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ORIGINAL PAPER

Coexisting ductal carcinoma in situ independently predicts lower tumor aggressiveness in node-positive luminal breast cancer

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Abstract Primary breast invasive ductal carcinoma coexisting with ductal carcinoma in situ (IDC-DCIS) is characterized by lower proliferation rate and metastatic propensity than size-matched pure IDC. IDC-DCIS is also more often ER-positive, PR-positive and/or HER2-positive. This analysis aims to clarify whether the presence of coexisting DCIS in IDC affects tumor aggressiveness in various biological subtypes of breast cancer, respectively. Tumor data obtained from 1,355 consecutive female patients undergoing upfront surgery for primary breast cancer were analyzed retrospectively; 196 patients with pure DCIS were excluded. Based on evidence that immunohistochemistry (IHC) provides a reasonable approximation of molecular phenotypes, the tumor samples were divided into 4 groups: (1) luminal A (ER and/or PR-positive, HER2-negative, Ki67 $\leq$ 12), (2) luminal B (ER and/or PR-positive, HER2-negative, Ki67 $>$ 12), (3) HER2 (HER2-positive) and (4) basal-like (triple-negative) disease. Ki67 expression and nodal involvement of IDC with or without DCIS in these groups were compared. The number of patients with luminal A, luminal B, HER2 and basal-like breast cancer were 396, 265, 258 and 117, respectively. Ki-67 was lower in IDC-DCIS than in size-adjusted pure IDC of both luminal A and luminal B subtypes ($P = 0.15$ and <0.005, respectively). In HER2 or basal-like tumors, there were no significant difference between pure IDC and IDC-DCIS. The presence of coexisting DCIS in IDC predicts lower biological aggressiveness in luminal cancers but not in the conventionally more aggressive HER2-positive and triple-negative subtypes.

Keywords Ductal carcinoma in situ (DCIS) · Invasive ductal carcinoma (IDC) · Ki67 · Luminal breast cancer

Introduction

In breast cancer, ductal carcinoma in situ (DCIS) often accompanies invasive ductal carcinoma (IDC). With the implementation of mammography screening and earlier detection of cancer, it is reported that up to 60% of invasive tumors contain both IDC and DCIS (IDC-DCIS) [1]. While currently available evidence supports a clonal relationship between the DCIS and IDC components of IDC-DCIS based on concordant expression of immunohistochemical [2–5] and genomic [6–8] markers, the clinical significance associated with the coexistence of DCIS in invasive disease has not been conclusively defined.

We have previously shown that IDC-DCIS is characterized by lower proliferation rate and metastatic propensity than size-matched pure IDC, especially if the ratio of DCIS to IDC size is high; IDC-DCIS is also more often estrogen receptor (ER)-positive, progesterone receptor (PR)-positive and/or human epidermal growth factor

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receptor 2 (HER2)-positive than is pure IDC [9]. Another study similarly reported more frequent ER and PR positivity in IDC-DCIS [1], although not confirmed by other smaller patient cohorts [10, 11]. Despite minor inconsistencies across studies, it can generally be recognized that IDC-DCIS represents a clinical and biological entity distinct from pure IDC. In fact, IDC-DCIS was associated with better metastasis-free survival [1] and a trend for better disease-free and overall survival which did not reach statistical significance [9, 12].

Breast cancer is thus a heterogeneous disease, which can otherwise be categorized by gene expression profiling into at least 5 major biologically and prognostically distinct subtypes, namely luminal A, luminal B, HER2-enriched, basal-like, and normal breast-like tumors [13]. Whether the association of DCIS with IDC predominantly corresponds to particular molecular subtype(s), and whether independent significance of such association exists within each subtype, have not been studied.

To date, the use of gene expression profiling assays in routine clinical practice is limited mainly by its cost, availability and technical complexity. On the other hand, expression patterns of ER, PR and HER2 as determined by immunohistochemistry (IHC) are more readily accessible, and may correlate with gene expression microarray categorization [14]. Recently, supplementation of these biomarkers with Ki67, a nuclear marker of cell proliferation, was shown to better separate the genetic subtypes [15]. Expression of cytokeratin (CK) 5/6, CK 8/18 and/or epidermal growth factor receptor (EGFR) were utilized to define the basal subtype [16, 17]. A variety of other IHC markers including androgen receptor (AR), p53, E-cadherin, MUC1 and nuclear BRCA1 are under evaluation [18].

In addition to its potential role in subtype prediction, Ki67 is an independent adverse prognostic factor for breast cancer survival [19, 20] and reportedly predictor of response to chemotherapy [21]. Its expression is independent of tumor size and nodal status [22–24]. There is currently no standard cutoff Ki67 value above which a high-risk group can be defined, although most data suggested a level of 10–14% [25].

This study aims to compare tumor aggressiveness, in terms of Ki67 expression, in IDC with and without coexisting DCIS stratified by biological subtypes and lymph node status, and to assess any independent significance of associated DCIS within each subgroup.

Methods

Patients and classification

Consecutive female patients undergoing upfront surgery for primary early ductal breast cancer in a single tertiary referral institute in Hong Kong between October 2000 and September 2008 were identified as previously described [9]. Data of a total of 1,355 tumor samples included in a prospectively maintained database were analyzed; 196 patients with pure DCIS were excluded. The remaining 1,159 tumor samples were classified according to ER, PR, HER2 and Ki67 expression, based on evidence that IHC provides a reasonable approximation of molecular phenotypes, into: (1) luminal A (ER and/or PR-positive, HER2-negative, Ki67 $\leq$ 12), (2) luminal B (ER and/or PR-positive, HER2-negative, Ki67 $>$ 12), (3) HER2-enriched (HER2-positive) and (4) basal-like (triple-negative) disease. In the subsequent analysis comparing Ki67 of IDC-DCIS versus pure IDC within each subtype, luminal cancers comprising of both luminal A and B subtypes were studied as one entity, to avoid possible confounder imposed by the predefined Ki67 cutoff. Tumor samples were further stratified by lymph node status into node-positive and -negative subgroups, which are often considered separately in the current literature due to their distinct prognosis and management implications.

Histopathological and immunohistochemical examination

Tumor histolopathology and the number of involved lymph nodes were evaluated by routine hematoxylin-eosin (H&E) staining. IHC analysis was performed with standard commercial kits on formalin-fixed, paraffin-embedded specimens. All the tumor histolopathology and IHC data were centrally reviewed in a central laboratory in Hong Kong. IHC of ER and PR were assessed using 6F11 and 1A6 antibodies, respectively, and detected by the polymer EnVision system (Dako, Glostrup, Denmark). Expression of ER and PR were graded by the semi-quantitative H-score, where a score of over 50 out of 300 was interpreted as positive [26]. The IHC assay used for HER2 was A0485 (polyclonal antibody; Dako). HER2 positivity was defined by IHC 3+ (strong positive staining on at least 10% of breast tissue specimen) and/or fluorescent in situ hybridization (FISH)-amplified (HER2 DNA to chromosome 17 centromere DNA ratio of at least 2) [27], the latter using PathVysion Vysis FISH (Abbott, Chicago, IL, USA). Finally, expression of Ki-67 was assessed by immunostaining with the antibody SP6 in all the analyzed cases. In cases of IDC-DCIS, Ki67 values of the invasive cancer component were used for analyses.

Statistical analyses

Summary statistics were used to describe patient demographics and pathological characteristics of tumor samples. Ki67 indices of tumors in different subtypes were

compared by the Mann Whitney U test. Spearman correlations were calculated to determine the association between variables. Adjustment for tumor size was performed by multivariate logistic regression. Univariate and multivariate analyses using a linear regression model were performed to assess factors affecting Ki67. Predictor variables evaluated included age, tumor size, grade, ER, PR and HER2 expression where applicable, and the presence of DCIS. Calculations were performed using the statistical software SPSS, version 18. Significance was assumed at $P < 0.05$.

Results

Patient demographics

The number of evaluable patients with luminal A, luminal B, HER2-enriched and basal-like breast cancer were 396, 265, 258 and 117, respectively. In particular, within our HER2-enriched cohort, HER2-positivity was defined by IHC 3+ in 245 patients (95%) while gene amplification was detected in the remaining 13 (5%).

The patients' demographic data are presented in Table 1. Median age in all subgroups were similar at 48–49 years, although there was a higher proportion of young patients (age ≤ 35) with basal-like cancers (Spearman Correlation $= -0.063$, $P = 0.033$). Most patients in all subgroups were premenopausal, consistent with the local epidemiology [28]. Approximately 10% of luminal and HER2-enriched tumors were screen-detected; a lower percentage was noted in basal-like tumors likely due to their aggressive natural history, but the difference was not statistically significant (Spearman correlation $= -0.034$, $P = 0.304$). Basal-like patients more often received breast conservation surgery as compared to those in other subgroups (OR $= 1.85$, $P = 0.003$) despite similar tumor sizes; the observation that these tumors were less associated with concomitant DCIS (Spearman correlation $= -0.083$, $P < 0.01$) suggested the latter might contribute to extensive disease indicating mastectomy. There was also a trend toward more frequent axillary dissection in the basal-like subtype (Spearman correlation $= -0.05$, $P = 0.109$).

Tumor characteristics of different biological subtypes

The luminal and HER2-positive subtypes were more frequently IDC-DCIS rather than pure IDC (Spearman correlation $= 0.002$ with $P = 0.935$; and Spearman correlation $= 0.112$ with $P = < 0.0005$, respectively), and vice versa for basal-like tumors (Spearman correlation $= -0.134$, $P < 0.01$), as illustrated in Table 2. In concordance with available evidence, HER2 and basal-like subtypes were more aggressive than their luminal counterparts, as reflected by higher Ki67 levels (Spearman correlation coefficient -0.31 and -0.46, respectively, $P < 0.01$); basal-like tumors were also more likely to be of higher histological grade (Spearman correlation $= 0.254$, $P < 0.01$).

Significance of coexisting DCIS within biological subtypes

On comparing size-adjusted IDC with or without DCIS within the same luminal subtype, Ki67 was found to be lower in IDC-DCIS than pure IDC (coefficient estimate $= -0.074$, $P < 0.01$). Moreover, in luminal patients with involved lymph nodes, DCIS emerged as an independent factor predicting Ki67 ($P = 0.03$); other factors included tumor grade ($P < 0.01$), ER ($P < 0.01$) and PR level ($P = 0.02$) (Table 3). On the other hand, in node-negative luminal cancers, the only factors after multivariate regression analysis were age ($P = 0.09$), tumor grade ($P < 0.01$) and ER score ($P < 0.01$). In the intrinsically more aggressive HER2 or basal-like tumors, however, the presence of associated DCIS did not correlate significantly with Ki67.

Discussion

Our study shows that the presence of coexisting DCIS in IDC is associated with significantly lower Ki67 levels in luminal breast cancers, but not in the conventionally more aggressive HER2-positive and triple-negative subtypes where apparent high-risk factors such as HER2-overexpression and high tumor grade play a predominant role. Particularly in luminal node-positive patients, the ability of associated DCIS to predict Ki67 is independent of ER level, tumor size and grade. As Ki67 in turn prognosticates survival and also predicts chemotherapy response, our findings may potentially aid risk stratification and adjuvant treatment decision in some luminal cancers where the benefit of chemotherapy beyond endocrine therapy is controversial [29].

In modern adjuvant treatment of breast cancer, research efforts have been directed to identify patient subgroups where unnecessary therapy can be safely withheld, thus a shift from a more inclusive strategy toward personalized medicine. Accompanying the development of this concept, the use of chemotherapy in hormone-positive node-negative patients was progressively reduced over recent years [30]. In ER-positive patients, the absolute increment in 10-year overall survival brought about by chemotherapy in addition to hormonal therapy is only approximately 2–10% depending on age and nodal status, implying

Table 1 Patient demographics of different biological subtypes of breast cancer

Characteristics	Luminal [% (no. of patients)]			HER2-positive [% (no. of patients)]	Basal-like [% (no. of patients)]
	All luminals	Luminal A	Luminal B		
Age at diagnosis					
Median (range)	48 (26–91)	49 (26–91)	47 (28–88)	48 (28–84)	49 (24–86)
≤35	5.9 (39)	4.8 (19)	7.5 (20)	9.3 (24)	12.0 (14)
36–50	56.0 (370)	53.8 (213)	59.2 (157)	53.9 (139)	42.7 (50)
≥51	37.7 (249)	41.2 (163)	32.5 (86)	36.4 (94)	43.6 (51)
Unknown	0.4 (3)	0.2 (1)	0.8 (2)	0.4 (1)	1.7 (2)
Menopausal status					
Pre-menopausal	63.5 (420)	60.4 (239)	68.3 (181)	66.7 (172)	60.7 (71)
Post-menopausal	36.5 (241)	39.6 (157)	31.7 (84)	33.3 (86)	39.3 (46)
Mode of discovery					
MMG screening	9.4 (62)	11.1 (44)	6.8 (18)	10.1 (26)	5.1 (6)
Self-detected	78.2 (517)	73.7 (292)	84.9 (225)	81.8 (211)	88.0 (103)
Others	12.4 (82)	15.2 (60)	8.3 (22)	8.1 (21)	6.9 (8)
Primary surgery					
Mastectomy	48.6 (321)	49.0 (194)	47.9 (127)	60.1 (155)	36.8 (43)
Breast conservation	49.2 (325)	48.7 (193)	49.8 (132)	38.4 (99)	60.7 (71)
Others	2.2 (15)	2.3 (9)	2.3 (6)	1.5 (4)	2.5 (3)
Axillary surgery					
Axillary dissection	31.6 (209)	29.0 (115)	35.5 (94)	29.5 (76)	38.5 (45)
Sentinel LN	59.3 (392)	62.4 (247)	54.7 (145)	59.3 (153)	52.1 (61)
Nil	9.1 (60)	8.6 (34)	9.8 (26)	11.2 (29)	9.4 (11)
Total number	661	396	265	258	117

Data are presented as percentages, with total case numbers following in parentheses

over-treatment in most patients, and at the expense of increased toxicities [31, 32]. Patients did not benefit equally from chemotherapy; subgroups deriving minimal benefit from chemotherapy could be observed regardless of nodal status [33, 34].

Multigene tumor assays correlated with prognosis and chemotherapy benefit in ER-positive node-negative [35, 36] and, more recently, in postmenopausal node-positive patients [36, 37]. Commercially available multigene assays include 21-gene recurrence score assay (OncotypeDx), 70-gene expression profile (MammaPrint) and 50-gene expression profile (PAM50). Although the recurrence score was reported to influence patient management decisions [38], the clinical use of these assays are still limited [29]. Moreover, the significance of an intermediate recurrence score, and whether all ER-positive patients are indicated for the assays are yet to be clarified. It is thus likely that the assays will not replace conventional clinicopathological parameters in prognosis and chemotherapy benefit prediction. It is also important to identify additional markers which are more easily accessible. The presence of associated DCIS in IDC, as shown in this study, may supplement the selection of patients in whom multigene assays should be performed or, in low-risk cases, where chemotherapy can be avoided.

Notably, in node-positive luminal patients, the coexistence of DCIS was an independent prognosticator and predictor, irrespective of the level of hormone receptor expression. Although IDC-DCIS is also more often ER and/or PR-positive [9], results of the present study suggest associated DCIS provides further information to predict lower tumor aggressiveness, in addition to its effect mediated through hormone receptor expression. On the other hand, node-negative luminal patients generally represent a low-risk and biologically less aggressive group. This may explain the finding in this study that concomitant DCIS, despite the trend to associate with lower Ki67, was not an independent factor predicting the latter.

We have postulated previously that the presence of DCIS in IDC indicates a carcinogenesis process where mild genetic deficits are acquired progressively, whereas pure IDC arising de novo is a result of one or more major (epi)genetic event [9]. While the genetic aberrations involved tumor progression from in situ carcinoma to invasiveness are minor and localized [39] and hence difficult to be identified [40–42], major mutations in specific

Table 2 Comparison of tumor characteristics of different biological subtypes

Characteristics	Luminal [% (no. of patients)]			HER2-positive [% (no. of patients)]	Basal-like [% (no. of patients)]
	All luminals	Luminal A	Luminal B		
Subgroups					
Pure IDC	46.7 (309)	43.9 (174)	50.9 (135)	36.8 (95)	66.7 (78)
IDC-DCIS	53.3 (352)	56.1 (222)	49.1 (130)	63.2 (163)	33.3 (39)
IDC size (cm)					
Median (range)	1.8 (0.07–10)	1.6 (0.07–10)	2.2 (0.09–10)	1.6 (0.01–9.5)	2.2 (0.2–9)
Grade					
1	15.9 (105)	23 (91)	5.3 (14)	2.7 (7)	1.7 (2)
2	45.1(298)	55.6 (220)	29.4 (78)	24.8 (64)	7.7 (9)
3	38 (251)	19.9 (79)	64.9 (172)	69.8 (180)	90.6 (106)
Unknown	1.1 (7)	1.5 (6)	0.4 (1)	2.7 (7)	0 (0)
Lymph node status					
Negative	60.1 (397)	62.9 (249)	55.8 (148)	61.2 (158)	61.5 (72)
1–3 lymph nodes	27.2 (180)	27.3 (108)	27.2 (72)	23.3 (60)	23.9 (28)
4–9 lymph nodes	7.9 (52)	6.3 (25)	10.2 (27)	10.1 (26)	7.7 (9)
>9 lymph nodes	3 (22)	1.8 (7)	5.7 (15)	4.3 (11)	5.1 (6)
Unknown	1.5 (10)	1.8 (7)	1.1 (3)	1.2 (3)	1.7 (2)
Lymphovascular invasion					
Negative	60.8 (402)	68.4 (271)	49.4 (131)	55.0 (142)	61.5 (72)
Positive	39.2 (259)	31.6 (125)	50.6 (134)	45.0 (116)	38.5 (45)
Ki-67 index (%)					
Median (range)	10 (0–95)	6 (0–12)	28 (13–95)	21 (0–80)	50 (0–95)
Total number	661	396	265	258	117

Data are presented as percentages, with total case numbers following in parentheses

Table 3 Univariate and multivariate model for prediction of node-positive breast cancer

Variable	Univariate			Multivariate		
	Beta	(95% CI)	*P* value	Beta	(95% CI)	*P* value
Age	2.71	(−1.40, 6.82)	0.20	–	–	–
Tumor size	1.10	(−0.53, 2.74)	0.18	–	–	–
Tumor grade	14.19	(10.74, 17.64)	<0.01	11.15	(7.70, 14.59)	<0.01
ER score	−0.09	(−0.12, −0.06)	<0.01	−0.05	(−0.8, −0.3)	<0.01
PR score	−0.07	(−0.09, −0.04)	<0.01	−0.03	(−0.5, −0.04)	0.02
Presence of DCIS	10.08	(5.32, 14.85)	<0.01	4.68	(0.35, 9.00)	0.03

tumor subtypes are more often described. For example, *BRCA1* and *TP53* mutations play a particular role in basal-like tumors [43, 44], and overexpression of HER2 encoded by *ERBB2* drives carcinogenesis of HER2-positive disease [45–48]. These genotypes/phenotypes are also negative prognosticators, if without targeted therapy in the case of HER2-positive disease [49–51]. Taken together, these considerations explain our finding in the present study that concomitant DCIS did not significantly affect Ki67 in the basal-like and HER2-positive subtypes, where mutated *BRCA1*/*TP53* and *HER2,* respectively are predominant factors overriding the otherwise lower biological aggressiveness reflected by concomitant DCIS.

Not all tumors harboring such major mutations arise de novo and become pure IDC as would have predicted by our initial postulation. It is also known that the hormone receptor and HER2 genotype and phenotype are predefined early and remain stable in the development of breast lesions [39], thus the acquisition of the aggressive basal-like or HER2-positive genotype/phenotype during stepwise tumor progression in IDC-DCIS is less likely. This observation exemplifies the heterogeneity of breast cancer even

within subtypes, and that the carcinogenesis pathway of individual tumor involves a complex interplay of various major and minor (epi)genetic factors.

There are several limitations in our study. It is a retrospective analysis of data, although the database was prospectively maintained. Also, molecular subtypes of breast cancer were defined by IHC surrogates rather than the gold standard of gene expression profiling; the IHC definitions in the present study were based on a 4-marker panel, without supplementation with less essential markers such as AR, EGFR, CK 5/6 and CK 8/18. Controversies exist in the IHC classification system; for example it remains unresolved whether ER and/or PR-positive and HER2-positive disease correlates with luminal B or HER2-enriched subtype [52]. Also, the normal breast-like subtype was not included in this study as in many other IHC studies due to the complex and variable expression patterns of this particular subtype [53]. The luminal A and B subtypes were analyzed in combination rather than separately, but the evaluation of semi-quantitative ER and PR scores as a continuous variable in multivariate analysis provided adequate assessment of the effect of hormone receptor levels. Finally, as matured survival data is not yet available at the time of this writing, the ability of coexisting DCIS to directly predict relapse and survival could not be evaluated, although Ki67 is an established marker of such outcomes.

In conclusion, the presence of associated DCIS signifies lower biological aggressiveness in luminal IDC, especially node-positive disease. Concomitant DCIS, determined during routine pathological examination, may represent a potential cost-effective consideration in the adjuvant management of breast cancer.

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