Expressions and Prognostic values of LPARs in human breast cancer

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Research Article

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Expressions and Prognostic values of LPARs in human breast cancer

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ABSTRACT

Background: LPA and its receptors play a major role in adjusting malignant behaviors in breast cancer (BC). Abnormal expression of certain LPA receptors in BC indicate that LPA receptors could be novel potential biomarkers in predicting prognosis and progression of BC. Further studies would focus on molecular mechanisms of LPA receptors in BC.

Results: In this study, we examined the transcription and survival data of BC patient LPARs from ONCOMINE, Kaplan-Meier plotter, GEPIA, bcGenEx-Miner and cBioPortal database. We revealed that LPAR2/3/5 expression levels in BC tissue were higher than that in normal breast tissue, whereas the expression levels of LPAR1/4/6 in BC tissue were lower than normal breast tissue. The expression levels of LPAR1/4/6 were associated with advanced-stage tumor. Survival analysis using the K-M plotter database showed that in all BC patients, high mRNA expression of

LPAR1/4/5/6 and the low mRNA expression of LPAR2/3 were correlated with the improved outcomes of BC patients. Subgroup analyses based on clinicopathological factors further revealed relationship between the expression levels of LPARs and the prognosis of BC patients with different types.

Conclusions: This study shows that LPAR2/3/5 are potential targets for precision treatment of BC patients, and six LPARs are new biomarkers for the prognosis of BC patients.

INTRODUCTION

Lysophosphatidic acid (LPA, 1-acyl-2-hemolyticsn-glycerin-3-phosphate) is a small glycerophosphatidic acid, naturally occurring lysophospholipid (LP), widely exists in human body[1]. lysophosphoslipids (LPS, LPE, and LPC) are hydrolyzed by autotaxin (ATX) to produce LPA in plasma, serum and adipocytes. It activates diverse cellular actions, such as promoting cell growth, differentiation, migration, division and survival on many cell types[2, 3]. LPA performs a wide range of biological functions by combining with a multiple of known G protein-coupled receptors (GPCRs)[4]. G-protein coupled receptors currently named lysophosphatidic acid receptors (LPARs), can be subdivided into six types, LPAR1-6, according to their homology. LPAR1-6 can be divided into two main subfamilies with obvious difference - endothelial differentiation gene family (LPAR1–3) and purinergic receptor family (LPAR4–6)[5]. LPAR1-3 belongs to the endothelium differentiation gene (EDG) receptor, meanwhile LPAR4-6 is a non EDG receptor[6, 7]. LPA receptors have seven transmembrane domains, three intracellular loops (intracellular loop-ICL1, ICL2 , ICL3) and three extracellular loops (extracellular loop-ECL1, ECL2, ECL3)[8, 9]. At least three kinds of G proteins, Now it's clear that the LPA receptors signal pathway involved at least two Gα subunits (Gαq/11，Gα12/13, Gαi/o and GαS) activating different downstream pathways, produce different results under different environments and cell types[10]. Gαq/11,Gα12/13, Gαi/o and GαS, activate multiple downstream signaling pathways: phospholipase C pathway, RhoA pathway, Ras-Raf-MEK-ERK

pathway, PI3K/PAK1/ERK pathway and Rac pathway[11]. Six LPA receptors have similar biological functions due to their similar G protein types[12]. Many research have revealed the key role of LPA and its receptors in various cancer tissues, such as lung cancer, breast cancer, pancreatic cancer, liver cancer, ovarian cancer, neuroblastoma, and thyroid cancer[12, 13].

Breast cancer (BC) is the most common malignant tumor in females and still a main cause of cancer-related mortality for females worldwide, especially in underdeveloped countries[14]. To date, the risk factors for BC are still complex, uncertain and heterogeneous factors involving reproduction, hormones overweight, physical inactivity, menopausal hormone therapy and alcohol intake[15]. The morbidity and mortality of BC remains high. Although improved diagnostic methods, advanced surgical techniques, as well as more and more anti-cancer drugs and targeted therapies have improved the clinical outcome of BC to a large extent, the recurrence or metastasis still often occurs and it is not optimistic of the long-term survival of patients with BC[16]. For this reason, further research is warranted to examining the underlying mechanism of occurrence and development of BC.

In BC, by the high expression of ATX and the combination with LPAR1-3, the autotaxin (ATX)-LPA axis promotes the proliferation, invasion and anti-apoptosis of BC cells, thereby causing breast inflammation and tumor formation[17, 18]. It activate LPA receptors, leading to the activation of downstream pathways ERK/MAPK ,PI3K/Akt and p38-PI3K , cytokines， wnt/β-catenin and estrogen receptor (ER) by the ATX-LPA axis [19]. The expression of LPA receptors has been proved relating with malignant behaviors of BC. Xu et al. suggest that LPA induced a temporary increase in cytosolic free Ca^{2+} in three BC cell lines[18]. Liu et al. have discovered that transgenic mice with high expression of ATX and LPAR1-3 was at a high risk to develop BC , and transgenic mice overexpressing LPAR3 was more easily metastasizes[20]. Our group has revealed that LPA promoted invasion and metastasis in a BC cell line(MCF-7), then, we knock down RhoA and found that LPA activated invasion of BC by RhoA/ROCK/MMP signaling [21].

Multiple research have proved the key role of LPA receptors in metastasis and invasion of BC [22]. However, there are few reports on the abnormal of LPARs expression levels and their relationship with clinicopathological characteristics and prognosis in BC. An deep-lying bioinformatics analysis related with LPARs and patients with BC is required [23]. Based on the analysis of thousands of gene expression or copy number variation published online, we analyzed the expression levels and mutation of different LPARs in BC patients to determine the mechanisms of LPARs in human BC. Although these data indicate that LPARs can be used as reliable markers of human BC, detailed molecular mechanisms of LPARs remains unclear. Therefore, the mechanism and clinical features of all six LPARs needs further investigated.

RESULTS

Transcriptional levels of LPARs in patients with BC

We used ONCOMINE databases to compare the mRNA expression levels of six LPARs in cancers with those in normal samples by (Figure 1). The expression levels of LPAR2, LPAR3, and LPAR5 were upregulated in patients with BC compared with normal breast tissues. In contrast, the expression of LPAR1, LPAR4, and LPAR6 were downregulated in BC patients compared with normal. The mRNA expression of LPAR1 in BC is lower than normal tissues in four datasets: In Richardson dataset, Ductal BC with a fold change of -3.074, and Invasive Ductal BC with a fold change of -2.270 in TCGA dataset. A similar trend is showed in LPAR1 in Curtis's dataset. The expression LPAR2 was higher in Invasive Lobular BC than in the normal samples with a fold change of 1.791 and in Invasive BC with a fold change of 1.756 in TCGA dataset [25]. The similar trend could be seen in Mixed Lobular And Ductal Breast and Medullary BC. Finak's dataset showed that LPAR3 has a fold change of 2.412 in patients with Invasive BC Stroma compared with that in patients with normal breast tissues (Table 1). In TCGA dataset and Curtis dataset, the transcription levels of LPAR4 in Invasive Ductal

BC are lower than those in breast normal tissues, and their fold changes are -1.059 and -1.067, respectively. LPAR5 is significantly upregulated in invasive BC and Invasive Lobular BC, with fold changes of 2.719 and 2.004 a in Finak's dataset and TCGA dataset, respectively (Table 1). And in TCGA dataset, LPAR5 is also overexpressed with a fold change of 2.337 in Mucinous BC than that in normal breast tissues. A similar trend can be seen in LPAR5 in Medullary BC. The mRNA expression of LPAR6 in Invasive Ductal BC and Invasive Lobular BC were significantly lower than that in normal breast tissues , with a fold change of -2.328， -3.812 in Ma' dataset , and a fold change of -3.316 , -2.376，-2.10 in TCGA. A similar trend is showed in LPAR6 in Richard 's dataset.

Relationship between the mRNA levels of LPARs and the clinicopathological characteristics of BC patients

We compared the mRNA expression of LPARs between BC and breast tissues by GEPIA (Gene Expression Profiling Interactive Analysis) dataset (http://gepia. cancer-pku.cn/). Findings suggest that the mRNA expression levels of LPAR2, LPAR3, LPAR5 were higher in whereas and the expression levels of LPAR1, LPAR4, LPAR6 were lower BC tissues than in normal breast tissues (Figure 2). Then e analyzed LPARs expression with BC stage. We found that LPAR1, LPAR4, and LPAR6 groups significantly varied, whereas LPAR2, LPAR3 and LPAR5 groups were no significant difference (Figure 3).

Relationship between the transcriptional levels of LPARs and clinical and molecular characteristics of patients with BC

We used the Welch's tests to evaluated the varied expression levels of LPARs with different clinical and molecular criteria for BC patients in the bcGenExMiner v4.1. For the criterion of age, these

findings suggest that，between >51 years old and ≤51 years old groups， upregulated expression of LPAR6 and downregulated expression of LPAR2, LPAR4 in >51 years old group ， whereas upregulated expression of LPAR3 and downregulated expression of LPAR5 was found in ≤51 years old group. there was no significant difference existed between >51 years and old ≤51 years old groups of LPAR1 expression. Positive nodal patients with BC showed higher mRNA expression level of LPAR2 than negative nodal patients, whereas the expression of LPAR6 was higher in negative nodal patients than in positive nodal status(Table 2). LPAR1, LPAR3, LPAR4 and LPAR5 was no significant difference between two groups.

ER status in BC patients showed a significant associated with the expression of LPARs : the expression of LPAR1, LPAR4,LPAR6 were downregulated in ER-negatived groups, whereas the expression of LPAR2, LPAR3 and LPAR5 were upregulated in ER-negative groups compared with ER-positive groups. A similar trend is showed with progesterone receptor(PR) status in BC patients: mRNA levels of LPAR1, LPAR4, LPAR6 were downregulated in PR-negatived groups, whereas mRNA levels of LPAR2, LPAR3 and were upregulated in PR-negatived groups. But between two groups, mRNA levels of LPAR5 did not show a significant difference. In HER2-positive groups of BC patients, LPAR2, LPAR3 expressions were higher than that in HER2-negative groups, but the expression levels of LPAR1 were significantly downregulated compared with HER2-negative groups. The expression levels of LPAR4, LPAR5 and LPAR6 did not show a significant difference between two groups. TNBC is the worst clinical outcome type of BC. The expression levels of LPAR2, and LPAR3 were strongly higher in TNBC patients, whereas LPAR1, LPAR4 and LPAR6 were downregulated expression in TNBC patients (Table 2). The mRNA expression of six LPARs was correlated with Scarff Bloom & Richardson (SBR) grade status (Figure 4).

Association of the increased mRNA expression of LPAR1/4/5/6 and the decreased mRNA expression of LPAR2/3 with the improved prognosis of BC patients

Our research further used publicly available datasets in KM Plotter tools to analyze the relationship between the expression levels of LPARs and the survival of BC patients in 6234 BCs (https://kmplot.com/analysis/index.php?p=service&cancer=breast). The desired Affymetrix IDs were as follows: 204036_at, LPAR1; 206723_s_at, LPAR2; 231192_at, LPAR3; 206960_at, LPAR4(P2Y9);230252_at, LPAR5; 218589_at, LPAR6. The KM curve and log-rank test analyses indicated that the increased LPAR1 and LPAR6 mRNA levels and the decreased LPAR2 mRNA levels were significantly correlated with the high overall survival (OS), high progression-free survival (RFS), and high distant metastasis-free survival(DMFS) (p<0.05) of all of the patients with BC, But show no significant correlation with the post-progression survival (PPS) ($p > 0.05$) (Figure 5) . The high expression of LPAR3 was significantly related with reduced PPS and DMFS rates for patients with BC (<0.05) (Figure 5) , whereas was no strongly correlation with OS and RFS rates in patients with BC ($p > 0.05$). High expression of LPAR4 was significantly correlated with improved RFS and DMFS rates (p<0.05), but no significant correlation with OS and DMFS ($p > 0.05$). Increased LPAR5 was significantly correlated with increased OS, RFS and PPS rates ($p < 0.05$), except DMFS rate ($p > 0.05$). All in all, The BC patients with high mRNA expression levels of the LPAR1/4/5/6 or low mRNA expression levels of LPAR2/3 were predicted to have a better prognosis.

Association of the expression of LPARs and OS rates in BC patients with different types.

We used the KM plotter to explore the relationship between LPARs and OS rates BC patients with different types. The desired Affymetrix IDs were as follows: 204036_at, LPAR1; 206723_s_at, LPAR2; 231192_at, LPAR3; 206960_at, LPAR4(P2Y9);230252_at, LPAR5; 218589_at, LPAR6. We based on clinicopathological characteristics, including ER, PR, HER-2 and lymph node status to

examining the prognostic values of LPARs in BC patients (Table 3). The results revealed that the high mRNA expression levels of LPAR1 and LPAR6 indicated improved OS rates in ER-positive patients($P<0.05$), meanwhile the overexpression of LPAR2 showed poor OS in this subgroup. Notably, high mRNA expression levels of LPAR5 and LPAR6 were significantly related with increased OS rates in ER-negatived patients($P<0.05$). Information of LPAR1, LPAR3 and LPAR5 with PR was limited to explored, so analysis of the prognostic significance of LPAR1,LPAR3,LPAR5 stratifying by PR status was not conducted. In the HER-2-positive subgroup, the high expression of LPAR2 and LPAR5 were marginally associated with increased OS rate ($P<0.05$).And in the HER-2-negative subgroup ,the high expression of LPAR5 was significantly associated with increased OS rate($P<0.05$). In addition, in lymph node-positive patients, the high expression of LPAR1, LPAR5 and LPAR6 were also significantly associated with improved OS rates ($P<0.05$). The high expression of LPAR6 was significantly associated with increased OS rate in lymph node-negative patients too. whereas lymph node-negative patients with the increased mRNA levels of LPAR3 showed poor OS.

Association of the mRNA expression of LPARs and RFS rates in BC patients with different types.

The association between LPARs and RFS rates BC patients with different types was determined by the KM plotter. The desired Affymetrix IDs were as follows: 204036_at, LPAR1; 206723_s_at, LPAR2; 231192_at, LPAR3; 206960_at, LPAR4(P2Y9);230252_at, LPAR5; 218589_at, LPAR6. Following this, we analyzed the prognostic values of LPARs in BC patients by stratifying patients into subgroups based on clinicopathological characteristics, including ER, PR, HER-2 and lymph node status (Table 4).In ER-positive BC patients, the expressions of LPAR1, LPAR4 and LPAR6 were significantly correlated with improved RFS rates(Table 3). By contrast, high mRNA levels of LPAR1 and LPAR4 predicted poor RFS in ER-negative patients. In patients with PR-positive BC, high mRNA levels of LPAR4, LPAR6 indicated high RFS, and the high expression of LPAR1 and LPAR6 showed

improved RFS in PR-negative patients (Table 4). With regards to HER-2 status , In the HER-2-positive subgroup, only the increased expression of LPAR5 was significantly correlated with reduced RFS rate. whereas, in the HER-2-negative subgroup ,the high mRNA levels of LPAR1, LPAR4 and LPAR6 indicated high RFS. All LPARs showed a significantly association with RFS rates in lymph node-positive BC patients, the high mRNA levels of LPAR1, LPAR4, LPAR5, LPAR6 were significantly correlated with improved RFS rates, by contrast, high expression levels of LPAR2, LPAR3 were associated with reduced RFS rates in BC patients with lymph node-positive. In lymph node-negative BC patients, high expression of LPAR3 and LPAR6 was determined to be associated with high RFS in this subgroup.

Association of the expression of LPARs and DMFS rates in BC patients with different types.

In patients with ER-positive BC, high mRNA expression of LPAR1, LPAR4 and LPAR6 were significantly associated with improved DMFS, whereas the high expression of LPAR2 showed poor DMFS in this subgroup. High mRNA expression of LPAR3 and LPAR5 was correlated with reduced DMFS rates in ER-negative patients (Table 5). Meanwhile, in ER-negative subgroup, high mRNA levels of LPAR4 showed significantly association with improved DMFS rate. Notably, the high mRNA levels of LPAR3 and LPAR5 was significantly correlated with an obviously reduced DMFS rate in patients with PR-positive BC .In PR-negative subgroup, only the increased mRNA expression of LPAR4 was associated with improved DMFS rates. High expression of LPAR3 demonstrated an obviously reduced DMFS rate for HER-2-positive patients and HER-2-negative patients, Notably, high mRNA levels of LPAR5 and LPAR6 was also correlated with poor DMFS in HER-2-positive patients. In HER-2-negative BC patients, high levels of LPAR1 and LPAR4 was significantly correlated with an improved DMFS rate .All LPARs showed a significantly association with DMFS rates in lymph node-positive BC patients, except LPAR5.the high expression of LPAR1, LPAR4, LPAR6 were significantly related with high DMFS, by contrast, high mRNA expression levels of LPAR2, LPAR3

were associated with reduced DMFS rates in lymph node-positive subgroup. And the high expression of LPAR1, LPAR4 and LPAR6 were also significantly correlated with an improved DMFS rate in lymph node-negative BC patients. But in lymph node-negative subgroup, high mRNA level of LPAR3 was significantly correlated with reduced DMFS rate.

Association of the expression of LPARs and PPS rates in BC patients with different types.

It was determined that high expression of LPAR2 and LPAR3 indicated reduced PPS rates in ER-positive BC patients (Table 6). On the contrary, high mRNA expression levels of LPAR1, LPA6 were significantly correlated with an improved PPS rate in ER-positive patients. In ER-negative patients, only the high level of LPAR6 showed a significant association with improved PPS rate. Because of limited number of patients, subgroup analysis in the PR-positive and PR-negative cohort was not conducted. In HER-2-positive BC patients, upregulated LPAR2 was significantly correlated with reduced PPS rate. However, high mRNA level of LPAR6 was also significantly correlated with reduced PPS rate in HER-2-positive subgroup. On the contrary, high mRNA expression level of LPAR5 was significantly correlated with an improved PPS rate in HER-2-negative patients. Furthermore, high PPS and lymph node-positive BC patients with elevated LPAR1 and LPAR6 had significantly relationship. Meanwhile, high expression of LPAR2 was significantly correlated with reduced PPS rate in lymph node-positive subgroup. However, no positive result was found in lymph node-negative BC patients (Table 6).

The correlations of each LPAR expression and LPARs alterations analysis in Breast invasive Carcinoma

We used the cBioPortal online tool to analyze the LPARs alterations, correlations in Breast Invasive Carcinoma (TCGA, Firehose Legacy, http://www.cbioportal.org/index.do?session_id=5a37ba8e498eb8b3d56242fb). LPARs were altered in

293 samples out of 1093 patients with BC (27.08%)，LPAR5 was altered in 8% of BC patients in this dataset. Multiple alterations were detected in 38 samples (3.48%) (Figure 6). Next, by using the cBioPortal online tool, our research explored the associations of each LPAR expression (RNA Seq V2 RSEM) for Breast Invasive Carcinoma (TCGA, Firehose Legacy), and included Pearson's correction. These findings suggested significant and positive associations in the following LPARs: LPAR1 with LPAR2, LPAR4, LPAR5 and LPAR6; LPAR2 with LPAR1, and LPAR5;LPAR4 with LPAR1 and LPAR5; LPAR5 with LPAR1, LPAR2, LPAR4 and LPAR6; LPAR6 with LPAR1 and LPAR5 (Figure 7). We next constructed the pathway for LPARs and the 10 most frequently altered neighbor genes. The findings showed that the mutation genes, including TP53, PIK3CA, HRNR, GATA3, TTN, CDH1, MUC16, DNMT3A, COL4A1 and LTBP2, were closely associated with LPARs alterations (Figure 7).

DISCUSSION

In recent years, mechanisms of LPARs in several cancers has been explored in some research[24, 25], nevertheless, bioinformatics analysis of BC has not yet been performed[26]. In this study, we firstly systematically explored the mRNA expression and prognostic (OS, RFS, DMFS and PPS) values of all six LPARs in human BC.

Among the LPARs, LPAR1 is the most studied in BC, expression and function of LPAR1 has been studied extensively in the BC. Some study observe overexpression of LPAR1 in BC cells. Horak et al. reveals that in Nm23-H1 negative mice, the high expression of LPAR1 enhance cell motility, then they suggest that Nm23-H1 may be a potential upregulator for LPAR1 [27]. The manipulation of LPAR1 level or function can change the survival and metastasis ability of BC cells in vivo and in vitro[28]. Some studies have also shown that LPAR1 promoting the metastatic ability of BC[29]. LPAR1 siRNA don't inhibited cancer cell metastasis, but also alters primary tumor size[30]. Overexpression of LPAR1 is significantly associated with positive lymph node and bone metastasis, implies that LPAR1 affects breast cancer progression[31]. However, some clinical studies indicate the expression of LPAR1

don’t show significantly change between normal and malignant breast tissues [32]. In our early research, LPAR1 expression levels show no significant difference between BC patients and normal tissues[33]. This inconsistency may result from tumor heterogeneity.

In our research, ONCOMINE datasets and Gepia datasets revealed the expression of LPAR1 in BC was lower than that in normal breast tissues, LPAR1 expression significantly varied with different tumor stage for BC. We found that the expression of LPAR1 was downregulated in ER-negatived groups compared with ER-positive groups. A similar trend was showed with PR status in BC patients. In HER2-positive groups, the transcription level of LPAR1 was significantly downregulated compared with HER2-negative groups. LPAR1 was also downregulated expression in TNBC patients. The high LPAR1 expression was significantly associated with improved OS, RFS, and DMFS in all BC patients. The subgroup analysis of the prognostic values for LPAR1 demonstrated that high expression of LPAR1 indicated improved OS, RFS, DMFS and PPS rates in ER-positive patients. Meanwhile, high mRNA levels of LPAR1 predicted poor RFS in ER-negative patients. In PR-negative patients, the high expression of LPAR1 showed improved RFS. In the HER-2-negative subgroup, the high mRNA levels of LPAR1 indicated high RFS and DMFS. In briefly, high expression of LPAR1 seemed to be associated with a good prognosis factors of BC, and overexpression of LPAR1 might bring better clinical outcomes for BC patients.

Although there are few studies on the expression and function of LPAR2 in BC, some reports confirm that the higher expression of LPAR2 in BC than in breast normal tissues[32]. Transgenic mice overexpressing LPA2 has a higher incidence of mammary tumors than mice overexpressing LPAR1 in the early stage, which suggests that LPAR2 was involved in the occurrence of BC[20]. Some studies confirm that LPAR2 regulated LPA-induced proliferation and migration of BC cells through the Erk or RhoA pathway in vitro[34, 35]. A literature also reports LPAR2 is significantly associated with LPA-induced IL-6 and IL-8 expression, which promoted TNBC progression[36]. Li et al. reveals that LPAR2 is higher expression in BC tissues than that in adjacent tissues, in particular in postmenopausal women.

This indicates that the overexpression of LPAR2 is significantly associated with postmenopausal breast canceration[37]. In our report, the expression of LPAR2 in BC tissues was higher than that in normal tissues. The expression of LPAR2 was lower in ≤51 years old than >51 years old groups. BC patients with positive nodal status showed higher mRNA level of LPAR2. The expression of LPAR2 was upregulated in ER/PR-negative and HER-2-positive groups compared with ER/PR-positive groups. The mRNA levels of LPAR2 was higher in TNBC patients. High expression of LPAR2 seemed to be associated with a poor prognosis factors of BC. High LPAR2 expression was significantly associated with poor OS, RFS, and DMFS in BC patients. The subgroup analysis of the prognostic values for LPAR2 showed that the overexpression of LPAR2 showed poor OS, DMFS and PPS in ER-positive patients. But in the HER-2-positive subgroup, the high expression of LPAR2 was marginally associated with increased OS rate and reduced PPS rate, this inconsistency may result from tumor heterogeneity. High expression levels of LPAR2 was significantly correlated with reduced RFS, DMFS and PPS rates in BC patients with lymph node-positive. All of them indicated that LPAR2 as a potential treatment target for BC, an BC patients with high expression of LPAR2 seemed to be higher degree of malignancy and poor prognosis .

Recent years some scholars focus on the expression and function patterns of LPAR3 in BCs. Liu et al. find that overexpression LPAR1-3 transgenic mice had an high risk of BC, while LPAR3 had the highest metastasis rate[20, 38]. Popnikolov et al. reveals LPA mediates tumor aggressiveness primarily through the LPAR3[39]. Nikolay et al. indicated that LPAR3 was overexpression in human BCs, and the overexpression of LPAR3 was related to absence of ER and PR[39]. Our early study found that LPAR3 could stimulate growing of TNBC cell lines and had dominant metastatic roles in TNBC[21, 33]. In this report, we demonstrated that the expression levels of LPAR3 was upregulated in patients with BC compared with that in patients with normal breast tissues, the mRNA expression levels of LPAR3 was upregulated in ≤51 years old group. The expression of LPAR3 was upregulated in ER/PR-negatived groups and HER-2-positve groups and was found to be significantly higher in TNBC patients, as the

expression trend of LPAR2. LPAR3 seemed to be associated with a poor prognosis factors of BC. The high expression levels of LPAR3 was strongly correlated with reduced PPS and DMFS rates for patients with BC. High mRNA expression of LPAR3 was strongly correlated with poor DMFS rates in ER-negative patients. Interestingly, the same trend was found in PR-positive BC. In ER-positive BC patients, high expression of LPAR3 indicated reduced PPS rates. These results implied that overexpression of LPAR3 predicted higher incidence of recurrence and metastasis, and worse prognosis. LPAR3 was also a potential treatment target for BC, and much further work is needed to clarify these issues.

LPAR4 is structurally more similar to P2Y purinergic receptors , but distinct from EDG family receptors (LPAR1, LPAR2, LPAR3)[40]. Some studies observe that LPAR4 can inhibits progression of human colon cancer and rat neuroblastoma [41, 42], indicate LPAR4 may inhibits cell migration and invasion. But studies on the expression and function of LPAR4 in BC are barely. In our research, we demonstrated that the mRNA expression levels of LPAR4 was downregulated in BC patients compared with normal. The expression of LPAR4 significantly varied with different tumor stage for BC. The expression of LPAR4 was downregulated in ER/PR-negatived groups and TNBC groups, as the expression trend of LPAR1. It seemed that, as LPAR1, LPAR4 was associated with good prognosis factors of BC. High expression of LPAR4 was determined to be significantly associated with improved RFS and DMFS. The overexpression of LPAR4 was significantly associated with improved RFS, DMFS rates in ER/PR-positive and HER-2-negative BC patients. Interestingly, high mRNA levels of LPAR4 predicted poor RFS in ER-negative patients. These results implied that high expression of LPAR4 in BC patients might have good clinical outcomes. The role of LPAR4 might as a tumor suppressor, same as LPAR1.

Few studies focused on the expression and function of LPAR5 in BC, however, its roles in other cancers have already been exploited. LPAR5 is validated to be negatively in cancer progressions[43]. Ishii et al. suggests PNAC-sh4 and PNAC-sh5 stimulating migration, invasion is associate with LPAR5

negatively promoted cancer progression [44]. LPAR5 is also found inhibiting invasion in human sarcoma and melanomas cells[45-47]. But in this study, we determined that the mRNA expression levels of LPAR5 was upregulated in patients with BC compared with normal breast tissues. The expression of LPAR5 was downregulated in ≤51 years old group, and was upregulated in ER-negatived groups. Although LPAR5 was upregulated in patients with BC, increased LPAR5 was significantly associated with increased OS, RFS and PPS rates, except DMFS rate. In particular, high expression of LPAR5 was significantly correlated with improved OS rates in ER-negative BC patients. High expression level of LPAR5 was significantly correlated with an improved PPS rate in HER-2-negative patients. In addition, in lymph node-positive patients, the high expression of LPAR5 was also significantly associated with improved OS and RFS rates. But in the HER-2-positive and PR-positive subgroup, the increased expression of LPAR5 was significantly related with reduced RFS and DMFS rate. These results implied that although increased LPAR5 was characterized by a relatively good prognosis, overexpression of LPAR5 still seemed to be associated with poor prognosis factors of BC. This inconsistency needs more work to be carried out in future.

The relationship of expression of LPAR6 and cancer progressions is unclear. But in recent years , Tao et al. indicate that LPAR6 might have a role in inhibiting BC development[48]. They suggests that the expression of LPAR6 in BC is lower than that in breast normal tissues, tumor cell proliferation and invasion is enhanced by knockdown of LPAR6[48]. In our research, we determined that the mRNA expression levels of LPAR6 was downregulated in BC patients compared with normal. The expression of LPAR6 significantly varied with different tumor stage for BC. The expression of LPAR6 was higher in negative nodal patients than in positive nodal status. The expression of LPAR6 was downregulated in ER/PR-negatived groups and TNBC groups. It indicated that LPAR6 was associated with good prognosis factors of BC. The KM curve and log-rank test analyses indicated that the high mRNA level of LPAR6 was significantly correlated with the high OS, RFS and DMFS of all of the patients with BC. In ER-positive BC patients, increased LPAR6 was significantly correlated with improved RFS and

DMF rates. Interestingly, in the HER-2-positive subgroup increased LPAR6 not only brought reduced DMFS and PPS rates, but also improved RFS rates. Moreover, we found a significant association between high PPS and patients with lymph node-positive BC with elevated LPAR6. In briefly, As LPAR1 and LPAR4, LPAR6 was a tumor suppressor gene in BC patients, high expression of LPAR6 seemed to be associated with a good prognosis factors of BC, and overexpression of LPAR6 might bring better clinical outcomes for BC patients.

In summary, it was concluded that mRNA expression levels of LPAR2, LPAR3 and LPAR5 were notably upregulated in BC, while the mRNA expression of LPAR1, LPAR4 and LPAR6 were downregulated in BC, compared with normal tissues. Furthermore, the increased mRNA expression of LPAR1/4/5/6 were significantly associated with improved prognosis of patients with BC, and high expression of LPAR1/4/6 seemed to be associated with a good prognosis factors of BC, as tumor suppressor gene. Meanwhile, the increased mRNA expression of LPAR2/3 were significantly associated with poor prognosis and poor prognosis factors of BC patients as oncogene. These results provided a better insight into the prognostic functions of LPARs mRNA genes in BC. Further research basing on LPARs molecular mechanisms might contribute to new methods of malignant BC diagnosis and treatment.

MATERIALS AND METHODS

ONCOMINE analysis

The online cancer microarray database ONCOMINE gene expression array dataset (www.oncomine.org) is used to analyze the transcription levels of LPARs in different cancers. The Student's t test was used to compare the mRNA expression of LPARs in breast cancer specimens and that in normal breast specimens, *p* value for difference. The cut-off of *p* value was defined as 0.05, and fold change was defined as 1.5.

GEPIA (Gene Expression Profiling Interactive Analysis) dataset

GEPIA is used to analyze the RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from TCGA and GTEx projects via standard processing pipelines. GEPIA provides customizable functions, such as tumor/normal differential expression analysis, profiling according to cancer type or pathological stages, correlation analysis, similar gene detection, dimensionality reduction analysis and patient survival analysis[49].

The BC Gene-expression Miner v4.1

The breast Cancer Gen-expression Miner v4.1 (bcGenExMiner v4.1) is a statistical mining tool that contains 36 published annotated genome data sets (5,861 patients in total), and has a statistical analysis function[50]. The expression module allows to compare the expression of candidate genes according to several clinical criterias (such as age, ER status, PR status, HER2 status, lymph node status, etc.). The prognostic module evaluates the prognostic value of candidate genes in human BC.

The Kaplan-Meier plotter

Kaplan-Meier plotter (KM plotter; http://kmplot.com/analysis/) is used to determine the prognostic values of LPARs in BC[51]. KM plotter is an online database, which containing microarray gene expression data and survival information derived from TCGA, Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/), and European Genome-Phenome Archive (https://ega.crg.eu/) containing a total of 6,234 patients with BC with survival data[52]. For each gene symbol, determine the required probe ID was determined according to the probe set files provided by the KM plotter[53]. According to the median mRNA expression level, patients were divided into high expression groups and low expression groups, and survival analysis was performed without follow-up restrictions. The desired probe IDs representing six genes were separately entered into the database to perform Kaplan-Meier survival analysis for OS, RFS, DMFS and PPS. Subgroup analysis was performed by separating

patients according to the following expression factors: ER, PR, HER-2 and lymph node status. Factors were defined as positive or negative. The number of cases, HRs, 95% CIs and log rank P-values were obtained from KM Plotter.

TCGA data and cBioPortal

The cBioPortal for Cancer Genomics provides analysis, visualization, and downloading of cancer genomics datasets[49]. By using the cBioPortal for Cancer Genomics (www.cbioportal.org), the BC dataset (TCGA, Firehose Legacy), which contains including histopathological data of 1,093 BC patients, was selected for LPARs analysis. The genomic profiles included mutations, mRNA expression z-scores (RNA Seq V2 RSEM), protein expression Z-scores (RPPA) and putative copy-number alterations (CNA) from GISTIC. Co-expression and pathway were calculated according to the online instructions of cBioPortal.

DECLARATIONS

Conflicts of interest

The authors declare that they have no competing interests.

Author contributions

K.S and T.W performed the analysis of the data. K.S and Z.L wrote the manuscript. J.L and R.C designed the study. All authors read and approved the manuscript.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable

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Availability of data and materials

All the datasets were retrieved from the publishing literature, so it was confirmed that all written informed consent was obtained. The online cancer microarray database ONCOMINE gene expression array dataset (www.oncomine.org) is used to analyze the transcription levels of LPARs in different cancers and with the accession numbers listed in Table 1. GEPIA (http://gepia.cancer-pku.cn/detail.php) is used to analyze the RNA sequencing expression data of LPARS from TCGA and GTEx projects via standard processing pipelines. Kaplan-Meier plotter (KM plotter; http://kmplot.com/analysis/) is used to determine the prognostic values of LPARs in BC. By using the cBioPortal for Cancer Genomics (www.cbioportal.org), the BC dataset (TCGA, Firehose Legacy), which contains including histopathological data of 1,093 BC patients, was selected for LPARs analysis.

Abbreviations

ATX: autotaxin; BC: breast cancer; Cis: confidence intervals; CNA: copy-number alterations; DMFS: distant metastasis-free survival; ECL: extracellular loop; EDG: endothelial differentiation gene; EGFR, Epidermal growth factor receptor; ER: Estrogen receptor; ERK: extracellular regulated protein kinases; ERK: extracellular regulated protein kinases; GEO: Gene Expression Omnibus; GEPIA: Gene Expression Profiling Interactive Analysis; GPCRs: G-protein coupled receptors; HER-2: human epidermal growth factor 2; HR, Hazard ratio; ICL: intracellular loop; KM plotter, Kaplan-Meier plotter; LP: lysophospholipid; LPA: lysophosphatidic acid; LPAR1-6: lysophosphatidic acid receptor1-6; MAPK: mitogen-activated protein kinase; OS, Overall survival; PI3K: phosphatidylinositol 3-kinase; PR: progesterone receptor; PPS: post-progression survival; RFS: progression-free survival; SBR: Scarff Bloom & Richardson; siRNA: Small interfering RNA; TNBC: triple-negative breast cancer.

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Table 1. transcription expression levels of LPARs between different types of BC and breast tissues (ONCOMINE database).

	Types of BC vs. Breast	Fold change	P value	t-test	Ref	Accession Number
LPA1	Ductal BC vs. Normal	-3.074	8.33E-7	-6.086	Richardson	AW269335
	Invasive Ductal BC vs. Normal	-2.270	5.98E-29	-14.405	TCGA	BC030615
	Medullary BC vs. Normal	-2.317	1.27E-9	--7.909	Curtis	NM_057159
	Mucinous BC vs. Normal	-2.101	7.90E-13	-9.037	Curtis	NM_057159
LPA2	Invasive Lobular BC vs.Normal	1.791	7.68E-9	6.202	TCGA	AF011466
	Invasive BC vs.Normal	1.756	1.07E-11	7.566	TCGA	AF011466
	Mixed Lobular And Ductal BC vs.Normal	1.889	3.13E-9	7.767	TCGA	AF011466
	Medullary BC vs.Normal	1.619	1.26E-7	6.174	TCGA	NM_004720
LPA3	Invasive BC Stroma vs.Normal	2.412	1.23E-7	8.441	Finak	NM_012152
LPA4	Invasive Ductal BC Stroma vs. Normal	-1.775	0.002	-3.161	MA	NM_005296
	Male Breast Carcinoma vs. Normal	-1.717	2.80E-5	-7.674	TCGA	NM_005296
LPA5	Mucinous BC vs.Normal	2.337	5.12E-5	9.772	TCGA	BC033571
	Invasive Lobular BC vs.Normal	2.004	1.83E-12	8.508	TCGA	BC033571
	Medullary BC vs.Normal	2.127	1.33E-11	9.446	Curtis	NM_020400
	Invasive BC Stroma vs.Normal	2.719	1.43E-16	14.515	Finak	NM_020400
LPA6	Invasive Ductal BC Stroma vs. Normal	-2.328	2.14E-5	-5.562	Ma	NM_005767
	Ductal BC in Situ Epithelia vs. Normal	-3.812	3.17E-5	-5.101	Ma	NM_005767
	Invasive Ductal BC vs. Normal	-3.316	1.00E-31	-18.350	TCGA	AF000546
	Invasive BC Stroma vs. Normal	-2.376	2.11E-21	-11.553	TCGA	AF000546
	Invasive Lobular BC vs.Normal	-2.104	1.16E-11	-7.823	TCGA	AF000546
	Ductal BC vs. Normal	-3.021	1.06E-7	-7.096	Richard	NM_005767

Table 2 The relationship between mRNA levels of LPARs and clinical and molecular criteria of BC patients

Criteria		LPAR1		LPAR2		LPAR3		LPAR4		LPAR5		LPAR6	
	No.	mRNA	P-value	mRNA	P-value	mRNA	P-value	mRNA	P-value	mRNA	P-value	mRNA	P-value
Age													
≤51	1099	–	0. 0715	–	<0. 0001	↑↑↑	<0. 0001	–	0. 0059	↓	<0. 0001	–	0.0127
>51	3208	–		↓		–		↓		–		↑	
Nodal status													
–	2415	–	0. 0561	–	0. 0180	–	0. 6281	–	0. 1828	–	0.6478	↑	0.0157
+	1646	–		↑		–		–		–		–	
ER (IHC)													
–	551	↓	<0. 0001	↑↑	<0. 0001	↑↑↑	<0. 0001	↓	<0.0001	↑	0.0198	↓	<0.0001
+	3911	–		–		–		–		–		–	
PR(IHC)													
–	828	↓	<0. 0001	↑↑	<0. 0001	↑↑↑	<0. 0001	↓	0. 0023	–	0.7104	↓	<0.0001
+	3498	–		–		–		–		–		–	
HER2 (IHC)													
–	3582	–	0. 0174	–	<0. 0001	–	0. 0078	–	0. 1391	–	0.4214	–	0.0027
+	661	↓		↑		↑		–		–		↓	
Triple-negative BC (TNBC)													
Not	4119	–	<0. 0001	–	<0. 0001	–	<0. 0001	–	<0. 0001	↑	0.1238	–	<0. 0001
TNBC	317	↓↓		↑↑		↑↑↑		↓		–		↓↓	

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Abbreviations: LPARs, lysophosphatidic acid receptors; BC, breast cancer; ER, estrogen receptor; PR, progesterone receptor; IHC, immunohistochemistry；TNBC，Triple-negative BC.

Table 3. The correlation between LPARs and OS for BC patients based on clinicopathological characteristics.

		Positive status			Negative status		
Clinicopathological factor	Gene symbol	Cases	HR (95% CI)	P-value	Cases	HR (95% CI)	P-value
ER	LPAR1	109	0.53 (0.37-0.76)	0.00047[a]	79	0.75 (0.44-1.29)	0.3
	LPAR2	548	1.8 (1.25-2.59)	0.0013 [a]	251	0.71 (0.44-1.15)	0.16
	LPAR3	109	2.02 (0.92-4.42)	0.073	79	0.57 (0.25-1.27)	0.16
	LPAR4	548	0.86 (0.59-1.25)	0.43	251	1.22(0.74-2.01)	0.43
	LPAR5	109	1.92 (0.66-5,54)	0.22	79	0.37 (0.18-0.76)	0.0047 [a]
	LPAR6	548	0.51 (0.35-0.72)	0.0014 [a]	251	0.59(0.37-0.95)	0.0027[a]
PR	LPAR1	N/A			N/A		
	LPAR2	83	0.23(0.03 -1.8)	0.12	89	2.64 (0.61-11.49)	0.18
	LPAR3	N/A			N/A		
	LPAR4	83	0.27 (0.07-1.09)	0.05[a]	89	2.1 (0.79-5.6)	0.13
	LPAR5	N/A			N/A		
	LPAR6	83	0.53 (0.11-2.57)	0.42	89	1.92 (0.72-5.12)	0.18
HER-2	LPAR1	26	1.43 (0.71-2.88)	0.31	130	1.48 (0.62-3.5)	0.37
	LPAR2	129	0.35 (0.14-0.85)	0.016[a]	130	0.58 (0.24-1.41)	0.22
	LPAR3	26	1.55 (0.47-5.13)	0.47	62	0.57 (0.18-1.83)	0.34
	LPAR4	129	0.81 (0.38-1.71)	0.58	130	2.53 (0.85-7.53)	0.084
	LPAR5	26	0.22 (0.06-0.77)	0.0095 [a]	62	0.27 (0.1-0.77)	0.0089 [a]
	LPAR6	129	1.78 (0.87-3.65)	0.11	130	0.35 (0.1-1.2)	0.082
Lymph node	LPAR1	177	0.54 (0.36-0.8)	0.0019 [a]	122	0.73 (0.49-1.09)	0.12
	LPAR2	313	1.3 (0.87-1.96)	0.2	594	1.34(0.92-1.96)	0.13
	LPAR3	177	1.4 (0.89-2.2)	0.14	122	3.75 (1.4-10.02)	0.0005 [a]
	LPAR4	313	0.67 (0.45-1.02)	0.059	594	1.35 (0.91-2.01)	0.14
	LPAR5	177	0.55 (0.32-0.94)	0.028 [a]	122	0.47 (0.19-1.17)	0.097
	LPAR6	313	0.42 (0.28-0.61)	5.1E-06 [a]	594	0.62 (0.43-0.9)	0.01 [a]

[a]P<0.05. HR, hazard radio; CI, confidence interval; N/A, not available; HER-2, human epidermal growth factor; ER, estrogen receptor; PR, progesterone receptor.

Table 4. The correlation between LPARs and RFS for BC patients based on clinicopathological characteristics.

		Positive status			Negative status		
Clinicopathological factor	Gene symbol	Cases	HR (95% CI)	P-value	Cases	HR (95% CI)	P-value
ER	LPAR1	762	0.72 (0.61-0.86)	0.00025[a]	347	1.32 (1.03-1.7)	0.029[a]
	LPAR2	2061	1.13 (0.96-1.33)	0.15	801	0.81 (0.65-1.02)	0.07
	LPAR3	762	0.8 (0.6-1.07)	0.13	347	1.24 (0.84-1.83)	0.28
	LPAR4	2061	0.75 (0.61-0.92)	0.0047[a]	801	1.28(1.02-1.61)	0.033[a]
	LPAR5	762	1.19 (0.88-1.61)	0.25	347	0.72 (0.51-1.02)	0.062
	LPAR6	2061	0.7 (0.59-0.82)	1.9E-0.5[a]	801	0.8(0.62-1.03)	0.083
PR	LPAR1	489	0.59 (0.4-0.86)	0.059	372	0.66 (0.49-0.88)	0.0048[a]
	LPAR2	589	1.38(0.97 -1.98)	0.075	549	1.18(0.89-1.58)	0.25
	LPAR3	489	0.81(0.55-1.19)	0.28	372	1.3(0.89-1.91)	0.18
	LPAR4	589	0.6 (0.41-0.87)	0.0074[a]	549	1.16(0.86-1.57)	0.32
	LPAR5	489	1.26 (0.86-1.85)	0.24	372	0.8(0.54-1.17)	0.24
	LPAR6	589	0.62(0.43-0.88)	0.0074[a]	549	0.66 (0.49-0.89)	0.0054[a]
HER-2	LPAR1	150	0.74 (0.48-1.15)	0.18	635	0.62 (0.47-0.82)	0.00073[a]
	LPAR2	252	0.7 (0.43-1.14)	0.15	800	1.26(0.95-1.69)	0.11
	LPAR3	150	1.77 (0.97-3.23)	0.058	635	1.17 (0.87-1.58)	0.28
	LPAR4	252	1.32 (0.85-2.06)	0.22	800	0.7 (0.53-0.92)	0.011[a]
	LPAR5	150	3.02 (1.71-5.35)	6.3E-0.5[a]	635	0.87 (0.63-1.21)	0.41
	LPAR6	252	0.72 (0.45-1.14)	0.16	800	0.59 (0.45-0.77)	0.00012[a]
Lymph node	LPAR1	724	0.68 (0.56-0.83)	0.00012[a]	496	0.76 (0.64-0.91)	0.0024[a]
	LPAR2	1133	1.35 (1.1-1.66)	0.004[a]	2020	0.92(0.77-1.09)	0.31
	LPAR3	724	1.3 (1-1.69)	0.049[a]	496	1.3 (0.88-1.93)	0.19
	LPAR4	1133	0.79 (0.64-0.96)	0.016[a]	2020	1.12 (0.94-1.32)	0.2
	LPAR5	724	0.69 (0.52-0.91)	0.0084[a]	496	0.82 (0.54-1.23)	0.32
	LPAR6	1133	0.64 (0.52-0.78)	8.5E-06[a]	2020	0.66 (0.55-0.79)	5.9E-06[a]

[a]P<0.05. HR, hazard radio; CI, confidence interval; N/A, not available; HER-2, human epidermal growth factor; ER, estrogen receptor; PR, progesterone receptor.

Table 5. The correlation between LPARs and DMFS for BC patients based on clinicopathological characteristics.

		Positive status			Negative status		
Clinicopathological factor	Gene symbol	Cases	HR (95% CI)	P-value	Cases	HR (95% CI)	P-value
ER	LPAR1	161	0.58 (0.41-0.82)	0.002[a]	68	1.73(0.95-3.16)	0.07
	LPAR2	664	1.86 (1.32-2.62)	0.00029[a]	218	0.68 (0.41-1.11)	0.12
	LPAR3	161	2.72 (0.62-11.82)	0.16	68	4.02 (0.94-17.23)	0.042[a]
	LPAR4	664	0.66 (0.45-0.95)	0.024[a]	218	0.6(0.38-0.97)	0.036[a]
	LPAR5	161	1.58(0.61-4.12)	0.34	68	3.08(1.33-7.16)	0.0058[a]
	LPAR6	664	0.56(0.4-0.78)	0.00058[a]	218	1.34(0.78-2.32)	0.29
PR	LPAR1	122	0.51 (0.22-1.22)	0.12	95	0.65 (0.36-1.17)	0.15
	LPAR2	192	2.1(0.9 -4.89)	0.08	154	0.63 (0.33-1.17)	0.14
	LPAR3	122	3.52(1.03-12.01)	0.032[a]	95	2.08(0.88-4.91)	0.086
	LPAR4	192	0.56 (0.25-1.28)	0.16	154	0.53(0.29-0.95)	0.031[a]
	LPAR5	122	309890121 (0-INF)	0.048[a]	95	1.86 (0.88-3.93)	0.1
	LPAR6	192	0.56(0.24-1.29)	0.16	154	1.68(0.94-3.01)	0.078
HER-2	LPAR1	66	0.55(0.29-1.06)	0.07	82	0.28 (0.12-0.66)	0.0018[a]
	LPAR2	126	1.53 (0.76-3.08)	0.23	150	2.15 (0.72-6.38)	0.16
	LPAR3	66	5.23 (1.2-22.78)	0.0014[a]	82	6.72 (1.43-31.66)	0.0053[a]
	LPAR4	126	0.56 (0.3-1.08)	0.078	150	0.34 (0.14-0.8)	0.0092[a]
	LPAR5	66	4.7 (1.74-12.72)	0.00084[a]	82	2.26 (0.58-8.74)	0.22
	LPAR6	126	1.91 (0.99-3.66)	0.0048[a]	150	0.39 (0.13-1.16)	0.08
Lymph node	LPAR1	172	0.59 (0.4-0.87)	0.0075[a]	162	0.66 (0.49-0.88)	0.004[a]
	LPAR2	382	1.67(1.14-2.46)	0.0083[a]	988	1.33(1-1.77)	0.052
	LPAR3	172	1.99 (1.06-3.71)	0.028[a]	162	3.89 (1.14-13.24)	0.019[a]
	LPAR4	382	0.54 (0.36-0.82)	0.0034[a]	988	0.71 (0.5-1)	0.047[a]
	LPAR5	172	1.44 (0.76-2.76)	0.26	162	1.48(0.61-3.6)	0.39
	LPAR6	382	0.57 (0.39-0.85)	0.0052[a]	988	0.64 (0.49-0.84)	0.0011[a]

[a]P<0.05. HR, hazard radio; CI, confidence interval; N/A, not available; HER-2, human epidermal growth factor; ER, estrogen receptor; PR, progesterone receptor.

Table 6. The correlation between LPARs and PPS for BC patients based on clinicopathological characteristics.

		Positive status			Negative status		
Clinicopathological factor	Gene symbol	Cases	HR (95% CI)	P-value	Cases	HR (95% CI)	P-value
ER	LPAR1	32	0.58 (0.37-0.9)	0.015 [a]	41	0.64 (0.36-1.12)	0.12
	LPAR2	173	1.78 (1.16-2.72)	0.0075 [a]	100	0.72 (0.43-1.23)	0.23
	LPAR3	32	3.07 (1.22-7.7)	0.012 [a]	41	0.68(0.29-1.58)	0.37
	LPAR4	173	0.8 (0.53-1.21)	0.29	100	132(0.77-2.26)	0.31
	LPAR5	32	0.44 (0.18-1.08)	0.066	41	0.58 (0.26-1.3)	0.18
	LPAR6	173	0.61 (0.37-1)	0.049 [a]	100	0.56(0.33-0.97)	0.037 [a]
PR	LPAR1	N/A			N/A		
	LPAR2	N/A			N/A		
	LPAR3	N/A			N/A		
	LPAR4	N/A			N/A		
	LPAR5	N/A			N/A		
	LPAR6	N/A			N/A		
HER-2	LPAR1	15	2.21(0.83-5.86)	0.1	27	0.38 (0.11-1.33)	0.11
	LPAR2	33	3.1 (1.04-9.22)	0.034 [a]	39	2.06 (0.76-5.56)	0.15
	LPAR3	15	0.61 (0.15-2.46)	0.48	27	0.36 (0.1-1.24)	0.096
	LPAR4	33	1.59 (0.59-4.23)	0.35	39	0.58(0.2-1.68)	0.31
	LPAR5	15	0.32 (0.09-1.15)	0.065	27	0.22 (0.08-0.65)	0.0027 [a]
	LPAR6	33	4.67(1.74-12.55)	0.00087 [a]	39	2.04 (0.56-7.39)	0.27
Lymph node	LPAR1	72	0.47 (0.29-0.76)	0.0014 [a]	20	0.78 (0.49-1.24)	0.29
	LPAR2	128	1.67 (1.05-2.64)	0.028 [a]	165	0.68(0.43-1.09)	0.11
	LPAR3	72	1.26 (0.68-2.33)	0.47	20	2.88 (0.92-9.02)	0.058
	LPAR4	128	0.67 (0.41-1.1)	0.11	165	0.8 (0.52-1.24)	0.32
	LPAR5	72	0.57 (0.31-1.05)	0.067	20	0.61 (0.19-2.01)	0.42
	LPAR6	128	0.55 (0.35-0.86)	0.0084 [a]	165	0.69 (0.44-1.1)	0.11

[a]P<0.05. HR, hazard radio; CI, confidence interval; N/A, not available; HER-2, human epidermal growth factor; ER, estrogen receptor; PR, progesterone receptor

Figures

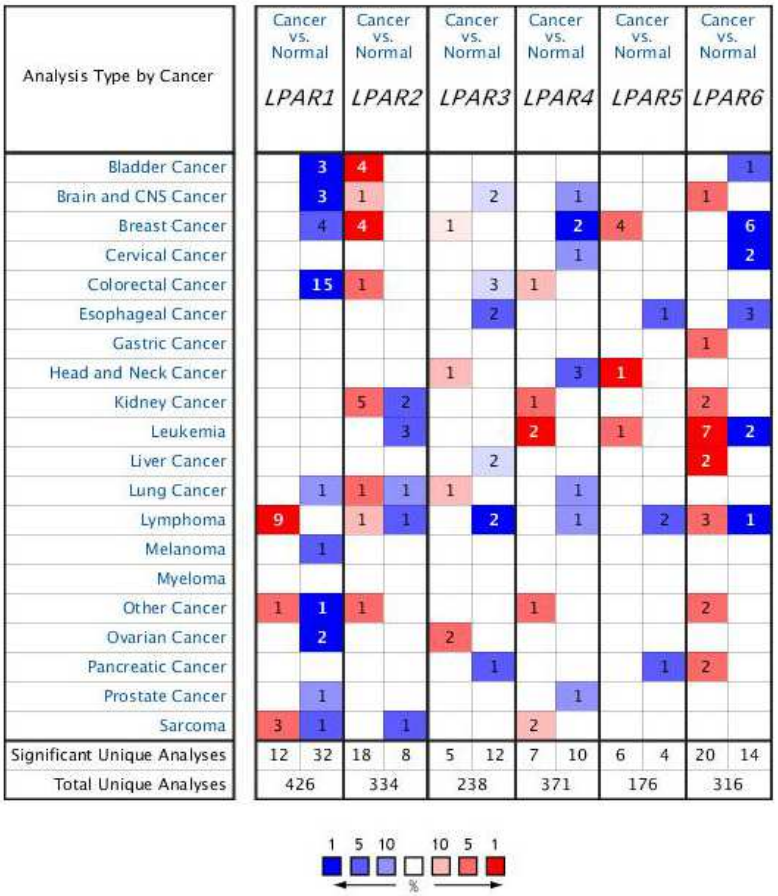

Figure 1. The transcription levels of LPARs in different types of cancers (ONCOMINE).

1

Figure 1

The transcription levels of LPARs in different types of cancers (ONCOMINE).

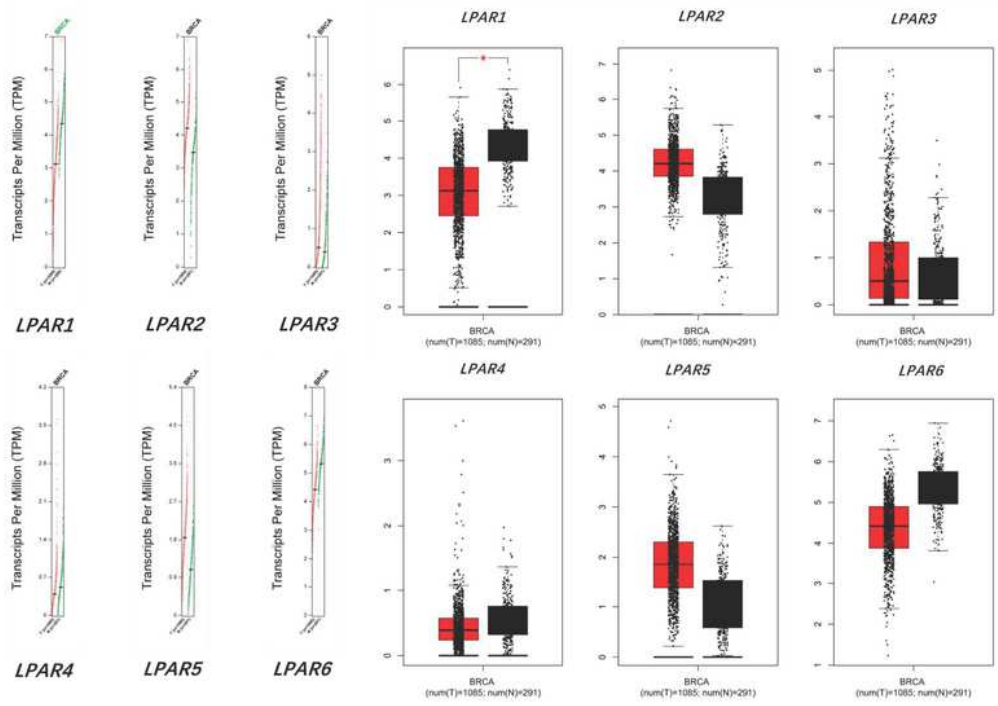

Figure 2. The expression of LPARs in BC (GEPIA).

Figure 2

The expression of LPARs in BC (GEPIA).

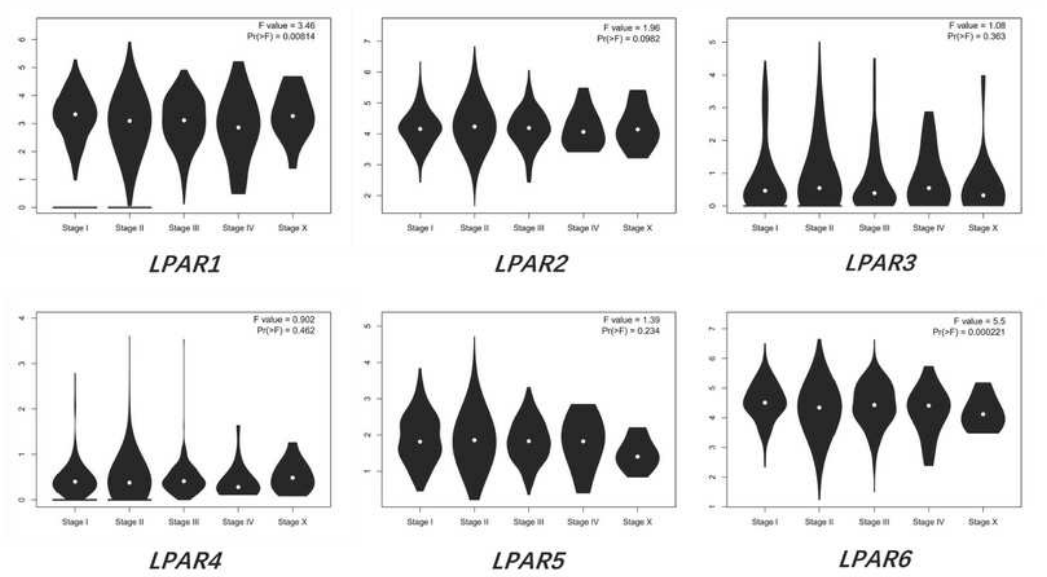

Figure 3. Correlation between LPARs expression and tumor stage in BC patients (GEPIA).

Figure 3

Correlation between LPARs expression and tumor stage in BC patients (GEPIA).

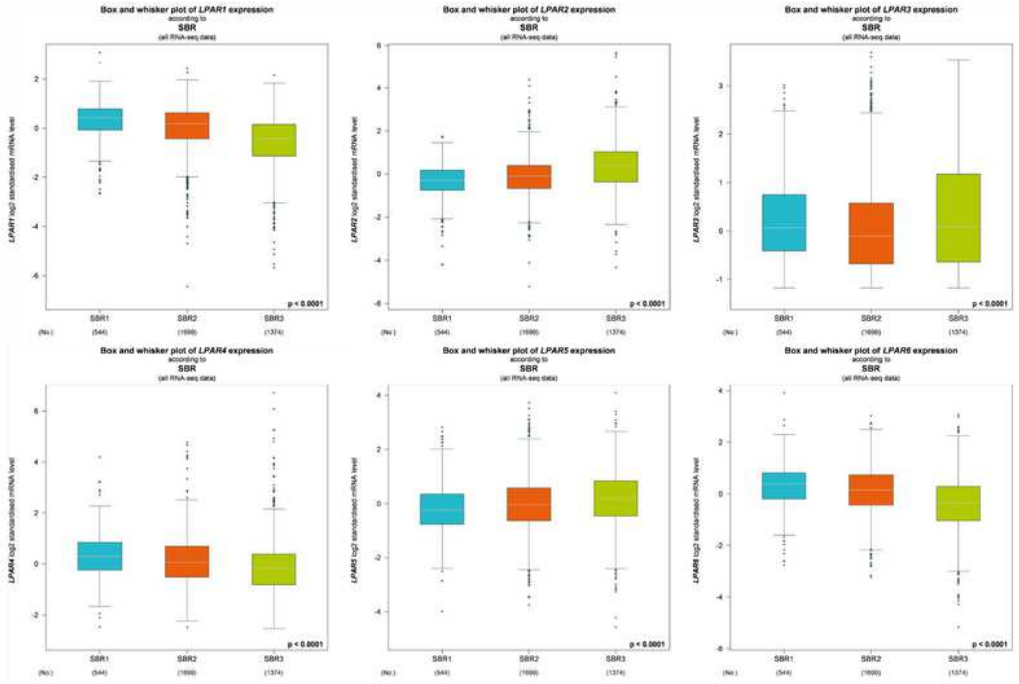

Figure 4 The relationship between mRNA levels of LPARs and SBR grade.

Abbreviations: LPARs, lysophosphatidic acid receptor; SBR, Scarff Bloom & Richardson

Figure 4

The relationship between mRNA levels of LPARs and SBR grade. Abbreviations: LPARs, lysophosphatidic acid receptor; SBR, Scarff Bloom & Richardson

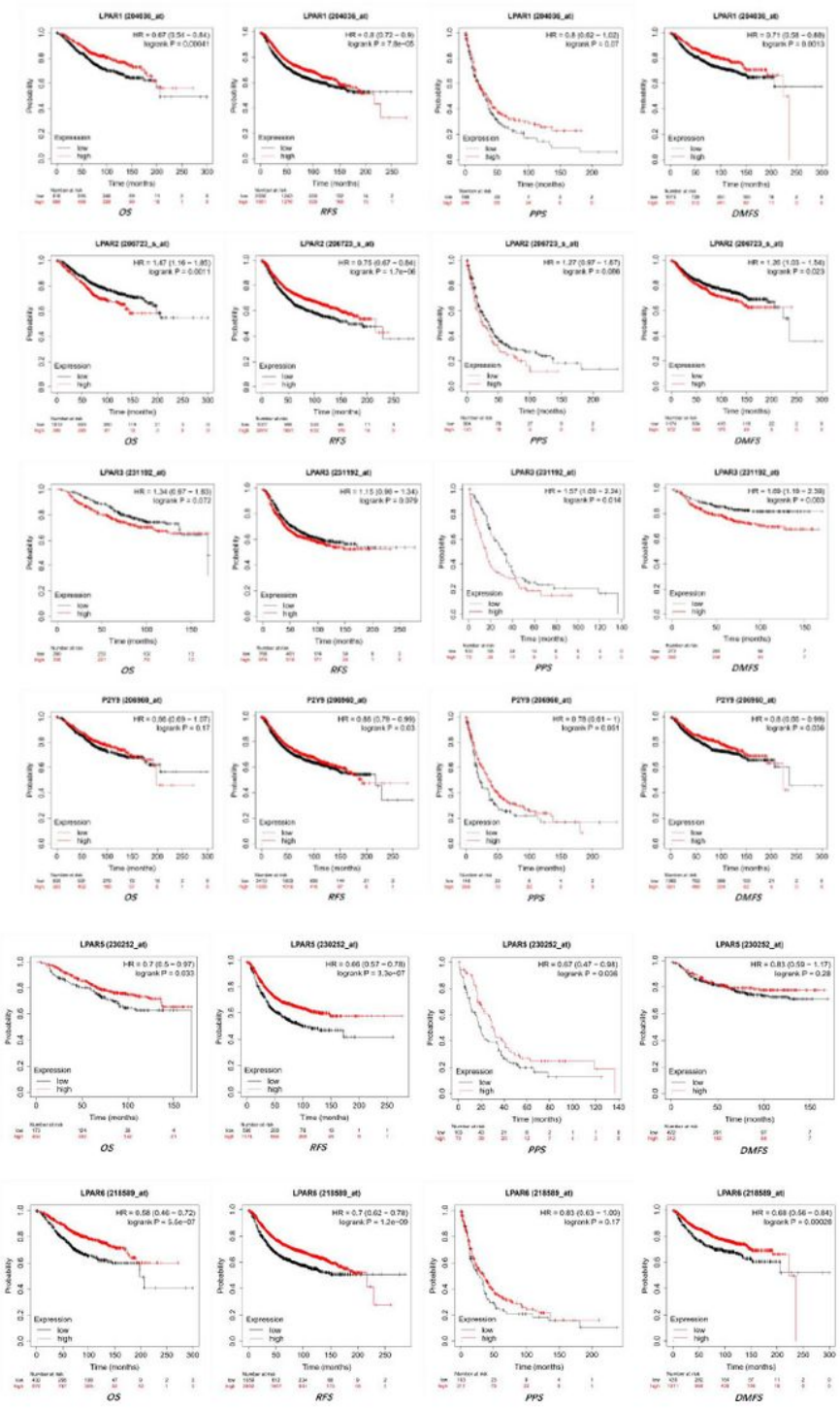

Figure 5 The relationship between mRNA levels of LPARs and prognosis of patients with BC.

Figure 5

The relationship between mRNA levels of LPARs and prognosis of patients with BC.

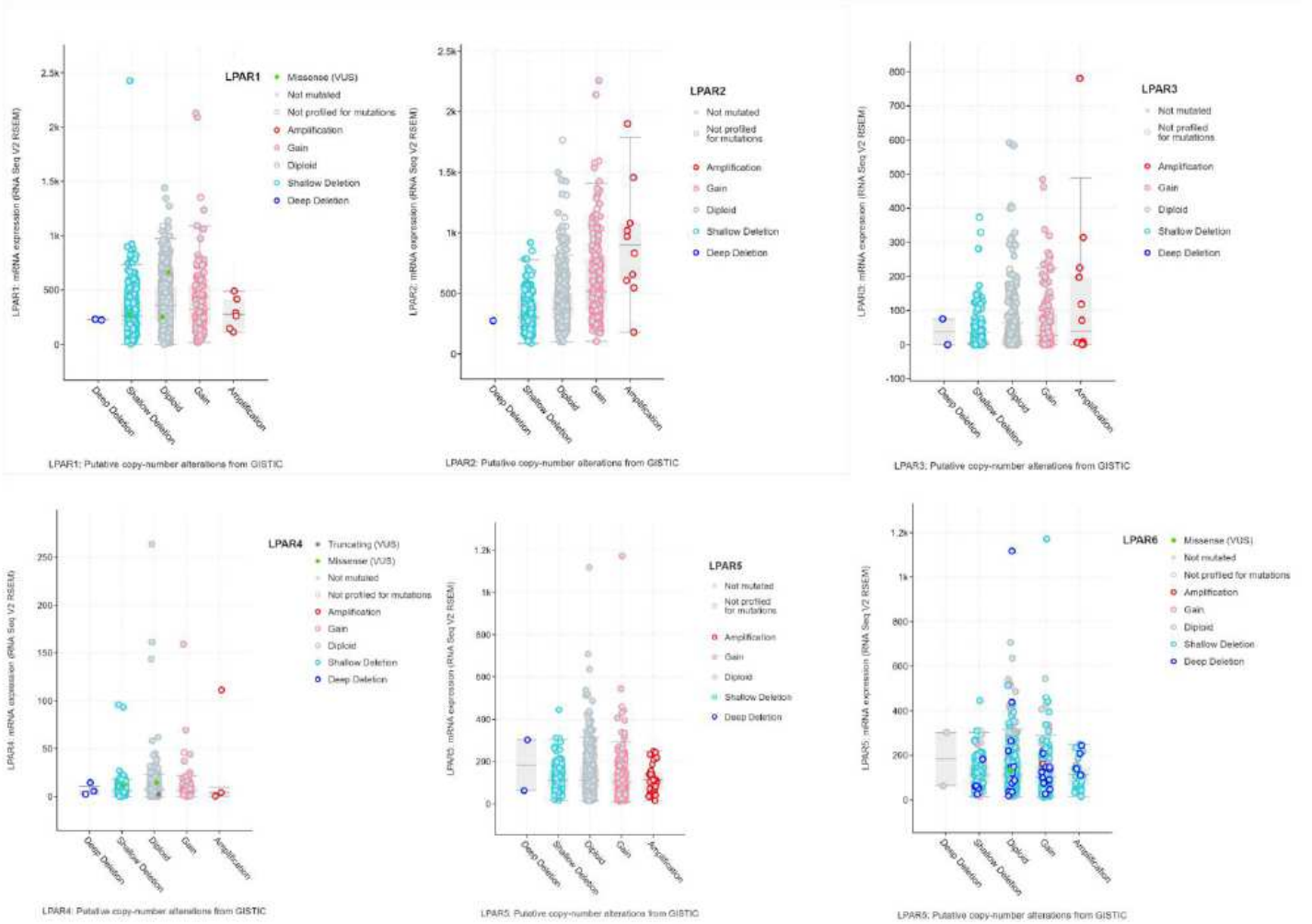

Figure 6. LPARs genes expression and mutation analysis in BC (cBioPortal).

Figure 6

LPARs genes expression and mutation analysis in BC (cBioPortal).

A	B	Neither	A Not B	B Not A	Both	Log2 Odds	p-Value	q-Value	Tendency
LPAR5	LPAR6	633	44	45	16	2.355	<0.001	<0.001	Co-occurr
LPAR1	LPAR5	647	31	49	11	2.228	<0.001	0.001	Co-occurr
LPAR1	LPAR6	646	31	50	11	2.197	<0.001	0.001	Co-occurr
LPAR1	LPAR2	666	34	30	8	2.385	<0.001	0.003	Co-occurr
LPAR1	LPAR4	663	35	33	7	2.007	0.005	0.016	Co-occurr
LPAR2	LPAR5	648	30	52	8	1.733	0.008	0.021	Co-occurr
LPAR4	LPAR5	646	32	52	8	1.635	0.011	0.025	Co-occurr
LPAR3	LPAR5	652	26	55	5	1.189	0.098	0.185	Co-occurr
LPAR2	LPAR4	664	34	36	4	1.118	0.144	0.24	Co-occurr
LPAR2	LPAR3	672	35	28	3	1.041	0.21	0.295	Co-occurr
LPAR4	LPAR6	642	35	56	5	0.712	0.229	0.295	Co-occurr
LPAR3	LPAR6	650	27	57	4	0.757	0.249	0.295	Co-occurr
LPAR1	LPAR3	668	39	28	3	0.876	0.256	0.295	Co-occurr
LPAR2	LPAR6	643	34	57	4	0.408	0.386	0.413	Co-occurr
LPAR3	LPAR4	668	30	39	1	-0.809	0.489	0.489	Mutual ex

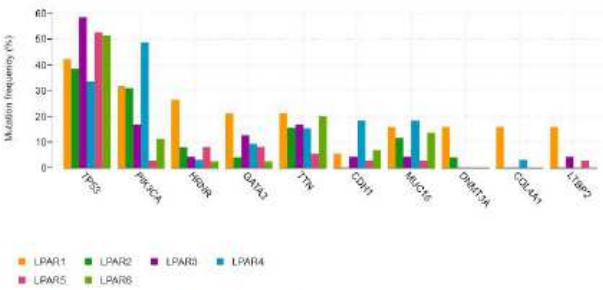

Figure 7. the correlations of LPARs and LPARs' pathways(cBioPortal).

Figure 7

The correlations of LPARs and LPARs' pathways(cBioPortal).