

Invasive ductal and invasive lobular carcinoma show a similar amplification profile of chromosomal markers prognostic for breast carcinoma recurrence.

Bonnie Carmichael, MD², Lisa Davis, PhD¹, Cole Harris, MS¹, Lei Tang, PhD¹, Patti Doherty, RN¹, John Alsobrook, PhD¹, Thomas M Williams, MD¹, Katie Doeden, MD², Bryan Hall, MS², Ian Rabinowitz, MD², John Hozier, PhD² and Therese Bocklage, MD². ¹Pathology, Exagen Diagnostics, Albuquerque, New Mexico, United States, 87102 and ²Department of Pathology, University of New Mexico, Albuquerque, New Mexico, United States, 87102



Abstract

Background: Previously, our group demonstrated that amplification of specific combinations of 17 genomic markers is highly predictive of breast cancer recurrence. Infiltrating lobular and ductal carcinomas have been considered distinct entities with differing prognoses and genetic abnormalities. We investigated whether these tumors can be distinguished by the amplification status of these 17 genomic sites.

Methods: Seventeen genomic markers predictive of cancer recurrence were discovered previously by our group using global mining of genome-wide RNA microarray data and high resolution DNA array comparative genomic hybridization data. In this study, we analyzed the amplification of these genomic markers in 44 ductal carcinomas (nuclear grade = 2) and 21 lobular carcinomas. The cases were histologically screened to represent pure ductal or lobular carcinomas and included tumor stages I through III, with non-local recurrences of 27% and 24% respectively. The lobular carcinoma cases were analyzed with e-cadherin staining which resulted in 12 negative and 9 positive cases for membranous e-cadherin.

Results: We found no statistically significant difference in the amplification of the 17 genomic markers of recurrence in ductal versus lobular carcinoma. The same pattern of amplification that is predictive of recurrence in ductal carcinoma was also predictive of recurrence in lobular carcinoma (p=7.0 x10⁻⁴, one-sided student's t-test). E-cadherin negative and e-cadherin positive lobular carcinomas showed similar amplification patterns.

Discussion: For decades, pathologists have histologically distinguished ductal from lobular carcinoma. Recently, the disparate expression of e-cadherin protein has supported such a histologic distinction. Furthermore, gene expression profile studies suggest the two tumors are different. Some studies suggest a better prognosis for patients with lobular carcinoma than similarly staged ductal carcinoma, although this is controversial. Our results indicate that the genomic markers we evaluated have similar amplification patterns in both tumor types. Thus, although there is histologic and molecular evidence that the tumors are distinctive, for these 17 genomic sites, no differences were noted. The markers most predictive of recurrence of ductal carcinoma also are most predictive of recurrence of lobular carcinoma.

Table 1
Genomic Markers Prognostic for Breast Cancer Evaluated in Ductal versus Lobular Carcinoma

Locus	Location
PDCD61P	3p23
IMPA1	8q21.13
AL080059	8q22.1
STK3	8q22.2
EXT1	8q24.11
RAD21	8q24.11
ANXA11	10q22.3
FANCA	16q24.3
ZNF207	17q11.2
GRB7	17q12
MLN64	17q12
ZNF144	17q12
NR1D1	17q21.1
SMARCE1	17q21.2
BIRC5	17q25.3
HEPSIN	19q13.12
CYP24	20q13.2

Background

Previously, our group developed an integrated approach to DNA marker discovery which uses a concurrent global search for predictive patterns in both gene expression and array comparative genomic hybridization (CGH) data. ¹ This statistical analysis identified combinations of genomic markers potentially predictive of breast cancer recurrence. Subsequently, we did a validation study using fluorescent in situ hybridization (FISH) probes manufactured to correspond to 17 potentially prognostic genomic loci. ² These 17 genomic markers are found on chromosomes 3, 8, 10, 16, 17, 19 and 20 and are listed in Table 1. The results demonstrated that an amplification pattern of specific subsets of the 17 genomic markers is highly predictive of breast cancer recurrence at the time of diagnosis. Interestingly, the genomic subset that formed the best predictor of recurrence for estrogen/progesterone receptor positive (ER/PR+) cancers was different than the subset for estrogen/progesterone receptor negative (ER/PR-) cancers. These are listed in Table 2. We derived an algorithm for computing a prognostic index (PI) from DNA copy numbers of each genomic marker subset, resulting in distinct PI scores for ER/PR+ and ER/PR- cancers. ³ Historically, lobular and ductal carcinoma have been considered distinct entities with differing prognoses and genetic abnormalities. In this study, we investigated whether lobular and ductal carcinomas can be distinguished by the amplification status of these 17 genomic sites.

Design

Breast cancer cases for this study were selected from a previous validation study of the prognostic significance of genomic markers for breast cancer which used a FISH assay format. ² This validation study was comprised of approximately 300 patients receiving care at the University of New Mexico that had at least a four-year follow-up period. Initial eligibility criteria for the previous study included a diagnosis of Stage I, II or III invasive breast carcinoma, availability of original clinical and pathological records and availability of archived tumor specimens. Additional criteria for our study included selection of cases with analyzable FISH signals and only hormone receptor positive cases since the great majority of lobular carcinomas are ER/PR+.

Of the 301 original cases, 70 cases were diagnosed as lobular carcinoma (with or without the presence of ductal carcinoma) and of these we identified 38 histologically pure lobular carcinoma cases. These 38 cases were stained with e-cadherin using microarray tissue blocks, which resulted in 16 negative, 15 positive and 7 equivocal cases. Of the 31 cases with satisfactory e-cadherin results, only 21 were both hormone receptor positive and had associated analyzable FISH signals. Of the 21 lobular carcinoma cases, there were 12 negative and 9 positive cases for membranous e-cadherin.

260 of the original 301 cases were diagnosed as ductal carcinoma from which 44 histologically pure ductal carcinoma cases with nuclear grade = 2, hormone receptor positivity and analyzable FISH data were selected for this study. Only nuclear grade 2 tumors were selected to match the corresponding nuclear grade of most lobular carcinomas.

Thus, we analyzed the amplification pattern of 17 genomic markers predictive of recurrence in 44 ductal carcinomas (nuclear grade = 2) and 21 lobular carcinomas. The cases included tumor stages I through III, with non-local recurrences of 27% for ductal and 24% for lobular carcinomas.

A student's t-test was used to evaluate the mean prognostic index (PI) between recurrent and non-recurrent cases for all lobular tumors, first by e-cadherin staining status, then as a combined group. In the e-cadherin negative cases (n=12), the PI was significantly different between recurrent (n=2) and non-recurrent (n=10) cases with p = 0.026. In the e-cadherin positive cases (n=9), the PI was still significantly different between recurrent (n=3) and non-recurrent (n=6) cases with p = 0.0064. When the two groups were combined (n=21, 5 with recurrence and 16 without recurrence), the PI was associated with recurrence with p = 0.0007.

Table 2
Specific genomic subsets found to be highly predictive of breast cancer recurrence

Estrogen/progesterone receptor positive cancers	Estrogen/progesterone receptor negative cancers
CYP24	NR1D1
PDCD61P	SMARCE1
BIRC5	BIRC5

Table 3
Patient demographic information segregated by cancer classification. Lobular carcinoma is categorized histologically, then by e-cadherin staining results.

Cancer Classification	Age range at diagnosis (years)	Stage 1	Stage 2	Stage 3	Cancer free at follow-up	Cancer recurred	Total cases
Invasive ductal	34 - 79	18	20	6	32	12	44
Invasive lobular - based on histology	36 - 81	8	5	8	16	5	21
e-cadherin negative	43 - 81	5	3	4	10	2	12
e-cadherin positive	36 - 74	3	2	4	6	3	9

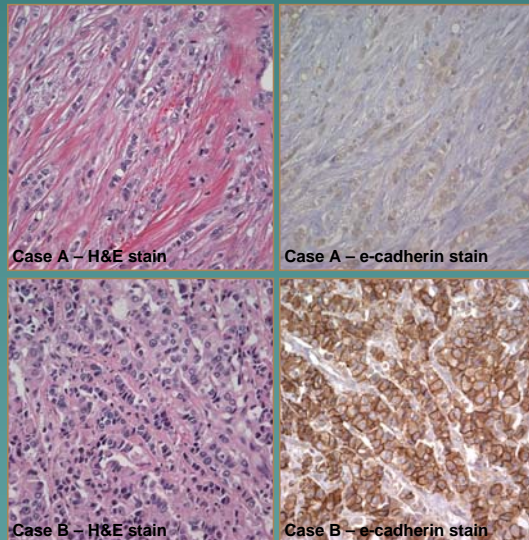


Figure 1
Comparison of two invasive lobular tumors with similar morphology. Case A is negative for membranous e-cadherin staining and Case B is positive for membranous e-cadherin staining.

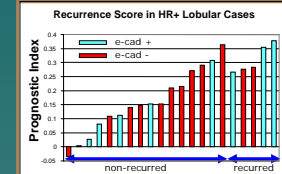


Figure 2
The PI is significantly different between cases which had recurrence and cases which did not have recurrence in both e-cadherin negative (p = 0.026) and e-cadherin positive groups (p = 0.0064).

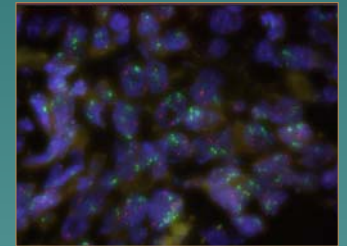


Figure 3
An example of breast cancer cells with fluorescent in situ hybridized probes showing amplification of the MLN64 gene region on chromosome 17 (green) and normal copy numbers of the PDCD61P gene region on chromosome 3 (red).

Discussion

For decades, pathologists have histologically distinguished ductal from lobular carcinoma. Recently, the disparate expression of e-cadherin protein has supported such a histologic distinction. Loss of the e-cadherin gene from chromosome 16q, thus, lack of staining in tissue sections, is a frequent finding in lobular carcinoma of the breast but is not typically found in low grade ductal carcinomas, which have positive e-cadherin staining. Furthermore, gene expression profile studies suggest the two tumors are different. Some studies suggest a better prognosis for patients with lobular carcinoma than similarly staged ductal carcinoma, although this is controversial. Our results indicate that the genomic markers we evaluated have similar amplification patterns in both tumor types. Thus, although there is histologic and molecular evidence that the tumors are distinctive, for these 17 genomic sites, no differences were noted. The markers most predictive of recurrence of ductal carcinoma also are most predictive of recurrence of lobular carcinoma.

Results

Initially, we compared the amplification patterns of 17 genomic markers of recurrence between ductal and lobular carcinoma of similar nuclear grade and to statistically significant difference was found. We then did a similar comparison using the amplification pattern of the three gene marker set (Prognostic Index) specific for recurrence prediction in ER/PR+ ductal carcinoma and found it was also predictive of recurrence in ER/PR+ lobular carcinoma (p = 7.0 x10⁻⁴, one-sided student's t-test). This is shown in Figure 2. E-cadherin negative and e-cadherin positive lobular carcinomas show similar amplification patterns. We conclude that, as for invasive ductal carcinoma, the Prognostic Index appears to be a strong predictor for recurrence in hormone receptor positive invasive lobular carcinoma cases.

REFERENCES

- Harris C. Discovery of multiplex genomic markers for predicting breast cancer recurrence. San Antonio Breast Cancer Symposium, December 2004.
- Harris C, Davis L, Doherty P, Hraber P, et al. Validation of multiplex genomic markers for predicting breast cancer recurrence in a FISH assay format. San Antonio Breast Cancer Symposium, December 2004.
- Davis L, Harris C, Tang L, Doherty P, Hraber P, Sakai Y, Bocklage T, Doeden K, Hall B, Rabinowitz I, Williams T, Hozier J. Amplification Patterns of Three Genomic Regions Predict Distant Recurrence in Breast Carcinoma, manuscript in submission at Diagnostic Molecular Pathology.