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Research Article

Pan-cancer Analysis Reveals SRC May Link Lipid Metabolism and Macrophages

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Background: SRC is a member of the membrane-associated non-receptor protein tyrosine kinase superfamily. It has been reported to mediate inflammation and cancer. However, the exact molecular mechanism involved is still not clear.

Objectives: The current study was designed to explore the prognostic landscape of *SRC* and further investigate the relationship between *SRC* and immune infiltration in pan-cancer.

Materials and Methods: Kaplan-Meier Plotter was used to detect the prognostic value of *SRC* in pan-cancer. Then using TIMER2.0 and CIBERSORT, the relationship between *SRC* and immune infiltration in pan-cancer was evaluated. Furthermore, the LinkedOmics database was used to screen *SRC* co-expressed genes, followed by functional enrichment of *SRC* co-expressed genes by Metascape online tool. STRING database and Cytoscape software were applied to construct and visualise the protein-protein interaction network of *SRC* co-expressed genes. MCODE plug-in was used to screen hub modules in the PPI network. The *SRC* co-expressed genes in hub modules were extracted, and the correlation analysis between interested *SRC* co-expressed genes and immune infiltration was conducted via TIMER2.0 and CIBERSORT.

Results: Our study demonstrated that SRC expression was significantly associated with overall survival and relapse-free survival in multiple cancer types. In addition, SRC expression was significantly correlated with the immune infiltration of B cells, dendritic cells, $CD4^+$ T cells, macrophages, and neutrophils in pan-cancer. The expression of SRC had shown to have close correlations with M1 macrophage polarisation in LIHC, TGCT, THCA, and THYM. Moreover, the genes that co-expressed with SRC in LIHC, TGCT, THCA, and THYM were mainly enriched in lipid metabolism. Besides, correlation analysis showed that SRC co-expressed genes associated with lipid metabolism were also significantly correlated with the infiltration and polarisation of macrophages.

Conclusion: These results indicate that SRC can serve as a prognostic biomarker in pan-cancer and is related to macrophages infiltration and interacts with genes involved in lipid metabolism.

Keywords. Immune cell infiltration, Lipid metabolism, Macrophages, Pan-cancer, SRC

1. Background

Cancer is the second most frequent cause of death globally and has become a significant public health concern (1). Recent studies have revealed that Tyrosine-Protein Kinase Src (*SRC*) -family protein tyrosine kinases (SFKs), a subfamily of non-receptor tyrosine kinases, is one of the most potential target for cancer treatment. The SFKs are the most prominent non-receptor tyrosine kinases, including Tyrosine-Protein Kinase Lyn (*LYN*), Tyrosine-Protein Kinase Fyn (*FYN*), Tyrosine-Protein Kinase Lck (*LCK*), Tyrosine-Protein Kinase Hck (*HCK*), Tyrosine-Protein Kinase Fgr (FGR), Tyrosine-Protein Kinase Blk (*BLK*), Tyrosine-Protein Kinase Yrk (*YRK*), Tyrosine-Protein Kinase Yes (*YES*), and *SRC* (2). *SRC*, also known as c-SRC, can be activated by multiple signal transduction pathways (3), such as the activated SRC kinase could phosphorylate the tyrosine residues of the corresponding target proteins, including MAPK, STAT, PI3K/AKT, and EGFR. In humans, the SRC has a multifaceted effect on cell morphology, adhesion, invasion, proliferation, and differentiation (4). The SRC protein that are abnormally activated has been linked to many tumors and studied to be closely related to the progression of multiple tumors (5). The specific mechanism through which SRC induce tumor morphology include promoting the proliferation of cancer cells, triggering metastasis, and inducing angiogenesis (6).

The tumor microenvironment (TME) is the surrounding microenvironment in which tumor cells exist and comprises of non-tumorous cells, cytokines, chemokines, and extracellular matrix (7, 8). The non-tumorous cells mainly include vascular endothelial cells, lymphatic endothelial cells, innate immune cells (Tumor-associated macrophages (TAMs), Dendritic cells (DCs), natural killer (NK) cells), acquired immune cells (T and B lymphocytes), and various stromal cells, such as cancer-associated fibroblasts (CAFs) (7, 8). The TME is a determinant of tumor heterogeneity and interactions within these cells can restructure the TME to regulate tumor progression (9). A constant tumor antigen stimulation and activation of immune system lead to the exhaustion or alteration of relevant effector cells in TME to the extent that these cells have defective function or even assist in tumor growth, resulting in an immunosuppressive microenvironment (8). Therefore, the correlation studies between immune cell infiltration and clinical outcome of tumor will help in finding drug targets to improve patients' survival.

Several studies have confirmed that tumor immuno-therapy combined with an autologous dendritic vaccine targeting T lymphocytes can improve the prognosis of patients with metastatic prostate cancer (9, 10). The tumor-promoting role of glioma-associated macrophages (GAMs), regulatory T cells (Treg), and the inactivation of natural killer (NK) cells in the TME weakens the anti-tumor effect and is closely related to the formation of glioma immunosuppressive microenvironment (7, 11). Knowledge of the proper functioning of infiltered immune cells will help in designing effective measures and strategies that will positively impact anti-tumour immunotherapy.

2. Objectives

The "basket clinical trial" is a treatment strategy under the new understanding of "treating different diseases with the same treatment." Patients who have a common altered molecular pathway for multiple diseases are treated under "basket clinical trial" (12). Generally, SRC kinase is hyperactivated in many types of cancer, including colon cancer and breast cancer, thus becomes a promising target for pan-cancer treatment. However, the mechanism underlying the hyperactivation of SRC in multiple cancers has not been understood fully. It has been hypothesized that this commonly expressed gene interacts with immune infiltration and may be a potential treatment target considering basket trials. Therefore, the present study aims to reveal role of *SRC* gene in pan-cancer, and potential pharmaceutical predictions. This study uses bioinformatic tools to analyze the relationship between *SRC* genes, the immune microenvironment, and the mechanisms regulating cancer progression.

3. Materials and Methods

3.1. The Relationship Between SRC and Survival in Pan-Cancer

Kaplan-Meier Plotter (13) was used to analyze the effect of *SRC* gene expression on the overall survival (OS) and relapse-free survival (RFS) of 20 cancer types, including kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), pancreatic adenocarcinoma (PAAD), testicular germ cell tumor (TGCT), thymoma (THYM), uterine corpus endometrial carcinoma (UCEC), bladder urothelial carcinoma (BLCA), stomach adenocarcinoma

(STAD), thyroid carcinoma (THCA), esophageal carcinoma (ESCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), esophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), pheo-chromocytoma and paraganglioma (PCPG), pancreatic adenocarcinoma (PAAD), and Sarcoma (SARC). Next, log-rank test was used to compare the OS and Recurrence-Free Survival (RFS) between low- and high-*SRC* expression groups. A p-value < 0.05 was considered statistically significant. The expression profile and clinical information of cancers were sourced from TCGA database.

3.2. Immune Infiltration Analysis of Pan-Cancer

The TIMER2.0 database (14) was applied to analyze tumor purity and the immune infiltration of neutrophils, macrophages, dendritic cells, $CD8^+$ T cells, $CD4^+$ T cells, and B cells. Correlation among *SRC* expressions and tumor purity and immune infiltration of these six immune cell types were calculated. The CIBERSORT algorithm was used to ensure their relationship and to evaluate the infiltration of neutrophils, macrophages, dendritic cells, $CD8^+$ T cells, $CD4^+$ T cells, and B cells, followed by their correlation analysis. A p-value < 0.05 was considered significantly correlated. Similarly, gene expression data of cancer samples were downloaded from TCGA database.

3.3. Analysis of SRC Co-Expressed Genes in Pan-Cancer

The LinkedOmics database (15) identified genes that co-expressed with *SRC* in different cancer using Pearson's correlation and were displayed in the heatmaps. Metascape online tool (https://metascape.org/gp/index.html#/main/step1) was used to analyse the biological function of the *SRC* co-expressed genes. The STRING database (https://string-db.org/) helped explore the interactions of *SRC* co-expressed genes, and Cytoscape software was used to construct and visualise the protein-protein interaction (PPI) network. Moreover, the MCODE plug-in was applied to screen hub modules in the PPI network. The clusterProfiler package analysed the biological function of genes in hub modules. Next, we chose the interesting candidate genes based on their biological function, and the correlation between their expressions and immune infiltration was calculated.

3.4. Statistical Analysis

R software (version 3.6.1) was used to conduct all statistical analyses. Log-rank test was used for comparing the OS and RFS between low- and high-*SRC* expression group. Correlations between gene expressions and proportion of immune cell infiltration were calculated by Spearman method. is seen as the significant difference.

4. Results

4.1. The Expression of SRC Is Related to OS and RFS in 11 Cancer Types

By Kaplan-Meier Plotter, we observed that patients with higher expressions of *SRC* had significantly better OS in BLCA, STAD, and THCA, while patients in KIRC, KIRP, LIHC, PAAD, TGCT, THYM, and UCEC had significantly worse OS (**Fig. 1A-1J**). Moreover, the patients in ESCA, KIRP, LIHC, PAAD, and THCA groups were observed to have markedly worse RFS with increased *SRC* expression (**Fig. 1K-1O**). This indicates that *SRC* may play an important role in the prognosis of BLCA, STAD, THCA, ESCA, KIRC, KIRP, LIHC, PAAD, TGCT, THYM, and UCEC.

4.2. The Expression of SRC Is Closely Correlated with M1 Macrophages in Multiple Cancers

Thus, we detected the relationship between *SRC* and immune cell infiltration in BLCA, STAD, THCA, ESCA, KIRC, KIRP, LIHC, PAAD, TGCT, THYM, and UCEC. Interestingly, both TIMER2.0 (**Fig. 2A**) CIBERSORT (**Fig. 2B**) databases revealed that *SRC* expressions in LIHC, TGCT, THCA were positively correlated with macrophage infiltration ($p<0.05$). However, for THYM, we found conflicted results that the expression of *SRC* was found to be negatively correlated with macrophage infiltration by TIMER, but positively correlated by CIBERSORT. Moreover, according to TIMER2.0, $CD4^+$ T cells and Dendritic Cell also demonstrated similar correlation patterns in LIHC, TGCT, THCA ($r<0$) and THYM ($r<0$) significantly.

Correlation studies between the expressions of *SRC* and marker genes of monocytes/M1/M2/ TAMs (16) were conducted to demonstrate the relationship between *SRC* and macrophages further. We found that in LIHC, TGCT, THCA and THYM, the expression of *SRC* was positively correlated with all the four macrophage subtypes using

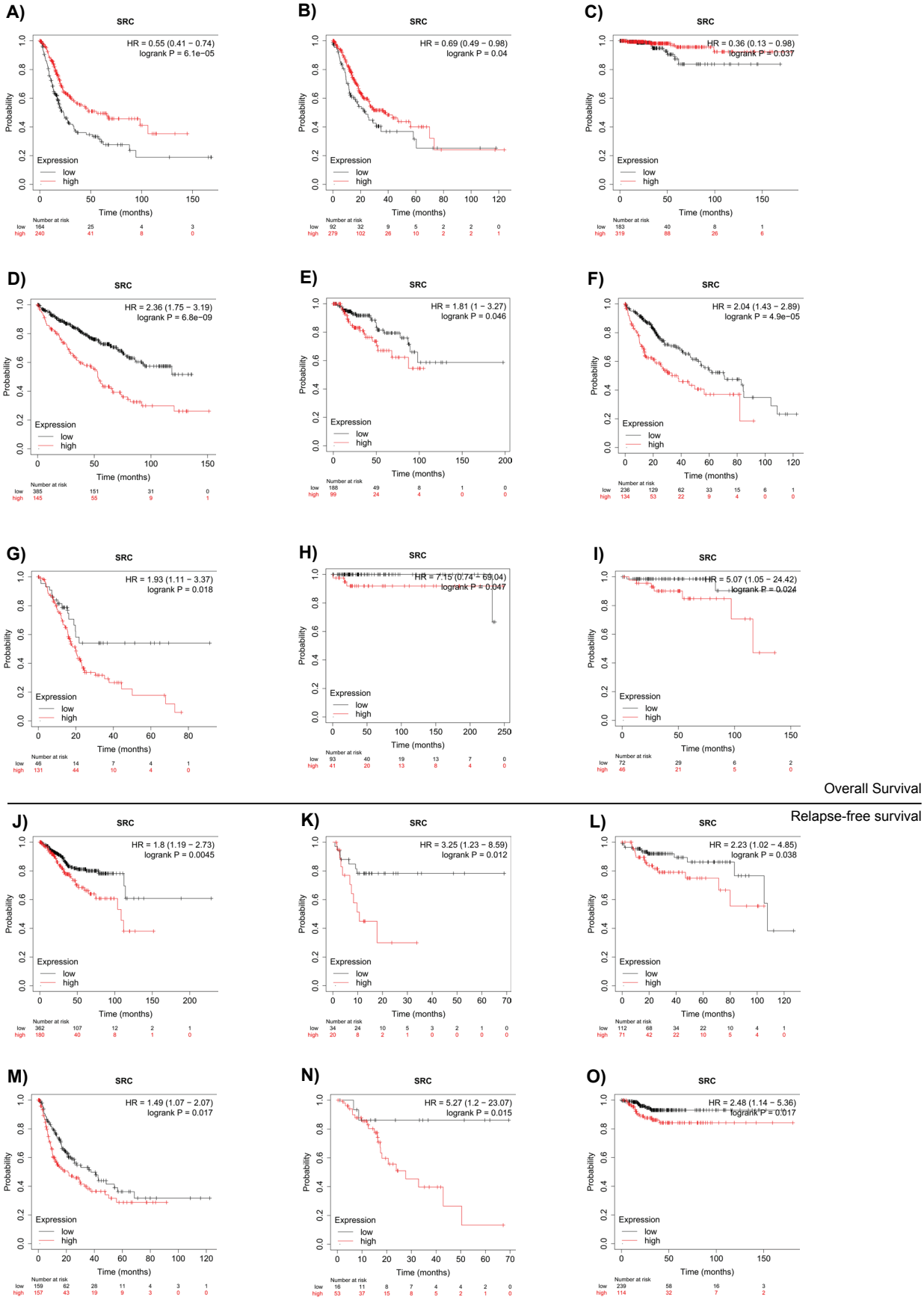

Figure 1. Legend continued on next page.

TIMER2.0 database (**Fig. 2C**) and GEPIA2 database (**Fig. 2D**), especially M1 macrophages (LIHC: IRF5, r = 0.37/ 0.34, PTGS2, r= 0.298/ 0.29; TGCT: NOS2, r = 0.52/ 0.5, IRF5, r = 0.53/ 0.38, PTGS2, r = 0.46/ 0.3; THCA: IRF5, r = 0.44/ 0.32; THYM: NOS2, r = 0.21/ 0.46, IRF5, r = 0.5/ 0.43, PTGS2, r = 0.52/ 0.26), indicating that *SRC* may contribute to the M1 macrophage polarisation in LIHC, TGCT, THCA, and THYM.

4.3. SRC Co-Expressed Genes Are Associated with Metabolism

Next, we investigated the genes that co-expressed with *SRC* in LIHC, TGCT, THCA, and THYM. We observed that the top 50 genes positively or negatively correlated with *SRC* in LIHC, TGCT, THCA, and THYM and were shown in the heatmaps (**Fig. 3 and Table S1-S4**).

The results of Metascape inferred that *SRC* co-expressed genes were mainly enriched in the biological functions associated with metabolism, including positive regulation of ion and anion transport, circulatory system process, lipid metabolic process, lipid biosynthesis process, and lipid metabolism (**Fig. 4A**). Next, the top 100 *SRC* co-expressed genes in LIHC, TGCT, and THCA and the top 200 *SRC* co-expressed genes in THYM were analysed using the STRING database to construct the PPI network (**Fig. 4B**). Further, module analysis was performed by MCODE plug-in which resulted in extraction of four hub modules from the PPI network (**Fig. 4B**). Functional enrichment analysis showed that genes in module 1 were significantly enriched into peroxisomal protein transport, peroxisomal lipid metabolism, and 7-oxo-C and 7-beta-HC pathways. Genes in module 2 were mainly involved in vesicle-mediated transport, exocytosis, post-translational protein phosphorylation, and complement and coagulation cascades. However, module 3 genes were closely associated with endocytosis, and module 4 genes participated in lipid synthesis and metabolism (**Fig. 4C**).

4.4. SRC May Link Lipid Metabolism and Macrophage Polarisation in Cancer

Functional enrichment studies of *SRC* and co-expressed genes indicated that lipid metabolism has an important role in LIHC, TGCT, THCA, and THYM. Considering the relationship between *SRC* and macrophages, we calculated the correlations between macrophages and *SRC* co-expressed genes (Nudix Hydrolase 19 (*NUDT19*), Protein Tyrosine Phosphatase Receptor Type J (*PTPRJ*), DAB Adaptor Protein 2 (*DAB2*), and CDP-Diacylglycerol Synthase 1 (*CDS1*)) involved in lipid metabolism.

We observed that the expressions of NUDT19, PTPRJ, DAB2 and CDS1 were significantly correlated with the infiltration of macrophages (**Fig. 4D**), i.e. CDS1-macrophage in THCA (cor = 0.385), DAB2-macrophage in THCA (cor = 0.594), NUDT19-macrophage in TGCT (cor = 0.321) and PTPRJ-macrophage in THCA (cor = 0.555) using TIMER. Similarly, CIBERSORT algorithm revealed that the expressions of NUDT19, PTPRJ, DAB2 and CDS1 has moderate correlations with macrophage infiltration in LIHC, TGCT, THCA or THYM (**Fig. 4E**), such as CDS1-macrophage in THYM (cor = 0.4731), DAB2-macrophage in TGCT (cor = 0.6142), NUDT19-macrophage in THYM (cor = 0.4717) and PTPRJ-macrophage in THYM (cor = 0.4751). Further correlation analysis demonstrated that these four *SRC*- and lipid-metabolism-related genes had a close relationship with M1 or M2 polarisation in LIHC, TGCT, THCA, and THYM (**Fig. 4F-4I**).

5. Discussion

Usually, the *SRC* is predominantly inactive in the cells. The activated *SRC* kinase is activated by phosphorylation tyrosine residues of the corresponding target protein, thus activating corresponding signaling pathways, including MAPK, STAT, PI3K / AKT and EGFR.

Figure 1. Survival analysis with SRC expression in diverse cancer types using Kaplan-Meier (KM) database. Overall Survival (OS) KM plots stating SRC a good prognosis in **A**) Bladder Urothelial Carcinoma (BLCA), **B**) Stomach Adenocarcinoma (STAD), **C**) Thyroid Carcinoma (THCA). SRC as a bad prognosis in **D**) Kidney Renal Clear Cell Carcinoma (KIRC), **E**) Kidney Renal Papillary Cell Carcinoma (KIRP), **F**) Liver Hepatocellular Carcinoma (LIHC), **G**) Pancreatic Adenocarcinoma (PAAD), **H**) Testicular Germ Cell Tumor (TGCT), **I**) Thymoma (THYM), and **J**) Uterine Corpus Endometrial Carcinoma (UCEC). Recurrence-Free Survival (RFS) KM plot in **K**) Esophageal Carcinoma (ESCA), **L**) Kidney Renal Papillary Cell Carcinoma (KIRP), **M**) Liver Hepatocellular Carcinoma (LIHC), **N**) Pancreatic adenocarcinoma (PAAD) and **O**) Thyroid Carcinoma (THCA).

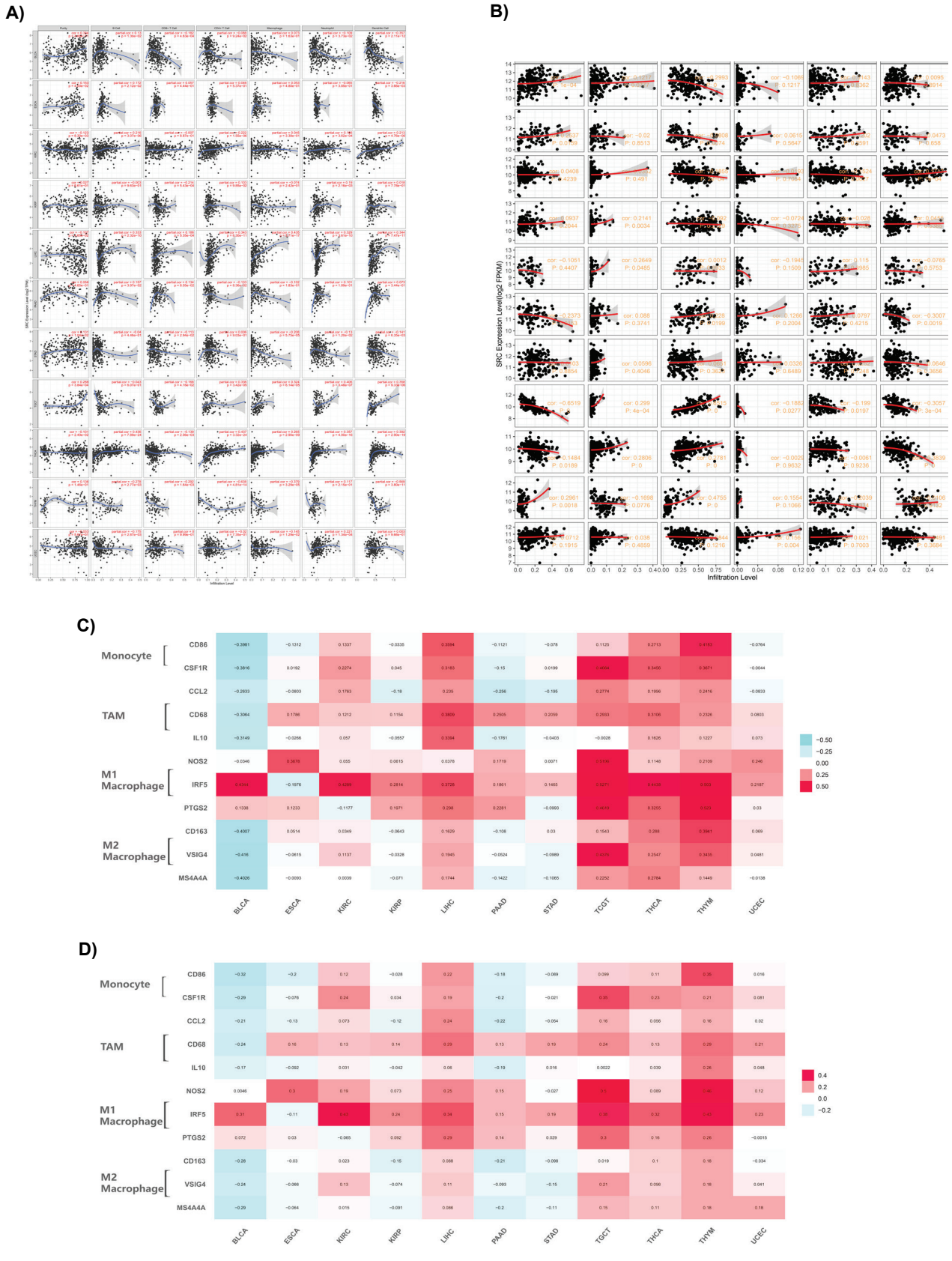

Figure 2. Correlation of SRC expression with immune infiltration analysis. Correlation of SRC expression with immune infiltration levels in the **A**) TIMER database and **B**) CIBERSORT database. Correlation of SRC expression with infiltration of M1 macrophages level in the **C**) TIMER and **D**) CIBERSORT database.

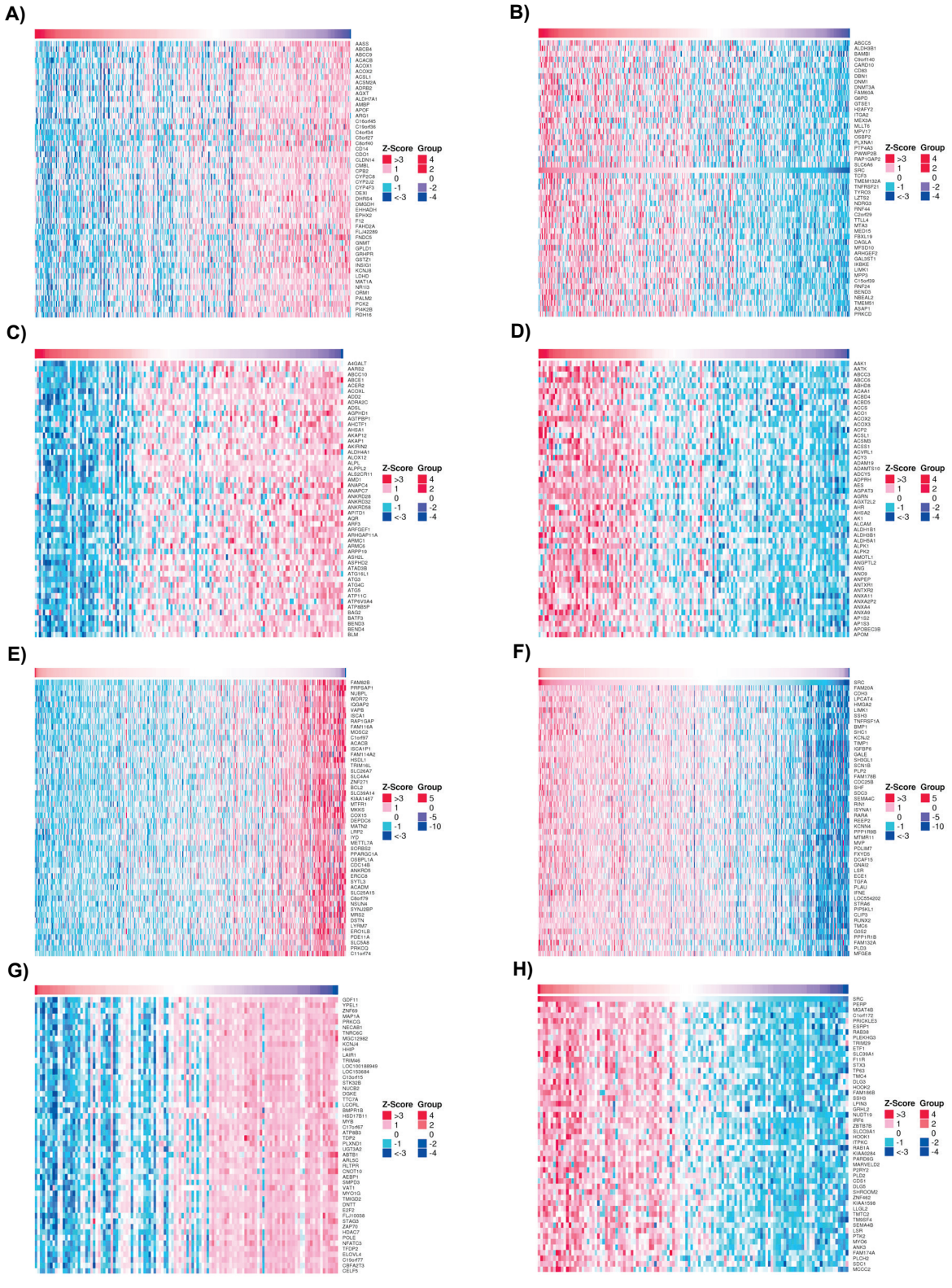

Figure 3. SRC co-expressed genes in pan-cancers. A) negatively, and **B**) positively correlated SRC and co-expressed genes in LIHC as analysed by Pearson test (LinkedOmics). **C**) negatively, and **D**. positively correlated SRC and co-expressed genes in TCGT as analysed by Pearson test (LinkedOmics). **E**) negatively, and **F**) positively correlated SRC and co-expressed genes in THCA as analysed by Pearson test (LinkedOmics). **G**) negatively, and **H**) positively correlated SRC and co-expressed genes in THYM as analysed by Pearson test (LinkedOmics).

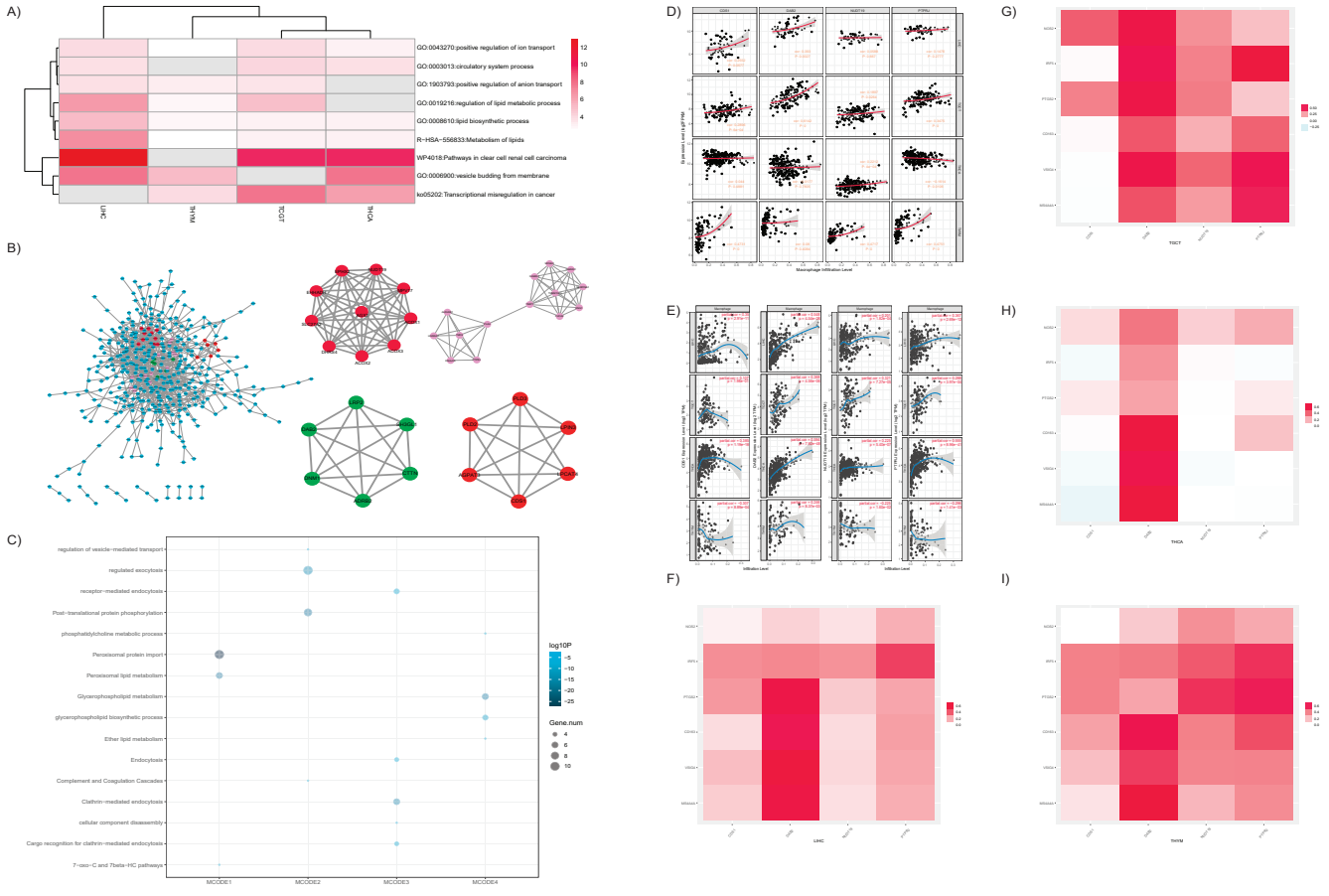

Figure 4. Network of the analysis of the co-expressed genes. A) Top ranking hits after an enrichment analysis (Metascape) against biological functions. **B**) PPI network of SRC co-expressed genes and the module as identified by MCODE. **C**) Functional enrichment analysis for the modules. The correlations between macrophage infiltration and 4 co-expression gene with SRC in diverse cancer types using the **D**) TIMER and **E**) CIBERSORT algorithm. The correlations between M1, M2 macrophage and four co-expression genes with SRC in diverse cancer types. **F**) LIHC, **G**) TGCT, **H**) THCA, and **I**) THYM. NOS2, IRF5, and PTGS2 are the marker genes for M1 macrophages. CS163, VSIG4, and MS4A4A are the marker genes for M1 macrophages.

These pathways are mostly related to metastasis and the proliferation of tumour cells. This study therefore firstly evaluated the prognosis in various cancers and for cancer patients. There was a significant difference in the OS of the *SRC* expression group among patients in 11 types of cancers. Under the conception of basket trials, it is hypothesized that the common role in the prognosis of *SRC* in multiple cancers might imply a common immune infiltration. Subsequently, the correlation analysis of immune infiltration found that the expression of the *SRC* gene is related to M1 macrophages in four cancers (LIHC, TGCT, THCA, THYM). Following this statement, these four cancer groups that strongly correlated with M1 macrophages were taken further for correlation studies with *SRC* co-expressed genes to understand the response mechanisms of biological signals in special physiological states such as cancers. The results indicate that genes co-expressed with the *SRC* gene may promote tumour growth by regulating lipid metabolism.

It seemed a consensus that upregulated expression of the *SRC* is a risk for the patients' survival (2, 17, 18). In contrast, our results have suggested that higher *SRC* expression was a good prognosis indicator in BLCA,

STAD, and THCA, which conflicts with the previous studies (19-21). To further explore the possible reason for the prognosis, we observed that *SRC* was positively associated with macrophages, especially M1 macrophages in LIHC, TGCT, THCA, and THYM. Our results were consistent with earlier reports that the *SRC* gene is involved massively in macrophages' activation (22). *SRC* gene and its elevated levels have been reported in LIHC (2), TGCT (18, 23), THYM (24), and THCA (25). The elevated *SRC* expression has been associated with the poor prognosis of LIHC patients (26). Upregulation of *SRC* or c-*SRC* is linked to the proliferation, migration, and invasion of TGCT cells (2, 18). For patients with THYM, the higher *SRC* gene is reported as a risk for survival (24, 27). Generally, TAMs are, referred to as macrophages in the solid TME, appear to be an essential bridge between inflammation and tumorigenesis, which is SRC kinase-dependent (28). The inflammation is supposed to drive the elevation of the *SRC* expression and other family members. The studies have demonstrated that *SRC* upregulates the production of multiple cytokines, such as IL-6, required for inflammation and cancer progression in inflammatory macrophages, especially the TAMs (28). This promoting effect of SRC on cytokines could aggregate malignant cells, resulting in tumor size growth. The increased level of *SRC* in macrophages might be seen as a biomarker for tumors growth (LIHC, TGCT, and THYM), and high tumor specificity for the *SRC* gene in macrophages would be effective for these cancers' diagnosis and treatment (2). In contrast, our results showed that THCA (**Fig. 1C**) is associated with good prognosis as compared to previous reports indicating higher expression of *SRC* gene association with poor OS (20). The clinical data from The Cancer Genome Atlas (TCGA) showed that most patients had been treated with pharmaceuticals and radiation, which might have changed the immune system and even TME (29). Thus, profiles of the *SRC* gene and the cells in the TME are changed subsequently. Since the *SRC* gene in THCA suggests a poor prognosis with RFS, we intend to believe *SRC* was a risk indicator for the patients with THCA. In TIMER2.0 we have found that, $CD4^+$ T cells and Dendritic Cells (DCs) are also positively correlated with levels of *SRC* expression in LIHC, TGCT, THCA and negatively correlated with it in THYM. The T-cells that express the CD4 antigen are also known as $CD4^+$ T-cells. It plays an important role in determining how immune responses develop (30). *SRC* gene is reported to asscociated with $CD4^+$ T cells (31). DC as an antigen-presenting cell, is capable of inducing naive T cell activation and effector differentiation. As SRC family is involved in dendritic cell responses, it is deduced that *SRC* gene might participate in DCs' activities (32, 33).

Next, we identified the co-expressed genes with *SRC* and found that these genes are mainly involved in metabolisms such as lipid metabolic process, lipid biosynthesis process, and lipid metabolism, in LIHC, TGCT, THCA, and THYM. Cancer cells are always linked to high demands for energy and material basis for rapid tumor growth. Metabolic reprogramming underlies macrophage activation, and TAMs often undergo metabolic reprogramming to ensure their survival and malignant tumor progression (34). It has been reported that purine metabolism is deregulated in liver cancer (35). Aberrant metabolism of steroids and Vitamin D is suggested to be involved in the progression of TGCT (36).

Similarly, enhanced metabolism of fatty acid and rapid aerobic glycolysis help in the survival of THCA cells(37). Moreover, it has been reported that intervention strategies that changed the metabolism of thymoma patients led to the increase in the active life of patients by three years, which is way longer than the similarly affected patients (26). Hence, there is consideration to studying altered metabolism to treat these cancers.

Subsequently, we constructed a protein interaction network and extracted key modules. We revealed that genes in key modules are closely related to lipid metabolism. Metabolic reprogramming resulting in alterations in lipid production has been reported in TGCT (38), THYM (39), and THCA (40). *De novo* lipogenesis re-activated or enhanced by cancer cells has been widely reported to resolve the lack of lipid resources (40-42). The dysregulation or alterations of lipid metabolism affects the growth of primary tumors and mediates tumor progression and metastasis in cancer (42). Therefore, targeting metabolic pathways has been considered as novel therapy in cancer.

By extracting the NUDT19, PTPRJ, DAB2, and CDS1 genes that co-expressed with *SRC* involved in lipid metabolism, we observed that they are closely related to the polarization of M1 and M2 macrophages. So far, the interactions among these genes are rarely reported. However, some functions of the four genes in lipid metabolism are found. *SRC* phosphorylates

Lipin 1, catalyzing the conversion of fatty acids taken up by cells into glycerolipids, especially phospholipids. Studies have shown that phosphorylation of Lipin 1 could accelerate cancer cell growth and promote tumor progression and metastasis in mouse models. The phosphorylation level of Lipin 1 was significantly increased in clinical breast cancer samples and has been shown to be closely related to the tumor size, lymph node metastasis, tumor recurrence time, and patient survival (43). PTPRJ is suggested to positively regulate *SRC* and drive oncogenic signaling (44). In addition, DAB2 has been shown to regulate the MAPK pathway and SRC activity (45). As NUDT19, PTPRJ, and CDS1 are co-expressed with *SRC*, the involvement of them in lipid metabolism by regulating SRC activity via the aforementioned pathways are thus inferred.

The interaction between lipid metabolism and macrophages is crucial for tumor cell growth. DAB2 has premetastatic activity and are highly expressed in tumor-infiltrating TAMs (46) for the premetastatic activity of TAMs. Higher expression of DAB2 in TAMs in patients with cancer correlates with a worse prognosis. It is then inferred that DAB2 might promote M2 polarization. It has been found that enhanced fatty acid oxidation capacity of TAMs in LIHC promotes the activation of M2 macrophages (47). The researchers also found that hepatocellular carcinoma-derived TAMs promoted IL-1β secretion by generating reactive oxygen species (ROS), thereby supporting the invasive ability of hepatoma cells *in vitro* and blocking fatty acid oxidation which could attenuate the effect of TAMs (48).

In thyroid cancer, TAM inhibits the synthesis of sphingomyelin and phosphatidylethanolamine and ROS production, thus attenuating the tumor-promoting activity of TAMs (42). Similarly, in THCA, TAMs are found with the increased synthesis of sphingomyelin and phosphatidylethanolamine along with increased M2-like macrophage phenotype markers and ROS expression, which contribute to the maintenance of genomic instability (41). There are few reports on the reprogramming of phospholipid metabolism of TAMs in TGCT and THYM, but the interaction between lipid metabolism and activation of TAMs is still a research hotspot for researchers.

The growth of the tumor requires a large volume of nutrients that results in nutrient-poor TME. The TME that is glucose-deficient has impaired T-cell function and anti-tumor immune responses. Thus, tumors evade immune surveillance. The nutrient-poor TME could result in DAB2-mediated (possibly other co-expressed genes *NUDT19*, *PTPRJ*, and *CDS1*) upregulation of *SRC* and, subsequently, metabolic reprogramming. Through reprogramming, TAMs avoid participating in nutrient "scramble" in the nutrient-poor microenvironment on the one hand and "passively" changes the functional phenotype and polarize TAMs towards the M2 type with pro-tumor effects on the other hand.

We demonstrated that the studies on *SRC* gene's association with macrophages polarization and lipid metabolism and the targeting of metabolism might be a treatment strategy for the pan-cancer. The results deduced by our study need further investigation and validation based on the experiments in molecular biology. Moreover, our primary focus was on a single gene (*SRC*) and we failed to find the key regulators of *SRC* upregulation in pan-cancers. Future studies in pan-cancer should examine the *SRC* gene profiles first *in vivo* that should be integrated with epigenetic analysis and include clinical cases.

6. Conclusion

A series of online database, including TIMER, CIBERSORT and Cytoscape software and its plug-in MCODE were applied to construct in silico work for *SRC* expression in Pan-cancer. The analysis decipher that *SRC* had a different prognostic role in pan-cancer and is related to macrophages infiltration and involved in lipid metabolism. In conclusion, our study has reported that *SRC* is associated with prognosis in multiple cancer types and may affect macrophage polarization through lipid metabolism. It has been established that different tumors have different molecular types, and there are varied immune cell infiltrations in the TME. The malignant phenotype of tumor cells is relatively uniform and the enrichment degree of various immune cells often determines the characteristics of TME, which further affects the response to targeted immunotherapy. Thus, our results indicate that cancer treatment targeting metabolism, such as reducing metabolism of fatty acid might be a novel direction for LIHC, TGCT, THCA, and THYM therapy, with the idea of basket trials.

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