Research Square

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Prognostic value of S1PR1 and Correlations with Immune Infiltrates in Breast and Lung Cancers

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Abstract

Sphingosine-1-phosphate receptor (S1PR1) is involved in vascular development, a key process in tumorigenesis. Our study evaluated its roles in tumor development and prognosis. In particular, S1PR1 expression data were obtained from the TIMER and Oncomine database. We used a bioinformatics approach to evaluate its relationship with prognosis, co-expressed and regulatory genes, correlations with tumor immune cell infiltration and correlations with immune infiltration markers. *S1PR1* was significantly lower expression in breast and lung cancer than in corresponding normal tissues. Lower *S1PR1* expression was related to poor overall survival and disease-free survival in breast and lung cancer. A functional network analysis suggested that S1PR1 regulates vasculogenesis. In addition, S1PR1 levels were significantly related to infiltrating $CD8^+$, $CD4^+$ T cells, macrophages, and neutrophils in breast invasive carcinoma; $CD8^+$ T cells, macrophages, neutrophils, and DCs in lung adenocarcinoma; and with B cells, $CD8^+$, $CD4^+$ T cells, macrophages, neutrophils and DCs in lung squamous cell carcinoma. Furthermore, S1PR1 levels were correlated with multiple immune marker sets in breast and lung cancer. The observed correlations between S1PR1 and both prognosis and immune cell infiltration provide a foundation for further research on its immunomodulatory role in cancer.

1. Introduction

Sphingosine-1-phosphate (S1P), produced by sphingosine kinase (Sphk), is a biologically active signaling lipid [1]. It is important that S1P regulates vascular development and function, including vascular maturation [2, 3]. Sphingosine-1-phosphate receptor (S1PR1) is a biologically active sphingolipid metabolite that mediates S1P activity, promotes cell proliferation and survival [4, 5]. S1PR1 is widely expressed in vascular endothelial cells and is required for embryonic vascular development and maturation [6]. Tumor progression requires new blood vessel growth, which is achieved by producing angiogenic factors that can activate vascular endothelial cells [7]. Tumor cells release angiogenic stimuli leading to angiogenesis and tumor growth, such as VEGF-a [8]. Studies have shown that S1PR1 inhibits VEGF signaling by promoting the interaction between VE-cadherin and VEGFR2, thereby inhibiting VEGF-induced vascular sprouting[9, 10]. However, the role of S1PR1 in tumorigenesis and its prognostic value are unclear. A preclinical study of human breast cancer cells found that S1PR1 antibody can enhance the cytotoxic effect of carboplatin on MDA-MB-231 cells and the anti-proliferative effect on SK-BR-3 (HER2 subtype) cells [11]. Lei et al. found that S1PR1 signaling has tumor-suppressive effects and survival benefits in breast cancer [12]. Therefore, it is necessary to clarify the role of S1PR1 in tumor development and progression. Transcriptome analysis can be used to predict important issues such as the intrinsic subtype of the primary tumor, tumor grade, drug reactivity, and recurrence risk [13–15].

In this study, we use publicly available data to assess the expression level of S1PR1 in various cancers. In addition, we test the prognostic significance of S1PR1 and its relationship with tumor-infiltrating immune cells. Our results shed light on the important role of S1PR1 in breast and lung cancer and identify a key relationship with tumor immunity and the underlying mechanisms.

2. Materials And Methods

2.1 Oncomine database analysis

The Oncomine database (https://www.oncomine.org/resource/login.html) was used to evaluate the expression level of *S1PR1* in various types of cancers [16]. The thresholds were a P-value of 0.0001 and fold change of 2.0.

2.2 PrognoScan database analysis

The PrognoScan database (http://www.abren.net/PrognoScan/) was used to test S1PR1 expression and survival in various types of cancers [17]. The threshold was an adjusted Cox P-value of < 0.05.

2.3 c-BioPortal database analysis

c-BioPortal (http://cbioportal.org) contains multidimensional cancer genomics data sets [18]. *S1PR1* mutations and copy number variation (CNV) in breast and lung cancer were analyzed using c-BioPortal. The OncoPrint tab was used to obtain an overview of genetic alterations for each sample.

2.4 Kaplan–Meier plotter

Kaplan-Meier Plotter (https://kmplot.com/) was applied to assess the prognostic value of S1PR1. Grouped according to the median expression of S1PR1 (high vs low expression), all patients were analyzed for overall survival (OS) and progression-free survival (PFS), and Kaplan-Meie was used to draw a survival chart. [19].

2.5 Immune infiltrate analysis using the TIMER

TIMER (https://cistrome.shinyapps.io/timer/) was used to analyze immune infiltrates across different types of cancer [20]. Especially the expression of S1PR1 in different cancer types, and the correlation between the expression of S1PR1 and the abundance of immune invasion was determined. In addition, the correlation between S1PR1 expression and tumor infiltrating immune cell gene markers was also explored through related modules.

24.6 Gene correlation analysis using GEPIA

GEPIA (http://gepia.cancer-pku.cn/index.html) was used to confirm the genes with significantly correlated expression levels in TIMER [21].

Using GEPIA (http://gepia.pku.cn/index.html) to verify genes with significantly related expression levels in the TIMER. The Spearman method was used to determine the correlation coefficients. The tumor tissue datasets were used for analysis.

2.7 LinkedOmics database analysis

The LinkedOmics database (http://www.linkedomics.org/login.php) was used to analyze S1PR1 co-expression based on Pearson's correlation coefficients. The results were visually evaluated using volcano plots and heat maps. The function module of LinkedOmics was used to analyze Gene Ontology (GO) biological processes (BP) and KEGG pathways by a gene set enrichment analysis (GSEA). The rank criterion was FDR < 0.05 and 500 simulations were performed [22].

3. Results

3.1 S1PR1 mRNA expression levels in different types of cancer in humans

The Oncomine database was used to analyze *S1PR1* mRNA levels in tumor tissues and normal tissues of various cancer types. *S1PR1* expression was lower in most tumor tissues, including sarcoma, bladder, brain, central nervous system, breast, colorectal, leukemia, lung, myeloma, and ovarian cancer tissues, than in normal tissues (**Figure 1a**). The mRNA-seq data from TCGA were analyzed using TIMER to verify these findings. Data from TCGA shown that the differential expression of S1PR1 between the tumor and adjacent normal tissues is shown in Figure 1b. Compared with adjacent normal tissues, *S1PR1* expression was significantly reduced in BLCA, BRCA, CHOL, COAD, ESCA, HNSC, KICH, KIRP, LIHC, LUAD, LUSC, PRAD, READ, SKCM, STAD, and UCEC. However, *S1PR1* expression was significantly higher in KIRC and THCA than in adjacent normal tissues (**Figure 1b**). These data showed that alterations in *S1PR1* expression depend on the tumor type, suggesting that this gene exerts diverse functions in various tumors.

3.2 Prognostic evaluation of S1PR1 in cancers

We investigated whether S1PR1 expression is related to prognosis. The effect of S1PR1 expression on survival was evaluated by PrognoScan. Two probes (204642_at and 239401_at) matching S1PR1 were detected. Notably, S1PR1 expression was significantly related to prognosis in two types of cancer, breast cancer and lung cancer (**Figure 2a–h**). In three breast cancer cohorts (GSE1456-GPL96, GSE7378, and GSE12276) (30, 31), low S1PR1 expression was significantly associated with a poorer prognosis (**Figure 2a–f**). We used the Kaplan-Meier plotter database to further examine the prognostic value of S1PR1 in breast cancer. Poor prognosis based on recurrence-free survival in breast cancer was significantly correlated with low S1PR1 expression (HR = 0.67, P = 7.1e-13), but a significant correlation was not observed for overall survival (HR = 0.86, P = 0.17) (**Figure 2g–h**). Its determined that the low expression of S1PR1 is an independent risk factor for poor prognosis of breast cancer.

In addition, low S1PR1 expression was also related to the poor prognosis in two cohorts of patients with lung cancer (GSE31210 and GSE8894), as determined using two probes (204642_at and 239401_at) (**Figure 2i–k**). Kaplan-Meier plotter database also shows that low expression of S1PR1 is an independent risk factor for poor prognosis of lung cancer (overall survival, HR = 0.7, P = 6.9e-08; progression-free survival, HR = 0.71, P = 0.00035) (**Figure 2l–n**). Furthermore, we found that low S1PR1 expression is associated with a poor prognosis in patients with soft tissue, blood, and brain cancers (Figure S1a–c). In contrast, low S1PR1 expression was an independent risk factor for a good prognosis in gastric cancer (Figure S1d–g). These results confirmed the prognostic value of S1PR1 in specific types of cancer; both high and low S1PR1 expression are associated with prognosis depending on the type of cancer. Based on the consistent results for the associations between S1PR1 expression and survival in lung and breast cancer, we focus on the precise effects of S1PR1 in these two cancer types as well as the underlying mechanisms.

3.3 Correlations between clinical characteristics and S1PR1 expression in breast and lung cancer

We used the Kaplan-Meier plotter to study the relationship between S1PR1 expression and clinical characteristics in patients with breast and lung cancer. Low expression of S1PR1was associated with worse

overall survival (OS) in male and female patients with lung adenocarcinoma (P < 0.05) (Table 1). In particular, low *S1PR1* mRNA expression was correlated with worse OS in stage 1 (P=9.20E-13) and early-stage (AJCC stage M) (P=0.013) lung cancer (Table 1). Low *S1PR1* mRNA expression was related to poor OS in patients with (P=0.023) or without (P=0.00075) smoking (Table 1). In addition, low *S1PR1* mRNA expression was related to worse OS in patients whose no received chemotherapy or radiotherapy. These findings strongly suggest that low *S1PR1* mRNA expression is correlated with poor OS in lung cancer (Table 1). In BRCA, low *S1PR1* mRNA expression was related to poor OS in ER-positive or HER2-negative patients and in the luminal androgen receptor subtype (Table 2). Taken together, high expression of *S1PR1* could be considered a good prognostic indictor for breast and lung cancer depending on the clinical characteristics.

3.4 Regulators of S1PR1 in breast and lung cancer

Use the LinkedOmics function module to detect the S1PR1 regulatory network to further understand the biological role of S1PR1 in breast and lung cancer. Figure 4a-c shows genes with significantly positive (dark red dots) and negative (dark green dots) correlations with S1PR1 (false discovery rate, FDR < 0.01). The top 50 positively and negatively related genes are shown in a heat map in Figure 3d-f. A Gene Ontology (GO)-based gene set enrichment analysis (GSEA) showed that genes that are co-expressed with S1PR1 are enriched for vasculogenesis and the purinergic receptor signaling pathway, while genes related to mitochondria and RNA transcript processing were inhibited in breast cancer (Figure 3g). Similarly, GO annotation results showed that genes co-expressed with S1PR1 are primarily associated with vasculogenesis, the purinergic receptor signaling pathway, and the phospholipase C-activating G protein coupled receptor signaling pathway, while tRNA metabolic process, RNA modification, and RNA transcript processing were inhibited in lung cancer (Figure 3h-i). A Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis showed enrichment for hematopoietic cell lineage, *Staphylococcus aureus* infection, and renin secretion pathways in both breast cancer and lung cancer. Spliceosome, DNA replication, and proteasome pathways were inhibited in both tumor types (Figure 3j-i). These results suggest that S1PR1 contributes to various processes in tumor development.

3.5 Genomic alterations in S1PR1 in breast cancer and lung cancer

cBioPortal database were used to determine the types and frequencies of S1PR1 alterations in BRCA, LUAD, and LUSC. *S1PR1* was altered in 4% of patients with BRCA. These alterations included mRNA missense mutations, amplifications, and deletions (Figure 4a). *S1PR1* was altered in 6% of patients with LUAD and 2.3% of patients with LUSC, including mRNA missense mutations, truncating mutations, amplifications, and deletions (Figure 4a). Finally, *S1PR1* CNV was associated with OS in LUAD but not with OS or DFS in BRAC and LUSC (Figure 4b–d). These results suggest that mutations in *S1PR1* are associated with prognosis in LUAD.

3.6 Relationship between immune and S1PR1 expression in breast cancer and lung cancer

Tumor infiltrating lymphocytes (TIL) are lymphocytes that leave the blood circulation and migrate to the vicinity of the tumor. The amount of TIL in the tumor is an important indicator to predict the prognosis of cancer patients and the response to immunotherapy [23, 24]. Tumor purity is a key factor in analyses of immune infiltration by genomic approaches [25]. Therefore, we use TIMER to investigate whether the

expression of S1PR1 in breast cancer and lung cancer is related to immune infiltration. Firstly, we found a significant negative correlation between the S1PR1 expression level and tumor purity, as determined using TIMER, in both breast cancer and lung cancer (Figure 4a-f, Left). S1PR1 is a determinant of immune infiltration in BRCA (tumor purity; r = −0.508, P = 1.76e-66), including subtypes of BRCA (BRCA-Basal: r = -0.5411, P = 1.28e-06; BRCA-Her2: r = -0.505, P = 4.44e-06 and BRCA-Luminal: r = -0.557, P = 9.15e-46). S1PR1 is related to immune infiltration in lung cancer, including LUAD (tumor purity; r = −0.353, P = 6.05e-16) and LUSC (tumor purity; r = −0.402, P = 5.20e-20).

Furthermore, the relationship between S1PR1 and specific immune infiltrates in breast cancer and lung cancer were analyzed. The S1PR1 expression level was significantly positively correlated with levels of infiltrating $CD8^+$ T cells (r = 0.38, P = 5.97e-35), $CD4^+$ T cells (r = 0.335, P = 1.03e-26), macrophages (r = 0.219, P = 3.67e-12), neutrophils (r = 0.168 P = 2.03e-07), and DCs (r = 0.208, P = 9.14e-11) in BRCA (Figure 5a). In BRCA-Basal, there were slight positive correlations between S1PR1 expression levels and levels of infiltrating $CD8^+$ T cells (r = 0.279, P = 1.76e-03) and $CD4^+$ T cells (r = 0.237, P = 8.52e-03). Similarly, there were positive correlations with infiltrating levels of $CD8^+$ T cells (r = 0.546, P = 1.13e-05), $CD4^+$ T cells (r = 0.529, P = 2.00e-05), neutrophils (r = 0.342, P =8.57e-03), and DCs (r =0.488, P = 1.35e-04) in BRCA-Her2. S1PR1 expression levels were positively correlated with levels of infiltrating $CD8^+$ T cells (r = 0.147, P = 3.43e-21), $CD4^+$ T cells (r = 0.316, P = 6.26e-14), macrophages (r = 0.151, P = 4.14e-04), neutrophils (r = 0.147, P = 6.67e-04), and DCs (r =0.213, P = 6.44e-07) in BRCA-Luminal tumors (**Figure 5a**). We also found that S1PR1 expression levels were positively correlated with levels of infiltrating $CD8^+$ T cells (r = 0.308, P = 3.61e-12), macrophages (r = 0.376, P = 1.01e-17), neutrophils (r = 0.246, P = 4.15e-08), and DCs (r =0.207, P = 4.16e-06) in LUAD. In addition, there were positive correlations with levels of infiltrating B cells (r = 0.358, P = 1.27e-15), CD8+ T cells (r = 0.459, P = 3.83e-26), $CD4^+$ T cells (r = 0.338, P = 3.98e-14), macrophages (r = 0.586, P = 2.61e-45), neutrophils (r = 0.453, P = 1.79e-25), and DCs (r =0.56, P = 2.12e-40) in LUSC. These results strongly suggest that S1PR1 plays a special role in the immune infiltration of breast cancer and lung cancer, and has a particularly strong effect on T cells, macrophages, neutrophils and DCs. Based on the observed correlations between S1PR1 and various types of immune cells in breast cancer and lung cancer indicated that S1PR1 may have high prognostic value.

3.7 Correlations between S1PR1 expression and immune marker sets

We further evaluated the correlations between S1PR1 and markers of various immune cells in breast cancer and lung cancer using TIMER and GEPIA databases (Sup Table 1). The correlations between S1PR1 expression and immune marker genes for different immune cell populations, including $CD8^+$ T cells, T cells (general), B cells, monocytes, TAMs, M1 and M2 macrophages, neutrophils, NK cells, DCs, and various functional T cells, such as Th1 cells, Th2 cells, Tfh cells, Th17 cells, and Tregs, as well as exhausted T cells were analyzed by TIMER. After adjusting for tumor purity, S1PR1 expression levels were significantly correlated with marker sets for various immune cells, except for NK cells, Th17, and T cell exhaustion in BRAC (Table 3 and Figure 6). However, S1PR1 expression levels were highly correlated with most immune marker sets and both T cell populations and exhausted T cells in LUAD and LUSC (Table 3 and Figure 6). We further analyzed the correlation between S1PR1 expression and the markers using the GEPIA database, including data for BRAC, LUAD, and LUSC. The results for correlations between S1PR1 and markers of immune

infiltrating cells were similar to those of the TIMER analysis (Sup Table 1). This further confirms that S1PR1 is significantly related to immune infiltrating cells in lung and breast cancer, suggesting that high levels of S1PR1 induce immune activity in the lung and breast cancer microenvironment.

4. Discussion

We systematically analyzed the expression levels of S1PR1 and the prognostic vaule in different types of cancers. Compared with levels in normal tissues, S1PR1 expression was significantly lower in BLCA, BRCA, CHOL, COAD, ESCA, HNSC, KICH, KIRP, LIHC, LUAD, LUSC, PRAD, READ, SKCM, STAD, and UCEC and was significantly higher in KIRC and THCA. Accordingly, S1PR1 expression patterns depend on the type of cancer, prognostic data from and Kaplan-Meier plotter showed that low levels of *S1PR1* are related to poor prognosis in breast and lung cancer. The down-regulation of *S1PR1* was associated with worse prognosis in breast and lung cancer and was significantly related to clinical characteristics, such as gender, population, smoking status, and stage. These results suggested that S1PR1 is a prognostic biomarker in breast and lung cancer.

The tumor microenvironment refers to non-cancer cells in and around tumors; infiltrated of immune cells in the tumor microenvironment plays a vital function in the occurrence and development of tumors [26, 27]. The evaluation of immune cell infiltration in breast and lung cancer using the TIMER database revealed strong correlations between S1PR1 and immune filtration in BRCA, LUAD, and LUSC. Furthermore, the S1PR1 expression level was positively correlated with levels of $CD8^+$ T, $CD4^+$ T, neutrophils, macrophages, and DCs in BRAC. The correlation between S1PR1 expression and immune cell marker genes suggests that S1PR1 regulates lung cancer tumor immunity through multiple immune cell populations. These results indicate that high levels of S1PR1 could increase the cytotoxicity of the immune system and immune activation in BRCA, LUAD and LUSC by increasing the infiltration of CTLs, CD4 + T cells, and DCs.

To further elucidate the molecular mechanisms underlying the role of S1PR1 in breast and lung cancer, we used GSEA to identify pathways that are enriched in in genes co-expressed with S1PR1. We found that S1PR1 was significantly associated with vasculogenesis, the purinergic receptor signaling pathway, and metabolism of nucleic acids in tumor conditions. Recent studies have provided potential explanations for the associations between S1PR1 expression, immune infiltration, and poor prognosis. Angiogenesis mimicry (VM) system is a blood vessel-like network in which tumor cells are co-expressed with endothelial cells and tumor markers [28]. VM is closely related to a variety of human malignancies, including breast cancer [29]. Angiogenesis mimicry leads to worse prognosis, increased tumor metastasis, low 5-year overall survival, and increased mortality [30]. This shows that S1PR1 defects promote the occurrence of VM, and the knockout of S1PR1 in breast cancer cells increases the number of VMs. More importantly, tumor cells with low S1PR1 expression receive nutrition through VM, and accelerate tumor growth in animal models [31]. Recent research has shown that S1PR signaling is an important vascular factor affecting tumor progression, metastasis, and responses to chemotherapy and immunotherapy [32]. Strategies to enhance S1PR1 function in the tumor vasculature may enhance the cytotoxic killing effect and chemotherapy effect of targeted anti-cancer therapy.

The limitations of our study is that no performed *in vitro* and animal experiments to confirm the role of S1PR1 in the growth and progression of breast and lung cancer and its relationship with the infiltration of immune

cells in the tumor microenvironment. Therefore, further research is needed to verify the role of S1PR1 in breast and lung cancer.

Contribution to the field statement

Our results provide multi-level evidence for the importance of S1PR1 in breast and lung cancer and its potential value as a biomarker. Low expression of S1PR1 was associated with poor prognosis in breast and lung cancer. In these two cancer types, the downregulation of S1PR1 may have profound effects on vasculogenic mimicry. In addition, our results suggest that S1PR1 may play a novel regulatory role in tumor immunity.

Declarations

Author Contributions: Data curation, Limei Zhong and Shaohua Song; Formal analysis, Limei Zhong and Donglin Cao; Funding acquisition, Yufeng Liu and Donglin Cao; Investigation, Limei Zhong and Lijuan Li; Methodology, Limei Zhong and Lijuan Li; Resources, Yufeng Liu; Software and Shaohua Song; Visualization, Lijuan Li; Writing – original draft, Donglin Cao; Writing – review & editing, Yufeng Liu. All authors have read and agreed to the published version of the manuscript.

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Ethical statement

Not Applicable

Conflicts of Interest:

The authors declare no conflict of interest

Availability of data and materials

All data generated or analyzed during this study are included in this published article

References

1. K S, Y Z, A DA, T N, A S, H W, H L, M A, A H, YE W *et al*: **Sphingosine-1-phosphate promotes erythrocyte glycolysis and oxygen release for adaptation to high-altitude hypoxia**. *Nature communications* 2016, **7**:12086.
2. Y A, L S, F H, K H, R S, G S, DK W, M T: **Endothelial Fli1 deficiency impairs vascular homeostasis: a role in scleroderma vasculopathy**. *The American journal of pathology* 2010, **176**(4):1983-1998.
3. ML A, T Y, RL P: **G-protein-coupled receptor S1P1 acts within endothelial cells to regulate vascular maturation**. *Blood* 2003, **102**(10):3665-3667.
4. S S, S M: **The outs and the ins of sphingosine-1-phosphate in immunity**. *Nature reviews Immunology* 2011, **11**(6):403-415.

5. S S, S M: **Sphingosine 1-phosphate, a key cell signaling molecule**. *The Journal of biological chemistry* 2002, **277**(29):25851-25854.
6. RL P, T H: **Emerging biology of sphingosine-1-phosphate: its role in pathogenesis and therapy**. *The Journal of clinical investigation* 2015, **125**(4):1379-1387.
7. J F: **Angiogenesis: an organizing principle for drug discovery?***Nature reviews Drug discovery* 2007, **6**(4):273-286.
8. N F: **Pathways mediating VEGF-independent tumor angiogenesis**. *Cytokine & growth factor reviews* 2010, **21**(1):21-26.
9. B J, H O, S G, K M, BS D, A S, B K, V B, S R, T E *et al*: **Flow-regulated endothelial S1P receptor-1 signaling sustains vascular development**. *Developmental cell* 2012, **23**(3):600-610.
10. K G, C N, K H, B L, BL S, L M, JJ H, L E, S N, S R *et al*: **The sphingosine-1-phosphate receptor S1PR1 restricts sprouting angiogenesis by regulating the interplay between VE-cadherin and VEGFR2**. *Developmental cell* 2012, **23**(3):587-599.
11. Xiao S, Yang J: **Preclinical study of the antitumor effect of sphingosine-1-phosphate receptor 1 antibody (S1PR 1-antibody) against human breast cancer cells**. *Investigational new drugs* 2019, **37**(1):57-64.
12. Lei FJ, Cheng BH, Liao PY, Wang HC, Chang WC, Lai HC, Yang JC, Wu YC, Chu LC, Ma WL: **Survival benefit of sphingosin-1-phosphate and receptors expressions in breast cancer patients**. *Cancer medicine* 2018, **7**(8):3743-3754.
13. Glinsky GV, Glinskii AB, Stephenson AJ, Hoffman RM, Gerald WL: **Gene expression profiling predicts clinical outcome of prostate cancer**. *The Journal of clinical investigation* 2004, **113**(6):913-923.
14. Chen B, Tang H, Chen X, Zhang G, Wang Y, Xie X, Liao N: **Transcriptomic analyses identify key differentially expressed genes and clinical outcomes between triple-negative and non-triple-negative breast cancer**. *Cancer management and research* 2019, **11**:179.
15. Tang H, Huang X, Wang J, Yang L, Kong Y, Gao G, Zhang L, Chen Z-S, Xie X: **circKIF4A acts as a prognostic factor and mediator to regulate the progression of triple-negative breast cancer**. *Molecular cancer* 2019, **18**(1):23.
16. Rhodes DR, Kalyana-Sundaram S, Mahavisno V, Varambally R, Yu J, Briggs BB, Barrette TR, Anstet MJ, Kincead-Beal C, Kulkarni P: **Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles**. *Neoplasia (New York, NY)* 2007, **9**(2):166.
17. Lánczky A, Nagy Á, Bottai G, Munkácsy G, Szabó A, Santarpia L, Győrffy B: **miRpower: a web-tool to validate survival-associated miRNAs utilizing expression data from 2178 breast cancer patients**. *Breast cancer research and treatment* 2016, **160**(3):439-446.
18. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E: **Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal**. *Sci Signal* 2013, **6**(269):pl1-pl1.
19. Szász AM, Lánczky A, Nagy Á, Förster S, Hark K, Green JE, Boussioutas A, Busuttil R, Szabó A, Győrffy B: **Cross-validation of survival associated biomarkers in gastric cancer using transcriptomic data of 1,065 patients**. *Oncotarget* 2016, **7**(31):49322.

20. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, Li B, Liu XS: **TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells**. *Cancer research* 2017, **77**(21):e108-e110.
21. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z: **GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses**. *Nucleic acids research* 2017, **45**(W1):W98-W102.
22. Vasaikar SV, Straub P, Wang J, Zhang B: **LinkedOmics: analyzing multi-omics data within and across 32 cancer types**. *Nucleic acids research* 2018, **46**(D1):D956-D963.
23. Ohtani H: **Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in human colorectal cancer**. *Cancer Immunity Archive* 2007, **7**(1):4.
24. Azimi F, Scolyer RA, Rumcheva P, Moncrieff M, Murali R, McCarthy SW, Saw RP, Thompson JF: **Tumor-infiltrating lymphocyte grade is an independent predictor of sentinel lymph node status and survival in patients with cutaneous melanoma**. *Journal of clinical oncology* 2012, **30**(21):2678-2683.
25. K Y, M S, E M, R V, H K, W T-G, V T, H S, PW L, DA L *et al*: **Inferring tumour purity and stromal and immune cell admixture from expression data**. *Nature communications* 2013, **4**:2612.
26. Zhu J, Petit P-F, Van den Eynde BJ: **Apoptosis of tumor-infiltrating T lymphocytes: a new immune checkpoint mechanism**. *Cancer Immunology, Immunotherapy* 2019, **68**(5):835-847.
27. P S, S H-L, JA W, A R: **Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy**. *Cell* 2017, **168**(4):707-723.
28. A V, G M, V A, MP P, M R, C R, A G, F N, AH C, GI O: **Fact or Fiction, It Is Time for a Verdict on Vasculogenic Mimicry?***Frontiers in oncology* 2019, **9**:680.
29. S EH, B B, F P, A R, C C, A I, Y M, K M, JL T, A E *et al*: **A new alternative mechanism in glioblastoma vascularization: tubular vasculogenic mimicry**. *Brain : a journal of neurology* 2010, **133**:973-982.
30. Z C, M B, L M, FH S, Z W, Q Z: **Tumour vasculogenic mimicry is associated with poor prognosis of human cancer patients: a systemic review and meta-analysis**. *European journal of cancer (Oxford, England : 1990)* 2013, **49**(18):3914-3923.
31. S L, C N, D Z, H S, X D, N C, X L, C C, F L, J B *et al*: **S1PR1 regulates the switch of two angiogenic modes by VE-cadherin phosphorylation in breast cancer**. *Cell death & disease* 2019, **10**(3):200.
32. A C, T L, CH L, T H: **Endothelial sphingosine 1-phosphate receptors promote vascular normalization and antitumor therapy**. *Proceedings of the National Academy of Sciences of the United States of America* 2020, **117**(6):3157-3166.

Tables

Table 1. Correlation between *S1PR1* mRNA expression and prognosis in lung cancer with respect to clinicopathological factors using Kaplan–Meier plotter

Clinicopathological characteristics		Overall survival		
		N	Hazard ratio	P-value
SEX:	Female	715	0.72(0.57-0.91)	**0.0064**
	Male	1100	0.72(0.61-0.84)	**4.90E-05**
Histology:	Adenocarcinoma	720	0.57(0.45-0.73)	**5.90E-06**
	Squamous cell carcinoma	524	0.85(0.67-1.07)	0.1677
Stage:	1	577	0.35(0.26-0.47)	**9.20E-13**
	2	244	0.74 (0.51-1.07)	1.13E-01
	3	70	1.03(0.6-1.77)	9.20E-01
	4	4	NA	NA
Grade:	I	201	1.19(0.83-1.71)	0.34
	II	310	0.83(0.6-1.13)	0.23
	III	77	0.61(0.32-1.19)	0.15
AJCC stage T:	1	237	1.01(0.76-1.34)	0.9527
	2	389	0.77(0.62-0.96)	0.019
	3	81	1.47(0.89-2.43)	0.13
	4	46	0.98(0.52-1.85	0.95
AJCC stage N:	0	781	0.85(0.68-1.04)	0.12
	1	56	1.78(0.89-3.57)	0.098
	2	111	1.27(0.84-1.9)	0.2515
AJCC stage M :	0	681	0.77(0.62-0.95)	**0.013**
	1	10	NA	NA
Smoking history:	Exclude those never smoked	820	0.79(0.64-0.94)	**0.023**
	Only those never smoked	105	0.37(0.21-0.68)	**0.00075**
Chemotherapy:	No	310	0.71(0.51-1)	**0.046**
	Yes	176	1.11(0.74-1.67)	0.62
Radiotherapy:	No	271	0.69(0.48-0.99)	**0.042**
	Yes	70	1.04(0.61-1.78)	0.8745

Bold values indicate P < 0.05; NA: none

Table 2. Correlations between *S1PR1* mRNA expression and clinical prognosis in breast cancer with respect to clinicopathological factors using Kaplan–Meier plotter.

Clinicopathological characteristics		Overall		
		N	Hazard ratio	P-value
ER status:	ER positive	2061	0.79(0.67-0.94)	**0.0057**
	ER negative	801	0.95(0.7-1.18)	0.62
PR status:	PR positive	589	0.91(0.64-1.29)	0.6024
	PR negative	549	1.02(0.76-1.36)	0.9124
HER2 status:	HER2 positive	252	1.13(0.73-1.75)	0.5743
	HER2 negative	800	0.75(0.57-0.96)	**0.0247**
Intrinsic subtype:	Basal	241	1.23(0.75-2.01)	0.41
	Luminal A	611	0.75(0.52-1.06)	0.1
	Luminal B	433	0.97(0.67-1.41)	0.88
	HER2+	147	0.67(0.35-1.28)	0.2235
Lymph node status:	Lymph node positive	313	0.94(0.64-1.38)	0.75
	Lymph node negative	594	1.07(0.73-1.55)	0.74
Grade:	1	345	0.68(0.4-1.15)	0.1461
	2	901	0.94(0.74-1.2)	0.63
	3	903	0.93(0.75-1.16)	0.5257
TP53 status:	Mutated	188	1.17(0.73-1.88)	0.52
	Wild type	273	0.81(0.42-1.54)	0.52
Pietenpol subtype:	Basal-like 1	58	1.69(0.55-5.17)	0.35
	Basal-like 2	38	0.96(0.28-3.34)	0.95
	Immunomodulatory	100	1.67(0.65-4.32)	0.28
	Mesenchymal	73	0.79(0.36-1.73)	0.56
	Mesenchymal stem -like	19	NA	NA
	Luminal androgen receptor	203	0.46(0.3-0.71)	**0.0002**
Systemically untreated patients:	Include	1402	0.86(0.69-1.07)	0.17
	Exclude	3951	0.67(0.6-0.75)	**7.1E-13**

Bold values indicate P < 0.05; NA: none

Table 3. Correlations between S1PR1 and related genes and markers of immune cells, as evaluated using TIMER

Description	Gene markers	BRAC Purity			LUAD Purity			LUSC Purity		
	varX	cor	p		cor	p		cor	p	
CD8+ T cell	CD8A	0.267	1.26E-17	***	0.166	2.19E-04	**	0.411	6.51E-21	***
	CD8B	0.176	2.42E-08	***	0.108	1.66E-02		0.378	1.22E-17	***
T cell (general)	CD3D	0.217	4.71E-12	***	0.112	1.28E-02		0.411	7.60E-21	***
	CD3E	0.276	7.15E-19	***	0.226	8.85E-07	***	0.459	2.82E-26	***
	CD2	0.202	3.20E-10	***	0.159	4.00E-04	**	0.438	7.99E-24	***
B cell	CD19	0.156	7.38E-07	***	0.181	5.37E-05	***	0.324	3.78E-13	***
	CD79A	0.177	1.98E-08	***	0.172	1.21E-04	**	0.325	3.29E-13	***
Monocyte	CD86	0.044	1.28E-01		0.228	2.97E-07	***	0.588	1.27E-45	***
	CD115 (CSF1R)	0.202	1.29E-10	***	0.264	3.10E-08	***	0.64	2.67E-56	***
TAM	CCL2	0.111	4.68E-04	**	0.093	3.86E-02		0.44	5.89E-24	***
	CD68	0.023	4.63E-01		0.289	5.86E-11	***	0.494	1.18E-30	***
	IL10	0.055	8.35E-02		0.27	1.10E-09	***	0.534	1.49E-36	***
M1 Macrophage	INOS (NOS2)	0.257	1.76E-16	***	0.374	7.93E-18	***	0.079	8.64E-02	
	IRF5	0.016	6.18E-01		-0.042	3.55E-01		-0.036	4.31E-01	
	COX2 (PTGS2)	0.338	4.90E-28	***	0.095	3.58E-02		0.214	2.37E-06	***
M2 Macrophage	CD163	0.056	7.72E-02		0.331	4.36E-14	***	0.645	1.52E-57	***
	VSIG4	0.08	1.14E-02		0.271	9.75E-10	***	0.625	4.77E-53	***
	MS4A4A	0.23	1.96E-13	***	0.365	5.39E-17	***	0.628	9.28E-54	***
Neutrophils	CD66b (CEACAM8)	0.04	2.03E-01		0.25	1.95E-08	***	0.212	2.99E-06	***
	CD11b (ITGAM)	0.007	8.24E-01		0.199	8.16E-06	***	0.491	2.66E-30	***
	CCR7	0.316	1.55E-24	***	0.321	2.57E-13	***	0.514	1.70E-33	***
Natural killer cell	KIR2DL1	0.011	7.27E-01		0.216	1.30E-06	***	0.146	1.36E-03	*
	KIR2DL3	0.051	1.10E-01		0.148	9.96E-04	**	0.233	2.63E-07	***
	KIR2DL4	-0.027	3.95E-01		-0.03	5.06E-01		0.152	8.45E-04	**
	KIR3DL1	0.095	2.63E-03	*	0.174	1.04E-	**	0.295	4.85E-	***

						04			11	
	KIR3DL2	0.068	3.19E-02		0.077	8.79E-02		0.217	1.68E-06	***
	KIR3DL3	-0.005	8.75E-01		0.025	5.81E-01		0.044	3.43E-01	
	KIR2DS4	0.035	2.68E-01		0.119	8.34E-03	*	0.221	1.05E-06	***
Dendritic cell	HLA-DPB1	0.237	3.89E-14	***	0.261	4.13E-09	***	0.621	3.86E-52	**
	HLA-DQB1	0.073	2.11E-02		0.089	4.79E-02		0.4	8.84E-20	***
	HLA-DRA	0.156	7.17E-07	***	0.219	8.69E-07	***	0.603	1.29E-48	**
	HLA-DPA1	0.21	2.26E-11	***	0.225	4.53E-07	***	0.622	1.87E-52	**
	BDCA-1(CD1C)	0.461	1.76E-53	***	0.271	1.00E-09	***	0.438	8.69E-24	***
	BDCA-4(NRP1)	0.484	1.58E-59	***	0.174	1.07E-04	**	0.473	6.69E-28	***
	CD11c (ITGAX)	0.087	6.21E-03	*	0.135	2.69E-03	***	0.445	1.58E-24	***
Th1	T-bet (TBX21)	0.227	4.72E-13	***	0.182	4.81E-05	***	0.403	5.17E-20	***
	STAT4	0.277	5.92E-19	***	0.131	3.66E-03	***	0.504	4.73E-32	***
	STAT1	0.116	2.61E-04	**	-0.046	3.10E-01		0.177	1.03E-04	**
	IFN-g (IFNG)	0.009	7.84E-01	***	-0.076	9.13E-02		0.108	1.85E-02	
	TNF-a (TNF)	0.193	8.08E+10	***	-0.076	9.30E-02		0.069	1.34E-01	
Th2	GATA3	0.078	1.43E-02		0.047	3.01E-01		0.232	3.00E-07	***
	STAT6	0.225	6.69E-13	***	0.138	2.20E-03	*	0.022	6.25E-01	
	STAT5A	0.165	1.81E-07	***	0.248	2.27E-08	***	0.413	4.22E-21	***
	IL13	0.048	1.27E-01		0.071	1.15E-01		0.199	1.20E-05	***
Tfh	BCL6	0.174	3.52E-08	***	0.119	8.01E-03	*	0.004	9.24E-01	
	IL21	0.001	9.77E-01		0.054	2.34E-01		0.207	4.92E-06	***
Th17	STAT3	0.043	1.75E-01		0.188	2.65E-05	***	0.158	6.09E-04	**
	IL17A	-0.053	9.29E-02		0.033	4.62E-01		-0.038	4.09E-01	
Treg	FOXP3	0.027	3.94E-01		0.058	1.98E-01		0.393	4.15E-19	***
	CCR8	0.014	6.71E-01		0.157	4.61E-04	**	0.464	7.27E-27	***
	STAT5B	0.283	8.58E-20	***	0.505	4.67E-12	***	0.138	2.47E-03	*

	TGFb (TGFB1)	0.321	3.21E-25	***	0.198	9.43E-06	***	0.064	1.64E-01	
T cell exhaustion	PD-1 (PDCD1)	0.112	4.12E-04	**	0.051	2.56E-01		0.361	3.80E-16	***
	CTLA4	0.018	5.75E-01		0.081	7.27E-02	***	0.404	3.88E-20	***
	LAG3	-0.109	6.00E-04	**	-0.035	4.39E-01		0.212	3.11E-06	***
	TIM-3 (HAVCR2)	0.039	2.19E-01		0.213	1.78E-06	***	0.589	8.44E-46	***
	GZMB	0.056	7.82E-02		0.024	5.99E-01		0.267	3.33E-09	***

Figures

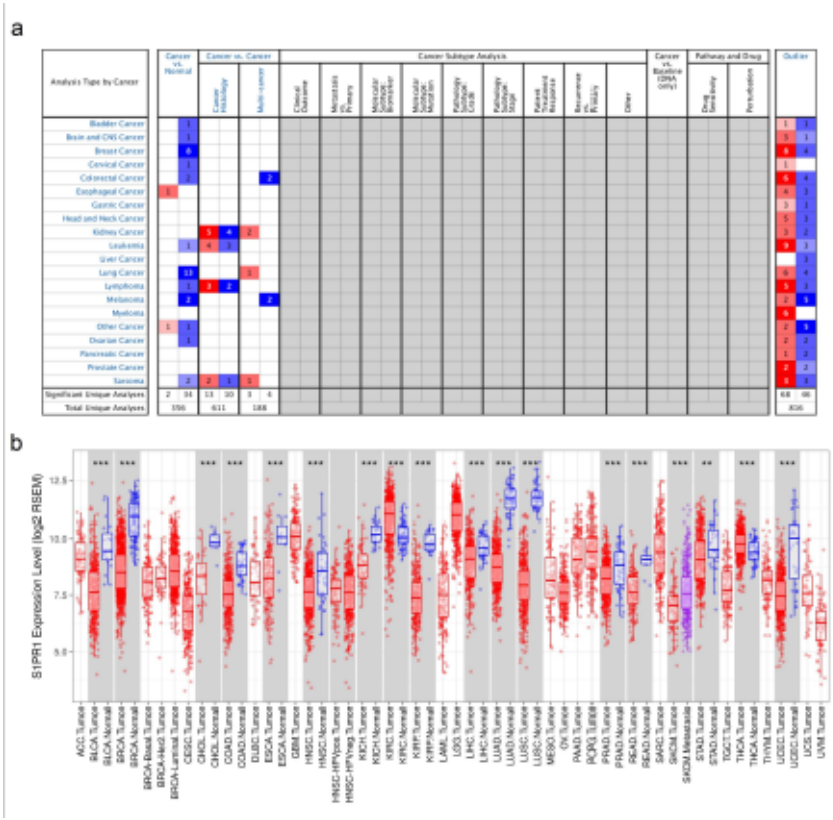

Figure 1

S1PR1 expression levels in different types of human cancers. (a) Differences in S1PR1 between cancer tissues and normal tissues based on data in the Oncomine database. (P = 1E-04, Fold change = 2, Data type = mRNA) (b) Human S1PR1 expression levels in different tumor types from TCGA database were determined using TIMER. BLCA (bladder urothelial carcinoma), BRCA (breast invasive carcinoma), CHOL (cholangiocarcinoma), COAD (colon adenocarcinoma), ESCA (esophageal carcinoma), HNSC (head and neck squamous cell carcinoma), KICH (kidney chromophobe), KIRP (kidney renal papillary cell carcinoma), LIHC (liver hepatocellular carcinoma), LUAD (lung adenocarcinoma), LUSC (lung squamous cell carcinoma), PRAD (prostate adenocarcinoma), READ (rectum adenocarcinoma), SKCM (skin cutaneous melanoma), STAD

(stomach adenocarcinoma), UCEC (uterine corpus endometrial carcinoma), KIRC (kidney renal clear cell carcinoma) and THCA (thyroid carcinoma). *P < 0.05, **P < 0.01, ***P < 0.001.

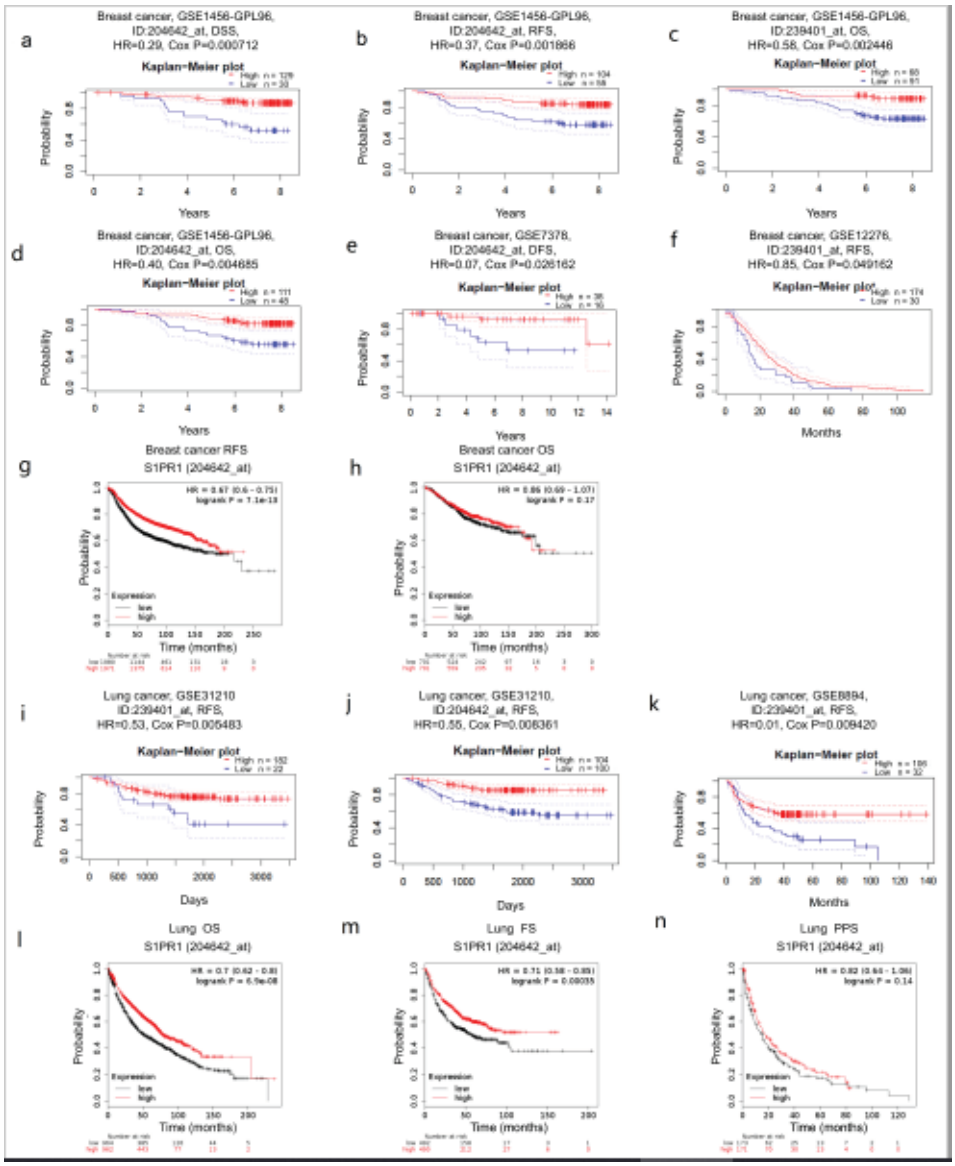

Figure 2

Prognostic value of S1PR1 in cancers. (a–f) Kaplan–Meier survival curves comparing high and low expression of S1PR1 in breast cancers using PrognoScan. Survival curves based on OS, DSS, and DFS in two colorectal cancer cohorts [GSE17536 (n = 177) and GSE14333 (n = 226)]. (g, h) Survival curves for breast cancers based on mRNA-seq data from TCGA of Kaplan–Meier plotter databases. (i–k) Kaplan–Meier survival curves comparing high and low expression of S1PR1 in lung cancers using PrognoScan. (l–n) Survival curves for lung cancers based on mRNA-seq data from TCGA of Kaplan–Meier plotter databases.

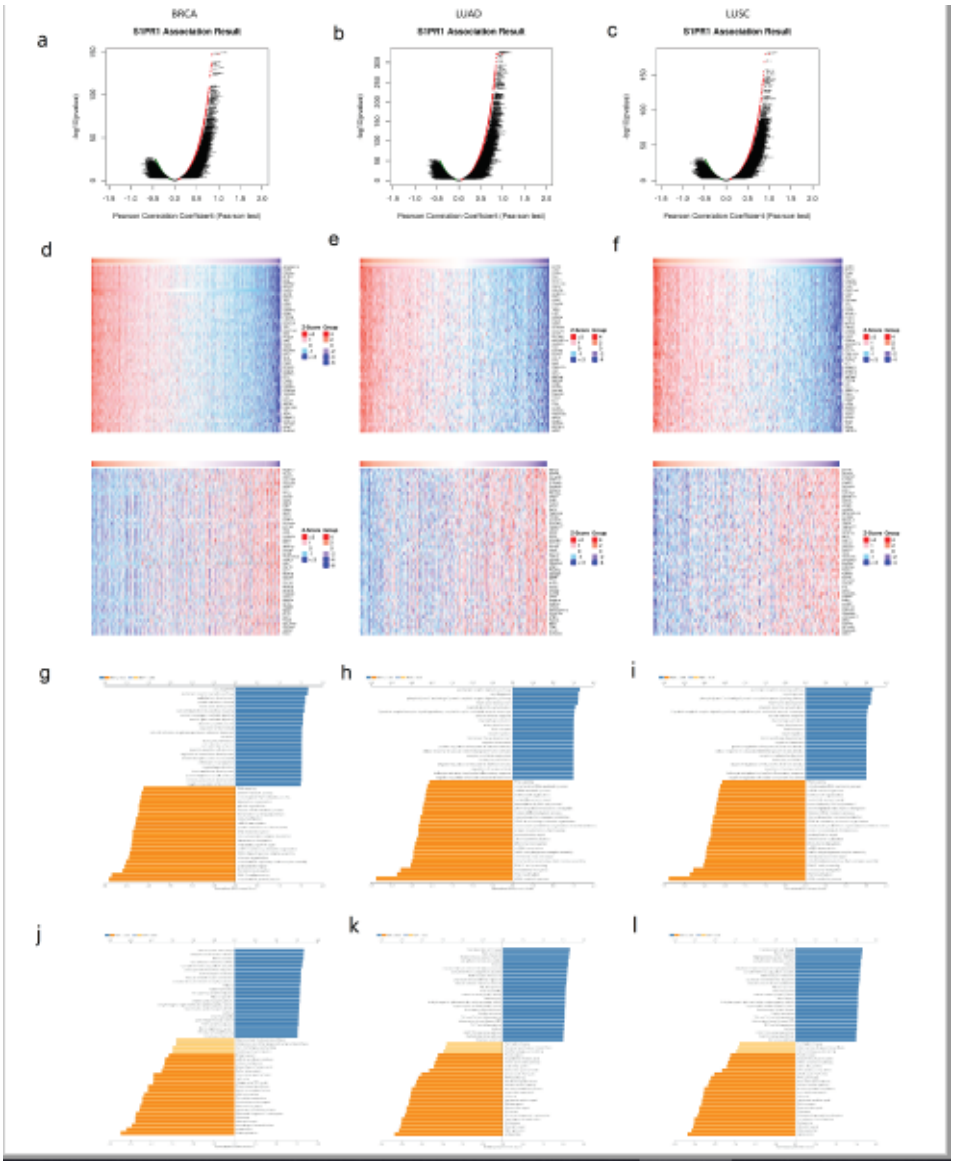

Figure 3

S1PR1 co-expression genes in breast and lung cancer. (a-c) The S1PR1 highly correlated genes -eidentified by Pearson test in BRCA (a), LUAD (b), and LUSC (c). (d-f) The heat map shows that in BRCA (d), LUAD (e), and LUSC (f), the first 50 genes are positively (red) and negatively (blue) correlated with S1PR1. (g-i) Significantly enriched GO annotations of S1PR1 in BRCA (g), LUAD (h), and LUSC (i). (j-l) Significantly enriched KEGG pathways of S1PR1 in BRCA (j), LUAD (l), and LUSC (l).

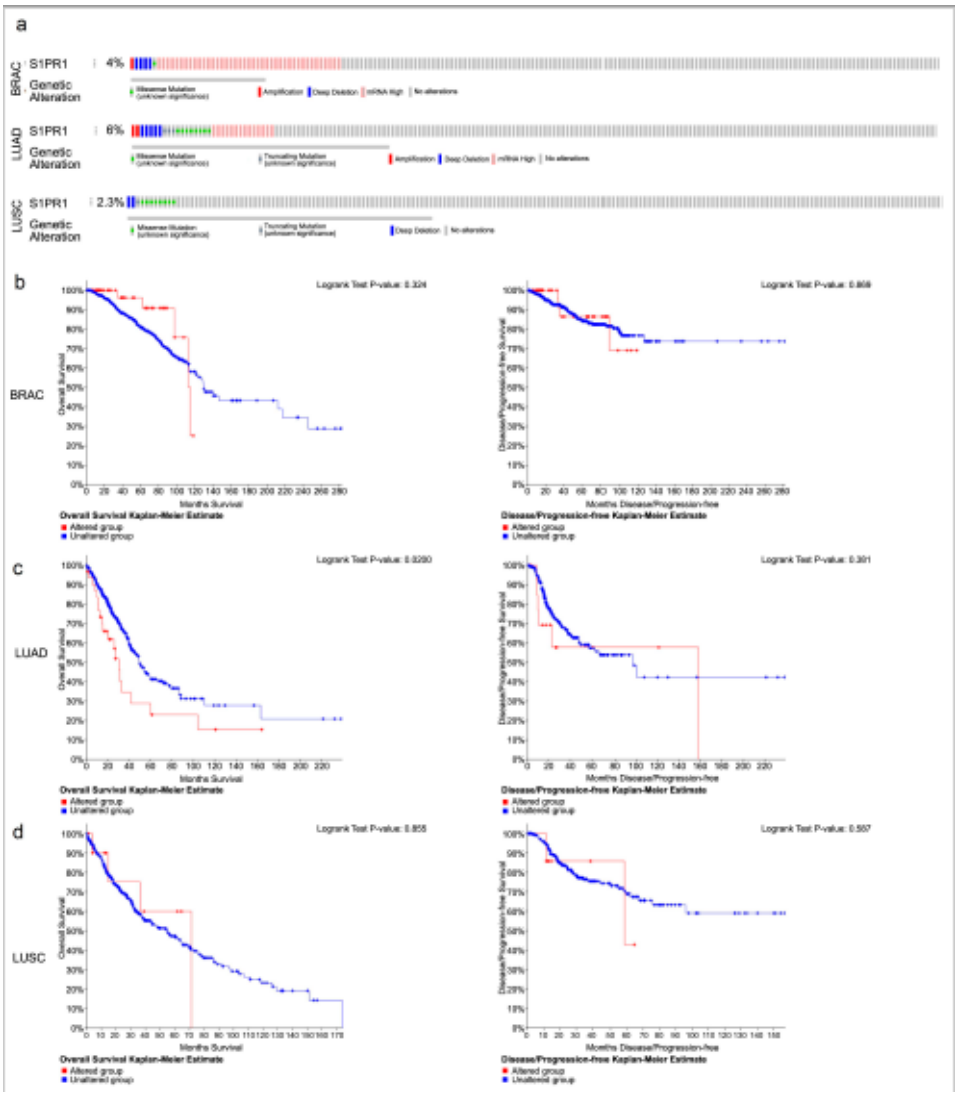

Figure 4

S1PR1 genomic alterations in breast cancer and lung cancer. (a) OncoPrint of S1PR1 alterations in BRCA, LUAD, and LUSC. Different types of genetic alterations highlighted in different colors. (b-d) The relationship between genetic alterations and S1PR1 (OS/DFS) in BRCA (b), LUAD (c), and LUSC (d). Logrank test was used in analysis of OS/DFS.

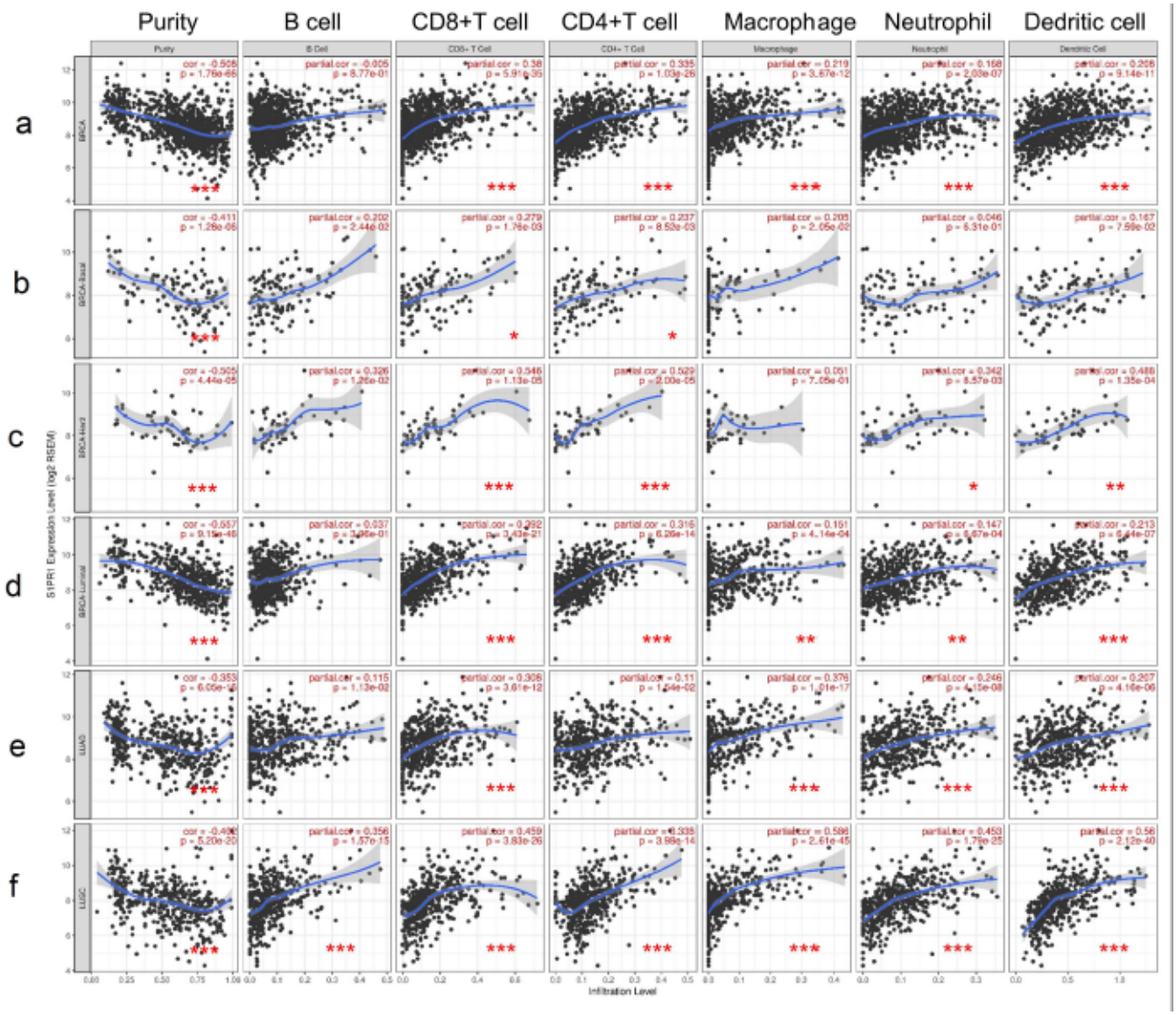

Figure 5

Correlations between S1PR1 expression and immune infiltration levels in breast cancer and lung cancer. (a) S1PR1 expression was significantly negatively related to tumor purity and significantly positively correlated with infiltrating levels of CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells in BRCA (n = 1093). (b) S1PR1 expression was significantly negatively related to tumor purity and was significant positively correlated with infiltrating levels of CD8+ T cells, CD4+ T cells, and dendritic cells in BRCA-Basal (n = 139). (c) S1PR1 expression was significantly negatively related to tumor purity and was significantly positively correlated with infiltrating levels of CD8+ T cells, CD4+ T cells, neutrophils, and dendritic cells in BRCA-Her2 (n = 67). (d) S1PR1 expression was significantly negatively related to tumor purity and was significantly positively correlated with infiltrating levels of CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells in BRCA-Luminal (n = 611). (e) S1PR1 expression was significantly negatively related to tumor purity and was significantly positively correlated with infiltrating levels of CD8+ T cells, macrophages, neutrophils, and dendritic cells in LUAD (n = 457). (f) S1PR1 expression was significantly negatively related to tumor purity and was significant positively correlated with infiltrating levels of B cells,

CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells in LUSC (n = 457). Spearman's correlation coefficients were used for analyses. * P < 0.01; ** P < 0.001; *** P < 0.0001.

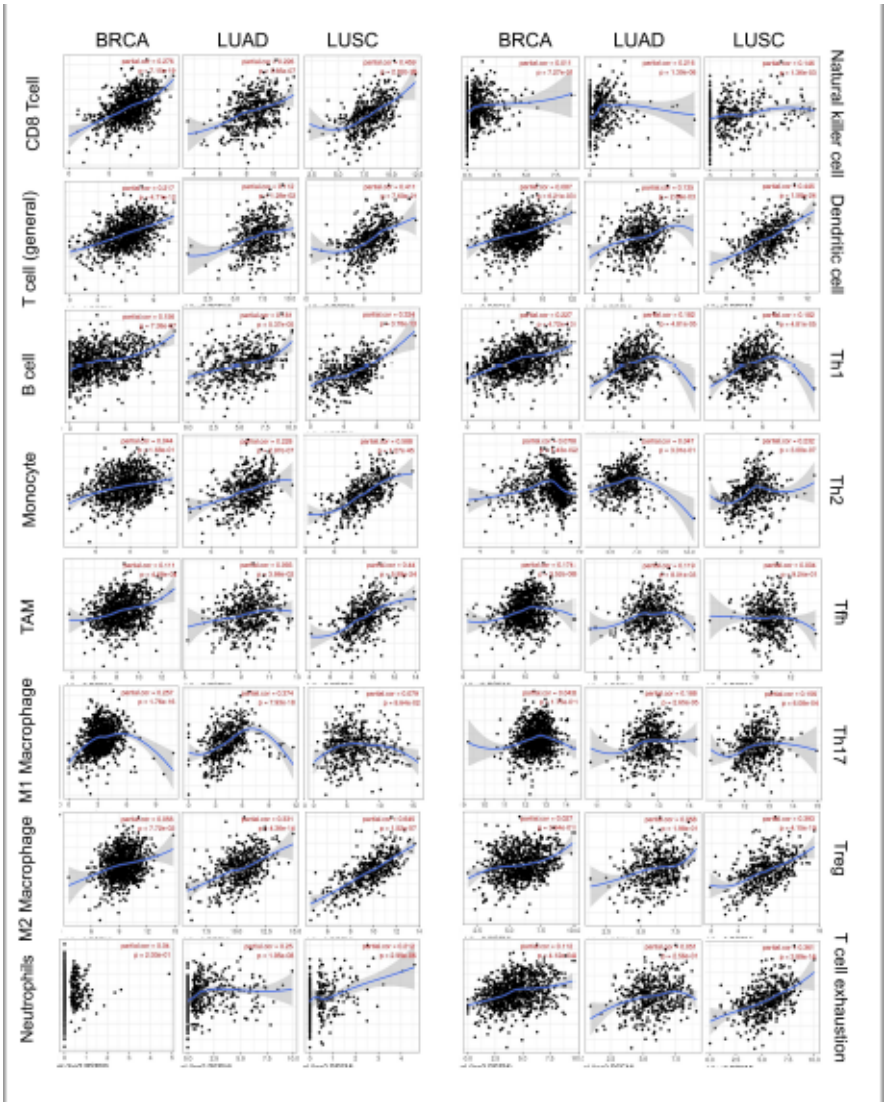

Figure 6

Correlations between S1PR1 expression and immune marker sets. Correlations between S1PR1 expression with markers of immune cells CD8+ T cell, T cell (general), B cells, monocytes, TAM, M1 macrophages, M2 macrophages, neutrophils, natural killer cells, dendritic cells, Th1, Th2, Tfh, Th17, Treg, and T cell exhaustion in BRCA, LUAD, and LUSC using TIMER.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SuppFigure1.tiff
- Suptable1.docx