Null Results in Brief

No Increased Ki67 Expression in Ductal Carcinoma *in Situ* Associated with Invasive Breast Cancer[1]

Ashraful Hoque, David G. Menter, Aysegul A. Sahin, Nour Sneige, and Scott M. Lippman[2]

Departments of Clinical Cancer Prevention [A. H., D. G. M., S. M. L.] and Pathology [A. A. S., N. S.], The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

Introduction

Several studies indicate that ∼50% of patients with positive biopsies for DCIS[3] of the breast may develop invasive breast cancer within 1–24 years of diagnosis (1). The biological mechanisms involved in the progression of DCIS to invasive cancer are not fully understood. Although Ki67 labeling indices increased in invasive breast cancer (2, 3), there are limited data on this marker in DCIS. Recently, Imamura *et al.* (4) reported that Ki67-associated proliferative activity of intraductal components (indicated by MIB1, an antibody that recognizes Ki67) is a significant prognostic determinant of disease-free survival in invasive ductal breast carcinoma. Allred *et al.* (5) found a differential expression of growth factor receptor HER-2/*neu* in DCIS with invasive disease *versus* DCIS without. On the basis of the above data, we conducted a case-control study of Ki67 in DCIS, with or without invasive breast cancer, testing the hypothesis that DCIS associated with invasive cancer would have a higher Ki67 expression than would DCIS alone.

Materials and Methods

Paraffin-embedded archival breast tissue specimens and surgical pathology reports of 100 consecutively examined controls (pure DCIS) and 100 consecutively examined cases (DCIS with invasive component) were identified from the pathology database of The University of Texas M. D. Anderson Cancer Center during the period of 1986–1999. H&E-stained slides of each DCIS case were reviewed and graded by a pathologist (A. A. S.) using standard grading criteria, as described previously (6). Cases and controls were frequency-matched according to nuclear grade. Sociodemographic characteristics and clinical variables were abstracted from patients' medical records. We determined Ki67 labeling indices by staining for KiS5 (Dako, Carpinteria, CA) binding

to Ki67. KiS5 is an antibody similar to MIB1 (4) in its ability to recognize Ki67 protein. On each slide, two or three fields with the strongest Ki67 staining were digitized (at ×200) via computerized image analysis techniques and quantified to determine the Ki67 labeling index of the lesion (*i.e.,* the mean percentage of stained area within the two or three fields). Student's t test was used to compare mean Ki67 indices of cases and controls. The χ^2 test was used to compare proportions of Ki67 scores among DCIS lesions graded 1, 2, or 3. Odds ratios and 95% confidence intervals were computed using logistic regression models. A probability value <0.05 was considered statistically significant. The sample size of 100 cases and 100 matched controls gave the study an 80% power to detect an odds ratio of 2.22 of increased Ki67 expression ($\alpha = 0.05$, two-sided test).

Results

The mean Ki67 labeling indices of DCIS associated with invasive breast cancer and DCIS alone were similar (Fig. 1), and the odds ratio for Ki67 was 0.92 (95% confidence interval, 0.79–1.06), which remained unaltered after adjusting for necrosis. We did find a correlation, however, between Ki67 labeling indices and nuclear grades among both cases and controls; higher nuclear grade correlated with higher Ki67 labeling index. This trend was stronger for DCIS alone ($P < 0.0001$) than for DCIS associated with invasive cancer ($P = 0.08$).

Discussion

We did not observe a statistically significant difference in Ki67 labeling indices between DCIS associated with invasive breast cancer and DCIS alone. In 157 specimens of invasive breast cancer with a DCIS component, Imamura *et al.* (4) found higher median MIB1 labeling indices (indicative of higher Ki67 expression) in invasive foci than in intraductal components. To extend this finding, we had hypothesized that DCIS associated with invasive cancer would have a higher Ki67 expression than would DCIS alone. A unique aspect of our study was in restricting Ki67 evaluations to only the DCIS component of cases or controls.

De Potter *et al.* (7) suggested that increased cell proliferation and enhanced cell motility associated with overexpressed c-erbB-2 may contribute to the development and progression of DCIS. As with our Ki67 findings, Allred *et al.* (5) reported that the incidence of HER-2/*neu* expression is lower in DCIS with invasive breast cancer than in DCIS alone. These findings suggest that the expression of c-*erb*B-2 and/or Ki67 may be down-regulated in a significant proportion of DCIS lesions as they progress to invasive cancer or that a subset of invasive breast cancers may develop *de novo* by mechanisms independent of c-*erb*B-2 and/or Ki67.
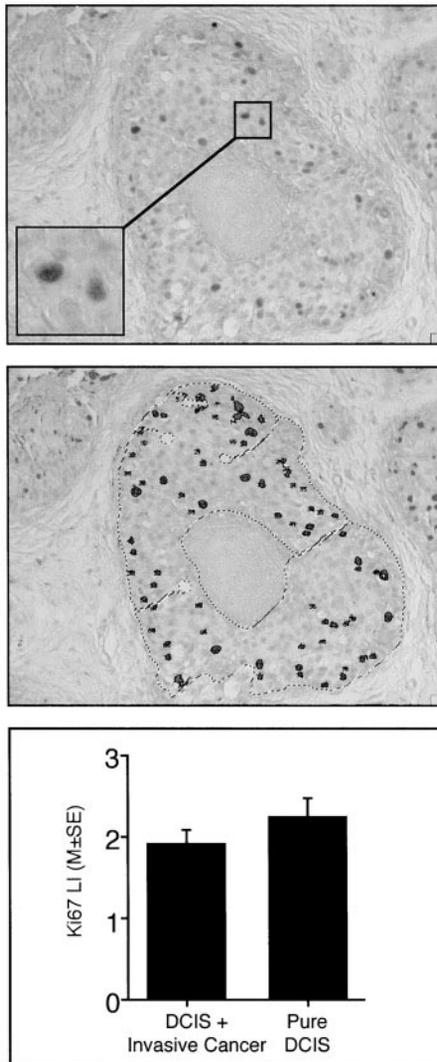
Fig. 1. An example of immunostaining (via DAB enzymatic signal enhancement) of Ki67 in a DCIS sample (at ×200). Ki67 staining was observed as a *black* signal on a *gray* background (*top panel*). A single DCIS-containing acinus within this sample was outlined with a digital stylus (represented by the *dotted lines*) to exclude noncellular material (*middle panel*). Segmentation of the nuclei (*small outlined areas within the acinus*) was performed and quantified, followed by the numbering of each nucleus (*middle panel*). *Columns*, means (*M*) of Ki67 labeling indices in DCIS alone (2.26 ± 0.22) and DCIS associated with invasive breast cancer (1.91 ± 0.18; *P* = 0.22; *bottom panel*); *bars*, SE.

Two limitations of our study involved the statistical or biomarker study design. The sample size of our study did not provide the power to detect Ki67 odds ratios <2.22 (see "Materials and Methods" above). Although cases and controls were matched by nuclear grade and adjusted for necrosis, we did not assess and so could not adjust for other potentially important cellular/molecular prognostic markers (1–3, 5, 8, 9). Ongoing studies will help further define the role of Ki67 in DCIS (8, 9), including its associations with c-*erb*B-2 and other cellular/molecular markers.

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