Research in Pharmaceutical Sciences, December 2025; 20(6): 789-799
Received: 19-04-2025
Peer Reviewed: 13-05-2025
Revised: 16-06-2025
Accepted: 28-06-2025
Published: 15-11-2025

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*Original Article*

# Expression of PPAR-alpha and gamma in breast cancer patients and their relationship with the expression of *FASN*, *ACSL4*, and *ACLY* genes

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## *Abstract*

**Background and purpose**: Peroxisome proliferator-activated receptors alpha and gamma (PPAR-α and PPAR-γ) are nuclear receptor proteins that play a crucial role in the regulation of cellular differentiation, development, metabolism, and tumorigenesis. Their expression levels have been implicated in the metabolic reprogramming of breast cancer cells, influencing their proliferation and survival. This study investigates the expression of PPAR-α and PPAR-γ in breast cancer and explores their relationship with key enzymes involved in fatty acid biosynthesis: fatty acid synthase (FASN), acyl-CoA synthetase long-chain family member (ACSL4), and ATP citrate lyase (ACLY).

**Experimental approach**: In this study, 28 pairs of fresh samples of breast cancer and adjacent non-cancerous tissue were analyzed to assess gene expression levels using quantitative real-time polymerase chain reaction (qRT-PCR) and immunohistochemistry staining.

**Findings/Results:** The expression of PPAR-α increased, while PPAR-γ decreased significantly in breast cancer tissues compared to adjacent normal tissues. The expression of PPAR-α was significantly associated with FASN mRNA expression. Additionally, a correlation was also observed between the expression levels of both PPAR-α and PPAR-γ with ACSL4 mRNA levels

**Conclusion and implications**: Given the obtained results, the involvement of PPARs in the regulation of lipid metabolism was substantiated. Moreover, the correlation of PPARs with ACSL4 highlights the possible role of PPAR-α and PPAR-γ in the regulation of tumor tissue ferroptosis and suggests that targeting these pathways could offer new therapeutic strategies for managing breast cancer. However, further studies are needed to understand the mechanism of action.

**Keywords:** ACSL4; Breast cancer; lipid metabolism; FASN; PPAR-α; PPAR-γ.

## INTRODUCTION

Cancer ranks among the leading causes of death worldwide. It is estimated that there will be approximately 2,001,140 new cancer cases in the United States in 2024, equivalent to about 5,480 diagnoses each day (1). Breast cancer (BC) stands as a significant contributor to female mortality associated with cancer (2).

One of the hallmarks of cancer is metabolic reprogramming, notably increased glucose uptake and glycolysis (the Warburg effect) (3-5). In parallel, alterations in lipid metabolism contribute to tumor growth, migration, invasion, and angiogenesis. Cancer cells can perform *de novo* lipogenesis at rates comparable to the liver, as fatty acids are essential for energy storage, membrane biosynthesis, and signaling (6, 7). Additionally, fatty acid oxidation supports cancer cell survival and proliferation (8). Key enzymes such as ATP citrate lyase (ACLY), acyl-CoA synthetase long chain (ACSL), and fatty acid synthase (FASN) are upregulated in various cancers (9,10).

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Website: http://rps.mui.ac.ir

DOI: 10.4103/RPS.RPS_64_25

The ACSL family is responsible for the conversion of the chain-length fatty acids (C12-C22) to fatty acyl-CoA esters (10), which is a prerequisite for the metabolism of fatty acids in several lipid metabolism pathways, including lipogenesis, glycerolipid synthesis, lipidation of proteins, and β-oxidation (10,11). ACSLs contain five isoforms, ACSL1, ACSL3, ACSL4, ACSL5, and ACSL6 (10, 12). ACSL4 is significantly upregulated in specimens of breast, prostate, liver, and colon cancer (13). In addition, ACAL3 and ACSL4 play a pivotal role in ferroptosis, a form of non-apoptotic cell death that is initiated by the accumulation of membrane lipid peroxides due to iron overload (14).

FASN, ACLY, and ACSL are among the target genes regulated by peroxisome proliferator-activated receptors (PPARs) (15,16). In lipid metabolism, these enzymes play a crucial role in the production and utilization of fatty acids. Therefore, PPAR activity can directly influence the activity of these enzymes, ultimately contributing to the regulation of lipid metabolism (17).

PPARs are ligand-inducible transcription factors that belong to the nuclear receptor (NR) superfamily. NRs have been implicated in a wide array of biological processes, such as regulating various aspects of lipid and glucose homeostasis, development, reproduction, immune function, growth, apoptosis, and cancer (18,19).

Three PPAR subtypes have been identified: PPAR-α (NR1C1), PPARb/d (NR1C2), and PPAR-γ (NR1C3) (20), which are encoded by distinct genes located on different chromosomes (21).

PPAR-γ plays a crucial role in lipid metabolism, facilitating adipogenesis and fatty acid storage (22). Its activation reduces circulating triglycerides and free fatty acids while promoting lipid uptake through adipogenesis and increased storage (23). PPAR-γ is essential in adipogenesis, regulating adipocyte differentiation, self-renewal, and mature adipocyte function (24). Additionally, PPAR-γ activation upregulates adipokines, which influence insulin sensitivity, inflammation, tumorigenesis, and overall metabolism (25). In BC, PPAR-γ expression is observed (25), and its activation by ligands is associated with tumor development, progression, and metastasis (26). Furthermore, PPAR-γ, amplified in advanced prostate cancer, supports cancer growth through fatty acid synthesis and mitochondrial biogenesis and cooperating with androgen receptor signaling (27).

PPAR-α is widely expressed in the body, with significant protein levels in metabolically active tissues (28), playing a role in fatty acid oxidation and affecting various aspects of metabolism, including inflammation and cancer progression (29,30). PPAR-α regulates lipid metabolism and homeostasis by modulating genes involved in lipoprotein lipase, apolipoproteins, fatty acid transport, fatty acid oxidation, and high-density lipoprotein metabolism (26,31). PPAR-α activation promotes fatty acid-binding protein 4 (FABP4) expression, supporting lipid metabolism and reducing lipotoxicity by enhancing fatty acid mobilization and utilization. In addition, PPAR-α induction of hepatic lipogenesis collaborates with insulin signaling through the regulation of the sterol regulatory element binding protein 1c-dependent pathway, impacting lipogenic enzyme expression (32). Also, inhibition of PPAR-α exhibits anti-proliferative effects on cancer cells, indicating its potential role in promoting tumorigenesis through modulation of antioxidant capacity and gene expression (28).

Despite previous studies highlighting the role of PPARs in lipid metabolism and tumor progression, limited data exist on the simultaneous expression patterns of PPAR-α and PPAR-γ alongside key lipogenic enzymes such as ACSL4, FASN, and ACLY. To address this gap, the present study evaluates the expression of PPAR-α and PPAR-γ in paired tumor and adjacent normal breast tissue samples, and investigates their correlation with FASN, ACLY, and ACSL4. Additionally, we assess the prognostic value of these factors through survival analysis. Our findings may provide new insights into the metabolic regulation of BC and suggest potential therapeutic targets.

## MATERIALS AND METHODS

### *Clinical samples*

Twenty-four pairs of BC and adjacent normal tissue samples were obtained from patients undergoing surgical resection at Ordibehesht Hospital in Isfahan, Iran, between 2016 and 2017. Following resection, all samples were promptly frozen in liquid nitrogen

and stored at -80 °C until RNA extraction. Histological confirmation was performed on all BC cases. This study received approval from the Ethics Committee of Isfahan University (ethics code: IR.MUI.PHANUT.REC.1402.092), and informed consent was obtained from all patients. The inclusion criteria were histologically confirmed primary BC, no prior chemotherapy, radiotherapy, or neoadjuvant treatment, and availability of complete clinical data. Patients with recurrent or metastatic disease or insufficient tissue were excluded.

### *RNA extraction and quantitative real-time polymerase chain reaction*

In this study, previously synthesized complementary DNA (cDNA) samples (BioFact™ RT-Kit, BioFACT, Daejeon, Korea) were used, which had been generated from total RNA extracted (BioFACT total RNA Prep Kit (Ver. 2.0, BioFACT, Daejeon, Korea)) from BC and adjacent normal tissues as part of our previous research protocol (33). All cDNA samples were stored at -80 °C until use. Before quantitative real-time polymerase chain reaction (qRT-PCR), the integrity and usability of the stored cDNA were assessed by amplification of a housekeeping gene (ACTB) to confirm successful reverse transcription and absence of degradation. Only samples showing clear amplification with consistent cycle threshold (Ct) values were included in the analysis.

qRT-PCR analyses were conducted using BioFact qPCR Master Mix Plus for SYBR Assay on an ABI StepOnePlus Real-time PCR system (Applied Biosystems, USA). Beta-actin (ACTB) served as an internal control for normalizing RNA input. The sequences of primers used for amplifying PPAR-α, PPAR-γ, and β-actin are indicated in Table 1.

The qRT-PCR reactions were conducted in three steps. Step 1 involved one cycle at 95 °C for 15 min. Step 2 comprised 40 cycles: DNA denaturation at 95 °C for 20 s, primer annealing at 58 °C for 30 s, and DNA extension at 72 °C for 30 s. Step 3, the melt curve stage, aimed to validate the specificity of the PCR product and included one cycle with three stages: 95 °C for 15 s, 60 °C for 1 min, then ramped to 95 °C at 0.3 °C per 5 s and held at 95 °C for 15 s. This procedure was performed for each sample in triplicate. The Ct value indicates the fractional cycle number at which the fluorescence intensity passes a fixed threshold above the baseline

The relative expression of each gene was determined using the ΔΔCt formula, comparing the expression of the target gene with the internal control gene β-actin ($\Delta Ct = Ct_{\text{target gene}} - Ct_{\text{internal control}}$). Group comparisons were based on the mean ΔCt ± SEM, and $2^{-\Delta\Delta Ct}$ was calculated. The fold change was estimated using the $2^{-\Delta\Delta Ct}$ method, representing the fold expression variation of the tumor group compared to their corresponding control group. PCR efficiency was evaluated through standard curve analysis using serial cDNA dilutions (34).

### *Immunohistochemistry*

Formalin-fixed, paraffin-embedded tissue sections (5 µm) were deparaffinized and rehydrated using standard xylene and ethanol series. Antigen retrieval was performed by heating the sections in 10 mM citrate buffer (pH 6.0) at 95 °C for 20 min. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide. The sections were then incubated overnight at 4 °C with primary antibodies: anti-PPAR-α (Abcam, ab24509, Cambridge, UK, dilution 1:100) and anti-PPAR-γ (Abcam, ab41928, Cambridge, UK, dilution 1:100). After washing, a secondary antibody conjugated with horseradish peroxidase (HRP) was applied, and signal detection was carried out using diaminobenzidine tetrahydrochloride (DAB). Slides were counterstained with hematoxylin and evaluated under an Olympus light microscope at 40× magnification. Negative controls were processed in parallel by omitting the primary antibody. Image analysis was performed using ImageJ software (version 1.52h), and the immunoreactivity of PPAR-α and PPAR-γ was quantified based on brown staining intensity (pixels/µm²). Data are presented as mean ± SD.

**Table1.** The primer sequences used for amplifying genes in this study.

| Genes | Forward sequences | Reverse sequences |
|---|---|---|
| **PPAR-α** | 5′-GAGCTATGGTATGTGGTTC-3′ | 5′-CATCTGGTCTGTTGGTC-3′ |
| **PPAR-γ** | 5′-GCAGGAGCAGAGCAAAGAG-3′ | 5′-GAGGAGAGTTACTTGGTCGTTC-3′ |
| **β-actin** | 5′-GTTGTCGACGACGAGCG-3′ | 5′-GCACAGAGCCTCGCCTT-3′ |

PPAR, Peroxisome proliferator-activated receptor.

### *Statistical analysis*

The result of gene expression was analyzed using Student's tests to compare the tumor with the control group, with data values presented as mean ± SEM, and statistical analysis conducted using SPSS 21 (IBM Corporation). The correlation between PPAR-α and PPAR-γ expression and clinicopathological parameters was demonstrated using one-way analysis of variance (ANOVA). Additionally, Pearson correlation (r) analysis was used to evaluate the linear relationship between gene expression levels (*e.g.*, PPAR-α, PPAR-γ, and ACSL4), and correlation plots were generated using SPSS version 26.

### *Assessment of the patient survival rate*

Assessment of the relevance of the PPAR-α and PPAR-γ mRNA expression to survival rate in patients with BC was performed through an online survival analysis tool named KM Plotter (35), which uses microarray gene expression information of 3951 individuals from Gene Expression Omnibus (GEO), European Genome-phenome Archive, and The Cancer Genome Atlas databases (http://kmplot.com/analysis/index.php?p=service&cancer=breast). Patient samples were first split into high and low expression groups on the basis of the auto-select best cutoff, then the Kaplan-Meier survival plots were obtained for the relapse-free survival rate with and without restriction subtypes, displaying hazard ratio and *P*-value.

## RESULT

### *PPAR-α and PPAR-γ mRNA expression in BC and adjacent normal tissue*

To evaluate the potential effect of PPAR-α and PPAR-γ on BC, the relative transcriptional levels of PPAR-α and PPAR-γ genes were evaluated in 28 paired human BC specimens and adjacent normal tissue by qRT-PCR.

Our analysis revealed that the expression of PPAR-α in BC tissue was significantly higher compared to adjacent normal tissue ($P < 0.05$); conversely, the expression of PPAR-γ was found to decrease in tumor tissue compared to normal tissue ($P < 0.05$). The relative expression levels of the PPAR-α and PPAR-γ genes in normal and tumor breast tissues are presented in Fig. 1.

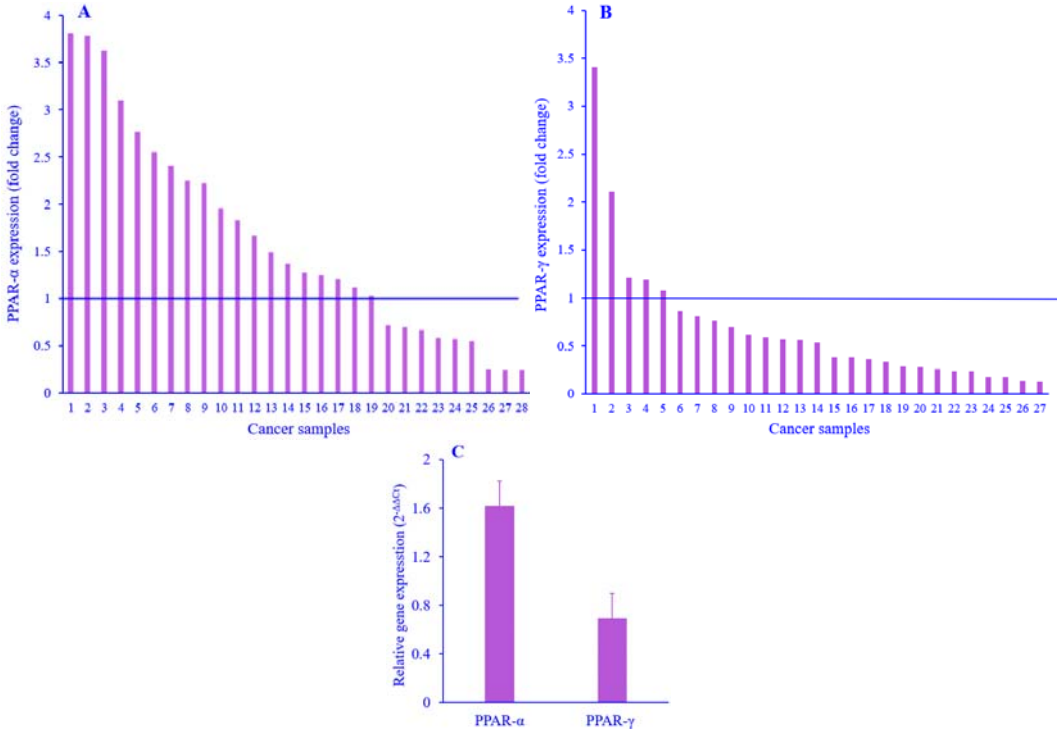


**Fig. 1.** The expression levels of PPAR-α and PPAR-γ in breast cancer tumors and adjacent normal tissues were assessed using qRT-PCR. The mRNA expression data were normalized to β-actin (ACTB) as the internal control and analyzed using the $2^{-\Delta\Delta Ct}$ chain-fold method. Panel (A) shows the relative fold change in PPAR-α expression for each tumor-normal tissue pair, while panel (B) displays the relative fold change for PPAR-γ. Panel (C) summarizes the average relative expression, showing a 1.61-fold increase in PPAR-α and a 0.69-fold decrease in PPAR-γ in tumor tissues compared to adjacent normal tissues. PPAR, Peroxisome proliferator-activated receptor.

The analysis of the results using the $2^{-\Delta\Delta Ct}$ method showed that the relative mRNA expression of PPAR-α was upregulated in 19 out of 28 BC samples (67.85%), with a fold change greater than 1.61 in tumor tissues compared to adjacent normal tissues ($P < 0.05$). In contrast, the relative mRNA expression of PPAR-γ was downregulated in 22 out of 27 samples (81.4%), with a fold change lower than 0.68 in tumor tissues compared to adjacent normal tissues (Fig. 1). One sample was excluded from the PPAR-γ analysis due to poor amplification quality.

### *PPAR-α and PPAR-γ protein expression in BC and adjacent normal tissue*

The protein expression levels of PPAR-α and PPAR-γ were examined in paired BC and adjacent normal tissue samples. Immunohistochemistry analysis revealed a significant upregulation of PPAR-α protein expression in BC tissue compared to normal tissue (Fig. 2A-C). In contrast, while PPAR-γ protein expression was reduced in BC tissue, this decrease was not statistically significant ($P > 0.05$; Fig. 2D-F).

### *Relationship between PPAR-α and PPAR-γ expression and clinicopathological features of the study population*

Using the $\chi^2$ test, indicated that the increased mRNA expression of PPAR-α and PPAR-γ in the BC tissues was negatively correlated to ACSL4 mRNA expression. The expression of PPAR-α was also significantly associated with FASN mRNA expression. There was no statistically significant difference between PPAR expression and age, tumor stage, estrogen receptor, progesterone receptor, and Ki-67 expression (Table 2).

Quantitatively significant inverse association between PPAR-α and PPAR-γ with ACSL4 was confirmed based on Spearman's correlation coefficient analysis (Fig. 3).

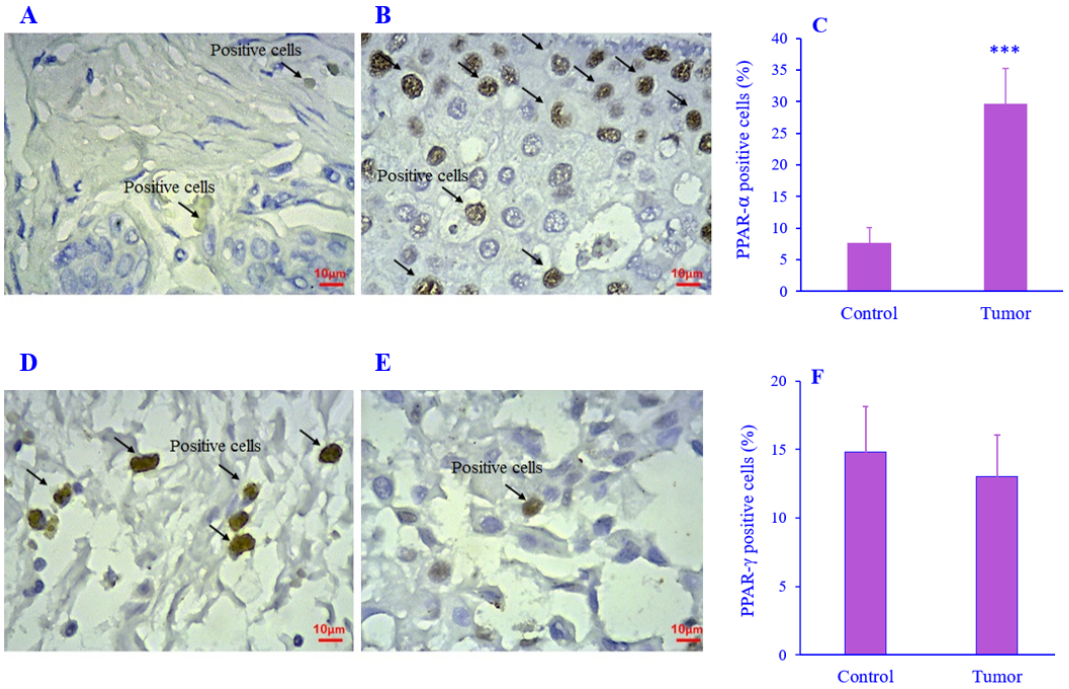


**Fig. 2.** Immunohistochemical staining and semi-quantitative analysis of PPAR-α and PPAR-γ protein expression in breast cancer and adjacent normal tissues. Panels A and D show adjacent normal tissues with a limited number of positively stained cells (brown color), indicating basal expression levels of PPAR-α and PPAR-γ, respectively. Panels B and E depict breast cancer tissues. Panel B demonstrates a substantial increase in PPAR-α-positive cells, indicating significant upregulation in tumor tissues, which was confirmed by (C) semi-quantitative analysis showing a statistically significant increase in PPAR-α protein expression in tumor samples compared to normal tissues (serving as the control group). In contrast, panel E shows a slight reduction in PPAR-γ-positive cells in tumor tissue compared to normal tissue. However, (F) the decrease in PPAR-γ expression was not statistically significant ($P > 0.05$). ***$P < 0.001$ indicates a significant difference compared to the control group. PPAR, Peroxisome proliferator-activated receptor.

**Table 2.** The relationship between the PPAR-α and PPAR-γ mRNA expression with ACSL4, FASN, ACLY, and clinicopathological variables of patients with breast cancer.

| Parameter | PPAR-α mRNA expression [a] | | | PPAR-γ mRNA expression [a] | | |
|---|---|---|---|---|---|---|
| | < 1.61 | ≥ 1.61 | *P*-value[b] | < 0.68 | ≥ 0.68 | *P*-value[b] |
| **Age (year)** | | | | | | |
| ≥ 50 | 5 (38.5%) | 8 (61.5%) | 0.116[#] | 7 (53.8%) | 6 (46.2%) | 0.420[#] |
| < 50 ( | 11 (73.3%) | 4 (26.7%) | | 11 (73.3%) | 4 (26.7%) | |
| **Tumor size** | | | | | | |
| < 2 cm | 2 (33.3%) | 4 (66.7%) | 0.365[##] | 5 (71.4%) | 2 (28.6%) | 0.668[##] |
| ≥ 2 cm | 14 (63.6%) | 8 (36.4%) | | 13 (61.9%) | 8 (38.1%) | |
| **Grade** | | | | | | |
| 1 | 0 (0.0%) | 1 (100%) | 0.313[#] | 0 (0.0%) | 1 (100%) | 0.323[#] |
| 2 | 11 (64.7%) | 6 (35.3%) | | 11 (68.8%) | 5 (31.3%) | |
| 3 | 5 (55.6%) | 4 (44.4%) | | 7 (70.0%) | 3 (30.0%) | |
| **Stage** | | | | | | |
| 1 | 1 (20.0%) | 4 (80.0%) | 0.295[##] | 4 (66.7%) | 2 (33.3%) | 0.864[#] |
| 2 | 12 (66.7%) | 6 (33.3%) | | 11 (61.1%) | 7 (38.9%) | |
| 3 | 3 (60.0%) | 2 (40.0%) | | 3 (75.0%) | 1 (25.0%) | |
| **Estrogen receptor** | | | | | | |
| Positive | 4 (50.0%) | 4 (50.0%) | 0.673[##] | 4 (57.1%) | 3 (42.9 %) | 0.647[##] |
| Negative | 12 (60.0%) | 8 (40.0%) | | 14 (66.7%) | 7 (33.3%) | |
| **Progesterone receptor** | | | | | | |
| Positive | 5 (45.5%) | 6 (54.5 %) | 0.408[##] | 7 (63.6%) | 4 (36.4%) | 0.999[##] |
| Negative | 11 (64.7 %) | 6 (53.3%) | | 11 (64.7%) | 6 (35.3%) | |
| **Ki67** | | | | | | |
| < 20 | 8 (61.5%) | 5 (38.5%) | 0.561[#] | 8 (66.7%) | 4 (33.3%) | 0.999[##] |
| ≥ 20 | 8(53.3%) | 7 (46.7%) | | 10 (62.5%) | 6 (37.5%) | |
| **ACSL4** | | | | | | |
| < 1 | 3 (25.0%) | 9 (75%) | **0.014**[#] | 5 (38.5%) | 8 (61.5%) | **0.047**[#] |
| ≥ 1 | 9 (75%) | 3 (25%) | | 9 (81.8%) | 2 (18.2%) | |
| **ACLY** | | | | | | |
| < 1.3 | 8 (47.1%) | 9 (52.9%) | 0.673[#] | 9 (50.0%) | 9 (50.0%) | 0.341[##] |
| ≥ 1.3 | 5 (62.5%) | 3 (37.5%) | | 5 (83.3%) | 1 (16.7%) | |
| **FASN** | | | | | | |
| < 1.4 | 9 (90.0 %) | 1 (10.0 %) | **0.004**[##] | 8 (80.0%) | 2 (20.0%) | 0.211[##] |
| ≥ 1.4 | 4 (26.7 %) | 11 (73.3%) | | 7 (46.7%) | 8 (53.3%) | |

PPAR, Peroxisome proliferator-activated receptor; ACSL4, long-chain acyl-coenzyme A synthetase 4; ACLY, ATP citrate lyase; FAS, fatty acid synthase; a, the PPAR-α and PPAR-γ mRNA expression was measured based on ACTB in tumor and adjacent normal tissues with $2^{-\Delta\Delta ct}$ in at least two experiments; b, All *P*-values < 0.05 were considered statistically significant; [#], Chi-Square tests; [##], Fisher´s exact test.

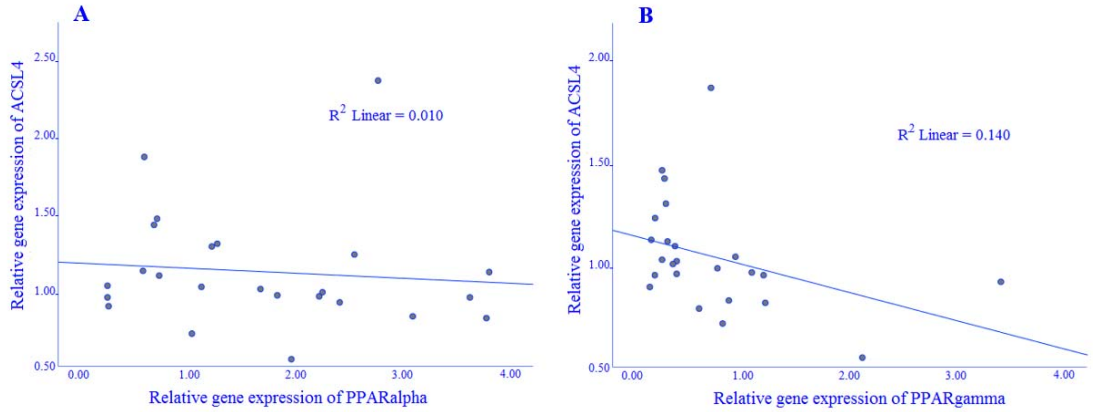


**Fig. 3.** Scatter plots showing the correlation between the expression levels of (A) PPAR-α and (B) PPAR-γ with ACSL4. Panel (A) indicates a very weak inverse correlation between PPAR-α and ACSL4 expression ($R^2 = 0.010$), while panel (B) shows a slightly stronger inverse correlation between PPAR-γ and ACSL4 ($R^2 = 0.140$). Pearson correlation analysis was performed, and the plots were generated using SPSS version 26. PPAR, Peroxisome proliferator-activated receptor; ACSL4, long-chain acyl-coenzyme A synthetase 4.

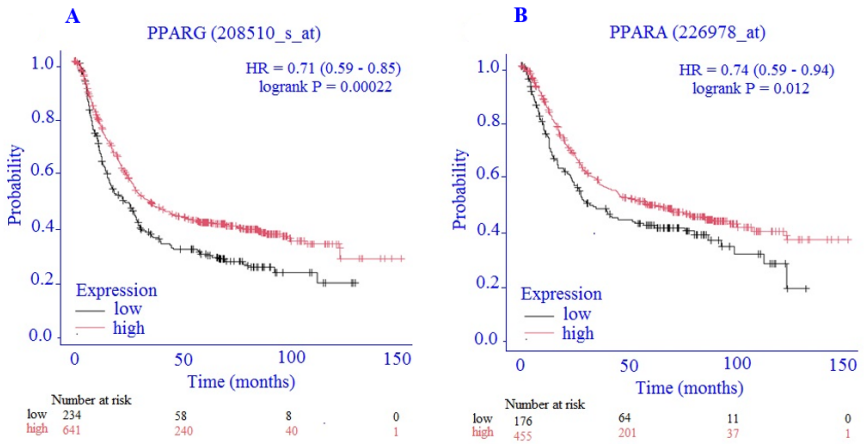


**Fig. 4.** Kaplan-Meier survival curves depicting the correlation between recurrence-free survival and mRNA expression levels of (A) PPAR-γ and (B) PPAR-α in breast cancer patients. Patients were stratified into high and low expression groups based on the optimal automatic cutoff point. The plot shows that higher expression of PPAR-γ and PPAR-α is associated with longer recurrence-free survival. Statistical significance was evaluated using the log-rank test. PPAR, Peroxisome proliferator-activated receptor.

### *PPAR-γ and PPAR-α as markers for predicting the survival of patients with BC*

The analysis of the Kaplan-Meier dataset indicates that higher expression of both PPAR-γ and PPAR-α is significantly associated with improved relapse-free survival. The data set for PPAR-γ and PPAR-α included data from 875 and 631 patients, respectively, and was obtained using the Kaplan-Meier plot for relapse-free survival (Fig. 4). These findings propose PPAR-γ and PPAR-α as potential novel prognostic biomarkers for predicting outcomes in BC patients.

## DISCUSSION

Changes in beta-oxidation and fatty acid synthesis pathways in cancer cells are crucial because they help tumor cells grow and survive by altering energy production and biosynthesis, and they also provide potential targets for therapy (36). The significance of PPARs in BC lies in their dual roles in regulating lipid metabolism and cellular differentiation, which can influence tumor growth and progression (37).

In this study, PPAR-γ expression showed a significant decrease in BC tumor tissue samples compared to the adjacent normal tissue. Additionally, significant associations were observed between the expression of PPAR-α and FASN, as well as a correlation between PPAR-α and PPAR-γ with ACSL4 in BC patients.

PPAR-α is a nuclear receptor involved in the regulation of lipid metabolism (15). It controls the expression of genes involved in fatty acid oxidation, transport, and energy homeostasis (38). The elevated expression of PPAR-α in BC has been linked to the promotion of lipid metabolism and proliferation of cancer cells, as it enhances the expression of genes involved in fatty acid oxidation and energy production (38). This regulation supports the rapid growth and survival of cancer cells under metabolic stress. Consistent with the concept, our study demonstrates high expression of PPAR-α in BC tissues compared to adjacent normal tissues. Chandran *et al*. also reported increased PPAR-α expression in BC tissue and highlighted its potential as a biomarker for disease prognosis. In addition, they showed that clofibrate, as a PPAR-α agonist, leads to high chemosensitivity in BC cells (39). Furthermore, some studies have reported that PPAR-α plays a role in regulating the tumor microenvironment through modulation of the phosphoinositol 3 kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling pathways, as well as nuclear factor-kappa B (NF-κB), thus promoting cell apoptosis and inhibiting angiogenesis (40). The study on PPAR-α activation using the agonist WY-14643 and its silencing with small-interfering RNA (siRNA) in goat mammary epithelial cells suggested that PPAR-α likely promotes monounsaturated fatty acid synthesis (41). However, we observed significant associations between the expression of PPAR-α and FASN. FASN is a key enzyme in *de novo* lipogenesis, responsible for converting acetyl-CoA into palmitate, a saturated fatty acid (42). FASN is often upregulated in cancer cells, including BC, where it supports rapid cell growth by supplying the necessary lipids for membrane synthesis and energy production (43).

Additionally, FASN is frequently considered a poor prognostic marker, as its overexpression is associated with reduced patient survival (44). Therefore, the observed correlation, in addition to showing the involvement of PPAR-α in regulating lipogenic pathways, is in alignment with the results obtained from Kaplan-Meier plots for relapse-free survival, which revealed a positive correlation between PPAR-α expression and patient survival rates (Fig. 3B).

Cancer cells with high PPAR-α and FASN expression might develop a "lipogenic phenotype", characterized by enhanced lipid synthesis and accumulation (44). This phenotype could contribute to tumor aggressiveness and metastasis, as the cells become more adaptable to various environmental stressors (42). PPAR-α and FASN are also involved in the regulation of inflammation, a key factor in the tumor microenvironment that affects cancer progression (43).

In this study, we also observed a significant negative correlation between PPAR-α and ACSL4 expression in BC patients. ACSL4 is critical for the activation of long-chain fatty acids, a step in fatty acid oxidation processes, and is also used for incorporation into cellular lipids, processes that are critical for the rapid proliferation of cancer cells (46). ACSL4 overexpression has been reported in several cancer cell lines, including breast, prostate, colon, gastric, and liver (15). We previously showed a negative correlation between ACSL4 and Ki-67 expression in BC patients; therefore, ACSL4 may be considered a tumor suppressor and a defense mechanism (33). On the other hand, some studies demonstrated that ACSL4 is associated with ferroptosis, a form of cell death driven by lipid peroxidation (46).

The observed negative correlation between PPAR-α and ACSL4 may indicate the role of PPAR-α in regulating lipid metabolism, and on the other hand, it may be due to the inhibitory role of PPAR-α in the ferroptosis process. Because some studies have shown that PPAR-α can inhibit ferroptosis through the regulation of glutathione peroxidase 4 (40). The results of this study revealed that PPAR-α may reduce the levels of lipid peroxides by decreasing ACSL4 activity. This could potentially decrease ferroptosis susceptibility in cancer cells by limiting the formation of lipid peroxides. Therefore, understanding the relationship between PPAR-α, ACSL4, and ferroptosis could lead to new therapeutic strategies, and targeting PPAR-α could potentially increase ferroptosis and improve treatment efficacy. Also, the significant correlation between PPAR-α and ACSL4 might be involved in resistance to cancer therapies. PPAR-α could modulate drug metabolism and detoxification pathways (47), while ACSL4 might contribute to maintaining cellular integrity and managing oxidative stress (48). Together, these factors could influence the ability of cancer cells to withstand conventional treatments, highlighting potential targets for overcoming drug resistance. Although the correlation coefficient for PPAR-α and ACSL4 ($R^2 = 0.01$) was weak and likely to be biologically insignificant, we included it for completeness and to reflect data variability. Only the correlation between PPAR-γ and ACSL4 ($R^2 = 0.140$) approached a biologically suggestive trend.

The results of this study also showed a significant alteration in PPAR-γ expression. In that, PPAR-γ expression significantly decreased in BC tumor tissue samples compared to adjacent normal tissue. Interestingly, despite the significant reduction in PPAR-γ mRNA expression, the corresponding decrease in protein levels, as assessed by immunohistochemistry, was not statistically significant. This discrepancy may be attributed to post-transcriptional regulatory mechanisms, differences in protein stability, or technical limitations associated with the immunohistochemistry method. Additionally, the lack of statistical significance at the protein level may reflect biological variability in PPAR-γ regulation.

PPAR-γ is another member of the PPAR family, known for its role in adipogenesis, glucose metabolism, and anti-inflammatory effects (49). Therefore, the reduced expression of PPAR-γ in BC seems to contribute to tumor progression and resistance to apoptosis (50). Porcuna and colleagues have shown that PPAR-γ plays a complex role in cancer biology, potentially influencing cancer cell differentiation and lipid metabolism (25). PPAR-γ generally acts as a tumor suppressor by promoting differentiation and apoptosis, and its downregulation may remove these inhibitory effects on cancer cell growth (51). The decreased levels of PPAR-γ may also be associated with resistance to certain chemotherapeutic agents, since PPAR-γ activation can sensitize cancer cells to apoptosis-inducing treatments (52).

Several studies have shown decreased expression of PPAR-γ in BC tissue (33). A study on BC tissue samples found that lower PPAR-γ expression

correlated with higher histological grades, increased lymph node metastasis, and poorer prognosis, suggesting its role in tumor aggressiveness (48). Some studies have also shown the role of PPAR-γ in inducing apoptosis of esophageal cancer cells (53). This result is consistent with our findings on the effect of PPAR-γ mRNA expression levels on the survival rate in patients with BC, using the online Kaplan-Meier plotter and data set. The data obtained from Kaplan-Meier plots for relapse-free survival revealed a positive correlation between PPAR-γ expression and patient survival rates, confirming the antiproliferative effect of PPAR-γ activation (Fig. 3).

According to the proposed role of PPAR-γ in studies conducted and the significant association between its expression and ACSL4 in the present study, it may be suggested that PPAR-γ and ACSL4 expression serve as defense mechanisms against tumors, acting as tumor suppressors that are selected based on the type of tissue or cancer. The upregulation of ACSL4 might compensate for the downregulation of PPAR-γ by ensuring a continued supply of activated fatty acids for essential cellular functions. This compensation could help maintain lipid metabolic processes critical for cancer cell survival and growth. With reduced PPAR-γ activity, cancer cells might become more reliant on ACSL4 to maintain lipid homeostasis and adapt to metabolic stress. This dependency could be a potential target for therapeutic intervention. Overall, the differential expression of PPAR-α and PPAR-γ in BC, along with their significant correlations with ACSL4 and FASN, highlights a complex regulatory network that influences cancer cell metabolism, growth, and survival. Assessing the expression levels of PPAR-α, PPAR-γ, FASN, and ACSL4 provides insights into the lipid metabolic profile of tumors. High FASN and ACSL4 expression, combined with low PPAR-γ expression, may reflect early metabolic reprogramming and could serve as a potential early detection marker. Therapeutically, targeting PPAR-α to disrupt its metabolic support to cancer cells, together with strategies aimed at restoring PPAR-γ expression, may offer a novel approach for BC treatment. Additionally, modulating ACSL4 activity may introduce another therapeutic layer by exploiting cancer cells' susceptibility to ferroptosis.

It is important to note that the therapeutic implications discussed are speculative and based on observed associations in a limited sample size (n = 28). Patient heterogeneity, tumor subtype differences, and other potential confounding clinical variables were not fully accounted for in this study. Therefore, to enhance the validity and generalizability of these findings, future investigations involving larger and more diverse patient cohorts are necessary to clarify the precise roles of PPAR-α and PPAR-γ in metabolic reprogramming and cancer progression, as well as to further explore the dual role of PPAR-α in BC.

## CONCLUSIONS

In conclusion, the differential expression of PPAR-α and PPAR-γ in breast cancer tissues compared to adjacent normal tissues suggests that these nuclear receptors may play a role in metabolic reprogramming during tumor progression. The observed associations between PPARs and key lipid metabolism-related genes such as FASN and ACSL4 may be a result of a potential regulatory network influencing lipid homeostasis and ferroptosis susceptibility. While these findings provide preliminary insight into the involvement of PPAR pathways in BC, further studies with larger cohorts and mechanistic experiments are necessary to validate these associations and explore their therapeutic relevance.

### *Acknowledgments*

This work was financially supported by Isfahan University of Medical Sciences, I.R. Iran (Grant No. 3402748), and the National Institute for Medical Research Development (NIMAD) Grant No. 958086.

### *Conflict of interest statement*

The authors declared no conflict of interest in this study.

### *Authors' contributions*

M. Pourfarzam conceived and supervised the study. M. Pourfarzam and N. Dinarvand designed experiments. P. Kiani performed experiments. N. Dinarvand and P. Kiani analyzed the data and wrote the first draft of the paper. All authors have read and approved the finalized article. Each author has fulfilled the authorship criteria and affirmed that this article represents honest and original work.

### *Data availability*

Additional data supporting the findings of this study are available upon reasonable request from the corresponding author.

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