Research Article

Bioinformatic assessment of the relationship between breast cancer and autophagy-related protein Ambra1 mutation

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Abstract

Objectives: Autophagy protein 1, regulated by Beclin 1 (Ambra1), promotes tumor formation and development by modulating autophagy. Therefore, in situ intervention in autophagy is a promising new strategy for tumor therapy. We aimed to evaluate the possible effects of changes in the Ambra1 gene on breast cancer (BC) treatment in the BRCA cohort.

Methods: The gene profile of a total of 996 patients with BC was examined using data obtained from the Cancer Genome Atlas database via cBioPortal. The effects of mutations on proteins were examined by scoring the Polymorphism Phenotyping v2, Mutation Assessor, and Sorting Intolerant from Tolerant databases. The association of genes with other genes was determined with the STRING database. Kaplan-Meier Plot database was used by evaluating the overall survival (OS). The promoter methylation was evaluated by the UALCAN database.

Results: Eleven mutations were detected. Four of these mutations were truncated proteins. Ambra1 tissue expression levels were upregulated compared to healthy tissue in the BRCA cohort; this was not statistically significant (p>0.05). Decreased Ambra1 expression levels were associated with a shorter OS (p=0.038). Ambra1 promoter region hypermethylation was significant in the BRCA cohort compared to healthy tissue (p<0.001).

Conclusion: To our best knowledge, our study is the first to examine the relationship between BC and Ambra1 using bioinformatic tools. Ambra1 may be a candidate target molecule within the treatment strategy due to the mutations evaluated in the BRCA cohort, hypermethylation status, and the association of Ambra1 with shorter OS. However, these situations need to be confirmed by further studies.

Keywords: Apoptosis, autophagy, breast cancer, breast medicine, genetics-cancer genetics, genetics-carcinogenesis

Breast cancer (BC) stands as the predominant malignancy affecting women globally, holding the position of the second most prevalent cause of cancer-related fatalities in the female population [1]. BC manifests as a heterogeneous and multifaceted disease influenced by diverse pathogenic factors. The prognosis of BC significantly improves with early-stage detection, evident in the notable enhancement of the 5-year survival rate. However, 15% of BC patients still have a poor prognosis due to being diagnosed at an advanced stage [2]. Furthermore, BC is characterized by distinct molecular subtypes and inherent biological properties, necessitating diverse therapeutic strategies tailored to each subtype, resulting in subtype-specific clinical outcomes. It is a global health problem due to the lack of effective treatment strategies that can be used for all disease subtypes [3]. Targeted therapies improve patient overall survival and reduce healthcare costs [3].

The regulatory protein Beclin1-regulated protein 1 (Ambra1) serves as an inherently dysregulated molecular protein exerting control over the viability and apoptosis of cancer cells

through the modulation of autophagic processes [4, 5]. Ambra1 protein has high plasticity. With this feature, it adapts very well to conformational changes. Current research reveals that Ambra1 is involved in multiple complex pathological processes. Therefore, Ambra1 protein becomes a molecule with high research potential. While Ambra1 protein plays a role as a tumor suppressor in the regulation of cell proliferation and tumor formation, it also functions as an oncogene in the regulation of tumor invasion and metastasis. This means that Ambra1 may play different roles in different genetic changes and different microenvironments [5].

Despite the contentious interpretations of Ambra1's involvement in cancer, the deliberate suppression of autophagy in specific circumstances emerges as a potentially beneficial foundational approach for cancer therapy [5]. Recent findings indicate that Ambra1 may exert inhibitory effects on the initiation, safeguarding, and advancement of cancer through the modulation of c-MYC and cyclins, which are commonly overexpressed in human cancer cells. Furthermore, Ambra1 exhibits elevated expression levels across diverse cancer types and demonstrates a significant association with the prognostic outcomes of patients. Consequently, the multifaceted roles played by Ambra1 endure potential implications for clinical oncology, particularly in the contexts of tumorigenesis and cancer progression [5].

Autophagy has been extensively studied in BC cells to understand its functions and mechanisms. Despite this, there is limited work to understand the specific role of a protein called Ambra1 in BC. Ambra1 plays a crucial role in autophagy induction, thus it may increase resistance or sensitivity to chemotherapeutic agents in cancer treatment. Studies report that Ambra1 inhibits paclitaxel-induced apoptosis and chemosensitivity via the AKT−FOXO1−BIM pathway in MCF-7 and MDA-MB-231 breast cancer cells. Additionally, Ambra1 is tightly associated with chemoresistance. During chemotherapy, cancer cells can reduce the cytotoxicity of chemotherapeutic agents through autophagy, thus promoting cancer survival. Therefore, autophagy inhibition by targeting Ambra1 may enhance the therapeutic target-achieving effect of agents [4].

In light of the above information, we evaluated whether Ambra1 gene mutations, promoter region methylation status, and overall survival (OS) could contribute to the possible future treatment strategy in our BRCA cohort.

Materials and Methods

Determination of the study group

The BRCA cohort to be evaluated in the study was obtained through the cBioPortal (https://www.cbioportal.org/) database. When accessing this database from the given internet address, breast tissue was selected as the tissue option. Then, the Breast Invasive Carcinoma (TCGA, PanCancer Atlas) option

was selected. Research was conducted in the BRCA cohort by selecting the relevant gene via the "Query By Gene" option.

Our BRCA cohort consists of a total of 996 cases. The BRCA cohort includes Breast Invasive Ductal Carcinoma (BIDC), Breast Invasive Lobular Carcinoma (BILC), Breast Invasive Carcinoma (BIC), and Invasive Breast Carcinoma (IBC) as cancer types. The results of these cases were obtained using the cBioPortal database (https://www.cbioportal.org/). The data were accessed on September 13, 2023, from The Cancer Genome Atlas (TCGA). The data used in this study were obtained from the public database TCGA; therefore, ethical approval was not required.

Analysis of gene mutations

The cBioPortal database (https://www.cbioportal.org/) was used to evaluate the mutations in Ambra1. Thanks to this database, the amino acid in which the mutation occurred, the cancer subtype, and clinical information about the cancer were accessed. It was determined whether there was a somatic mutation or not thanks to the COSMIC (https://cancer.sanger.ac.uk/cosmic) database.

Survival prognosis of Ambra1 gene

In conducting prognosis analysis for overall survival (OS), the Kaplan-Meier Plotter (KM) tool, accessible at https://kmplot.com/analysis/, was employed. This tool systematically examines the associations between gene expressions and corresponding cancer survival rates, providing valuable insights into overall survival outcomes [6]. Moreover, the application of this tool facilitated an in-depth comprehension of the prognostic significance associated with the expression levels of the Ambra1 gene in BC patients. The KM plotter can evaluate the correlation between the expression of Ambra1 (mRNA) and survival in BC. The KM plotter uses Cox proportional hazards regression and the computation of the False Discovery Rate.

Gene-gene interaction

The STRING database (https://string-db.org/) systematically compiles and integrates information on protein-protein interactions, encompassing both physical associations and functional relationships. The dataset is derived from various sources, including automated text mining of scientific literature, computational predictions based on co-expression and conserved genomic context, information from interaction experiments databases, and established complexes/pathways sourced from curated references. Rigorous assessment and scoring of these interactions are performed [7].

Gene expression

Gene Expression Profiling Interactive Analysis, version 2 (GEPIA2.0), was used to evaluate the expression of the Ambra1 gene between the tumor tissues and the adjacent normal tissues. GEPIA2.0 uses TCGA database and genotype-tissue expression dataset (GTEx) samples to perform this analysis. The screening criteria used in GEPIA2.0 were p<0.05

and |Log2FC| the cutoff point was 0.1. These criteria were used to filter out genes that were not significantly differentially expressed between the two datasets [8]. In the dataset used for gene expression, the number of breast cancer tissue samples is 1085, and the number of adjacent healthy tissue samples that do not contain cancerous tissue is 291, provided from the GEPIA2.0 database.

Pathogenicity of mutations

We used Polymorphism Phenotyping v2 (PolyPhen-2), Mutation Assessor (MA), and Sorting Intolerant from Tolerant (SIFT) tools' algorithms on cBioPortal to investigate the possible pathogenicity and clinical effects of mutations detected in the Ambra1 gene. PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) uses a machine learning approach to classify genetic variants as benign or harmful [9]. This tool is automated, meaning it can analyze large datasets of rare genetic variants quickly and efficiently. Predictions made by PolyPhen-2 are essential for interpreting the impact of rare genetic variants on human health and disease. The output of PolyPhen-2 can classify the substitution effect as benign (score≤0.5), possibly damaging (0.5<score≤0.9), or possibly damaging (score>0.9) [10]. Mutation Assessor (http://mutationassessor.org/r3/) functions as a server/tool that predicts the functional impact of amino acid substitutions in proteins. This tool is particularly useful for identifying mutations discovered in cancer or missense polymorphisms. The functional impact of these mutations is evaluated based on the evolutionary conservation of the affected amino acid in protein homologues, that is, how similar the amino acid is between different species and how important it is for the function of the protein. The set used for validation contains 60,000 variants associated with diseases listed in the Online Mendelian Inheritance in Humans (OMIM) database. The evaluation results are given as low, medium, high, and neutral.

Sorting Intolerant from Tolerant (SIFT) (https://sift.bii.a-star.edu.sg/) is a computational tool used to predict the potential impact of amino acid substitutions on protein function. These substitutions can have varying effects on protein function, ranging from no effect to complete loss of function. SIFT uses a combination of sequence and structural information to predict the effect of these substitutions on protein function. The algorithm compares the amino acid sequence of the protein in question against a database of known protein sequences. Subsequently, SIFT assigns a score to each substitution indicating its likelihood of having a significant effect on protein function.

Promoter methylation status

UALCAN is an interactive open-access webpage for OMICS data analysis (http://ualcan.path.uab.edu/index.html). This database is built on PERL-CGI and can be used to analyze approximately 6000 gene methylation levels [11]. In this study, the promoter methylation level of Ambra1 in the BRCA dataset was examined.

Results

The cBioPortal web tool was used to analyze changes in the Ambra1 protein in BC patients. Among 996 cases, 11 cases (1.1%) of BC patients had genetic changes in the Ambra1 gene. The types of mutations encountered in BC in the Ambra1 gene are shown in Table 1. In the BRCA cohort, the most detected mutation type in the Ambra1 gene was a missense mutation (4 mutations, 36.3%), while the other mutations were 1 fusion gene mutation (9.1%), 2 frame shift deletions (18.2%), 2 splice region mutations (18.2%), 1 frame shift insertion (9.1%), and 1 nonsense mutation (9.1%). All mutations were confirmed to be of somatic origin. The Ambra1 gene contains the WD40 domain, which is 100% conserved throughout evolution, and three different types of motifs: two PxP (aa 275–281 and 1206–1212), two TQT (aa 1104–1106 and 1116–1118), and one LIR (aa 1043–1052). Ambra1 is cleaved by caspases in the D482 region. While no mutations were detected before the WD40 domain and the D482 region, 7 different mutations were found in the regions covering PxP (aa1206–1212) and other motifs. Additionally, in the BRCA cohort, there are 4 mutations in the Ambra1 gene that can cause truncated protein (p. I1256Ffs17, p. D1287Tfs78, p. E523*, p. S629Lfs*11). Additionally, there were recurrent hotspot (statistically significant) mutations along with these mutations in the Ambra1 gene. All recurrent hotspot mutations accompanying other Ambra1 mutations are shown in Table 2.

The OS results we obtained from the KM plotter analysis are shown in Figure 1. According to the analysis results, we recognized that decreased expression levels of Ambra1 were associated with shorter survival (p=0.038).

The examination of gene-gene interactions was conducted through the utilization of the STRING database software program. The outcomes of this analysis are visually represented in Figure 2, encapsulating the data derived from our investigation. The other genes with which the Ambra1 gene interacts most frequently are ATG14, BECN1, CUL4A, CUL4B, DDA1, DDB1, PIK3C3, PIK3R4, TRAF6, and UVRAG, respectively, according to the relationship scoring, and it consists of a total of 10 nodes. Apart from this, according to the gene relationships examined in different publications, it was also found to be associated with the ANXA5, GABARAPL2, SQSTM1, ATG5, CASP9, BCL2L11, FOXO1, AKT1, and ATG12 genes (Fig. 2).

Ambra1 gene expression levels of BC (n=1085) patients were higher than the healthy control group (n=291), but this increase was not statistically significant (p>0.05) (Fig. 3).

The scores of PolyPhen-2, MA, and SIFT are shown in Table 2. Based on the outcomes of the analysis and scoring metrics, the mutation identified in the Ambra1 gene (p. L1090F) was ascertained as the most impactful missense mutation, inducing substantial alterations in both protein structure and function. This mutation was consequently deemed the most pathogenic among the identified variants.

Table 1. Ambra1 Gene Mutation Analysis and treatment option in cases

No	Gen	Nucleotit change	Somatic status	Type of cancer	Subtype	American joint committee on cancer metastasis stage code	Neoplasm disease lymph node stage American joint committee on cancer code	Neoplasm disease stage American joint committee on cancer code	Diagnosed age	Overall survival (months)	Treatment option
M-1	Ambra1	NA	Confirmed somatic	BIDC	Luminal A	M0	N1	T2	47	50.0	Doxorubicin, Cyclophosphamide, Paclitaxel, Radiation, Tamoxifen, Anastrazole
M-2	Ambra1	c.3765del	Confirmed somatic	BIDC	Luminal B	M0	N1A	T2	42	17.0	Epirubicin, Cyclophosphamide, Docetaxel, Zoledronic Acid, Radiation, Tamoxifen
M-3	Ambra1	c.3859del	Confirmed somatic	BILC	Luminal B	M0	N0	T3	83	0	NA
M-4	Ambra1	c.2822-2A>G	Confirmed somatic	BILC	Luminal B	M0	N0	T3	83	0	NA
M-5	Ambra1	c.1648A>C	Confirmed somatic	IBC	Basal-like	M0	N0 (I-)	T1C	82	23.0	NA
M-6	Ambra1	c.3691G>C	Confirmed somatic	BIDC	Basal-like	M0	N0	T2	62	7.5	NA
M-7	Ambra1	c.3268C>T	Confirmed somatic	BIDC	Luminal A	M0	N2	T2	67	0.4	NA
M-8	Ambra1	c.3608C>A	Confirmed somatic	BIDC	Basal-like	M0	N1	T2	51	125.1	NA
M-9	Ambra1	c.1567G>T	Confirmed somatic	BIDC	Luminal A	M0	N0	T1	62	71.0	Radiation, Trastuzumab, Anastrazole
M-10	Ambra1	c.1884dup	Confirmed somatic	BIDC	Luminal A	M0	N1	T2	31	74.7	NA
M-11	Ambra1	c.2976+1del	Confirmed somatic	BIC (NOS)	Luminal A	MX	N3A	T3	39	25.9	Docetaxel, Doxorubicin, Cyclophosphamide, Tamoxifen, Goserelin, Radiation

BIDC: Breast Invasive Ductal Carcinoma; BILC: Breast Invasive Lobular Carcinoma; IBC: Invasive Breast Carcinoma; BIC: Breast Invasive Carcinoma; NA: not applicable

Table 2. Mutation profile of Ambra1 gene and recurrent hotspot mutations

No	Gen	Nucleotit change	Protein change	Variation type	Mutation assesor score	SIFT score	PolPhen-2 score	Recurrent hotspot mutations (statistically significant)
M-1	Ambra1	NA	PEX16-AMBRA1 Fusion	Fusion	–	–	–	PIK3CA (p. H1047R missense)
M-2	Ambra1	c.3765del	I1256Ffs*17	FS del	–	–	–	PIK3CA (p.E545K ve p.E453K missense), TP53 (p.X187_splice site mutation)
M-3	Ambra1	c.3859del	D1287Tfs*78	FS del	–	–	–	–
M-4	Ambra1	c.2822-2A>G	X941_splice	Splice	–	–	–	–
M-5	Ambra1	c.1648A>C	T550P	Missense	0.60 (neut.)	0.12 (tole.)	0.00 (benign)	TP53 (p. S241C missense), IL6ST (p. S279C missense)
M-6	Ambra1	c.3691G>C	A1231P	Missense	1.10 (low)	0.01 (delet.)	0.19 (benign)	FGFR2 (p. N549K missense, TP53 (p. X225_splice), NCKAP1L (p. R21H missense), PACSIN1 (p. R389H missense)
M-7	Ambra1	c.3268C>T	L1090F	Missense	2.21 (med.)	0.00 (delet.)	1.00 (Prob.D.)	BAX (p. I133M missense)
M-8	Ambra1	c.3608C>A	T1203N	Missense	0.00 (neut.)	0.02 (tole.)	0.25 (benign)	–
M-9	Ambra1	c.1567G>T	E523*	Nonsense	–	–	–	PIK3CA (p.N345K missense), PGR (p.E723K missense)
M-10	Ambra1	c.1884dup	S629Lfs*11	FS ins	–	–	–	PIK3CA (p.K111E missense), ERBB2 (p.D769Y missense), TP3 (p.N247I missense)
M-11	Ambra1	c.2976+1del	X992_splice	Splice	–	–	–	PIK3CA (p.H1047R missense)

SIFT: Sorting Intolerant from Tolerant; FS del: Framashift Deletion; FS ins: Frameshift Insertion; Neut: Neutral; Delet: Deleterious; med: Medium; Prob.D: Probably Damage; NA: Not applicable

Ambra1 promoter region methylation status was found to be statistically hypermethylated in BRCA samples compared to healthy tissue (p<0.001) (Fig. 4).

Discussion

Ambra1 has been shown to increase metastasis in BC mouse models [12]. Although the results need to be confirmed, upregulation of Ambra1 is thought to be associated with tumorigenesis and BC progression by affecting some vital pathways [2]. The sensitivity of cells to chemotherapeutic agents and the regulation of chemosensitivity are quite important in BC patients. Ambra1 is a key protein controlling the switch between autophagy and apoptosis and has been shown to modulate paclitaxel-induced apoptosis in BC cells via the BIM/mitochondrial pathway [13]. Studies have argued that Ambra1 regulates BIM expression at the transcriptional level through the Akt-FoxO1 pathway, and this regulation could be considered a potential target for BC treatment [13, 14].

Post-translational modifications in the Ambra1 gene generally focus on phosphorylation and ubiquitylation. It is stated that these modifications are closely related to autophagy [15]. Two important autophagy-related kinases, unc-51 like autophagy activating kinase 1 (ULK1) and mTOR complex 1, are activated through the phosphorylation of Ambra1. As a result of phosphorylation at the Ser52 and Ser465/Ser635 regions of Ambra1, it becomes a substrate for mTORC1 and ULK1, respectively [16]. In our BRCA cohort, unstable small polypeptide chains may occur as a result of the E523* nonsense mutation. In this case, the polypeptide chain may terminate at this point, and the Ser 635 region will not be formed. Thus, Ser635 region phosphorylation of Ambra1 may not occur. This phosphorylation is required for the association of ULK1 with Ambra1, as well as for regulating the dissociation of AmbRa1-Vps34-beclin-1 from the dynein complex [17]. When autophagy is induced, activated ULK1 phosphorylates Ambra1 at Ser465 and Ser635 sites. If these reactions do not occur, autophagy may be disrupted.

In the BRCA cohort, four truncated protein-forming mutations were found in the Ambra1 gene. These mutations do not include the W40 region, which is a 100% conserved domain, and the D482 region, which is cleaved by cas-
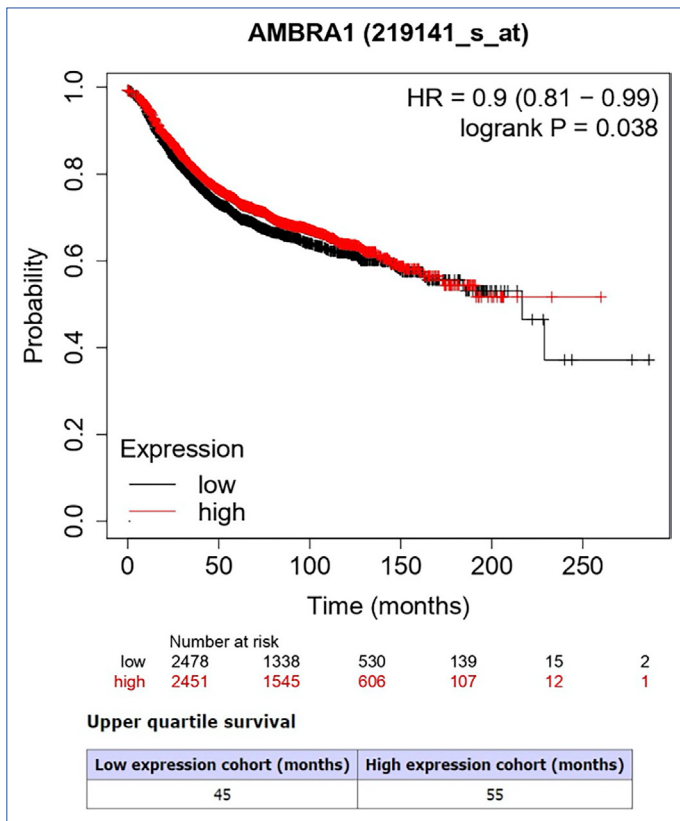
Figure 1. Different expressions of Ambra1 in BC patients in the overall survival (OS) curve (using the Kaplan-Meier plotter). The red line represents the survival rate curve of patients with BC who expressed the gene, and the black line represents the survival rate curve of BC patients who did not express the gene.
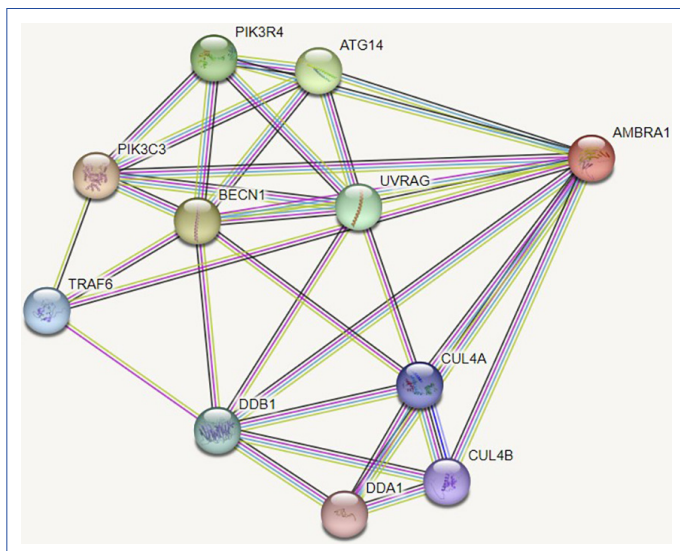
HR: Hazard ratio; BC: Breast cancer.

Figure 2. Schematic representation of known and predicted protein-protein interactions with the *Ambra1* gene. Each line has features. (Red line-indicates the presence of fusion evidence; Green line-neighborhood evidence; Blue line- cooccurrence evidence; Purple line-experimental evidence; Yellow line- textmining evidence; Light blue line-database evidence; Black line-coexpression evidence.).
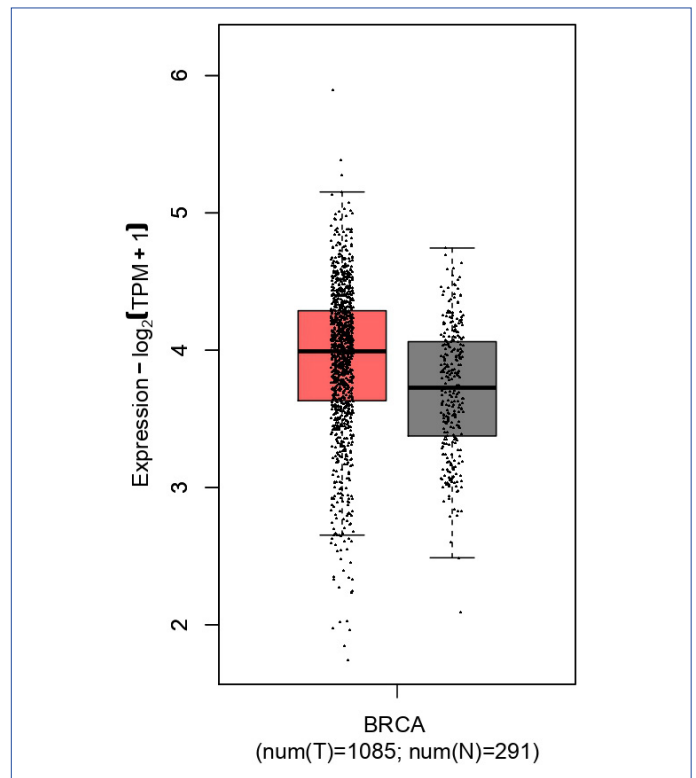

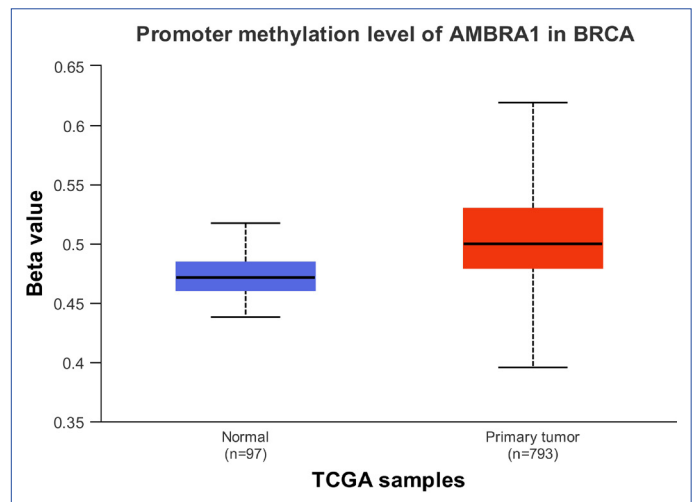**Figure 3.** mRNA expression of Ambra1 in BC (red) and normal breast tissues (gray).

Figure 4. Promoter methylation level analysis of Ambra1.

TCGA: The Cancer Genome Atlas.

pases during apoptosis. However, they may affect functional motifs located after the splicing site. The remaining part after cleaving the D482 region is also called the BH3-like domain. The pro-apoptotic segment within Ambra1 is designated as the BH3-like domain. Specifically, the PxP motifs situated at amino acid positions 275–281 and 1177–1183 within Ambra1, which reside within this pro-apoptotic region, play a crucial role in binding to the catalytic subunit of protein phosphatase 2A. This binding interaction serves to regulate the activity of c-MYC [18].

The interaction between Ambra1 and dynein light chain 1 is facilitated through the mediation of TQT motifs located within the C-terminal sequence of Ambra1. Furthermore, they have a role in binding Ambra1 to the dynein motor complex in the absence of autophagy induction [19]. Ultimately, the binding interaction between Ambra1 and autophagy-associated protein 8 family proteins, specifically light chain 3 beta, is contingent upon the critical involvement of the LIR motif situated in the C-terminal region of Ambra1 [20]. The p.I1256Ffs17, p.D1287Tfs78, p.E523*, and p.S629Lfs*11 truncated mutations occurring in this region in our BRCA cohort may cause early termination of the polypeptide and the formation of a dysfunctional protein. As a result, loss of function in the BH3-like domain may occur. In this case, the balance between autophagy and apoptosis may be disrupted, and negative consequences may occur for cancer pathogenesis.

In addition, cases with these truncated changes (except p.D1287Tfs*78) are accompanied by mutations in the PIK3CA gene as recurrent hotspot changes. These mutations frequently occur in BC patients and have an oncogenic character. Additionally, mutations in this gene often appear to be an important genetic change that promotes cancer growth and may play a role in resistance to treatment [21]. In our BRCA cohort, the four proteins most associated with the Ambra1 protein are PIK3C3, PIK3R4, BECN1, and ATG14. PIK3C3 and PIK3R4 are involved in the maturation, initiation, and endocytosis processes of autophagosomes. Ambra1 interacts with these proteins at the initiation stage of autophagy, and autophagy is induced [22].

Beclin 1-associated autophagy-related key regulator (ATG14 gene) is required for both basal and inducible autophagy. It determines the localization of the autophagy-specific PI3-kinase complex PI3KC3-C1. ATG14 plays a role in autophagosome formation and MAP1LC3/LC3 conjugation to phosphatidylethanolamine [22]. It promotes BECN1 translocation from the trans-Golgi network to autophagosomes and enhances PIK3C3 activity in a BECN1-dependent manner [23]. ATG14 is essential for the autophagy-dependent phosphorylation of BECN1. It stimulates the phosphorylation of BECN1, but suppresses the phosphorylation of PIK3C3 by AMPK [24]. After the autophagy initiation phase, nucleation and phagophore formation are very important for the regulation of autophagy. With the formation of the ULK1 complex, the nucleation phase of autophagic membranes begins. The molecules that play a key role in the nucleation stage are Beclin 1 (BECN1) and Bcl-2. Autophagy is suppressed by the binding of Bcl-2 to BECN1. On the other hand, interaction of BECN1 with the lipid kinase vacuolar sorting 34 protein (VPS34) promotes membrane nucleation. The ULK1 complex phosphorylates the class III phosphatidylinositol-3-kinase (PtdIns3K) complex, enabling its activation. The PtdIns3K complex includes BECN1, NRBF2, ATG14, PIK3C3, PIK3R4, and Ambra1. Phagophores are formed by the fusion of membranes formed by nucleation from ER membranes. The ATG9 system, includ-

ing ATG9, ATG2A/B, and WDR45, also plays an important role in phagophore formation [25]. In this case, loss of function in Ambra1 as a result of mutations in the Ambra1 protein may prevent the induction of autophagy as it will disrupt the above protein-protein interaction mechanisms. In the treatment options related to these proteins, PIK3C3 inhibitors (Lapatinib) are used to inhibit proliferation in BC [26].

Extant literature indicates a reported association of BECN1 and ATG14 with potential implications in cancer progression or resistance to chemotherapeutic interventions. BECN1 plays a pivotal role as a key regulator within the PIK3C3 complex, influencing autophagosome nucleation and participating in endocytic trafficking processes. ATG14, as an additional regulatory subunit within the PIK3C3-C1 complex, participates in the process of autophagosome nucleation and facilitates the fusion of autophagosomes with lysosomes [27]. ATG14 demonstrates the capacity to modulate the responsiveness to targeted therapeutic agents, such as gemcitabine, cisplatin, and sorafenib, through its influence on the expression profiles of microRNAs (miRNAs) in pancreatic, ovarian, colorectal, and hepatic cancers [28–30].

It is argued that the Ambra1 gene, like ATG14 and BECN1, may be involved in chemoresistance and may support cancer survival by reducing the cytotoxicity of chemotherapeutic agents through autophagy during chemotherapy. It is considered that when autophagy inhibition is performed by targeting Ambra1, the therapeutic effectiveness of the agents used in treatment may increase [4].

Studies conducted on different cancer types have tried to reveal the relationship between Ambra1 expression levels and OS [31]. A study conducted in patients with gastric cancer stated that high Ambra1 expression levels were an independent factor in predicting poor OS in patients [31]. In addition, two studies have demonstrated that high expression levels of Ambra1 correlate with poorer survival in pancreatic ductal adenocarcinoma and cholangiocarcinoma patients [32, 33]. While this is the case in other types of cancer, in vivo, Ambra1 has been demonstrated to be an important protein that determines whether epirubicin-treated BC cells undergo apoptosis or autophagy [5]. Although Ambra1 tissue expression levels were upregulated compared to healthy tissue in our BRCA cohort, this was not statistically significant. However, decreased Ambra1 expression levels were associated with poor OS.

Epigenetic mutation changes gene expression in a heritable manner, without any change in the DNA sequence, and is as effective as genetic changes in cancer formation. In cancer cells, hypermethylation in CpG islands in promoter regions is observed along with widespread hypomethylation in the genome. Promoter region hypermethylation causes subsequent gene silencing, which is especially important in inactivating tumor suppressor genes. DNA methylation is considered a potential marker for early detection of cancer. Promoter hypermethylation has been identified as a poten-

tial marker and has been shown to be able to detect established BC. Changes in promoter methylation status are common events that occur in the early stages of tumorigenesis and can be detected with minimally invasive measures. A number of cancer-associated genes have been found to be frequently methylated in BC. These markers are promising in distinguishing between malignant disease and benign disease or normal tissue and may inform the detection of lobular carcinomas [34].

When we examined the Ambra1 gene in the BRCA cohort, the promoter region methylation level of Ambra1 was hypermethylated in BC patients compared to healthy controls. Although this situation is thought to lead to gene silencing and decreased expression levels, it is contrary to the upregulation of the Ambra1 gene in our BRCA cohort.

Normally, promoter sequence methylation typically results in chromatin becoming more densely packed. As a result, transcription is negatively affected. In this process, the methyl-CpG (mCpG)-binding domain specifically binds to the methylated sequences of proteins. It can be explained by the classical model that it recruits repressor complexes such as the histone deacetylase complex. Histone deacetylation causes chromatin to become condensed, thereby inhibiting transcription. However, this model does not account for how promoter hypermethylation could lead to increased expression rather than the expected reduction.

In the first scenario, recent findings propose a competitive mechanism for methylation-dependent transcription regulation, wherein methylated sequences might also attract transcription factors (TFs) that specifically recognize methylated binding motifs. This process could then lead to the initiation of transcription [35]. In the second scenario, this hypermethylation situation may be caused by post-translational modification. These may explain the increased expression levels of Ambra1 in our BRCA cohort.

Considering the above possible first scenario, the increase in expression occurring with hypermethylation in the Ambra1 gene may put a different perspective on treatment options targeting the Ambra1 molecule.

Limitation: There were some limitations in our study. The most important of these limitations is the promoter region methylation status. Significant differences in normal and tumor tissue sample sizes in data obtained from UALCAN databases may cause bias in the results. For this reason, we believe that prospective studies are needed in groups with homogeneously distributed sample sizes in order to obtain more meaningful information about promoter region methylation. The same situation occurs in the data obtained from GEPIA2, where Ambra1 gene expression is evaluated. Therefore, more reliable results can be obtained by reducing the serious difference between sample sizes when evaluating data obtained from databases and making statistical comparisons.

Conclusion

To our best knowledge, our study is the first to examine the relationship between BC and Ambra1 using bioinformatic tools. In summary, changes in autophagy-related genes can be used as potential treatment targets. It is not clear how promoter region hypermethylation occurs in the autophagy-related Ambra1 gene in the BRCA cohort. Once this situation is clarified, it may be investigated to determine whether there is a potential treatment target. However, in order for Ambra1 to be evaluated as a potential treatment target, mutations occurring in the Ambra1 gene need to be clarified in population-based prospective studies both in breast cancer and other types of cancer. For this, geneticists and clinical biochemists will need to collaborate and carefully evaluate possible changes in the Ambra1 gene.

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