Original Study

Stromal Clusterin Expression Predicts Therapeutic Response to Neoadjuvant Chemotherapy in Triple Negative Breast Cancer

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Abstract

Expression of clusterin correlates with tumor progression and therapeutic response in breast cancer. We explored whether clusterin expression was predictive of response to neoadjuvant chemotherapy in 72 triple negative breast cancers. We found low stromal clusterin was associated with a good pathologic response, suggesting a potential role for the assessment of stromal clusterin as a predictive biomarker for outcome.

Background: Expression of clusterin correlates with tumor progression and therapeutic response in several human malignancies, including breast cancer. However, its predictive value in the neoadjuvant setting in breast cancer remains unexplored. The objective of this explorative study was to determine whether clusterin expression in breast cancer correlated with clinical pathologic characteristics and whether its expression was predictive of response to neoadjuvant chemotherapy (NAC). **Materials and Methods:** We determined the clusterin expression pattern in 72 triple negative breast cancers (TNBC) treated with NAC before surgery. Clusterin expression was evaluated by immunohistochemistry and was correlated with pathologic characteristics and response to NAC using residual cancer burden score. **Results:** Immunohistochemistry analysis revealed a differential pattern of expression between tumor and stroma. Clusterin expression in the tumor associated stroma as opposed to expression by the neoplastic epithelium was significantly associated with neoadjuvant-treated TNBC. Low stromal clusterin, low stromal content, and high tumor-infiltrating lymphocytes were associated with a significantly greater likelihood of achieving a good pathologic response as reflected by lower residual cancer burden scores ($P = .002$, $P = .003$, and $P = .001$, respectively). Tumor and/or stromal clusterin expression were not associated with patient age, tumor histologic grade, stage, and lymph node status. **Conculsion:** This study suggests a potential role for the assessment of stromal clusterin as a predictive biomarker for response of TNBC to neoadjuvant therapy. Further validation of this biomarker in a large study is needed.

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Introduction

Triple-negative breast cancers (TNBCs) are a heterogeneous group characterized by the lack of expression of hormonal receptors and the absence of human epidermal growth factor receptor 2 (HER2) overexpression. TNBC represents approximately 15% of all breast cancers and has an aggressive clinical behavior, with a higher risk of both local and distant relapses that frequently present as visceral and/or brain metastases.[1] The majority of deaths occur in the first 5 years following initial diagnosis. Few systemic treatment options exist for TNBC besides the use of chemotherapy. Treatment responses to systemic therapy in TNBC are heterogeneous, and long-term outcomes differ in this subgroup of patients. Some patients have a robust response to neoadjuvant chemotherapy (NAC) and present with a pathologic complete response (pCR) at the time of surgery, whereas other patients lack this response and suffer from early relapse after surgery.[2,3] Unfortunately, no predictive biomarkers exist that allow us to identify which patients will achieve a pCR from chemotherapy at the time of diagnosis.

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Clusterin is a ubiquitous secretory heterodimeric disulfide-linked glycoprotein, which is implicated in a number of biological processes, including lipid transport, membrane recycling, cell adhesion, programmed cell death, and complement cascade, representing a truly multifunctional protein.[4,5] A large number of studies have focused on clusterin gene products as mediators of cell cycle progression and cell death induction. Abnormal distribution and expression of clusterin have been demonstrated in a variety of human malignancies including non−small-cell lung, ovarian, gastric, pancreatic, colon, prostate, and breast cancers.[3,6-12] The biological function of clusterin has been puzzling scientists since its discovery. The clusterin gene is found to encode more than 1 mRNA, and several protein forms derive from the unique gene. Isoform 1 has a prevalent cytoplasmic/nuclear localization and may account for the existence of an intracellular form of clusterin escaping the secretory pathway.[13] Conversely, isoform 2 encodes the protein of 449 amino acids targeted to secretion, which is the most extensively studied form of clusterin.[14-16] There is now substantial evidence that secretory clusterin has a chaperone action much like that of small heat shock proteins and is one of the first described extracellular chaperones.[17-20] It is not known if clusterin proteins of different mass result from alternative splicing, transcription initiation, or simply representing species at different stages of maturation (eg, cleaved or uncleaved, at different stages of glycosylation). Nevertheless, it seems reasonable that the biological role of clusterin outside and inside the cell is different. Clusterin is found to act primarily as a tumor suppressor in the early stages of carcinogenesis; however, high expression of secreted or cytoplasmic clusterin may represent a pro-survival stimulus, as it confers an increased resistance to killing by anti-cancer drugs and enhances tumor cell survival in specific niches.[21] This discrepancy may be explained by the existence of different clusterin forms, and they may undergo specific changes of expression during the different phases of neoplastic transformation.

In order to assess the prognostic and predictive value of clusterin, we evaluated clusterin expression in patients with TNBC who were treated with NAC. Immunohistochemical staining was used for determining clusterin expression, and a residual cancer burden (RCB) score was used for the pathologic response and clinical outcome analysis. The immunohistochemical study of 72 TNBC specimens demonstrated 3 differential expressed clusterin patterns in tumor and tumor associated stroma. We found that tumor-associated stromal clusterin expression and not tumor clusterin expression significantly correlated with NAC treatment response in TNBC.

Materials and Methods

Tissue Collection

The selection of patients and analysis was approved by the Lifespan Medical Center Institutional Review Board, approval #467617, and the Women and Infants Hospital Institutional Review Board, #14-0090. Among patients who received NAC at the Lifespan or at Women and Infants Hospital between 2007 and 2014, we identified those with TNBC who received NAC and for whom sufficient tissue was available for analysis.

Pathologic Evaluation

Tumor specimens were evaluated for tumor type, size, extent of the disease, lymph node status, and histologic grade using the Nottingham combined histologic grading system. Immunohistochemistry of estrogen receptor (ER), progesterone receptor (PR), and HER2 expression were classified according to the College of American Pathologists/American Society of Clinical Oncology guidelines used at the time of diagnosis.[22-24] For patients treated with NAC, pretreatment core biopsy specimens were utilized. Pathologic response to NAC was assessed by the American Joint Committee on Cancer cancer staging and RCB score.[25,26] The RCB system stratifies patients with residual invasive cancer by size and invasive cellularity of the residual tumor bed, number of involved lymph nodes, and largest focus of cancer in an involved node into classes I, II, and III. RCB class 0 is synonymous with having achieved a pCR; an on-line calculator is available at http://www.mdanderson.org/breastcancer_RCB. Patients who achieved a pCR or minimal residual disease (RCB class I) were considered good pathologic responders, whereas patients with more significant residual disease (RCB class II-III) were considered poor pathologic responders.

Tumor-associated Stroma and Tumor-infiltrating Lymphocyte (TIL) Analysis

The amount of tumor-associated peri- and intra-tumoral stroma and TILs was morphologically evaluated on pretreated biopsy samples, which commonly consisted of 2 to 5 needle cores of average 1.5 cm in length obtained with either a 14 gauge spring-loaded biopsy device or a 12 gauge vacuum-assisted biopsy device as previously described.[27] Briefly, the amount of tumor-associated stroma was scored as 0 to 2: 0 for absent or minimal stroma (< 10%), 1 for mild to moderate amount of stroma (10%-40%) and 2 for abundant stroma (≥ 40%). Stromal TILs (sTILs) were evaluated based on criteria published by Denkert et al.[28] Briefly, intraepithelial tumor-infiltrating lymphocytes (iTILs) were defined as lymphocytes in direct contact with the tumor cells, whereas sTILs were defined as lymphocytes in the surrounding stroma, with the percent of the tumor or stromal volume comprised of infiltrating lymphocytes, as opposed to tumor or other stromal tissues, on an hematoxylin and eosin-stained biopsy section estimated by the reading pathologists, with results reported in increments of 10 (0%-1% was scored as 0, with all other estimates rounded up to the next highest decile [ie, 11%-20% was scored as 20]). sTILs and iTILs were totaled to calculate TILs. The trends were similar for each lymphocyte fraction (data not shown). sTILs were chosen to analyze as they are considered to the most consistent metric as recommended by the International TILs Working Group.[29] The histologic evaluation was graded independently by 2 pathologists (Y.W. and J.X.), who were blinded to clinical information including the posttreatment outcome, at the time of analysis, with the summary score representing the mean of the 2 separate scores.

Immunohistochemistry

Anti-clusterin α chain (human) antibody (1:200, clone 41D, EMD Millipore, Billerica, MA), ER (1:50, clone 1D5, DAKO, Glostrup Municipality, Denmark), PR (1:400, clone 1A6, DAKO, Glostrup Municipality, Denmark), and HER2/neu (Dako HercepTest) antibodies were used for immunohistochemistry. Four-micron sections were cut from formalin-fixed paraffin-embedded tissue blocks, heated at 60°C for 30 minutes, deparaffinized, rehydrated, and subjected to antigen retrieval by heating the slides in epitope retrieval buffer in a water bath at 95°C for 45 minutes.

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