

Original Article

Large palpable ductal carcinoma in situ is Her-2 positive with high nuclear grade

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Abstract: Ductal carcinoma in situ (DCIS) of the breast is a heterogeneous group with variable clinical presentation. The exact molecular mechanism is not known why some ductal carcinomas may reach to such a large size but still remains in situ. Although, molecular classification of DCIS lesions and nuclear grading are important for identification of more aggressive lesions but it is not sufficient. Our aim was to examine the expression pattern of immunohistochemical (IHC) markers of ER, PR, HER-2 in palpable DCIS lesions and compare with clinicopathological findings. Our center is referral hospital from South of Iran. Samples were obtained from fifty four patients with a diagnosis of palpable DCIS. Equivocal (2+) case in HER-2 IHC testing was more characterized by chromogenic in situ hybridization. The positive frequency of HER2, ER, and PR was 92%, 48%, and 37% respectively. Palpable DCIS lesions were significantly more HER-2 positive (92%). The DCIS cases were more likely to be of high nuclear grade (grade III) and Her-2 positive cases were more likely to be of high nuclear grade than intermediate grade. All ER negative tumors had high nuclear grade. The Her-2 positivity is suggested as the most important factor responsible for marked in situ proliferation and production of palpable mass.

Keywords: Ductal carcinoma in situ, palpable, Her-2, grading

Introduction

Recent studies using microarray and unsupervised cluster analysis have provided new insights into classification of invasive breast cancers [1-3]. The molecularly distinct breast cancer subgroups which vary in their gene expression signature and clinical course include luminal subtype A and B, the Her2 subtype and a basal-like subgroup [3]. Nevertheless, some recent reports suggest that the breast carcinogenesis is a series of stochastic genetic events that lead to distinct and divergent pathways toward invasive carcinoma, so, several molecular markers in addition to ER, PR, and Her2/neu appear to be needed to predict clinical outcome precisely and devise optimal individualized therapy [4-6].

Although less sensitive, immunohistochemical staining of paraffin embedded tissue sections has been shown to be a reliable surrogate for molecular classification of invasive breast cancers as categorized by gene expression profil-

ing studies. Application of immunohistochemistry to archival tissues from existing studies also provides the opportunity to correlate tissue marker data with long term exposures and follow up data [7].

While new information regarding the molecular heterogeneity of invasive cancer is rapidly emerging, far less is known about the spectrum of molecular phenotypes among cases of ductal carcinoma in situ (DCIS). In addition, several existing reports have studied mainly screening detected, non palpable cases of DCIS which compose more than 75-80% of DCIS lesions in these studies [3].

During the past years we have faced several cases of DCIS which remained in situ despite reaching large sizes and they usually came to medical attention by palpable masses with no or only foci of microinvasion. These appear to be somehow different from non palpable ones at molecular level. The assumption was that they were of either high proliferative activity or

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Table 1. Characteristics of the primary antibodies

Antibody	Clon	Company source	Dilution
ER	ID5	DAKO	1/65
PR	PgR636	DAKO	1/120
HER2	Polyclonal Rabbit	DAKO	1/800
SMA	IA4	DAKO	ready to us
CK5/6	D516B4	DAKO	1/40
CD10	CD10 SS2/36	Novocastra	1/100

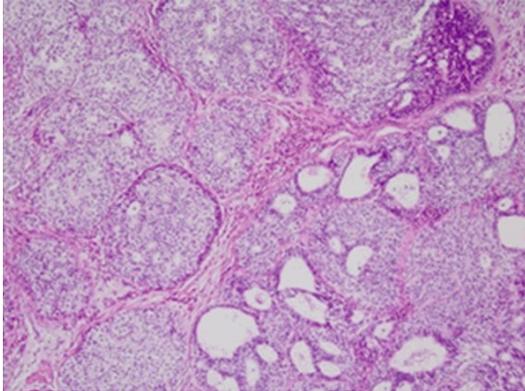


Figure 1. Ductal carcinoma in situ (DCIS), (H&E × 100).

low invasion capability that could bring them to such sizes without significant invasion to basement membrane. Beside this, even though total embedding of non palpable breast masses is a rule, there is no consensus regarding how many sections should be taken from different parts of a palpable DCIS lesion to exclude invasive component definitely, and also whether using immunohistochemistry will give any more help in this regard?

This study was conducted to determine the expression pattern of immunohistochemical markers in palpable DCIS lesions to determine whether these cases were molecularly distinct from others.

Our other objective was to evaluate the adequacy of standard specimen sectioning and immunostaining for myoepithelial markers in palpable DCIS lesions for excluding foci of invasive component.

Methods

Study population

All symptomatic patients who presented with palpable breast masses and diagnosed as

either pure ductal carcinoma in situ (DCIS) or DCIS with microinvasion, based on routine standard sectioning and histopathologic examination, were included in the study.

These cases had been referred to hospitals affiliated with Shiraz University of Medical Sciences (SUMS) in the South part of Iran and undergone modified radical mastectomy or quadrantectomy as well as axillary lymph node dissection in the period from April 2004 to December 2012. Our department is a referral center in breast pathology from South of Iran and more than 5000 cases of breast cancer in different stages was registered.

After routine handling and sectioning of the surgical specimens, one section per every 0.5-1 cm of tumor diameter, based on published recommended guidelines [8] and histopathologic examination of the specimens resulting in diagnosis of DCIS, the remained tumoral tissues were cut into consecutive slices of approximately 0.3 cm thick and were totally embedded and examined more precisely for this study by each of 3 reviewing pathologists. A total number of 75 cases identified, 54 of them who had a good regular follow up were included in the study. Review of records and archived slides showed that 30 and 24 cases had pure DCIS (without any obvious co-existing invasive component) and DCIS with micro-invasive component (i.e. invasion < 1 mm in the non specialized stroma around the ducts) respectively.

None of the patients had history of infiltrating carcinoma in the contralateral breast. All the slides were fully described regarding architectural features, nuclear grade, presence or absence of necrosis, tumor size, multicentricity and status of lymph node involvement. Nuclear grade assessment of tumoral tissues was based on the consensus conference committee recommendation for nuclear grading of DCIS [9, 10].

Immunohistochemistry

Immunohistochemical staining for ER, PR, HER-2, P63, SMA, CK5/6, and CD10 was done on representative sections of the paraffin embedded tumoral tissues (5 micrometer in thickness). After quenching of endogenous peroxidases and heat induced antigen retrieval, sections were incubated with monoclonal mouse antibodies against ER, PR, P63, SMA, CK5/6

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Table 2. ER, PR and HER2 in DCIS (n = 54)

IHC Marker	Positive (%)	Negative (%)
ER	26 (48.2)	28 (51.8)
PR	20 (37.1)	34 (62.9)
HER2	50 (92.5)	4 (7.5)

Table 3. Prevalence of breast cancer subtype in DCIS

Subtype	Patients with DCIS (n = 54)
Luminal A	4 (7.5)
Luminal B	23 (42.5)
HER2+/ER-	27 (50)
Basal-like	0

(Dako Corp, Carpinteria, CA, USA), polyclonal rabbit antibodies against HER-2 (Dako Corp, Carpinteria, CA, USA) and antibodies against CD10 (Novocastra Lab. Ltd., UK) (**Table 1**). Basal cell markers (P63, SMA, CK5/6) were used for better evaluation of suspected micro-invasive regions. The bound primary antibodies were reacted with secondary antibodies conjugated with horse radish peroxidase. Visualization of the complexes was accomplished with DAB as chromogen. Sections were also lightly stained with Mayer's hematoxylin and interpreted with only one expert pathologist to avoid interobserver variability. Any nuclear staining in ER and PR IHC testing was regarded as positive.

Regarding HER-2 testing, it was scored 0 to 3, according to criteria recommended by the American Society of Clinical Oncology/College of American Pathologists guidelines, using a staining intensity scoring system [11]. Intense and uniform circumferential membrane staining (chicken wire) in at least 10% was scored as 3+ and interpreted as positive. Equivocal (2+) case in HER-2 IHC testing was more characterized by chromogenic in situ hybridization (Zytovision, Germany).

Cases with ratio of HER-2/centromere 17 copy number greater than 2 were considered positive for gene amplification, 1.8 to 2 were equivocal, and less than 1.8 were considered as negative. Based on IHC and CISH findings, tumors were simply classified in "Luminal A"(ER+/PR+/HER-2 neg), "Luminal B" (ER+/PR+/HER2+), "HER-2+" (ERneg/PRneg/HER-2+), "Triple Neg" (ERneg/PRneg/HER-2 neg)

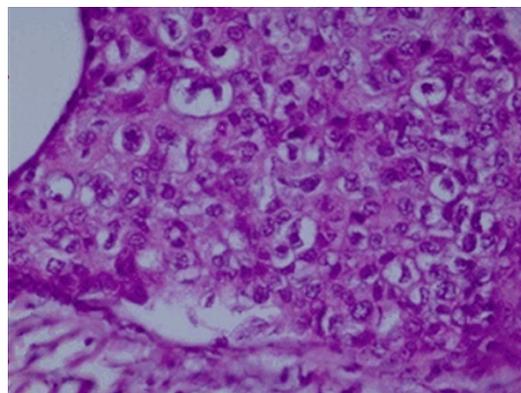


Figure 2. DCIS with high nuclear grade (Grade III), (H&E × 400).

as it is usually applied for invasive ductal carcinoma, NOS.

Statistical analysis

Statistical analyses were performed using the SPSS software package, version 18.0 (SPSS Inc., Chicago, IL, USA). For IHC findings, the results were compared by the Mann-Whitney U test for continuous data and Fisher exact test for dichotomous data. Results were considered significant if they met the threshold of *P* value ≤ 0.05.

Results

Clinicopathological characteristics

A total of fifty four patients with a diagnosis of DCIS were included. Mean age of the patients was 45 years (age range 26-70, 77% ≤ 50). Thirty seven cases (68.5%) had left breast mass and 17 patients (31.5%) were right sided. All patients underwent modified radical mastectomy or quadrantectomy with at least 15mm free margin. None of them received intraoperative radiotherapy. Tumor sizes ranged from 1.5 to 7.0 cm with mean size of 3.8 cm ± 1.27.

Ten (20%) had multi centric tumor in the same breast. Overall, six patients (11%) had involvement of lymph nodes, that proved later to be of microinvasive category. Patients had 2-8 years follow up. Four patients experienced local recurrence and one distant metastasis. The primary mode of detection was palpation in all cases. As tumoral tissues were totally embedded after initial diagnosis, mean number of slides increased to 28 slides per case (range

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Table 4. Comparison of DCIS nuclear grade and hormone receptor (n = 54)

	Grade III (n = 39)	Grade II (n = 15)	Grade I (n = 0)	p-value
Her2/neu				
Positive	36 (92)	14 (93)	0	1.000
Negative	3 (8)	1 (7)	0	1.000
ER				
Positive	11 (28.2)	15 (100)	0	0.001*
Negative	28 (71.8)	0 (0)	0	0.001*
PR				
Positive	7 (17.9)	14 (93)	0	0.001*
Negative	32 (82.1)	1 (7)	0	0.001*

*P<0.05 is consider significant.

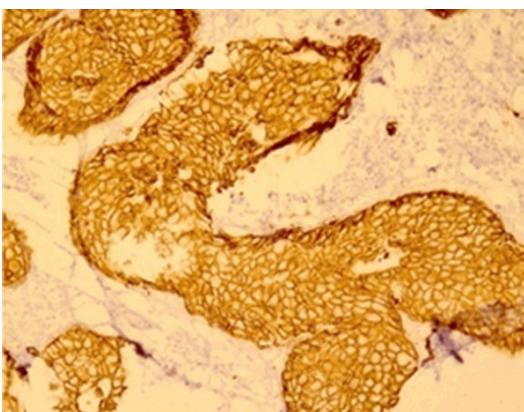


Figure 3. Her-2 positive malignant cells (3+) (IHC, × 400).

16-44, SD ± 6.58). Twenty four cases of DCIS showed co-existing microinvasion in initial pathologic examination with standard number of sections taken. Total embedding of tumoral tissues did not change the diagnosis i.e., no additional invasive parts detected. That was also true for the 30 cases of pure DCIS (**Figure 1**).

Immunohistochemistry

The positive frequency of HER2, ER, and PR in our cases was 92%, 48%, and 37% respectively (**Table 2**). Palpable DCIS lesions were significantly more HER-2 positive (92%) than what expected in invasive ductal carcinoma, NOS (**Table 2**).

In molecular classification made by IHC, the majority of tumor subtype in DCIS was HER2+/ER- (50%) followed by luminal B (42.5%) and luminal A (7.5%) respectively. (No triple nega-

tive/basal cell type detected (**Table 3**).

In respect of nuclear grade the DCIS cases were more likely to be of high nuclear grade (grade III) (**Figure 2**) than intermediate grade (grade II). No DCIS lesion with low nuclear grade was identified (**Table 4**).

Her-2 positive cases were more likely to be of high nuclear grade than intermediate grade (**Figure 3**). While all of the ER negative DCIS lesions

are of high nuclear grade, ER positive cases are exclusively to be of intermediate nuclear grade (100%). ER and PR negativity are shown to be significantly associated with high nuclear grade (70-80%) (**Table 4**).

Comparison of clinicopathological findings between pure DCIS and DCIS with microinvasion showed the similar results (**Table 5**) with no significant difference between two groups. Looking at the morphology, great majority of DCIS lesions with high nuclear grade are HER-2 positive, HER-2 type and luminal B type. Moreover, myoepithelial markers nicely stained myoepithelial cells in all foci that a diagnosis of DCIS was suspected on H&E slide examination but they were negative or disrupted in microinvasive regions, so using basal cell markers did not add any more information to the previous diagnosis. Careful examination of H&E slides was adequate to determine the status of invasiveness.

Discussion

Ductal carcinoma in situ (DCIS) of the breast is a heterogeneous disease with variable malignant potential and clinical presentation. It is not known why some ductal carcinomas may reach to such a large size but still remain in situ. There seems to be some defects in the power of invasiveness and some additional genetic damages maybe needed for tumoral cells to become invasive. Also higher grade makes them more proliferative rather than invasive [12, 13].

Our study describes symptomatic cases of DCIS (both pure DCIS and those with microinvasion) which presented with palpable breast

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Table 5. Comparison of clinicopathologic parameters between pure DCIS and DCIS with microinvasion

	Pure DCI (n = 30)	DCIS with microinvasion (n = 24)	p-value
Age (Mean ± SD)	46.35 ± 8.38	43.63 ± 10.28	0.301
Size (Mean ± SD)	4.00 ± 0.84	3.60 ± 1.60	0.301
Grade			
I	0	0	
II	6 (20)	9 (37.5)	
III	24 (80)	15 (62.5)	
L.N. involvement			
Yes	0 (0)	6 (25)	0.020*
No	30 (100)	18 (75)	0.020*
Her2/neu			
Positive	27 (90)	23 (95.8)	1.000
Negative	3 (10)	1 (4.2)	1.000
ER			
Positive	12 (40)	14 (58.4)	0.343
Negative	18 (60)	10 (41.6)	0.343
PR			
Positive	10 (35)	10 (41.6)	0.748
Negative	20 (65)	14 (58.4)	0.748
Multicentricity			
Yes	8 (26)	3 (12.5)	0.695
No	22 (74)	21 (87.5)	0.695

*P<0.05 is consider significant.

masses. The other studies mainly evaluated the screen detected non-palpable DCIS lesions (Table 6).

Palpable DCIS lesions in our study are classified into 3 groups including luminal A, luminal B and HER-2 molecular phenotypes. Prevalence of these molecularly defined phenotypes differed significantly from that of screen detected DCIS lesions as shown in other studies. Palpable DCIS in our study are significantly more likely to have HER-2 and luminal B phenotypes than cases studied by Tamimi et al (Table 6). Moreover, Her-2 positive cases in our study are more likely to be of high nuclear grade than intermediate grade. This is nearly the same as what seen in Tamimi's study [3], in which 69.8% of screen detected Her-2 positive cases are of nuclear grade 3 and 28.7% are of nuclear grade 2. Great majority of palpable DCIS cases with nuclear grade 3 are Her-2 positive (92.9%) which is nearly 2 times more prevalent than that in Tamimi's report (48.1%). This data indicate that HER-2 positivity is strongly associated

with higher nuclear grade and nuclear grade 3 is more consistently associated with HER-2 positivity in palpable DCIS lesions than screen detected cases of DCIS.

By considering these findings together, it may be concluded that Her-2 positivity, as the most important factor responsible for marked in situ proliferation and production of palpable mass.

Enhanced proliferation of tumoral cells induced by HER-2 positivity is more likely to cause additional genetic abnormalities to be accumulated within tumoral cells. Therefore, tumor behavior may be changed and may get capability for invasion.

This concept is supported by both higher frequency of chromosome 17q12 amplification in poorly differentiated DCIS lesions studied by Buerger et al. [14] and gain of 17q12 which is shown to be associated with the pathway toward high grade DCIS studied by Moore and colleagues [15], 17q12 is the region

on long arm of chromosome 17 which harbors HER-2 gene locus. Amplification of HER-2 gene located at 17q12 is almost always the underlying cause of HER-2 protein over expression in tumoral cells.

Nearly half (51%) of screen detected cases of DCIS lesions with nuclear grade 3 in Tamimi's report [3] show HER-2 protein over expression. This fraction correlates well with the fraction of non-palpable high grade cases of DCIS (56%) in Moore et al study [15], which showed gain of 17q12 in tumoral cells of their cases. Accordingly, we also expect our HER-2 positive cases to show gain of 17q12 by molecular cytogenetic methods implying HER-2 gene amplification. The 1:1 relationship between HER-2 gene amplification and HER-2 protein overexpression is also strongly supported by the fact that no alternative mechanism giving equivalent expression level of HER-2 have yet been actually demonstrated [15, 16].

Although, molecular classification of DCIS lesions as well as nuclear grade may be impor-

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Table 6. Frequency of DCIS subtype among our study and others

	Luminal A n (%)	Luminal B n (%)	HER2 type n (%)	Basal n (%)	Unclassified N (%)	Total
Our study	4 (7.5)	23 (42.5)	27 (50)	0	0	54
Tamimi et al, 2008	170 (62.5)	36 (13.2)	37 (13.6)	21 (7.7)	8 (2.9)	272
Meijnen et al, 2008	63 (38.6)	21 (12.8)	43 (26.3)	8 (4.9)	28 (17.1)	163
Livasy et al, 2006	149 (61)	23 (9)	38 (16)	19 (8)	16 (6)	245

tant in identifying more aggressive lesions [3], they are not sufficient by themselves to affect clinical course and prognosis. Great majority of palpable DCIS lesions in our study are HER-2 positive (92.3%) and show tumoral tissue with nuclear grade 3 but they lack the capability to produce invasive component despite their remarkable growth and palpable size. These data suggest that reliance on HER-2 over expression as well as nuclear grade to guide clinical management and to predict clinical outcome is far from satisfactory and additional molecular markers are needed to predict tumor behavior and clinical outcome.

The ER and PR expression pattern of DCIS lesions in our study relatively differs from others. About 75% of DCIS lesions in Barnes's study [17] were ER positive, but only 48.3% of our cases are positive for ER. Consistent with their study, our cases show that comedo-type intra ductal carcinoma is more likely than other variants to be ER negative. Moreover, Bur et al. showed that 80% of DCIS lesions were ER positive with higher frequency of receptor positivity in non-comedo type [18].

All ER negative tumors in our study had high nuclear grade ($P < 0.001$) and majority of high nuclear grade tumors were ER and PR negative. These findings are consistent with Bur et al. study which showed that large cell size, nuclear pleomorphism and necrosis are the cellular features that are associated with the absence of estrogen receptor reactivity [18].

Prevalence of lymph node metastasis in DCIS lesions with microinvasion in our study (5 of 19) is 26.3% which is higher than that (5%) reported by both Solin et al. [19].

In our study, lymph node involvement is more frequently occurred with luminal B phenotype than HER-2 type lesions. Similar finding has been reported by Kumar et al. regarding invasive tumors of luminal B phenotype that were more likely to have lymph node metastasis [20].

Hwang et al. [21], studied on genomic and phenotype alterations of large and small high-grade DCIS. They believed that specific subgroup of DCIS presented in premenopausal woman as large mass with high grade morphology and without concurrent invasive component. On the other hand, another group of small DCIS associated with invasive cancer. In comparison of these groups, they revealed that large DCIS were reduced expression of ER and cyclin D1, with fewer copy number gains of c-myc on chromosome 8q and zinc-finger protein 217 on chromosome 20q. However, the frequency of ERBB2 amplifications was not different in the 2 groups. The expression of ER in these cases was inversely linked to comedo carcinoma and nuclear grade in DCIS similar to previous reports.

Claus et al [22] evaluated the pure DCIS and found a positive correlation between tumor size, nuclear grade comedo morphology and HER-2 overexpression.

Accordingly, it may be concluded that in addition to HER-2 positivity, some other molecular markers such as ER status as well as additional genetic abnormalities may have great influence on tumoral cell behavior, reminding that HER-2 positivity, although, pathophysiologically important, is not sufficient by itself to affect the prognosis significantly.

Another important factor that promotes the progression of DCIS to invasive cancer is micro-environmental factors [21]. Interactions between carcinoma-associated fibroblasts (CAFs) and epithelial cells lead to alterations in the gene expression profile of both cell types [23]. Secretion of CXCL12 by CAFs may promote cell proliferation through interactions with CXCR4 expressed by tumor cells [24].

Total embedding of tumoral tissues added no more information regarding diagnosis of microinvasion. Therefore, routine recommended sectioning i.e., one section from each 0.5-1 cm of tumor seems to be adequate for interpretation.

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Moreover, evaluation of myoepithelial markers P63, SMA, CD10, CK5/6 while supporting the in situ status of tumoral tissues, added no more information regarding the status of invasive component in palpable DCIS.

Disclosure of conflict of interest

None.

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References

- [1] Perou CM, Jeffrey SS, van de Rijn M, Rees CA, Eisen MB, Ross DT, Pergamenschikov A, Williams CF, Zhu SX, Lee JC, Lashkari D, Shalon D, Brown PO, Botstein D. Distinctive gene expression patterns in human mammary epithelial cells and breast cancers. *Proc Natl Acad Sci U S A* 1999; 96: 9212-7.
- [2] Brenton JD, Carey LA, Ahmed AA, Caldas C. Molecular classification and molecular forecasting of breast cancer: ready for clinical application? *J Clin Oncol* 2005; 23: 7350-60.
- [3] Tamimi RM, Baer HJ, Marotti J, Galan M, Galaburda L, Fu Y, Deitz AC, Connolly JL, Schnitt SJ, Colditz GA, Collins LC. Comparison of molecular phenotypes of ductal carcinoma in situ and invasive breast cancer. *Breast Cancer Res* 2008; 10: R67.
- [4] Steinman S, Wang J, Bourne P, Yang Q, Tang P. Expression of cytokeratin markers, ER-alpha, PR, HER-2/neu, and EGFR in pure ductal carcinoma in situ (DCIS) and DCIS with co-existing invasive ductal carcinoma (IDC) of the breast. *Ann Clin Lab Sci* 2007; 37: 127-34.
- [5] Nofech-Mozes S, Spayne J, Rakovitch E, Hanna W. Prognostic and predictive molecular markers in DCIS. *Adv Anat Pathol* 2005; 12: 256-64.
- [6] Gasparini G, Longo R, Torino F, Morabito A. Therapy of breast cancer with molecular targeting agent. *Ann Oncol* 2005; 16 (Supp 4): iv28-iv36.
- [7] Nielsen TO, Hsu FD, Jensen K, Cheang M. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. *Clin Cancer Res* 2004; 10: 5367-5374.
- [8] Rosai J. Rosai and Ackermans Surgical Pathology. 10th edition. Mosby Elsevier Inc; 2011.
- [9] Pinder SE. Ductal carcinoma in situ (DCIS): pathological features, differential diagnosis, prognostic factors and specimen evaluation. *Mod Pathol* 2010; 23 Suppl 2: S8-13.
- [10] Consensus Conference on the classification of ductal carcinoma in situ. The Consensus Conference Committee. *Cancer* 1997; 80: 1798-802.
- [11] Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM, Hayes DF; American Society of Clinical Oncology; College of American Pathologists. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol* 2007; 25: 118-45.
- [12] Van Bockstal M, Lambein K, Denys H, Braems G, Nuyts A, Van den Broecke R, Cocquyt V, De Wever O, Libbrecht L. Histopathological characterization of ductal carcinoma in situ (DCIS) of the breast according to HER2 amplification status and molecular subtype. *Virchows Arch* 2014; 465: 275-89.
- [13] Carraro DM, Elias EV, Andrade VP. Ductal carcinoma in situ of the breast: morphological and molecular features implicated in progression. *Biosci Rep* 2013; [Epub ahead of print].
- [14] Buerger H, Otterbach F, Simon R, Poremba C, Diallo R, Decker T, Riethdorf L, Brinkschmidt C, Dockhorn-Dworniczak B, Boecker W. Comparative genomic hybridization of ductal carcinoma in situ of the breast- evidence of multiple genetic pathways. *J Pathol* 1999; 187: 396-402.
- [15] Moore E, Magee H, Coyne J, Gorey T, Dervan PA. Widespread chromosomal abnormalities in high-grade ductal carcinoma in situ of the breast. Comparative genomic hybridization study of pure high-grade DCIS. *J Pathol* 1999; 187: 403-9.
- [16] Kokate P, Sawaimoon S, Bhatia S, Mandava S. Evaluation of genetic status of HER-2/neu and aneusomy 17 by fluorescence in situ hybridization and comparison with immunohistochemistry assay from Indian breast cancer patients. *Genet Test Mol Biomarkers* 2012; 16: 239-45.
- [17] Barnes R, Masood S. Potential value of hormone receptor assay in carcinoma in situ of breast. *Am J Clin Pathol* 1990; 94: 533-537.
- [18] Bur ME, Zimarowski MJ, Schnitt SJ, Baker S, Lew R. Estrogen receptor immunohistochemistry in carcinoma in situ of the breast. *Cancer* 1992; 69: 1174-1181.
- [19] Solin LJ, Fowble BL, Yeh IT, Kowalyszyn MJ, Schultz DJ, Weiss MC, Goodman RL.

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- Microinvasive ductal carcinoma of the breast treated with breast-conserving surgery and definitive irradiation. *Int J Radiat Oncol Biol Phys* 1992; 23: 961-8.
- [20] Kumar V, Tewari M, Singh U, Shukla HS. Significance of Her-2/neu protein over expression in Indian breast cancer patients. *Indian J Surg* 2007; 69: 122-8.
- [21] Hwang ES, Lal A, Chen YY, DeVries S, Swain R, Anderson J, Roy R, Waldman FM. Genomic alterations and phenotype of large compared to small high-grade ductal carcinoma in situ. *Hum Pathol* 2011; 42: 1467-75.
- [22] Claus EB, Chu P, Howe CL, Davison TL, Stern DF, Carter D, DiGiovanna MP. Pathobiologic findings in DCIS of the breast: morphologic features, angiogenesis, HER-2/neu and hormone receptors. *Exp Mol Pathol* 2001; 70: 303-16.
- [23] Schnitt SJ. The transition from ductal carcinoma in situ to invasive breast cancer: the other side of the coin. *Breast Cancer Res* 2009; 11: 101.
- [24] Rozenchan PB, Carraro DM, Brentani H, de Carvalho Mota LD, Bastos EP, e Ferreira EN, Torres CH, Katayama ML, Roela RA, Lyra EC, Soares FA, Folgueira MA, Góes JC, Brentani MM. Reciprocal changes in gene expression profiles of cocultured breast epithelial cells and primary fibroblasts. *Int J Cancer* 2009; 125: 2767-77.