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Upregulation of Src by lncRNA Modulates FAK-dependent ErbB Signaling in Hepatocellular Carcinoma

Xiuli Mao[1], Wenxian Lin[2,*], Yueyong Li[3*], Lizhu Tang[4,5], Xiamin Zhang[1]

1 Graduate School, Youjiang Medical University for Nationalities, Baise, 533000, China. 2 Department of Phase I Clinical Research, The People's Hospital of Guangxi Zhuang Autonomous Region,Nanning 530016,Guangxi,China. 3 Department of Oncology, The Affiliated Changsha Central Hospital, Hengyang Medical School, University of South China, Changsha 410004, China. 4 Ji'nan University, 510632, Guangzhou, China. 5 Department of Interventional oncology, Affiliated Hospital of Youjiang Medical University for Nationalities, Baise 533000, China.

*Corresponding to: Wenxian Lin, Yueyong Li

Abstract: Objective: To investigate Src expression in hepatocellular carcinoma (HCC) using bioinformatics approaches, focusing on its modulation of focal adhesion kinase (FAK/PTK2) via ErbB signaling and the regulatory role of long non-coding RNAs (lncRNAs). Methods: Fifty-five EGFR pathway genes extracted from QuickGO were uploaded to the BGI Multi-Omics Platform to generate FPKM matrices of tumor and adjacent tissue. Differentially expressed genes (DEGs) were defined by $q < 0.05$ and $|\log_2 FC| > 1$. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment were performed with DAVID; a protein–protein interaction (PPI) network was constructed in STRING and Cytoscape, and the top 12 hub genes were selected by degree. The same platform generated a hierarchical clustering heatmap and GO level-2 classification. Tumor Immune Estimation Resource 2.0 (TIMER2.0) was used to profile Src expression across cancers, HCC survival, and immune infiltration. Src/miRNA/lncRNA expression matrices were exported; transcripts with $q < 0.05$ were filtered in Excel, and a lncRNA-miRNA-Src competing endogenous RNA (ceRNA) network was built and visualized. Results: Src was overexpressed in HCC. It enhanced FAK activity and ErbB signaling, thereby accelerating HCC initiation and progression. Src activity correlates tightly with tumor aggressiveness. lncRNAs up-regulated Src and positively associate with angiogenesis, proliferation, and metastasis in HCC. Conclusion: High Src expression predicts poor prognosis in HCC. LncRNAs modulate Src levels and ErbB pathway activity.

Keywords: HCC, Src, lncRNA, ErbB signaling pathway

1 INTRODUCTION

China accounted for 35.9% of global liver cancer cases, with incidence and mortality rates exceeding global averages. Males experienced a significantly higher burden than females. Hepatitis B, hepatitis C, and alcohol use were the primary etiologies. The number of cases doubled over the past 30 years, primarily driven by population aging[1]. The hepatocellular carcinoma (HCC) treatment faces a triple dilemma of pronounced chemoresistance, extremely high postoperative recurrence/metastasis rates, and dismal systemic therapy outcomes, making it one of the most challenging solid malignancies to treat[2-4]. Even after curative resection or ablation, early recurrence and distant metastasis remain frequent, yielding poor prognosis. HCC proliferation, invasion, and metastasis are tightly controlled by multiple signaling pathways and complex molecular networks[5]. Clarifying the core drivers of relapse, drug resistance, and dissemination will uncover new therapeutic targets and provide a rational basis to overcome resistance, block recurrence, and improve clinical outcome.

The Src family comprises nine non-receptor tyrosine kinases. Among them, the proto-oncogene Src is the best characterized and is linked to multiple human diseases. Upon upstream activation, Src autophosphorylates and amplifies proliferative, pro-survival, and migratory pathways. Active Src phosphorylates focal adhesion kinase (FAK) at multiple tyrosines. The resulting Src-FAK positive feedback loop markedly accelerates tumor cell invasion and metastasis[6, 7]. Emerging evidence shows that non-coding RNAs drive HCC progression[8]. The long non-coding RNA (lncRNA) DBH-AS1 promotes HCC via the miR-138/FAK/Src/ERK axis, providing a molecular rationale for its clinical targeting[9]. Combining PARP1 and Src inhibitors elicits synthetic lethality in HCC. This strategy may benefit patients with high Src activity or intrinsic PARP1-inhibitor resistance[10]. Recent work has focused on

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lncRNAs that regulate Src. Several lncRNAs contain small open reading frames (ORFs). The resulting micropeptides tether Src/YES1 to the membrane, trigger downstream signaling, and fuel hepatocarcinogenesis[11]. Thus, lncRNA-mediated control of Src expression is tightly linked to HCC progression[12]. Growing attention is directed toward lncRNAs as diagnostic markers and therapeutic targets for HCC. Here, we used bioinformatics to map Src-driven signaling in HCC, built a protein–protein interaction (PPI) network, and explored how lncRNAs modulate Src and thereby affect FAK-ErbB signaling. The goal was to identify novel molecular targets and biomarkers for HCC therapy.

2 Tools and Methods

2.1 Retrieval of Pathway-Specific Genes

The keyword "epidermal growth factor" (EGF) was queried in QuickGO (https://www.ebi.ac.uk/QuickGO/GTerm?id=GO:0048471). The Gene Ontology (GO) term "EGFR signaling pathway" was selected, restricted to Mus musculus, and 55 official gene symbols (SYMBOL) were exported.

2.2 Identification of Differentially Expressed Genes

The 55 SYMBOL were uploaded to the BGI Multi-omics Platform (https://biosys.bgi.com/#/main) to obtain Gene IDs. Expression matrices for tumor (H) and adjacent non-tumor (C) liver were downloaded. Genes with $q < 0.05$, $|\log_2 FC| > 1$ and FPKM > 1 in either H or C were retained, yielding up- and down-regulated sets.

2.3 Pathway Enrichment Analysis

The 55 candidate genes were subjected to KEGG pathway enrichment analysis using the DAVID database to verify significantly enriched biological pathways. The EGFR signaling pathway, as a classical cell signal transduction pathway, has its molecular interaction network systematically curated in multiple authoritative databases (including KEGG, Reactome, and PANTHER), which integrate multi-dimensional information encompassing genomics, biological pathways, and disease associations[13], thereby providing a standardized annotation framework for pathway enrichment analysis. GO terms were grouped into molecular function (MF), cellular component (CC), and biological process (BP).

2.4 PPI Network Construction

Differentially expressed genes (DEGs) were uploaded to STRING to build a protein–protein interaction network. The network was imported into Cytoscape 3.9.1 and the top 12 hub genes were selected by degree centrality to generate the core PPI map.

2.5 Clustering Heatmap of DEGs

The 29 DEGs were re-uploaded to the BGI Multi-omics Platform. Expression values were $\log_2(\text{value} + 1)$ transformed and samples were split into tumor and adjacent non-tumor groups. A hierarchical clustering heatmap was generated.

2.6 GO Level-2 Classification

The DEGs were re-uploaded to the BGI Multi-omics Platform. GO enrichment was run and results were parsed to level-2 terms.

2.7 Functional Annotation Chart for Src

DEGs were uploaded to DAVID. The Functional Annotation Chart containing Src and FAK was downloaded. Entries with FDR (Benjamini) < 0.001 were filtered in Excel and plotted as bar charts of enrichment score or gene count.

2.8 Pan-Cancer Src Expression

Src expression across tumors was queried with Tumor Immune Estimation Resource 2.0 (TIMER2.0) [14].

2.9 Survival Curve

Src was entered into TIMER, disease set to HCC, and the overall-survival curve was generated.

2.10 Immune-Infiltration Map

TIMER 2.0 Immune-Gene module was used. Spearman partial correlation, adjusted for tumor purity, between Src expression and immune-cell infiltration in HCC was computed; partial cor and p values are reported[15].

2.11 Src Competing Endogenous RNA (CeRNA) Network

Src, miRNA and lncRNAs matrices were exported from the BGI Multi-omics Platform. miRNAs down-regulated in tumor with $q < 0.05$ and lncRNAs up-regulated in tumor with $q < 0.05$ were selected in Excel. Validated miRNA-Src targeting interactions were integrated to build the lncRNA–miRNA–Src competing endogenous RNA network and visualized in Cytoscape.

3 Results

3.1 Pathway Analysis of Src

GO and KEGG enrichment analyses revealed that Src acts upstream of FAK activation. Elevated Src activates FAK and thereby potentiates ERBB signaling, driving angiogenesis, proliferation, and metastasis (Figure 1).

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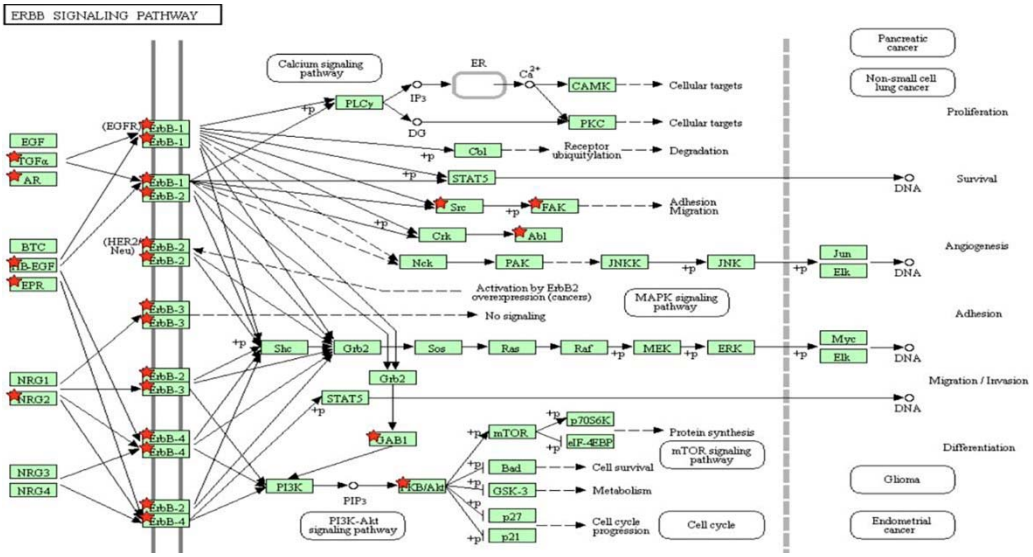

FIGURE 1. ERBB SIGNALING PATHWAY

Figure Legend: Red stars denote key genes of the signaling pathway (https://david.ncifcrf.gov/).

3.2 PPI NETWORK AND HUB GENES IN HCC

DEGs identified with the BGI Multi-omics Platform were imported into STRING and the top 12 hubs were selected by degree in Cytoscape. The network contains 24 nodes (Figure 2); Hbegf, Akt1, Src, FAK, Adam17, Areg, Erbb2, Erbb3 and Erbb4 occupy the centre, indicating dense inter-connectivity. Figure 3 narrows the list to 12 hubs: Hbegf, Src, Areg, Erbb2, Erbb3, Erbb4, Ereg, Egfr, Gab1, FAK, Btc and Nrg2. Together with the ERBB pathway map (Figure 1), these genes form a sequential cascade whose encoded proteins interact directly and may drive HCC progression.

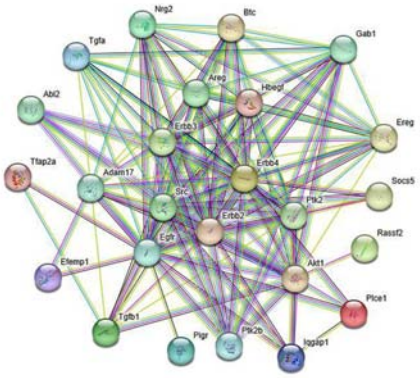

FIGURE 2. PPI NETWORK OF DIFFERENTIALLY EXPRESSED GENES

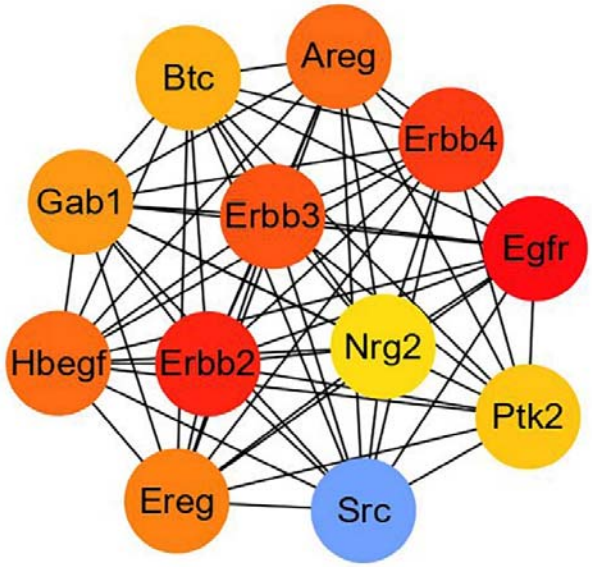

FIGURE 3. NETWORK OF TOP 12 SYMBOL

3.3 CLUSTERING HEATMAP OF DEGS

A hierarchical heatmap generated with the BGI Multi-omics Platform shows coordinated up-regulation of upstream genes (HB-EGF, ERBB, Src) and the downstream target FAK in tumor tissue (Figure 4). The tight co-clustering indicates synchronous activation of this signaling axis in HCC.

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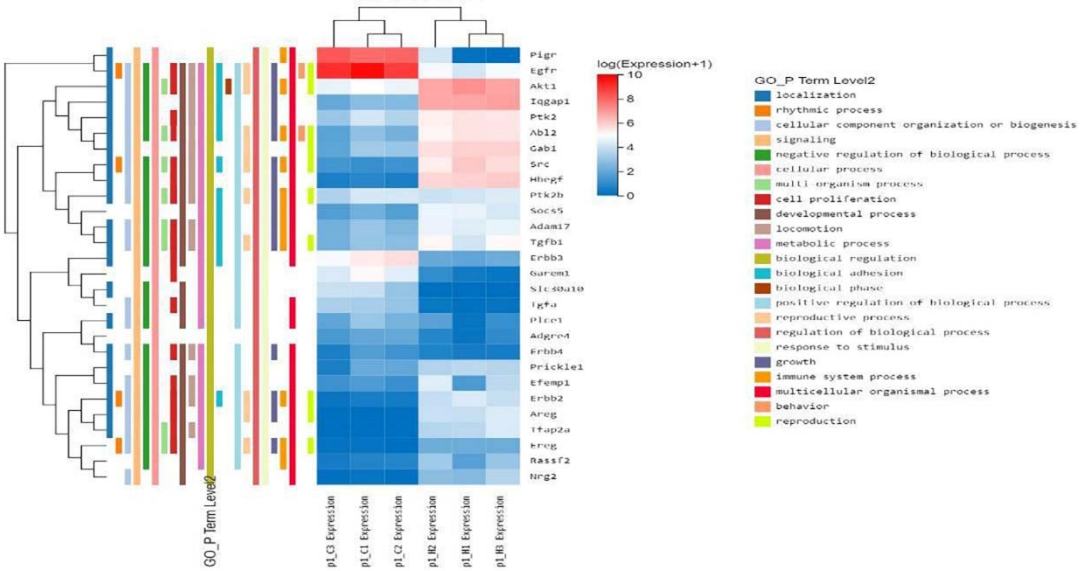

FIGURE 4. HIERARCHICAL CLUSTERING HEATMAP OF DIFFERENTIALLY EXPRESSED GENES

Figure Legend: Clustering heatmap of DEG expression with biological process annotation. The central heatmap shows the expression patterns of differentially expressed genes. Gene names are listed on the right. The left panel indicates the associated biological processes (BP). The rightmost color bar represents cluster assignment, with distinct colors denoting different clusters.

3.4 *GO LEVEL-2 CLASSIFICATION*

The numbers and enrichment scores of DEGs across GO level-2 terms were calculated with the BGI Multi-omics Platform; results are displayed in Figure 5.

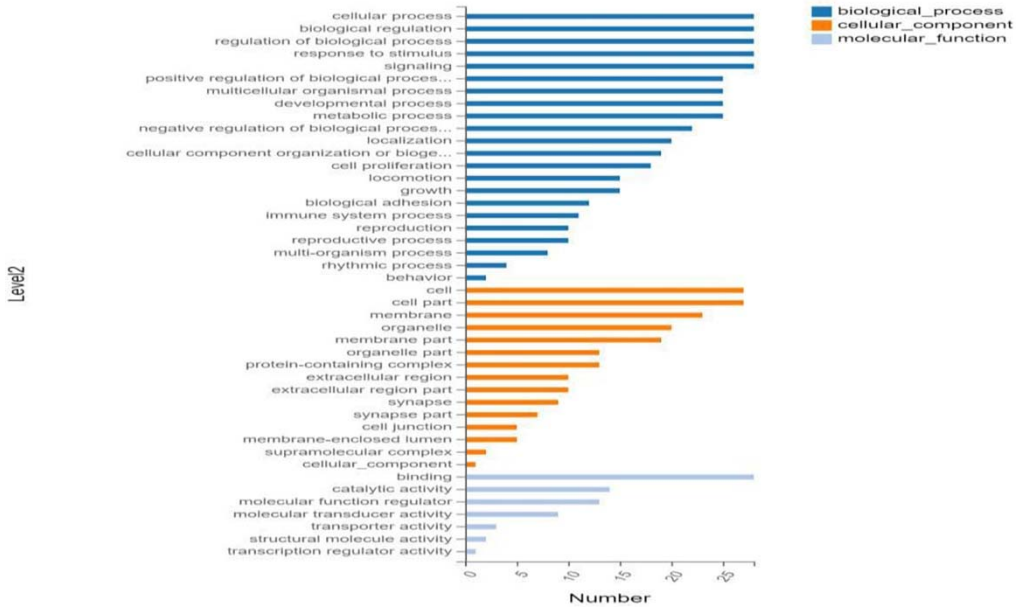

FIGURE 5. LEVEL-2 GENE ONTOLOGY (GO) CLASSIFICATION

3.5 *FUNCTIONAL ENRICHMENT OF SRC*

DAVID analysis (Figure 6) assigned Src-related DEGs to three KEGG pathways, ten biological processes (BP), one cellular component (CC) and seven molecular functions (MF). Src localizes to the plasma membrane and activates downstream signals via phosphorylation. Aberrant Src activity drives HCC by inducing angiogenesis and proliferation, primarily through the ErbB and EGFR signaling pathways.

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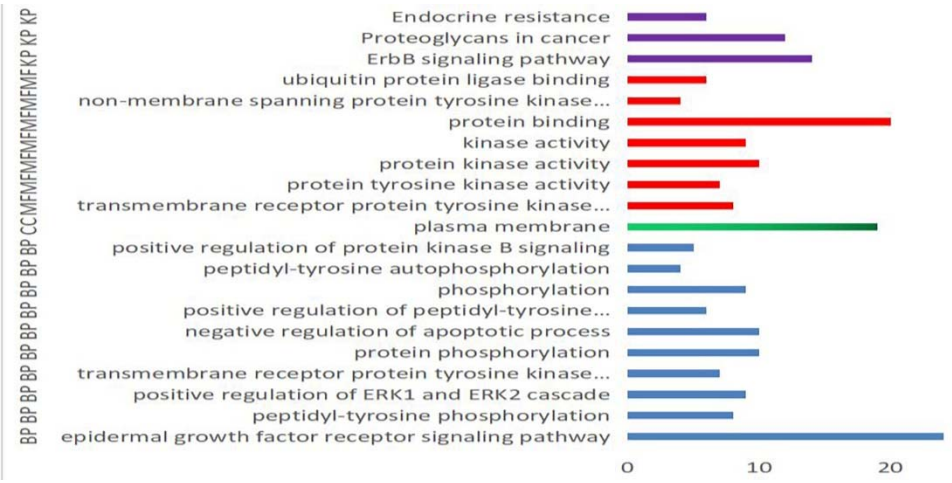

FIGURE 6. FUNCTIONAL ANNOTATION CHART OF SRC

3.6 *PAN-CANCER AND HCC EXPRESSION OF SRC*

TIMER2.0 analysis revealed Src up-regulation across multiple tumors, including BLCA, CHOL, ESCA, KICH, LIHC, THCA, READ and LUSC (Figure 7). In HCC, Src levels were markedly higher than in adjacent liver ($P < 0.001$), indicating a positive association between Src over-expression and HCC.

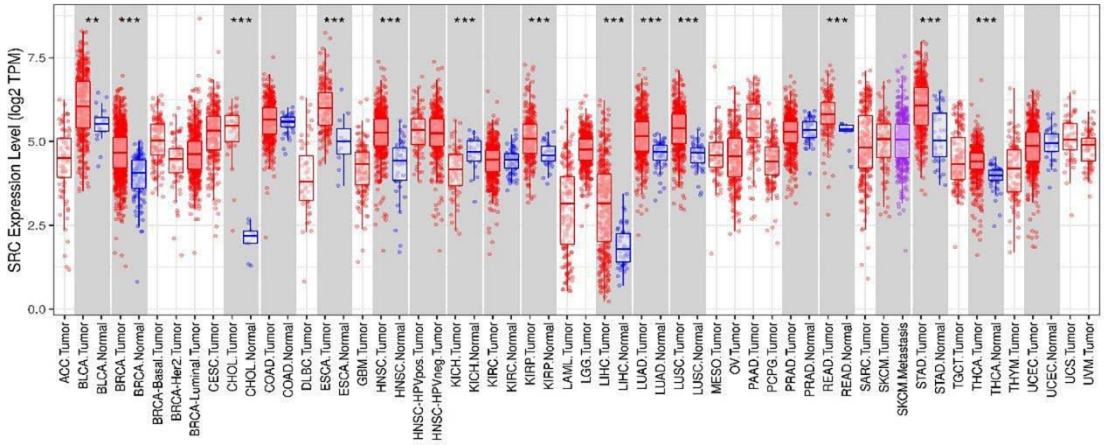

FIGURE 7. PAN-CANCER EXPRESSION PROFILE OF SRC

Figure Legend: P-value significance codes: $0 \leq$ *** $< 0.001 \leq$ ** $< 0.01 \leq$ * $< 0.05 \leq \cdot < 0.1$; $P < 0.05$ was considered statistically significant.

3.7 *SURVIVAL ANALYSIS OF SRC IN HCC*

Kaplan-Meier analysis with the survival module of TIMER2.0 (Figure 8) showed $P = 0.03$. Patients with low Src expression had higher 5-year (60-month) survival than those with high expression, indicating that elevated Src predicts poor prognosis in HCC.

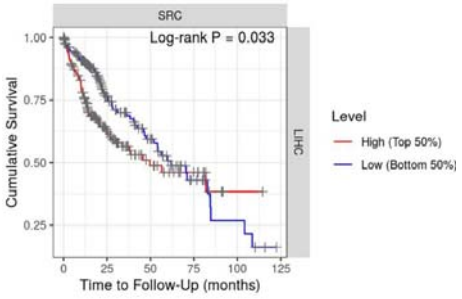

FIGURE 8. SURVIVAL CURVE OF HEPATOCELLULAR CARCINOMA

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Figure Legend: X-axis, time to follow-up (months); Y-axis, cumulative survival. Samples were split into Src-high (red) and Src-low (blue) groups using the 50 % cut-off. Log-rank P = 0.033, statistically significant.

3.8 *Immune-Infiltration Analysis Of Src In HCC*

TIMER2.0 gene module revealed positive correlations between Src expression and infiltration of B cells, CD8+ T cells, CD4+ T cells, macrophages, dendritic cells, and neutrophils in HCC (all $P < 0.05$, Figure 9).

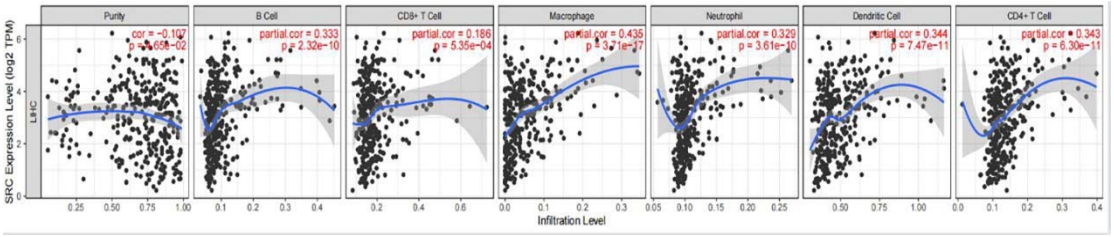

Figure 9. Immune Infiltration Profile Of Src In Hepatocellular Carcinoma

3.9 *PPI Network Analysis Of Src Mrna, Mirna, And Lncrna In Hepatocellular Carcinoma*

Figure 10 PPI network of Src-related miRNAs and lncRNAs in hepatocellular carcinoma constructed using Cytoscape. (A) Network visualization showing interactions between Src (yellow node), miRNAs (red nodes), and lncRNAs (blue nodes). (B) Regulatory mechanism: upregulated miRNAs inhibit Src expression by degrading Src mRNA, whereas upregulated lncRNAs promote Src expression through miRNA sponging, leading to activation of MAPK, PI3K/AKT, and EGFR pathways and subsequent HCC progression.

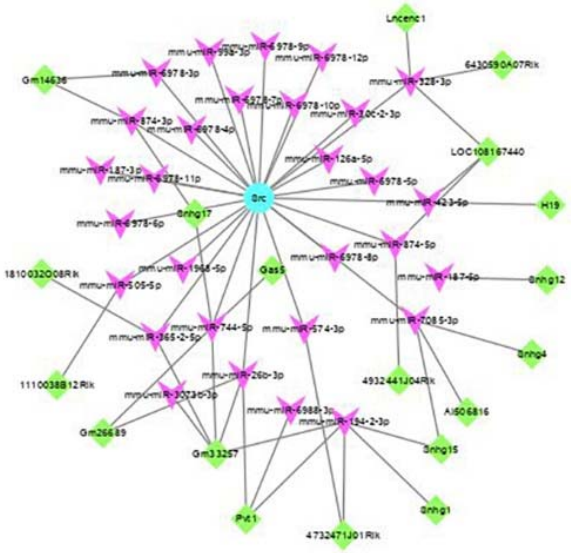

Figure 10. CERNA Network Diagram

4 Discussion

HCC is common and highly vascular. Early tumors are resected; intermediate or advanced cases receive trans-arterial chemoembolization, ablation, iodine-125 brachytherapy, or targeted/immune agents[14, 16, 17]. Outcomes remain poor, resistance is frequent, and five-year survival is low[18]. The pathogenesis of HCC remains unclear. Alpha-fetoprotein (AFP), the only widely used marker, lacks sensitivity and specificity, and can be elevated in many diseases. Thus, specific biomarkers for diagnosis and disease monitoring are still missing. Recent intensive research has improved our understanding of HCC development, especially at the molecular level. Therefore, further investigation of HCC progression mechanisms and the discovery of novel therapeutic targets and biomarkers are essential for improving diagnosis, treatment, and prognosis. Recent studies have focused on non-coding RNAs that regulate Src. For example, miRNA-3064-5p suppresses angiogenesis by targeting the FOXA1/CD24/Src axis. Downregulation of miRNA-3064-5p enhances Src expression and promotes angiogenesis, while its upregulation inhibits the expression of vascular endothelial growth factor (VEGF) and angiopoietin, thereby suppressing blood vessel formation in HCC[12]. By base-pairing with complementary sequences, miRNAs direct Src mRNA to degradation or translational repression; thus, reduced levels of Src-targeting miRNAs correlate positively with angiogenesis, proliferation, and metastasis in HCC. lncRNAs are now under intensive investigation. Multiple reports show that non-coding RNAs modulate vascularization, growth, invasion, and dissemination of HCC[9, 12, 19], highlighting several lncRNAs as potential biomarkers for diagnosis and disease monitoring and as novel therapeutic targets. This study primarily investigated the signaling pathways involving Src in HCC through bioinformatics databases. Functional enrichment analysis of Src was performed using annotation and functional enrichment tools in the DAVID database. The results revealed that Src-related differentially expressed genes in HCC were mainly enriched in three signaling pathways, ten biological processes (BP), one cellular component (CC), and seven molecular functions (MF). Src primarily functions at the plasma membrane, where it undergoes phosphorylation to activate downstream signaling pathways, thereby promoting tumor angiogenesis, cancer cell proliferation,

and metastasis. Cytoscape mapped the ERBB PPI network; Src ranked among the top 12 hubs, confirming its central role. Using the BGI Multi-omics Platform, we identified Src-related miRNAs and lncRNAs and built a ceRNA network. In HCC, down-regulated miRNAs fail to effectively suppress Src, while up-regulated lncRNAs further relieve this repression by sponging residual miRNAs, collectively leading to Src overexpression. Phosphorylated Src activates MAPK, PI3K/AKT, and EGFR pathways, driving proliferation, invasion, metastasis, and angiogenesis. TIMER2.0 showed Src expression higher in HCC than adjacent. Multiple bioinformatics analyses, including GEPIA ($P = 0.016$), Kaplan-Meier Plotter ($P = 0.0023$) and UALCAN ($P = 0.00049$), consistently demonstrated that high SRC mRNA expression is significantly associated with shortened overall survival in HCC[20]. TIMER2.0 survival curves (Figure 8) showed that high Src expression predicts poor prognosis in HCC. Src activates FAK and the ErbB pathway, while lncRNAs modulate Src levels to fuel tumor growth. Thus, Src and its lncRNA regulators may serve as biomarkers and therapeutic targets for HCC.

5 Conclusion

Bioinformatics analysis showed that Src expression correlates with HCC development and prognosis, indicating its potential as a biomarker. lncRNA-mediated up-regulation of Src is positively associated with angiogenesis, proliferation, and metastasis, and targeting these oncogenic lncRNAs may provide a new therapeutic strategy. Our in-silico study identifies candidate Src-related drivers of HCC, but experimental validation in larger cohorts is required.

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References

[1]Li M, He H, Zhao X, et al. Burden of liver cancer and underlying etiologies in China from 1990 to 2021: A systematic analysis from the Global Burden of Disease Study 2021. Chin Med J (Engl). 2026. 139(3): 415-423.

[2]Zou Y, Wan X, Zhou Q, et al. Mechanisms of drug resistance in hepatocellular carcinoma. Biol Proced Online. 2025. 27(1): 19.

[3]Papaconstantinou D, Tsilimigras DI, Pawlik TM. Recurrent Hepatocellular Carcinoma: Patterns, Detection, Staging and Treatment. J Hepatocell Carcinoma. 2022. 9: 947-957.

[4]Herrero A, Toubert C, Bedoya JU, et al. Management of hepatocellular carcinoma recurrence after liver surgery and thermal ablations: state of the art and future perspectives. Hepatobiliary Surg Nutr. 2024. 13(1): 71-88.

[5]Das J, Barman B, Sarma P, Das BK, Chetia R, Kalita PP. Signalling pathways in hepatocellular carcinoma (HCC) metastasis and invasion: Molecular mechanisms and therapeutic implications. Biochem Biophys Rep. 2026. 45: 102403.

[6]Mezquita B, Reyes-Farias M, Pons M. Targeting the Src N-terminal regulatory element in cancer. Oncotarget. 2023. 14: 503-513.

[7]Bolós V, Gasent JM, López-Tarruella S, Grande E. The dual kinase complex FAK-Src as a promising therapeutic target in cancer. Onco Targets Ther. 2010. 3: 83-97.

[8]Tian Y, Zhang M, Liu LX, et al. Exploring non-coding RNA mechanisms in hepatocellular carcinoma: implications for therapy and prognosis. Front Immunol. 2024. 15: 1400744.

[9]Bao J, Chen X, Hou Y, Kang G, Li Q, Xu Y. LncRNA DBH-AS1 facilitates the tumorigenesis of hepatocellular carcinoma by targeting miR-138 via FAK/Src/ERK pathway. Biomed Pharmacother. 2018. 107: 824-833.

[10]Luo X, Zheng E, Wei L, et al. The fatty acid receptor CD36 promotes HCC progression through activating Src/PI3K/AKT axis-dependent aerobic glycolysis. Cell Death Dis. 2021. 12(4): 328.

[11]Pang Y, Liu Z, Han H, et al. Peptide SMIM30 promotes HCC development by inducing SRC/YES1 membrane anchoring and MAPK pathway activation. J Hepatol. 2020. 73(5): 1155-1169.

[12]Zhang P, Ha M, Li L, Huang X, Liu C. MicroRNA-3064-5p sponged by MALAT1 suppresses angiogenesis in human hepatocellular carcinoma by targeting the FOXA1/CD24/Src pathway. FASEB J. 2020. 34(1): 66-81.

[13]Kirouac DC, Saez-Rodriguez J, Swantek J, Burke JM, Lauffenburger DA, Sorger PK. Creating and analyzing pathway and protein interaction compendia for modelling signal transduction networks. BMC Syst Biol. 2012. 6: 29.

[14]Chen Z, Xiao Y, Yang P, Wang R. Pan-cancer Analysis Reveals SRC May Link Lipid Metabolism and Macrophages. Iran J Biotechnol. 2023. 21(2): e3325.

[15]Cui X, Zhang X, Liu M, et al. A pan-cancer analysis of the oncogenic role of staphylococcal nuclease domain-containing protein 1 (SND1) in human tumors. Genomics. 2020. 112(6): 3958-3967.

[16]European Association for the Study of the Liver, . EASL Clinical Practice Guidelines on the management of hepatocellular carcinoma. J Hepatol. 2025. 82(2): 315-374.

[17]Singal AG, Llovet JM, Yarchoan M, et al. AASLD Practice Guidance on prevention, diagnosis, and treatment of hepatocellular carcinoma. Hepatology. 2023. 78(6): 1922-1965.

[18]Liu S, Liu Y, Sha M, Li Y, Sheng J. Editorial: Advances in chemotherapy-resistant hepatocellular carcinoma, volume II. Front Med (Lausanne). 2025. 12: 1594854.

[19]Pei S, Chen Z, Tan H, Fan L, Zhang B, Zhao C. SLC16A1-AS1 enhances radiosensitivity and represses cell proliferation and invasion by regulating the miR-301b-3p/CHD5 axis in hepatocellular carcinoma. Environ Sci Pollut Res Int. 2020. 27(34): 42778-42790.

[20]Yao D, Deng Y, Zhang S, et al. Comprehensive Analysis of Prognostic Value and Immune Infiltration of Src Family Kinases in Hepatocellular Carcinoma. Front Biosci (Landmark Ed). 2023. 28(5): 90.