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C-KIT expression in ductal carcinoma in situ of the breast: co-expression with HER-2/neu

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Summary The proto-oncogene c-KIT (CD117) is highly expressed in normal breast epithelium and is decreased in invasive breast cancer. In this study, we analyzed the protein expression and the mutational status of c-KIT in ductal carcinoma in situ (DCIS) of the breast and correlated these findings with nuclear grade, architectural pattern, and expression of HER-2, estrogen receptor (ER)–α, and progesterone receptor (PR). C-KIT, HER-2, ER, and PR expression were analyzed immunohistochemically in 106 cases of paraffin-embedded DCIS (85 pure DCIS and 21 DCIS with concurrent carcinoma). Direct sequencing of exons 9 and 11 of the c-KIT gene was performed to analyze the hot spot mutational regions in representative cases. C-KIT expression was found in 55 (52.8%) of all DCIS, correlating with high nuclear grade ($P < .0001$), comedonecrosis ($P < .0001$), and solid growth pattern ($P = .001$). Furthermore, c-KIT expression was strongly associated with HER-2 positivity ($P < .0001$) and was significantly lower in ER- or PR-positive cases ($P = .001$ and $P = .006$, respectively). C-KIT expression alone or co-expression with HER-2 in pure DCIS did not differ significantly from DCIS with invasive component ($P = .09$). Mutational analysis in 6 c-KIT–positive DCIS revealed no activating mutations in exons 9 or 11. Our findings suggest that the expression of c-KIT protein might define a subset of poorly differentiated, HER-2–positive DCIS with decreased expression of steroid hormone receptors, comedonecrosis, and a solid growth pattern. The implications of c-KIT and HER-2 co-expression for breast carcinogenesis should be further evaluated.
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1. Introduction

The proto-oncogene c-KIT (CD117), located on chromosome 4q11-21, encodes for a surface membrane tyrosine

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kinase receptor that is structurally related to the platelet-derived growth factor and the colony-stimulating factor-1 receptors, with 5 immunoglobulin-like extracellular domains and a split tyrosine kinase domain [1]. C-KIT's ligand is stem cell factor (SCF), alternatively termed steel factor or KIT ligand [2]. In normal tissues, c-KIT functions within hematopoietic cells, germ cells, and neural crest–derived melanocytes [3]. Interaction of c-KIT receptor and its ligand results in activation of the KIT kinase domain. The consequent phosphorylation of tyrosine residues is the prerequisite for activation of a variety of signal transduction pathways involved in proliferation, apoptosis, and tumorigenesis [4]. C-KIT protein expression has been found in a wide variety of malignant tumors including myeloid leukemia [5], small cell lung cancer [6], seminomas [7], and gastrointestinal stromal tumors (GISTs) [8] but is frequently diminished or lost in other tumors, including malignant melanoma [9].

In 1998, Hirota et al [8] reported that some GISTs contained activating mutations in the KIT gene that are associated with overexpression (activation) of c-KIT protein. Most GISTs harbor activating mutations, mainly clustered in exons 9, 11, and 13 [10,11]. As a direct result of these mutations, c-KIT proteins in GISTs are constitutionally activated in a ligand-independent manner. This SCF-independent activation can be blocked by means of a tyrosine kinase inhibitor known as imatinib mesylate (formerly STI-571, now known as Gleevec in the United States and Glivec in Europe; Novartis, Basel, Switzerland).

Several studies have shown that c-KIT is highly expressed in normal breast epithelium but is present at only low levels or is completely lost in primary invasive breast cancer or breast cancer metastases [12-15]. To date, the expression of c-KIT in premalignant breast disease such as ductal carcinoma in situ (DCIS) has been evaluated in only very small numbers regardless of nuclear grading or other histopathological parameters [16]. Furthermore, there are no data about activating mutations of the c-KIT gene in DCIS.

Therefore, we investigated c-KIT expression in 106 DCIS of the breast (33 cases of low grade, 22 cases of intermediate grade, and 51 cases of high grade) by immunohistochemical methods. Furthermore, we searched for activating gene mutations in a representative number of c-KIT–positive DCIS in exons 9 and 11 of the c-KIT gene. The detection of c-KIT in DCIS was compared with nuclear grading, architectural pattern, and other histopathological markers of tumor aggression, including estrogen receptor (ER), progesterone receptor (PR), and HER-2/neu expression.

2. Material and methods

2.1. Case selection

One hundred six cases of DCIS were collected from the files of the Institute of Pathology at the University of Duesseldorf from 2002 through 2004. Patients' mean age was 58 (range, 18-89 years). All cases were independently reviewed by two experienced pathologists (R. D. and C. P.) to define the nuclear grade and the architectural patterns of DCIS according to the consensus conference on the classification of DCIS [17]. Eighty-five of these cases were "pure" DCIS and 21 DCIS were associated with concurrent invasive ductal carcinoma. The DCIS with invasion group (n = 21) were examined for the purpose of detecting any major differences from pure DCIS in expression of c-KIT.

2.2. Immunohistochemistry

The formalin-fixed, paraffin wax–embedded breast tissues were immunostained for c-KIT, ER, PR, and HER-2/neu using standard methods. The following primary rabbit monoclonal antibodies were used: anti–ER-α antibody (DCS, Hamburg, Germany, clone SP1; dilution 1:800) and anti-PR antibody (DCS, clone SP2; dilution 1:800). Rabbit polyclonal antibodies were used for HER-2/neu (DAKO Diagnostica GmbH, Hamburg, Germany, anti-human c-erbB2-oncoprotein, code no. A0485; dilution 1:500) and for c-KIT (DAKO Diagnostica GmbH, code no. A4502; dilution 1:200).

Sections were pretreated by heat antigen retrieval in 0.01 mol/L sodium citrate buffer (pH 6.0) in a steamer at 110°C for 15 minutes. The sections were then cooled for 5 minutes and were rinsed in tap water. After blockage of biotin and peroxidase, immunohistochemical staining was performed on an automated immunostainer (Biogenex, i6000, San Ramon, Calif) using a standard labeled streptavidin-biotin method (UltraTek Reagent Detection Kit, Scy Tek, Logan, Utah) followed by 3,3′ -diaminobenzidine enzymatic development. Sections were counterstained blue with hematoxylin.

Substitution of the primary antibodies with a matched concentration of nonimmune rabbit IgG served as negative controls. Sections of a GIST tissue with previously documented c-KIT status was used as positive control.

Immunostained slides were scored after the entire slide had been evaluated by light microscopy. The expression of ER and PR was scored by assigning a proportion score and an intensity score according to Ref. [18]. Any nuclear staining of DCIS epithelium was counted toward the proportion score. Tumors with scores of 4 or greater were considered to be positive for ER and PR expression.

The c-KIT expression level was scored as described previously [19]: score 1+: the cytoplasm was discretely and weakly to moderately stained in 10% or more of the constituent carcinoma cells; score 2+: the cytoplasm was strongly stained with or without membrane staining in 10% or more of the constituent carcinoma cells. If no staining was observed or staining was observed in less than 10% of the constituent carcinoma cells, a score of 0 was given. Cases with a score of 1+ and 2+ were considered positive.

Immunohistochemical expression of HER-2/neu oncoprotein was categorized into three groups: score 0: no

staining at all or membrane staining in less than 10% of the tumor cells; score 1+: faint, incomplete membranous staining; 2+: moderate, complete membranous staining; 3+: strong membranous staining, observed in more than 10% of the tumor cells, respectively. A score of 3+ was designated as positive [20]. Cases with 2+ scores were further evaluated by fluorescence in situ hybridization (FISH) to confirm HER-2/neu gene amplification.

2.3. Fluorescence in situ hybridization analysis of HER-2/neu

All cases with a 2+ score in the HER-2/neu immunohistochemistry were analyzed by FISH for HER-2 gene amplification. The tissue conditioning was carried out using the Tissue Conversion Kit (Qbiogene, Heidelberg, Germany) and followed by an overnight hybridization with a dual-color probe (HER-2/neu gene/Alphasatellite 17, Qbiogene). Assay and scoring were strictly performed according to manufacturer's protocol and standardized methods.

Table 1 Correlation of various histopathological features with nuclear grading of DCIS

	Low grade (n)	Intermediate grade (n)	High grade (n)	P
DCIS (n)				
Total	33	22	51	
Pure	24	19	42	
With carcinoma	9	3	9	
Comedonecrosis				
Present	4	10	31	<.0001
Absent	29	12	20	
Growth pattern				
Solid	6	6	30	.012
Micropapillary	1	–	5	NA[b]
Cribriform	15	7	4	.015
Intraductal papillary	1	1	1	NA
Mixed type	10	8	11	.310
ER				
Negative	2	6	31	<.0001
Positive	31	16	20	
ND[a]	0	0	0	
PR				
Negative	6	11	41	<.0001
Positive	27	11	10	
ND	0	0	0	
HER-2/neu				
Negative	32	17	26	<.0001
Positive	1	5	25	
ND	0	0	0	
C-KIT				
Negative	22	14	13	<.0001
Positive	9	8	38	
ND	2	0	0	

Abbreviations: ND[a], not determined; NA[b], not applicable because of low numbers in these subgroups.

2.4. Laser capture microdissection, c-KIT mutation analysis

Six DCIS were selected for laser capture microdissection to obtain tumor cells for the analysis of c-KIT mutational status (PixCell II, Arcturus, Moerfelden-Walldorf, Germany). Pure cell populations of each case were gained by dissection of at least three consecutive 10-μm-thick hematoxylin-stained tissue sections. DNA extraction was performed according to standard protocols using proteinase K digestion (1 mg/mL) followed by column purification step using the QIAamp DNA purification kit (QIAGEN, Hilden, Germany).

Amplification of c-KIT exons 9 and 11 was achieved using a primer system published elsewhere (exon 9 forward: 5′ -TTTGGAAAGCTAGTGGTTCA, reverse: 5′ -ATGGTAGACA-GAGCCTAAAC; exon 11 forward: 5′ -CTATTTTTCCCTTTCTCCCC, reverse: 5′ -TACCCA-AAAAGGTGACATGG) [21].

For bidirectional cycle sequencing, the BigDyeTerminator system (PE-Biosystems, Weiterstadt, Germany) was used with the respective amplification primers and analysis of the reaction products was performed on an ABI PRISM 310 sequencer (PE-Biosystems).

3. Statistical analysis

To compare expression values between c-KIT with grading of DCIS and the other immunohistochemical markers (HER-2/neu, ER, PR), we performed the χ^2 (Yates correction if necessary) and Fisher exact tests. The overall threshold of significance was .05. All P values were 2-sided.

4. Results

4.1. DCIS classification

The DCIS were classified according to the consensus conference on the classification of DCIS [17], which stratifies DCIS primarily by nuclear grade (nuclear size and morphology) into low, intermediate, and high grades. Furthermore, the presence of necrosis (comedo versus noncomedo) and architectural pattern is presented. Of the 106 DCIS, 33 (31%) cases were classified as low grade, 22 (21%) cases as intermediate grade, and 51 (48%) cases as high grade. Classification according to the architectural histology was as follows: 42 (40%) pure solid, 6 (6%) pure micropapillary, 26 (25%) pure cribriform, 3 (3%) intraductal papillary, and 29 (26%) mixed type. The presence of comedonecrosis and a solid growth pattern correlated well with high nuclear grade (P < .0001 and P = .012, respectively). Vice versa, cribriform architecture was significantly associated with low nuclear grade (P = .015) (Table 1).

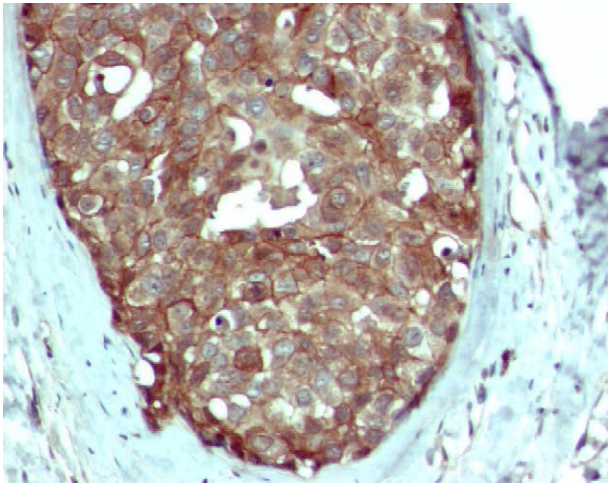

Fig. 1 Ductal carcinoma in situ, high grade: membranous and cytoplasmic staining for c-KIT, ×200.

4.2. c-KIT

Analysis of c-KIT expression was evaluable in 104 of 106 cases. Fifty-five (52.8%) of these cases were positive for c-KIT. The positive immunohistochemical staining of c-KIT in DCIS was observed diffusely in the cytoplasm and/or on the cell membrane. c-KIT expression was associated with DCIS grade including 9 (29%) of 31 low-grade, 8 (36.3%) of 22 intermediate-grade, and 38 (74.5%) of 51 high-grade DCIS ($P < .0001$) (Table 1). A representative example of 2+ c-KIT immunostaining is shown in Fig. 1. Furthermore, c-KIT expression correlated well with the presence of comedonecrosis and a solid growth pattern ($P < .0001$ and $P = .001$, respectively) whereas cribriform growth pattern was negatively associated with c-KIT expression ($P = .002$). There was no significant correlation between nuclear grade and the distribution of 1+- and 2+-positive DCIS cases ($P = .385$). c-KIT expression was absent in the stroma of normal breast whereas the epithelium of normal ductal and lobular breast cells showed a strong and most often membranous staining pattern. The staining was found to be heterogeneously distributed in atrophic breast lobules and in benign breast diseases such as sclerosing adenosis or cystic breast disease. Furthermore, some macrophages and smooth muscle cells of the vascular walls were positive for c-KIT. Negative controls showed no staining for c-KIT at all.

No mutations were detected in exons 9 or 11 (hot spot regions of c-KIT) in 6 of 6 randomly selected c-KIT–positive DCIS cases.

4.3. HER-2/neu

HER-2/neu expression was distributed according to the staining score as follows: 3+: 29 (27.3%) cases; 2+: 10 (9.3%); 1+: 24 (22.5%); 0: 44 (41.5%). Fluorescence in situ hybridization analysis of the cases with score 2+ revealed a HER-2/neu gene amplification in two DCIS. Based on both immunohistochemical data and FISH analyses, 31 (29.2%) cases of DCIS were scored positive for HER-2/neu (score 3 or score 2/FISH positive) including 1 (3%) low-grade, 5 (22.7%) intermediate-grade, and 25 (49%) high-grade DCIS cases ($P < .0001$) (Table 1).

4.4. ER/PR status

A total of 67 of 106 cases of DCIS were ER positive (63.2%), including 31 (93.9%) low-grade, 16 (72.7%) intermediate-grade, and 20 (39.2%) high-grade DCIS cases ($P < .0001$) (Table 1). Progesterone receptor–positive test results were observed in 48 (45.2%) of 106 cases, including 27 (81.8%) low-grade, 11 (50 %) intermediate-grade, and 10 (19.6 %) high-grade lesions ($P < .0001$) (Table 1).

4.5. Correlation between c-KIT, HER-2/neu, ER, PR, and DCIS grade

A significant correlation was found between DCIS grade and the expression of c-KIT, HER-2/neu, ER, and PR. c-KIT– and HER-2/neu–positive test results were associated with high-grade DCIS ($P < .0001$, respectively). Positivity of ER and PR was significantly associated with low nuclear grade in DCIS ($P < .0001$, respectively).

4.6. Correlation between c-KIT, HER-2/neu, ER, and PR

c-KIT–positive DCIS were significantly associated with HER-2/neu expression ($P < .0001$) (Fig. 2). Of the 55 cases expressing c-KIT, 26 (47.2%) co-expressed HER-2/neu. In contrast, of the 48 cases that did not show c-KIT expression, only 5 (10.4%) overexpressed HER-2/neu. Furthermore, c-KIT-expression was significantly decreased in ER- or PR-positive DCIS ($P = .001$ and $P = .006$, respectively) (Fig. 3).

Finally, co-expression of c-KIT and HER-2/neu depended strongly on high nuclear grade because 23 (88.4%) of 26 c-KIT/HER-2/neu–positive DCIS showed high nuclear

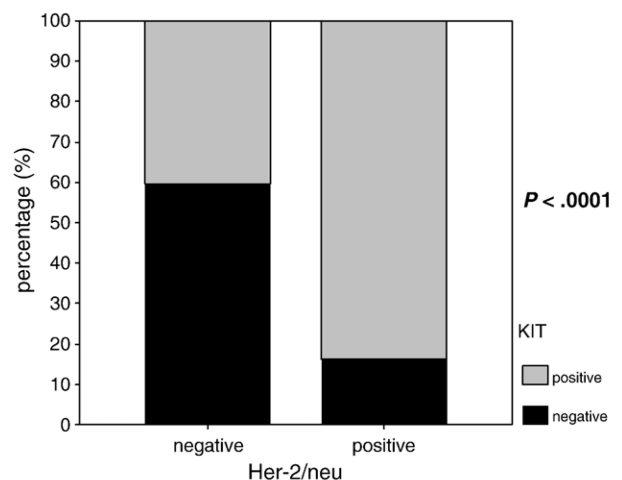

Fig. 2 Correlation of c-KIT expression with HER-2/neu overexpression in DCIS.

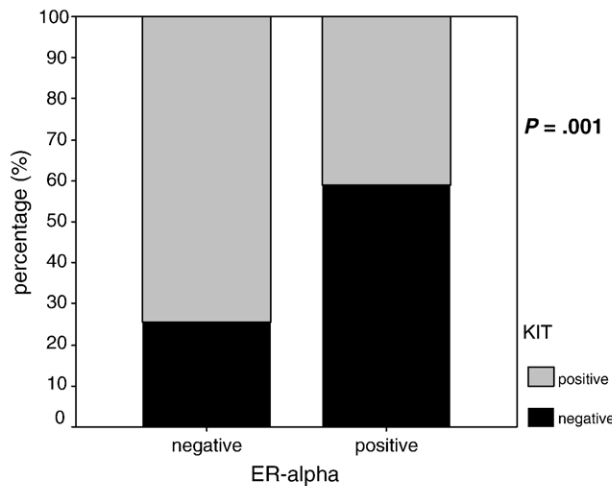

Fig. 3 Correlation of c-KIT expression with ER-α in DCIS.

grade (grade 3) ($P < .0001$) and 22 (84.6%) of these DCIS were ER or PR negative ($P < .0001$).

HER-2/neu protein overexpression was negatively correlated with ER and PR expression ($P < .0001$, respectively) and significantly depended on nuclear grade because 18 (94.7%) of 19 HER-2/neu–positive/ER- and/or PR-negative DCIS showed high nuclear grade (grade 3) ($P < .0001$).

4.7. Correlation between c-KIT expression in DCIS associated with invasive carcinoma versus pure DCIS

In contrast to pure DCIS, in which 48 (56.4%) of 85 exhibited c-KIT expression, in DCIS with concurrent invasive carcinoma, 7 (33.3%) of 21 cases showed a relatively lower frequency of c-KIT expression. Three of c-KIT–positive DCIS with carcinoma showed co-expression with HER-2/neu and 4 of those did not. The comparison of all these rates reached no statistically significant difference ($P = .09$).

In the 7 c-KIT–positive DCIS associated with invasive carcinomas, 3 of these 7 carcinomas were positive for c-KIT, 1 was positive for HER-2/neu, 3 were positive for ER, and 2 were positive for PR.

5. Discussion

Ductal carcinomas in situ of the breast comprise a heterogeneous group of lesions, covering a wide spectrum of clinical conditions and histopathological changes. With respect to clinical behavior, features of DCIS range from biologically aggressive lesions with a substantial risk of progression into invasive cancer to lesions with a very low malignant potential. Large nuclear size in DCIS correlates significantly with DNA aneuploidy, high proliferation activity, low steroid receptor content, and overexpression of HER-2/neu and p53 indicating an aggressive behavior [22]. The influencing factors for transformation of DCIS to invasive cancer are still widely unknown. c-KIT is one of the factors that may play a role in breast carcinogenesis because several studies have shown that c-KIT is expressed in normal mammary epithelium and is almost lost in invasive breast carcinoma [12,15]. In a very recent study by Ulivi et al [16], a progressive decrease in c-KIT expression from normal mammary epithelium to carcinoma in situ (n = 16) and an almost complete undetectability of the proto-oncogene expression in invasive breast carcinoma were found. Moreover, a significant co-expression of c-KIT and its ligand SCF has been reported in normal breast epithelium suggesting that an autocrine stimulation of c-KIT by SCF is important in the maintenance of differentiation of mammary epithelium whereas a gradual decrease of both factors accompanies malignant transformation.

To date, no data have been available on c-KIT expression in different subgroups of DCIS. Therefore, we analyzed c-KIT expression in 106 DCIS of the breast and investigated whether c-KIT expression is associated with nuclear grade or other common breast cancer–associated immunohistochemical markers such as ER, PR, and HER-2/neu.

In the present study, c-KIT expression was observed in 52.8% of all DCIS. Ulivi et al [16] reported c-KIT immunoreactivity in 44% and Tsuda et al [19] in 13% of DCIS, which might be attributed to the low number of cases in their studies (n = 16 and n = 15, respectively), different staining conditions, or scoring methods.

The mechanism of c-KIT expression in breast tissues is still unknown. In GIST, acute myelogenous leukemia, and mastocytosis, c-KIT is activated by somatic mutation [23]. The lack of activating c-KIT gene mutations in the 6 cases of DCIS in the present study sequenced for exons 9 and 11 of the c-KIT gene does not provide evidence for KIT activation in DCIS. This lack of activating c-KIT gene mutations is also seen in invasive breast cancer [24] and in phyllodes tumors of the breast [25,26]. In ovarian cancers, paracrine or autocrine activation is postulated [27].

According to the consensus conference on the classification of DCIS [17], the in situ lesions in the present study were classified by architectural pattern and nuclear grade into high, intermediate, and low grade. We found a significant correlation between positive ER and PR status and low nuclear grade. Furthermore, the overexpression of HER-2/neu in DCIS was significantly associated with high nuclear grade, which is in agreement with findings from previous studies [28]. Our data demonstrate that c-KIT expression is significantly associated with high nuclear grade, comedonecrosis, and solid growth pattern and is significantly decreased in ER- and/or PR-positive DCIS. These results confirm observations of Tsuda et al [19] in a small number of DCIS (n = 15) that c-KIT expression is a frequent finding in comedo-type DCIS that are more often poorly differentiated. Taken together, these findings indicate a c-KIT expression in poorly differentiated DCIS with decreased expression of steroid hormone receptors.

Similar findings have been described for invasive breast cancer. Several studies found c-KIT protein expression in only 0 to 14% of invasive breast cancers [12,13,19,24,29]. However, in these c-KIT–positive breast cancers, c-KIT expression correlates significantly with high tumor grade [19,24] and is detected in 82% of progressive metastatic breast cancer [30]. Furthermore, a recent study reported a relationship between c-KIT expression and the basal-like breast cancer subtype with most c-KIT–positive breast tumors belonging to the basal-like breast cancer subtype [29], which is characterized by ER negativity, high tumor grade, and poor prognosis [31]. Therefore, once expressed in neoplastic breast lesions, c-KIT seems to be significantly associated with high tumor grade and steroid hormone receptor negativity in invasive carcinoma as well as in DCIS. The reason for this is still unclear and might be a sign of dedifferentiation with a different meaning than c-KIT expression in normal breast epithelium or benign breast lesions.

To the best of our knowledge, we report here for the first time a significant correlation between c-KIT and HER-2/neu protein expression in DCIS. Expression of either c-KIT or HER-2/neu rather than co-expression of both receptor-type tyrosine kinases has been reported in several malignancies [32,33]. To date, co-expression of c-KIT and HER-2/neu has merely been described in stage I adenocarcinomas and squamous cell carcinomas of the lung and identified a peculiar subset of highly proliferative and aggressive tumors [34]. Palmu et al [30] found expression of HER-2/neu in 35% and of c-KIT in 82% of progressive metastatic breast cancers but there was no correlation between c-KIT and HER-2/neu. Nevertheless, a previous in vitro study suggested a close relationship between these two tyrosine kinase receptor proteins in the breast. Kauraniemi et al [35] could demonstrate that Herceptin (HER-2/neu antibody) treatment induced in vitro down-regulation of c-KIT expression in c-KIT–transfected MCF-7 breast cancer cell lines. Furthermore, a close relationship among c-KIT and a number of other tyrosine kinase receptor proteins has been described especially between c-KIT and EGFR in breast cancer [19]. Hines et al [36] could demonstrate in vitro that epidermal growth factor (EGF)- and heregulin-stimulated growth is enhanced by co-expression of SCF and KIT. Furthermore, von Ruden et al [37] reported that expression of normal EGF receptor in the bone marrow cells of W/W^{v} mice, which carry inactivating mutations in c-KIT, can partially compensate for the defective c-KIT receptor in mast cell development. They postulated that the EGF and c-KIT receptors may have partially overlapping signal transduction pathways.

In summary, our results indicate that the expression of c-KIT protein might define a subset of poorly differentiated, HER-2/neu–positive DCIS with decreased expression of steroid hormone receptors, comedonecrosis, and a solid growth pattern and that c-KIT expression in these DCIS cases is not the result of activating mutations in exons 9 or 11 of the c-KIT gene as is described for GISTs. Furthermore, the co-expression of c-KIT and HER-2/neu in this subgroup of DCIS cases suggests that these receptors may be interdependent. The implications of c-KIT and HER-2/neu co-expression for breast carcinogenesis should be further evaluated.

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References

[1] Yarden Y, Kuang WJ, Yang-Feng T, et al. Human proto-oncogene c-KIT: a new cell surface receptor tyrosine kinase for an unidentified ligand. EMBO J 1987;6:3341-51.

[2] Zsebo KM, Williams D, Geissler E, et al. Stem cell factor is encoded at the S/locus of the mouse and is the ligand for the c-KIT tyrosine kinase receptor. Cell 1990;63:213-24.

[3] Nocka K, Majumder S, Chabot B, et al. Expression of c-KIT gene products in known cellular targets of W mutations in normal and W mutant mice—evidence for an impaired c-KIT kinase in mutant mice. Genes Dev 1989;3:816-26.

[4] Heinrich MC, Rubin BP, Longley BJ, Fletcher JA. Biology and genetic aspects of gastrointestinal stromal tumors: KIT activation and cytogenetic alterations. HUM PATHOL 2002;33:484-95.

[5] Wang C, Curtis J, Geissler E, McCulloch E, Minden M. The expression of the proto-oncogene c-KIT in the blast cells of acute myeloblastic leukemia. Leukemia 1989;3:699-702.

[6] Plummer H III, Catlett J, Leftwich J, et al. c-*myc* expression correlates with suppression of c-KIT proto-oncogene expression in small cell lung cancer cell lines. Cancer Res 1993;53:4337-42.

[7] Tian Q, Frierson HF, Krystal GW, Moskaluk CA. Activating c-KIT gene mutations in human germ cell tumors. Am J Pathol 1999;154:1643-7.

[8] Hirota S, Isozaki K, Moriyama Y, et al. Gain-of-function mutations of c-KIT in human gastrointestinal stromal tumors. Science 1998;279:577-80.

[9] Lassam N, Bickford S. Loss of c-KIT expression in cultured melanoma cells. Oncogene 1992;7:51-6.

[10] Miettinen M, Lasota J. Gastrointestinal stromal tumors—definition, clinical, histological, immunohistochemical, and molecular genetic features and differential diagnosis. Virchows Arch 2001;438:1-12.

[11] Lux ML, Rubin BP, Biase TL, et al. KIT extracellular and kinase domain mutations in gastrointestinal stromal tumors. Am J Pathol 2000;156:791-5.

[12] 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.

[13] 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.

[14] 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.

[15] 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.

[16] 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.
[17] Consensus Conference on the classification of ductal carcinoma in situ. The Consensus Conference Committee. Cancer 1997;80: 1798-802.
[18] Remmele W, Stegner HE. Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue. Pathology 1987;8: 138-40.
[19] Tsuda H, Morita D, Kimura M, et al. Correlation of KIT and EGFR overexpression with invasive ductal breast carcinoma of the solid-tubular subtype, nuclear grade 3, and mesenchymal or myoepithelial differentiation. Cancer Sci 2005;96:48-53.
[20] Bankfalvi A, Giuffre G, Ofner D, et al. Relationship between HER2 status and proliferation rate in breast cancer assessed by immunohistochemistry, fluorescence in situ hybridisation and standardised AgNOR analysis. Int J Oncol 2003;23:1285-92.
[21] Yamamoto H, Oda Y, Kawaguchi K, et al. c-kit and PDGFRA mutations in extragastrointestinal stromal tumor (gastrointestinal stromal tumor of the soft tissue). Am J Surg Pathol 2004;28:479-88.
[22] Ottesen GL. Carcinoma in situ of the female breast. A clinico-pathological, immunohistological, and DNA ploidy study. APMIS 2003;(Suppl):1-67.
[23] Heinrich MC, Blanke CD, Druker BJ, Corless CL. Inhibition of KIT tyrosine kinase activity: a novel molecular approach to the treatment of KIT-positive malignancies. J Clin Oncol 2002;20:1692-703.
[24] 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.
[25] Carvalho S, e Silva AO, Milanezi F, et al. c-KIT and PDGFRA in breast phyllodes tumours: overexpression without mutations? J Clin Pathol 2004;57:1075-9.
[26] Sawyer EJ, Poulsom R, Hunt FT, et al. Malignant phyllodes tumours show stromal overexpression of c-myc and c-kit. J Pathol 2003;200: 59-64.
[27] Wilczynski SP, Chen YY, Chen W, Howell SB, Shively JE, Alberts DS. Expression and mutational analysis of tyrosine kinase receptors c-kit, PDGFRalpha, and PDGFRbeta in ovarian cancers. Hum Pathol 2005;36:242-9.
[28] Baqai T, Shousha S. Oestrogen receptor negativity as a marker for high-grade ductal carcinoma in situ of the breast. Histopathology 2003;42:440-7.
[29] Nielsen TO, Hsu FD, Jensen K, et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res 2004;10:5367-74.
[30] Palmu S, Soderstrom 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;22:411-4.
[31] Sorlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001;98:10869-74.
[32] Araki K, Ishii G, Yokose T, et al. Frequent overexpression of the c-KIT protein in large cell neuroendocrine carcinoma of the lung. Lung Cancer 2003;40:173-80.
[33] Bar-Sela G, Kuten A, Ben-Eliezer S, Gov-Ari E, Ben-Izhak O. Expression of HER2 and C-KIT in nasopharyngeal carcinoma: implications for a new therapeutic approach. Mod Pathol 2003;16: 1035-40.
[34] Pelosi G, Barisella M, Pasini F, et al. CD117 immunoreactivity in stage I adenocarcinoma and squamous cell carcinoma of the lung: relevance to prognosis in a subset of adenocarcinoma patients. Mod Pathol 2004;17:711-21.
[35] Kauraniemi P, Hautaniemi S, Autio R, et al. Effects of Herceptin treatment on global gene expression patterns in HER2-amplified and nonamplified breast cancer cell lines. Oncogene 2004;23: 1010-3.
[36] Hines SJ, Litz JS, Krystal GW. Coexpression of c-kit and stem cell factor in breast cancer results in enhanced sensitivity to members of the EGF family of growth factors. Breast Cancer Res Treat 1999;58: 1-10.
[37] von Ruden T, Stingl L, Ullrich A, Wagner EF. Rescue of W-associated mast cell defects in W/Wv bone marrow cells by ectopic expression of normal and mutant epidermal growth factor receptors. Blood 1993; 82:1463-70.