FOXA1 Expression in Breast Cancer—Correlation with Luminal Subtype A and Survival

Sunil Badve,[1,2] Dmitry Turbin,[8] Mangesh A. Thorat,[1] Akira Morimiya,[1] Torsten O. Nielsen,[8] Charles M. Perou,[7] Sandi Dunn,[9] David G. Huntsman,[8] and Harikrishna Nakshatri[3,4,5,6]

Abstract

Purpose: FOXA1, a forkhead family transcription factor, is essential for optimum expression of ~50% of estrogen receptor α (ERα):estrogen responsive genes. FOXA1 is expressed in breast cancer cells. It segregates with genes that characterize the luminal subtypes in DNA microarray analyses. The utility of FOXA1 as a possible independent prognostic factor has not been determined in breast cancers.

Materials and Methods: A tissue microarray comprising tumors from 438 patients with 15.4 years median follow-up was analyzed for FOXA1 expression by immunohistochemistry. Interpretable FOXA1 expression obtained in 404 patients was analyzed along with other prognostic factors like tumor grade, size, nodal status, ER, progesterone receptor (PR), and HER2/*neu*.

Results: FOXA1 expression (score >3) was seen in 300 of 404 breast cancers and it correlated with ER (P = 0.000001), PR (P = 0.00001), and luminal A subtype (P = 0.000001). Loss of expression was noted with worsening tumor grade (P = 0.001). Univariate analysis showed nodal status (P = 0.0000012), tumor size (P = 0.00001), FOXA1 (P = 0.0004), and ER (P = 0.012) to be predictors of breast cancer–specific survival. Multivariate analysis showed only nodal status (P = 0.001) and tumor size (P = 0.039) to be significant prognostic factors, whereas FOXA1 (P = 0.060) and ER (P = 0.131) were not significant. In luminal subtype A patient subgroup, FOXA1 expression was associated with better cancer-specific survival (P = 0.024) and in ER-positive subgroup, it was better predictor of cancer-specific survival (P = 0.009) than PR (P = 0.213).

Conclusion: FOXA1 expression correlates with luminal subtype A breast cancer and it is significant predictor of cancer-specific survival in patients with ER-positive tumors. Prognostic ability of FOXA1 in these low-risk breast cancers may prove to be useful in clinical treatment decisions.

Estrogen receptor (ER) expression is a good predictive and prognostic factor in breast cancer. However, not all ER-positive breast cancers behave alike. Knowing why and how some ER-positive breast cancers behave differently than others is important from both research and clinical viewpoint. A clinician will certainly be more pleased to know which patients with ER-positive breast cancers need to be treated aggressively and a research scientist would be interested in knowing the pathways involved in these different behaviors and if any of those can serve as therapeutic targets.

Estrogen plays an important role in the growth, proliferation, and differentiation of mammary epithelium. ERα and ERβ mediate the biological action of estrogen by functioning as estrogen-activated transcription factors (1, 2). ERα is expressed in 10% to 15% of luminal epithelial cells of normal breast and these cells are generally considered slowly proliferating and well-differentiated cell types (3). However, >50% of breast cancers express ERα at the time of initial diagnosis (1). These findings suggest a distinct role for ERα in the growth of normal, immortalized, and transformed mammary epithelial cells. Identifying signaling pathways that control these distinct functions of ERα may provide a unique opportunity to develop

Authors' Affiliations: Departments of [1]Pathology and Laboratory Medicine, [2]Internal Medicine, [3]Surgery, and [4]Biochemistry and Molecular Biology; [5]Walther Oncology Center, Indiana University School of Medicine; [6]Walther Cancer Institute, Indianapolis, Indiana; [7]Departments of Genetics and the Pathology and Laboratory Medicine, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; [8]Genetic Pathology Evaluation Centre and [9]Child and Family Research Institute, University of British Columbia, British Columbia, Vancouver, Canada

Received 1/16/07; revised 4/4/07; accepted 4/17/07.

Grant support: NIH grants RO1 CA89153 (H. Nakshatri), RO1 CA101227-01 (C.M. Perou), and National Cancer Institute/Eastern Cooperative Oncology Group grant CA37403 (S. Badve). The Genetic Pathology Evaluation Centre is supported by an unrestricted educational grant from Sanofi-Aventis. T.O. Nielsen and D.G. Huntsman are scholars of the Michael Smith Foundation for Health Research.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Requests for reprints: Harikrishna Nakshatri, R4-202, Indiana Cancer Research Institute, 1044 West Walnut Street, Indianapolis, IN 46202 and Sunil Badve, Department of Pathology, Indiana University School of Medicine, 635 Barnhill Drive, MS-A128, Indianapolis, IN 46202. Phone: 317-274-2053; Fax: 1-317-278-2018; E-mail: sbadve@iupui.edu.

©2007 American Association for Cancer Research.

doi:10.1158/1078-0432.CCR-07-0122

agents that prevent transformation of ERα-positive luminal epithelial cells from a slowly proliferating and well-differentiated phenotype to a rapidly proliferating aggressive phenotype.

Recent gene expression profiling studies have classified breast cancer into five distinct subtypes with unique molecular characteristics and prognostic significance (4, 5). These include luminal subtypes A and B, HER2+/ER-, basal-like, and normal-like subtypes. Luminal subtypes A and B are ERα-positive breast cancers with subtype A expressing higher levels of ERα and having a better prognosis than subtype B (5). Why patients with luminal subtype A tumors have better prognosis than patients with luminal subtype B tumors is not known. One possible explanation is that ERα functions differently in luminal A versus luminal B cancers, which may be due to the influence of additional factors, including transcription factors, coactivators, and corepressors that modulate ERα activity.

Transcription factors that are coexpressed with ERα in luminal subtype A include GATA3 and XBP-1 (4, 6). FOXA1/HNF3α, a forkhead family transcription factor, is receiving considerable attention with respect to ERα function because it interacts with *cis*-regulatory regions in heterochromatin and enhances the interaction of ERα with chromatin (7). Recent studies have shown the requirement of FOXA1 for optimum expression of ~50% of ERα-regulated genes and estrogen-induced proliferation (7–9). Thus, estrogen:ERα dependency of breast cancers for survival or proliferation may be related to the expression levels of FOXA1. Although previous studies have shown the coexpression of FOXA1, GATA3, XBP-1, and ERα in cell lines and breast cancer samples at the mRNA level (6, 10, 11), a detailed protein analysis relating FOXA1 to ERα and other disease markers such as progesterone receptor (PR), HER2, nodal status, and histologic grade and prognostic significance of such association have not been reported. We did immunohistochemistry for FOXA1 expression in breast carcinoma tissues of 438 patients with median follow-up of 15.4 years and compared FOXA1 expression with various established disease markers and breast cancer–specific survival. We show that FOXA1 expression is associated with ERα positivity, the luminal subtype A, and better breast cancer–specific survival.

Materials and Methods

Patient information and tissue microarray. A tissue microarray comprising duplicate cores of tumors from 438 patients with a median follow-up of 15.4 years was analyzed for FOXA1 expression. These samples were obtained from archival cases at Vancouver General Hospital between 1974 and 1995. Patient information, tumor pathology, tissue microarray preparation, and the expression of a number of biomarkers have been reported previously (Supplementary Information; ref. 12) and summarized in Table 1. Complete clinical treatment details are not available for all patients, which is a limitation of this cohort. In addition to the tissue microarrays, analysis of FOXA1 expression was done on normal breast tissue obtained from reduction mammoplasty specimens. As all personal identifiers had been removed from these samples, information about age or coexisting disease was not available for the normal samples. The study was approved by institutional review boards at Vancouver General Hospital and Indiana University School of Medicine.

Immunohistochemical staining for FOXA1. Expression of FOXA1 was analyzed using goat anti-human FOXA1 antibody (Santa Cruz Biotechnology) by immunohistochemistry. After dewaxing and hydration, 4 μm sections from formalin fixed paraffin-embedded tissue were treated with 0.05% citraconic anhydride (pH 7.40) in a decloaking chamber (BioCare) with chamber settings of SP1: 98°C for 45 min, and SP2: 0 s. The slides were then cooled for 20 min at room temperature. Endogenous peroxidase activity was blocked by Peroxoblock (Invitrogen Corporation) for 2 min. Nonspecific binding of antibodies were blocked with serum-free protein block (Dako) for 15 min. The slides were then incubated with polyclonal goat FOXA1 antibodies (1:250, Santa Cruz Biotechnology) for 1 h at room temperature. The sections were incubated with anti-goat horseradish peroxidase polymer conjugate (Invitrogen) according to the manufacturer's instructions. The stain was visualized using 3,3′-diaminobenzidine plus (Dako) and hematoxylin QS (Vector Laboratories) counterstain. To verify the specificity of staining, nonimmune goat serum and PBS controls were used. Interpretable staining was obtained in 404 of 438 patients. Percentage of staining was categorized as "0" if there was no nuclear expression, "1" for up to 10% positive tumor nuclei, "2" for 11% to 20% and so on till a maximum score of "10." Intensity was scored as "1+," "2+," and "3+" for weak, moderate, and strong staining, respectively. Percentage (P) and intensity (I) of nuclear expression were multiplied to generate numerical score ($S = P \times I$).

Immunohistochemical staining for ER, PR, and HER-2/neu. Immunohistochemical staining was done on serial sections for ER and PR (Clones SP1, NeoMarker and PR636, DAKO, respectively,) and Hercept Test using protocols recommended by the manufacturer (Dako). ER and PR status was assessed from immunohistochemistry stained sections using a 10% cutoff. HER-2/neu expression was scored as 0, 1+, 2+, and 3+ as recommended by the manufacturer (DAKO). In case of discrepant scores from two cores, the higher score of two was taken for the statistical analysis.

Table 1. Description of the patients and characteristics of their tumors

Characteristics	Data
Age at diagnosis, y	
Mean	61.01
Median	63
Range	59.03
Lymph node status, *n* (%)	
Negative	266 (60.7)
Positive	126 (28.8)
Unknown	46 (10.5)
ER status, *n* (%)	
Negative	93 (21.5)
Positive	216 (49.3)
Unknown	129 (29.4)
Tumor grade, *n* (%)	
I	94 (21.5)
II	236 (53.9)
III	108 (24.6)
Tumor size, *n* (%)	
≤5 mm	4 (2.2)
≤1 cm	64 (14.6)
≤2 cm	142 (32.4)
>2 cm	147 (33.6)
Unknown	81
Histology, *n* (%)	
DCIS	16 (3.6)
IDC, NOS	353 (80.5)
IDC, variants	24 (5.5)
ILC	43 (10)
IDC and ILC	2 (0.4)

Abbreviations: DCIS, ductal carcinoma *in situ*; IDC, infiltrating ductal carcinoma; ILC, infiltrating lobular carcinoma; NOS, not otherwise specified.

Table 2. Bivariate analysis of FOXA1 (n = 404) with other disease markers

Variable	Correlation coefficient	Significance (two-tailed)	No. patients
ER	0.510	0.000001	303
Luminal type A*	0.415	0.000001	259
PR	0.235	0.00001	337
Histology grade	-0.176	0.001	347
Nodal status	0.025	0.643	354
Her2	0.015	0.787	317

NOTE: Complete information on three variables (i.e., ER, PR, and FOXA1) was available for 294 patients.
*Luminal type A is defined ER and/or PR positive and Her2 negative.

Statistical methods. FOXA1 expression was compared with tumor grade, nodal status, and expression of ER, PR, HER2, and the luminal subtype A (ref. 13; defined as ER and/or PR+, and HER2-neg) in Spearman's two-tailed correlation tests. Information regarding each of these variables was not available for all patients, a known limitation of tissue microarray based studies and old cohort studies. Number of patients for whom information was available is given variable-wise in Table 2. Kaplan-Meier analysis was done using log-rank test for comparison of linear trends with cancer-specific survival as primary end point. Cox proportional hazard ratio (HR) model was used for the multivariate survival analysis. We used univariate Cox regression to calculate HRs for the subgroups. All tests were two-sided. We used 5% α level to determine significance. SPSS 14.0 (SPSS, Inc.) statistical package was used to perform the analysis.

We used X-tile version 3.5.0 (14) to define the optimal cutoff point for the negative and positive scores for FOXA1. On a training set of patients (n = 202) generated by the program, the greatest difference in linear survival trends was achieved comparing scores 0 to 3 versus 4 to 30 (χ^2 = 5.1, P = 0.024). This X-tile determined cutoff was confirmed on the validation set (n = 202; χ^2 = 7.2, P = 0.007). This algorithm uses a training validation approach to define optimal prognostic cutoffs from continuous or ordinal tumor biomarker scoring data. Using this approach, scores of 0 to 3 were defined as $FOXA1_{low}$ and 4 to 30 as $FOXA1_{high}$. Our attempts of analysis with different scoring variables such as intensity or percentage of cells with intensity 3 yielded poorer discrimination ability with respect to survival than the cutoff values obtained by X-Tile analysis. Hence, we used cutoffs obtained by X-tile analysis for all subsequent analysis.

Results

FOXA1 expression in normal breast and breast cancer

Representative FOXA1 immunostaining of normal breast and breast cancers is shown in Fig. 1. FOXA1 expression was observed in a few luminal epithelial cells of the normal breast. The expression was restricted to the nucleus with little or no cytoplasmic staining. Invasive cancers showed variable expression; none, weak, moderate, and strong. Staining was nuclear in both normal and cancerous tissues. Thus, it seems that FOXA1 is expressed in a specific subpopulation of normal luminal epithelial cells. High level of FOXA1 expression ($FOXA1_{high}$; score >3) was seen in 300 of 404 interpretable breast cancers.

Correlation of FOXA1 expression with other disease markers

We compared FOXA1 expression with ERα, PR, HER2/neu, histologic grade, nodal status, and luminal subtype A (Table 2). FOXA1 expression correlated significantly with ERα (P = 0.000001), PR (P = 0.00001), and luminal subtype A (P = 0.000001). Eighty-four percent (205 of 244) of hormone receptor–positive patients showed high FOXA1 expression compared with only 32% (16 of 50) of hormone receptor negative patients. Similarly, 84% (168 of 200) of luminal subtype A patients showed high FOXA1 expression compared with only 40.67% (24 of 59) of luminal subtype B. Worsening histologic grade also showed loss of FOXA1 expression (P = 0.001). FOXA1 expression did not correlate with nodal status or HER2/neu expression.

Survival analysis

Univariate analysis. Univariate analysis showed (Table 3; Fig. 2) nodal status, tumor size, FOXA1, and ER as significant predictors of cancer-specific survival. HER-2/neu and tumor grade did not significantly affect cancer-specific survival.

Multivariate analysis. Multivariate analysis was done using a Cox regression model, which included nodal status, ER, FOXA1, and tumor size as variables (n = 259, patients with complete information available on all included variables). It showed nodal status [HR, 2.32; 95% confidence interval (95% CI), 1.44-3.74; P = 0.001] and tumor size (HR, 1.95; 95% CI, 1.16-3.28; P = 0.039) to be the significant predictors of cancer-specific survival, whereas FOXA1 [HR, 0.58; 95% CI, 0.33-1.02; P = 0.060 not significant (NS)] and ER (HR, 0.63; 95% CI, 0.34-1.15; P = 0.131 NS) did not significantly affect survival.

Subgroup analysis. We did two post hoc subgroup analyses; luminal subtype A subgroup and ER-positive lymph node–negative subgroup. Luminal subtype A patients expressing high FOXA1 had significantly better survival with relative risk of death due to breast cancer being 0.60 (P = 0.024; Fig. 3). ER-positive lymph node–negative patients expressing high FOXA1 also showed a trend toward better survival with relative risk of death due to breast cancer being 0.63 (P = 0.215 NS). Five and 10-year cancer-specific survival in ER-positive lymph node–negative patients expressing high FOXA1 was 92.6 ± 2.5% and 85.4 ± 3.5%, respectively, compared with 80.0 ± 10.3% and 72.7 ± 11.7% in $FOXA1_{low}$ patients.

Prognostic significance of FOXA1 in combination with ERα and PR

At present, PR has been used as a marker for predicting response to endocrine therapy and event-free survival of patients with ERα-positive breast cancers (15). Endocrine therapy–resistant ERα-positive tumors often show loss of PR expression, which is suggested to indicate switching of cell survival from ERα:estrogen–dependent signaling to ERα:growth factor

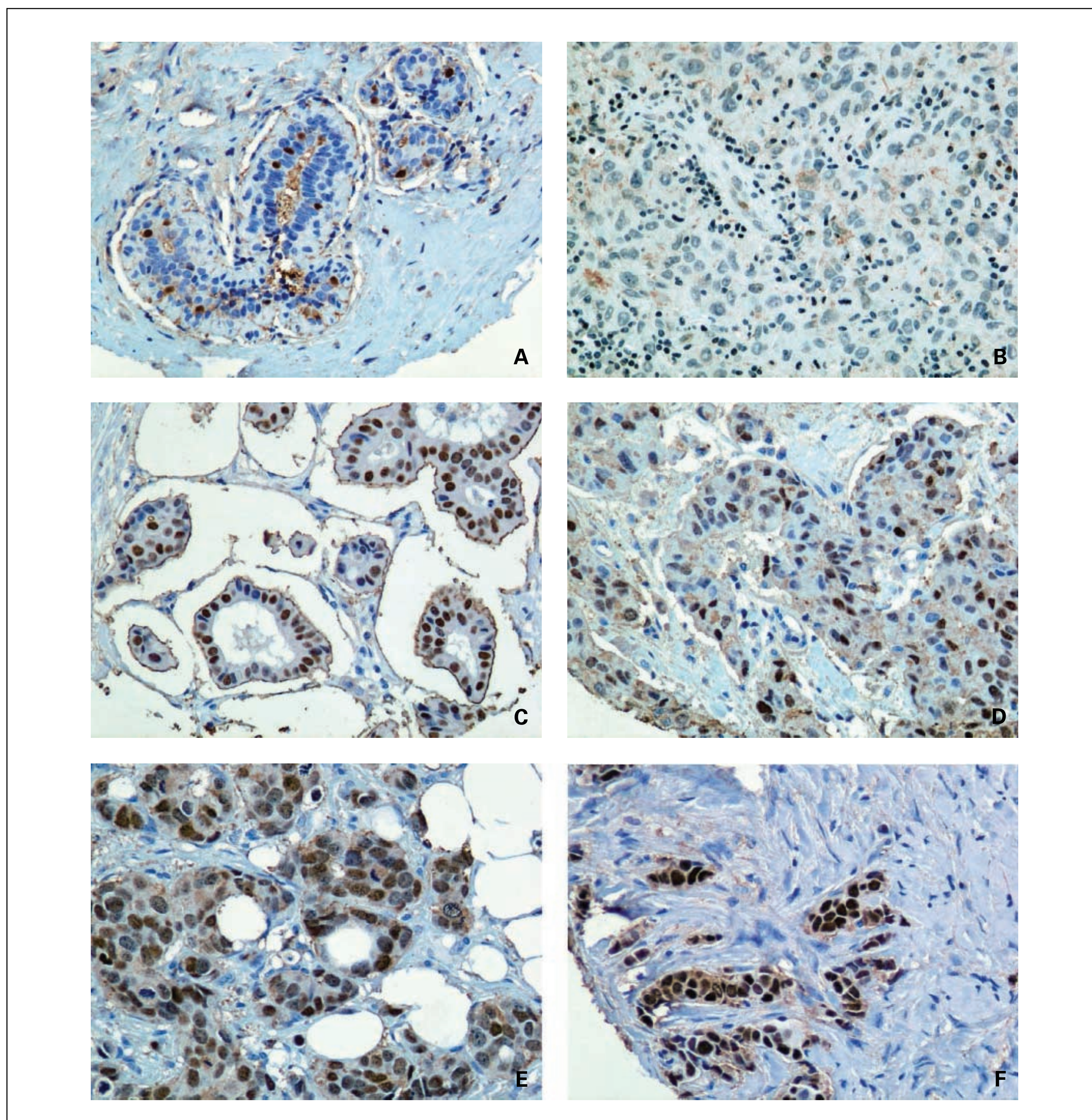

Fig. 1. Immunohistochemical analysis of FOXA1 in breast cancer. Normal breast contains rare FOXA1-positive cells (*A*), whereas invasive carcinomas show three distinct patterns of expression: no expression (*B*), weak expression (*C* and *D*), and strong expression (*E* and *F*). Numerical scores: *A* = 3, *B* = 0, *C* = 6, *D* and *E* = 16, and *F* = 30.

cross-talk–dependent signaling (15). To determine whether FOXA1 expression has prognostic significance within the context of PR expression, we analyzed effect of loss of PR (ER+/PR+ versus ER+/PR-), loss of FOXA1 (ER+/FOXA1+ versus ER+/FOXA1-), and loss of both PR and FOXA1 (ER+/PR+/FOXA1+ versus ER+/PR-/FOXA1-) on survival. Loss of PR expression did not affect survival with relative risk of death due to breast cancer being 1.36 (95% CI, 0.79-2.34; $P = 0.273$), whereas loss of FOXA1 expression independently affected survival with relative risk of death due to breast cancer being 2.16 (95% CI, 1.21-3.85; $P = 0.009$; Fig. 4). Loss of FOXA1 expression is a better predictor of therapy resistance compared with loss of PR expression. Relative risk due to combined loss of PR and FOXA1 (HR, 2.29) was similar to that of loss of FOXA1 alone (HR, 2.16).

Discussion

We conducted this study with few specific goals. One of the goals was to improve our understanding of ER/FOXA1

Table 3. Univariate analysis of various variables

Variable	Comparison	HR (95% CI)	Significance
Nodal status	N_0 vs N_+	2.63 (1.79-4.00)	0.0000012
Tumor size	T_1 vs T_2	1.95 (1.31-2.91)	0.00001
	T_1 vs T_3	3.47 (2.00-6.00)	
FOXA1	$FOXA1_{low}$ vs $FOXA1_{high}$	0.51 (0.36-0.75)	0.0004
ER	ER- vs ER+	0.54 (0.34-0.85)	0.012
Tumor grade	Grade 1 vs grade 2	1.51 (0.63-3.62)	0.065
	Grade 1 vs Grade 3	2.20 (0.95-5.08)	
HER-2/neu	HER-2/neu- vs HER-2/neu+	1.59 (0.97-2.56)	0.077

relationship in breast cancer patients. The other goal was to see if FOXA1 expression can be studied by a clinically reproducible method (immunohistochemistry) and whether such expression analysis can provide a clinically useful prognostic factor especially in low-risk breast cancer patients. We found FOXA1 to be a positive prognostic factor in breast cancer (HR, 0.51; univariate analysis), particularly among ERα-positive cases (HR, 0.46). FOXA1 is associated with luminal subtype A breast cancers (P = 0.000001) and not with luminal subtype B.

FOXA1 is a ''winged helix'' transcription factor, which has recently been dubbed as a ''pioneer factor'' responsible for the recruitment of ERα to the genome (7). Depletion of FOXA1 protein in MCF-7 breast cancer cells leads to reduced estrogen-dependent gene expression and proliferation, which is consistent with its role in mediating the effects of estrogen (8, 9). The COOH-terminal region of FOXA1 interacts with histones H3 and H4 and this interaction is responsible for opening compacted chromatin (16). By opening chromatin, FOXA1 may permit efficient interaction of ERα with its response elements and subsequent interaction of ERα-associated histone modifying enzymes with histones. Consistent with this possibility, about half of estrogen-regulated genes contain binding sites for FOXA1 (8). Optimum expression of these estrogen-regulated genes may occur only in cells that coexpress ERα and FOXA1 and only these cells may be addicted to estrogen-dependent survival and proliferation signaling pathways.

PR negativity is often considered as a marker for the gain of hormone-independent growth properties by ERα-positive breast cancers through increased cross-talk between ERα and growth factor signaling pathways (15). We observed that patients who are ER+/FOXA1-/PR- have worse prognoses than patients who are ER+/FOXA1+/PR+. It is possible that ER+/FOXA1-/PR- tumors have acquired hormone-independent growth properties because of the shift in ERα function from traditional ERα:FOXA1:estrogen regulated pathway to a ERα:growth factor–activated signaling pathways. Thus, simultaneous analysis of ERα, FOXA1, and PR may provide the earliest indication for hormone independence and/or ERα:growth factor signaling pathway cross-talk in ERα-positive breast cancers.

We also did an exploratory subgroup analysis in ER-positive lymph node–negative patients. We aimed at testing possible utility of FOXA1 as a classifier in this so-called low-risk patient group, which is toughest to deal with when it comes to taking adjuvant treatment decisions, especially decisions like whether to give chemotherapy. A prognostic marker that can further identify patients with very low risk of recurrence will be very useful to avoid toxic therapies in patients who are not likely to derive any additional benefits. Although FOXA1 expression

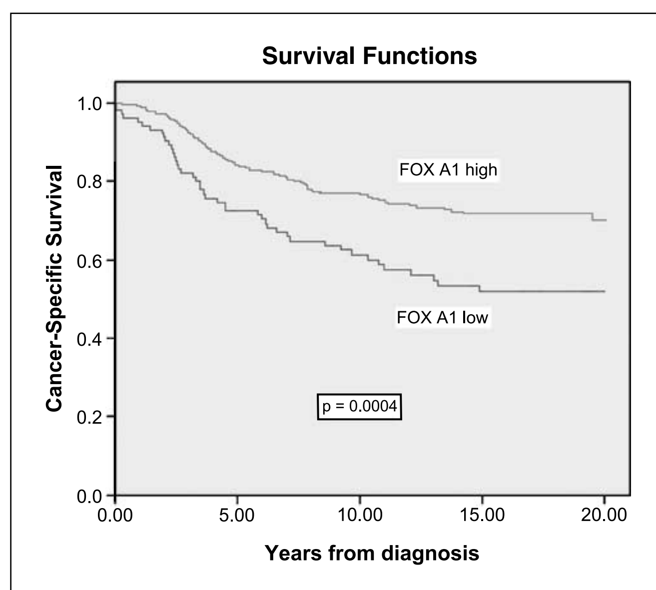

Fig. 2. Kaplan-Meier analysis of cancer-specific survival (all patients).

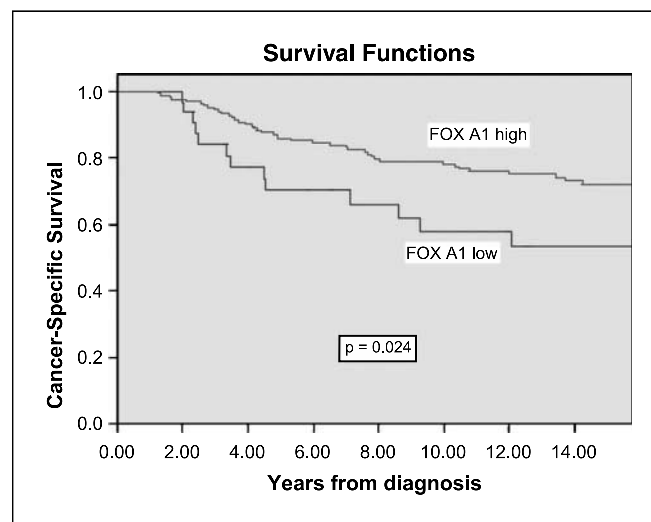

Fig. 3. Kaplan-Meier analysis of cancer-specific survival (luminal subtype A patients).

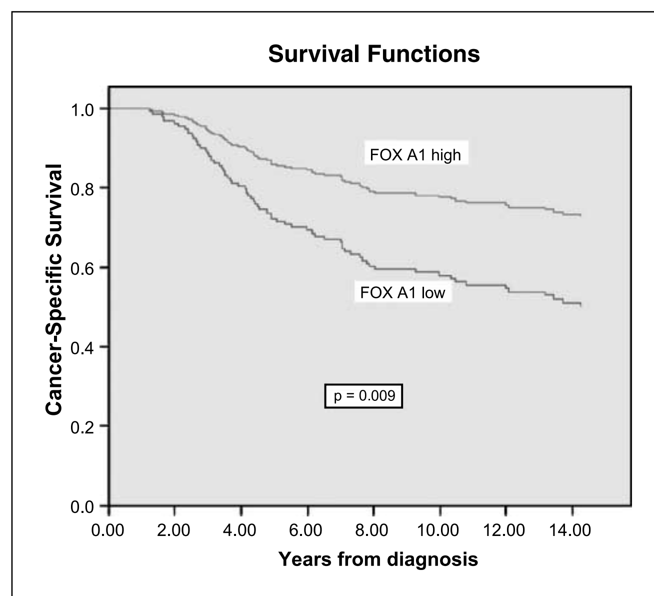

Fig. 4. Kaplan-Meier analysis of cancer-specific survival (ER+ patients).

was not a statistically significant predictor of survival in this subgroup, a 10-year cancer-specific survival of 85.4 ± 3.5% in $FOXA1_{high}$ patients (in this cohort with >50% T_2 or larger tumors from early systemic therapy days) seems promising. It is certainly worth further exploration in larger studies to see if FOXA1 can be used as a prognostic marker in small ER-positive lymph node–negative tumors to avoid toxic therapies in patients with high FOXA1 expression.

We did not have complete clinical treatment details available for all patients in this old cohort (1974-1995). However, because this is a patient cohort spreading over many years, during which breast cancer treatment changed significantly, treatment information in any case cannot be analyzed meaningfully. Because this is a tissue microarray–based study, information regarding all variables is not available for all 404 patients with interpretable FOXA1 staining. This resulted in individual analyses having lower number of patients than 404, decreasing statistical power of the study.

Expression patterns of FOXA1 in normal breast and tumors are strikingly similar to that of ERα. In normal breast, ERα expression is observed only in 10% to 15% of luminal epithelial cells (3), which is similar to the FOXA1 expression that we observed in the normal breast (Fig. 1). Most of the ERα-positive normal cells are nondividing and do not express Ki67, a proliferation marker (3). To determine whether FOXA1 is coexpressed with ERα or is excluded from Ki67-positive cells, we attempted dual immunohistochemistry, which was not successful. The intensity of FOXA1 staining in luminal epithelial cells of normal breast and tumors that are highly positive for FOXA1 is similar; an identical finding has also been observed for ER and GATA3 (17). Hence, FOXA1 expression in breast tumors seems not due to cancer-specific overexpression but rather reflects initiation of cancers from luminal cells that express FOXA1.

The functional role of FOXA1 in normal breast is yet to be determined. FOXA1-/- mice are viable for 2 weeks and these animals have been analyzed extensively for prostate development (18). FOXA1-/- animals show a severely altered ductal pattern lacking differentiated or mature luminal epithelial cells. It has been suggested that FOXA1 in concert with the androgen receptor is involved in differentiation of prostate epithelium by regulating the expression of genes such as *Nkx3.1*, *Shh*, and *FOXA2* (18). By analogy, FOXA1 in concert with ERα may be involved in mammary ductal morphogenesis and differentiation. ERα-expressing cells have previously been shown to control proliferation and gene expression pattern in stromal and ERα-negative luminal cells through paracrine mechanisms (19–21). This function of ERα may be dependent on FOXA1. Interestingly, FOXA1 expression is regulated by estrogen (9). Thus, proliferation and differentiation of normal breast epithelium may be under the control of the FOXA1:ERα axis. Because of their mutual dependency for normal function, signaling events that alter the expression levels or function of either of them may be sufficient for initiating transformation of FOXA1+/ERα+ cells or acquisition of estrogen-independent growth properties.

Emerging data based on molecular signatures clearly shows the existence of subclasses within ER-positive tumors, which have different clinical courses and different likelihoods of response to therapy (5, 22, 23). The differences between these classes involve multiple pathways and are not limited to differences in proliferation rates. This feature, thus far, has been difficult to duplicate using simple protein/immunohistochemistry markers. In this light, it is of interest to note that FOXA1 is an ER-regulated transcription factor that additionally controls downstream transcription of estrogen:ER–regulated genes as well as exerts direct control on cell proliferation possibly via regulation of $p27^{Kip1}$ (8, 9, 24).

FOXA1 is a prognostic factor in ER-positive breast cancers and is associated with luminal subtype A. Loss of FOXA1 expression may mean shifting of ERα function from traditional ERα:FOXA1:estrogen–regulated pathway to a ERα:growth factor activated signaling pathway resulting in resistance to hormonal therapy. It will be worthwhile to study FOXA1 in a more recent and larger cohort with smaller tumors to validate the utility of FOXA1 expression analysis in identifying and stratifying ER positive patients and to define its exact clinical utility as a prognostic marker.

Acknowledgments

We thank P. Bhat-Nakshatri for technical assistance.

References

1. Ali S, Coombes RC. Endocrine-responsive breast cancer and strategies for combating resistance. Nat Rev Cancer 2002;2:101–12.
2. Deroo BJ, Korach KS. Estrogen receptors and human disease. J Clin Invest 2006;116:561–70.
3. Clarke RB, Howell A, Potten CS, Anderson E. Dissociation between steroid receptor expression and cell proliferation in the human breast. Cancer Res 1997; 57:4987–91.
4. Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature 2000;406: 747–52.
5. Sorlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001;98:10869–74.
6. Oh DS, Troester MA, Usary J, et al. Estrogen-regulated genes predict survival in hormone

receptor-positive breast cancers. J Clin Oncol 2006; 24:1656–64.

7. Carroll JS, Brown M. Estrogen receptor target gene: an evolving concept. Mol Endocrinol 2006;20:1707–14.

8. Carroll JS, Liu XS, Brodsky AS, et al. Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1. Cell 2005;122:33–43.

9. Laganiere J, Deblois G, Lefebvre C, Bataille AR, Robert F, Giguere V. From the cover: location analysis of estrogen receptor α target promoters reveals that FOXA1 defines a domain of the estrogen response. Proc Natl Acad Sci U S A 2005;102:11651–6.

10. Lacroix M, Leclercq G. About GATA3, HNF3A, and XBP1, three genes co-expressed with the oestrogen receptor-α gene (ESR1) in breast cancer. Mol Cell Endocrinol 2004;219:1–7.

11. Doane AS, Danso M, Lal P, et al. An estrogen receptor-negative breast cancer subset characterized by a hormonally regulated transcriptional program and response to androgen. Oncogene 2006;25:3994–4008.

12. Sutherland BW, Kucab J, Wu J, et al. Akt phosphorylates the Y-box binding protein 1 at Ser102 located in the cold shock domain and affects the anchorage-independent growth of breast cancer cells. Oncogene 2005;24:4281–92.

13. Carey LA, Perou CM, Livasy CA, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA 2006;295:2492–502.

14. Camp RL, Dolled-Filhart M, Rimm DL. X-tile: a new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization. Clin Cancer Res 2004;10:7252–9.

15. Cui X, Schiff R, Arpino G, Osborne CK, Lee AV. Biology of progesterone receptor loss in breast cancer and its implications for endocrine therapy. J Clin Oncol 2005;23:7721–35.

16. Cirillo LA, Lin FR, Cuesta I, Friedman D, Jarnik M, Zaret KS. Opening of compacted chromatin by early developmental transcription factors HNF3 (FoxA) and GATA-4. Mol Cell 2002;9:279–89.

17. Usary J, Llaca V, Karaca G, et al. Mutation of GATA3 in human breast tumors. Oncogene 2004; 23:7669–78.

18. Gao N, Ishii K, Mirosevich J, et al. Forkhead box A1 regulates prostate ductal morphogenesis and promotes epithelial cell maturation. Development 2005;132:3431–43.

19. Mueller SO, Clark JA, Myers PH, Korach KS. Mammary gland development in adult mice requires epithelial and stromal estrogen receptor α. Endocrinology 2002;143:2357–65.

20. Zeps N, Bentel JM, Papadimitriou JM, D'Antuono MF, Dawkins HJ. Estrogen receptor-negative epithelial cells in mouse mammary gland development and growth. Differentiation 1998;62:221–6.

21. Anderson E, Clarke RB. Steroid receptors and cell cycle in normal mammary epithelium. J Mammary Gland Biol Neoplasia 2004;9:3–13.

22. Fan C, Oh DS, Wessels L, et al. Concordance among gene-expression-based predictors for breast cancer. N Engl J Med 2006;355:560–9.

23. O'Shaughnessy JA. Molecular signatures predict outcomes of breast cancer. N Engl J Med 2006;355: 615–7.

24. Williamson EA, Wolf I, O'Kelly J, Bose S, Tanosaki S, Koeffler HP. BRCA1 and FOXA1 proteins coregulate the expression of the cell cycle-dependent kinase inhibitor p27(Kip1). Oncogene 2006;25:1391–9.