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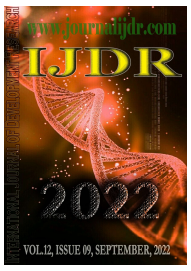

RESEARCH ARTICLE OPEN ACCESS

IN SILICO ANALYSIS REVEALED ARP2/3 COMPLEX MOLECULE AS A POTENTIAL PROGNOSTIC AND PREDICTIVE BIOMARKER IN BREAST CANCER

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ABSTRACT

We can stratify breast cancer into different clinically relevant molecular subtypes. The ARPC3 molecule can significantly contribute to the process of metastasis formation due to its expression in actin cytoskeleton remodeling and has been arousing the interest of many researchers. In this work, we sought to evaluate *in silica* the role of ARPC3 gene expression and its correlation with prognostic and predictive factors in breast cancer, using several datasets deposited in public repositories. Our data showed that ARPC3 showed higher gene expression in breast tumor samples regardless of clinical stages compared to adjacent normal tissue. Additionally, we identified higher expression of mRNA for ARPC3 in hormone-dependent tumors and less expressed in triple negative cases. Our sample evidence allowed us to infer that there is a correlation between the differential expression of ARPC3 with lymph node status, SBR classification, molecular subtype and HER2 oncoprotein. Regarding prognosis, high expression of ARPC3 in human breast tumor samples conferred worse recurrence-free survival for patients. Finally, among patients who were diagnosed with luminal tumors and who did not respond to hormone therapy, they had higher expression of ARPC3. Together, our data point to ARPC3 as a potential prognostic and predictive biomarker in breast cancer.

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INTRODUCTION

Breast cancer (BC) is a malignant neoplasm of heterogeneous characteristics that represents the highest incidence and mortality rates among women worldwide (Lima, Kehm, and Terry 2021). In Brazil alone in 2019, more than 18.000 women died from this malignant neoplasm and the estimates for the triennium 2020-2022, according to INCA (National Cancer Institute José Alencar Gomes da Silva), is 66.280 new cases, being the most frequent breast cancer in this population (Schilithz *et al*. 2019). Regarding the molecular characteristics of breast cancer, the genes commonly evaluated to subtype the disease are: gene encoding human epidermal growth factor receptor 2 (HER2), genes encoding hormone receptors for estrogen (ER) and progesterone (PR) and the Ki-67 cell proliferation regulator gene (Harbeck *et al*. 2019). Thus, the clinically relevant

molecular subtypes are: Luminal A, Luminal B, HER2 enriched (HER2+) and Triple Negative (TN) (Nascimento and Otoni 2020). Thus, systemic therapies for the treatment of breast cancer are directly related to these subtypes, so that Luminal A tumors are commonly treated with endocrine therapy associated or not with chemotherapeutic agents, while Luminal B tumors are treated with endocrine therapy, targeted therapy according to HER2 status and chemotherapy. HER2+ cases, on the other hand, show a good response to monoclonal antibodies against this tyrosine kinase receptor. Finally, TNBC represent a challenge for clinical oncology due to the lack of molecular targets, leaving as a first-line treatment the use of taxanes and anthracyclines associated or not with PARP enzyme inhibitors, depending on the mutation status of BRCA1 and 2 (Fragomeni, Sciallis and Jeruss 2018; Nascimento and Otoni 2020).
As a result, discoveries about biomarkers in breast cancer have contributed greatly to early detection, differential diagnosis, monitoring, metastasis prediction, clinical management of the disease and selection of increasingly accurate therapies and, as a result, there are currently 25 target-molecular therapies were approved for clinical use based on biomarkers (Califf 2018; Liu, Pandya and Afshar 2021). However, there are many gaps in understanding drug resistance mechanisms and accurate diagnosis. Thus, studies on individual biomarkers or by genetic signatures have become increasingly important in an attempt to elucidate the intrinsic characteristics of the tumor at different levels (Liu, Pandya and Afshar 2021).

Importantly, invasion and metastasis occur through the ability of cancer cells to bypass a series of molecular and biophysical obstacles (Suhail *et al*. 2019). In order for a cell to move and invade other tissues, several mechanisms are necessary, such as an increase in receptors for detecting extracellular signals, protrusions towards the signal source or microenvironment conducive to its maintenance, resistance to programmed cell death and death by loss of adhesion, contraction and retraction of the cell tail (Wang *et al*. 2004). Therefore, for many of these processes to be possible, reorganization of the actin cytoskeleton must occur (Mondal, Di Martino and Bravo-Cordero 2021). Furthermore, during the cell motility cycle, resting cancer cells receive external chemoattractant signals that make them polarized, consequently favoring binding to cell surface receptors and stimulating intracellular signaling pathways (Yamaguchi and Condeelis 2007). Thereby, they allow nucleating promoting factors (NPF) – such as the WASP-family verprolin-homologous protein (WAVE) and Wiskott-Aldrich (WASP) – to activate the actin-related protein 2/3 complex (Arp2/3), with the purpose of binding to a preexisting actin filament and initiating the nucleation and polymerization of actin forming microfilaments (Pandit *et al*. 2020). Thus, actin assembly is used to form protrusions known as lamellipodia, filamepodia, and invadopodia at the edge of the cell membrane of mobile cells, facilitating invasion into the extracellular matrix and migration into blood vessels during intravasation (Pandit *et al*. 2020; Suraneni *et al*. 2012).

It is understood that the Arp2/3 complex in humans is made up of the accessory subunits p40 (ARPC1A), p41 (ARPC1B), p34 (ARPC2), p21 (ARPC3), p20 (ARPC4), p16 (ARPC5) and two related proteins to actin: ARP2 (ACTR2) and ARP3 (ACTR3) (Cooper, Wear, and Weaver 2001). Notably, in the last decades many studies have focused on the relevance of the biological role and the organization of the Arp2/3 complex subunits regarding the processes of actin nucleation, microfilament formation, extension and formation of protrusions, promotion, migration and invasion of cancer cells, as well as the correlation between prognosis, histological grade and clinical outcome of patients (Chen *et al*. 2019; Iwaya, Norio and Mukai 2007; Kaneda *et al*. 2004; Rauhala *et al*. 2013). Among these studies, there is one that used *in silico* bioinformatics tools on the identification of Arp2/3 as a prognostic biomarker in hepatocellular carcinoma, revealing that the increased expression of this complex is related to patients with more advanced stages of cancer and worse survival rates (Huang *et al*. 2021). Notably, it is understood that the ARPC3 subunit can contribute significantly to the process of metastasis formation due to its expression in actin cytoskeleton remodeling (Dombkowski *et al*. 2011). Furthermore, studies show that ARPC3 is highly expressed in laminepodia of motile fibroblasts and is dynamically associated with peripheral regions of the actin cytoskeleton, including when in motion, participating in lamellipodial protrusion (Machesky *et al*. 1997; Welch *et al*. 1997). However, there is currently a need for more robust evidence that might indicate ARPC3 as a prognostic marker in breast cancer. Therefore, this study sought to evaluate *in silica* the role of ARPC3 gene expression and its correlation with prognostic and predictive factors in breast cancer.

METHODOLOGY

Experimental Design: In our descriptive, quantitative study, conducted *in silica*, we performed several analyzes using the web platforms *KM Plotter*, *PrognoScan*, *bc-GenExMiner*, *UALCAN* and *ROC Plotter*. We evaluated the relevance of ARPC3 (actin related protein 2/3 complex subunit 3) gene expression in samples from breast cancer patients, in terms of demographic, epidemiological, clinicopathological and response to currently used therapies.

UALCAN: *UALCAN* (http://ualcan.path.uab.edu/) is a web resource that presents an easy-to-handle transcriptomic dataset that generates high-quality graphs with built-in biostatistics. In our study, we investigated the levels of ARPC3 gene expression as a function of tumor or healthy breast tissue, race, molecular subtypes and finally the methylation profile of the ARPC3 promoter region in tumor and normal breast samples (Chandrashekar *et al*. 2017).

Brest Cancer Gene Expression Miner: We analyzed ARPC3 expression according to different clinicopathological parameters, regarding classic breast cancer biomarkers and different molecular subtypes using the online transcriptomic data mining tool *Breast Cancer Gene-Expression Miner* v4. 5 (http://bcgenex. centregauducheau.fr/). We used the median expression as a cut-off point and p values <0.05 as statistically significant (Jézéquel *et al*. 2012).

KM Plotter: To evaluate the correlation of the gene expression level of ARPC3 with its potential prognostic value, we used the open access algorithm *Kaplan-Maier Plotter* (https://kmplot.com/ analysis/). This online tool provides us with the possibility to calculate the correlation between the expression of different types of genes with the overall, disease-free and metastasis-free survival, using datasets that include *GEO* (Gene Expression Omnibus) and *TCGA* (The Cancer Genome Atlas). We considered p-values <0.05 as statistically significant using the Log-rank test (Lánczky and Győrffy 2021).

Progno Can: To assess the relationship of survival in breast cancer patients as a function of ARPC3 expression, we used datasets from *PrognoCan* (http://www.prognoscan.org/). This platform has a public dataset of microarrays, which help us to assess the biological relationships between prognostic information and the gene expression of various biomarkers. Cox P values and hazard ratio (HR) with 95% confidence intervals (CI) were calculated automatically (Mizuno *et al*. 2009).

ROC Plotter: Finally, we investigated and evaluated the possible relationship of the differential expression of ARPC3 with the response or not of breast cancer patients to the different treatments used, using the *ROC Plotter* database (http://www. rocplot.org/) (Fekete and Győrffy 2019).

RESULTS

Initially, we performed analyzes using transcriptomic data provided by the TCGA consortium to assess ARPC3 expression in different contexts. We found that, in tumor tissue samples, ARPC3 is more expressed compared to normal breast tissues ($p<0.0001$) (Figure 1A). In addition, we observed greater methylation in the ARPC3 promoter region in breast tumor samples when compared to adjacent normal tissue ($p<0.0001$) (Figure 1D).

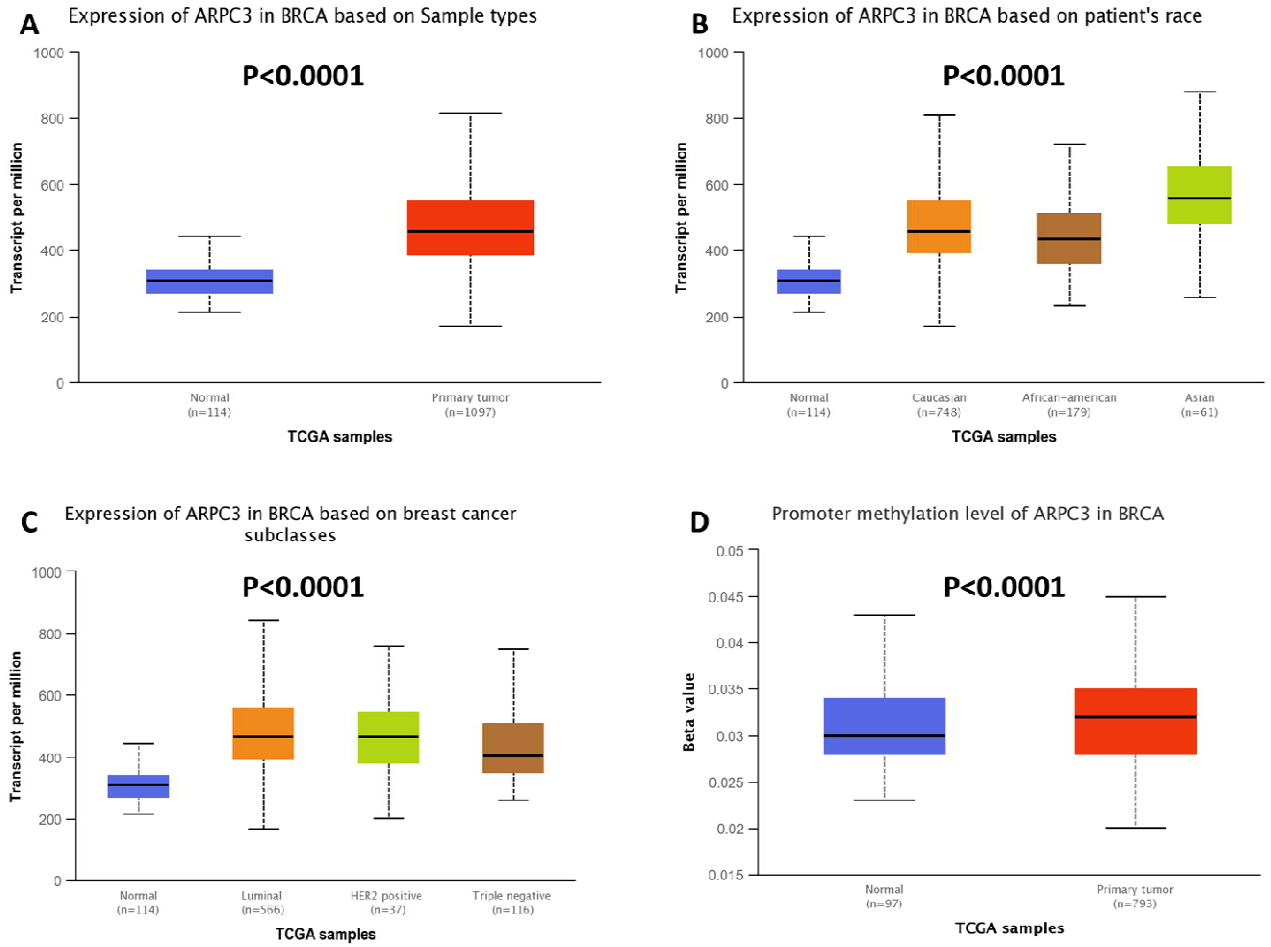

Figure 1. ARPC3 gene expression in samples from breast cancer patients. A) Expression of ARPC3 in tumor and normal breast samples. B) ARPC3 expression profile according to ethnicity. C) ARPC3 expression in different molecular subtypes of breast cancer. D) Methylation profile of the ARPC3 promoter region in tumor and normal breast samples

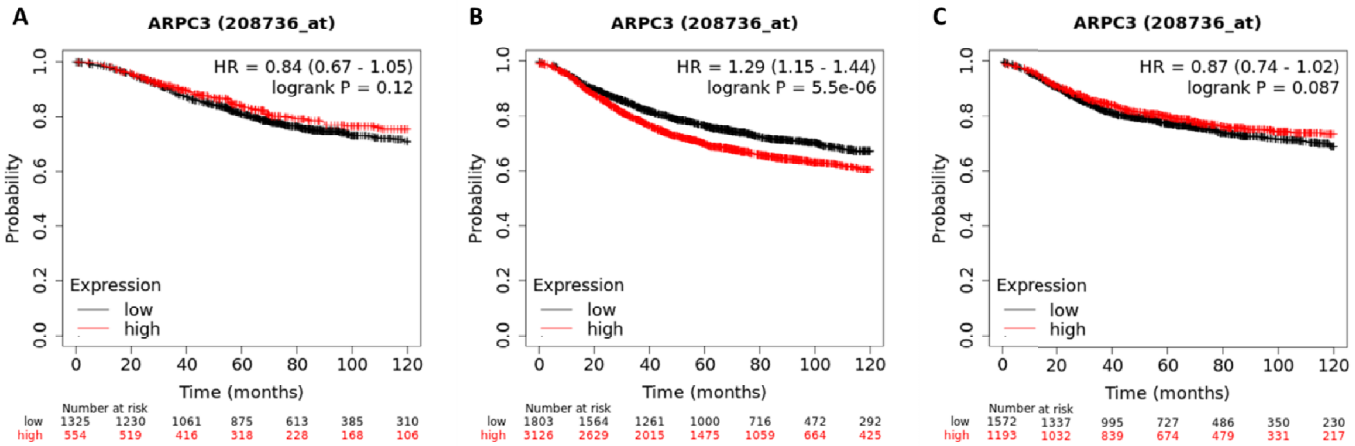

Figure 2. Kaplan-Meier Plotter-derived survival curves assessing the prognostic significance of ARPC3 in breast cancer. According to the different levels of ARPC3 gene expression in samples from breast cancer patients, rates of (A) overall survival, (B) recurrence-free survival and (C) metastasis-free survival were evaluated

Surprisingly, regarding the racial factor, we found that Asian patients with breast malignancy had higher levels of ARPC3 expression when compared to Caucasian and African-American patients ($p<0.0001$) (Figure 1B). Regarding the different molecular subtypes, the Luminal and HER2+ subtypes showed higher transcript levels of ARPC3 when compared to the TN breast cancer subtype ($p<0.0001$) (Figure 1C).

In order to estimate the relationship between ARPC3 expression and clinical and pathological parameters of breast cancer patients, we used the bc-GenExMiner database. Our investigation pointed to a statistically significant association between the differential expression of this gene with the following conditions: lymph node status ($p<0.0001$), SBR grade (Scarff-Bloom-Richardson) ($p<0.0001$) and with the HER2 receptor tyrosine kinase ($p<0.0001$) (Table 1). Despite investigating the prognostic significance of ARPC3 in breast cancer, we used survival curves derived from the online Kaplan-Meier Plotter software. According to survival analysis, we identified that increased ARPC3 gene expression in breast tumor samples, regardless of molecular subtype, was a factor that conferred worse recurrence-free survival (RFS) ($p<0.0001$) for these patients (Figure 2B). Additionally, there was no statistically significant correlation between the differential expression of this biomarker and overall ($p=0.120$) and metastasis-free ($p=0.087$) survival (Figures 2A and 2C).

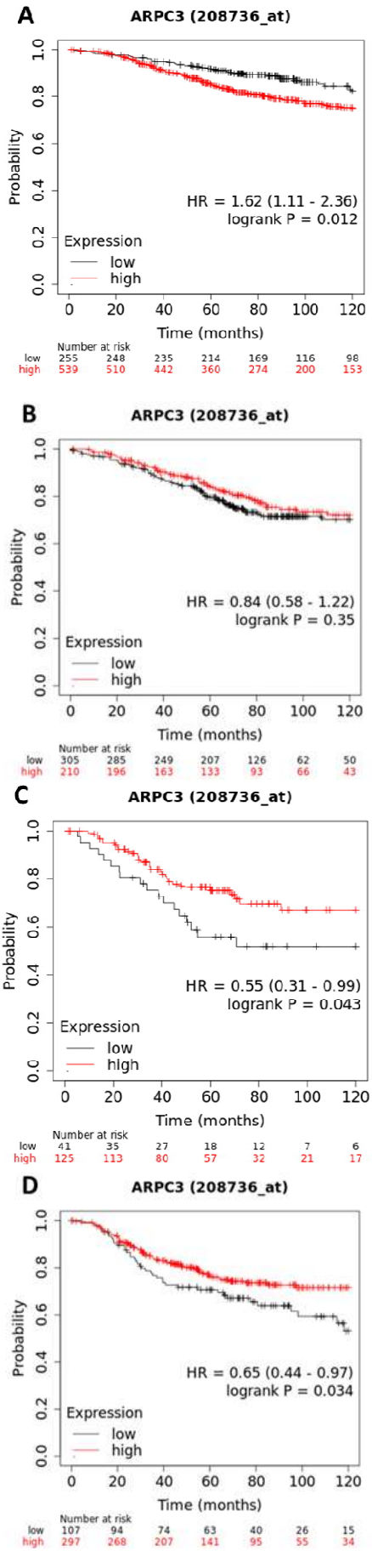

Figure 3. Overall survival curves of breast cancer patients as a function of ARPC3 gene expression. Overall survival of breast cancer patients according to the difference in ARPC3 gene expression levels in tumors classified as (A) Luminal A, (B) Luminal B, (C) HER2+ and (D) Triple Negative

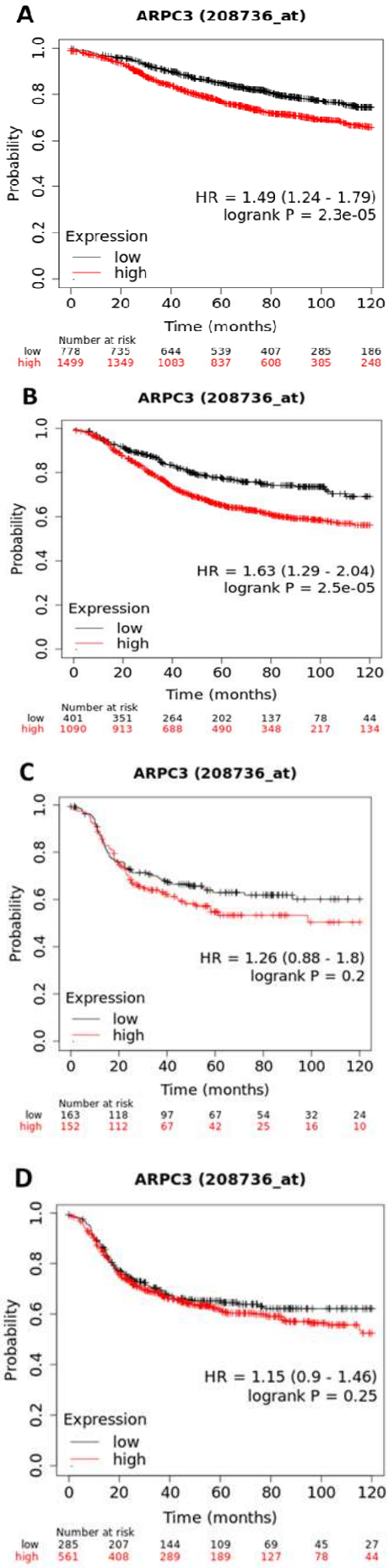

Figure 4. Recurrence-free survival curves of breast cancer patients as a function of ARPC3 gene expression. Relapse-free survival of breast cancer patients according to the difference in ARPC3 gene expression levels in tumors classified as (A) Luminal A, (B) Luminal B, (C) HER2+ and (D) Triple Negative

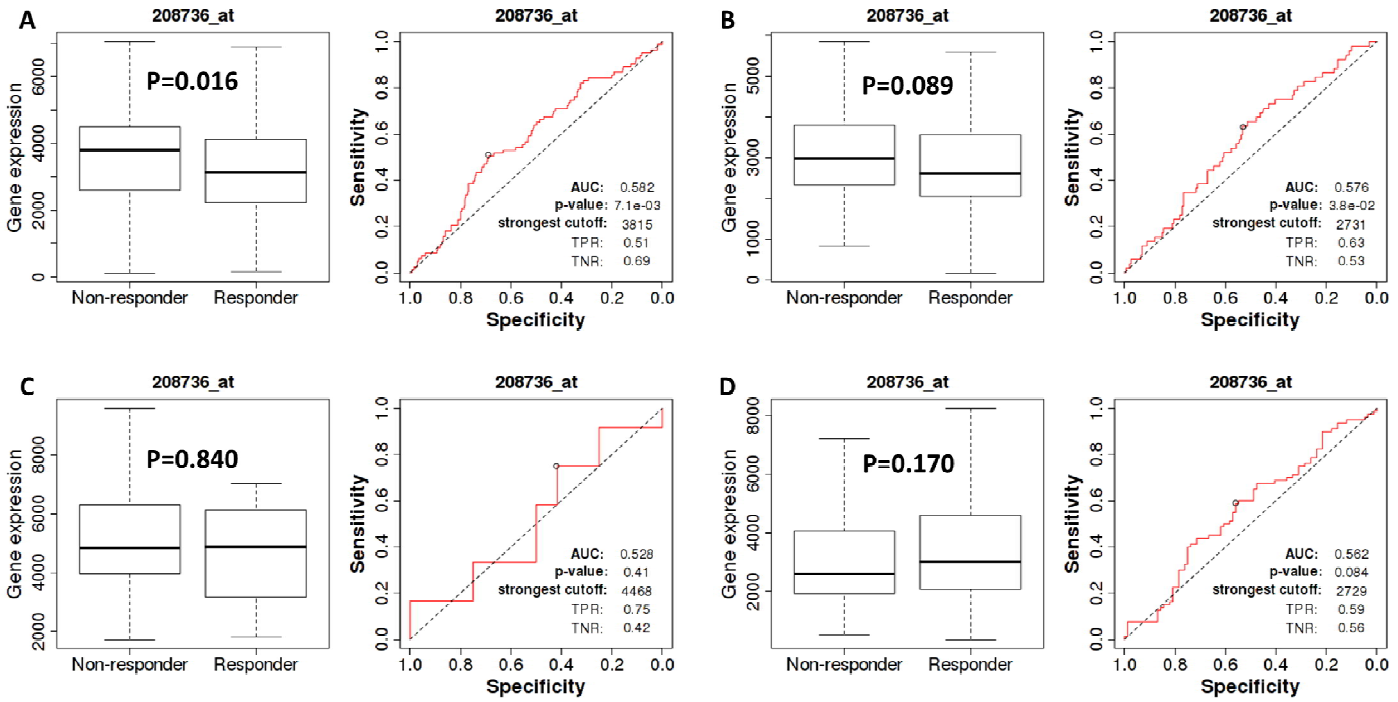

Figure 5. Association between ARPC3 gene expression and the response of breast cancer patients to different therapies. (A) Cases of breast cancer classified as Luminal A and treated with endocrine therapy. (B) Cases of breast cancer classified as Luminal B and treated with endocrine therapy. (C) Cases of breast cancer classified as HER2+ and treated with monoclonal antibodies. (D) Cases of breast cancer classified as Triple Negative and treated with chemotherapeutic agents

To support our initial findings, we sought information on differential expression of ARPC3 transcripts with survival data using the PrognoScan database. The results showed that the differential expression of ARPC3 may be a determining factor in the overall, disease-free, disease-specific and metastasis-free survival in patients with breast cancer, using different probes for this gene (Table 2). Subsequently, we also evaluated the survival of breast cancer patients according to the levels of ARPC3 gene expression in different molecular subtypes. Based on our findings, we noted a clear association between high levels of ARPC3 with worse overall survival (OS) in patients with Luminal A breast cancer (p=0.012) (Figure 3A). However, there was no statistical association when we evaluated the Luminal B subtype (p=0.350) (Figure 3B). Unlike the cases of patients with hormone-dependent tumors, for the more aggressive subtypes of breast cancer HER2+ and TN, reduced expression of ARPC3 was correlated with worse overall survival (p=0.043 and p=0.034, respectively) (Figure 3C and 3D, respectively). Additionally, analyzing the recurrence-free survival curves (RFS) and we noticed that upregulation of ARPC3 gene expression was able to confer a worse prognosis for patients with breast tumors subtyped as Luminal A and B (p<0.0001) (Figure 1). 4A and 4B). There was no statistically significant correlation in recurrence-free survival based on differential ARPC3 expression in the HER2+ (p=0.200) and TN (p=0.250) subtypes (Figure 4C and 4D, respectively). Finally, we sought data on the differential expression of ARPC3 associated with the response of breast cancer patients to different therapies commonly used in clinical practice. Thus, we found that patients diagnosed with Luminal A tumors who did not respond to hormone therapy had higher expression of ARPC3 (p=0.016) (Figure 5A). The same profile was observed for cases classified as Luminal B, although this value was not statistically significant, most likely due to the small number of cases (p=0.089) (Figure 5B). Additionally, cases of breast cancer classified as HER2+ and treated with monoclonal antibodies did not present relevant data on the basis of our statistical analysis (p=0.840) (Figure 5C). Similar to the HER2+ cases, patients with tumors classified as TN and treated with chemotherapeutic agents, the differential expression of ARPC3 was not a determining factor to confer differences in the patients' response to treatment (p=0.170) (Figure 5D).

Table 1. Relationship between ARPC3 gene expression and clinicopathological parameters of breast cancer patients using the bc-GenExMiner database

Variables	Patient Number	ARPC3 microarray	*P* value
Age			0.5529
≤51	2819	-	
>51	4683	-	
Nodal Status			<0.0001
Negative	4413	-	
Positive	3471	Incresead	
SBR			<0.0001
1	987	Decreased	
2	3177	-	
3	3309	Incresead	
Status TP53			0.3826
Wild-type	638	-	
Mutated	284	-	
Estrogen Receptor			0.1514
Negative	2437	-	
Positive	6816	-	
Progesterone Receptor			0.1453
Negative	2455	-	
Positive	3187	-	
HER2			<0.0001
Negative	4581	-	
Positive	778	Incresead	
Molecular Subtypes			<0.0001
Luminal A	3243	-	
Luminal B	2924	-	
HER2	1204	Incresead	
Triple Negative	2002	Decreased	

DISCUSSION

Breast cancer is the most common among women and is characterized by intertumoral and intratumoral heterogeneity, different prognostic profiles and treatment response rates that make it a challenging neoplasm (Harbeck *et al*. 2019).

Table 2. ARPC3 gene expression and survival data from breast cancer patients using the PrognoScan database

Dataset	Probe name	End point	Patient number	Cox *P*-value	HR
GSE11121	208736_at	Distant Metastasis Free Survival	200	0.001265	0.14 [0.04 - 0.46]
GSE1378	12416	Relapse Free Survival	60	0.018323	2.88 [1.20 - 6.93]
GSE9893	3464	Overall Survival	155	0.010174	1.31 [1.07 - 1.61]
GSE1456-GPL96	208736_at	Relapse Free Survival	159	0.013128	3.77 [1.32 - 10.75]
GSE1456-GPL96	208736_at	Disease Specific Survival	159	0.049699	3.43 [1.00 - 11.73]
GSE3494-GPL96	208736_at	Disease Specific Survival	236	0.043821	2.41 [1.02 - 5.66]
GSE4922-GPL96	208736_at	Disease Free Survival	249	0.001894	2.95 [1.49 - 5.84]
GSE2990	208736_at	Relapse Free Survival	125	0.016172	2.64 [1.20 - 5.83]

Due to this complexity, the use of biomarkers has been a method of aid in diagnosis, prediction of therapeutic response, determination of conducts and disease surveillance throughout the process of the oncological chain (Esparza-López *et al*. 2017). The ARPC3 subunit is part of the Arp2/3 complex and is an important actin nucleator to generate branched networks that promote the projection of the plasma membrane into protrusive structures with phenotypic characteristics of migration and invasion called lamellipodia, filamepodia and invadopodia (Jacquemet, Hamidi and Ivaska 2015; Molinie and Gautreau 2018). These structures function in edge projection, environmental probing, extracellular matrix degradation, and cell displacement (Crosas-Molist *et al*. 2022). It is precisely this remodeling process that enables, in some cases, the metastasis of cancer cells (Huang *et al*. 2021; Otsubo *et al*. 2004). It is important to note that pioneering exploratory studies on the function of the Arp2/3 complex in animal cells using Swiss 3T3 fibroblasts showed that ARPC3 was distributed along the thin actin-rich lamellipodia at the ends of these mobile and stationary cells (Welch *et al*. 1997). In addition, ARPC3 was strategically located during actin polymerization providing stabilization in the geometry of the complex (Egile *et al*. 2005). For the formation of these phenotypic structures to occur, it is necessary that the Rho GTPases regulate the NPF and this can occur in different ways. The first, through RAC1 for ENA/WASP-mediated polymerization. Another way is that binding of RAC1 to Sr1 stimulates the Scar/WAVE regulatory complex. The third, that PAK1 and MLK3 stimulate the WAVE complex. Finally, let Cdc42 through N-WASP activate Arp2/3 (Crosas-Molist *et al*. 2022). Furthermore, RAC1 controls an important inhibitory circuit of the Arp2/3 complex through the engagement and refolding of the Arp2/3 complex inhibitor protein (Arpin) (Liu *et al*. 2016).

Some studies confirm the importance of these NPF in cancer progression and their relationship with the Arp2/3 complex when, for example, WAVE3 was identified as a marker in the increased risk of specific mortality from breast cancer with metastatic potential in cases classified as TN and as a potential therapeutic target (Kulkarni *et al*. 2012). Likewise, the highest expression of the WAVE2 signal to the Arp2/3 complex was detected in cell lines derived from breast cancer that had HER2 gene amplification (Yokotsuka *et al*. 2011). Furthermore, studies show that 17β-estradiol induces focal adhesion kinase (FAK) phosphorylation, since, when excited, it recruits GTPase Cdc42, so that N-WASP relays this signaling to the Arp2/3 complex and with it, restarts actin branching in the formation of lamellipodia at sites related to cell movement for migration, invasion and metastasis in breast cancer (Sanchez *et al*. 2010). In fact, the role of this complex in cancer is so relevant that different studies have shown that some of its molecules are involved in carcinogenesis in several types of cancers, including prostate, stomach, lung, head and neck, multiple myeloma, liver and colorectal (Gamallat *et al*. 2022; Kinoshita *et al*. 2012; Lv *et al*. 2018; Semba *et al*. 2006; Su *et al*. 2018; Xiong and Luo 2018; J. Zhang *et al*. 2017). In the process of breast carcinogenesis, it is known that the ARPC3 paragole, that is, the ARPC2 molecule, is related to progression, proliferation and metastasis (Chen *et al*. 2019; Cheng *et al*. 2019). Arp2, on the other hand, is related to a poor prognosis in invasive breast cancer and its co-expression with WAVE2 resulted in ductal carcinoma with higher histological grade, lymph node metastasis and lower disease-free survival (Iwaya, Norio and Mukai 2007). Also, studies indicate that the expression of transcripts and protein of the inhibitor of the Arp2/3 complex (Arpin) in cancerous tissues is lower compared to normal breast tissues (Liu *et al*. 2016). However, even in cancer cases in which any of the different subunits may be more or less expressed, it is possible to hypothesize that the presence of a subunit is indicative of total or partial activity of the entire Arp2/3 complex. Although we have many studies focused on certain subunits of the complex, little is known about the correlation between ARPC3 mRNA expression and dysregulation in breast cancer. Therefore, this study used searches in several transcriptomic databases to evaluate the differential gene expression in different types of samples and the impact of gene expression in different clinical and treatment response contexts in breast cancer. Initially, the data obtained through *TCGA* allowed us to infer that ARPC3 is more expressed in breast tumor tissues when compared to adjacent normal tissues. To date, there are no data in the literature that can be compared with our findings. However, this same expression pattern was found in another subunit of the complex, the ARPC2 molecule, where Zhang and colleagues identified higher levels of ARPC2 protein using immunohistochemistry in gastric cancer tissues than in healthy tissues (Zhang *et al*. 2017). Corroborating these findings, two studies with bioinformatics analyzes using various public resources, one of which used 371 samples of liver tumor tissues and 50 samples of normal tissues, showed that ARPC3 and other subunits of the Arp2/3 complex were positively regulated in hepatocellular carcinoma tissues (Huang *et al*. 2021; Zhang *et al*. 2021). Together, these data indicate that the increased expression of ARPC3 and other subunits of the Arp2/3 complex is associated with tumorigenesis in different models (Molinie and Gautreau 2018).

Based on our results, ARPC3 is hypermethylated in breast tumor samples when compared to healthy tissues, which may characterize a possible regulatory mechanism in cancer, which allows us to have some insights into the epigenetic mechanisms of this marker. Growing evidence supports the proposition that analogous epigenetic changes may contribute to the acquisition of hallmark capabilities during tumor development and malignant progression (Joo *et al*. 2018; Severi *et al*. 2014; van Veldhoven *et al*. 2015). Interestingly, Dombrowski and colleagues used miR-AT computational methods to predict the combined activity of genes at target sites of microRNAs that were repressed in metastatic breast cancer cells, and identified that ARPC3 was highly regulated, placing it as a potential oncogene (Dombkowski *et al*. 2011). Furthermore, we identified that Asian patients with malignant breast cancer have higher levels of ARPC3 gene expression when compared to Caucasian and African-American patients. Research shows that breast cancer is the fastest growing malignancy and causes the highest numbers of fatalities in women in some parts of Asia, this is also noticed in other countries that harbor this population, so we were intrigued about the relationship of ARPC3 in Asian women and the possibility of further retrospective and prospective investigations at the transcriptomic or proteomic level that may support this relationship and, perhaps, suggest it as a biomarker for population screening (Navarro *et al*. 2022; Rajkumar *et al*. 2022). It is considered that, in breast cancer, the axillary lymph nodes are research targets because they are the initial site of micro and macro metastasis, converging on a worse prognosis proportionally to the number of lymph nodes involved (Paula *et al*. 2017). Therefore, we performed several analyzes using the public Bc-GenExMiner database to assess the relationship of ARPC3 gene expression with lymph node status and several other clinicopathological parameters. We evidenced a statistically

significant association between the differential expression of this gene and lymph node status. The same behavior can be seen in other members of the Arp2/3 family that showed a relationship between the higher expression of subunits of this complex with lymph node invasion (J. Zhang *et al*. 2017). A study that evaluated changes in cellular behavior and gene expression in neoplastic cells, using mammary tumors derived from the medium T oncogene (PyMT), observed that the genes encoding the subunits of the Arp2/3 complex are positively expressed in invasive cells, in this context, ARPC3 and other subunits of the complex were highly regulated (Wang *et al*. 2007). In general, these findings are in line with the role of Arp2/3 that provides cell movement mediated by the formation and disassembly of actin filaments, enabling a phenotype that makes cells more mobile with the ability to determine sites of protrusion and cellular direction for tissue invasion and lymphovascular system (Crosas-Molist *et al*. 2022; Otsubo *et al*. 2004).

Furthermore, we observed that ARPC3 was significantly more expressed in grade 3 of the SBR classification. Note that the SBR classification system assesses the histological grade of tumor tissue based on tumor size, lymph node status and vascular invasion status, and the higher the SBR grade, the worse the patient's prognosis (Amat *et al*. 2002). Thus, these findings may suggest a possible oncogenic role for ARPC3. Subsequently, we sought to understand the prognostic role of ARPC3 in breast cancer using the KM Plotter web platform. We showed that the differential expression of ARPC3 was not determinant for the overall and metastasis-free survival of patients with breast cancer. However, high expression of this marker was related to reduced disease-free survival. Corroborating these findings, Moazzam and colleagues using a cohort of 127 samples of malignant breast tumors found that increased expression of both ARP2/3 transcripts and protein was significantly associated with reduced rates of disease-free survival of patients (Moazzam *et al*. 2009). With regard to molecular subtypes, we identified that elevated ARPC3 expression is linked to a worse prognosis for patients with hormone-dependent breast tumors correlated with a worse overall survival for Luminal A cases and disease-free survival with tumors subtyped in Luminal A and B. In general, hormone-dependent subtypes have a favorable prognosis, slow evolution, high response rates to endocrine therapies and a lower frequency for the development of metastasis (Hashmi *et al*. 2018). On the other hand, Sanchez *et al*. showed that the E2-induced signaling cascade with its respective receptor can lead to drastic changes in cell membrane morphology, actin cytoskeleton rearrangement and formation of focal adhesion complexes, culminating in increased breast cancer cell motility through the FAK/cdc42/N-WASP/Arp-2/3 signaling cascade (Sanchez *et al*. 2010).

Furthermore, in the cases of patients with breast tumors subtyped as HER2+ and TN, we observed a simple correlation between the decrease in ARPC3 expression with worse overall survival of patients with breast cancer, but there was no statistical relationship with disease-free survival. However, we have to be cautious in inferring our findings as the sample cohort for HER2+ and NT tumors were significantly lower when compared to hormone-dependent tumors. To date, little is known about how molecules of the Arp2/3 complex could be acting on these two clinically more aggressive subtypes of breast cancer. A study carried out by Yokotsuka's group showed that HER2 may be correlated with the formation of lamellipodia, in addition, WAVE2–Arp2/3 signaling may be induced by HER2 overexpression, leading to increased migration in breast cancer cells (Yokotsuka *et al*. 2011). Subsequently, we investigated the relationship between ARPC3 gene expression and the response rates of breast cancer patients who used different therapies according to the molecular profile of the tumor. We found that tumors from hormone-dependent breast cancer patients who did not respond to endocrine therapy had a higher expression of ARPC3, although in the Luminal B subtype there was almost statistical significance, most likely due to the low sample number for this subtype. Our findings are intriguing, as the presence of estrogen and progesterone hormone receptor expression in the Luminal subtypes generally confers benefits on patients with the use of selective estrogen receptor modulators and aromatase inhibitors (Moccia and Haase 2021). Thus, our findings indicate that the activity of molecules of the Arp2/3 complex may be one of the resistance mechanisms in hormonal breast cancer, requiring further investigation of the mechanisms underlying the binding of these signaling pathways. In fact, the altered expression of members of the Arp2/3 complex subunits in different types of cancer and the correlation with aggressive and metastatic behaviors, poor outcomes and prognoses, in addition to therapeutic alternatives, has been investigated by several authors, relating them to possible biomarkers. for future therapeutic targets (Algayadh, Dronamraju, and Sylvester 2016; Kazazian *et al*. 2017; Liu *et al*. 2016). Thus, reinforcing the possibility of the potential oncogenic role of ARPC3, a study performed in hepatic metastatic breast cancer tissues showed that there was significant expression of ARPC3 in all human samples examined. Thus, they are connected to the functional role of this marker, with regard to the motility of cancer cells in the process of co-opting vessels in vivo to carry out metastasis. It is known that breast cancer metastases in various organs such as lymph nodes, brain, skin, lung and liver use vessel cooptation and that in some cases antiangiogenic therapy is ineffective, reducing the clinical benefit and impacting the overall survival of patients (Frentzas *et al*. 2017). Furthermore, other studies using invasion assays have pointed to significant expression of ARPC3 in invasive breast cancer cells (Dombkowski *et al*. 2011). In addition, the knockdown of ARPC3 in cases of metastatic hepatic breast tumors decreased the ability of these tumors to co-opt pre-existing hepatic vessels (Frentzas *et al*. 2017). Finally, in view of the above, we suggest that more studies be developed in order to mechanistically explore the ongenic potential of ARPC3, regarding the promotion of motility and invasion of breast cancer cells, in addition to deepening the evaluation of its potential value as a biomarker for clinical and response prediction to therapies.

CONCLUSION

The results of this study suggest ARPC3 as a potential prognostic and predictive biomarker in breast cancer. However, in vitro studies are needed for a better understanding of its role in the motility and invasiveness of breast cancer cells and which signaling pathways this molecule is acting to influence the impaired clinical outcome and resistance to multiple anti-cancer drugs.

Approval by the research ethics committee: For this type of work, the approval of the Research Ethics Committee is not necessary.

Informed consent: Informed consent was not required for this study.

Conflict of interest: The authors declare that there is no conflict of interest in this project.

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