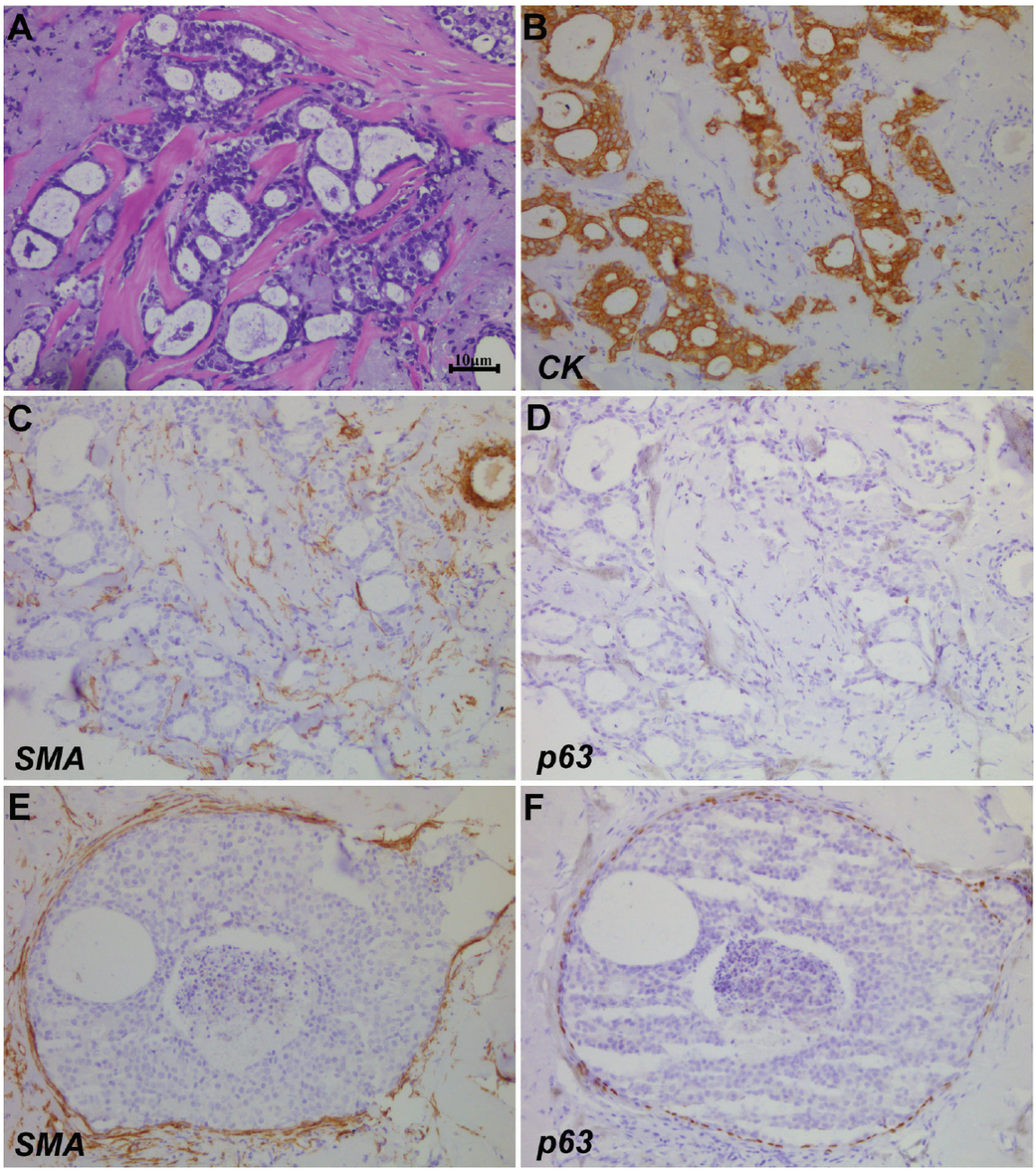

Figure 3. The infiltrating nature and luminal origin of ICC. A: The HE staining image of the cribriform foci in ICC case. B-D: Immunohistochemistry staining of luminal epithelium marker CK5/6 (B), myoepithelium markers of SMA (C) and p63 (D) are shown in the same area as A. The tumor cell of cribriform carcinoma was CK-positive, but SMA- and p63-negative. Note that the fibroblast in the stroma and the blood vessel are also SMA-positive (C) but p63-negative (D). E, F: The DCIS in ICC case is SMA- (E) and p63-positve (F). CK, cytokeratin; ICC, invasive cribriform carcinoma; SMA, smooth muscle actin. Scale bars = 10μm in A-F.

34-77), respectively. The mean age of the patients with tumor containing cribriform components seemed to be slightly younger than those with LG-IDC cases, although there was no significant difference among these three groups (**Table 2**). The laterality and family history were not obviously different among these three groups. Among 139 cases of three groups whose clinical data were complete, all patients accepted the surgery. Surgical procedures consisted of radial mastectomy (22.3%), modified mastectomy (65.5%) and breast-conserving

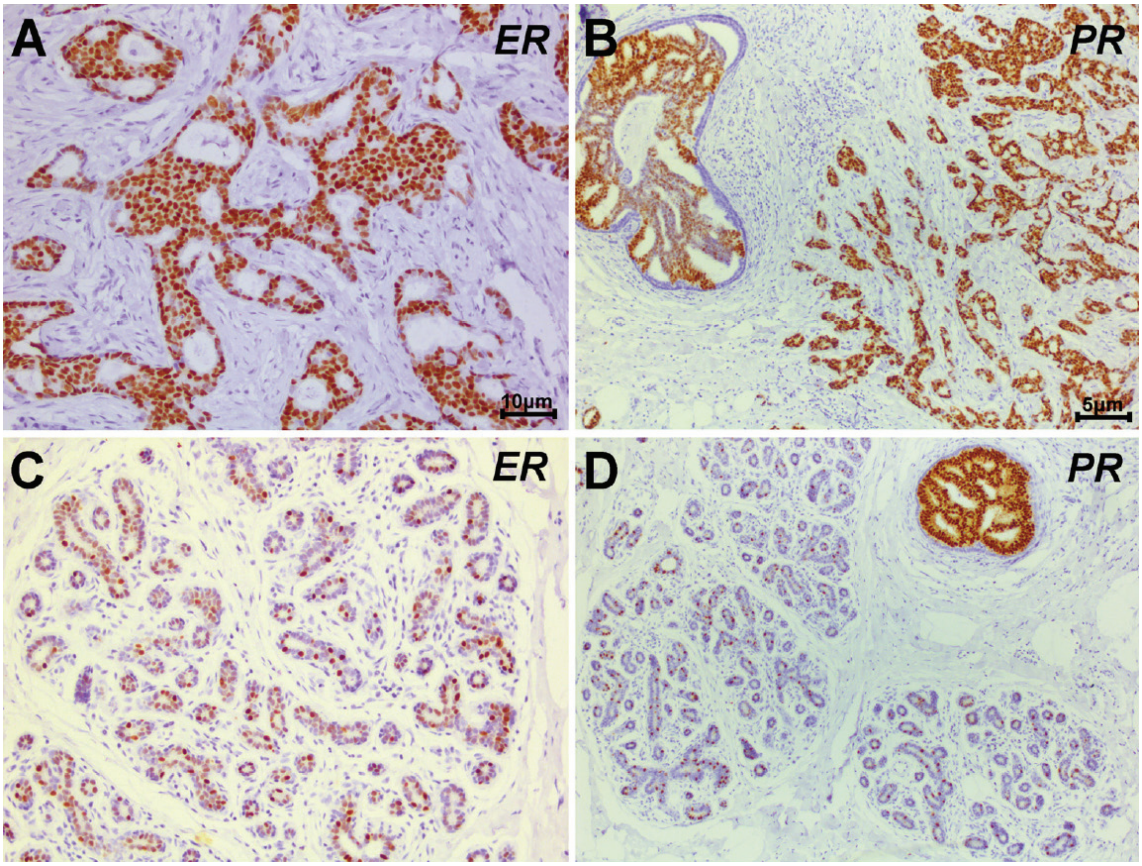

Figure 4. The expression of ER and PR in ICC. A, B: The majority of tumor cells in ICC are strong ER- (A) and PR-positive (B), with a similar density for their adjacent DCIS. C, D: The paraneoplastic normal mammary lobule shows weak or moderate ER (C) and PR expression to a less proportion (D). DCIS, ductal carcinoma in situ; ER, estrogen receptor; ICC, invasive cribriform carcinoma; PR, progestogen receptor. Scale bars = 5μm in B and D; and 10μm in A and C.

surgery (12.2%). Thirty-nine patients had received adjuvant radiotherapy including those who underwent breast-conserving surgery as a routine. 134 patients (96.4%) underwent postoperative systemic therapy, including endocrine therapy and chemotherapy. And 8 cases received neoadjuvant chemotherapy.

Some factors closely related to prognosis were evaluated among ICC, IBC and LG-IDC groups, as seen in **Table 2**. 58% ICC patients had smaller tumor size (≤2cm) and lower tumor stage (T1), which is more frequent than those in IBC and LG-IDC groups (52.5% and 12.2%, respectively). Notably the tumor in one pure ICC patient untreated for thirteen years had grown to 10 cm in size and also invaded the skin (T4 stage). There was another case whose tumor reached T4 stage in ICC group (4%), while the patients at T4 stage in IBC and LG-IDC were two (5%) and five (10.2%) cases. The axilla was staged in all the patients with a median of twenty lymph nodes (range from 3 to 81) sampled. 74.5% (38/50) ICC patients was histologically negative lymph node (LN), statistically more than that in IBC and LG-IDC cases, with approximately half in the latter two groups. Only one (2%) ICC patient was found to have metastasis of over than three lymph nodes (six LNs), while there were seven (17.5%) and twelve (24.5%) patients in IBC and LG-IDC, respectively. The maximal number of metastatic LN in LG-IDC and IBC patients was 27 and 22, respectively. The majority of ICC patients (96.1%) were positive in ER of tumors, as compared with 90% and 62.5% in IBC and LG-IDC group, respectively. The expression of PR did not change accordingly between ICC and IBC groups, but its positive rate in both groups was distinctly higher than LG-IDC group. HER2 was positive in 2% of ICC and 8.3% LG-IDC patients, but we did not find that in IBC group. ICC dis-

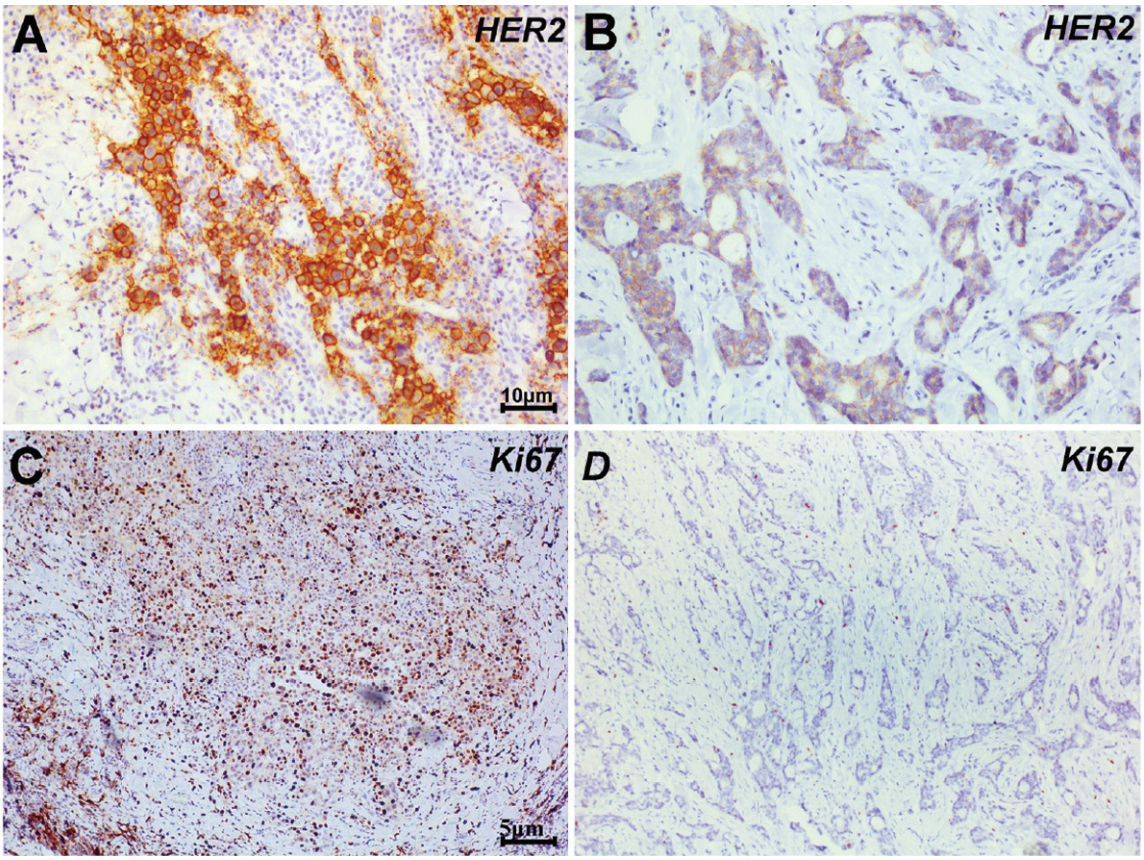

Figure 5. The expression of HER2 and Ki67 in ICC and LG-IDC. A: Over than 30% of tumor cells in LG-IDC show strong and complete membrane staining of HER2, while infiltrating tumor cells in ICC group display negative or 1+ HER2 positive (B). C: Some LG-IDC cells show high Ki67 proliferation index, but only sporadic cancer cells in ICC are Ki67 positive in (D). HER2, human epidermal growth factor receptor 2; ICC, invasive cribriform carcinoma; LG-IDC, low-grade invasive ductal carcinoma. Scale bars = 5μm in C and D; and 10μm in A and B.

played obviously lower proliferation index than the other two groups, as indicated by Ki67 detection.

All cases in ICC and IBC groups survived without tumor occurrence and no death was found at the end of the follow-up, while thirteen cases in LG-IDC had disease occurrence and two cases died of breast cancer.

Clinicopathological comparison within the tumors containing cribriform pattern

According to the different contents of cribriform components in the tumor, we compared the clinicopathological characteristics of the pure ICC, mixed ICC and IBC (**Table 3**). The mean age at diagnosis in both pure and mixed ICC was 51 years (range, 36-71 and 37-76, respectively). The median tumor size was 1.8 cm in pure ICC and 2.0 cm in mixed ICC. There was no distinct difference of tumor size and T stage among the pure, mixed ICC and IBC group. Pure ICC showed less frequency of axillary lymph node involvement than mixed ICC (20% VS. 33.3%, P=0.011). However, the maximal number of positive lymph node in pure ICC was 6 lymph nodes, which was more than that in mixed ICC (3 lymph nodes). All patients in pure ICC group were ER positive, while 20/21 (95.2%) in mixed group. PR was positive in 26/30 (86.7%) pure ICC, and 19/21 (90.5%) mixed ICC, compared with 90% and 95% in ER and PR of IBC, respectively. When the percentage of ER or PR expression was calculated as a continuous variance, the mean percentage of ER in pure ICC seemed to be higher than mixed ICC and IBC (66.7% VS. 58.4% and 60.0%, respectively), although that of PR might be slightly lower than mixed ICC (57.5% VS. 61.7%). But no statistical difference was found among these three groups (P=0.491

Table 2. Clinicopathological characteristics and treatment patterns among the patients of ICC, IBC with less cribriform components (<50%) and LG-IDC-NOS

Characteristics	ICC (%) n=51	IBC (%) n=40	LG-IDC-NOS (%) n=49	*P* value
Age (years)				0.297
< 40	7 (14.0)	3 (7.5)	4 (8.2)	
40-55	29 (58.0)	26 (65.0)	25 (51.0)	
> 55	14 (28.0)	11 (27.5)	20 (40.8)	
Laterality				0.886
Left	20 (40.0)	17 (42.5)	22 (44.9)	
Right	30 (60.0)	23 (42.5)	27 (55.1)	
Menopausal status				0.308
premenopausal	31 (62.0)	23 (57.5)	23 (46.9)	
postmenopausal	19 (38.0)	17 (42.5)	26 (53.1)	
Family history				0.952
yes	3 (6.0)	3 (7.5)	3 (6.1)	
no	47 (94.0)	37 (92.5)	46 (93.9)	
Nipple involvement				0.670
yes	4 (8.0)	5 (12.5)	9 (18.4)	
no	46 (92.0)	35 (87.5)	40 (81.6)	
T stage				0.000
T1 (≤2cm)	29 (58.0)	21 (52.5)	6 (12.2)	
T2 (>2cm, ≤5cm)	19 (38.0)	17 (42.5)	34 (6.4)	
T3 (>5cm)	0 (0)	0 (0)	4 (8.2)	
T4 (skin or chest muscle involvement)	2 (4.0)	2 (5.0)	5 (10.2)	
Lymph node status				0.004
negative	38 (74.5)	19 (47.5)	25 (51.0)	
1-3 positive	12 (23.5)	14 (35)	12 (24.5)	
≥4 positive	1 (2.0)	7 (17.5)	12 (24.5)	
Median number nodes sampled (range)	24 (8-32)	30 (3-46)	23 (8-42)	
TNM stage				0.000
Stage I	28 (54.9)	9 (22.5)	9 (18.4)	
Stage II	20 (39.2)	23 (57.5)	31 (63.3)	
Stage III	3 (5.9)	8 (20)	9 (18.4)	
ER				0.000
negative	2 (3.9)	4 (10)	18 (37.5)	
positive	49 (96.1)	36 (90)	30 (62.5)	
PR				0.002
negative	6 (11.8)	2 (5)	15 (31.2)	
positive	45 (88.2)	38 (95)	33 (68.8)	
HER2				0.001
negative	50 (98.0)	40 (100)	39 (81.3)	
positive	1 (2.0)	0 (0)	4 (8.3)	
equivocal	0 (0)	0 (0)	5 (10.4)	
Ki67 status				0.000
≤14%	37 (72.5)	15 (37.5)	18 (37.5)	
>14%	14 (27.5)	25 (62.5)	30 (62.5)	
Endocrine therapy				0.229
No	18 (36)	17 (42.5)	26 (53.1)	
Yes	32 (64)	23 (57.5)	23 (46.9)	
Chemotherapy				0.627
No	5 (10)	2 (5)	5 (10.2)	
Yes	45 (90)	38 (95)	44 (89.8)	
Radiotherapy				0.670
No	38 (76)	27 (67.5)	35 (71.4)	
Yes	12 (24)	13 (32.5)	14 (28.6)	

P-values were calculated to compare among ICC, IBC and LG-IDC-NOS groups.ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IBC, invasive breast cancer; ICC, invasive cribriform carcinoma; LG-IDC-NOS, low-grade invasive ductal carcinoma-not otherwise specified; PR, progesterone receptor.

Table 3. Comparison among the pure ICC, mixed ICC and invasive breast cancer (IBC) with less cribriform components (<50%) group

Characteristics	pure ICC n=30	mixed ICC (>50%) n=21	IBC (<50%) n=40	*P* values
Age (years)				0.891
mean	51	51	52	
range	36-71	37-76	34-77	
Tumor size (cm)				0.788
mean	2.38	2.19	2.19	
median	1.80	2.00	2.00	
range	1.00-10.00	0.90-4.00	0.80-5.00	
T stage, n (%)				0.914
T1b (0.5-1 cm)	1 (3.4)	3 (14.3)	4 (10)	
T1c (1-2 cm)	16 (55.2)	9 (42.9)	18 (45)	
T2 (2-5 cm)	11 (37.9)	9 (42.9)	18 (45)	
T3 (>5 cm)	0 (0)	0 (0)	0 (0)	
T4 (skin or chest muscle involvement)	1 (3.4)	0 (0)	0 (0)	
Lymph node status, n (%)				0.011
negative	24 (80)	14 (66.7)	19 (47.5)	
1-3 positive	5 (16.7)	7 (33.3)	14 (35.0)	
≥4 positive	1 (3.3)	0 (0)	7 (17.5)	
ER (%)				0.491
average	66.7	58.4	60.0	
range	15-95	0-90	0-95	
PR (%)				0.378
average	57.5	61.7	51.9	
range	0-95	0-90	0-95	
Ki67				
negative, n (%)	14 (46.7)	8 (38.1)	0 (0)	0.000
positive, n (%)	16 (53.3)	13 (61.9)	40 (100)	
average (%)	6.7	6.8	19	0.000
range	0-40	0-30	2-40	

P-values were calculated to compare among distinct ICC subtypes and IBC groups. ER, estrogen receptor; IBC, invasive breast cancer; ICC, invasive cribriform carcinoma; PR, progesterone receptor.

and 0.378, respectively). Fourteen cases of pure ICC (46.7%) did not show any signs of Ki67 proliferation (negative), as compared with 8/21 (38.1%) in mixed ICC (*P*=0.000). However, in the patients with Ki67 proliferation, the mean percentage of Ki67 in pure ICC was slightly lower than that in mixed ICC, and obviously lower than in IBC group (6.7% VS. 6.8% and 19%, respectively, *P*=0.000).

Discussion

In this study, we retrospectively investigated the clinicopathological characteristics and prognostic factors of ICC in a Chinese population, which was compared with the tumor containing less cribriform elements and LG-IDC-NOS without any cribriform elements. Although both ICC and LG-IDC-NOS belong to a family of low-grade breast neoplasia, we still found that ICC was associated with a series of favorable prognostic factors, such as smaller tumor size, less frequent axillary lymph node metastasis, lower tumor stage, higher positive rate of ER and/or PR expression and lower proliferation index. Morphological results demonstrated ICC was a well-differentiated neoplasm, such as extensive gland lumen formation, the presence of apical snout on the surface of the sieve pore and mucin-like secretion, and relatively low pleomorphism. Calcification could be found in our ICC cases and Ahmed [16] suggested that calcification might be the result of an active secretory process by the tumor cells in his electron microscopic observation. The immunohistochemical results indicated the luminal phenotypes for most of ICC cases because of the presence of ER. In our study, we found only 2 of

51 ICC cases were negative for ER, both in mixed subtype, but positive for PR instead. Some publications proposed the consistent expression of ER contributed to the excellent prognosis of ICC, but it is unclear whether hormone receptor discordance would occur in the process of ICC development [3, 17, 18]. In our series, we found one pure ICC case with distant metastasis (bone) for the first time as untreated for thirteen years. ER and PR in this case were still positive after 13 years of disease development, given the tumor at initial mass was positive for ER, since no publications of negative ER for pure ICC had been reported till now [1-3]. The patient was still alive with free disease after seven years post surgery, accepting conventional adjuvant therapy. We believed the persistent expression of hormone receptor in pure ICC may be a factor resulting in its excellent prognosis.

In ICC, the prognosis of mixed ICC is generally considered to be less favorable than that of the pure form [1-3, 6]. In our series, we found distinctly higher histologically positive lymph nodes in mixed ICC than the pure form (33.3% *VS*. 20%), which was consistent with Page's results, although the percentage reported were relatively lower (14.3% VS. 25%, respectively). We supposed that it was caused by the different surgery selection, since the majority of patients in our cohort underwent radical mastectomy or modified radical mastectomy, while those in Page's investigation underwent simple mastectomy, which made more lymph nodes be sampled and detected. In addition, there was a similar trend of the tumor's proliferation index detected by Ki67 between these two groups. Mixed ICC displayed higher proliferation index than pure ICC, no matter the positive rate or the average percentage of Ki67. This result seemed to prove more frequent lymph node metastasis in mixed ICC. In thirty pure ICC cases, we found one case whose maximal number of metastatic lymph node was more than three (6 positive lymph nodes), inconsistent with Venable's conclusion [3], while there was not the case in the mixed ICC. Therefore the number of metastatic lymph node may be not the proper prognostic factor for evaluating the different subtypes of ICC. Thus the pathologist must be careful to differentiate these two subtypes of ICC, since pure ICC may be associated with some favorable prognostic factors in contrast to ICC of mixed type.

A molecular classification of breast cancer based on gene array profiles has been proposed for a better understanding of its biology and treating guidelines. However, gene array analysis would not be possible in all patients and then the immunohistochemical subtyping has been proposed and accepted, which was best matched with the gene expression patterns. According to the different contents of cribriform pattern in the tumor, we found nearly all the cases in ICC and IBC groups were luminal A, while one case in ICC group was luminal B due to the concomitant expression of ER and HER2. As Colleoni proposed, in luminal breast cancer with negative node, several special types displayed better prognosis than those of no special types, especially for tubular, cribriform and mucinous carcinoma [19]. Current published data indicate that when compared with "grade 1" IDC, TC is associated with longer disease-free survival and breast cancer-specific survival close to normal life expectancy [20]. An up-regulation of "estrogen receptor signaling pathway" at transcriptional level being observed in TC may explain the distinction of prognosis within these two tumors [15]. As genomic similarity with TC, ICC also exhibited significantly higher positive rate of estrogen receptor than IDC containing less cribriform components and LG-IDC in our study. Thus, whether adjuvant chemotherapy is suitable in ICC cases is questionable, since the efficacy of adjuvant or neoadjuvant chemotherapy in patients with ER-positive disease is lacked [21-23]. Colleoni recommended that favorable histotypes (e.g. tubular, cribriform, mucinous, papillary types) with luminal tumors may be suitable for no therapy or endocrine therapy alone [19]. However, it must be cautious to make such a decision, because we found one pure ICC case was still possible to have distant metastasis (bone) if not treated for a long time (13 years). Tailored treatment research for ICC through international cooperation is key to make progress and solidify consensus on how to treat individual patients with special types of breast cancer such as invasive cribriform carcinoma.

In summary, invasive cribriform carcinoma shows more favorable prognostic factors than invasive breast carcinoma with less cribriform patterns and IDC-NOS with low histological grade, especially for classical ICC. Considering the tumor biology, and the benign course of pure ICC studied, chemotherapy may not be

indicated in the typical case. One defect of our study is the deficiency of ICC patients' follow-up time due to its rare occurrence. However, we had found that the patients whose tumors contained cribriform structures had a more favorable prognosis than those with LG-IDC, although no survival differences presented between ICC and IBC groups. Increasing follow-up time will help to elucidate this distinction.

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Conflict of interest statement

All authors declare no conflicts of interest.

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