Research article

Annexin 2 protein expression is associated with breast cancer subtypes in African American women

Desta A. Beyene [a,e,g], Norma F. Kanarek [b], Tammey J. Naab [c], Luisel L. Ricks-Santi [d], Tamaro S. Hudson [e,f,g,*]

[a] Department of Biochemistry and Molecular Biology, Howard University, Washington, DC, USA
[b] Department of Environmental Health and Engineering, Johns Hopkins Bloomberg School of Public Health and Department of Oncology, Johns Hospital School of Medicine, USA
[c] Department of Pathology, College of Medicine, Howard University, Washington, DC, USA
[d] Department of Biological Sciences, Cancer Research Center, Hampton University, Hampton, VA, USA
[e] Department of Research, Veteran Affairs Medical Center, Washington, DC, USA
[f] Department of Pharmacology, College of Medicine, Howard University, Washington, DC, USA
[g] Howard University Cancer Center, Washington, DC, USA

ARTICLE INFO

ABSTRACT

Background: A review of literature on the expression of Annexin 2 in cancer has shown that there is very limited research work on the association of this protein with breast cancer aggressiveness in African Americans. In the present study, TMA breast tissues from African American women were stained with Annexin 2 antibody to determine the association between the molecular subtypes and Annexin 2 protein expression.

Method: An annotated case series of 135 breast cancer tissues archived from 2000 to 2010 was acquired from the Howard University Tumor Registry. The association between ANX2 expression and survival by molecular subtypes Luminal A, Luminal B, HER2, and Triple Negative (TN) was assessed using Multinomial regression, chi-square analysis, and Kaplan-Meir graphs (Stata 11).

Results: Our findings show a marked association between ANX2 protein expression in Luminal B and HER2 subtypes unadjusted and when adjusted for age. Borderline differences in tumor grade were found in TN only. Univariately, age (<50, 50 + years) and metastases were highly significant for overall survival, disease-free survival and recurrence-free survival. Stage, tumor size, and nodal involvement were of borderline or greater significance for overall and disease-free survival. ANX2 expression was not significant. Kaplan Meier tests of ANX2 showed significant separation of overall survival by ANX2 protein expression in all breast tumor subtypes. In multivariate analyses comparing TN to Luminal A, ANX2 was not important while controlling for age and grade.

Conclusion: ANX2 might be a biomarker of aggressiveness and a relevant candidate biomarker in high risk African American women with Luminal B and HER2 breast cancer.

1. Introduction

Breast cancer is the most common cancer diagnosis and the second leading cause of death among American women [1]. African American breast cancer patients of all ages are more likely to have advanced disease at diagnosis, an increased risk of recurrence and a poorer prognosis compared with their Caucasian counterparts [2, 3]. Tumor stage, tumor grade and lymph node metastasis are commonly used as prognostic factors for breast cancer [4]. However, these are not enough for

accurately predicting the prognosis of breast cancer. Therefore, biomarkers are needed to more accurately predict the level of disease aggressiveness and survival outcome. ANX2 has been implicated in tumorigenesis and metastasis of breast cancer [5]. Therefore, this protein could have a potential use as a prognostic biomarker for predicting the disease progress in breast.

Annexin 2 (ANX2) belongs to a family of Ca2+ dependent phospholipid and membrane binding proteins called annexins and contains a conserved repeating domain of approximately 70 amino acids. ANX2

(also called p36, annexin II, or ANXA2) is a 36 kDa protein [6] and is located on chromosome 15q22.2 [7]. ANX2 is highly conserved and ubiquitously distributed in various body cell types and accounts for about 0.5–2% of the total cell protein [8]. ANX2 plays a major role in tumorigenesis, drug resistance, and metastasis [9]. Overexpression of ANX2 is frequently observed in a broad spectrum of cancer cells, including breast cancer [10, 11], colorectal carcinoma [12], and lung cancer [13] while under expressed in others, such as prostate cancer[14].

In breast cancer, ANX2 is undetectable in normal and hyperplastic ductal tissue samples but is consistently expressed in invasive breast cancer and ductal carcinoma in situ [15]. ANX2 gene expression is associated with tumors with selected poor prognostic characteristics such as grade characterized by poor differentiation, enriched expression of human epidermal growth factor receptor 2 (HER2), and triple negative (TN) subtypes [16]. According to Sortie et al. [17] Luminal A tumors have the more favorable prognosis and make up approximately 40% of all breast cancer cases. Luminal B tumors are very similar to Luminal A tumors but have higher expression of proliferative genes in comparison to Luminal A. They make up 20% of breast cancer cases and tend to be diagnosed at higher tumor grades than Luminal A tumors. HER2 tumors are often aggressive and have poor prognosis. TN breast cancer makes up approximately 15–20% of breast cancer diagnoses. TN is the most aggressive breast cancer subtype [18] and is unresponsive to anti-hormonal and HER2-targeted therapies due to the absence of hormone receptors and HER2 expression. The upregulation of ANX2 in cancer has several clinical applications, including as a diagnostic marker for early detection, a predictive factor for prognosis, or a marker for drug resistance.

African American women continue to have high rates of breast cancer mortality compared with other ethnicities and have a limited number of predictive markers for the different subtypes. Therefore, the aim of this study is to investigate the use of ANX2 protein expression for predicting disease progression for breast cancer subtypes in African American women. Commensurately, low ANX2 will be positively associated with overall and disease-free survival.

2. Materials and methods

2.1. Study design

This study was reviewed and formally exempted by the Howard University Institutional Review Board. Our cases originated primarily from the community population of women served who are predominantly minority and of low to moderate income. We analyzed 135 sequential invasive breast ductal carcinomas (IDC) from African-American women diagnosed and treated at the Howard University Hospital between 2000 and 2010 where adequate tumor tissue was available and obtained. Demographic and clinical information was case information from the Howard University Cancer Center Tumor Registry. The hormone receptor status of HER2, ER and PR were used to classify four breast cancer subtypes as described by others [16, 19]. Breast cancer subtype was abstracted from hospital reports: Luminal A, Luminal B, HER2 positive, and TN. Tumor characteristics collected included grade (categorized as Grade (I-II/III-IV) and stage (I-II/III-IV), tumor size, metastases (yes/no), and nodal involvement (yes/no)). Age at diagnosis and menopausal status (pre/post) were obtained from the medical record.

2.2. Tissue samples

Formalin-fixed paraffin embedded (FFPE) tissues in diagnosed women were assessed using tissue microarrays (TMAs, Pantomics, Inc, Richmond, CA). The TMAs consisted of 10×16 arrays of 1.0 mm tissue cores from well preserved morphologically representative tumor cells in archived FFPE surgical blocks from primary IDCs in 135 African-American women. A precision tissue arrayer (Beecher Instruments, Silver Spring, MD) with two separate core needles for punching the donor

and recipient blocks was used. The device also had a micrometer-precise coordinate system for tissue assembly on a multi-tissue block. Two separate tissue cores of IDC represented each surgical case in the TMA. Each tissue core was assigned a unique TMA location number, which was subsequently linked to an Institutional Review Board-approved database containing demographic and clinical data.

2.3. Preparation of tissue microarrays (TMA)

TMA is a useful tool for identifying the signature profiles of proteins in the different breast cancer subtypes. The TMA paraffin blocks were constructed in our lab as described by Hewitt SM [20]. The TMA blocks with core samples were tempered by placing in the incubator at 37 °C overnight, cut on microtome to obtain 5-μm thick slices and float mounted on super frost plus micro slides. Slides were stained with hematoxylin-eosin (H&E) and representative areas with invasive tumor were identified and marked on the H&E slide by the pathologist. The individually marked slides were placed on top of each donor paraffin block and carefully aligned to locate the corresponding tumor sites where the core samples are to be collected. Three separate 1.0 mm tissue cores were obtained from each donor block and mounted in the recipient TMA (Beecher, Inc., Pathological Devices).

2.4. Immunohistochemistry

Immunohistochemistry (IHC) was performed on three TMA breast tumor sections of FFPE tissue. The TMA slides were deparaffinized in xylene twice for 5 min each, rehydrated in absolute ethanol (2 times 5 min each) followed by 95% and 70% ethanol for 5 min each. Deparaffination was completed manually with xylene washes and serial rehydration through alcohol-water series. Further deparaffination, rehydration and heat-induced antigen retrieval at pH 9.0 was performed on DAKO PT-Linker (Carpinteria, CA). Antibody detection was carried out using an anti-ANX2 (clone C-10, monoclonal mouse antihuman, 1:10k dilution). IHC was then completed using the DAKO Autostainer Link Chamber (Carpinteria, CA) according to manufacturer's protocol. The binding of the primary antibody was visualized using the Avidin Biotin Complex method (ABC kit, Vector Lab). The chromogen substrate was diaminobenzidine (DAB kit, Invitrogen). Stained slides were counterstained with hematoxylin (Invitrogen) and finally treated with 70%, 95% and absolute ethanol and xylene. Slides were cover slipped with an automatic unit (Tissue-Tek SCA, Thermo- Fisher Scientific) and examined by a pathologist under the light microscope.

2.5. Evaluation of immunohistochemical staining

Tissue samples were analyzed as positive or negative for ANX2 antibody. Immunohistochemically stained sections were scored by two independent observers blinded to the clinical outcome using a laboratory grade binocular light microscope. Individual tissue cores were scored for intensity of reactivity (0, no staining; 1+, weak cytoplasmic and membrane staining; 2+, moderately intense staining; and 3+, strong staining) and the percentage of reactive cells. The results were entered into a secure research database. An H-score was derived from the results of these measurements by multiplying intensity score by extent/percentage of stained area. All samples were categorized based on the H-score [21, 22]. H-score ≥ 200 cells stained with ANX2 were recorded as positive and those with H-score < 200 cells stained as negative. All samples were scored while blinded to tissue phenotype. Estrogen Receptor (ER), Progesterone Receptor (PR) and Human Epidermal Growth Factor Receptor (HER2) scores were obtained from the Tumor Registry medical records. The specific DAKO antibodies used were: ER-alpha, rabbit monoclonal, cloneSP1, 1:200 dilution; PR, mouse monoclonal, clone PgR636, 1:100 dilution; HER2, rabbit polyclonal, clone e-erb-2, 1:500 dilution.

2.6. Statistical analysis

A case series of 135 breast tumors with pathology specimens and complete case information at Howard University Hospital were analyzed for ANX2 levels. All statistical analyses were conducted in Stata 11 and included tests of significance by Chi Square, ANOVA, multinomial regression and Kaplan Meier analysis of survival.

3. Results

3.1. Annexin 2 is highly expressed in HER2 subtype

A visual comparison of the expression of ANX2 shows distinct features that distinguish three of the four breast cancer subtypes (Figure 1). ANX2 is highly expressed in HER2 (A) but is marginally expressed in TN (B) and shows limited expression in luminal B (C).

3.2. Demographics and pathological features of African American women diagnosed with breast cancer at Howard University

The demographics and pathological characteristics showed that approximately 75% (101/135) of the patients were older than 50 years. Positive expression status for ER, PR and HER2 was 39%, 48% and 90%, respectively. The percentages for luminal A, Luminal B, HER2+ and TN were 41%, 15%, 11% and 33%, respectively. Most of the cancers (77%) were classified as stage I-II. Sixty percent of tumors were of size greater than 20 mm. Ninety percent of the patients showed no distant metastasis but 57% had affected lymph nodes (Table 1).

High expression of ANX2 was observed in 23% (31/135) of the patients. Across, cancer type, significant or near significant differences were observed for ANX2 expression (p = 0.055), no metastases (p = 0.055), early stage (p = 0.066), high grade (p = 0.096), smaller tumor size (p = 0.030) and nodes present (p = 0.029) (Table 1). Though there were no age differences detected, post-menopausal status (p = 0.070) by cancer

subtype was borderline (Table 1). Tumor size differences in those with tumors less than 20 mm differed significantly across tumor subtype (p = 0.030). There were no significant differences in low grade, later disease stage, larger tumor size, absence of affected nodes, or distant metastasis.

3.3. Low and high Annexin 2 expression associated with recurrence free survival

Table 2 shows t-test analysis results of ANX2 expression and clinical characteristics in relation to overall survival, disease-free and recurrence-free survival. Univariately, a nonsignificant difference (p = 0.128) was observed between the low and high expression of ANX2 in recurrence free survival only. The age difference between patients less than 50 years and 50 years or older was highly significant for all three survival outcomes (p < 0.002) while only a borderline significant difference was observed between pre- and post-menopausal status in disease free survival only (p = 0.091). Differences in stage (I-II vs III-IV), tumor size (<20mm vs > 20mm) and lymph node (presence or absence) were significantly or borderline different for overall and disease-free survival. There was also a significant difference between patients who had metastasis and those who did not have any (<0.0002) for all three-survival outcomes.

3.4. Protein expression of annexin 2 is associated with different breast cancer subtypes

Multinomial regression analysis showed significant and positive differences in ANX2 expression for HER2 and Luminal B but not for TN type compared to Luminal A (Table 3). Age at diagnosis was a significant factor in HER2 and TN subtypes though of borderline significance in the Luminal B subtype. The age differences across the different breast cancer subtypes is confirmed in Table 4. High grade cancer was a significant factor for TN alone Table 3.
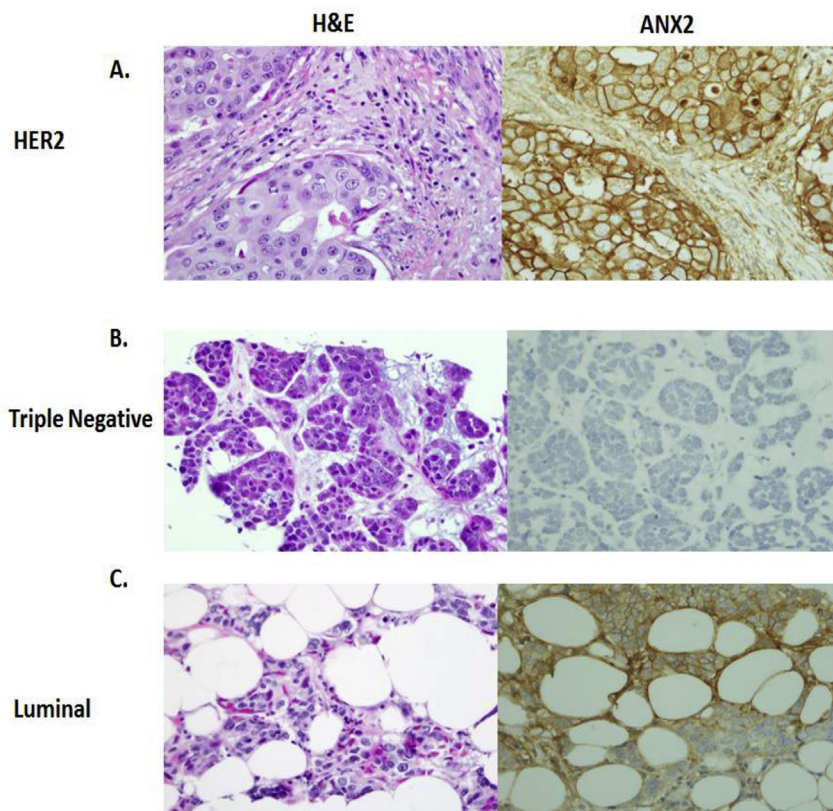

Figure 1. Comparison of ANX2 expression in Luminal, Triple Negative and HER2 carcinoma. A. The IHC staining of ANX2 in HER2 presents with strong circumferential membranous staining in all cells and the H&E demonstrates Invasive poorly differentiated mammary carcinoma (ductal, not otherwise specified) and Infiltrating solid collections of pleomorphic large cells with vesicular nuclear chromatin, prominent nucleoli, and abundant eosinophilic cytoplasm and mitotic activity. B. The IHC staining of ANX2 in Triple Negative breast cancer subtype presents as negative, while the H&E shows Invasive poorly differentiated mammary carcinoma (ductal, not otherwise specified) and Infiltrating small collections of medium sized cells displaying prominent nucleoli and other cells demonstrating hyperchromatic nuclei with mitotic activity. C. IHC staining of ANX2 in Luminal B breast cancer subtype which presents with a patchy weak to moderate membranous staining, while H&E demonstrates an invasive moderately differentiated mammary carcinoma (ductal, not otherwise specified) with medium sized cells that have rare small nucleoli and no tubule formation infiltrating fat.

Table 1. Annexin 2 expression overall, by age, menopause status and clinical characteristics across breast cancer subtypes.

ANNEXIN 2		Luminal A (N = 55)	Luminal B (N = 20)	HER2 (N = 15)	Triple Negative (N = 45)	P*
Overall (N = 135)	Mean	174.1	215	211.7	161.3	**0.055**
	SE	11.7	17	18.5	13.7	
	CI	151.0 to 197.3	181.3 to 248.7	175.2 to 248.2	134.2 to 188.5	
AGE						
Age <50 (N = 34)	Mean	168.5	220.1	232.5	124.9	0.168
	SE	27.94	42.6	36.4	29.9	
	CI	113.2 to 223.7	135.8 to 304.3	161.2 to 303.8	65.8 to 184.0	
Age 50+ (N = 101)	Mean	175.9	212.9	199.9	176.4	0.322
	SE	12.7	19.3	18.8	14.3	
	CI	150.7 to 201.0	174.7 to 251.1	162.8 to 237.0	148.1 to 204.7	
MENOPAUSAL STATUS						
Menopausal status-post (N = 34)	Mean	172.7	217.5	231.7	129.6	**0.070**
	SE	27.5	42.7	36.4	30.9	
	CI	118.2 to 227.2	133.1 to 301.9	159.6 to 303.7	68.4 to 190.8	
Menopausal status-pre (N = 101)	Mean	174.2	198.3	214.4	174.5	0.391
	SE	14.4	19.6	19.2	13.1	
	CI	145.8 to 202.6	159.5 to 237.1	176.4 to 252.3	148.6 to 200.4	
GRADE						
Grade high (N = 91)	Mean	144.7	190.5	211.7	164.8	0.096
	SE	19.5	22.4	18.5	13.8	
	CI	106.2 to183.2	146.5 to 234.4	175.2 to 248.2	137.5 to 192.1	
Grade Low (N = 44)	Mean	195.3	245	-	113.3	0.457
	SE	13.5	23.9	-	78.4	
	CI	168.6 to 222.0	197.8 to 292.2	-	0 to 183.2	
STAGE						
Stage I-II (N = 104)	Mean	169.7	206.7	226.7	155.3	0.066
	SE	12.6	19.6	24.9	15.6	
	CI	144.9 to 194.6	167.9 to 245.4	177.4 to 275.9	124.5 to186.0	
Stage III-IV (N = 31)	Mean	194	240	189.2	182.5	0.691
	SE	31.6	35.6	26.9	19.6	
	CI	131.5 to 256.5	169.5 to 310.5	135.9 to 242.4	123.9 to 241.1	
TUMOR SIZE						
Tumor size <20 mm (N = 54)	Mean	161.3	234.5	165	153	**0.030**
	SE	16	18.6	24.7	18.6	
	CI	129.7 to 193.0	197.7 to 271.3	116.2 to 213.8	110.6 to 195.4	
Tumor size >20 mm (N = 81)	Mean	184.8	195.5	223.3	165.5	0.681
	SE	16.8	28.2	21.3	17.8	
	CI	151.6 to 218.1	139.8 to 251.2	181.3 to 265.4	130.4 to 200.6	
NODES						
Nodes Present (N = 58)	Mean	172.6	234.2	183.3	161	**0.029**
	SE	15.4	15.1	36.6	14.8	
	CI	142.2 to 202.9	204.4 to 264	110.9 to 255.8	131.7 to 190.2	
Nodes none (N = 77)	Mean	176.5	186.3	230.6	161.8	0.503
	SE	18.5	35.1	18	25.9	
	CI	139.9 to 213.1	116.7 to 255.8	195 to 266.1	110.5 to 213.1	
METASTASES						
Metastases Present (N = 14)	Mean	172.7	215	212.7	161.9	0.495
	SE	12	19.3	21	14.6	
	CI	149 to 196.5	176.8 to 253.2	171.1 to 254.3	133 to 190.8	
Metastases None (N = 121)	Mean	211.5	215	205	157.5	**0.055**
	SE	58.5	41.3	35	43	
	CI	95.8 to 327.3	133.3 to 274.2	135.8 to 274.2	72.4 to 242.6	

*P value was based on t-test.

3.5. Annexin 2 expression is associated with overall and disease-free survival

The Kaplan-Meir disease-free survival and overall survival by ANX2 expression are shown in Figures 2, 3, and 4. Lower ANX2 expression was associated with statistically borderline (p = 0.10) higher overall survival and disease-free survival time (Figure 2). The expression of ANX2 in relation to overall and disease-free survival for the four subtypes is shown in Figure 3; none was statistically significant those Luminal A and B with low ANX2 appear to have the worse overall and disease-free survival. In these two figures, high ANX2 expression was associated with higher overall and disease-free survival time for Luminal B, HER2 and TN.

Figure 4 shows ANX2 expression in relation to overall and disease-free survival for the four subtypes. ANX2 expression for HER2 subtype showed a higher overall and disease-free survival compared to the other three types, though across all combinations there was no statistical significance.

4. Discussion

There is very limited research work on the association of ANX2 protein with the different breast cancer subtypes in African Americans. Most reported results address ANX2 gene expression in TN subtypes alone. In the present study, ANX2 protein expression was evaluated in relation to

Table 2. Evaluation of Annexin 2, age, and clinical characteristics association with overall, disease-free, and recurrence-free survival.

		Overall Survival	Disease-free Survival	Recurrence-free Survival
OVERALL				
	Mean	70.7	65.9	17.0%
	SE	3.0	3.2	3.2%
	CI	64.9 to 76.6	59.5 to 72.3	10.6%–23.5%
ANNEXIN2				
Low (N = 104)	Mean	69.7	67.07	10.9%
	SE	5.04	5.47	4.6%
	CI	59.7 to 79.6	56.3 to 77.9	1.7%–20.0%
High (N = 31)	Mean	71.3	65.3	20.2%
	SE	3.66	4.01	4.3%
	CI	64.1 to 78.5	57.3 to 73.2	11.8%–28.7%
		P = 0.795	P = 0.792	P = 0.128
AGE				
<50 years (N = 34)	Mean	37.8	34.1	0%
	SE	2.26	3.03	-
	CI	33.2 to 42.4	28.0 to 40.3	-
≥50 years (N = 101)	Mean	81.9	76.6	22.8%
	SE	3.2	3.61	4.21%
	CI	75.5 to 88.2	69.4 to 83.7	14.5%–31.1%
		P < 0.0000	P < 0.0000	P < 0.002
MENOPAUSAL STATUS				
Post (N = 101)	Mean	73.21	69	14.80%
	SE	3.4	3.7	3.60%
	CI	66.5 to 80.0	61.6	7.8–21.9%
Pre (N = 34)	Mean	63.4	56.5	23.50%
	SE	5.8	6.2	7.40%
	CI	51.9 to 74.9	44.3 to 68.7	8.9–38.1%
		p = 0.150	p = 0.091	p = 0.248
GRADE				
High (N = 91)	Mean	70.8	65.8	19.80%
	SE	3.7	4	4.20%
	CI	63.5 to 78.1	57.9	11.5–28.1%
Low (N = 44)	Mean	70.6	66.1	11.40%
	SE	4.9	5.6	4.80%
	CI	60.9 to 80.3	55.1 to 77.0	1.8%–20.9%
		p = 0.971	p = 0.967	p = 0.226
STAGE				
I-II (N = 104)	Mean	75.3	69.7	16.35%
	SE	3.2	3.61	3.64%
	CI	68.9 to 81.7	62.6 to 77.0	9.13–23.6%
III-IV (N = 31)	Mean	55.5	52.8	19.35%
	SE	6.2	6.6	7.21%
	CI	68.9 to 81.7	39.6 to 65.8	5.09%–33.6%
		p = 0.004	p = 0.025	p = 0.698
TUMOR SIZE				
<20 (N = 54)	Mean	79.6	72.6	13.0%
	SE	4.3	5.2	4.6%
	CI	71.0 to 88.1	62.3 to 82.9	3.8–22.1%
20+ (N = 81)	Mean	64.9	61.4	19.8%
	SE	3.9	4.1	4.5%
	CI	57.2 to 72.6	53.4 to69.4	10.9%–28.6%
		p = 0.014	p = 0.088	p = 0.308
NODES				
YES (N = 77)	Mean	64.8	60.8	18.9%
	SE	4.9	5.1	5.2%
	CI	55.2 to 74.4	50.6 to 70.9	8.7–29.2%
NO (N = 58)	Mean	75.2	69.7	15.6%
	SE	3.6	4.1	4.2%
	CI	68.1 to 82.4	61.6 to 77.8	7.4–23.8%
		p = 0.080	p = 0.088	p = 0.608
METASTASES				
YES (N = 14)	Mean	39.4	21.1	85.7%
	SE	8.3	4.2	9.7%
	CI	22.9 to 55.8	12.9 to 29.4	66.5–100%

Table 2 (*continued*)

		Overall Survival	Disease-free Survival	Recurrence-free Survival
NO (N = 121)	Mean	74.4	71	9.10%
	SE	3.0	3.3	2,6%
	CI	68.5 to 80.3	64.6 to 77.5	3.9%–14.3%
		p = 0.0002	p = 0.0000	p = 0.0000

*P is based on t test.

Table 3. Multinomial models: Annexin 2 and clinical characteristics regressed on breast cancer subtypes.

	Breast Cancer Subtypes			
	Luminal A (N = 55)	Luminal B (N = 20)	HER2 (N = 15)	Triple Negative (N = 45)
ANNEXIN2				
Coefficient	Reference	0.0078	0.0097	0.002
CI		0.0007 to 0.0149	0.0012 to 0.0182	-0.0035 to 0.0076
P		0.032*	0.025*	0.473
Age at Diagnosis				
Coefficient	Reference	-0.0407	-0.0603	-0.0619
CI		-0.0865 to 0.0050	-0.1180 to -0.0026	-0.1045 to -0.0193
P		0.081**	0.04*	0.004*
High Grade				
Coefficient	Reference	0.9163	18.2	3.2
CI		-0.2136 to 2.0463	-2949.6 to 2950.9	1.832 to 4.567
P		0.112	0.99	<0.000*

*Indicates correlation is significant at *p < 0.05; **p < 0.10.

Table 4. Average age across breast cancer subtypes.

		Luminal A	Luminal B	HER2	Triple Negative	
		(N = 55)	(N = 20)	(N = 15)	(N = 45)	P
AGE	Mean	61.3	56.2	54.7	54.2	
	SE	1.8	2.1	2.8	1.9	
	CI	57.8 to 64.9	52.8 to 60.2	49.2 to 60.1	50.5 to 57.8	0.024

Luminal B, HER2 and TN breast cancer relative to Luminal A. ANX2 expression was a significant predictor by breast cancer subtypes Luminal B and HER2, independent of age at diagnosis and tumor grade, which were also significant factors. The multinomial analysis using Luminal A as a reference showed significant increase in ANX2 expression for Luminal B and HER2. For the TN compared to Luminal A, significant ANX2 was not observed when high grade was controlled for. According to Noor et al. [9] and Gibbs et al [18] increased ANX2 expression is related to higher levels of HER2 at mRNA and protein levels [14], an observation made in this case series.

Reports from analysis of breast cancer subtypes and normal tissues have shown that ANX2 is highly expressed in TN compared to its low to negligible expression in the other subtypes and normal tumors [15, 19]. Here, Luminal A and TN did not differ on ANX2. It is also reported that ANX2 expression is associated with poor survival outcome and prognosis. This relates to the fact that ANX2 promotes TN progression through angiogenesis and metastasis [8] which in this study is exhibited by appositive relationship between TN and high grade. Moreover, there was a strong association between ANX2 and Luminal B as well as HER2 indicating the specific association of ANX2 with the aggressive behavior
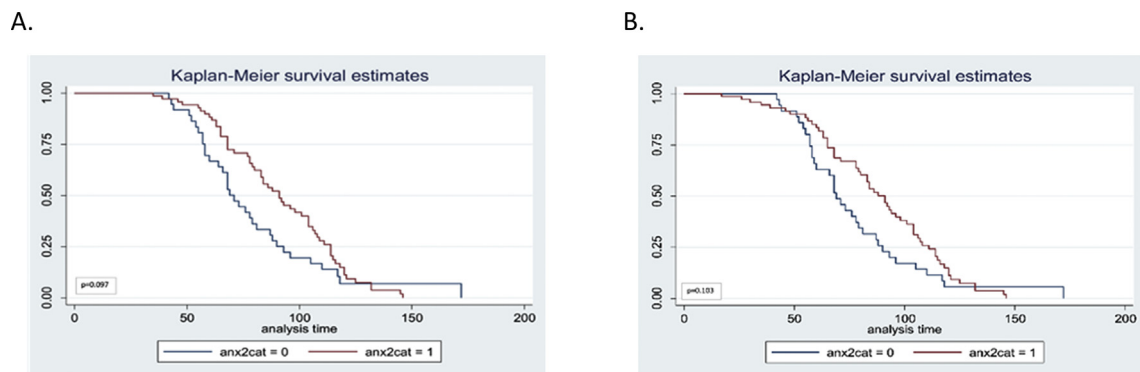
A.

B.

Figure 2. Illustrates overall (A) and Disease-free (B) Survival by Annexin 2 Status. Log-rank test for equality of survivor functions yielded borderline significance, p = 0.0972 (A) and p = 0.1025 (B).
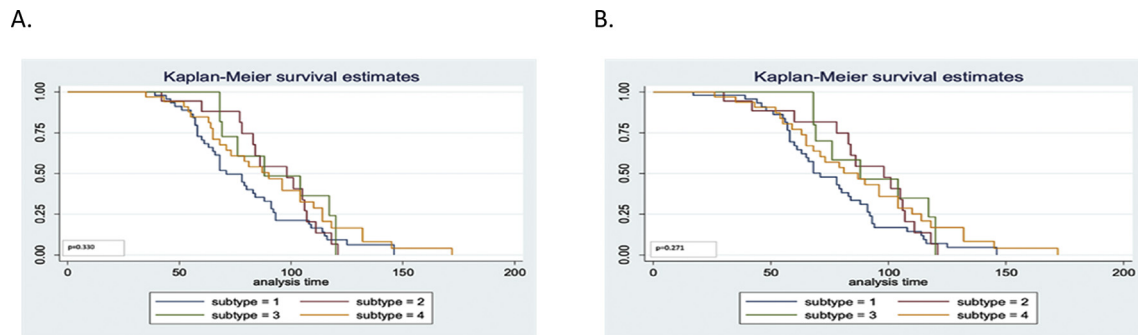
A.

B.

Figure 3. Kaplan Meier Overall and Disease-Free Survival by Breast Cancer Subtype: Luminal A (1), Luminal B (2), HER2 (3), and Triple Negative (4). Log-rank test for equality of survivor functions yielded p = 0.3301 (A) and p = 0.2711.
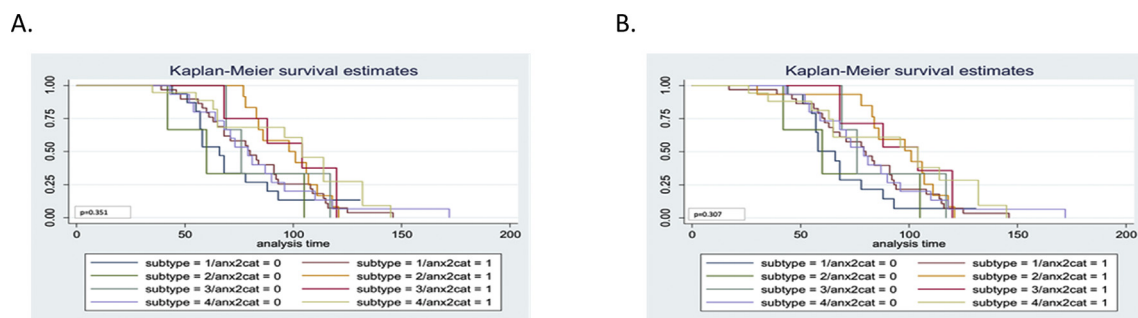
A.

B.

Figure 4. Kaplan Meier Graphs of Overall (A) and Disease-Free (B) Survival for Combinations of Annexin 2 and Molecular Subtype. Log-rank test for equality of survivor functions yielded p = 0.3512 (A) and p = 0.3072.

of HER2 relative to Luminal A. The aggressive biology of ANX2 has been confirmed by Gibbs et al. [18] which demonstrated that ANX2 contributes to the aggressive biology of TN breast cancer in African American women.

In the prior literature, ANX2 overexpression is associated with racial variation and is a potential prognostic and diagnostic candidate for TN [16, 18]. A gene expression study of African American women indicated that ANX2 expression was significantly elevated compared to Caucasian and Hispanic women. Furthermore, the elevated ANX2 gene expression was significantly associated with TN as well as with reduced overall survival and reduced recurrence-free survival [16, 17]. Gibbs et al [18] reported that ANX2 gene expression was correlated with poor survival in patients with TN but not with the other breast cancer types. This correlation between ANX2 expression and poor survival in TN was not found in our study. This is to be expected since significant ANX2 expression in TN was observed in the high-grade tumors only. The effect of disease stage and grade on survival may also have been confounded by age differences and the relatively few patients with high expression of ANX2.

A limitation in our study is that we did not have a large sample size, which may limit the assessments among breast cancer subtypes. Most reported gene studies of ANX2 displayed larger sample sizes. In addition, we were unable to distinguish between basal-like breast tumors and TN. The number of TN patients who expressed ANX2 was much lower than among those who did not. Moreover, we note that a majority of our patients were older than 50 years and about 41% of them had high expression of Luminal A subtype. Despite these limitations, this preliminary study has shown clearly that ANX2 expression is elevated in Luminal B and HER2 subtypes and in the case of TN, with high grade tumors only. In conclusion, the expression of ANX2 correlates with the aggressiveness of breast cancer and substantiates its prospect as a prognostic marker for molecular breast cancer subtypes in African Americans.

5. Conclusion

This study highlights a significant association between ANX2 protein expression and the subtypes Luminal B (p = 0.032) and HER2 (p = 0.025). Our survival analysis showed that ANX2 protein expression in breast tumors might be a biomarker candidate for breast cancer outcome prediction in high risk groups such as Luminal B and HER2 cases. Further confirmatory studies will be needed to examine the correlation between ANX2 expression, grade and survival using a larger sample size in a biracial group of women.

Declarations

Author contribution statement

Desta Beyene: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Norma Kanarek, Luisel Ricks-Santi: Analyzed and interpreted the data; Wrote the paper.

Tammey Naab: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Tamaro Hudson: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Competing interest statement

Additional information

Acknowledgements

References

[1] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, Ca - Cancer J. Clin. 68 (1) (2018) 7–30, 2018.

[2] F.O. Ademuyiwa, O.I. Olopade, Racial differences in genetic factors associated with breast cancer, Canc. Metastasis Rev. 22 (1) (2003) 47–53.

[3] R.T. Chlebowski, Z. Chen, G.L. Anderson, et al., Ethnicity and breast cancer: factors influencing differences in incidence and outcome, J. Natl. Cancer Inst. 97 (6) (2005) 439–448.

[4] I. Soerjomataram, M.W. Louwman, J.G. Ribot, et al., An overview of prognostic factors for long-term survivors of breast cancer, Breast Canc. Res. Treat. 107 (3) (2008) 309–330.

[5] S.E. Moss, R.O. Morgan, The annexins, Genome Biol. 5 (4) (2004) 219.

[6] K.A. Hajjar, S. Krishnan, Annexin II: a mediator of the plasmin/plasminogen activator system, Trends Cardiovasc. Med. 9 (5) (1999) 128–138.

[7] K. Huebner, L.A. Cannizaro, A.Z. Frey, et al., Chromosomal localization of the human genes for lipocortin I and Lipocortin II, Oncogene Res. 2 (4) (1988) 299–310.

[8] Schlaepfer DD and Haigler HT Expression of Annexins as a function of cellular growth state, JCB (J. Cell Biol.) 111 (1) (1990) 229–238.

[9] N.A. Lokman, M.P. Ween, M.K. Oehler, C. Ricciardelli, The role of Annexin A2 in tumorigenesis and cancer progression, Canc. Microenviron. 4 (2011) 199–208.

[10] M. Sharma, R.T. Ownbey, M.C. Sharma, Breast cancer cell surface annexin II induces cell migration and neoangiogenic via tPA dependent plasmin generation, Exp. Mol. Pathol. 88 (2) (2010) 278–286.

[11] S. Deng, B. Jing, T. Xing, et al., Overexpression of annexin A2 is associated with abnormal ubiquitination in breast cancer, Dev. Reprod. Biol. 10 (3) (2012) 153–157.

[12] R. Duncan, B. Carpenter, L.C. Main, et al., Characterization and protein expression profiling of annexins in colorectal cancer, Br. J. Canc. 98 (2) (2008) 426–433.

[13] J.W. Jia, K.L. Li, J.X. Wu, S.L. Guo, Clinical significance of annexin II expression in non-small lung cancer, Tumor Biol. 34 (3) (2013) 1767–1771.

[14] D.A. Beyene, T.J. Naab, N.F. Kanarek, et al., Differential Expression of Annexin2, SPINK1 and Hsp60 predict progression of prostate cancer through bifurcated WHO Gleason score categories in African American Men, The Prostate 78 (2018) 801–811. March 27.

[15] L.A. Carey, C.M. Perou, C.A. Livasy, et al., Race, breast cancer subtypes, and survival in the Carolina breast cancer study, J. Am. Med. Assoc. 295 (2006) 2492–2502.

[16] A.A. Onitilo, J.M. Engel, R. Greenlee, B.N. Mukesh, Breast cancer subtypes based on ER/PR and Her2 expression: comparison of clinopathologic features and survival, Clin. Med. Res. 7 (1-2) (2009) 4–13.

[17] T. Sorlie, C.M. Perou, R. Tibshirani, et al., Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications, Proc. Natl. Acad. Sci. 98 (2001) 10869–10874.

[18] L.D. Gibbs, P. Chaudhary, K. Manshein, R.J. Hare, et al., ANAXA2 Expression in African American Triple-Negative Breast Cancer Patients, Breast Cancer Research and Treatment, 2018. (Accessed November 2018).

[19] P.L. Nguyen, A.G. Taghian, M.S. Katz, A. Niemierko, et al., Breast cancer subtype approximated by estrogen receptor, progesterone receptor, and Her-2 is associated with local and distant recurrence after breast-conserving therapy, J. Clin. Oncol. 26 (2008) 2373–2378.

[20] S.M. Hewitt, Tissue microarrays as a tool in the discovery and validation of predictive biomarkers, Methods Mol. Biol. 823 (2012) 201–214.

[21] H. Ma, Y. Lu, P.A. Marchbanks, et al., Quantitative measures of estrogen receptor expression in relation to breast cancer-specific mortality risk among white women and black women, Breast Canc. Res. 15 (5) (2013), R90.

[22] R. Diallo-Danebrock, E. Ting, O. Gluz, A. Herr, et al., Protein expression profiling in high-risk breast cancer patients treated with high-dose or conventional dose–dense chemotherapy, Clin. Canc. Res. 13 (2 Pt1) (2007) 488–497.