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Original contribution

Adenoid cystic carcinomas of the breast have low Topo IIα expression but frequently overexpress EGFR protein without EGFR gene amplification☆

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Summary Adenoid cystic carcinoma of the breast is a rare subtype of breast cancer with basal-like features. Published studies on breast adenoid cystic carcinoma are limited, resulting in relatively scarce information on the value of predictive tumor markers. We studied 20 primary cases of adenoid cystic carcinoma of the breast for expression of estrogen receptor, progesterone receptor, androgen receptor, epidermal growth factor receptor, HER-2/neu, and topoisomerase IIα using immunohistochemistry and fluorescent in situ hybridization methods. Estrogen and progesterone receptor expression were detected in 1 case each. All tumors were uniformly negative for Her-2/neu expression. Androgen receptor and topoisomerase IIα expression were weakly positive in three cases and 7 cases, respectively. Epidermal growth factor receptor overexpression was detected in 13 cases (65% of all cases). Amplification of *TOP2A* or *HER-2/neu* gene was not detected in any of the cases. Our study shows that the majority of adenoid cystic carcinomas of the breast do not overexpress Her-2/neu, topoisomerase IIα, or estrogen receptor, and thus, they are unlikely to respond to therapies targeting these proteins. However, these tumors frequently over-express epidermal growth factor receptor, indicating a potential benefit from anti–epidermal growth factor receptor therapy for patients with advanced adenoid cystic carcinomas of the breast.

1. Introduction

Adenoid cystic carcinoma (ACC), a salivary gland–like subtype of breast carcinoma, constitutes approximately 0.1%

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of all breast carcinomas [1]. The typical immunohistochemical profile for ACC is negative for estrogen receptor (ER), progesterone receptor (PR) and Her-2/neu but is positive for basal cell markers including basal cytokeratins (CK5/6, CK14, or CK17) and/or epidermal growth factor receptor (EGFR), p63, SMA, and C-Kit [2,3].

The paucity of reliable predictive markers for advanced cases of triple negative breast carcinomas including ACC remains as a problem.

EGFR (HER-1) belongs to the ErbB family of receptor tyrosine kinases, and its activation has been implicated in breast cancer cell growth and progression. It stimulates a number of different signaling pathways including Ras/mitogen–activated protein kinase pathway, the phosphoinositide-3-kinase/Akt pathway, and the phospholipase-Cγ/protein kinase C pathway [4]. Anti-EGFR therapies are available for certain tumors which over-express EGFR, such as colorectal carcinoma, glioblastoma, and lung cancer. EGFR expression in breast cancer is common [5], particularly in basal-like breast carcinomas [6], which raises the possibility of using targeted anti-EGFR treatment strategies for breast carcinomas overexpressing this protein [7].

TOP2A gene encodes the enzyme topoisomerase IIα (Topo IIα) that catalyzes the breakage and reunion of double-stranded DNA leading to relaxation of DNA supercoils [8]. It is included in a number of fundamental nuclear processes including DNA replication, transcription, chromosome structure, condensation, and segregation. Topo IIα is a molecular target for anthracyclines and sensitivity to the drugs is related to the TOP2A levels, particularly TOP2A gene amplification.

Patients with ACC characteristically present without axillary lymph nodes metastases, but late visceral metastases are not uncommon, and targeted therapies are not currently considered in such cases due to the paucity of data on predictive molecular characteristics in these tumors. Our study provides additional clinical information in a series of breast ACC and explores novel predictive markers not previously investigated in this tumor type.

2. Materials and methods

2.1. Breast tumor samples and patients data

The study included 20 patients (19 female and one male) diagnosed with primary adenoid cystic carcinoma of the breast. The cases were retrieved from the files of Institute of Oncology (Ljubljana, Slovenia), Thomas Jefferson University Hospital (Philadelphia, PA), Creighton University Medical Center (Omaha, NE), and Clinical Center of the University of Sarajevo (Sarajevo, Bosnia, and Herzegovina). All cases were initially diagnosed at these institutions and confirmed upon the central review. Institutional review board of the Creighton University Medical Center has approved the study.

2.2. Methods

2.2.1. Pathologic assessment

Routinely stained hematoxylin and eosin tumor sections were re-examined (Z.G.), and diagnoses were confirmed. Additional relevant histopathologic parameters recorded included histologic grade, angiolymphatic, and perineural invasion, and the presence of tumor necrosis.

2.2.2. Immunohistochemical assays

Immunohistochemical assays for estrogen receptor α (ER; clone 6F11, Ventana Medical Systems, Inc, Tucson, AZ; ER; clone SP1, Lab Vision, Fremont, CA), progesterone receptor (PR; clone 16, Ventana Medical Systems, Inc; PR; clone PgR636, Dako, Glostrup, Denmark), androgen receptor (AR; Clone AR441, DakoCytomation, Inc, Carpinteria, CA), Topo IIα (Clone Ki-S1, DakoCytomation, Inc), EGFR (DAKO EGFR PharmDX diagnostic kit, DakoCytomation, Inc), Her-2/neu (Clone CB11, Ventana Medical Systems, Inc), and cytokeratin 5/6 (D5/16B4, Dako) expression were performed on the formalin-fixed, paraffin-embedded tissue sections using the commercially available kits and automated staining procedures with 3,3′-diaminobenzidine tetrahydrochloride chromogen. All immunohistochemical stains were performed at the central laboratory (Creighton Medical Laboratories and Creighton University Medical Center).

The tumor was regarded as positive for steroid receptors (ER, PR, and AR) if more than 5% of the cells showed nuclear staining [9,10].

For Topo IIα expression only nuclear staining was considered specific. Immunostaining frequency of the tumor cells was scored on a scale ranging from 0 to 4+ (1+ for 1%-5% positive tumor cells; 2+ for 6%-25%; 3+ for 26%-75%; 4+ for more than 75%) [11,12].

Her-2/neu protein expression results were scored according to the American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations [13]. Briefly, cases showing no membrane immunostaining or staining in less than 10% tumor cells were scored 0; cases with weak and incomplete membrane staining in more than 10% of tumor cells were scored 1+; cases with complete membrane staining that was either nonuniform or weak in intensity but with obvious circumferential distribution in more than 10% of the cells were scored 2+; and cases with strong membrane staining in more than 30% tumor cells were scored 3+ [13].

Scoring of EGFR expression was performed according to the manufacturers' instructions: only membranous staining was considered positive. Weak (1+) intensity is defined as faint and incomplete membrane staining. Moderate (2+) and strong (3+) intensity are both varying degrees of circumferential staining of membranes. For statistical purposes,