Expression of c-kit in Adenoid Cystic Carcinoma of the Breast

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Abstract

Breast adenoid cystic carcinoma (BACC) is a biologically distinct tumor with morphologic mimickers, which might make accurate classification problematic. Because c-kit expression has been reported in adenoid cystic carcinoma of various anatomic sites, we evaluated BACC for c-kit by immunohistochemical analysis, comparing the findings to similarly stained mimickers. Tested cases included 6 BACCs, 15 low-grade infiltrating ductal carcinomas (LGIDCs) chosen as potential mimickers, and 15 head-neck adenoid cystic carcinomas (HNACCs).

All BACCs showed plasma membranous and cytoplasmic staining equal to or greater than that of adjacent benign epithelium. Five BACCs (83%) expressed c-kit in more than 50% of tumor cells. Only 2 of 15 LGIDCs expressed low-intensity, focal c-kit staining. Of the 15 HNACCs, 10 (67%) expressed c-kit. Hormone receptors were consistently negative in BACCs.

All BACCs expressed c-kit, whereas LGIDCs infrequently expressed low-intensity c-kit. Immunohistochemical evaluation for c-kit might aid in accurately classifying carcinomas with histologic features overlapping adenoid cystic carcinoma and LGIDC.

Adenoid cystic carcinomas (ACCs) constitute approximately 0.1% of breast malignant neoplasms (breast ACCs [BACCs]) and, similar to salivary gland ACCs (head-neck ACCs [HNACCs]), represent a morphologically heterogeneous tumor type. BACCs typically are hormone receptor-negative,1 and although limited literature is available on their natural behavior, the vast majority of reported cases had a favorable long-term outcome.2 Because BACC is rare, it might be difficult to distinguish from more common breast tumors, such as tubular and infiltrating cribriform carcinomas and from benign mimickers such as collagenous spherulosis.

With the wide introduction of immunohistochemical analysis, numerous basic research and clinical pathologic studies are ongoing in the evaluation of cellular markers for tumor type characterization and the applicability of such in supporting (or redefining) morphologic tumor categorization. In an early BACC study, Due et al3 evaluated immunohistochemical markers such as collagen type IV, vimentin, epithelial membrane antigen, steroid receptors, and proliferative index and concluded that steroid receptors were the best discriminators between infiltrating cribriform carcinoma and ACC; ACC was consistently negative.

Unlike the rare BACC, HNACC represents a relatively common salivary gland malignant neoplasm, and as such, studies evaluating genetic alterations of tumor suppressor genes and/or oncogenes in these tumors are numerous. The c-kit oncogene, originally isolated as a transduced transforming gene in a feline sarcoma virus,4 is expressed in many benign tissue types and in a variety of solid tumors. ACCs of major and minor salivary glands have been reported to express c-kit protein in immunohistochemical studies.5,6 Thus, c-kit has been shown to aid in the differentiation of salivary gland ACC.
from polymorphous low-grade adenocarcinoma, a frequent mimicker of ACC in the head and neck, recently shown to be negative for c-kit. In addition, immunohistochemical expression of c-kit might be associated with heterogeneous c-kit mutations in tumor cells. In gastrointestinal stromal tumors (GISTs), these mutations have been shown to correlate with more aggressive tumor behavior. The goals of this study were to examine the immunohistochemical pattern of c-kit expression in BACC and in other subtypes of morphologically similar infiltrating breast carcinomas and to review the literature for documented evidence of the potential significance of c-kit expression in BACC and in breast tumorigenesis.

Materials and Methods

A search of the pathology files at Baystate Medical Center, Springfield, MA, from January 1987 to December 2002 for BACC and HNACC revealed 10 and 23 cases, respectively. Archival paraffin-embedded tissue blocks were available for 6 BACC and 15 HNACC cases. Fifteen cases of low-grade infiltrating ductal carcinoma (LGIDC) also were retrieved, representing morphologic mimickers of BACC. The total infiltrating breast carcinomas included the following types: adenoid cystic, 6; pure tubular, 7; mixed cribriform-tubular, 4; pure cribriform, 1; cribriform-micropapillary, 1; and not otherwise specified, 2. Representative tissue blocks were retrieved for each case, and a freshly H&E-stained slide was examined from each tissue block.

Immunohistochemical study was performed on all cases using the DakoCytomation LSAB2 System-HRP kit (DAKO, Carpinteria, CA). Microwave antigen retrieval was performed using DAKO Target Retrieval Solution (citrate buffer, pH 6.0). Slides were loaded on the DakoCytomation Autostainer and stained according to the manufacturer description. The primary antibodies used were c-kit (clone 104D2, rabbit antihuman CD117; dilution 1:100; DAKO), a myoepithelial antibody cocktail constituted by calponin (clone CALP-1; dilution 1:800; DAKO) and p63 (clone Y4A4; dilution 1:160; BP Pharmingen, San Diego, CA), estrogen receptor (clone 1D5; dilution 1:35; DAKO), progesterone receptor (clone PgR636; dilution 1:50; DAKO), and c-erb-B2 (clone aPS1113; dilution 1:100; Novus Biologicals, San Antonio, TX). Sections were finally stained with diaminobenzidine chromogen for 7 minutes, counterstained in hematoxylin for 5 minutes, and dehydrated in ethyl alcohol and xylene before manual coverslipping.

The percentage of c-kit–immunoreactive tumor was defined by examining the entire tumor area of each specimen. The intensity of immunoreactivity was determined as negative, weak, moderate, or strong. In each case, staining characteristics of benign breast epithelium and/or mast cells were evaluated. Plasma membranous staining and/or cytoplasmic staining were considered positive. Estrogen and progesterone receptor immunoreactivity was evaluated semiquantitatively, with positive staining defined as greater than 10% at least moderately staining tumor cell nuclei.

HER-2/neu immunoreactivity was graded according to the HercepTest (DAKO) staining criteria.

Results

Table I summarizes the characteristics of the BACCs and LGIDCs. A total of 6 BACCs and 15 LGIDCs were studied. All BACCs and LGIDCs were in women with an age range from 37 to 93 years (mean, 61.6 years), with no laterality predilection. Positive internal control samples included stained mast cells and/or stained benign ductal or lobular epithelium. The morphologic patterns of the BACCs were cribriform and/or cylindromatous (3 cases), solid/cystic (2 cases), and solid/basaloid (1 case). The pathologic tumor size was T1c in 5 and T3 in 1; 3 tumors were grade I, and 3 were grade III. The LGIDC pathologic tumor sizes were as follows: T1a, 2; T1b, 6; T1c, 6; and T2, 1. Of the tumors, 14 were histologic grade I, and 1 was grade II.

The HNACCs were from 11 women and 4 men with an age range of 32 to 81 years (mean, 45.5 years). Of these cases, 12 were primary and 3 were metastatic. The morphologic pattern was cribriform/cylindromatous in 13 cases and solid pattern grade III in the remaining 2 cases.

The immunohistochemical profile of the BACCs and LGIDCs is shown in Table 1. Regardless of morphologic pattern, c-kit was present in all BACCs. Of 6 BACCs, 5 cases (83%) expressed c-kit in more than 50% of tumor cells, with the stain intensity equal to or greater than that of adjacent benign breast epithelium. Plasma membranous (predominant pattern) and cytoplasmic staining were seen, varying in intensity from weak to strong. Similarly, benign breast ducts and lobules (not present in 1 case) showed patchy staining with varying intensities Image 1K. Tissue mast cells were identified in all cases and were uniformly positive (Image 1K). One solid-pattern BACC expressed weak cytoplasmic c-kit staining in 15% of the tumor. In BACCs with classic or solid cystic patterns, c-kit expression was localized to the inner cell layer Image 1A vs Image 1B, Image 1C vs Image 1D, Image 1F, and Image 1G. In the solid basaloid pattern BACC, c-kit expression was seen in all cell layers Image 1H and Image 1I. In this latter case, the liver metastasis developed 4 years after the initial diagnosis; the metastatic tumor was identical morphologically to the primary BACC and expressed c-kit (not shown). Myoepithelial cell stain, performed on 2 cases, showed an inverse pattern of staining compared with c-kit (Image 1E vs Image 1D, respectively).

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associated closely with a benign adenomyoepithelioma, which also expressed c-kit in the epithelial component only (not shown).

Among the 15 cases of LGIDC subtypes, 13 were negative for c-kit and 2 showed focal, weak to moderate expression of c-kit in a small subset of tumor cells (5%) (Figure 1J and Image 1K). Benign breast epithelium (not identified in 1 case) and tissue mast cells were consistently positive in these cases (Image 1K).

Estrogen receptor, progesterone receptor, and HER-2/neu immunoreactivity were consistently negative in BACCs, whereas the majority of the LGIDCs showed positive hormone receptor immunostaining and no evidence of HER-2/neu overexpression. One LGIDC case showed 2+ HER-2/neu staining by immunohistochemical analysis; however, fluorescence in situ hybridization showed no overamplification.

Of the 15 HNACCs (12 primary and 3 metastatic), 10 (67%) showed c-kit expression. The 5 negative cases were identified as follows: high-grade solid pattern primary ACC, 2; non–solid pattern primary ACC, 1; and non–solid pattern metastatic ACC, 2 (data not shown). In 1 case of metastatic tumor, the primary tumor was not available. The remaining metastatic case showed negative c-kit staining in the original primary tumor and in lung metastases, whereas liver metastases showed positive, albeit weak, cytoplasmic immunoreactivity for c-kit.

**Discussion**

ACC is characterized by an infiltrative growth pattern of admixed glandular and stromal elements. Criteria for the diagnosis of ACC include a biphasic pattern of true lumina and pseudolumina and a biphasic cellular population of myoepithelial and epithelial cells. The microscopic growth patterns are similar irrespective of site of origin, ie, cribriform, solid, tubular, reticular (trabecular), or basosolid. In tumors with a solid growth pattern, the cellular components might be mixed epithelial myoepithelial, as shown by Ro et al by ultrastructural analysis, or predominantly epithelial, as seen in solid basosolid ACC.

BACC is a rare tumor and is graded according to the relative proportion of solid growth pattern, such that tumors with greater than 30% solid growth are considered high grade.
Image II  Breast adenoid cystic carcinoma (BACC) and low-grade infiltrating ductal carcinoma (LGIDC) with CD117 immunohistochemical analysis. Histologic features of classic (A and C, H&E, ×20) and solid cystic (F, H&E, ×20) patterns in BACC and corresponding CD117 immunohistochemical analysis (B and D, ×60; G, ×40) with plasma membranous and cytoplasmic staining of the inner cell layer. Myoepithelial cell immunohistochemical analysis (E, ×60) with nuclear and cytoplasmic staining of the outer cell layer.
Overall, BACC has a more favorable prognosis than more common types of infiltrating mammary carcinomas, when controlled for grade and stage. When the glandular elements predominate, these tumors can mimic LGIDCs, particularly tubular and cribriform carcinomas.

ACCs of major and minor salivary glands have been reported to express c-kit protein by immunohistochemical analysis. In addition, c-kit has been shown to aid in the morphologic differentiation of salivary gland ACC from polymorphous low-grade adenocarcinoma, a frequent mimicker of ACC in the head and neck.

The present study demonstrated c-kit immunopositivity in all 6 BACCs tested, with staining limited to the epithelial component. The pattern of immunostaining was heterogeneous, with plasma membranous and cytoplasmic staining patterns. Heterogeneous c-kit positivity was seen in benign breast epithelium, with mast cells consistently positive, as previously described. To compare BACC with its morphologic mimickers, we preferentially examined low-grade variants of infiltrating ductal carcinoma and demonstrated absent c-kit expression in all but a small percentage of these tumors. The 2 cases that expressed c-kit did so in a very limited subset (5%) of tumor cells. As others, we confirm that BACCs typically are hormone receptor-negative and do not overexpress HER-2/neu oncoprotein. In regard to benign lesions that might mimic BACC, collagenous spherulosis has been shown to be negative for c-kit.

Hill reported positive c-kit expression in 3 cases of BACC, with comparison with the pattern of cytokeratin staining. Of note, c-kit expression has been evaluated on breast carcinoma...
tissue microarrays, showing similar findings. Among the breast tumors that expressed c-kit in the studies by Simon et al. and Went et al., preferential expression was seen in medullary carcinoma, a high-grade tumor with a known overall good prognosis. Although further studies are needed in this area, the relationship of c-kit expression to high-grade but good-prognosis tumors (ie, medullary carcinoma and a subset of BACCs) potentially might relate to a distinct biologic pathway in these tumors compared with breast carcinomas with a poor prognosis.

The c-kit proto-oncogene encodes a transmembrane receptor tyrosine kinase (TK) that maps to chromosome 4 (q11-12) and is related structurally to the platelet-derived growth factor/colony stimulating factor-1 receptor family of proteins. Previously, c-kit was examined in a variety of cell and tissue types, including mast cells and epithelial cells of the breast. In the latter, a heterogeneous plasma membranous and cytoplasmic staining pattern was reported, as similarly observed in our study.

Although the role of c-kit in mast cell development and proliferation is well established, its role in human breast epithelium is not fully understood. Chui et al demonstrated that the c-kit gene product correlates with growth control and differentiation of normal breast epithelium. Lammie et al found c-kit and c-kit ligand proteins to be expressed in breast tissue, within the epithelial and myoepithelial components, respectively. They suggested an autocrine kit ligand-receptor function and possibly a role for these molecules in cell-cell or cell-stromal interaction.

A number of studies have looked at c-kit expression in breast carcinoma, the majority of which have found decreased c-kit expression in malignant breast epithelium. Early studies by Natali et al demonstrated decreased expression of c-kit in primary breast tumors. The decrease was observed more so in conventional infiltrating ductal carcinomas and metastatic breast tumors and less so in special-type variants of infiltrating carcinoma, such as tubular, cribriform, mucinous, and medullary carcinomas. They first suggested that malignant transformation was associated with decreased to absent c-kit protein and significantly decreased c-kit–specific messenger RNA transcripts. This suggestion was supported further by Chui et al, who examined normal breast tissue, benign proliferative lesions, and breast carcinomas and observed gradual loss of c-kit expression during malignant transformation. In separate studies Tsuura et al demonstrated rare c-kit staining in infiltrating ductal carcinomas of the breast, by immunohistochemical analysis and in situ hybridization. No relationship was found between the expression of c-kit and the clinicopathologic parameters of the breast cancers studied, with epithelium of the included benign proliferative lesions found to constitutively express c-kit gene product. Most recently, an immunohistochemical study by Yared et al showed similar findings.

In contrast with the aforementioned findings, Palmu et al showed c-kit immunoreactivity in a cytoplasmic pattern in 82% of primary tumors in patients with progressive metastatic disease. However, these differences might be attributable to the type and specificity of the antibody used.

Among nonepithelial breast tumors, c-kit is expressed in stromal cells of malignant phyllodes tumors but not in stromal cells of the benign counterpart. Recently, Dabiri et al demonstrated that the presence of c-kit–positive mast cells in peritumoral stroma correlated with a good prognosis in breast cancers with long-term follow-up. These findings further support a role for c-kit in cell-stromal interactions.

Although some breast carcinomas express c-kit in immunohistochemical analysis, the results of mutational analysis studies have been negative in these tumors and in malignant and borderline phyllodes tumors. Similarly,HNACC has not been shown to have c-kit mutations. Although c-kit mutational analysis has not been reported in BACCs, from the aforementioned data, it is unlikely that gene alterations would be present. Because the expression of wild-type c-kit is conserved in some breast tumors such as ACC, it is likely that the immunohistochemical detection of c-kit expression in these tumors does not necessarily reflect gene up-regulation. As such, conservation of c-kit expression in these tumors might be due in part to autocrine-paracrine stimulation of the receptor ligand or cross-activation of other kinases.

The clinical value of certain TK inhibitors, such as the HER-2 TK inhibitor, trastuzumab (Herceptin) used in HER-2neu–overexpressing breast tumors, is well documented. The TK inhibitor imatinib mesylate (Gleevec) has been used for the treatment of GIST and mast cell disease, both known to express c-kit and to have c-kit mutations. However, preliminary data failed to show significant response to imatinib in other tumors expressing c-kit. These nonresponding tumors, similar to the few GIST tumors that do not respond to imatinib, failed to express activating intragenic mutations in the c-kit TK.

BACC seems to maintain c-kit expression, paralleling benign breast epithelium. Our results, as seen in other small series, indicate that immunohistochemical detection of c-kit in BACC might be diagnostically useful in differentiating it from its more common morphologic mimickers, which infrequently express c-kit. The combination of c-kit, hormone receptor, and HER-2neu immunohistochemical analysis aids accurate classification, and the addition of c-kit immunohistochemical analysis warrants consideration in any breast lesion with ACC in the differential diagnosis.

Data suggest that c-kit might have a protective role in breast cancer pathogenesis, and the loss of inhibition of c-kit might impair this growth control. Continued molecular studies are needed to further define the role of c-kit proto-oncogene expression in nonclassic mammary tumors, ie, ACC and medullary carcinoma. Understanding the interaction and role
of c-kit, c-kit ligand, and the platelet-derived growth factor family of receptors in breast tumorigenesis potentially might lead to new insights and possibly therapeutic strategies for the treatment of malignant neoplasms of the breast.

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