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ARTICLE

Biomarker Expression and Risk of Subsequent Tumors After Initial Ductal Carcinoma In Situ Diagnosis

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Background Studies have failed to identify characteristics of women who have been diagnosed with ductal carcinoma in situ (DCIS) and have a high or low risk of subsequent invasive cancer.

Methods We conducted a nested case–control study in a population-based cohort of 1162 women who were diagnosed with DCIS and treated by lumpectomy alone from 1983 to 1994. We collected clinical characteristics and information on subsequent tumors, defined as invasive breast cancer or DCIS diagnosed in the ipsilateral breast containing the initial DCIS lesion or at a regional or distant site greater than 6 months after initial treatment of DCIS (N = 324). We also conducted standardized pathology reviews and immunohistochemical staining for the estrogen receptor (ER), progesterone receptor, Ki67 antigen, p53, p16, epidermal growth factor receptor-2 (ERBB2, HER2/neu oncoprotein), and cyclooxygenase-2 (COX-2) on the initial paraffin-embedded DCIS tissue. Competing risk models were used to determine factors associated with risk of subsequent invasive cancer vs DCIS, and cumulative incidence survival functions were used to estimate 8-year risk.

Results Factors associated with subsequent invasive cancer differed from those associated with subsequent DCIS. Eight-year risk of subsequent invasive cancer was statistically significantly (P = .018) higher for women with initial DCIS lesions that were detected by palpation or that were p16, COX-2, and Ki67 triple positive ($p16^+COX\text{-}2^+Ki67^+$) (19.6%, 95% confidence interval [CI] = 18.0% to 21.3%) than for women with initial lesions that were detected by mammography and were p16, COX-2, and Ki67 triple negative ($p16^-COX\text{-}2^-Ki67^-$) (4.1%, 95% CI = 3.4% to 5.0%). In a multivariable model, DCIS lesions that were $p16^+COX\text{-}2^+Ki67^+$ or those detected by palpation were statistically significantly associated with subsequent invasive cancer, but nuclear grade was not. Eight-year risk of subsequent DCIS was highest for women with DCIS lesions that had disease-free margins of 1 mm or greater combined with either $ER^-ERBB2^+Ki67^+$ or $p16^+COX\text{-}2^-Ki67^+$ status (23.6%, 95% CI = 18.1% to 34.0%).

Conclusion Biomarkers can identify which women who were initially diagnosed with DCIS are at high or low risk of subsequent invasive cancer, whereas histopathology information cannot.

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Ductal carcinoma in situ (DCIS) has become a relatively common diagnosis (1,2), yet the clinical and biological significance of DCIS lesions is not fully understood. It appears that 5%–10% of women diagnosed with DCIS who are treated by lumpectomy alone develop a subsequent invasive cancer within 5 years, and a similar proportion develops a subsequent DCIS lesion (3–7). Adjuvant radiation and tamoxifen have been shown to decrease the rate of subsequent tumors (3,8,9), but not to influence breast cancer mortality (4–6,10–12).

Clinical trials and population-based studies have failed to consistently identify which women will be at high vs low risk of subsequent invasive cancer among those diagnosed with DCIS (10,13), thereby creating a dilemma for physicians in choosing the intensity of their treatment (14). Identification of biomarkers that can accurately predict subsequent invasive cancer and/or DCIS could aid in stratifying an individual's risk for subsequent tumors. A few studies have examined biomarkers including the estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 oncoprotein (HER2/neu, also known as ERBB2), human epidermal growth factor receptor-4 oncoprotein (HER4/neu), Ki67, and cyclooxygenase-2 (COX-2) as predictors of subsequent tumors in women diagnosed with DCIS, but the results have been inconsistent (15–20). These studies were based primarily on follow-up of nonpopulation-based case series of women in whom DCIS had been managed with a variety of treatment modalities, making it difficult to know whether the results

CONTEXT AND CAVEATS

Prior knowledge

Current biomarkers have been inadequate to distinguish which women with a diagnosis of ductal carcinoma in situ (DCIS) have a high risk of subsequent invasive breast cancer.

Study design

Clinical data and breast tissue specimens were collected from 1162 surgically treated DCIS patients from 1983 to 1994, and DCIS tissue was subjected to immunohistochemical staining. Women whose DCIS tissue expressed various combinations of biomarkers were compared for 5- and 8-year risks of invasive cancer or subsequent DCIS.

Contribution

Eight-year risk of invasive cancer was highest among women whose DCIS lesions were detectable by palpation or were p16, cyclooxygenase-2, and Ki67 positive. Eight-year risk of further DCIS was highest among women with different specific biomarker combinations.

Implications

These biomarker combinations may be useful to women and their physicians as prognostic indicators.

Limitations

All data were collected retrospectively, subject to tissue availability. Patients had been treated by lumpectomy alone, so biomarker combinations could not be used to predict responsiveness to adjuvant therapies.

From the Editors

were a function of biomarkers or treatment or both. In addition, most of the studies were small, conducted at a single institution, had short length of follow-up, tested only individual markers, and did not stratify by type of subsequent tumor. These study design restrictions limit the ability of published results to be generalized.

The primary purpose of this study was to identify clinical, histopathologic, and molecular characteristics of initial DCIS lesions that are associated with subsequent invasive cancer or DCIS. We studied a large population-based cohort of women with DCIS who were treated by lumpectomy alone to determine risk of subsequent disease as a function of these factors.

Subjects and Methods

Subjects

The study sample and methods have been previously described (5). In brief, we used data from the Surveillance, Epidemiology, and End Results (SEER) program of Northern California to identify women who were aged 40 years or older when diagnosed with DCIS and who were treated by lumpectomy alone in one of the nine San Francisco Bay Area counties from January 1, 1983, to December 31, 1994. From an initial pool of 1568 women, we excluded 142 women who had DCIS treated by mastectomy or by lumpectomy plus radiation within 6 months of the initial diagnosis, 19 women who had a prior diagnosis of breast cancer, 18 women who died within 6 months of the initial diagnosis, 37 women whose initial DCIS lesion was found to have invasive cancer on standardized pathology review, and 20 women whose DCIS diagnosis could not be confirmed. Of the 1332 eligible participants, 29 women could not be located; 18 women did not speak fluent English, Cantonese, Spanish, or Russian (the languages we used to conduct the telephone interviews); 118 women refused to participate; and five women had a doctor's request not to be contacted. The study cohort consisted of 1162 women with an overall participation rate of 87%. This study was reviewed and approved by the University of California, San Francisco Committee on Human Research. Study participants provided verbal and/or written informed consent.

Telephone Interviews and Vital Status

We obtained demographic information and a breast health history from each woman during a telephone interview on average 7.5 years after initial diagnosis, as previously described (5). In brief, the interview included questions about breast procedures a woman had undergone, family history of breast cancer, detection method at diagnosis, and menopausal status. To obtain information for 206 women who were either deceased or not able to participate in an interview because of illness, we interviewed a proxy and/or conducted medical record review. We obtained data regarding vital status and underlying cause of death including breast cancer as of December 31, 2005, from the California Department of Vital Statistics and/or death certificates.

Standardized Pathology Review for Nested Case–Control Study

Paraffin-embedded tissue samples and/or hematoxylin- and eosin-stained slides of initial DCIS tissue from women who had subsequent tumors (case subjects) and women with DCIS who did not have subsequent tumors (control subjects) were retrieved from pathology laboratories. Control subjects were randomly selected and frequency matched to case subjects by year of diagnosis before retrieval of their DCIS tissue. We could not obtain paraffin-embedded tissue blocks from some hospitals that had discarded the tissues, had insufficient staff to collect the tissues, and/or refused to provide tissue for research (80 case subjects and 93 control subjects). Subsequent tumors were defined as DCIS or invasive breast cancer that was diagnosed in the ipsilateral breast (that had contained the initial DCIS lesion) or at a regional or distant site (bone, brain, liver, lung, and skin) more than 6 months after the initial diagnosis and treatment of DCIS. Women who had both DCIS and invasive cancer in subsequent tissue samples were categorized as having a subsequent invasive cancer. To classify a woman as having had a subsequent tumor event as defined above, we investigated the nature of all breast procedures reported by women during the telephone interview by obtaining and reviewing pathology reports for breast biopsies performed after the initial diagnosis and linking to the Northern California SEER program in 2002 and 2008. Pathology reports were available on 94% of breast biopsies performed after the initial diagnosis. Women who developed only contralateral breast cancer during the study period were included in the study as control subjects.

As previously described (5), study pathologists blinded to the clinical outcome reviewed the slides stained with hematoxylin and

eosin (N = 502) of the original DCIS lesions from 114 women who had a subsequent invasive cancer event, 109 women who had a subsequent DCIS event, and 279 control subjects who did not have a subsequent tumor event to verify initial diagnoses of DCIS; to verify diagnoses of subsequent disease; and to determine nuclear grade, type, and quantity of necrosis, tumor size, and margin width of the initial DCIS diagnosis. Our pathologists established at least 80% agreement on identification of histopathologic characteristics in a training set of women with DCIS before reviewing study case and control subjects. Disagreements were resolved by consensus.

Measurement of Biomarkers for Nested Case–Control Study

We used immunohistochemical staining to identify DCIS phenotypes using slides from formalin-fixed paraffin-embedded tumor blocks (N = 329) from 72 women who had a subsequent invasive cancer event, 71 women who had a subsequent DCIS event, and 186 control subjects who did not have a subsequent tumor event. We scored the index lesion for the presence of the following proteins using the indicated mouse monoclonal antibodies: for ER using a 1:400 dilution of antibody 1D5 (DAKO, Carpinteria, CA), for PR using a 1:25 dilution of antibody 1A6 (Novocastra, Bannockburn, IL), for Ki67 antigen (MKI67 [FHA domain] interacting nucleolar phosphoprotein) using a 1:100 dilution of antibody MIB-1 (DAKO), for p53 (TP53) using a 1:200 dilution of antibody PAb 1801 (Neomarkers, Fremont, CA), for human epidermal growth factor receptor-2 (ERBB2) using a 1:200 dilution of antibody TAB250 (Invitrogen, Grand Island, NY), for COX-2 using a 1:200 dilution of antibody M3617 (DAKO), and for p16 (cyclin-dependent kinase inhibitor 2A) using a 1:200 dilution of antibody MS218 (Neomarkers) (21,22). Staining with primary antibodies was followed by staining with biotinylated labeled secondary antibodies and detection with an avidin–biotin–horseradish peroxidase system. Specimens were counterstained with hematoxylin. Positive and negative control tissues were used for assessment of each marker as follows: ER, breast tumor case and cell line MCF-7; PR, breast tumor case and cell line T47D; Ki67, breast tumor case; p53, colon tumor case and cell line T47D; ERBB2, breast tumor case and cell line SKBR3; COX-2, a DCIS case; and p16, normal breast tissue and colon case.

One investigator (J . Bennington) scored ER, PR, ERBB2, and p53 stains, and two investigators (M. L. Gauthier and H. K. Berman) scored p16, COX-2, and Ki67 stains; all were blinded to clinical outcomes. For p53, ERBB2, ER, and PR, the percentage of tumor cells that showed staining of any intensity was estimated and recorded. The marker p53 was considered to be overexpressed, and ER and PR were considered to be present when 10% or more tumor cells showed staining. Similarly, ERBB2 was considered to be overexpressed when 10% or more tumor cells showed moderate or strong membrane staining (+2 or higher); these were criteria previously used for scoring DCIS lesions for ERBB2 (23).

Using a condensed Allred score (24), COX-2 staining was evaluated on a scale of 0, 1, 2, or 3, with each value corresponding to a combination of Allred classes (0 = Allred class 0; 1 = Allred classes 2, 3, and 4; 2 = 5 and 6; 3 = 7 and 8; see Supplementary Material, available online). Scoring of p16 was evaluated on a scale of 0, 1, 2, or 3 based on the percentage of positively staining tumor cells, irrespective of staining intensity (0 = no staining, 1 = fewer than 25% of cells stained, 2 = 25%–75%, 3 = more than 75% of cells stained) (22). Tissues with a score of at least 2 were considered to overexpress COX-2 or p16. For Ki67 scoring, a minimum of 1000 tumor cells were counted from at least three high-powered (×40) fields in areas that showed the highest labeling. The labeling index was expressed as a percentage and was calculated as the number of positive cells divided by the number of positive plus negative cells. Tissues were considered to have high Ki67 expression if more than 10% of tumor cells were stained, which was more than the median value for all tumors evaluated. In a random sample of 45 specimens, a comparison of two independent scorers of select immunohistochemical assays yielded a *k* statistic of 0.93 and concordance of 98% for p16, a *k* statistic of 0.73 and concordance of 87% for COX-2, and a *k* statistic of 0.82 and concordance of 91% for Ki67 (see Supplementary Material, available online, for representative staining).

Statistical Analysis

We used Cox proportional hazards models to determine univariate and multivariable hazard ratios (HR) for various clinical and histopathologic characteristics and biomarkers among women in the cohort who had a subsequent tumor compared with women who did not. We examined combinations of biomarkers that were found as individual markers in univariate analyses to be statistically significantly associated with invasive cancer and/or DCIS or were previously shown to have a biological basis for association with subsequent tumors after a DCIS diagnosis (22) or were previously reported to be associated with breast cancer survival (25). For inclusion in the multivariable models, we considered individual and combinations of factors that were statistically significantly associated with invasive cancer and/or DCIS in univariate analyses. For multivariable models, margin width was considered as an ordinal variable (ordered as ≥10 mm, 2 to <10 mm, 1 to 1.9 mm, uncertain, and positive). The validity of the proportional hazards assumption was verified by log-cumulative hazard plots and, where appropriate, inclusion of a time-dependent variable. Subsequent invasive cancer, DCIS, and death from causes other than breast cancer were competing events. To calculate the appropriate hazard ratio, we used the competing risk package cmprsk in R (http://cran.rproject.org/doc/packages/cmprsk.pdf) to estimate coefficients in the "proportional subdistribution hazards" regression model described by Fine and Gray (26). This model can be used to directly assess the effect of covariates on the subdistribution of a particular type of outcome, in this case invasive cancer or DCIS, in a competing risk setting. In a sensitivity analysis, we excluded women who developed contralateral breast cancer and results were very similar to the results we present from the final models.

To estimate the risk of subsequent tumor events (invasive cancer or DCIS), we generated a standard Kaplan–Meier survival curve. To estimate the 5- and 8-year probability of subsequent tumor events for the population-based cohort by histopathologic characteristics and biomarkers that were collected only for case and control subjects, the results of the case–control study were

converted to survival curves. To do so, we imputed histopathologic characteristics and biomarker measurements for those women in the cohort who were not included in the nested case–control study. The imputed values were based on the observed prevalence of the individual histopathologic and biomarkers in the nested study stratified by case and control subject status as well as by the type of subsequent tumor event as previously described (5). To estimate the risk of subsequent invasive cancer with a DCIS event and death from causes other than breast cancer (N = 125) as competing risks, and to estimate risk of subsequent DCIS with an invasive cancer event and death from causes other than breast cancer as competing risks, we used the code from Pepe and Mori (27) to estimate the marginal distribution, that is, the cumulative incidence function. This process was repeated 2500 times, each time generating a new imputed value for each woman for whom we had missing data for a marker of interest. For each time point *t*, the 2500 Kaplan–Meier or cumulative incidence function survival estimates were averaged and the 95% confidence interval (CI) was reported as the 0.025 and 0.975 quantiles of those survival estimates.

Four risk groups (ie, lowest, low, intermediate, and high risk) were defined separately for subsequent invasive cancer and DCIS based on statistically significant univariate and multivariable associations as well as level of risk associated with clinical and histopathologic characteristics and molecular markers and subsequent invasive cancer or DCIS. Groups were defined by combining clinical and histopathologic characteristics and molecular markers that have similar strength associations and level of risk for subsequent tumor events.

All statistical tests were two-sided. *P* values less than .05 were considered statistically significant.

Results

From January 1, 1983, to May 1, 2008, 324 of the 1162 women in the study cohort (27.9% overall or 3% per year) developed a subsequent breast tumor (median follow-up = 98.0 months or 8.2 years [range = 6.3 to 299.1 months or 0.5 to 25 years]). Of the 1162 women, 154 (13.3%) had subsequent local DCIS lesions, 170 had subsequent invasive cancer (of these, 120 [10.3%] had local disease, 33 [2.8%] regional disease, eight [0.7%] distant disease, and nine [0.7%] disease of unknown location), and 125 (10.8%) died of a cause other than breast cancer. Among the women who had subsequent invasive cancer, 34 (2.9%) died of breast cancer. The 8-year risk of subsequent invasive cancer was similar to the 8-year risk of subsequent DCIS (11.1% vs 11.6%, respectively).

Univariate Results of Factors Associated With Subsequent Invasive Cancer vs DCIS

The risk of subsequent invasive cancer was increased among women whose initial DCIS was detected by palpation compared with that for women whose DCIS was detected by mammography (HR = 2.0, 95% CI = 1.3 to 2.9). The proportional incidence of DCIS by mode of detection did not vary by year of diagnosis (data not shown). We observed that risk of subsequent DCIS varied by age: There was increased risk for women aged 40–49 years compared with women aged 70 years and older (HR = 2.2, 95% CI = 1.4 to 3.4). Race and/or ethnicity, family history of breast cancer, and menopausal status were not associated with incidence of subsequent tumors (Table 1), and neither was oral contraceptive or postmenopausal hormone therapy use or body mass index (data not shown).

Whereas histopathologic characteristics were not associated with subsequent invasive cancer (Table 2), several such characteristics were associated with an increased risk of subsequent DCIS: initial DCIS lesions that were larger than 10 mm, had positive or uncertain margins, were of high nuclear grade, or had extensive necrosis (Table 2).

Whereas DCIS lesions with individual expression of the biomarkers ER, PR, p53, ERBB2, and COX-2 were not statistically significantly associated with subsequent invasive cancer, p16 and selected combinations of markers did provide stratification of risk (Table 3). Women whose initial DCIS lesions were p16 positive ($p16^+$) or p16 and Ki67 positive ($p16^+Ki67^+$) or p16, COX-2, and Ki67 positive ($p16^+COX\text{-}2^+Ki67^+$) had an increased risk of subsequent invasive cancer compared with women whose DCIS lesions did not express these combinations of markers (Table 3). Of note, Ki67 in combination with ER, PR, p53, or ERBB2 was not associated with subsequent invasive cancer nor was p16 in combination with ER, PR, p53, or ERBB2 (data not shown).

Markers associated with subsequent DCIS differed from those associated with subsequent invasive cancer. Women whose initial DCIS lesions were ER negative (ER^-), ERBB2 positive ($ERBB2^+$), or Ki67 positive ($Ki67^+$) among individual markers, or were ER^-ERBB2^+ or ER^-Ki67^+ among marker combinations, had an increased risk of subsequent DCIS compared with women who had lesions that did not express these individual markers or combinations of markers. Subsequent DCIS also was associated with initial DCIS lesions that were $p16^+Ki67^+$ or $p16^+COX\text{-}2^-Ki67^+$.

Distributions by tumor size, margin status, and nuclear grade according to case–control status were similar for women for whom we could obtain tumor blocks and those we could not (data not shown).

Multivariable Results of Factors Associated With Subsequent Invasive Cancer vs DCIS and Risk of Subsequent Tumors by These Factors

In a multivariable competing risk model, we found both DCIS lesions that were detected by palpation and those that were $p16^+COX\text{-}2^+Ki67^+$ were statistically significantly associated with subsequent invasive cancer, whereas nuclear grade was not (Table 4). When we examined the subgroup of women whose initial DCIS was detected by mammography, the independent association of $p16^+COX\text{-}2^+Ki67^+$ lesions with subsequent invasive cancer remained (HR = 2.3, 95% CI = 1.0 to 5.3). Among DCIS lesions associated with a subsequent invasive cancer, 25% were detected by palpation and 23% were $p16^+COX\text{-}2^+Ki67^+$; only two case subjects had both these traits. The 5- and 8-year risks of subsequent invasive cancer were high for women whose initial DCIS lesions were detected by palpation (13.2% and 17.8%, respectively; Table 5) and highest for women whose initial DCIS lesions were $p16^+COX\text{-}2^+Ki67^+$ (19.6% and 27.3%, respectively).

Table 1. Prevalence of risk factors among women initially treated for ductal carcinoma in situ (DCIS) by lumpectomy alone according to the type of subsequent tumor event (invasive cancer or DCIS)*

Variable†	No subsequent tumor event‡ (N = 838), % (No.)	Invasive event (N = 170), % (No.)	DCIS event (N = 154), % (No.)
Age at diagnosis, y			
40–49	18 (154)	26 (44)	34 (53)
50–59	23 (194)	22 (38)	23 (35)
60–69	24 (198)	22 (38)	21 (33)
≥70	35 (292)	29 (50)	21 (33)
P§	Referent	.6	<.001
Race and/or ethnicity			
White	77 (643)	77 (131)	82 (125)
African American	7 (58)	9 (15)	5 (8)
Hispanic	8 (65)	8 (14)	6 (9)
Asian	8 (64)	6 (10)	7 (10)
P§	Referent	.6	.4
Family history of breast cancer‖			
Negative	74 (459)	70 (97)	73 (95)
Positive	26 (164)	30 (42)	27 (36)
P§	Referent	.4	.9
Menopausal status¶			
Postmenopausal	96 (791)	93 (150)	93 (143)
Premenopausal	4 (32)	7 (12)	7 (11)
P§	Referent	.13	.8
Detection method			
Mammography	81 (519)	73 (97)	88 (112)
Palpation#	19 (120)	27 (37)	12 (16)
P§	Referent	<.001	.06

* Excludes women with a history of breast cancer and women who had radiation therapy or mastectomy.

† There was no race and/or ethnicity data missing. However, 22.7% of subjects had missing data for family history, 2.0% for menopausal status, and 22.5% for detection method.

‡ Control subjects were women with ductal carcinoma in situ who did not have a subsequent tumor event.

§ Wald test calculated from the proportional subdistribution hazards regression coefficients; age-adjusted two-sided test.

‖ Defined as at least one first-degree relative (mother, sister, or daughter) with breast cancer.

¶ Women were considered to be postmenopausal if both ovaries had been removed, if they reported their periods had stopped permanently for reasons other than hysterectomy, if they were currently using postmenopausal hormone therapy, or if they were aged 55 or older.

Palpable mass found by the woman or by her physician upon physical examination at the time of diagnosis.

Factors that were independently associated with subsequent DCIS included positive or uncertain margins, DCIS lesions that were $p16^+COX\text{-}2^-Ki67^+$, and those that were $ER^-ERBB2^+Ki67^+$, whereas nuclear grade was no longer statistically significant. The 5- and 8-year risks of subsequent DCIS were highest for women with defined molecular subtypes of DCIS (Tables 4 and 5). The 5- and 8-year risks of subsequent DCIS were lowest for women who had disease-free surgical margins of 10 mm or larger (Table 5).

Risk of Subsequent Invasive Cancer or DCIS by Risk Group

We next estimated the 5- and 8-year risks of subsequent invasive cancer and DCIS for four risk groups based on the statistically significant univariate and multivariable factors reported in Tables 3 and 4, and 5- and 8-year risks reported in Table 5. Among women who were initially diagnosed with DCIS, 17.3% were in the lowest-risk group, which had an 8-year risk of subsequent invasive cancer of 4.1%, and 26.8% were in the next to lowest-risk group, which had an 8-year risk of 6.9% (Table 6). Over a quarter (27.6%) of the women were in the high-risk group, which had an 8-year risk of subsequent invasive cancer of 19.6%. The 8-year risk of subsequent invasive cancer was statistically significantly (P = .018) higher for women with initial DCIS lesions that were detected by palpation or that were $p16^+COX\text{-}2^+Ki67^+$ (19.6%, 95% CI =18.0% to 21.3%) than for women with initial lesions that were detected by mammography and were $p16^-COX\text{-}2^-Ki67^-$ (4.1%, 95% CI = 3.45 to 5.0%).

Women with DCIS could also be divided into groups according to the risk for further DCIS. Here, 19.9% of the women initially diagnosed with DCIS were in the lowest-risk group and had an 8-year risk of subsequent DCIS of 3.9%, and 21.2% were in the low-risk group, with an 8-year risk of 10.2% (Table 6). In this case, only 5.1% of these women were in the high-risk group and had an 8-year risk of subsequent DCIS of 23.6%.

Discussion

We examined the clinical characteristics of women with DCIS who were treated by lumpectomy alone and determined the histopathologic and molecular characteristics of their breast lesions to identify factors associated with the occurrence of subsequent

Table 2. Univariate results of histopathologic factors associated with type of subsequent tumor event (invasive cancer or ductal carcinoma in situ [DCIS])*

Factor†	No subsequent tumor event‡ (N = 279), % (No.)	Invasive event (N = 114), % (No.)	Risk of invasive event, HR§ (95% CI)	DCIS event (N = 109), % (No.)	Risk of DCIS event, HR§ (95% CI)
Tumor size, mm					
>10	30 (194)	39 (70)	1.2 (0.8 to 1.8)	41 (64)	1.4 (1.0 to 2.1)
≤10	70 (85)	61 (44)	1.0 (referent)	59 (45)	1.0 (referent)
Margins					
Positive	23 (62)	36 (39)	1.6 (0.9 to 2.7)	37 (38)	3.6 (1.8 to 7.2)
Uncertain‖	22 (58)	22 (24)	1.1 (0.6 to 2.1)	24 (25)	2.7 (1.3 to 5.8)
1–1.9 mm disease free	22 (57)	15 (16)	0.8 (0.4 to 1.6)	20 (20)	2.5 (1.1 to 5.4)
≥2 to <10 mm disease free	10 (26)	9 (10)	1.1 (0.5 to 2.3)	10 (10)	2.3 (0.9 to 5.5)
≥10 mm disease free	23 (62)	18 (19)	1.0 (referent)	9 (9)	1.0 (referent)
Nuclear grade¶					
High	35 (92)	44 (47)	1.2 (0.8 to 2.1)	60 (61)	2.7 (1.5 to 4.8)
Intermediate	33 (85)	35 (38)	1.3 (0.8 to 2.2)	26 (26)	1.4 (0.8 to 2.7)
Low	32 (83)	21 (22)	1.0 (referent)	14 (14)	1.0 (referent)
Necrosis type					
Comedo	39 (100)	45 (48)	1.1 (0.8 to 1.6)	45 (46)	1.1 (0.8 to 1.6)
Focal punctate	61 (159)	55 (59)	1.0 (referent)	55 (56)	1.0 (referent)
Quantity of necrosis					
Extensive	18 (48)	25 (26)	1.2 (0.8 to 1.8)	28 (29)	1.5 (1.0 to 2.3)
Moderate/scant	82 (212)	75 (80)	1.0 (referent)	72 (73)	1.0 (referent)

* CI = confidence interval; HR = hazard ratio.

† Here, 4.0% of specimens had missing data regarding margins, 6.8% for nuclear grade, type of necrosis, and extent of necrosis.

‡ Control subjects were a random sample of women with DCIS who did not have a subsequent tumor event and were frequency matched by year of diagnosis to the case subjects who were women who had a subsequent tumor event.

§ Adjusted for diagnosis age.

‖ Unknown or could not be assessed.

¶ For lesions with more than one type of nuclear grade, an overall grade was assigned according to the highest grade present.

tumors and to determine the risk of subsequent tumors as a function of these factors. We found initial DCIS lesions that were detected by palpation or had $p16^+COX\text{-}2^+Ki67^+$ expression were the two factors most strongly associated with risk of subsequent invasive cancer; however, these factors were not associated with risk of subsequent DCIS. A little more than a quarter of these women (27.6%) were categorized as having a high risk of subsequent invasive cancer (ie, 19.6% at 8 years). Importantly, many women (44.1%) who did not demonstrate one of these two factors were categorized as having a low risk of subsequent invasive cancer at 8 years (4.1% and 6.9% for the lowest- and low-risk groups, respectively). In addition, we developed the ability to distinguish factors associated with risk of subsequent invasive cancer vs risk of subsequent DCIS, an important clinical goal that could guide initial therapeutic decisions. We found that initial lesions that were $ER^-ERBB2^+Ki67^+$, lesions that were $p16^+COX\text{-}2^-Ki67^+$, and lesions that had positive or uncertain surgical margins were strongly associated with risk of subsequent DCIS; however, these factors were not associated with risk of subsequent invasive cancer.

Recent molecular studies on DCIS lesions may provide insights about the biological contributions of p16, COX-2, and Ki67 expression in $p16^+COX\text{-}2^+Ki67^+$ lesions. Molecular studies have identified markers that distinguish different subtypes of DCIS (22,28–31) that may relate, in an unknown fashion, to molecularly defined subtypes of invasive breast cancer. Previously, we reported in a pilot study that DCIS lesions that express p16 and COX-2 and have a high proliferative capacity share characteristics with basal-like invasive tumors (22). Overexpression of p16 has been validated as a basal-like marker in two recent studies (32,33). In this report, we demonstrated that expression of these markers results in a high risk of subsequent invasive cancer but not DCIS. This is similar to studies of invasive breast cancer where a basal-like subtype is associated with worse clinical outcomes (25). Furthermore, the established role of COX-2 in promoting invasive potential (34–36) provides a biological rationale for why the $p16^+COX\text{-}2^+Ki67^+$ lesions tend to recur as invasive carcinomas, whereas $p16^+COX\text{-}2^-Ki67^+$ lesions tend to recur as DCIS. Moreover, the $p16^+COX\text{-}2^+Ki67^+$ phenotype is independent of risk conferred by DCIS lesions detected by palpation. Palpable DCIS lesions accounted for 15%–20% of DCIS lesions in this study, consistent with recent studies of women undergoing screening mammography (1). That palpable DCIS lesions appear to be more aggressive than mammography-detected lesions is consistent with the observation that palpable invasive cancer lesions tend to be more aggressive than mammography-discovered invasive lesions (37).

Attempts to predict risk of subsequent invasive cancer vs DCIS using a woman's age at diagnosis and nuclear grade of the DCIS lesion have met with limited success (5,38), in part, because there is only moderate agreement in assessing histopathologic characteristics, such as nuclear grade (39,40). We combined biomarker data with data pertaining to diagnosis age and nuclear grade to predict

Table 3. Univariate results of molecular markers associated with type of subsequent tumor event (invasive cancer or ductal carcinoma in situ [DCIS])*

Factor†	No subsequent tumor event‡ (N = 186), % (No.)	Invasive event (N = 72), % (No.)	Risk of invasive event, HR§ (95% CI)	DCIS event (N = 71), % (No.)	Risk of DCIS event, HR§ (95% CI)
ER					
Negative	20 (35)	20 (13)	0.8 (0.4 to 1.5)	31 (21)	1.7 (1.0 to 2.9)
Positive	80 (143)	80 (53)	1.0 (referent)	69 (47)	1.0 (referent)
PR					
Negative	21 (36)	31 (20)	1.3 (0.7 to 2.1)	33 (21)	1.5 (0.9 to 2.5)
Positive	79 (138)	69 (45)	1.0 (referent)	67 (42)	1.0 (referent)
p53					
Positive	10 (17)	10 (6)	0.8 (0.4 to 1.9)	17 (10)	1.8 (0.9 to 3.5)
Negative	90 (153)	90 (57)	1.0 (referent)	83 (49)	1.0 (referent)
ERBB2 oncoprotein					
Positive	13 (25)	19 (14)	1.1 (0.6 to 1.9)	30 (21)	2.0 (1.2 to 3.2)
Negative	87 (161)	81 (58)	1.0 (referent)	70 (50)	1.0 (referent)
Ki67					
Positive‖	36 (62)	59 (38)	1.7 (1.0 to 2.7)	67 (40)	2.3 (1.3 to 4.1)
Negative	64 (109)	41 (26)	1.0 (referent)	33 (20)	1.0 (referent)
p16					
Positive	30 (43)	57 (37)	2.3 (1.4 to 3.8)	41 (26)	1.1 (0.7 to 1.8)
Negative	70 (98)	43 (28)	1.0 (referent)	59 (38)	1.0 (referent)
COX-2					
Positive	46 (68)	50 (34)	1.3 (0.8 to 2.0)	34 (22)	0.6 (0.4 to 1.1)
Negative	54 (79)	50 (34)	1.0 (referent)	66 (42)	1.0 (referent)
p16/Ki67					
Positive/positive	11 (14)	34 (18)	2.1 (1.2 to 3.8)	33 (18)	2.0 (1.1 to 3.6)
All other groupings	89 (111)	66 (35)	1.0 (referent)	67 (36)	1.0 (referent)
COX-2/Ki67					
Positive/positive	18 (24)	33 (18)	1.8 (1.0 to 3.2)	25 (14)	1.1 (0.6 to 2.1)
All other groupings	82 (106)	67 (37)	1.0 (referent)	75 (41)	1.0 (referent)
p16/COX-2/Ki67					
Positive/positive/positive	8.5 (10)	23 (12)	2.2 (1.2 to 4.2)	15 (8)	1.2 (0.5 to 2.5)
All other groupings	91.5 (107)	77 (40)	1.0 (referent)	85 (44)	1.0 (referent)
p16/COX-2/Ki67					
Positive/negative/positive	2.6 (3)	12 (6)	1.5 (0.6 to 3.6)	19 (10)	3.2 (1.5 to 6.9)
All other groupings	97.4 (114)	88 (46)	1.0 (referent)	81 (42)	1.0 (referent)
ER/PR/ERBB2					
Negative/negative/negative	5 (9)	6 (4)	1.1 (0.4 to 3.3)	6 (4)	1.1 (0.4 to 3.0)
All other groupings	95 (172)	94 (64)	1.0 (referent)	94 (65)	1.0 (referent)
ER/ERBB2					
Negative/positive	6.4 (11)	5 (3)	0.5 (0.2 to 1.5)	19 (12)	3.0 (1.6 to 5.7)
All other groupings	93.6 (161)	95 (61)	1.0 (referent)	81 (53)	1.0 (referent)
ER/Ki67					
Negative/positive	9 (14)	13 (7)	0.8 (0.4 to 1.8)	28 (15)	2.8 (1.5 to 5.2)
All other groupings	91 (136)	87 (48)	1.0 (referent)	72 (39)	1.0 (referent)
ERBB2/Ki67					
Positive/positive	7 (11)	18 (10)	1.6 (0.8 to 3.2)	21 (12)	1.9 (1.0 to 3.5)
All other groupings	93 (146)	82 (46)	1.0 (referent)	79 (46)	1.0 (referent)
ER/ERBB2/Ki67					
Negative/positive/positive	2.7 (4)	6 (3)	0.9 (0.3 to 2.7)	15 (8)	3.6 (1.7 to 7.8)
All other groupings	97.3 (143)	94 (50)	1.0 (referent)	85 (45)	1.0 (referent)

* CI = confidence interval; COX-2 = cyclooxygenase-2; ER = estrogen receptor; ERBB2 = human epidermal growth factor receptor-2 (HER2/neu-oncoprotein); HR = hazard ratio; PR = progesterone receptor.

† Missing data: 5.2% for ER status, 7.9% for PR status, 10.6% for p53 status, 0% for ERBB2, 10.3% for Ki67, 17.6% for p16, and 15.2% for COX-2.

‡ Control subjects were a random sample of women with DCIS who did not have a subsequent tumor event and were frequency matched by year of diagnosis to the case subjects who were women who had a subsequent tumor event.

§ Adjusted for diagnosis age.

‖ More than 10% positive cells.

risk of invasive cancer. Only initial DCIS lesions that had been detected by palpation and those mammography-detected lesions that were $p16^{+}COX\text{-}2^{+}Ki67^{+}$ retained a statistically significant association with invasive cancer in a multivariable analysis. We have previously reported an association between high nuclear and subsequent invasive cancer at a median follow-up of about 6 years

Table 4. Hazard ratios (HRs) and 95% confidence intervals (CIs) from final multivariable models of clinical and histopathologic characteristics and molecular markers independently associated with subsequent tumor events*

Variable	Invasive cancer, HR (95% CI)
Age at diagnosis, y	1.0 (0.8 to 1.3)
Detection by palpation (vs mammography)†	2.7 (1.4 to 5.5)
Nuclear grade	
High vs low	1.0 (0.4 to 2.3)
Intermediate vs low	1.9 (0.8 to 4.3)
p16/COX-2/Ki67	
Positive/positive/positive	2.2 (1.1 to 4.5)
All other groupings	1.0 (referent)
Variable†	**DCIS*, HR (95% CI)**
Age at diagnosis, y	0.9 (0.7 to 1.1)
Margins ordinal (per category increase)‡	1.3 (1.1 to 1.7)
Nuclear grade	
High vs low	1.7 (0.6 to 4.8)
Intermediate vs low	1.3 (0.4 to 4.1)
p16/COX-2/Ki67	
Positive/negative/positive	3.7 (1.7 to 7.9)
All other groupings	1.0 (referent)
ER/ERBB2/Ki67	
Negative/positive/positive	5.8 (2.4 to 14)
All other groupings	1.0 (referent)

* COX-2 = cyclooxygenase-2; DCIS = ductal carcinoma in situ; ER = estrogen receptor; ERBB2 = human epidermal growth factor receptor-2 (HER2/neu-oncoprotein).

† Palpable mass found by the woman or by her physician upon physical examination.

‡ Margins ordinal defined as margin ≥10 mm disease free = 0; margin ≥2 to <10 mm disease free = 1; margin 1–1.9 mm disease free = 2; margin uncertain = 3; margin positive = 4.

(5). We did not observe an association between nuclear grade and invasive cancer at a median follow-up of about 8 years, consistent with a study that reported 10-year subsequent tumor rate was not statistically significantly different between women with high nuclear grade and all other grades (41). One explanation for this observation is that the nuclear grade of the initial DCIS lesion may be associated with short-term epithelial proliferation but not long-term proliferation. The contribution of Ki67 to risk of subsequent invasive cancer may capture, in part, the previously observed association of nuclear grade and subsequent invasive cancer (5) and has the benefit of signifying short- and long-term risk of subsequent tumor in this cohort.

Factors associated with subsequent DCIS differed from those associated with subsequent invasive cancer. Disease-free surgical margins have been strongly associated with a lower risk of subsequent tumors, in particular DCIS (5,42). In this study, when biomarkers were combined with data pertaining to margin status and nuclear grade, positive margins remained a strong predictor of subsequent DCIS, suggesting that persistence of neoplastic cells from the original DCIS lesion may contribute to subsequent DCIS. Margin status did not predict subsequent invasive cancer, implying that most subsequent invasive cancer is an independent process from any residual nonsurgically removed DCIS. In addition to margin status, we found that certain combinations of molecular markers are present in a very small number (5.1%) of DCIS lesions that are statistically significantly associated with a high risk of subsequent DCIS. The high-risk lesions include lesions that are $ER^-ERBB2^+Ki67^+$ or $p16^+COX\text{-}2^-Ki67^+$. The striking differences in lesion characteristics associated with subsequent invasive cancer compared with subsequent DCIS suggest biological heterogeneity among DCIS lesions.

Our study has several strengths. First, it is a large population-based study of women with DCIS treated by lumpectomy alone that has measures of clinical, histopathologic, and molecular characteristics by type of subsequent tumor with a median follow-up of about 8 years. Our results are directly applicable to women with different standard histological types of DCIS because this study included sufficient numbers of women within each category. Second, we collected DCIS case subjects from 63 hospitals, thereby minimizing the chance of selection bias because of specific clinical practices at some hospitals. Third, our large sample size allowed us to assess the combinations of biomarkers that independently associated with subsequent invasive cancer vs DCIS by using a multivariable model.

The study also has possible limitations. Clinical factors were assessed retrospectively, raising the possibility of recall bias. However, factors that a woman might attribute as causes of subsequent tumors and thus remember more readily when questioned, such as presence of family history of breast cancer, were not associated with subsequent tumors, suggesting that recall bias did not greatly affect our results. Because we studied women treated by lumpectomy only, we could not determine whether various biomarker profiles are more likely to respond to adjuvant therapies. Additionally, we were only able to measure biomarkers on a subset of women based on the availability of tumor blocks at participating hospitals. Our imputation of missing biomarker data may have resulted in a small overestimation or underestimation of risk of subsequent tumors. Similar to challenges presented by assessment of ERBB2 expression, the immunohistochemical interpretation of COX-2 and p16 expression can be challenging because of heterogeneity within DCIS. Refinement of immunohistochemical methods and validation in additional cohorts and independent laboratories are required to validate our results. Likewise, identification of additional markers may further refine risk groups and increase the robustness of risk assessment.

In conclusion, our study adds to the literature in that we identified combinations of biomarkers in DCIS lesions whose expression patterns improve estimation of a woman's risk for subsequent invasive cancer. Our results suggest that among initial DCIS lesions $p16^+COX\text{-}2^+Ki67^+$ expression or the ability to be detected by palpation are the two most important factors that predict higher risk of subsequent invasive cancer. Conversely, mammographically detected Ki67-negative DCIS lesions, in particular those that are also p16 and COX-2 negative, are associated with a lower risk of subsequent invasive cancer that is similar to the risk of contralateral invasive cancer in women after their first primary invasive breast cancer (43). Of note, women in the lowest-risk group have an 8-year risk of invasive breast cancer comparable to an average-risk 60-year-old woman's 10-year risk of invasive breast cancer.

Table 5. Estimate of 5- and 8-year risks of invasive cancer vs ductal carcinoma in situ (DCIS) for characteristics of women initially diagnosed with DCIS that were independently associated with subsequent invasive cancer or DCIS events*

Variable	5-y risk of invasive cancer, % (95% CI)	8-y risk of invasive cancer, % (95% CI)
Overall	7.8 (6.2 to 9.4)	11.1 (9.2 to 13.0)
Detection method		
Palpation†	13.2 (12.3 to 14.3)	17.8 (16.2 to 19.4)
Mammography	6.5 (6.3 to 6.6)	9.3 (9.2 to 9.6)
p16/COX-2/Ki67		
Positive/positive/positive	19.6 (16.6 to 23.4)	27.3 (22.9 to 33.9)
All other groupings	6.8 (6.6 to 7.0)	9.5 (9.2 to 9.8)
Variable	**5-y risk of DCIS, % (95% CI)**	**8-y risk of DCIS, % (95% CI)**
Overall	9.7 (7.9 to 11.4)	11.6 (9.7 to 13.5)
Margins		
Positive or uncertain	12.4 (11.7 to 13.0)	14.6 (13.8 to 15.4)
1 to <10 mm disease free	10.2 (9.4 to 10.9)	11.7 (10.4 to 12.3)
≥10 mm disease free	2.8 (2.5 to 3.3)	4.4 (3.9 to 5.2)
p16/COX-2/Ki67		
Positive/negative/positive	20.8 (17.3 to 25.3)	24.9 (20.3 to 33.4)
All other groupings	8.6 (8.4 to 8.8)	10.4 (10.1 to 10.7)
ER/ERBB2/Ki67		
Negative/positive/positive	37.2 (29.3 to 49.0)	40.5 (31.7 to 54.3)
All other groupings	8.8 (8.7 to 9.0)	10.6 (10.4 to 10.8)

* CI = confidence interval; COX-2 = cyclooxygenase-2; ER = estrogen receptor; ERBB2 = human epidermal growth factor receptor-2 (HER2/neu-oncoprotein).

† Palpable mass found by the woman or by her physician upon physical examination.

We also confirmed margin status as a strong predictor of subsequent DCIS and identified expression of novel combinations of biomarkers predicting subsequent DCIS, which differ from those of that predict subsequent invasive cancer. These markers, compared with nuclear grade, improve the estimation of risk for subsequent DCIS.

Many women who have been diagnosed with DCIS have an inaccurate perception of their risk of subsequent invasive cancer

Table 6. Stratification of women into low, intermediate, and high 5- and 8-year risk by type of subsequent tumor event

Risk category*	Prevalence in cohort, %†	5-y risk of invasive cancer, % (95% CI)	8-y risk of invasive cancer, % (95% CI)
Lowest‡	17.3	2.1 (1.9 to 2.6)	4.1 (3.4 to 5.0)
Low§	26.8	4.4 (4.0 to 5.0)	6.9 (6.1 to 8.0)
Intermediate‖	28.3	7.7 (7.0 to 8.5)	11.5 (10.3 to 12.8)
High¶	27.6	14.1 (13.1 to 15.3)	19.6 (18.0 to 21.3)
Risk category*	**Prevalence in cohort, %†**	**5-y risk of DCIS, % (95% CI)**	**8-y risk of DCIS, % (95% CI)**
Lowest#	19.9	2.7 (2.4 to 3.2)	3.9 (3.3 to 4.8)
Low**	21.2	7.8 (6.8 to 8.7)	10.2 (8.1 to 12.7)
Intermediate††	53.8	12.0 (11.4 to 12.6)	14.4 (13.6 to 15.2)
High‡‡	5.1	19.2 (15.3 to 23.9)	23.6 (18.1 to 34.0)

* Risk groups were defined separately for subsequent invasive cancer and ductal carcinoma in situ (DCIS) based on multivariable associations in Table 4 as well as level of risk associated with factors in Table 5. CI = confidence interval.

† Average prevalence estimated among 2500 cohorts of 1162 women with missing measures imputed as described in the statistical section.

‡ DCIS mammographically detected plus Ki67, cyclooxygenase-2 (COX-2) and p16 triple negative (Ki67–COX-2$^-$p16$^-$).

§ DCIS mammographically detected plus Ki67 negative and either COX-2 positive (Ki67$^-$COX-2$^+$) or p16 positive (Ki67$^-$p16$^+$) or both positive (Ki67$^-$COX-2$^+$p16$^+$).

‖ DCIS mammographically detected plus Ki67 positive and either COX-2 positive (Ki67$^+$COX-2$^+$) or p16 positive (Ki67$^+$p16$^+$) or COX-2-negative/p16-negative (Ki67$^+$COX-2$^-$p16$^-$).

¶ Detected by palpation or p16, Ki67, and COX-2 triple positive (p16$^+$Ki67$^+$COX-2$^+$).

DCIS with margins of 1 mm or greater disease free plus estrogen receptor (ER) positive and HER2/neu-oncoprotein (ERBB2) negative and Ki67 negative (ER$^+$ERBB2$^-$Ki67$^-$).

** DCIS with margins of 1 mm or greater disease free plus either ER negative, ERBB2 negative (ER$^-$ERBB2$^-$) or p16 and Ki67 positive (p16$^+$Ki67$^+$) or COX-2 negative, Ki67 positive (COX-2$^-$Ki67$^+$) or COX-2 positive, Ki67 positive (COX-2$^+$Ki67$^+$) or ERBB2 positive, Ki67 positive (ERBB2$^+$Ki67$^+$).

†† Positive or uncertain margins or ER negative, Ki67 positive (ER$^-$Ki67$^+$) or ER negative, ERBB2 positive (ER$^-$ERBB2$^+$).

‡‡ DCIS with margins of 1 mm or greater disease free plus ER negative/ERBB2 positive/Ki67 positive (ER$^-$ERBB2$^+$Ki67$^+$) or p16/Ki67 positive and COX-2 negative (p16$^+$COX-2$^-$Ki67$^+$).

(44). Here, we show that the mode of detection and the biomarkers p16, COX-2, and Ki67 may be used to help stratify a woman's risk of subsequent invasive cancer and to help her decide whether she should undergo adjuvant therapies. In addition, these factors may provide insight for targeted interventions.

References

1. Ernster VL, Ballard-Barbash R, Barlow WE, et al. Detection of DCIS in women undergoing screening mammography. *J Natl Cancer Inst.* 2002;94(20):1546–1554.
2. Smigal C, Jemal A, Ward E, et al. Trends in breast cancer by race and ethnicity: update 2006. *CA Cancer J Clin.* 2006;56(3):168–183.
3. Fisher B, Costantino J, Redmond C, et al. Lumpectomy compared with lumpectomy and radiation therapy for the treatment of intraductal breast cancer. *N Engl J Med.* 1993;328(22):1581–1586.
4. Fisher B, Land S, Mamounas E, Dignam J, Fisher E, Wolmark N. Prevention of invasive breast cancer in women with ductal carcinoma in situ: an update of the national surgical adjuvant breast and bowel project experience. *Semin Oncol.* 2001;28(4):400–418.
5. Kerlikowske K, Molinaro A, Cha I, et al. Characteristics associated with recurrence among women with ductal carcinoma in situ treated by lumpectomy. *J Natl Cancer Inst.* 2003;95(22):1692–1702.
6. Julien JP, Bijker N, Fentiman IS, et al. Radiotherapy in breast-conserving treatment for ductal carcinoma in situ: first results of the EORTC randomised phase III trial 10853. *Lancet.* 2000;355(9203):528–533.
7. Fisher B, Dignam J, Wolmark N, et al. Lumpectomy and radiation therapy for the treatment of intraductal breast cancer: findings from national surgical adjuvant breast and bowel project B-17. *J Clin Oncol.* 1998;16(2):441–452.
8. Warren JL, Weaver DL, Bocklage T, et al. The frequency of ipsilateral second tumors after breast-conserving surgery. *Cancer.* 2005;104(9):1840–1848.
9. Fisher B, Dignam J, Wolmark N, et al. Tamoxifen in treatment of intraductal breast cancer National Surgical Adjuvant Breast and Bowel Project B-24 randomised controlled trial. *Lancet.* 1999;353(9169):1993–2000.
10. Schwartz GF, Solin LJ, Olivotto IA, Ernster VL, Pressman PIthe consensus Conference Committee. The consensus conference on the treatment of in situ ductal carcinoma of the breast. *Cancer.* 2000;88(4):946–954.
11. Ernster VL, Barclay J, Kerlikowske K, Wilkie H, Barbash R. Mortality among women with ductal carcinoma in situ of the breast in the population-based SEER Program. *Arch Intern Med.* 2000;160(7):953–958.
12. Solin L, Fourquet A, Vicini F, et al. Long-term outcome after breast-conservation treatment with radiation for mammographically detected ductal carcinoma in situ of the breast. *Cancer.* 2005;103(6):1137–1146.
13. Ringberg A, Nordgren H, Thorstensson S, et al. Histopathological risk factors for ipsilateral breast events after breast conserving treatment for ductal carcinoma in situ of the breast—results from the Swedish randomised trial. *Eur J Cancer.* 2007;43(2):291–298.
14. Silverstein MJ. Ductal carcinoma in situ of the breast: 11 reasons to consider treatment with excision alone. *Womens Health.* 2008;4(6):565–577.
15. Ringberg A, Anagnostaki L, Anderson H, Idvall I, Ferno M. Cell biological factors in ductal carcinoma in situ (DCIS) of the breast-relationship to ipsilateral local recurrence and histopathological characteristics. *Eur J Cancer.* 2001;37(12):1514–1522.
16. Provenzano E, Hopper JL, Giles GG, Marr G, Venter DJ, Armes JE. Biological markers that predict clinical recurrence in ductal carcinoma in situ of the breast. *Eur J Cancer.* 2003;39(5):622–630.
17. Cornfield DB, Palazzo JP, Schwartz GF, et al. The prognostic significance of multiple morphologic features and biologic markers in ductal carcinoma in situ of the breast. *Cancer.* 2004;100(11):2317–2327.
18. Roka S, Rudas M, Taucher S, et al. High nuclear grade and negative estrogen receptor are significant risk factors for recurrence in DCIS. *Eur J Surg Oncol.* 2004;30(3):243–247.
19. Barnes NL, Khavari S, Boland GP, Cramer A, Knox WF, Bundred NJ. Absence of HER4 expression predicts recurrence of ductal carcinoma in situ of the breast. *Clin Cancer Res.* 2005;11(6):2163–2168.
20. Barnes N, Haywood P, Knox WF, Bundred NJ. Survivin expression in in situ and invasive breast cancer relates to COX-2 expression and DCIS recurrence. *Br J Cancer.* 2006;94(2):253–258.
21. Gauthier M, Pickering C, Miller C, et al. p38 regulates cyclooxygenase-2 in human mammary epithelial cells and is activated in premalignant tissue. *Cancer Res.* 2005;65(5):1792–1799.
22. Gauthier ML, Berman HK, Miller C, et al. Abrogated stress response distinguishes basal-like tumors and DCIS lesions associated with subsequent tumor events. *Cancer Cell.* 2007;12(5):479–491.
23. Tamimi R, Baer H, Marott J, et al. Comparison of molecular phenotypes of ductal carcinoma in situ and invasive breast cancer. *Breast Cancer Res.* 2008;10(4):R67.
24. Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. *Mod Pathol.* 1998;11(2):155–168.
25. Carey LA, Perou CM, Livasy CA, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *JAMA.* 2006;295(21):2492–2502.
26. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc.* 1999;94(446):496–509.
27. Pepe MS, Mori M. Kaplan-Meier, marginal or conditional probability curves in summarizing competing risks failure time data? *Stat Med.* 1993;12(8):737–751.
28. Allred D, Wu Y, Mao S, et al. Ductal carcinoma in situ and the emergence of diversity during breast cancer evolution. *Clin Cancer Res.* 2008;14(2):370–378.
29. Bryan B, Schnitt S, Collins L. Ductal carcinoma in situ with basal-like phenotype: a possible precursor to invasive basal-like breast cancer. *Mod Pathol.* 2006;19(5):617–621.
30. Dabbs D, Chivukula M, Carter G, Bhargava R. Basal phenotype of ductal carcinoma in situ: recognition and immunohistologic profile. *Mod Pathol.* 2006;19(11):1506–1511.
31. Livasy C, Perou C, Karaca G, et al. Identification of a basal-like subtype of breast ductal carcinoma in situ. *Hum Pathol.* 2007;38(2):197–204.
32. Subhawong A, Subhawong T, Nassar H, et al. Most basal-like breast carcinomas demonstrate the same Rb-/p16+ immunophenotype as the HPV-related poorly differentiated squamous cell carcinomas which they resemble morphologically. *Am J Surg Pathol.* 2008;33(2):163–175.
33. Herschkowitz J, He X, Fan C, Perou C. The functional loss of the retinoblastoma tumour suppressor is a common event in basal-like and luminal B breast carcinomas [published online ahead of print September 9, 2008]. *Breast Cancer Res.* 2008;10(5):R75.
34. Crawford YG, Gauthier ML, Joubel A, et al. Histologically normal human mammary epithelia with silenced p16(INK4a) overexpress COX-2, promoting a premalignant program. *Cancer Cell.* 2004;5(3):263–273.
35. Hu M, Peluffo G, Chen H, Gelman R, Schnitt S, Polyak K. Role of COX-2 in epithelial-stromal cell interactions and progression of ductal carcinoma in situ of the breast. *Proc Natl Acad Sci U S A.* 2009;106(9):3372–3377.
36. Minn AJ, Gupta GP, Siegel PM, et al. Genes that mediate breast cancer metastasis to lung. *Nature.* 2005;436(7050):518–524.
37. Silverstein MJ, Skinner KA, Lomis TJ. Predicting axillary nodal positivity in 2282 patients with breast carcinoma. *World J Surg.* 2001;25(6):767–772.
38. Vicini FA, Kestin LL, Goldstein NS, et al. Impact of young age on outcome in patients with ductal carcinoma-in-situ treated with breast-conserving therapy. *J Clin Oncol.* 2000;18(2):296–306.
39. Wells WA, Carney PA, Eliassen MS, Grove MR, Tosteson ANA. Pathologists' agreement with experts and reproducibility of breast ductal carcinoma-in-situ classification schemes. *Am J Surg Pathol.* 2000;24(5):651–659.
40. Douglas-Jones AG, Morgan JM, Appleton MAC, et al. Consistency in the observation of features used to classify duct carcinoma in situ (DCIS) of the breast. *J Clin Pathol.* 2000;53(8):596–602.
41. Solin LJ, Kurtz J, Fourquet A, et al. Fifteen-year results of breast-conserving surgery and definitive breast irradiation for the treatment of ductal carcinoma in situ of the breast. *J Clin Oncol.* 1996;14(3):754–763.
42. Solin LJ, Fourquet A, Vicini FA, et al. Mammographically detected ductal carcinoma in situ of the breast treated with breast-conserving surgery and definitive irradiation: long-term outcome and prognostic significance of

patient age and margin status. *Int J Radiat Oncol Biol Phys.* 2001;50(4):991–1002.
43. Chen Y, Thompson W, Semenciw R, Mao Y. Epidemiology of contralateral breast cancer. *Cancer Epidemiol Biomarkers Prev.* 1999;8(10):855–861.
44. Partridge A, Adloff K, Blood E, et al. Risk perceptions and psychosocial outcomes of women with ductal carcinoma in situ: longitudinal results from a cohort study. *J Natl Cancer Inst.* 2008;100(4):243–251.

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