ARTIGO ORIGINAL

c-kit immunohistochemical expression in breast carcinomas and lymph node metastasis. Lack of prognostical significance and review of literature

Expressão imuno-histoquímica de c-kit em carcinomas mamários e metástases para linfonodos. Ausência de significado prognóstico e revisão de literatura

Fiorita G. L. Mundim[1,2], Angela F. Logullo[2], Miriam F. B. Engelman[1], Suely Nonogaki[3], Luciana Logullo[4], Fernando Augusto Soares[5]

[1] Department of Pathology, Universidade do Vale do Sapucaí (Univás).
[2] Department of Pathology, Universidade Federal de São Paulo – Escola Paulista de Medicina (Unifesp-EPM).
[3] Department of Pathology, Adolfo Lutz Laboratory.
[4] GANEP Research Department.
[5] Department of Pathology, Hospital A. C. Camargo – Hospital do Câncer, São Paulo, Brazil.
Address: Fiorita G. L. Mundim, Rua Minas Gerais, 50, B Medicina, 37550-000, Pouso Alegre, MG, Brazil. Phone/Fax: 55-35-3423-6027, e-mail: hjmundim@uol.com.br
Recebido em: 26/6/2009. **Aceito após modificações em:** 4/8/2009

Keywords
c-kit; Breast carcinomas; Immunohistochemistry.

ABSTRACT

Objective: To evaluate the expression of c-kit in breast invasive ductal carcinomas (IDC) and metastasis to lymph nodes considering epithelial and stromal components separately and correlate this variable to others clinical, pathological and biological markers (EGFR, Her-2, ER, PR, Ki-67 and p53 expression). **Methods:** We analysed 80 IDC, stage T2-T4 Nx Mx, in TMA of epithelial, stromal component and lymph nodes. Statistical analysis considered significant a p value < 5%. **Results:** c-kit expression was founded in 9 cases in epithelial component (11.3%) and in 10 cases (12.5%) of stromal component. The 43 samples of lymph nodes metastasis were negative. EGFR and Her-2 were predominantly negative, both in epithelial (77.5% and 73.75%, respectively), as stromal (97.5% and 95.0%) components and metastasis to lymph nodes (83.7% and 62.8%). While ER, PR, Ki-67 and p53 were positive in 49 (61.0%), 40 (53.0%), 67 (83.75%) and 59 cases (73.75%) in the epithelial component. Stromal cells have proved negatives. c-kit epithelial expression correlated to presence of *in situ* component ($p = 0.044$) and stromal c-kit expression correlated to presence of necrosis ($p = 0.002$). There was no association between c-kit expression and staging and biological markers. Transformed epithelial cells at the lymph nodes metastasis stained for ER, PR, Ki-67 and p53 in 27 (62.8%), 16 (37.5%), 41 (95.0%) and 28 cases (65.1%), respectively. **Conclusions:** The expression of c-kit is mostly negative in primary IDC both in the epithelial and stromal component, as well as in lymph node metastasis. The lack of correlation between c-kit and others tyrosine kinase proteins suggest that they are independently regulated. Metastasis for lymph nodes were not c-kit positive and further studies of mutations of *c-kit* and his family, correlate with other prognostic factors and survival required to reveal the exact mechanism of action of this molecule in breast cancer.

Palavras-chaves
c-kit; Carcinomas de mama; Imuno-histoquímica.

RESUMO

Objetivo: Avaliar a expressão de c-kit em células epiteliais, estromais e metástases para linfonodos de carcinomas ductais mamários invasivos (CDI) e correlacionar essa variável com os outros marcadores clínicos, patológicos e biológicos (EGFR, HER-2, RE, RP, Ki-67 e p53). **Métodos:** Analisaram-se 80 CDI, estádios T2-T4 Nx Mx, em TMA de componente epitelial, estromal e linfonodos. O valor de $p < 5\%$ foi considerado significante. **Resultados:** A expressão de c-kit foi

encontrada em 9 casos no componente epitelial (11,3%) e em 10 casos (12,5%) do componente estromal. As 43 amostras de metástases para linfonodos foram negativas. EGFR e Her-2 foram predominantemente negativos, tanto em epitélio (77,5% e 73,75%, respectivamente), como estroma (97,5% e 95,0%) e metástases para linfonodos (83,7% e 62,8%), enquanto RE, RP, Ki-67 e p53 foram positivos em 49 (61,0%), 40 (53,0%), 67 (83,75%) e 59 casos (73,75%) no componente epitelial. Células do estroma se mostraram negativas. A expressão de c-kit epitelial correlacionou-se com a presença do componente *in situ* (p = 0,044) e a expressão de c-kit no estroma se associou com a presença de necrose (p = 0,002). Não houve associação entre a expressão de c-kit com estadiamento e marcadores biológicos. Células epiteliais transformadas de metástases para linfonodos coraram para RE, RP, Ki-67 e p53 em 27 (62,8%), 16 (37,5%), 41 (95,0%) e 28 casos (65,1%), respectivamente. **Conclusões:** A expressão de c-kit é majoritariamente negativa em CDI primário tanto no componente epitelial quanto no estromal, assim como em metástases linfonodais. A falta de correlação entre o c-kit e outras proteínas tirosina quinases sugere que elas sejam reguladas de forma independente. Metástases para linfonodos não foram positivas para c-kit, e estudos posteriores de mutações do c-kit e sua família, correlacionando com outros fatores prognósticos e sobrevida, são necessários para revelar o exato mecanismo de ação dessa molécula no câncer de mama.

Introduction

In breast cancer, c-kit expression remains a controversial issue. It has been associated to the basaloid phenotype profile and, in another hand, described as highly attenuated during breast carcinogenesis[9]. While regularly expressed at the normal breast lobule, it is not clear when and why proliferative ductal epithelium loses c-kit production. Moreover, the majority of studies evolved in c-kit assessment in neoplastic tissue is not concerned about with component is expressing c-kit. The Cajal intestinal cell, for instance, originator of GIST and responsive to Gleevec®, is a non-epithelial element. Therefore, it would be important to assess with group of cells *do* express c-kit in breast carcinomas and if neoplastic tissue in lymph nodes metastasis would show any difference in c-kit expression profile[10,11].

We aimed to evaluate c-kit expression in mammary infiltrative ductal carcinomas (IDC) and the respective compromised lymph nodes at epithelial and mesenchymal components separately; and correlate these results to the clinical, pathological and immunohistochemical prognostic variables. We also correlated c-kit expression to other kinase protein receptors (EGFR and Her-2 expression) and classical prognostic tools (KI-67, ER, PR and p53 expression) in primary carcinomas and compromised lymph nodes.

Material and methods

After pathological review, 80 breast carcinomas cases with adequate specimens were included. All cases were diagnosed and treated from 1997 to 2005. The age of the patients varied from 23 to 88 years with a median age of 57, 35 years. In most cases, the presence of a lump in the breast was the first symptom; diagnosis was confirmed in two thirds with a mammogram or ultrasound exam. Two pathologists (FGLM and MFBE) reviewed all cases independently and the classification was confirmed according to TNM diagnostic criteria (AJCC, 2002). The tumor width varied from 0.7 to 7.5 cm. All cases were treated surgically; a local excision was performed in 1 case, quadrantectomy in 21 cases and mastectomy was performed in the remaining 47 cases. Recurrence occurred in three patients, and most cases remained without notice.

Construction of tissue micro arrays (TMA)

Four distinct TMAs were constructed. Two contained each 80 samples of consecutive stromal or epithelium of IDC and the third and forth TMAs consisted on 43 samples of consecutive stromal or epithelium tissue from representative compromised lymph nodes of the same cases studied at the IDC TMAs. The constructed TMA series consisted of 8 to 12 arrays of 1.5 mm tissue cores from archived formalin-fixed, paraffin embedded surgical blocks of the patient's primary tumor or lymph nodes. Two separate tissue cores of invasive carcinoma or lymph node metastasis, totaling a surface area of 3.5 mm^2, represented each surgical case in the TMA series. Donor blocks that met previously described inclusion criteria were selected and reviewed. One of us (FGLM) selected portions of included cases containing tumor. The arrays were produced as described previously using a manual tissue arrayer (Beecher Instruments, Silver Spring, MD)[14]. Five-µm sections were cut from the array blocks using tape-sectioning

materials from Instrumedics (Hackensack, NJ). Every fiftieth slide was stained with H&E for quality control review.

Each separate tissue core was made anonymous and assigned a unique TMA location number, which was subsequently linked to an approved database containing corresponding clinical and pathological data.

Immunohistochemical essay

Eighty consecutive cases of IDC tumors were fixed in formalin, routinely processed and embedded in paraffin wax. Sialinized slides containing 3 µm sections underwent a immunohistochemical essay including antigen retrieval performed using a pressure cooker (Eterna, Nigro™) and 10 mM citrate buffer pH 6.0 as described elsewhere[14]. Incubation with primary antibody was performed overnight at 4° C according to the following dilutions: polyclonal rabbit antihuman c-kit (1:5000, Dako-A4502), EGFR (1:400 Novocastra – NCL-EGFR384; EGFR-25), polyclonal rabbit c-erbB-2 (1:500, Dako-A0485), monoclonal anti-ER (1:1000, Neomarkers, SP1), monoclonal anti-PgR (1:400, Dako, PgR636), Ki-67 (1:200, Dako M7240, MIB-1), and p53 (1:200, Dako M7240, DO7). The following day, slides were rinsed with PBS and incubated with the secondary antibody (biotinylated goat anti-Mouse/Rabbit immunoglobulin, diluted 1:200, for 30 min at 37° C) followed by and PBS rinse and incubated with streptavidin – biotinylated – peroxidase – complex (Duet mouse/rabbit HRP, DakoCytomation A/S, cat# 0492, diluted 1:200, for 30 min at 30° C).

The slides were developed with 0.06% DAB as chromogen, with 0.06% hydrogen peroxide, and counterstained with Harris hematoxylin.

Positive and negative control slides were included in all reactions. The negative control was a slide where the primary antibody was omitted and positive controls consisted on previously known positive cases.

IHC interpretation

Her-2 expression analysis was performed according to the DAKO system for IHC method[15,17,18].

Results

Two pathologists (FGLM and MFBE) reviewed the surgical pathology reports. All clinical and pathological data were displayed at table 1.

We analyzed c-kit expression in stromal and epithelial cells separately.

Table 1. Clinical and pathological variables of 80 IDC included in the study

Clinical and pathological variables		
Variable	**Number of cases**	**Per cent**
Menopause		
Present	58	72.50
Absent	22	27.50
Laterality		
Right	33	41.25
Left	47	58.75
Type surgery		
Mastectomy	47	58.75
Nodulectomy	1	1.25
Quadrantectomy	21	26.25
Setorectomy	11	13.75
TNM staging		
0	2	2.50
I	17	21.25
IIA	20	25.00
IIB	12	15.00
IIIA	10	12.50
IIIB	4	5.00
IIIC	15	18.75
IV	0	0.00
Treatment		
Chemotherapy	58	72.50
Radiotherapy	22	27.50
Undetermined	39	48.75
Recurrence		
Absent	14	17.50
Present	0	0.00
Unknown	66	82.50
Tumor size		
< 2 cm	40	50.00
2-5 cm	34	42.50
≥ 5 cm	6	7.50
Carcinoma *in situ*		
Present	38	47.50
Absent	42	52.50
Histologic grade		
1-low grade	18	22.50
2-intermediary grade	29	36.25
3-high grade	33	46.25
Lymphatic invasion		
Present	42	52.50
Absent	38	47.50
Vascular invasion		
Present	1	1.25
Absent	79	98.75
Perineural invasion		
Present	8	10.00
Absent	72	90.00
Atypical ductal hyperplasia		
Absent	76	95.00
Typical	2	2.50
Atypical	2	2.50

Table 1. Clinical and pathological variables of 80 IDC included in the study (continued)

Clinical and pathological variables		
Columnar cell hyperplasia		
Absent	73	91.25
Without atypia	6	7.50
With atypia	1	1.25
Necrosis		
Absent	37	46.25
Light	16	20.00
Moderate	19	23.75
Accentuated	8	10.00
Lymph nodes status		
With metastasis	43	53.75
Without metastasis	26	32.50
Lymphadenectomy not held	11	13.75
Tumor edge		
Infiltrative	44	55.00
Expansive	36	45.00

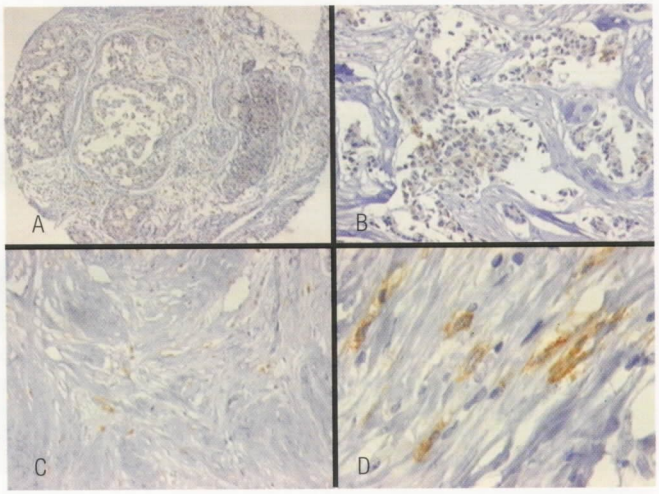

Figure 1. A – c-kit positive in epithelial component (40x); B – c-kit positive in epithelial component (100x); C – c-kit positive in stromal component (40x); D – c-kit positive in stromal component (100x).

Stromal component

Surprisingly, among all tumors, only 10 cases of IDC contained stromal cells positive for c-kit staining. In all other cases, fibroblasts were completely negative for c-kit expression. EGFR and Her-2 were totally negative in the malignant stromal component. The same results were obtained when the immunoassay was repeated using ER, PR, p53 or Ki-67 antigens. There was no particular morphological feature in the 10 positive cases compared to the other negative lesions. Lymph nodes metastases were completely negative to kit expression.

Epithelial component

C-kit was also sparsely positive in the glandular component. Reactive cells showed a diffuse staining in the cytoplasm that was moderately intense and comparable to normal breast epithelial expression. In 9 of the 80 tumors studied, the epithelial component was reactive to c-kit antibody (11.25%). Ductal hyperplasia and in situ carcinoma when present within the tumors showed completely negative for c-kit expression (Figure 1).

However, the 10 positive cases had not more than 10% positive fibroblasts scattered among the negative cells, and the distribution in the most positive cases showed 1% to 40% positive stromal cells. Although they expressed the protein within a smaller larger amount (less than 10%) of fibroblasts, the pattern was not in the large, strong staining pattern commonly seen in GIST tumors. The majority of other cases did not show any reactivity at all. Myofibroblasts and endothelial cells were also negative for c-kit. We assessed c-kit expression in the epithelial cells separately, and expression of c-kit was more common in this particular component. In 9 of 80 cases, the epithelial component was positive for c-kit. Usually, the intensity of staining in the epithelial cells was weaker than in normal tissue. When IDC exhibited epithelial hyperplasia, typical or atypical was present or associated with the lesion, it was frequently negative for c-kit.

ER, PR, EGFR, Her-2, p53 and ki-67 expression followed standardized distribution among epithelial cells and frequencies were listed in table 2.

When we analyzed c-kit expression in epithelial cells compared to the 6 other proteins studied (ER, PR, Her-2, EGFR, p53 and ki-67) there was not any evident statistical correlation. Stage and prognostic variables as tumor width, lymph node status, and histological grade were not correlated to c-kit expression.

Stromal expression was very low to allow any statistical correlation.

Considering both components together, 58 cases were totally negative and 5 showed c-kit expression restricted to epithelial cells. Only 4 cases exhibited c-kit expression in both glandular and stromal cells and 9 cases showed expression in one component but not the other.

Discussion

In normal breast gland, c-kit protein is regularly expressed in ductal and alveolar breast cells[28] in a membrane and cytoplasmatic pattern[29]. We analyzed whole-slide slices of normal breast tissue along IDC in 16 cases in order to validate c-kit expression detected in our TMA samples according to literature. Acinar epithelium was frequently and regularly positive

Table 2. Immunohistochemical expression of EGFR, erB-2, ER, PR, Ki-67 and p53 in epithelial component, stromal component and lymph nodes

Biomarker	Epithelium			Stroma			Lymph nodes		
	Positive	Negative	Missing	Positive	Negative	Missing	Positive	Negative	Missing
EGFR	16 (20.0%)	62 (77.5%)	2 (2.5%)	0	78 (97.5%)	2 (2.5%)	5 (11.6%)	36 (83.6%)	2 (4.8%)
erbB-2	19 (23.75%)	59 (73.75%)	2 (2.5%)	0	76 (95%)	4 (5%)	15 (34.8%)	27 (62.8%)	1 (2.4%)
ER	49 (61.25%)	31 (38.75%)	-	0	71 (88.75%)	9 (11.25%)	27 (62.8%)	15 (34.8%)	1 (2.4%)
PR	40 (50%)	40 (50%)	-	0	77 (96.25%)	3 (3.75%)	16 (37.5%)	11 (25.0%)	16 (37.5%)
Ki-67	67 (83.75%)	7 (8.75%)	6 (7.5%)	0	76 (95%)	4 (5%)	41 (95.2%)	1 (2.4%)	1 (2.4%)
p53	59 (73.75%)	21 (26.25%)	-	0	75 (93.75%)	5 (6.25%)	28 (65.1%)	13 (30.1%)	2 (4.8%)

and ductal cells showed a less evident and scattered staining pattern. Interestingly, stromal cells from the normal breast parenchyma were not reactive to c-kit. Although there is plenty of demonstration of c-kit reactivity in breast neoplastic tissue, the accurate analysis performed by pathologists focused in breast pathology enhanced the detailed description and identification of c-kit breast expression. We could report acinar, ductal, stromal and lymph node expression separately and verify that c-kit expression was seldom present in neoplastic tissue. These results were in concordance of many previous reports that indicate a reduction of c-kit expression during breast carcinogenesis. The diminution of c-kit protein was also associated to tumor progression[7]. However, as shown in table 3, presence and amount of c-kit protein in IDC remains controversial. Besides the differences of assessment methodology, the accuracy in immunohistochemical evaluation seems a major role in this issue.

Recently a technical explanation for "false positive" stromal cells was demonstrated by Djordjevic B and Hanna WM (2008) in fibroepithelial breast tumors[30]. When toluidine blue and tryptase staining was performed along c-kit immunostaining, the staining pattern matched that of c-kit in the number of cells and their distribution, with confirmed and characterized the latter as mast cells and excluding any appreciable level of true stromal c-kit staining[30].

Since the last decade, TMA methodology has been utilized to improve the range of samples and antibodies in a given essay, improving standard sensibility and correlation among cases[32]. Immunohistochemical sensibility reaches over 95% when compared to classical whole cut histological samples[33-36]. We found the TMA technology very useful to obtain reproducible data. One of the major problems in c-kit expression is to compare the several diverse results available in literature. We think that TMA results will restrain immunohistochemical results to a standard level that will improve the value of metanalysis in this field.

The description of basaloid type of IDC and its characterization as a group of aggressive tumors that, along cytokeratin 5/6, often express c-kit and EGFR[9]; associated c-kit to myoepithelial and mesenchymal differentiation[16] and aggressive profile[9]. In a large series of 1654 cases, Simon *et al.*[10] demonstrated a low incidence of c-kit expression in IDC (2.6%) and strong association to aggressive phenotype ($p < 0.0001$). Although the majority of our cases cursed with lymph node metastasis or larger tumors, prognostic variables were not associated to c-kit expression as in Simon[10] series. KIT staining was very low and among pathological variables only presence of necrosis reached statically significance ($p = 0.002$). Although evidence of association between c-kit expression and basaloid profile was reached by other studies[9] as long as to undifferentiated tumors[16] and p53 expression[31], these results were not reproduced by others[19]. Some parameters of basaloid immunohistochemical profile, as Her-2, PR, ER and EGFR positive did not correlate to c-kit expression as expected.

Maybe only a small subset of aggressive IDC and a minority of the basaloid tumors, as defined by ER, PR and Her-2 negative cases, are really c-kit expressing tumors. It seems that c-kit expression, when involved in breast carcinogenesis, plays an early role in transformation[16] and thereby is lost during carcinogenesis. Therefore, putative positive cases would be those with a maintained c-kit expression.

Another concern was to determine if premalignant lesions could express any level of c-kit. At the adjacent normal tissue around the role-cut slides, columnar cells and atypical hyperplasia lesions were not reactive for c-

Table 3. c-kit expression in breast tissue. Main results available (adapted Tsutsui *et al.*)[19]

		c-kit expression				
Author (year)	Method	Normal breast tissue	Benign tumours	*In situ* cancers	Invasive cancers	Metastatic cancers
Natali *et al.* (1992)[20]	IHC[a]	6/6/ (100%)	28/36 (78%)		10/80 (13%)	1/40 (3%)
Matsuda *et al.* (1993)[21]	IHC				2/10 (20%)	
Hines *et al.* (1995)[7]	mRNA[b] IHC				9/11 (82%)	
Chui *et al.*	IHC	6.22 + 2.11	3.33 + 2.44		0.43 + 1.27	
(1996)[22]	(IRS)[c]	*(n = 20)*	*(n = 58)*		(n = 57)	
Tsuura *et al.* (2002)[23]	IHC	338/338 (100%)	131/141 (93%)	0/11 (0%)	2/171 (1%)	
Palmu *et al.* (2002)[24]	IHC				33/40 (82%)	
Ko CD *et al.* (2003)[25]	IHC (IRS)	5.90 + 1.37 *(n = 20)*	4.05 + 1.82 *(n = 20)*		0.90 + 1.79 (n = 40)	1.06 + 1.86 (n = 18) 0.20 + 0.63 (n = 10)
Yared *et al.* (2004)[26]	IHC	21/21 (100%)	16/24 (88%)	3/29 (10%)	4/41%)	0/4 (0%)
Ulivi *et al.* (2004)[27]	IHC mRNA	14/14 (100%) 14/14 (100%)		7/16 (44%) 12/16 (75%)	7/75 (9%) 3/14 (21%)	
Simon *et al.* (2004)[10]	IHC				43/1654 (2.6%)	
Azoulay *et al.* (2005)[11]	IHC				18/18 (100%)	
Tsutsui *et al.* (2006)[19]	IHC				59/217 (27%)	
Diallo *et al.* (2006)[5]	IHC			55/104 (52.8%)		
Roussidis *et al.* (2007)[12]	IHC	52/179 (29%)	17/101 (17%)	12/50 (24%)	13/28 (46%)	
Nalwoga *et al.* (2008)[9]	IHC			5/65 (7.7%)		

[a]*Immunohistochemistry.* [b]*Messenger ribonucleic acid.* [c]*Immunoreactive score.*

kit. Other authors registered heterogeneous pattern of c-kit expression in columnar cell lesions[29], decreasing with progressive atypia.

However, we demonstrated a statistical correlation between presence of c-kit expression and identification of *in situ* component of ductal carcinoma ($p = 0.044$). It is not clear if *in situ* component confers a worst or better prognosis to IDC[37]; and basaloid type is not necessarily associated to CDIS. C-kit evaluation in DCIS has been performed in smaller groups of lesions[5,9,16]. The role of tyrosine kinase transmembrane receptors in DCIS must be addressed in further analysis since it seems not always be coincident to the invasive counterpart. Other tyrosine kinase receptors also show a non-linear accumulation during carcinogenesis. Her-2 expression, for instance, is more prevalent in DCIS than in IDC[38].

One interesting contribution was the opportunity to evaluate the metastasis present in axilar lymph nodes. Previous reports indicate that c-kit expression is variable among primary IDC and distant metastasis[16,19,22,24,25].

Impressively, all 43 cases evaluated were totally negative, in stromal or in epithelial cells present at the neoplastic tissue. It seems that c-kit expression is not a *sine qua non* condition for distant metastasis. Moreover, the few positive cases produced negative axilar metastasis, suggesting

that maybe c-kit expression in those tumors were not a major but rather a transient feature.

Recently, the expression of c-kit in malignant tumors became interesting to oncologists. The c-kit gene encodes a receptor tyrosine kinase that is differentially expressed in gastrointestinal stromal tumors (GIST), but not in other stromal neoplasm, such as smooth muscle and neural tumors. The novel chemotherapeutic agent containing STI571, an active inhibitor of c-kit tyrosine kinase activity, enables treatment of those tumors after surgery[12]. Therefore; identification of other possible targets of STI571 is very appealing. However, the protein expression profile of IDC appears to be very heterogeneous, and according to other studies reported in the literature, few human IDC tumors express c-kit at the clinically relevant levels found in GIST. Indeed, Ayse Polat[29] and Yared *et al.*[26] reported that only 0% and 10% of IDC were c-kit positive, respectively. Although initially it seemed to function as a potential prognostic value in Phyllodes tumors of the breast, these results were not reproduced by others[31].

In IDC, we could verify that in our cases stromal component is not reactive for many antigens evaluated. There are previous reports of stromal cells expressing steroidal receptors, such as desmoids tumors[5] and p53 positive in sarcomas. Apparently, stromal component of IDC seems to be quiescent, since even proliferation rate, according to Ki-67 expression, is very low. It is clear that molecular reactivity of mesenchymal component of IDC are not in parallel of epithelium-transformed cells and more investigations are needed to delineate the stromal profile of IDC. Evidence indicates equivalent results at the stromal cells of lymph node metastasis.

Considering the hypothesis that c-kit positive IDC cases are members of the basaloid type, the aggressiveness and worst evolution of this group could justify the possibility of STI571 therapy. However, one prerequisite for effectiveness would be over expression of c-kit protein. Recently Imatinib was tested in 13 patients with advanced disease with no therapy response. Twelve patients died and the median overall survival was 7.7 months. No adverse effects were registrated, but all cases were immunohistochemically c-kit negative[39].

The identification of proper c-kit over expression in a subset of aggressive IDC cases could turn possible to consider the STI571 therapy a potential agent in advanced disease[16]. We conclude that c-kit immunohistochemical expression is mostly negative in IDC regardless of component evaluated; lymph node metastasis of IDC also do not express c-kit; and prognostic variables (ER, PR, Her-2, Ki-67, tumor width and stage) are not associated to c-kit expression in IDC.

References

1. Ross JS, Fletcher JA. Targeted therapy in breast cancer. The HER-2/neu gene and protein. Mol Cell Proteomics. 2004;3(4):379-98.
2. Perou CM, Sorlie T, Eisen MB, Van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature. 2000;406:747-52.
3. DeMatteo RP, Heinrich MC, El-Rifai WM, Demetri G. Clinical management of gastrointestinal stromal tumors: before and after STI571. Hum Pathol. 2002;33(5):466-77.
4. Akin C, Metcalfe DD. The biology of Kit in disease and the application of pharmacogenetics. J Allergy Clin Immunol. 2004;114(1):13-9; quiz 20.
5. Diallo R, Rody A, Jackisch C, Tinge E, et al. C-KIT expression in ductal carcinoma in situ of the breast: co-expression with HER-2/neu. Hum Pathol. 2006;37:205-11.
6. Cohen PS, Chan JP, Lipkunskaya M, Biedler JL, Seeger RC. Expression of stem cell factor and c-kit in human neuroblastoma. The Children's Cancer Group. Blood. 1994;84(10):3465-72.
7. Hines SJ, Organ C, Kornstein MJ, Krystal GW. Coexpression of the c-kit and stem cell factor genes in breast carcinomas. Cell Growth Differ. 1995;(6):769-79.
8. Joensuu H, Puputti M, Tynninen O, Sihto H, Blom T, Mäenpää H, et al. Amplification of KIT, PDGFRA, VEGFR2, and EGFR in gliomas. Mol Cancer Res. 2006;4(12):927-34.
9. Nalwoga H, Arnes JB, Wabinga H, Akslen LA. Expression of EGFR and c-kit is associated with the basal-like phenotype in breast carcinomas of African women. APMIS. 2008;116(6):515-25.
10. Simon R, Panussis S, Maurer R, et al. KIT (CD117)-positive breast cancers are infrequent and lack KIT gene mutations. Clin Cancer Res. 2004;10:178-83.
11. Azoulay S, Laé M, Fréneaux P, Merle S, Al Ghuzlan A, Chnecker C, et al. KIT is highly expressed in adenoid cystic carcinoma of the breast, a basal-like carcinoma associated with a favorable outcome. Mod Pathol. 2005;18(12):1623-31.
12. Roussidis AE, Theocharis AD, Tzanakakis GN, Karamanos NK. The importance of c-Kit and PDGF receptors as potential targets for molecular therapy in breast cancer. Curr Med Chem. 2007;14(7):735-43.
13. Cristofanilli M, Morandi P, Krishnamurthy S, Reuben JM, Lee BN, Francis D, et al. Imatinib mesylate (Gleevec) in advanced breast cancer-expressing C-Kit or PDGFR-beta: clinical activity and biological correlations. Ann Oncol. 2008;19(10):1713-9.
14. Hornick JL, Fletcher CD. Immunohistochemical staining for KIT (CD117) in soft tissue sarcomas is very limited in distribution. Am J Clin Pathol. 2002;117:188-93.
15. Ko CD, Son BH, Ahn SH, Ka IW, Gong GY, Kim JC. Significance of mismatch repair protein expression in the chemotherapeutic response of sporadic invasive ductal carcinoma of the breast. Breast Journal. 2004;10(1):20-6.
16. Tsuda H, Morita D, Kimura M, Shinto E, Ohtsuka Y, Matsubara O, et al. Correlation of KIT and EGFR overexpression with invasive ductal breast carcinoma of the solid-tubular subtype, nuclear grade 3, and mesenchymal ormyoepithelial differentiation. Cancer Sci. 2005;96:48-53.
17. Allred DG, Harvey JM, Berardo M, Clark GM. Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response do adjuvant endocrine therapy in breast cancer. J Clin Oncol. 1999;17:1474-81.

18. Bankfalvi A, Boecker W, Reiner A. Comparison of automated and manual determination of HER2 status in breast cancer for diagnostic use: a comparative methodological study using the Ventana BenchMark automated staining system and manual tests. Int J Oncol. 2004;25(4):929-35.
19. Tsutsui S, Yasuda K, Suzuki K, Takeuchi H, Nishizaki T, Higashi H, et al. A loss of c-kit expression is associated with an advanced stage and poor prognosis in breast cancer. Br J Cancer. 2006;94:1874-8.
20. Natali PG, Nicotra MR, Sures I, Mottolese M, Botti, C, Ullrich A. Breast cancer is associated with loss of the c-kit oncogene product. Int J Cancer. 1992;52:713-7.
21. Matsuda R, Takahashi T, Nakamura S, Sekido Y, et al. Expression of the c-kit protein in human solid tumors and in corresponding fetal and adult normal tissues. Am J Pathol. 1993; 142:339-46.
22. Chui X, Egami H, Yamashita J, et al. Immunohistochemical expression of the c-KIT proto-oncogene product in human malignant and non-malignant breast tissues. Br J Cancer. 1996;73:1233-6.
23. Tsuura Y, Suzuki T, Honma K, Sano M. Expression of c-KIT protein in proliferative lesions of human breast: sexual difference and close association with phosphotyrosine status. J Cancer Res Clin Oncol. 2002;128:239-46.
24. Palmu S, Söderström KO, Quazi K, Isola J, Salminen E. Expression of c-KIT and HER-2 tyrosine kinase receptors in poor-prognosis breast cancer. Anticancer Res. 2002;22(1A):411-4.
25. Ko CD, Kim JS, Ko BG, et al. The meaning of the c-KIT proto-oncogene product in malignant transformation in human mammary epithelium. Clin Exp Metastasis. 2003;20:593-7.
26. Yared MA, Middleton LP, Bernstam FM, Cristofanilli M, Sahin AA. Expression of c-kit proto-oncogene product in breast tissue. Breast J. 2004;10(4):323-7.
27. Ulivi P, Zoli W, Medri L, et al. C-KIT and SCF expression in normal and tumor breast tissue. Breast Cancer Res Treat. 2004;83:33-42.
28. Maffini MV, Soto AM, Sonnenschein C, Papadopoulos N, Theoharides TC. Lack of c-kit receptor promotes mammary tumors in N-nitrosomethylurea-treated Ws/Ws rats. Cancer Cell Int. 2008;8:5.
29. Polat A. c-KIT expression in columnar cell lesions of the breast accompanied by benign and malignant breast diseases. Pathol Res Pract. 2007;203:765-9.
30. Djordjevic B, Hanna WM. Expression of c-kit in fibroepithelial lesions of the breast is a mast cell phenomenon. Mod Pathol. 2008;21(10):1238-45.
31. Tan PH, Jayabaskar T, Yip G, Tan Y, Hilmy M, Selvarajan S, et al. p53 and c-kit (CD117) protein expression as prognostic indicators in breast phyllodes tumors: a tissue microarray study. Mod Pathol. 2005;18(12):1527-34.
32. Kononen J, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med. 1998;4(7):844-7.
33. Camp RL, Charette LA, Rimm DL, et al. Validation of tissue microarray technology in breast carcinoma. Lab Invest. 2000; 80(12):1943-9.
34. Zhang D, et al. Reliability of tissue microarrays in detecting protein expression and gene amplification in breast cancer. Mod Pathol. 2003;16(1):79-84.
35. Loring P, et al. HER2 positivity in breast carcinoma: a comparison of chromogenic in situ hybridization with fluorescence in situ hybridization in tissue microarrays with targeted evaluation of intratumoral heterogeneity by in situ hybridization. Appl Immunohistochem Mol Morphol. 2005;13(2):194-200.
36. Henriksen KL, et al. Semi-quantitative scoring of potentially predictive markers for endocrine treatment of breast cancer: A comparison between whole sections and tissue microarrays. J Clin Pathol. 2006;14-8.
37. Logullo AF, Godoy AB, Mourão-Neto M, Simpson AJ, Nishimoto IN, Brentani MM. Presence of ductal carcinoma in situ confers an improved prognosis for patients with T1N0M0 invasive breast carcinoma. Braz J Med Biol Res. 2002;35(8):913-9.
38. Selvarajan S, et al. c-erbB-2 (HER-2/neu) immunohistochemistry in invasive breast cancer: is there concordance between standard sections and tissue microarrays? Pathology. 2006;38(4):316-20.
39. Cristofanilli M, Morandi P, Krishnamurthy S, Reuben JM, Lee BN, Francis D, et al. PDGFR-beta: clinical activity and biological correlations. Ann Oncol. 2008;19(10):1713-9.