Loss of Retinoblastoma (RB) Tumor Suppressor Expression in Breast Cancer Correlates with Better Response to Neoadjuvant Chemotherapy

Jeanne M McFalls MD[1], Jessica Kline BA[1], Gordon F Schwartz MD, MBA, FACS[2] and Agnieszka K Witkiewicz MD[1]

[1]Department of Pathology; [2]Department of Surgery, Thomas Jefferson University, Philadelphia, PA

BACKGROUND:

Neoadjuvant chemotherapy followed by surgery is standard of care for locally advanced breast cancer. However, breast cancers show a wide variation in response to neoadjuvant chemotherapy, with some achieving pathological complete response while others progress unabated. The aim of this study was to investigate whether expression of the retinoblastoma tumor suppressor (RB), p16, estrogen receptor (ER), progesterone receptor (PR), and HER2 in pre-treatment breast cancers predicts the response to neoadjuvant chemotherapy.

DESIGN:

We retrospectively reviewed the medical records and pathology of 131 patients with breast cancer who were treated with neoadjuvant chemotherapy followed by surgical excision at our institution from 1982 to 2011. Patients for whom no tissue was available prior to neoadjuvant treatment were excluded (33 patients). Overall demographic and clinicopathologic features of this cohort are summarized in Table 1. Positivity for ER, PR, and Her2 were determined by immunohistochemistry on formalin-fixed, paraffin-embedded tissue sections from pretreatment biopsies. RB and p16 staining was performed using monoclonal antibodies against antigens retrieved from deparaffinized pre-treatment tissue sections. P16 staining was graded as 0 (no cells staining), 1+ (weak nuclear or cytoplasmic blush or <25% of cells showing strong staining), 2+ (25 to 75% of cells strongly staining), or 3+ (>75% of cells strongly staining). For RB, only nuclear staining was considered. Cases were considered negative for RB when no neoplastic nuclei stained but there was staining in surrounding stromal and endothelial cells. Medical records were reviewed for pre-treatment tumor size and stage. Post-surgical excision pathology slides were reviewed and response to neoadjuvant therapy was assessed by pathological complete response (pCR), modified Miller-Payne score, and clinical-pathologic staging (CPS) systems. Maximum one-dimensional tumor sizes were used as proxy for cellularity to determine the modified Miller-Payne score, where grade 1 represented no reduction of tumor size, grade 2 represented a less than 30% decrease in tumor size, grade 3 represented a 30-90% reduction in tumor size, grade 4 represented a greater than 90% reduction in tumor size, and grade 5 represented no residual tumor. The CPS system is a composite score based on the patient's pre-treatment clinical stage, post-treatment pathologic stage, estrogen receptor status, and nuclear grade.

Table 1: Clinical and pathological features of neoadjuvant cohort

Factor	No.	%
Age, years		
Median	55	
Range	17-90	
Clinical tumor size, cm		
Median	4.0	
Mean	4.3	
Clinical stage at presentation		
I	1	1
IIA	25	26
IIB	22	22
IIIA	20	20
IIIB	29	30
IIIC	1	1
Pathologic tumor size, cm		
Median	0.9	
Mean	1.2	
Range	0-6	
Pathologic stage		
0	20	20
I	31	31
IIA	19	19
IIB	9	9
IIIA	12	12
IIIB	0	0
IIIC	7	7
ER status		
Positive	30	30
Negative	68	69
PR status		
Positive	48	49
Negative	50	51
HER2 status		
Positive	21	21
Negative	50	51
Indeterminate	10	10
Unknown	17	17
Nuclear grade		
1	11	11
2	44	45
3	40	41
Unknown	3	3
Neoadjuvant therapy		
AC	14	14
ACT	14	14
AT	8	8
CMF	34	35
FAC	7	7
CT	5	5
Other	9	9
Unknown	5	5

RESULTS:

There is a well-established reciprocal relationship between RB and p16, and the majority of cases that exhibited loss of RB were strongly positive for p16 (Figure 1). Of the 98 cases evaluated, 27 were characterized by RB loss. The rate of pathologic complete response (pCR) among these cases was 40.7% (Figure 2A, left panel). In contrast, in the remaining 71 cases that were clearly RB positive the pathological response rate was 12.8% (Figure 2A, left panel). Additionally, loss of RB significantly predicted an improved response to neoadjuvant chemotherapy as measured by modified Miller-Payne score ($p = 0.0004$) and CPS score ($p = 0.0015$) (Figure 2B & C). RB loss was seen in 16% of ER positive and 50% of ER negative breast cancers. The association of RB loss with pathological complete response to neoadjuvant chemotherapy held in both ER-positive and ER-negative cases, as well as across various neoadjuvant chemotherapy regimens (5-fluorouracil/Adriamycin/cytoxan (FAC), Taxane/Adriamycin (TA), and Taxane/5-Fluorouracil/Adriamycin/Cytoxan (TFAC)) (data not shown).

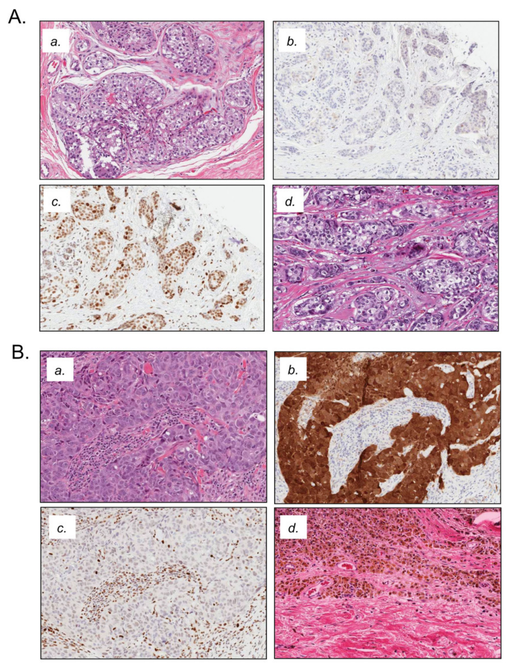

Figure 1: Representative non-responder (A) and good responder (B). Pre-treatment biopsy H&E (a), pre-treatment biopsy p16 (b), pre-treatment RB (c), post-treatment surgical excision H&E (d).

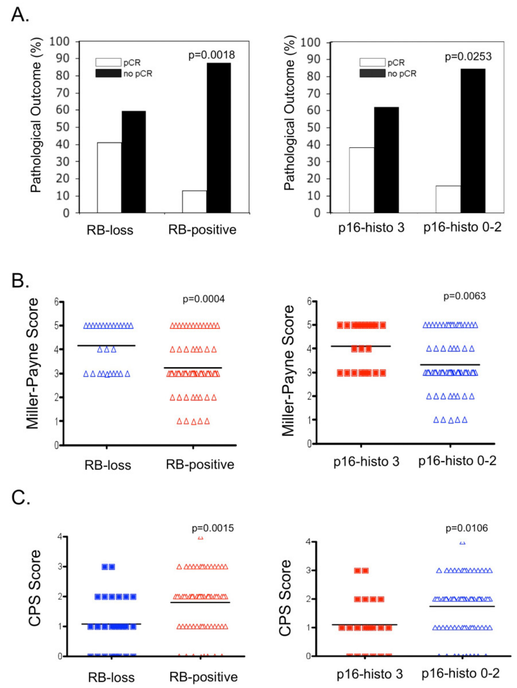

Figure 2: Association of (A) pathological complete remission (pCR), (B) modified Miller-Payne score, and (C) CPS score with RB and p16 status.

CONCLUSION:

Loss of RB tumor suppressor staining in pre-treatment breast cancer biopsies can be used prior to initiation of neoadjuvant chemotherapy to predict good tumor response. This association between RB loss and good neoadjuvant response holds regardless of ER status and across various chemotherapy regimens. Assessment of RB status in the pre-treatment biopsy could be a useful clinical tool to define patients who are most likely to benefit from neoadjuvant chemotherapy.