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Exploring the Potential of ATP Synthase F1 (ATP5F1) subunits as Biomarker for Breast Cancer: A Bioinformatic Study

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ABSTRACT

Mitochondrial dysfunction is common in cancer, and the mitochondrial electron chain is often affected during carcinogenesis. This study provides a comprehensive analysis of the ATP5F1 gene family to explore *ATP5F1A*, *B*, *C*, *D*, and *E* genes for their expression and prognostic values in breast cancer. Gene expression data were obtained from GEO dataset and TCGA, enabling the assessment of differential expression, survival correlations, and tumor grade-specific expression. Protein expression data was obtained from the Human Protein Atlas, mutation frequencies were retrieved from cBioPortal, and functional enrichment was performed using Enrich. Correlations with immune cell infiltration were also evaluated using TIMER. Notably, our investigation revealed that *ATP5F1C*, *ATP5F1D*, and *ATP5F1E* were highly upregulated, while *ATP5F1A* was downregulated in cancerous tissue. Conversely, decreased expression of *ATP5F1B*, *C*, and *E* was associated with better overall and recurrence-free survival, while high expression of *ATP5F1A* and *D* was associated with good prognosis. Moreover, mutation analysis revealed amplification and over-expression of *ATP5F1C* and *E*, which was further validated by protein-level analysis in tumor tissues. Enrichment analysis demonstrated oxidative phosphorylation and ATP metabolism, while TIMER analysis identified subtype-specific immunometabolic roles. Our results reveal ATP5F1C and ATP5F1E as promising prognostic biomarkers in breast cancer and suggest that differential ATP5F1 expression shapes tumor bioenergetics and the immune microenvironment. Crucially, further experimental and clinical validation is essential to clarify mechanistic contributions to breast cancer pathogenesis and to explore therapeutic targeting. These insights may guide personalized interventions exploiting mitochondrial vulnerabilities.

Keywords: ATP5F1 subunits, Breast cancer, Biomarker, Bioinformatics methods

INTRODUCTION

Globally, breast cancer ranks as one of the most prevalent malignancies and substantially contributes to cancer-related mortality. Universal efforts are required to mitigate its rising burden, particularly in developing

Despite advances in early clinical diagnosis and treatment strategies, the foresight of breast cancer treatment is poor; thus, identifying sensitive and specific prognostic biomarkers is urgently required [2]. Research has demonstrated that adenosine triphosphate

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(ATP) metabolic pathways are closely associated with the initiation and progression of various human cancers [3]. Cancer cells often reprogram the expression of some metabolic pathways to enhance ATP synthesis, supporting growth and survival. Moreover, extracellular ATP plays a critical role shaping tumor microenvironment through modulating immune and inflammatory responses, leading to tumor progression [4]. ATP, the primary cellular energy carrier molecule, is synthesized from adenosine diphosphate (ADP) and inorganic phosphate (Pi) via a fundamental biochemical process. This conversion is done by ATP synthase which is a ubiquitous mitochondrial enzyme complex. Located at the terminal step of the oxidative phosphorylation pathway, ATP synthase anabolizes ATP through utilizing proton gradient across inner mitochondrial membrane [5]. Structurally, ATP synthase consists of two components F0 and F1. F0, embedded in the inner mitochondrial membrane and responsible for proton translocation. F1, located in the mitochondrial matrix, serves as a soluble catalytic core responsible for ATP synthesis. The peripheral stalk connects F0 and F1. These two are connected by a peripheral stalk which stabilizes the complex. The F1 consists of five subunits: three α, three and one each of β, γ, δ, and ε [6,7]. α and β subunits form a hexamer ring with the central cavity; while the γ, δ, and ε subunits form the central stalk. These subunits are encoded by *ATP5F1A, ATP5F1B, ATP5F1C, ATP5F1D*, and *ATP5F1E* which are located on 18q21.1, 12q13.3, 10p14, 19p13.3, and 20q13.32, respectively. Mutations and dysregulation of these genes have been reported in various complications, including mitochondrial complex V deficiencies and hydronephrosis [8–13]. Energy metabolism, particularly mitochondrial ATP synthesis, has gathered more attention recently. Research has found multiple cancer types in which mitochondrial bioenergetics has been altered to meet the high metabolic demands of tumor progression [14]. Understanding the ATP synthase dysregulation may offer novel opportunities for therapeutic interventions in breast cancer.

The objective of the present study is to provide a comprehensive analysis of the ATP5F1 subunit genes to determine their potential as prognostic biomarker in breast cancer. We investigated the expression patterns and clinical relevance of *ATP5F1A, B, C, D*, and *E* in breast cancer by leveraging publicly available transcriptomic datasets. Our findings offer new insights into ATP synthase subunits in tumor biology and magnify their possible biomarker potentiation in breast cancer diagnosis.

MATERIALS AND METHODS

Gene Expression Data Collection and Preprocessing

Gene expression data were obtained from Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). Four microarray datasets were selected (GSE17907, GSE9574, GSE6883, and GSE31448). Chosen GSEs included normal and tumor samples from various histopathological grades (grades 1-3), enabling comparative analysis of *ATP5F1A, B, C, D*, and *E* gene expressions. Then, all data were normalized through transcripts per million (TPM) method. Differential expression analysis was done by GEO2R tool, incorporating the GEO query and Limma packages from Bioconductor in R. Mean values of each sample was calculated and subsequently averaged across GSEs to assess the overall expression pattern of each interested gene.

Survival analysis

The prognostic values of ATP5F1 subunit genes were evaluated using Kaplan–Meier Plotter (http://kmplot.com/analysis) [15]. Overall survival (OS) and relapse-free survival (RFS)

curves of designated genes were generated using breast cancer mRNA data from TCGA. The log-rank p-value < 0.05 was considered statistically significant.

Expression and Clinical Association Analyses

The UALCAN portal (http://ualcan.path.uab.edu/analysis.html), a comprehensive web resource utilizing TCGA and MET500 [16], was used to evaluate gene expression differences between breast cancer and normal tissue. Furthermore, expression patterns across cancer stages and molecular subtypes were explored. P<0.05 indicates a statistically significant difference. Moreover, the mutation rate of our gene family was evaluated separately. Bc-GenExMinerv4.1 (http://bcgenex.centregauducheau.fr) [15] was used to investigate the gene expression correlation with multiple clinicopathological parameters, i.e. age, nodal status, molecular subtypes (basal-like, triple negative (TNBC)) and receptor status (ER, PR, HER2). Subtype-specific expression was analyzed by PAM50 classification. Data were in log2 format. Moreover, the different grades of cancer were individually examined for each gene with the aid of the DNA microarray database. P-value and standardization with Log2 was performed for these data. Tumor grade–based gene expression analysis was evaluated by Scarff–Bloom–Richardson (SBR) grading system, which classifies breast tumors into grades 1 (well-differentiated), 2 (moderately differentiated), and 3 (poorly differentiated). Expression levels of *ATP5F1A, B, C, D*, and *E* were compared between the grades to evaluate whether their expression is associated with tumor histological differentiation. The analysis was conducted on SBR annotated datasets and compared using GEO2R and bc-GenExMiner v4.1.

Protein Expression Analysis

To investigate protein-level expression, the Human Protein Atlas (HPA) database (proteinatlas.org) [15] was used. Immunohistochemical data were reviewed to compare ATP5F1 subunit protein expression between breast tumor and normal tissue.

Co-expression and Functional Enrichment Analysis

Co-expression networks and protein-protein interactions (PPIs) of ATP5F1 subunits were determined by (https://www.cbioportal.org). After identifying the functionally associated genes, their interaction was visualized applying integrated PPI data.

To further gain insights into the biological processes and molecular pathways involving ATP5F1 subunits enrichment analysis was performed by Enrichr (https://maayanlab.cloud/Enrichr/). The analysis included the Gene Ontology (GO) terms for biological process (BP), molecular functions (MF), KEGG pathways, and transcription factor (TF).

Analysis of Immune Cell Infiltration

In order to investigate the correlation between *ATP5F1A–E* gene expression and immune cell infiltration of breast cancer, we utilized the TIMER (Tumor Immune Estimation Resource) web server (http://timer.cistrome.org/). TIMER predicts six major immune cell abundance, B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells, from the RNA-seq expression profiles of The Cancer Genome Atlas (TCGA). Correlation between immune infiltration level and gene expression was analyzed using Spearman's correlation coefficient. $p < 0.05$ was set as the statistical significance. This analysis provided insights into the potential of ATP5F1 subunits in modulating the tumor microenvironment of immune infiltration.

RESULTS

Distinct expression of ATP5F1 members in breast cancer tissues

We further compared mRNA expression levels of *ATP5F1A–E* genes between breast cancer and normal adjacent tissues according to TCGA datasets. The levels of expression for *ATP5F1C, ATP5F1D,* and *ATP5F1E* were significantly higher in cancer tissues ($p<0.001$; Figure 1C–E), whereas *ATP5F1A* expression was significantly lower in cancer samples compared to normal breast tissues ($p<0.001$; Figure 1A). *ATP5F1B* showed no statistical difference between cancerous and normal tissues (Figure 1B).

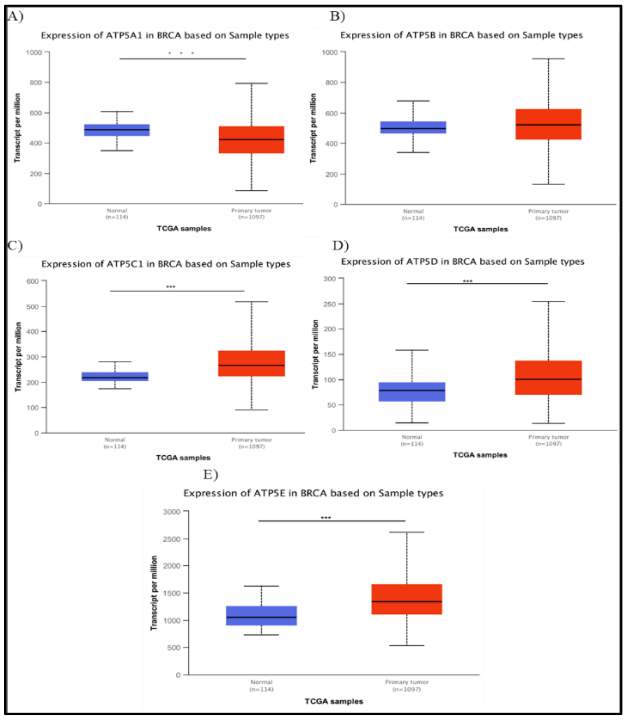


Figure 1. Expression of the ATP5F1 subunits in breast cancer (UALCAN Analysis). (A–E) Box plot of subunit gene transcripts in normal and primary tumor (BRCA) tissues. The box plot shows comparisons of the expressions of TCGA data from the ATP5F1 subunits in breast cancer, between normal samples (n = 114) and primary tumors (n = 1097). Statistical significance is represented by $p < 0.05$.

Mutation landscape of ATP5F1 subunits in breast cancer

We searched the genomic alterations of ATP5F1 subunits in breast cancer samples using the cBioPortal tool (Figure 2). The five genes had varying degrees of alteration. The highest rate of mutations was observed in *ATP5F1E* (11%), which was followed by *ATP5F1C* (8%), *ATP5F1A* (6%), *ATP5F1D* (5%), and *ATP5F1B* (4%). The most frequent types of alterations were amplification and mRNA high expression, particularly observed in *ATP5F1C, D*, and *E*. There were fewer instances of missense mutations of unknown significance, mainly in *ATP5F1A* and *ATP5F1B*. Deep deletions and mRNA low expression were observed in *ATP5F1B* and *ATP5F1D*. These findings suggest that ATP5F1 genes, namely *ATP5F1E* and *ATP5F1C*, are extremely mutated in breast cancer and potentially involved in metabolic reprogramming and tumor development.

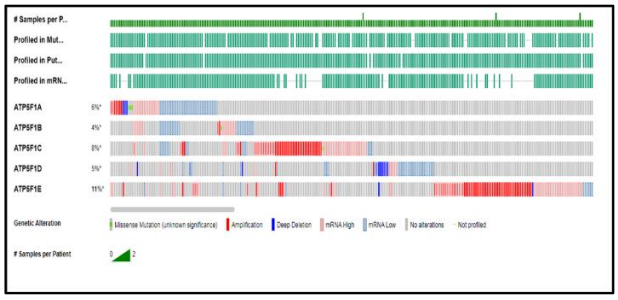
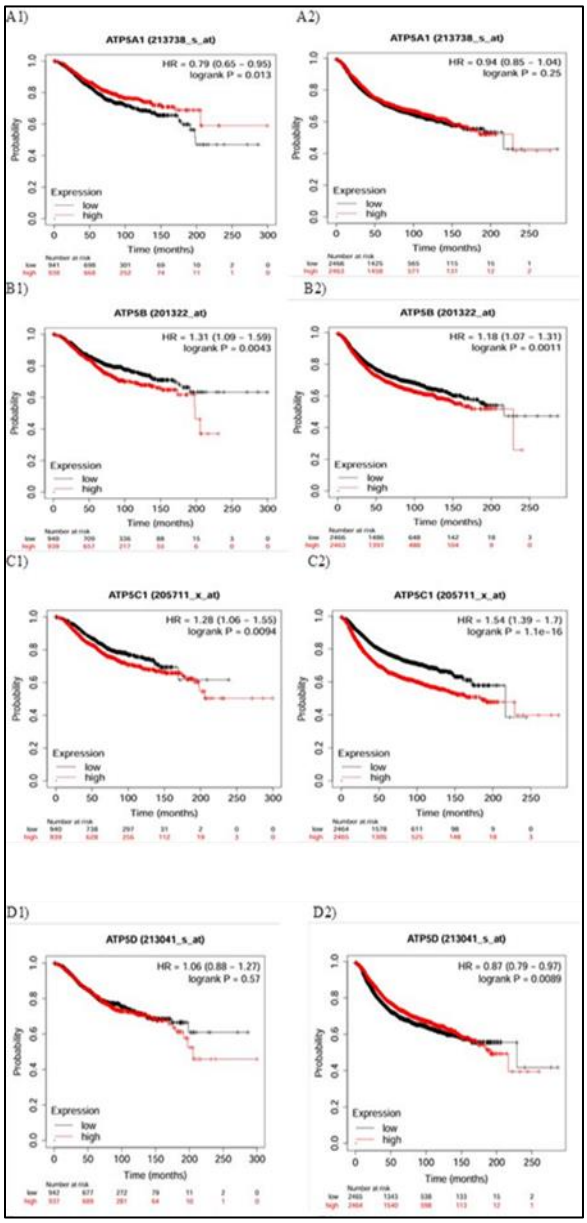


Figure 2. cBioPortal OncoPrint of mutation profile of ATP5F1A-E in breast cancer.

Prognostic value of ATP5F1 subunits in breast cancer

To determine the prognostic value of *ATP5F1A-E*, we employed the KM Plotter program to test their association with survival OS and RFS. Of interest, low mRNA expression of *ATP5F1B* (HR=1.31), *ATP5F1C* (HR=1.28), and *ATP5F1E* (HR=1.35) were significantly associated with increased OS. On the other hand, increased *ATP5F1A* (HR=0.79) expression was associated with improved survival. Apart from that, downregulation of

ATP5F1B (HR=1.18), *ATP5F1C* (HR=1.54), and *ATP5F1E* (HR=1.44) was also strongly correlated with enhanced RFS, and upregulation of *ATP5F1D* (HR=0.87) also played a positive part in recurrence-free prognosis (Figure 3A–E). These findings suggest differential expression of ATP5F1 subunits are most likely to play variable roles in breast cancer progression and prognosis in patients.



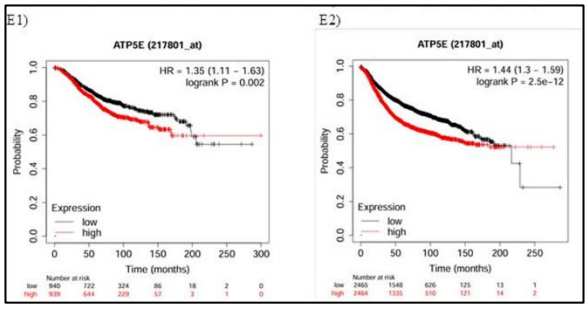


Figure 3. The prognostic effect of the expression of *ATP5F1A, B, C, D*, and *E* genes in www.kmplot.com. Analysis indicated for (A1) the overall survival (OS) rate of *ATP5F1A* (p=0.013), (A2) the relapse-free survival (RFS) rate of *ATP5F1A* (p=0.25), (B1) the OS rate of *ATP5F1B* (p=0.0043), (B2) the RFS rate of *ATP5F1B*(p=0.0011), (C1) the OS rate of *ATP5F1C* (p=0.0094), (C2) the RFS rate of *ATP5F1C* (p=1.1e-16), (D1) the OS rate of *ATP5F1D* (p=0.57), (C2) the RFS rate of *ATP5F1D* (p=0.0089), (E1) the OS rate of *ATP5F1E* (p=0.002), and (E2) the RFS rate of *ATP5F1E* (p=2.5e-12).

Correlation with ATP5F1 Gene Expression and Clinical Parameters

In order to analyze clinical importance more effectively, we explored ATP5F1 gene expression with respect to breast cancer clinical features according to bc-GenExMiner and PAM50 subtyping. Among all the subunits, the greatest expression differences were noticed for *ATP5F1C* for different clinicopathological features like nodal status, ER and PR status, three-negative breast cancer (TNBC) and basal-like subtype (Table 1). Most importantly, *ATP5F1B, C,* and *E* had profoundly altered expression in all PAM50 molecular subtypes versus normal tissue. *ATP5F1A* expression was relatively lower in the HER2 subtype, while *ATP5F1D* had enhanced expression particularly in HER2-positive and basal-like tumors (Figure 4).

Table 1. The relationship between mRNA expression of ATP5F1subunit genes and clinicopathological parameters of breast carcinoma.

Gene Name	Criteria	Age		Nodal status		ER		PR		HER2		TNBC		Basal-like BC	
	(n.)	<51 (476)	≥51 (267)	(–) (332)	(+) (358)	(–) (187)	(+) (530)	(–) (243)	(+) (470)	(–) (396)	(+) (109)	Non (578)	TNBC (87)	Non (605)	Basal -like (136)
ATP5F1A	mRNAexpression status	–	–	up	–	–	–	–	–	up	–	–	–	–	–
	P-value [a]	0.9767		**0.0057***		0.5917		0.9643		**0.0390***		0.5410		0.1957	
ATP5F1B	mRNAexpression status	–	–	–	–	–	–	up	–	–	up	–	–	–	–
	P-value	0.4130		0.3057		0.7163		**0.0406***		**0.0017***		0.1290		0.8084	
ATP5F1C	mRNAexpression status	–	–	up	–	up	–	up	–	–	–	–	up	–	up
	P-value	0.1316		**0.0491***		**0.0001***		**0.0001***		0.1337		**0.0001***		**0.0001***	
ATP5F1D	mRNAexpression status	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	P-value	0.5626		0.6556		0.3656		0.3168		0.1977		0.7239		0.7799	
ATP5F1E	mRNAexpression status	–	–	–		–		–		–		–	up	–	–
	P-value	0.0892		0.8457		0.1658		0.2610		**0.0054***		0.6921		0.9289	

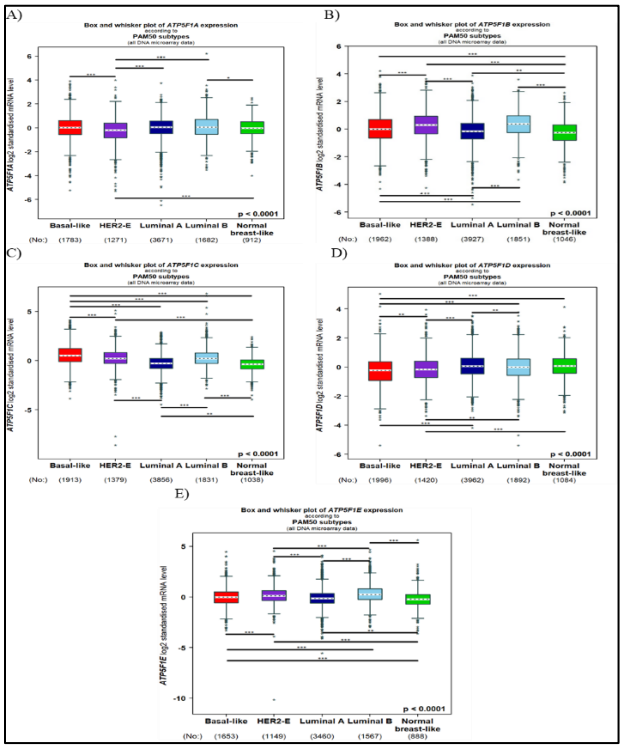


Figure 4. Association between mRNA expression of *ATP5F1A, B, C, D*, and *E* genes and different clinical parameters using the bc-GenExMiner database.

In addition, all ATP5F1 genes exhibited statistically significant alteration in expression in SBR tumor grades 1–3, suggesting a potential role in breast cancer differentiation and formation. In more detail, the SBR results revealed that *ATP5F1A* and *ATP5F1D* tend to alleviate their expression in higher grades. *ATP5F1C, D*, and *E* demonstrated sequential upregulation from grade I to grade III tumors, corroborating their possible involvement in the development and aggressive phenotypes of breast cancer (Figure 5A–E). These findings show the prospective utility of ATP5F1 subunits as biomarkers for tumor aggressiveness and grading stratification.

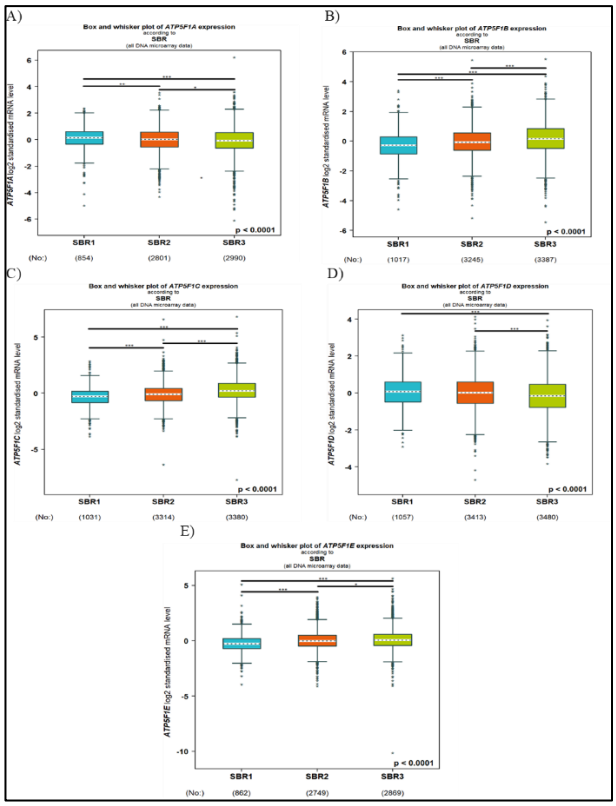
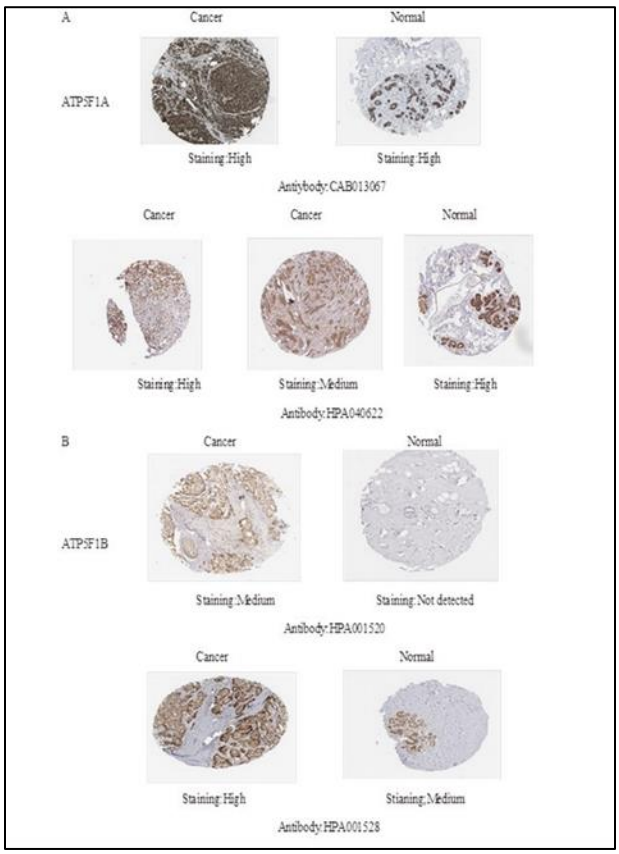


Figure 5. mRNA expression levels of ATP5F1 genes across three tumor grades based on SBR classification in breast cancer.

Protein-Level Validation of Atp5f1 Gene Expression

To confirm transcriptional data at the protein level, we quantified immunohistochemical

(IHC) staining profiles of ATP5F1 subunits in the HPA. ATP5F1A, B, and D proteins were expressed at intermediate levels in normal breast tissue with drastically higher and more consistent expression in tumor tissue (Figure 6). Most importantly, ATP5F1C was not detected in normal tissue but weakly expressed in breast cancer samples, suggesting a cancer-specific induction.



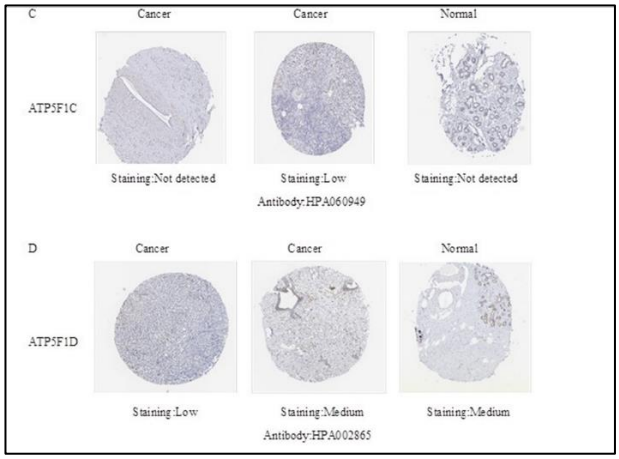


Figure 6. ATP5F1 protein expression was determined using the Human Protein Atlas database

Enrichment Analysis of ATP5F1 Co-Expressed Genes

To explore the biological significance of *ATP5F1A–E* and their co-expressed genes, we performed enrichment analysis using the Enrichr database. From Biological Processes point of view, as predicted, the co-expressed genes were significantly enriched in ATP metabolic processes, mitochondrial ATP synthesis-coupled proton transport, and oxidative phosphorylation, emphasizing the mitochondrial energy dependence of breast cancer progression. Molecular Functions revealed proton-transporting ATP synthase activity, ATPase activity, and NADH dehydrogenase activity were significantly enriched. Additionally, genes were enriched in metabolic pathways including oxidative phosphorylation, Parkinson's disease (a classical model of mitochondrial diseases), and the citrate cycle. Transcription Factors enriched in the current analysis were YY1, NRF1, and TFAM, which are important for mitochondrial biogenesis and energy homeostasis (Figure 7A–D). These enrichment results emphasize the role of ATP5F1 subunits in energy metabolism and suggest mitochondrial regulators as potential upstream drivers.

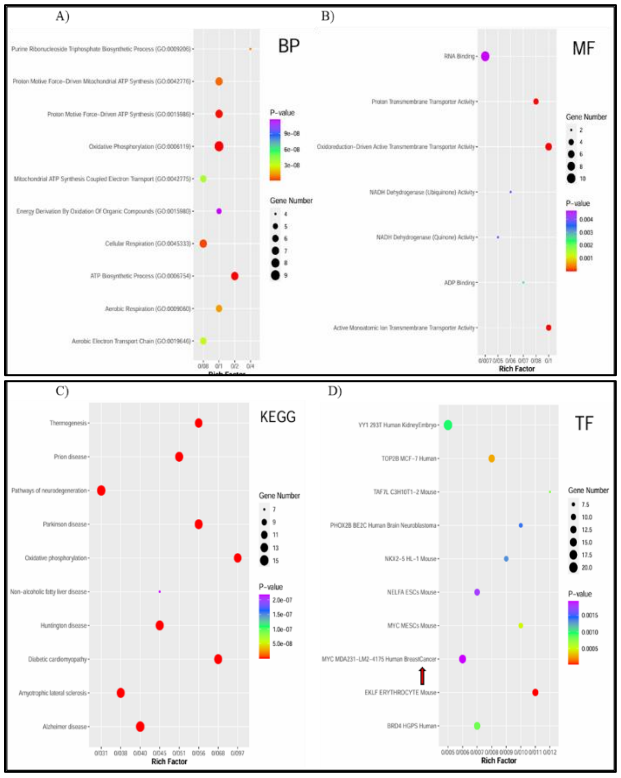


Figure 7. Functional enrichment analysis of ATP5F1 subunits in breast cancer. A) Biological processes, B) Molecular functions, C) KEGG pathways, and D) Transcription factors

Correlates between Immune Cell Infiltration and ATP5F1 Subunit Gene Expression

The ATP5F1 mitochondrial complex possesses distinctive immune-modulatory properties in breast cancer, as its subunits exhibit divergent correlations with tumor-infiltrating immune cells (Figure 8A–E). *ATP5F1A* is weakly correlated with CD4+ T cells (r=0.128, p=6.13e−05) and B cells (r=0.116, p=2.58e−04), while *ATP5F1B* is highly correlated with B cells (r=0.183, p=5.74e−09), CD8+ T cells (r=0.124, p=1.00e−04), and CD4+ T cells (r=0.141, p=9.89e−06), suggesting a pro-immune property. *ATP5F1C* correlates most highly with B cells (r=0.196, p=4.45e−10) but inhibits macrophages (r=−0.117, p=2.81e−04) and neutrophils (r=−0.083, p=8.95e−03). *ATP5F1D* is unexpectedly very immunosuppressive with strong negative correlations towards CD4+ T cells (r=−0.40, p<1e−38), neutrophils (r=−0.282, p=2.06e−19), dendritic cells (r=−0.177, p=4.04e−08), and B cells (r=−0.127, p=6.62e−05). *ATP5F1E* weakly supports B cells (r=0.10, p=1.63e−03) but represses macrophages (r=−0.191, p=2.29e−09) and dendritic cells (r=−0.103, p=1.40e−03). These signatures imply that *ATP5F1B/C* may induce anti-tumor immunity, while *ATP5F1D/E* may trigger immune evasion. The opposing action of subunits indicates their potential role as biomarkers of immune-hot (ATP5F1B/C-high) versus immune-cold (ATP5F1D-high) tumors and suggests metabolic-immune crosstalk in the TME.

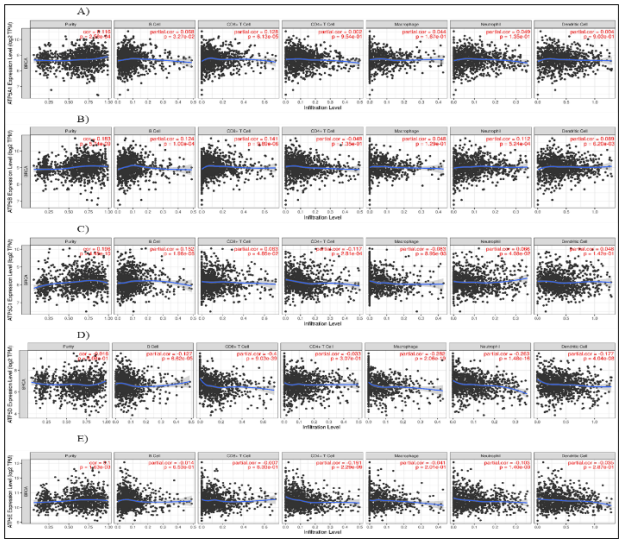


Figure 8. Scatterplot of correlations among expressions of the ATP5F1 gene family and levels of immune infiltration in breast cancer. Correlation of ATP5F1 gene family, namely A) *ATP5F1A*, B) *ATP5F1B*, C) *ATP5F1C*, D) *ATP5F1D*, and E) *ATP5F1E* with levels of immune infiltration (purity, B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells). The correlation measurement is indicated by the partial correlation value using Spearman's partial rho and the statistical significance of the p value.

DISCUSSION

Breast cancer remains among the most prevalent and lethal cancers in women globally [1].

Mitochondrial ATP synthase is an essential enzyme for oxidative phosphorylation that secures cellular energy homeostasis. Dysregulation of ATP synthase subunits may be responsible for disrupting metabolic homeostasis and oncogenic transformation as well as tumor growth [4,5,17]. Alterations in energy metabolism, with enhanced glycolysis and increased requirements for ATP, are a characteristic of the majority of cancers, including breast cancer [18,19]. In this study, we analyzed five subunits of the F1 sector of the ATP synthase complex (ATP5F1A, ATP5F1B, ATP5F1C, ATP5F1D, and ATP5F1E) and expression modes and prognostic significance in breast cancer. Whereas these subunits belong to the same enzyme complex, new evidence is developing that they may have alternative regulatory mechanisms and functional activities in cancer biology [4,20].

In the current study, high expression of the *ATP5F1A* gene (encoding subunit α) was associated with enhanced overall survival (Figure 3A1 and Table 1). Its mRNA level was significantly lower in cancer compared to controls (Figure 1A). Interestingly, of all the breast cancer subtypes characterized by PAM50, the subtype with the least *ATP5F1A* mRNA was HER2-enriched (Table 1). This observation may be contradictory, given that HER2-overexpressing cancers tend to be more aggressive and are associated with poorer prognosis, particularly in the absence of HER2-targeted therapy. This suggests that while high *ATP5F1A* expression is broadly associated with good prognosis in all samples of breast cancer, this association is likely the outcome of subtype-specific oncogenic drivers such as HER2 amplification. In HER2-positive cancers, the survival benefit achieved by increased *ATP5F1A* expression may be counteracted by the oncogenic activity of HER2 itself. The prognostic value of *ATP5F1A* may therefore be context-specific, with both molecular subtype and overall tumor microenvironment contributing. In addition, whereas *ATP5F1A* mRNA was reduced in tumors, the corresponding protein level was also elevated in cancer tissue (Figure 6A). One of the reasons for this discordance is the higher half-life of the protein or heightened post-transcriptional regulation. Shedding light on this, Chouhan et al. indicated that phosphorylated ATP5F1A was vastly greater in cancer prostate tissues compared to normal ones, albeit found in barely any normal cells, suggesting post-translational modifications would be a cause behind its stabilization or buildup in malignantly affected cells [21].

ATP5F1B mRNA analysis revealed that lower expression levels were significantly associated with improved overall survival and higher recurrence-free survival in breast cancer patients, suggesting a potential role as a negative prognostic marker (Figure 3B1 and 2). At the protein level, *ATP5F1B* (β subunit) showed moderate expression across samples (Figure 6B). Interestingly, its expression was reduced in progesterone receptor (PR)-positive tumors, whereas higher expression levels were observed in more aggressive subtypes, including HER2-enriched, Luminal B, and basal-like breast cancers (Table 1). This pattern suggests that *ATP5F1B* may be implicated in the metabolic reprogramming characteristic of more proliferative or treatment-resistant tumor phenotypes. These findings also raise the possibility of *ATP5F1B* serving as a molecular marker for PR-negative tumors, where fewer biomarkers are currently available. Supporting this, a study by Slater et al. on uveal melanoma identified elevated *ATP5F1B* expression as a poor prognostic indicator, reinforcing its broader relevance in tumor progression and mitochondrial metabolism across cancer types [22].

ATP5F1C, encoding the γ subunit of mitochondrial ATP synthase, is an essential

component within the catalytic core of the enzyme complex to integrate proton translocation with the production of ATP. Herein, *ATP5F1C* expressed significantly more mRNA in breast cancer tissues compared to adjacent normal tissues (Figure 1C), a pattern in agreement with findings from Fiorillo et al., which demonstrated ATP5F1C is an oncogenic substrate for the FDA-approved drug Bedaquiline, inhibiting mitochondrial ATP production and metastasis in vivo [9]. Furthermore, increased *ATP5F1C* expression was associated with poorer overall survival and lower recurrence-free survival (Figure 3C1 and 2), highlighting its potential as a prognostic biomarker for breast cancer. Protein-level information also confirmed the transcriptomic findings, reflecting moderate to high levels of ATP5F1C protein in breast tumor tissues, particularly in aggressive subtypes (Figure 6C). Notably, higher ATP5F1C expression correlated with nodal status and hormone receptor (ER and PR) status and basal-like molecular subtype (Table 1), which is indicative of poor prognosis and limited treatment options. These results support the hypothesis that ATP5F1C overexpression might be an adaptive metabolic mechanism to compensate for the overwhelming bioenergetic demands of rapidly proliferating tumor cells. Of particular note, ATP5F1C has been implicated in earlier studies as a cancer mitochondrial vulnerability whose inhibition leads to bioenergetic collapse and a suppression of metastatic potential [9,10]. Its central position in oxidative phosphorylation pathways further identifies it as a target of interest for interrupting metabolic pathways in basal-like or triple-negative breast cancers.

ATP5F1D is the gene that encodes the δ subunit of mitochondrial ATP synthase and plays an essential role in the stabilization of the F1 complex catalytic core. In the current research, mRNA levels of *ATP5F1D* were significantly elevated in breast tumor tissue when compared to normal tissue (Figure 1D), signifying transcriptional activation in tumorigenesis. However, paradoxically, low levels of ATP5F1D protein were observed in tumor tissues (Figure 6D), which indicates post-transcriptional or post-translational regulation. This disparity could be due to increased breakdown of proteins, reduced translation efficiency, or adaptation mechanisms for compensating ATP synthesis in very glycolytic cancer cells. Our findings are consistent with a recent paper by Wang et al. [23], which studied subunits of the ATP synthase, including ATP5F1D, as causatives of drug resistance and poorer prognosis in various forms of cancers like glioblastoma, ovarian carcinoma, and breast cancer. These subunits were proposed as metabolic vulnerabilities in cancer cells with enhanced oxidative phosphorylation. Furthermore, expression of *ATP5F1D* was found to be highest among the Luminal A subtype of breast cancer (Table 1), a group that is typically found to have a good prognosis but is known to develop endocrine resistance in some cases. Upregulation of *ATP5F1D* mRNA in this subtype may reflect a subpopulation of tumors that consume more mitochondrial respiration than glycolysis, and would be potential targets for mitochondrial-targeting therapies. Together, these findings indicate *ATP5F1D* to be a metabolically important gene in breast cancer with subtle regulatory modes at the transcript and protein levels, and as a possible prognostic marker and therapeutic target.

ATP5F1E encodes the regulatory subunit ε responsible for coupling proton flow with ATP synthesis in mitochondrial ATP synthase. In this research, *ATP5F1E* mRNA was found to be expressed at remarkably higher levels in breast tumor tissues compared with normal tissues (Figure 1E) indicating its transcriptional activation in cancer cells. Despite this overexpression, reduced *ATP5F1E* mRNA level

was profoundly correlated with improved overall survival and heightened recurrence-free survival rates (Figure 6E), proposing that overexpression of *ATP5F1E* could whether promote a more aggressive phenotype or resistance to treatment. From molecular subtype perspective, the highest *ATP5F1E* expression occurred in the HER2-enriched subtype (Table 1), which is a type with high proliferation rate, poor prognosis, and prevalent metabolic reprogramming. As mentioned previously, HER2-positive tumors have a tendency to use mitochondrial metabolism to support rapid growth, and this is possibly why *ATP5F1E* is overexpressed in this category. This makes an intriguing suggestion regarding a potential link between HER2 signaling and ATP synthase regulation. Moreover, our findings are in line with the Lin and Huang research that evidenced increased expression of *ATP5F1E* in colorectal cancer via the hsa_circ_0079480/miR-498 axis, which leads to enhanced cell proliferation, migration, and invasion [24]. Although this axis has not been explored specifically in breast cancer, simultaneous upregulation of *ATP5F1E* expression and poor prognosis both indicate a conserved oncogenic process across different tumor types. In general, these results support the hypothesis that *ATP5F1E* is a putative oncogene in breast cancer, particularly in HER2-positive carcinoma, and would be a promising candidate to further investigate as a prognostic biomarker or therapeutic target in aggressive and metabolically active breast cancers.

Our mutation analysis identified that *ATP5F1E* and *ATP5F1C* has have the highest frequencies of genomic mutations among ATP5F1 subunits in breast cancer, with DNA amplification and mRNA overexpression being the predominant events (Figure 2). Our findings are in line with our gene expression data, where both *ATP5F1C* and *ATP5F1E* were significantly overexpressed in breast cancer tissues, suggesting their additional implicated roles in tumor growth. *ATP5F1E* has been implicated in colorectal cancer by the hsa_circ_0079480/miR-498/*ATP5E* axis, and our findings suggest a comparable mechanism in breast cancer [24]. Although missense mutations were rare, their presence in *ATP5F1A* and A*TP5F1B* warrants further study to determine their functional effect. Notably, the consistent amplification of *ATP5F1E* and *C* suggests that these subunits are metabolic or therapeutic candidates in breast cancer, particularly in tumors with high oxidative metabolism. Further functional studies are needed to establish how these alterations affect ATP synthase activity, mitochondrial dynamics, and energy production in breast cancer cells, and if they can serve as diagnostic biomarkers or therapeutic targets.

Our TIMER-based immune infiltration analysis (Figure 8) revealed novel associations between ATP5F1 subunits and tumor-infiltrating immune cells. ATP5F1A showed weak but significant positive associations with CD4+ T cells and B cells. Our ATP5F1B expression was correlated to B cells and T cell populations. Notably, ATP5F1D had far-reaching immunosuppressive trends (CD4+ T cells and neutrophils). These data may strengthen developing knowledge on the role of ATP5F1D in immune evasion for cancers. Interestingly, ATP5F1C was correlated to B cells, its negative correlation with macrophages deviates from noted pro-tumorigenic activation of macrophages in glioblastoma, and indicates potential cancer-type dependent effects. These findings provide an illustration of synchronized but alternative immunomodulatory roles within the complete ATP5F1 complex in breast tumor microenvironment, with ATP5F1B/C likely mediating adaptive immunity and ATP5F1D/E promoting an environment of immunosuppression.

This comprehensive bioinformatics analysis highlights the diversified roles of ATP5F1A–E subunits in breast cancer. Their expression

difference, survival correlation, mutation landscape, and immune interactions imply their biological significance in tumor metabolism, growth, and immune regulation. In particular, *ATP5F1C* and *ATP5F1E* are potential oncogenic drivers and biomarkers considering their high frequency of overexpression and correlation with poor patient prognosis. On the other hand, *ATP5F1A* may act as a tumor suppressor, while *ATP5F1D* and *ATP5F1B* play complicated immunomodulatory roles. The findings offer new perceptions into cancer mitochondrial bioenergetics and create prospects for therapeutic strategies targeting metabolic processes and the tumor immune microenvironment. Future experimental confirmation is required to continue elucidating the regulatory mechanisms and translational significance of ATP5F1 subunits in breast cancer.

CONCLUSION

In this study, it is revealed that ATP5F1 subunits play multi-dimensional and critical roles in breast cancer, particularly ATP5F1C and ATP5F1E, associated with poor prognosis and potentially can be considered oncogenic biomarkers. The evidence suggests the role of mitochondrial metabolism and immune modulation in cancer progression and identifies the ATP5F1 subunits as potential targets for future drug therapy.

COMPETING INTERESTS

The authors declare no conflict of interest.

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