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FOXA1 protein expression in ER+ and ER− breast cancer in relation to parity and breastfeeding in Black and White women

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Abstract

Background: Forkhead box protein A1 (FOXA1) promotes luminal differentiation, and hypermethylation of the gene can be a mechanism of developing estrogen receptor-negative (ER−) breast cancer. We examined FOXA1 in breast tumor and adjacent normal tissue in relation to reproductive factors, particularly higher parity and no breastfeeding, that are associated with ER− tumors.

Methods: We performed immunohistochemistry for FOXA1 in breast tumors (n=1,329) and adjacent-normal tissues (n=298) in the Women's Circle of Health Study (949 Blacks and 380 Whites). Protein expression levels were summarized by histology (H) scores. Generalized linear models were used to assess FOXA1 protein expression in relation to reproductive factors by ER status.

Results: ER+ vs. ER− tumors had higher FOXA1 protein expression (P<0.001). FOXA1 expression was higher in tumor versus paired adjacent-normal tissue in women with ER+ or non-triple-negative cancer (both P<0.001), but not in those with ER− or triple-negative cancer. Higher number of births (1, 2, and 3+) was associated with lower FOXA1 protein expression in ER+ tumors (differences in H score, or β= −8.5, 95% CI= −15.1 to −2.0), particularly among parous women who never breastfed (β= −10.4, −19.7 to −1.0), but not among those who breastfed (β =

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−7.5, 95% CI= −16.9 to 1.8). The associations for ER− tumors were similar, although they were not statistically significant.

Conclusions: In this tumor-based study, higher parity was associated with lower FOXA1 expression in ER+ tumors, and breastfeeding may ameliorate the influence.

Impact: These findings contribute to our understanding of FOXA1 methylation and breast cancer etiology.

Keywords

FOXA1; breast cancer; reproductive factors; African Americans/Blacks

Introduction

In the United States, African American/Black women are more likely than White women to be diagnosed with poor prognosis breast cancers, particularly estrogen receptor-negative (ER −) breast cancer.[1,2] Until recently, little was known with respect to biological risk factors for ER− breast cancer in Black women. Data from the African American Breast Cancer Epidemiology and Risk (AMBER) Consortium provide convincing evidence that, while having children is associated with reduced risk of ER+ breast cancer, it is linked to increased risk of ER− breast cancer in Black women.[3] Notably, breastfeeding may modulate the association, as the increased risk of ER− cancer was not observed among those who breastfed,[3] a finding that is consistent with data from predominately White women.[4] There also appeared to be differential risk relationships by ER status with age at menarche.[5,6] Together, these findings suggest that hormonal exposure in early years could affect later development of ER− breast cancer in Black women, who are more likely to experience menarche at a younger age than White women, have more children, and not breastfeed.[7]

The mechanisms underlying relationships between reproductive factors and development of ER− breast cancer are largely unknown. It is possible that early reproductive events and hormone perturbation could influence breast cancer subtypes through effects on progenitor cells in the mammary gland.[8] One possible mechanism whereby reproductive events could influence whether luminal progenitor cells give rise to ER− versus ER+ breast tumors is through DNA methylation, an epigenetic modification that occurs in specific patterns throughout development. DNA methylation may be affected by the milieu of hormonal changes that occur during puberty, pregnancy, and lactation.[9,10] Our group compared methylation patterns between Black and White women and found that one of the top differentially methylated loci in ER− breast tumors was within the Forkhead box A1 (*FOXA1*) gene.[11] As a pioneer transcription factor, FOXA1 promotes the differentiation of luminal progenitors to mature luminal cells while repressing the basal phenotype.[12–16] *BRCA1*-deficient breast tumors, the vast majority of which exhibit a ER− and basal-like phenotype, also have increased DNA methylation and silencing of the *FOXA1* gene.[17] Our analysis also showed that *FOXA1* DNA methylation levels in ER− tumors may differ by number of births and breastfeeding behaviors,[11] suggesting that FOXA1 may be an important link between reproductive exposures and the rise of ER− tumors.

To further elucidate the potential mechanism that reproductive factors modulate ER− breast cancer risk through FOXA1, we examined FOXA1 protein expression in breast tumor and adjacent normal tissue from 949 Black women and 380 White women in relation to reproductive factors. We hypothesized that the change of FOXA1 protein expression from adjacent normal to tumor tissue may predict ER status. Also, we predicted that FOXA1 protein expression in tumor tissues would be lower in women with more children and no history of breastfeeding.

Materials and Methods

Patient Samples

Breast tumor tissue samples were from participants in the Women's Circle of Health Study (WCHS), a case-control study conducted in metropolitan New York City and 10 counties in eastern New Jersey which was designed to investigate risk factors for aggressive breast cancer in Black and White women. Details on study recruitment and participation rates have been described elsewhere.[18,19] This study was approved by the Institutional Review Boards of all participating institutions and all study participants provided written informed consent prior to the baseline interview. In-home interviews were conducted to obtain data on known and suspected risk factors for breast cancer. As part of the informed consent, participants were asked to sign a release for pathology reports and archived tumor specimens, with more than 95% of patients agreeing. Formalin-fixed paraffin-embedded tumor and matched adjacent-normal tissue blocks were requested from hospitals where the diagnostic surgical procedure was performed; for a subset of cases (44%) where hospitals would not release blocks, whole sections were requested. Hematoxylin and Eosin stained sections of tissue specimens were reviewed by a study pathologist (T. K.) for annotating tumor-dense regions for the construction of TMAs. Three 0.6 mm cores from a tumor tissue block and if available, two cores from an adjacent-normal tissue block were placed into TMA blocks for analysis. Completed TMAs and whole sections were stored in nitrogen-filled desiccators at room temperature to preserve antigenicity. Clinical and tumor characteristics, including the expression status of hormone receptors (HR, i.e., ER and progesterone receptor [PR]) and human epidermal growth factor receptor 2 (HER2), were based on patients' pathology reports. The statistical analysis included 1,329 cases with conclusive FOXA1 staining results from either invasive cancer or ductal carcinoma *in situ* (DCIS), including 298 who also had adjacent normal tissue.

Immunohistochemistry and image analysis

TMAs and whole sections containing breast tumor samples were stained for FOXA1 using the monoclonal primary antibody HNF-3α (Santa Cruz Biotechnology, Catalog No. sc-101058, Dallas, TX), which we had previously optimized.[11] Stained slides were digitally imaged at ×20 magnification using the Aperio ScanScope XT (Aperio Technologies, Vista, CA). Automated image analysis of immunohistochemistry staining was performed with Aperio GENIE, a computer-assisted classifier to identify tumor regions.[20] Whole section slides were annotated manually to identify tumor epithelial regions for the image analysis. Adjacent-normal tissue sections followed the same manual annotation process as tumor tissue sections to identify regions of lobule and ducts. Tissue cores or whole sections with

low cellularity (<25 cells) were excluded. The percent of cells stained was recorded in each intensity category: 0, 1+ (only partial or weak staining), 2+ (moderate and complete staining), and 3+ (intense and complete staining). Tumor cores on TMAs were collapsed into patient-level data using a cellularity-weighted approach, as previously described.[21] A histological score (H-score) at the patient level was calculated by the formula: [1 × (% cells 1+) + 2 × (% cells 2+) + 3 × (% cells 3+)] × 100 (Figure 1).[22] Tumor and adjacent-normal tissue followed the same scoring protocol. Distributions of FOXA1 protein expression according to specimen and tissue types in ER+ and ER− tumors are presented in Supplemental Table 1.

Reproductive characteristics

Data on reproductive history, including age at first menstrual period (age at menarche), number of pregnancies, and the outcome of each pregnancy were collected as a part of a comprehensive in-person interview. For each birth, participants were asked about the status and duration of breastfeeding. Women were defined as postmenopausal if they reported that they had ceased menstruation naturally at least one year before the reference date, or if they had both ovaries removed.

Statistical analysis

We examined the difference in FOXA1 protein expression between the paired tumor and adjacent normal tissue by paired t-tests, overall and within molecular subtypes. The molecular subtype was defined as hormone receptors (ER and PR) positive (HR+)/HER2−, HR+/HER2+, HR−/HER2+, or HR−/HER2−. We derived the difference of H-score between the tumor and adjacent normal tissue and modeled the difference for predicting ER+ (vs. ER −) tumors using logistic regressions, adjusting for age, race, grade, and stage.

FOXA1 H-scores were examined in relation to clinicopathological and reproductive characteristics using analysis of variance (ANOVA). For reproductive characteristics, the associations were examined separately for ER+ and ER− tumors because their influences on breast cancer risk vary by ER status.[7,23] Associations of reproductive characteristics with FOXA1 protein expression in tumors were assessed using generalized linear models with the gamma distribution with log link, adjusted for age at diagnosis and race. The model fit was assessed based on the Bayesian information criterion. We performed two sets of sensitivity analyses to evaluate the potential influence of confounding bias. First, because 102 (7.7%) and 17 (1.2%) participants had a missing value on grade and stage, respectively, these variables were not included in the final model. Regression models were fit with additional adjustment for breast cancer stage and tumor grade to evaluate their influence on the associations. Second, we further included specimen type (TMA vs. whole section tissue) in the regression models, as FOXA1 protein expression levels tended to be higher in whole sections than TMAs (Supplemental Table 1). Because the association of parity with ER− tumor risk differed by breastfeeding in Blacks,[3] we evaluated the difference in the association of parity with FOXA1 protein expression stratified by ever versus never breastfeeding and then race. Statistical interactions were assessed by examining a product term of parity and breastfeeding among parous women, using Wald tests in regression

models. The analyses were performed with SAS v9.4. All tests of statistical significance were two-sided; a P-value of less than 0.05 was considered statistically significant.

Results

The univariate results showed that FOXA1 protein expression was lower in tumors of Black (versus White) women and of more aggressive characteristics, with expression lower in tumors that were higher grade, larger in size, and more advanced stage (Table 1). Invasive ductal carcinoma had lower FOXA1 protein expression than DCIS, but invasive lobular carcinoma showed the highest levels among the histological types. ER− tumors had lower FOXA1 protein expression than ER+ tumors, and triple-negative breast cancer (TNBC), i.e., HR−/HER2− (H-score = 45), had the lowest expression among the molecular subtypes. Comparisons between paired tumor and adjacent normal tissues showed significant upregulation of FOXA1 protein expression in tumor tissues among HR+/HER2+, HR+/HER2−, and HR−/HER2+ subtypes (all P<0.001), but downregulation in tumor tissues among TNBC (P=0.005) (Figure 2). In multivariable models adjusting for age, race, grade, and stage, the increase of FOXA1 protein expression from adjacent normal to tumor tissue significantly predicted ER+ vs. ER− subtypes (odds ratio = 1.13 95% CI= 1.09– 1.18, P<0.001) and non-TNBC vs. TNBC subtypes (odds ratio= 1.22, 95% CI= 1.14–1.31, P<0.001 for each 10 point increase in H-score; Supplemental Table 2).

Table 2 shows the univariate results for associations between FOXA1 protein expression and reproductive characteristics in tumors according to ER status, and in adjacent normal tissue. Among parous women, later age at first live birth was associated with higher levels of FOXA1 protein expression in ER− breast cancer (P = 0.019). Having more children was associated with lower FOXA1 H-scores in ER+ tumors (P = 0.046). A similar reduction of FOXA1 protein expression with increased parity was also observed for ER− tumors and adjacent normal tissue, but the differences were not statistically significant. FOXA1 protein expression did not appear to differ by history of breastfeeding among parous women.

In multivariable analyses (Table 3), there were suggested associations that parous women had lower FOXA1 protein expression in ER+ and ER− tumors compared to nulliparous women, but the estimates (β = −5.3 [P=0.32] and −13.4 [P=0.27], respectively) were not significant. Among parous women with ER+ tumors, a higher number of births was associated with lower FOXA1 protein expression in tumors (β = −8.5, 95% CI= −15.1 to −2.0, P=0.010). When stratified by breastfeeding, the association between number of births and FOXA1 protein expression in ER+ tumors was significant in those who never breastfed (β = −10.4, 95% CI= −19.7 to −1.0, P=0.029), but not among those who reported having breastfed (β = −7.5, 95% CI= −16.9 to 1.8, P=0.11) (P-interaction = 0.57). Additional adjustment for tumor grade, breast cancer stage, and specimen type did not change the associations (Supplemental Table 3). Among women with ER− breast cancer, a higher number of births was also associated with lower FOXA1 protein expression overall (β = −10.3, P=0.13) and in those who never breastfed (β = −13.9, P=0.20). The associations were not statistically significant, likely due to reduced sample size.

When further stratified by race (Table 4), a borderline-significant inverse association between parity and FOXA1 protein expression in ER− tumors was evident in samples from Black women (β = −11.8, P=0.07), but not in samples from White women (β = 5.7, P=0.84). In ER− tumors from Black women, the inverse association was stronger in women who had never breastfed compared to those who had breastfed (β = −16.9 [P=0.11] vs. −3.8 [P=0.38]), although the estimates were not significant. Age at menarche was not associated with FOXA1 protein expression in ER+ or ER− tumors in multivariable models (Supplemental Table 4).

Discussion

We observed that ER+ vs. ER− tumors had higher FOXA1 protein expression, and the comparisons of FOXA1 expression between tumor and adjacent normal tissue suggest that FOXA1 is upregulated in ER+ and other non-TN breast cancers, but downregulated in TNBC. These findings are consistent with the role of FOXA1 in promoting the luminal differentiation but suppressing the basal differentiation of the progenitor cells during breast tumorigenesis. Also, a higher number of births was associated with lower FOXA1 protein expression in ER+ tumors, and the association was attenuated in women who breastfed. For ER− tumors, consistent associations of parity and breastfeeding with FOXA1 were observed overall and in Black women, although the estimates were not significant possibly due to reduced sample size. Our race-specific findings should be interpreted with caution because of the smaller sample size of White women compared to Black women. We were unable to examine the association between FOXA1 protein expression in ER− tumors and parity by breastfeeding in White women. Another limitation was that we were unable to eliminate potential confounding bias, as a limited number of confounders were adjusted. In addition, the tissue samples were not from a single source, as a large proportion of patients' tissue samples were only available as whole section slides. However, the difference would not lead to systematic bias because the specimen type (whole section versus TMA) was unlikely related to the reproductive factors and its inclusion in the multivariable models did not change the results.

The biological action of FOXA1 is key to the function of ER in breast cancer.[12–15] FOXA1 is a pioneer factor that renders chromatin accessible to transcription factors, including ER, so that ER can promote the transcription of its target genes.[24] Most breast tumors, including ER− or basal-like tumors, are thought to arise from luminal progenitor cells.[25] Together with ER and GATA-3, another transcription factor, FOXA1 induces luminal cell differentiation and suppress the basal phenotype.[16] Studies have shown that FOXA1 and ER are co-expressed in breast tumors and lower FOXA1 expression levels correlate with higher tumor grade,[26,27] a feature of ER− tumors and TNBC. From our previous work[11] and others,[10] we speculate that, in many cases, the reproductive factor-associated changes in FOXA1 protein levels reflect the levels of DNA methylation at the *FOXA1* gene. Interestingly, Gong et al. showed that the BRCA1 protein can regulate the expression of *FOXA1* by impeding EZH2 methyltransferase activity, and propose that silencing or mutation of the *BRCA1* gene abrogates this inhibitory effect ultimately leading DNA methylation at *FOXA1*. They further suggested that the resultant repression of *FOXA1* allows cells to acquire a basal-like phenotype,[17] consistent with earlier findings that most BRCA1-deficient breast tumors are

basal-like TNBC.[28] This suggests that parity-associated methylation of *FOXA1* may predispose transformed cells to develop into TNBC.

Our findings on FOXA1 protein expression in ER− breast cancer are consistent with epidemiological and tumor DNA methylation data. Epidemiological evidence of parity in relation to ER− breast cancer in Black women has been confirmed by the AMBER Consortium.[3] In the consortium, which consisted of 1,252 cases of ER− tumors and 14,180 controls, Black women who had more children were at higher risk of ER− breast cancer compared to those with fewer children. In addition, the positive association of parity with ER− tumors was attenuated by breastfeeding, that is, among women who breastfed, their risk of ER− tumors did not increase with the higher number of births.[3,4,23] A consistent finding was observed in the Breast Cancer Family Registry with predominately White women.[4] In our earlier investigation, higher parity was associated with DNA hypermethylation of *FOXA1* in ER− tumors, particularly for Black women who never breastfed.[11] The evidence is further supported by the current study that FOXA1 protein expression in ER− tumors was lower, which may result from hypermethylation, among Black women with a higher number of births and no breastfeeding. Although the association of parity with FOXA1 protein expression in ER− tumors was not significant, the estimates of beta coefficients were similar or somewhat stronger in ER− compared with ER+ tumors. The findings warrant confirmation with a larger sample of women with ER− tumors.

In AMBER, ever versus never breastfeeding was associated with a decreased risk of ER− breast cancer. However, we did not find an association of breastfeeding itself with FOXA1 protein expression. The reason for this null finding is unclear. Pregnancy can alter DNA methylation in mammary gland epithelial cells and “epigenetic memory” of pregnancy has been observed in animal models.[29,30] The pregnancy-related DNA methylation changes may help prime the mammary gland for lactation. During lactation, prolactin and mammary gland epithelial cells enhance milk protein and lipid synthesis, and the process may promote DNA demethylation of lactation-specific genes, including DNA methyltransferases, which is an important driver of DNA methylation.[31,32] However, the potential contribution of lactation on DNA methylation in the FOXA1 signaling axis is unclear. Also, there may be mechanisms other than modulation of FOXA1 whereby parity and breastfeeding affect breast cancer risk. It has been hypothesized that postpartum involution promotes remodeling of terminal duct lobular units, with immune and inflammatory reactions, which are hallmarks of ER− tumors,[33,34] and breastfeeding can ameliorate these processes.[35,36] Studies directly examining FOXA1 and related factors in postpartum breast tissue in women with and without breastfeeding, or animal studies mimicking parity with and without breastfeeding, may further elucidate the mechanisms.

Another risk factor for ER− breast cancer in Black women is early age at menarche.[5] However, we did not observe an association of age at menarche with FOXA1 protein expression in breast tumors. Studies have suggested radiation exposure in puberty as a mechanism of developing ER− tumors related to early age at menarche. Early versus late age at menarche often leads to a longer duration between menarche and first live births, a period during which undifferentiated ductal cells may be highly susceptible to DNA damage caused

by carcinogens.[37,38] The role of FOXA1 in promoting luminal cell differentiation between menarche and a full-term pregnancy is unclear and warrants investigations.

Our study has several strengths. To our knowledge, this study is the first reporting relationships between reproductive characteristics and FOXA1 protein expression in breast tumors and adjacent normal tissue. A subset of our samples provided both tumor and adjacent normal tissue, allowing for modeling to what extent that the change of FOXA1 from normal to tumor tissue was associated with the rise of ER+ tumors and non-TNBC. Because of a relatively small sample size of those with adjacent normal tissue, this sub-analysis should be considered exploratory. Other strengths include that our study population comprised a large number of Black women, a population with higher risk of ER− tumors than Whites. Also, we used automated imaging analysis to derive an objective assessment of FOXA1 protein expression.

In conclusion, this tumor-based study showed that FOXA1 protein expression in ER+ breast tumors was inversely associated with parity, and the association was attenuated in women who breastfed. Results were largely consistent for ER− tumors although a larger sample is required for confirmation. The observation is in line with our previous findings of *FOXA1* DNA hypermethylation in breast tumors, and further supports the potential roles of FOXA1 in the mechanism of parity and breastfeeding influencing the risk of ER− tumors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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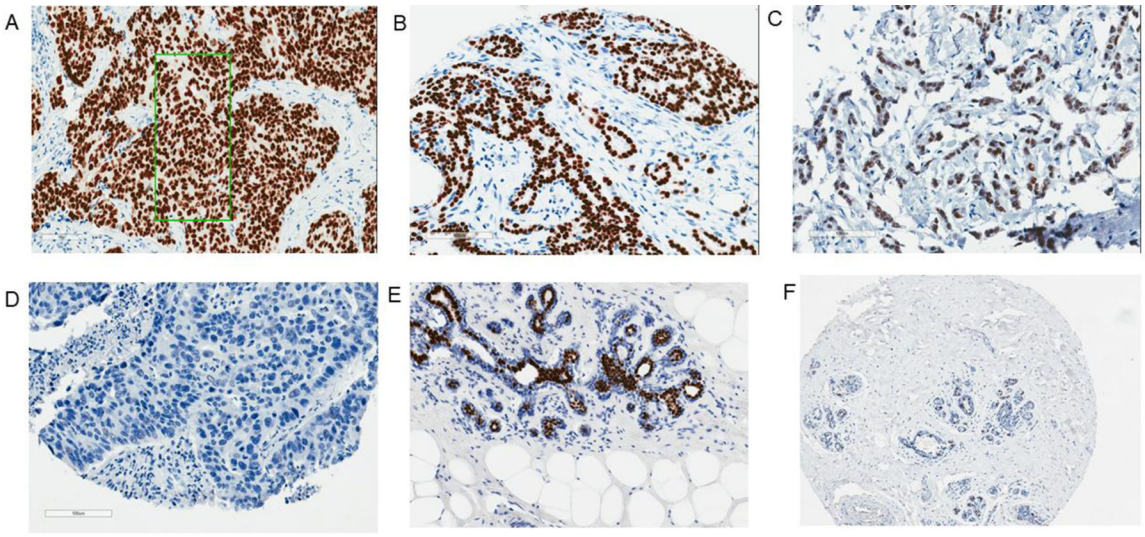

Figure 1.

FOXA1 IHC in breast tissue. A. whole section tumor tissue (H-score = 275) (x20, the green frame was the annotated area for automatic scoring); B–D. TMA tumor cores (H-score = 203, 74, and 0, respectively); E. whole section adjacent-normal tissue (H-score = 55); F. TMA adjacent-normal core (H-score = 34)

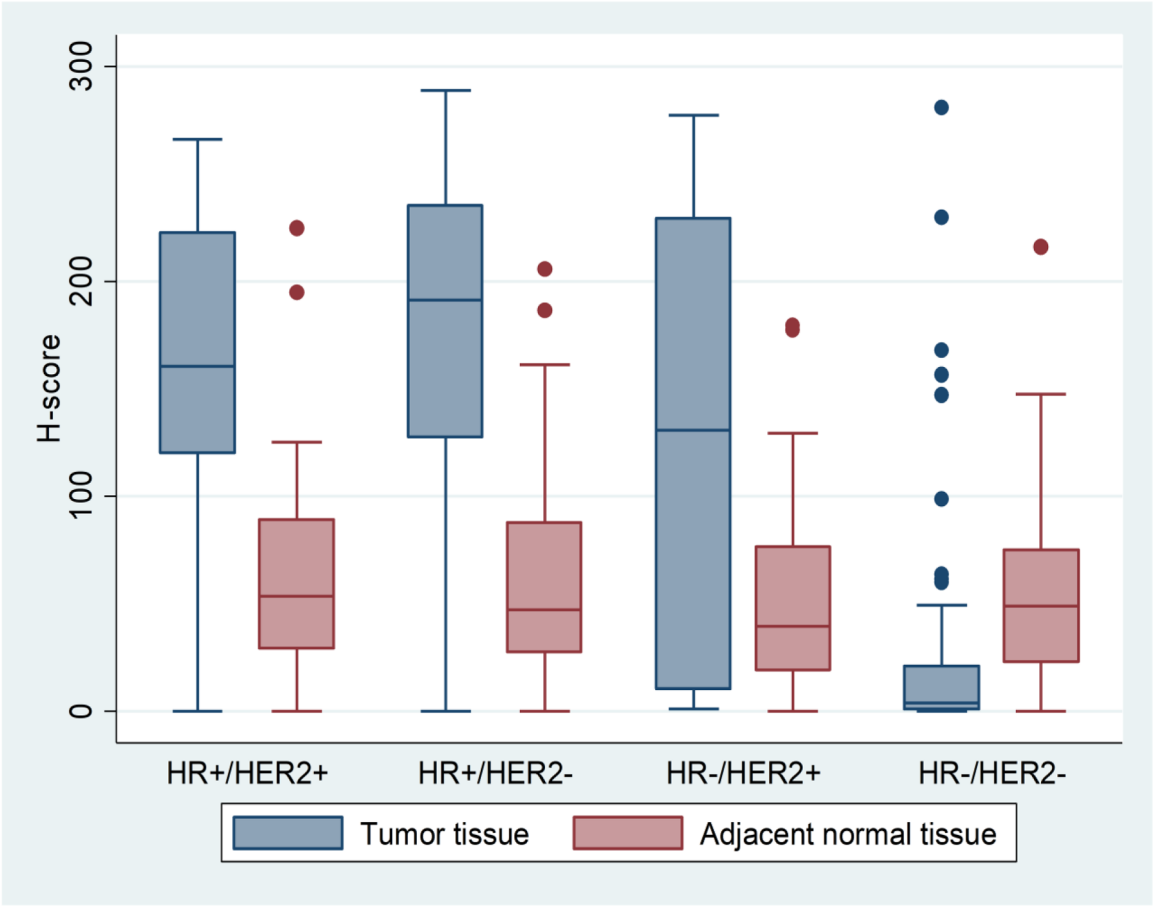

Figure 2.

Box plot of FOXA1 protein expression in tumor and adjacent-normal tissue by molecular subtype of breast cancer.

Note: A total of 298 patients with both tumor and adjacent-normal tissue; among them 254 with subtype information. The H-score of FOXA1 protein expression were different between tumor and adjacent-normal tissue overall (P<0.001) and within each subtype (P=0.005 for HR−/HER2− and P<0.001 for the other three subtypes; paired t-tests). The FOXA1 protein expression in adjacent-normal tissue were similar between the subtypes (P=0.78, ANOVA).

Table 1.

FOXA1 protein expression according to demographic and tumor characteristics of study participants

Characteristic	N	H-score, means (SD)	P-value[1]
Total	1329	157 (90)	–
Race			0.001
Black	949	151 (95)	
White	380	170 (74)	
Age			0.74
<40	153	154 (93)	
40–49	366	154 (93)	
50–59	445	157 (88)	
≥60	365	161 (88)	
Histology			<0.001
Ductal carcinoma in situ	217	171 (80)	
Invasive ductal carcinoma	968	151 (92)	
Invasive lobular carcinoma	94	191 (63)	
Invasive mammary carcinoma and other invasive	47	135 (86)	
Tumor grade			
Low	180	175 (65)	<0.001
Intermediate	480	183 (72)	
High	567	125 (102)	
Tumor size (cm)			0.005
<1.0	227	163 (78)	
1.0 – 1.9	408	160 (87)	
≥2.0	478	143 (100)	
AJCC Stage			0.001
0, I	731	165 (84)	
II	418	143 (97)	
III, IV	163	151 (93)	
Lymph node status			0.41
Negative	813	155 (90)	
Positive	388	159 (91)	
ER status			<0.001
Positive	960	185 (69)	
Negative	348	75 (92)	
PR status			<0.001
Positive	854	182 (72)	
Negative	426	102 (100)	
HER2 status			0.001
Positive /equivocal	246	172 (85)	
Negative	989	149 (92)	
Molecular subtype			<0.001

Characteristic	N	H-score, means (SD)	P-value[1]
HR+[2]/HER2+	156	186 (70)	
HR+/HER2−	696	179 (74)	
HR− /HER2+	79	136 (105)	
HR− /HER2−	200	45 (70)	

[1] ANOVA

[2] Hormone receptor positive included ER+/PR+, ER+/PR−, and ER−/PR+ tumors

Table 2.

Associations of reproductive characteristics with FOXA1 protein expression in ER+ tumors, ER− tumors, and adjacent normal tissue

	ER+ tumors			ER− tumors			Adjacent normal tissue[2]		
Reproductive characteristics	**N**	**H-score, mean (SD)**	**P-value[1]**	**N**	**H-score, mean (SD)**	**P-value[1]**	**N**	**H-score, mean (SD)**	**P-value[1]**
Age at menarche, years			0.55			0.61			0.70
<11	117	190 (67)		38	62 (75)		32	56 (42)	
11–12	391	183 (69)		134	75 (90)		125	59 (46)	
≥13	449	187 (70)		176	79 (97)		140	63 (45)	
Age at first live birth, years			0.87			0.019			0.49
<24	442	185 (72)		189	63 (88)		135	59 (44)	
25–29	169	182 (65)		53	74 (86)		46	65 (52)	
≥30	145	185 (69)		47	105 (102)		45	68 (46)	
Parity			0.42			0.13			0.20
Nulliparous	204	189 (65)		59	92 (96)		72	54 (43)	
Parous	756	185 (70)		298	72 (91)		226	62 (46)	
Number of births (among parous women)			0.046			0.28			0.50
1	198	193 (68)		68	87 (92)		61	68 (48)	
2	282	186 (67)		111	65 (89)		83	62 (47)	
≥3	276	177 (74)		110	70 (92)		82	59 (44)	
Breastfeeding (among parous women)			0.93			0.77			0.16
Never	393	185 (71)		150	73 (94)		120	66 (51)	
Ever	363	184 (70)		139	70 (88)		106	58 (40)	
Duration of breastfeeding (among women who breastfed), months			0.22			0.74			0.77
<12	213	188 (69)		84	68 (83)		66	57 (39)	
≥12	150	179 (70)		55	73 (94)		40	59 (41)	
Menopausal status			0.85			0.39			0.18
Premenopause	438	185 (70)		167	71 (90)		163	57 (45)	
Postmenopause	522	186 (69)		181	79 (94)		135	64 (46)	

[1] ANOVA

[2] Among participants who had adjacent normal tissue (n=298)

Table 3.

FOXA1 protein expression in relation to parity and history of breastfeeding

	ER+ tumors			ER− tumors		
	N	β (95% CI)[1]	P-value	N	β (95% CI)[1]	P-value
Parous vs. Nulliparous (ref.)	960	−5.3 (−15.6, 5.1)	0.32	348	−13.4 (−37.4, 10.7)	0.27
Number of births (1, 2, 3+)	756	−8.5 (−15.1, −2.0)	0.010	289	−10.3 (−23.5, 2.9)	0.13
Number of birth stratified by history of breastfeeding (BF)						
Never BF	393	−10.4 (−19.7, −1.0)	0.029	150	−13.9 (−35.1, 7.3)	0.20
Ever BF	363	−7.5 (−16.9, 1.8)	0.11	139	−3.6 (−21.9, 14.7)	0.70
		P-interaction[2] = 0.57			P-interaction[2] = 0.30	

[1] Generalized linear models adjusting for age and race.

[2] Wald tests for the interaction between number of births and breastfeeding

Table 4.

FOXA1 protein expression in relation to parity and history of breastfeeding by race

		ER+ tumors			ER− tumors		
	Race	**N**	**β (95% CI)[1]**	**P-value**	**N**	**β (95% CI)[1]**	**P-value**
Parous vs. Nulliparous (ref.)	Black	661	−7.1 (−21.5, 7.3)	0.33	281	−22.0 (−49.7, 5.8)	0.12
	White	299	−1.9 (−16.4, 12.5)	0.79	67	3.5 (−49.8, 56.7)	0.90
Number of births (1, 2, 3+)	Black	549	−6.8 (−14.4, 0.8)	0.08	247	−11.8 (−24.6, 1.0)	0.07
	White	207	−14.1 (−26.7, −1.5)	0.028	42	5.7 (−50.7, 62.0)	0.84
Number of birth stratified by history of breastfeeding (BF)							
Never BF	Black	306	−8.7 (−19.4, 2.1)	0.11	135	−16.9 (−37.3, 3.5)	0.11
Ever BF		243	−5.5 (−16.5, 5.5)	0.33	112	−3.8 (−21.6, 14.1)	0.68
			P-interaction[2] = 0.64			P-interaction = 0.32	
Never BF	White	87	−17.8 (−35.5, −0.2)	0.047	15	_[3]	
Ever BF		120	−13.0 (−30.8, 4.9)	0.16	27	_[3]	
			P-interaction = 0.58				

[1] Generalized linear models adjusting for age.

[2] Wald tests for the interaction between number of births and breastfeeding

[3] Estimates not shown because small number of participants in the stratum (N <30).