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Original contribution

Dicer expression in estrogen receptor–positive versus triple-negative breast cancer: an antibody comparison☆,☆☆

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Summary Dicer is an RNase III enzyme responsible for cleaving double-stranded RNAs into small interfering RNAs and microRNAs, which either target messenger RNA transcripts for degradation or inhibit translation. Dicer protein levels have been examined in breast cancer with contradictory results. Our goal was to resolve whether Dicer levels differ in breast cancer versus normal breast epithelium and between estrogen receptor–α–positive (ER+) or estrogen receptor–α–negative (ER−) primary breast cancers. We compared 3 different Dicer antibodies: Abcam 4A6, Abcam ab5818, and Sigma HPA000694, using immunohistochemistry and Western blot analyses. All 3 Dicer antibodies detected higher levels of Dicer in ER+ breast cancer cell lines versus ER−, and all 3 recognized exogenous overexpressed Dicer. In clinical specimens, all 3 antibodies detected higher Dicer in ER+ breast cancers versus triple-negative breast cancer (TNBC) but had very different staining patterns by immunohistochemistry on the same tumor samples. Using the optimal antibody, ab5818, selected for its sensitivity and specificity, Dicer protein expression was significantly higher in ER+ versus TNBC clinical specimens of primary tumor ($P < .0001$, unpaired t test). Dicer was also significantly higher in adjacent normal breast epithelium versus TNBC ($P < .0001$, paired t test; n = 18 pairs). Differences in antibody performance may explain contrasting results observed in the literature regarding Dicer protein in breast cancer. If Dicer becomes more clinically relevant as a prognostic indicator, further antibody optimization and standardization will be critical.

1. Introduction

Human Dicer is an RNase III enzyme responsible for cleaving double-stranded RNAs into small interfering RNAs or microRNAs (miRNAs). These small RNAs are then incorporated into a multiprotein RNA-induced silencing complex, which uses them as templates for targeting specific messenger RNAs (mRNAs) leading to their degradation or inhibiting their translation. miRNAs play major roles in both development and disease [1].

Dicer is a haploinsufficient tumor suppressor because dysfunctional miRNA processing leads to enhanced transformation

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and tumorigenesis [2–4]. However, studies of Dicer levels in relation to tumor progression and outcome in various cancers have demonstrated conflicting results [5–8]. For example, in breast cancer, a gradual loss of Dicer protein with disease progression was detected by immunohistochemistry (IHC) in clinical breast cancer specimens (normal > ductal carcinoma in situ > invasive carcinoma > nodal metastases) [9], whereas other studies have reported higher Dicer expression in nodal metastases compared to primary tumors by quantitative real-time polymerase chain reaction (qRT-PCR) [10] and IHC [11].

Overall, estrogen receptor–α–positive (ER+) breast cancers have a better prognosis than triple-negative breast cancers (TNBCs) that, by definition lack ER, progesterone receptor, and amplification of human epidermal growth factor receptor 2 [12–14]. Our group previously published that Dicer protein was higher in ER+ versus TNBC cell lines and that increasing microRNA 200c (miR-200c) in TNBC cell lines to levels found in ER+ lines resulted in dramatically increased Dicer protein, as well as increased expression of the miRNAs characteristically higher in ER+ cell lines [15].

To validate our findings in clinical specimens of ER+ and TNBC, we evaluated the performance of Dicer antibodies for IHC on formalin-fixed, paraffin-embedded (FFPE) specimens. Dicer monoclonal antibodies 13D6, 4A6 (made from the same immunogen as 13D6) (Abcam, Cambridge, MA), and polyclonal antibodies ab5818 (Abcam) and HPA000694 (Sigma-Aldrich, St Louis, MO) were compared using Western blot (WB) and IHC analyses. We also examined endogenous Dicer, exogenous Dicer from an expression vector, and manipulation of Dicer via restoration of miR-200c. Lastly, we tested ER+ and TNBC clinical specimens for Dicer protein by IHC. Although the antibodies used performed similarly for WB analyses, major differences were observed in cellular staining patterns of Dicer by IHC using the various antibodies, highlighting the necessity for rigorous antibody performance evaluation before drawing definitive conclusions regarding Dicer protein in breast or any other cancer, developmental state, or tissue. Based on results obtained with the highest performing Dicer antibody, we conclude that Dicer is significantly lower in TNBC than in ER+ breast cancer or adjacent noninvolved breast epithelium.

2. Materials and methods

2.1. Human tissues

In ER+ breast cancer, postmenopausal women (n = 25) with newly diagnosed ER+ breast cancer, grade 1 to 3, stage II/III were included in this study. The protocol (01-627) was approved by the Colorado Multiple Institutional Review Board, and informed consent was provided by all patients. In TNBC, patients (n = 21) ranged in age from 19 to 72 years old with a mean age of 47.44 ± 12.03 years. All tumors were grade 3 and negative for ER, progesterone receptor, and human epidermal growth factor receptor 2 (Colorado Multiple Institutional Review Board protocol 04-0066).

2.2. Cell culture

T47D breast cancer cells, which are ER+ and belong to the luminal A subtype, were grown in minimum essential medium, 5% fetal bovine serum (FBS), nonessential amino acids, and insulin. The TNBC cell line BT549 was grown in Roswell Park Memorial Institute medium, 10% FBS, and insulin. HEY ovarian cancer cells were grown in Roswell Park Memorial Institute medium, 10% FBS, and L-glutamine. HEK293FT human embryonic kidney cells were grown in Dulbecco Modified Eagle Medium containing 10% FBS. All cells were maintained at 37°C and 5% carbon dioxide and fingerprinted for authenticity using the Identifiler DNA profiling kit (ABI, Grand Island, NY) at the University of Colorado Cancer Center Sequencing Core Facility.

2.3. miR-200c-inducible cells

BT549 cells were transduced with a doxycycline (DOX)-inducible lentiviral vector (pTRIPz) encoding the precursor sequence for miR-200c (pTRIPz-200c) and stably selected using puromycin. A clone of BT549-TripZ-200c, demonstrating robust expression of miR-200c upon induction with little background, was used in all subsequent experiments. BT549-TripZ-200c cells were plated at a density of 8×10^5 cells per 10-cm dish, and miR-200c expression was induced with 1 μg/mL DOX for 48 hours.

2.4. miR-200c-inducible xenograft tumors

These methods are previously described [16].

2.5. Antibodies

Primary antibodies to Dicer were optimized and used at the following concentrations: mouse monoclonal 13D6 (1:25 for IHC) and 4A6 (made from the same immunogen as 13D6, and selected over 13D6) (1:25 for IHC, and 1:50 for WB), rabbit polyclonal ab5818 (1:50 for IHC and WB) (Abcam, Cambridge, MA) (note: ab5818 is currently sold as PA5-19437 [Thermo Fisher, Grand Island, NY]), and rabbit polyclonal HPA000694 (1:50 for IHC, 1:500 for WB) (Sigma-Aldrich, St. Louis, MO). Additional antibodies used for WB include topoisomerase I (TOPOI) (C-21) (Santa Cruz Biotechnology, Dallas, TX), α-tubulin clone B-5-1-2, and glyceraldehyde-3-phosphate dehydrogenase (Sigma).

2.6. Blocking peptide

A blocking peptide was commercially available for antibody ab5818 (Abcam ab24556). Peptide at 10× the