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Original Article

Tubular carcinoma and grade 1 (well-differentiated) invasive ductal carcinoma: Comparison of flat epithelial atypia and other intra-epithelial lesions

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The distinction between tubular carcinomas (TC) and invasive well-differentiated (grade 1) ductal carcinoma (IDC) is important given treatment and prognostic differences. Studies have described a strong association between flat epithelial atypia (FEA) and TC. The incidence of FEA associated with grade 1 IDC is not well established. The aim of the present study was to assess morphology and intra-epithelial lesions between 14 TC and 18 grade 1 IDC matched for size. Of 14 TC, eight (57%) had associated FEA, seven (50%) had micropapillary atypical ductal hyperplasia (ADH), three (21%) had low nuclear grade ductal carcinoma *in situ* (DCIS), and four (29%) had lobular neoplasia. Notably, only two of 18 (11%) grade 1 IDC had associated FEA. Three of 18 (16%) grade 1 IDC had ADH, two (11%) had lobular neoplasia, and seven (39%) had DCIS. All tubular carcinomas were estrogen receptor (ER) positive and negative for *Her-2/neu* overexpression. All grade 1 IDC were ER positive but 5% also overexpressed *Her-2/neu*. Axillary lymph node metastasis was present in 11% of grade 1 IDC and absent in TC. A strong association was found between TC, FEA, and micropapillary ADH, which may reflect a biological progression. Despite matching for tumor size, grade 1 IDC have a higher incidence of lymph node metastasis and may have Her-2-neu overexpression compared to TC.

Key words: flat epithelial atypia, grade 1 (well-differentiated) invasive ductal carcinoma, tubular carcinoma

Tubular carcinomas (TC) of the breast are uncommon tumors, accounting for <2% of all invasive breast carcinomas. They are low-grade carcinomas characterized by prominent glandular differentiation with fewer reported genetic alterations (recurrent loss of 16q, gain of 1 q) compared to invasive ductal carcinoma.[1,2] The differences between TC and well-differentiated grade 1 invasive ductal carcinomas (grade 1 IDC) warrant further study.

The importance of differentiating TC from IDC is underscored by data showing that TC have a lower incidence of axillary lymph node metastasis and fewer recurrences.[3–6] Recent studies have documented an association between TC and micropapillary and cribriform ductal carcinoma *in situ* (DCIS) as well as flat epithelial atypia (FEA).[7,8] FEA is a descriptive term for an intraductal alteration characterized by replacement of native epithelial cells by a single or 3–5 layers of monotonous atypical cuboidal to columnar cells with apical snouts, and is distinguished from columnar cell change and columnar cell hyperplasia by the presence of mild cytological atypia, and from atypical ductal hyperplasia (ADH) and DCIS by the absence of architectural atypia.

The incidence of intraductal proliferations occurring in association with grade 1 IDC is not well-established. We systematically assessed the pathological features and concomitant intra-epithelial lesions associated with TC and grade 1 IDC matched for size to better understand the histopathology of these lesions and identify features that may help in the differential diagnosis.

MATERIALS AND METHODS

Study population

We identified all TC diagnosed at University of Michigan School of Medicine between January 1998 and August 2006 following excision biopsy. Grade 1 IDC diagnosed on excisions and matched for size, were also retrieved from the surgical Pathology files. All cases were retrospectively

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evaluated by the authors (LPK and CGK). We applied strict criteria for the diagnosis of TC i.e. the tubular component accounted for at least 90% of tumor as recommended by the World Health Organization (WHO) Working Group on the Pathology and Genetics of Tumors of the Breast.[9] The grade 1 IDC selected for study were well-differentiated, with low-grade nuclei, no prominent specialized pattern, and failed to exhibit characteristics diagnostic of a specific histological type. Importantly, the grade 1 IDC included in the present study were matched for size with TC (median size 0.8 cm and 0.9 cm for TC and grade 1 IDC, respectively) in order to avoid the bias of disparity in carcinoma dimension between the two groups.

Morphological review

Morphological parameters evaluated in the present study included (i) presence of angulated glands with open lumina, apical snouts, nuclear grade, and stromal cellularity; (ii) presence of associated intra-epithelial lesions including FEA, atypical lobular hyperplasia (ALH), lobular carcinoma *in situ* (LCIS), ADH and DCIS. Although at present there is no unequivocal agreement on whether quantitative criteria should be applied to separate ADH from low-grade DCIS, in the present study ADH was diagnosed following the criteria established by WHO.[9] Thus, ADH was diagnosed when there was a proliferation of evenly distributed monomorphic cells growing in different patterns (micropapillary, cribriform) but that coexisted with usual ductal hyperplasia, and/or there was partial involvement of duct.

All the available slides of excision biopsies were reviewed by two study authors (LPK and CGK). The slides per case ranged from six to 13 slides. Statistical analyses were performed by the biostatistician in our group (YD) using Pearson's χ^2 test. $P < 0.05$ was considered statistically significant.

Clinical information

Clinical information including hormone receptors (estrogen receptor, ER; progesterone receptor, PR) and *Her-2/neu* status as well as axillary lymph node involvement were reviewed for all cases when available.

Immunohistochemistry (ER, PR and Her-2/neu)

Hormone receptors (ER and PR) as well as *Her-2/neu* were assessed on immunohistochemistry using a refined labeled streptavidin–biotin technique (LSAB+ Kit, HRP; Dako Cytomation, Carpinteria, CA, USA) for ER and PR. Following paraffin removal and hydration, slides were treated with Tris (0.25 mol/L)-EDTA (0.1 mmol/L) buffer, pH 9.0 in a microwave pressure cooker for 15 min for optimal antigen retrieval before immunostaining. Cases were stained with monoclonal antibodies against ER (clone 6F11, 1:100 dilution; Ventana Medical Systems, Tucson, AZ, USA), PR (clone 636, 1:400 dilution; Dako Cytomation) and *Her-2/neu* ((clone CB11; Ventana Medical Systems) for 32 min at 42°C on 4 μm-thick sections obtained from formalin-fixed paraffin-embedded blocks. A Ventana Basic DAB Detection Kit was used according to manufacturer's specifications for *Her-2/neu*. Staining was performed on the Ventana BenchMark XT platform (Ventana Medical Systems).

Only nuclear staining for ER and PR was evaluated quantitatively (>5% tumor cells staining was considered positive). *Her-2/neu* immunoreactivity was semiquantified according to the following established grading scheme: negative or 0, no or any detectable membrane staining in <10% of tumor cells; 1+, weak, incomplete membrane staining in >10% of tumor cells; 2+, moderate staining (proportion and intensity of staining between 1+ and 3+ scores) i.e. weak to moderate complete staining in >10% of tumor cells; and 3+, strong, complete membrane staining in >30% of the tumor cells. Cytoplasmic staining was not included in the grading scheme. On immunohistochemistry a score of 2+ or 3+ was considered Her-2-neu overexpression.

Fluorescence *in situ* hybridization (*Her-2/neu*)

Fluorescence *in situ* hybridization (FISH) for confirmation of *Her-2/neu* overexpression was performed on all cases with 2+ immunohistochemistry score at the Mayo Clinic, Rochester, MN, USA according to previously described established procedure. Briefly, FISH was performed using PathVysion DNA Probe Kit (Vysis, Downers Grove, IL, USA). Unstained 4 μm slides were deparaffinized, dehydrated and air-dried. Following an average of 60 min of protease digestion and denaturation, tissue sections were hybridized for 16 h at 37°C with prewarmed probes for the *Her-2* gene and chromosome 17 centromere (*Her-2/neu*/CEP 17, Abbott Molecular/Vysis, Des Plaines, IL, USA). After hybridization the slides were washed, counterstain was applied and slides were coverslipped and sealed. *Her-2* signals were quantified in the area of invasive tumor and at least 30 non-overlapping interphase cell nuclei were evaluated. In each nucleus the number of *Her-2* and chromosome 17 centromere signals were counted and the Her-2 : CEP 17 ratio was calculated. A ratio >2.2 was considered a positive result (amplification of *Her-2/neu* by FISH).

RESULTS

Fourteen TC and 18 grade 1 IDC matched for size were retrieved for analysis. Table 1 summarizes the patient char-

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Table 1 Morphological features of TC and grade 1 IDC

	Tubular carcinoma *n* (%)	Grade 1 IDC *n* (%)	*P*
n	14	18	
Median age (range) (years)	56.7 (36–81)	60 (39–94)	
Median size (range) (cm)	0.8 (0.2–1.5)	0.9 (0.4–1.3)	
Angulated glands with open lumina			
>90%	14 (100)	0	
50–90%	0	3 (17)	
<50%	0	15 (83)	
			<0.0001
Fused glands and/or cords	0	13 (72)	
			<0.0001
Apical snouts			
>50%	14 (100)	2 (11)	
25–49%	0	3 (17)	
5–24%	0	8 (44)	
<5%	0	5 (28)	
			<0.0001
Stromal characteristics			
Myxoid	7 (50)	6 (33)	
Fibrotic	7 (50)	12 (67)	
			NS

IDC, invasive ductal carcinoma; TC, tubular carcinoma; NS, not significant.

acteristics and morphological features. The median age of the patients was 56.7 years (range, 36–81 years) for TC and 60 years (range, 39–94 years) for grade 1 IDC. The median tumor size was 0.8 cm (range, 0.2–1.50 and 0.9 (range, 0.4–1.3 cm) for TC and grade 1 IDC, respectively.

All TC had >90% angulated glands with open lumina (Fig. 1a). Of the 18 grade 1 IDC, 17% ($n = 3$) had prominent tubular architecture, with angulated glands accounting for 50–75% of the tumor, and was regarded as 'mixed type carcinoma' as per the WHO classification of breast tumors. The remaining 83% of grade 1 IDC (15/18) had <50% angulated glands. Fused glands and/or cords of tumor cells were seen in 72% of IDC (13/18; Fig. 1d) and were absent in all TC. Prominent apical snouts (involving >50% of tumor cells) were present in the vast majority of TC (93%, 13/14) while they were rare in grade 1 IDC (11%, 2/18). Furthermore, the stroma was myxoid and/or fibroblastic in both TC and grade 1 IDC. Presence of angulated glands, fused glands/cords and apical snouts was significantly more common in TC when compared to grade 1 IDC ($P < 0.0001$). No significant differences were found in the stromal characteristics of the tumors.

Table 2 lists the frequency of FEA and other intra-epithelial lesions in TC and grade 1 IDC. FEA was almost an exclusive feature of TC. In the TC group FEA was present in 57% of TC (8/14) while it was seen in 11% of grade 1 IDC (2/18; $P = 0.005$). The most common morphological pattern of FEA (7/8) was that of a lobular well-circumscribed configuration of variably distended terminal ducts (blunt duct adenosis pattern on low power (10×); Fig. 1c) whereas the large cystically dilated ducts with flocculent secretions in lumen was uncommon (1/8). In all cases, regardless of the low-power appearance, the cells lining the acini and ducts had low-grade atypia characterized by epithelial cells with round to ovoid nuclei, slightly increased nuclear–cytoplasm ratio, and loss of polarity with disorganization of nuclei relative to the basement membrane. The ADH and DCIS lesions associated with TC had a cribriform and/or micropapillary growth pattern in all cases (Fig. 1b). TC was associated with FEA and micropapillary ADH in 50% (7/14), of which cribriform low-grade DCIS was also present in 21% (3/14).

FEA was rare in grade 1 IDC, being present in 11% (2/18). Interestingly, the two grade 1 IDC tumors with associated FEA had tubular features but did not meet the criteria for pure TC, because the tubular differentiation was <90% of tumor. The reminder of the grade 1 IDC (16/18) did not contain FEA. Ten of 18 grade 1 IDC tumors (55%) had ADH and DCIS, with most having cribriform architecture (8/10; Fig. 1e,f). A solid pattern of growth was less common (2/10). Micropapillary ADH or DCIS was focally present in one of three grade 1 IDC with tubular features.

Lobular intra-epithelial lesions, ALH and/or LCIS, were observed in 29% of TC (4/14)and were less frequently associated with grade 1 IDC (11%, 2/18 cases), but this was not statistically significant ($P = 0.21$). TC was associated with FEA and ALH/LCIS in 15% (2/14).

As shown in Table 3, although lymph node metastasis developed in 11% of patients with grade 1 IDC (2/18), they were absent in all patients with TC. All TC were positive for

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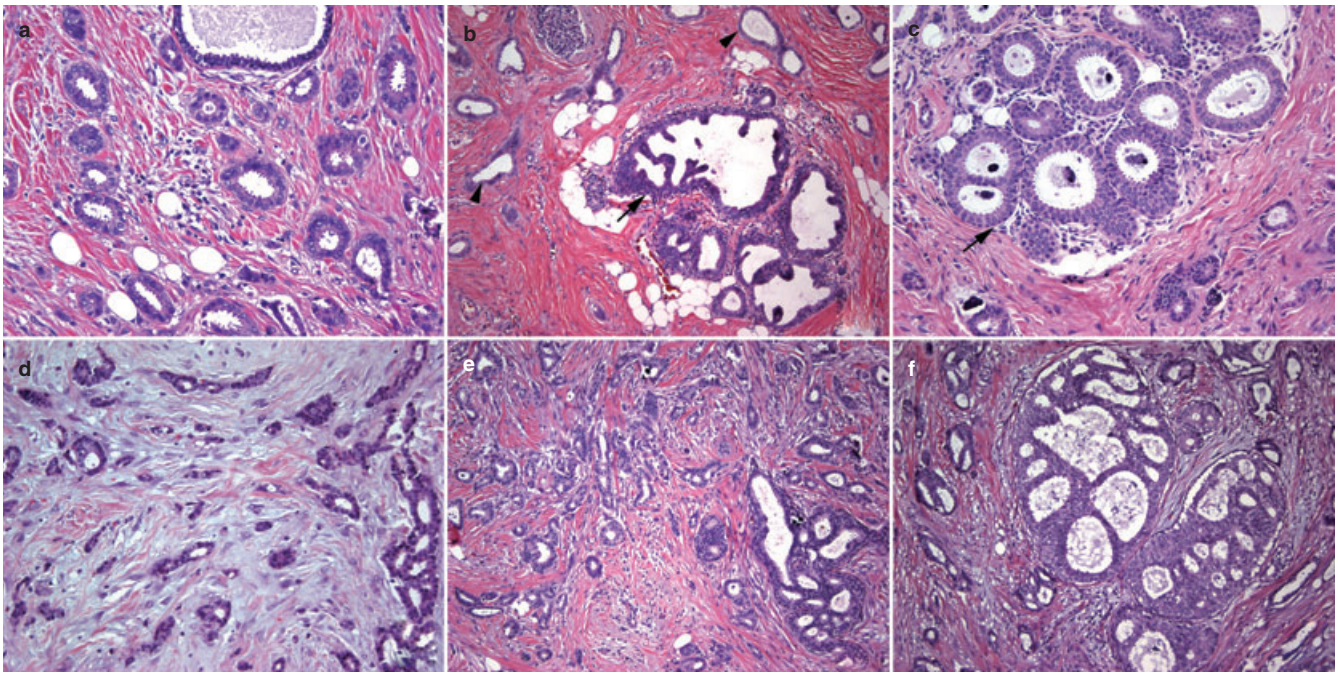

Figure 1 Intra-epithelial lesions including flat epithelial atypia (FEA) in tubular carcinoma (TC) and grade 1 invasive ductal carcinoma (IDC). (**a**) TC composed of haphazardly arranged round and angulated glands with open lumens and apical snouts surrounded by fibroblastic stroma (HE). (**b**) Micropapillary atypical ductal hyperplasia (ADH; arrow) with adjacent TC (arrowheads; HE). (**c**) FEA with blunt adenosis pattern (arrow) characterized by flat growth pattern (no complex architecture, unlike ADH) and cytological atypia similar to low nuclear grade ductal carcinoma *in situ* (DCIS). The cells of FEA lack polarity with respect to basement membrane and have round to ovoid nuclei with occasional nucleoli. Note adjacent TC. (**d**) Well-differentiated (grade 1 IDC) composed of predominantly fused glands as well as cords of cells with few single glands in a myxoid stroma (HE). (**e**) Cribriform ADH in a single duct characterized by cribriform proliferation of relatively monomorphic cells with occasional overlapping of cells (note that cells are not as uniform as they would be in low-nuclear grade DCIS) with adjacent grade 1 IDC (HE). (**f**) Cribriform low-nuclear grade DCIS with grade 1 IDC characterized by uniform cells with round nuclei and sharp punched out lumina (HE).

Table 2 Distribution of intra-epithelial lesions

Associated intra-epithelial lesions	Tubular carcinoma ($n = 14$) *n* (%)	Grade 1 IDC ($n = 18$) *n* (%)
Flat epithelial atypia	8 (57)	2 (11) ($P = 0.005$)
Lobular neoplasia (ALH/LCIS)	4 (29)	2 (11) (NS)
Atypical ductal hyperplasia	7 (50)	3 (16) ($P = 0.04$)
Ductal carcinoma *in situ*	3 (21)	7 (39) (NS)

ALH, atypical lobular hyperplasia; IDC, invasive ductal carcinoma; LCIS, lobular carcinoma *in situ*; NS, not significant.

Table 3 Hormone receptors, Her-2/neu and axillary lymph node status

	ER positive *n* (%)	PR positive *n* (%)	Her-2/neu overexpression IHC *n* (%)	Her-2/neu over-expression FISH *n* (%)	Axillary lymph node status *n* (%)
TC ($n = 14$)	14 (100)	9 (64)	1 (7)	0	0
Grade 1 IDC ($n = 18$)	18 (100)	15 (83)	3 (17)	1 (5)	2 (11) (NS)

ER, estrogen receptor; FISH, fluorescence *in situ* hybridization; IHC, immunohistochemistry; NS, not statistically significant; PR, progesterone receptor; TC, tubular carcinoma

ER, and 64% (9/14) were positive for PR. All grade 1 IDC were positive for ER and 83% (15/18) were also positive for PR. Of note, *Her-2/neu* overexpression was detected on immunohistochemistry in 7% of TC (1/14) and in 17% of grade 1 IDC (3/18). FISH confirmed the amplification of *Her-2/neu* in 5% of grade 1 IDC (1/3) while the TC, as well as two grade 1 IDC with *Her-2/neu* overexpression on immunohistochemistry, were negative on FISH.

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DISCUSSION

In the present study we analyzed morphological characteristics, precursor intra-epithelial lesions, hormone receptor, *Her-2/neu* overexpression and lymph node status of TC in comparison with size-matched grade 1 IDC tumors, with the aim of identifying pathological characteristics that could be applied to daily surgical pathology practice.

TC has an excellent prognosis, with the survival of patients with tubular carcinoma being similar to that of the general population.[10] Furthermore, because of the rare incidence of metastasis, adjuvant systemic therapy has been recommended for large tumors, usually >3 cm, and adjuvant radiation therapy following breast conservation therapy is not recommended by some groups.[10,11] Therefore, it is imperative that a definitive diagnosis of TC is made following strict histological criteria. Furthermore, defining the intraductal putative precursor lesions associated with TC that may help in the differential diagnosis with grade 1 IDC would be helpful in daily pathology practice.

Historically, there has been a lack of consensus among pathologists concerning the proportion of tubular structures required to establish the diagnosis of TC. Although it is inherently assumed that all TC have a prominent tubular configuration, several early studies did not have specific cut-offs, while other authors have used percentages of tubular structures ranging from 70% to 100%.[8,12–14] Thus, although there is a body of literature on the features of TC, it is difficult to compare results among these studies given the different definitions used. Recently, the WHO Working Group on the Pathology and Genetics of Tumors of the Breast made the recommendation that 90% of tubules are needed to establish the diagnosis of TC.[9] This definition was used in the present study. Once the definition is applied, TC are usually small; the median size of TC in the present series was 0.8 cm with the majority (10/14) being <1 cm. We noted that even matching the tumors for size, TC had less lymph node metastasis than grade 1 IDC. Not surprisingly, we found that TC do not overexpress *Her-2/neu*, in contrast with a very small group of grade 1 IDC.

Goldstein and O'Malley reported that 'cancerization of small ectatic ducts by DCIS cells with apocrine snouts', a term that now is designated as FEA, is associated with TC in 43.7% of cases.[15] Recent studies, as well as the present series (57% of TC associated with FEA, $P = 0.005$), have confirmed the strong association between FEA and TC, which may reflect a biological progression.[7,8] The present findings are in agreement with those of Fernandez-Aguilar *et al*. and Goldstein and O'Malley (47.8% and 43.7%, respectively),[8,15] in that we found that FEA is significantly associated with TC.

To the best of our knowledge only two studies have attempted to compare precursor lesions between TC and grade 1 IDC.[7,15] Both studies are in agreement with the present findings that FEA is more commonly associated with TC than with grade 1 IDC. In the present study we noted FEA in two grade 1 IDC that had tubular features (50–75% of tubules) but which did not meet the criteria for TC.

We found that the majority of DCIS lesions in both TC and grade 1 IDC are of low nuclear grade, supporting the fact that a good correlation exists between the histological grade of DCIS and the grade of the associated invasive carcinoma.[8,16] Interestingly, ADH and DCIS lesions arising in association with TC commonly show micropapillary and/or cribriform architecture, while a solid growth pattern of DCIS is uncommon in the setting of TC.[7,15,17] We noted that the triad of TC, FEA, and micropapillary ADH/DCIS is present in 50% of TC, and postulate that there may be a biological progression. We also found an association between TC and ALH, LCIS, and FEA.

In summary, the present data validate the fact that TC is a distinct tumor entity that has lower incidence of axillary lymph node metastasis and no *Her-2/neu* overexpression when compared to size-matched grade 1 IDC. Although in isolation, morphological features including the presence of angulated glands and/or apical snouts are not helpful morphological features in distinguishing TC from grade 1 IDC, the combination of these features in the absence of fused glands and/or cords is a strong diagnostic tool for TC. FEA is strongly associated with TC and with the tubular component of mixed tumors. The strong association between TC, micropapillary ADH and FEA found in the present study underscore the investigation of these lesions in the pathobiology of TC.

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