International Journal of Radiation Oncology biology • physics

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Biology Contribution

Low p53 Binding Protein 1 (53BP1) Expression Is Associated With Increased Local Recurrence in Breast Cancer Patients Treated With Breast-Conserving Surgery and Radiotherapy

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Received Apr 12, 2011, and in revised form Jan 27, 2012. Accepted for publication Jan 31, 2012

Summary

In this study we provide a retrospective analysis of a large cohort treated with breast-conserving surgery and whole-breast irradiation in whom low p53 binding protein 1 (53BP1) expression correlated significantly with worse outcome in all endpoints assessed. Among others, it correlated with worse local outcomes. Additionally, we found 53BP1 expression to correlate with triple-negative phenotype

Purpose: To investigate whether the expression of p53 binding protein 1 (53BP1) has prognostic significance in a cohort of early-stage breast cancer patients treated with breast-conserving surgery and radiotherapy (BCS+RT).

Methods and Materials: A tissue microarray of early-stage breast cancer treated with BCS+RT from a cohort of 514 women was assayed for 53BP1, estrogen receptor, progesterone receptor, and HER2 expression by immunohistochemistry. Through log−rank tests and univariate and multivariate models, the staining profile of each tumor was correlated with clinical endpoints, including ipsilateral breast recurrence−free survival (IBRFS), distant metastasis−free survival (DMFS), cause-specific survival (CSS), recurrence-free survival (RFS), and overall survival (OS).

Results: Of the 477 (93%) evaluable tumors, 63 (13%) were scored as low. Low expression of 53BP1 was associated with worse outcomes for all endpoints studied, including 10-year IBRFS (76.8% vs. 90.5%; $P=.01$), OS (66.4% vs. 81.7%; $P=.02$), CSS (66.0% vs. 87.4%; $P<.01$), DMFS (55.9% vs. 87.0%; $P<.01$), and RFS (45.2% vs. 80.6%; $P<.01$). Multivariate analysis incorporating various clinico-pathologic markers and 53BP1 expression found that 53BP1 expression was again an independent predictor of all endpoints (IBRFS: $P=.0254$; OS: $P=.0094$; CSS: $P=.0033$; DMFS: $P=.0006$; RFS: $P=.0002$). Low 53BP1 expression was also found to correlate with triple-negative (TN) phenotype ($P<.01$). Furthermore, in subset analysis of all TN breast cancer, negative 53BP1 expression trended for lower IBRFS (72.3% vs. 93.9%;

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This research was supported by the Breast Cancer Research Foundation (B.G.H.) and the Radiological Society of North America (H.J.R.N.).

Presented in part at the 93rd Annual Meeting of the Radium Society, 2011, and the American Society for Radiation Oncology 3B Research Forum, 2011.

Conflict of interest: none.

Supplementary material for this article can be found at www.redjournal.org.

Int J Radiation Oncol Biol Phys, Vol. 83, No. 5, pp. e677−e683, 2012
0360-3016/$ - see front matter
doi:10.1016/j.ijrobp.2012.01.089

and furthermore to predict worse outcomes among patients with triple-negative tumors.

$P = .0361$) and was significant for worse DMFS (48.2% vs. 86.8%; $P = .0035$) and RFS (37.8% vs. 83.7%; $P = .0014$).

Conclusion: Our data indicate that low 53BP1 expression is an independent prognostic indicator for local relapse among other endpoints in early-stage breast cancer and TN breast cancer patients treated with BCS+RT. These results should be verified in larger cohorts of patients to validate their clinical significance. © 2012 Elsevier Inc.

Keywords: p53 binding protein 1 (53BP1), Radiotherapy, Breast cancer, Molecular markers, Breast-conserving surgery

Introduction

The DNA damage response pathway plays a key role in determining how individual cancers respond to radiation and chemotherapy. Defects in specific DNA repair pathways play a key role in the pathogenesis of some subsets of breast cancer that can be exploited therapeutically (1). For example, BRCA1-deficient cancers display a defect in homology-mediated DNA repair that renders them exquisitely sensitive to cross-linking agents such as cis-platinum and poly (ADP-ribose) polymerase (PARP) inhibitors (2-5). The breast cancers that arise in BRCA1 mutation carriers are mostly high grade with a "triple-negative" (estrogen receptor [ER]−, progesterone receptor [PR]−, HER2−) phenotype and profile as basal-like breast cancers (6, 7). This has led to the hypothesis that a subset of sporadic triple-negative breast cancers (TNBC) may also harbor defects in the BRCA1-associated repair pathway and thus may also share a similar sensitivity to cross-linking agents and PARP inhibitors (8, 9).

P53 binding protein 1 (53BP1) is a protein involved in DNA-damage checkpoint activation and DNA repair that is involved in both nonhomologous end-joining (NHEJ) and homology-mediated repair of double-strand DNA breaks (10-13). It is postulated to transmit DNA damage signals from sensor proteins to transducer proteins in the DNA damage response pathway (10). Recent studies demonstrate that the defect in homology-mediated DNA repair seen in BRCA1-deficient cells can be alleviated by concomitant loss of 53BP1 expression (14-16). This loss of 53BP1 leads to resistance to cis-platinum and PARP inhibitors in BRCA1-deficient cells, suggesting that 53BP1 is a critical modulator of repair choice in BRCA1 mutant cells (14, 15, 17). An analysis of two independent breast cancer cohorts demonstrated that a subset of both BRCA1-associated breast cancer and TNBC had lost 53BP1 protein expression (14). Triple-negative breast cancers with decreased 53BP1 expression have worse distant metastasis−free survival (DMFS) than TNBC with intact 53BP1 expression. This suggests that 53BP1 expression may have prognostic significance in breast cancer.

The purpose of this study was to determine the clinical significance of 53BP1 expression for local outcome, among other endpoints, in a cohort of women with early-stage breast cancer treated with breast-conserving surgery and radiotherapy (BCS+RT).

Methods and Materials

Tissue microarray and patient characteristics

The protocol was reviewed and approved by the Human Investigations Committee. Patients selected for the study were treated between 1970 and 2005. Breast cancer tissue cores were stored in paraffin and subsequently compiled into a tissue microarray in 2009. Of the more than 2000 patients treated, only the patients for whom paraffin-embedded tissue was available in the tissue archives of Yale hospital were included for analysis. Of this group of 514 patients, 477 had evaluable tumor cores for staining of 53BP1. Information about the patients' clinical history was obtained from the patients' database, as previously described (18). All patients had histologic evidence of invasive breast carcinoma with early-stage (I/II) disease and were treated with BCS+RT. After surgery, patients received standard whole-breast irradiation to a total median dose to the breast of 48 Gy and a total tumor bed dose of 64 Gy; regional nodes were treated to a median dose of 46 Gy, as clinically indicated (19). Adjuvant systemic chemotherapy and hormone therapy were administered as clinically indicated in accordance with standard practices during this time interval.

Ipsilateral breast recurrence−free survival (IBRFS) was defined as clinically and biopsy-proven relapse in the ipsilateral breast; distant metastases were defined as clinical evidence of distant disease according to clinical and/or radiographic evidence. Overall survival (OS) was defined as the time from the initial diagnosis to time of death due to any cause, and cause-specific survival (CSS) as the time from the initial diagnosis to death with disease. Ipsilateral breast recurrence−free survival was defined as the time from the initial diagnosis to ipsilateral breast tumor relapse; DMFS was defined as the time from the initial diagnosis to the time of distant relapsed; and relapse-free survival (RFS) was defined as the time from the initial diagnosis to any local, regional, or distant relapse.

Immunohistochemical study

Immunohistochemical analysis was performed on 5-μm-thick tissue sections prepared from formalin-fixed, paraffin-embedded tissue from the constructed tissue microarray block. Tissue sections were deparaffinized and then quenched in 2% hydrogen peroxide−methanol solution. Samples were then pretreated to promote antigen retrieval with the DAKO Target Retrieval Solution (DAKO, Carpinteria, CA). Slides were then incubated with 53BP1 antibody (#IHC-00001, Bethyl Laboratories, Montgomery, TX). The antibody was diluted at 1:500 and 1:250. One slide of the array was stained with antibody at 1:500 dilution and another slide at 1:250 dilution. After incubation, slides were washed in phosphate-buffered saline, and a biotinylated secondary antibody was applied. Samples were applied with DAKO streptavidin−horseradish peroxidase. DAKO DAB (3,3-diaminobenzidine tetrahydrochloride dehydrate) was applied as a chromogenic substrate. A known positive case was included as positive control. For the negative control, the primary antibody was replaced with