Pan-cancer analysis of the oncogenic effects of G-protein-coupled receptor kinase-interacting protein-1 and validation on liver hepatocellular carcinoma

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Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med.* 2023;32(10):1139–1147

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Funding sources
The study was supported by the major provincial science and technology projects of Yunnan (grant No. 202002AA100007).

Conflict of interest
None declared

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Received on May 18, 2022
Reviewed on June 13, 2022
Accepted on February 12, 2023

Published online on March 30, 2023

Cite as
Wang T, Su K, Wang L, et al. Pan-cancer analysis of the oncogenic effects of G-protein-coupled receptor kinase-interacting protein-1 and validation on liver hepatocellular carcinoma. *Adv Clin Exp Med*. 2023;32(10):1139–1147. doi:10.17219/acem/161157

DOI
10.17219/acem/161157

Abstract

Background. Despite G-protein-coupled receptor kinase-interacting protein-1 (GIT1) being recognized as a new promoter gene in some types of cancer, its effect on human pan-cancers and liver hepatocellular carcinoma (LIHC) remains unclear.

Objectives. To elucidate the molecular mechanisms of GIT1 in pan-cancer and LIHC.

Materials and methods. Various bioinformatics approaches were utilized to elucidate the oncogenic effects of GIT1 on human pan-cancers.

Results. The GIT1 was aberrantly expressed in pan-cancers and associated with the clinical stage. Moreover, the upregulation of GIT1 expression was indicative of poor overall survival (OS) in patients with LIHC, skin cutaneous melanoma (SKCM) and uterine corpus endometrial carcinoma (UCEC), as well as of poor disease-free survival (DFS) in patients with LIHC and UCEC. Furthermore, GIT1 levels were correlated with cancer-associated fibroblasts (CAFs) in adrenocortical carcinoma (ACC), cervical squamous cell carcinoma (CESC) and LIHC. The analysis of single-cell sequencing data revealed an association of GIT1 levels with apoptosis, cell cycle and DNA damage. In addition, multivariate Cox analysis indicated that high GIT1 levels were an independent risk factor for shorter OS in patients with LIHC. Finally, the gene set enrichment analysis revealed INFLAMMATORY_RESPONSE pathway and IL2_STAT5_SIGNALING to be the most enriched in LIHC.

Conclusions. Our data demonstrate the oncogenic effects of GIT1 on various cancers. We believe that GIT1 can serve as a biomarker for LIHC.

Key words: pan-cancer analysis, GIT1, oncogene, liver hepatocellular carcinoma

Background

Liver cancers are associated with elevated mortality rates across the world,[1–3] and while significant advancements have been made in surgical techniques, chemotherapy and other treatment approaches, the 5-year survival rate remains far from satisfactory.[4–6] Moreover, liver cancer is the most common type of cancer in China. Specifically, cancer recurrence at the intermediate or advanced stage occurs in approx. half of the patients. Considering the increase in the incidence and mortality rates of liver cancer, it is crucial to identify new prognostic biomarkers.

G-protein-coupled receptor kinase-interacting protein-1 (GIT1) has been shown to repress the β2-adrenergic receptor pathway and stimulate receptor phosphorylation. Many proteins interact with GIT1 via its various domains. Notably, GIT1 is essential for focal cell migration, adhesion and the development of lamellipodia. The principal roles of GIT1 include focal adhesion remodeling,[7] receptor internalization and transmission of cellular signals.[8] The GIT1 is widely expressed in the brain, liver, lungs, nerves, and blood vessels.[9,10] The expression of GIT1 is upregulated in breast cancer, while its downregulation has been found to regulate the cell progression of breast cancer.[11] The GIT1 can stimulate tumor development by activating extracellular signal-regulated kinase signaling in hepatocellular carcinoma.[12,13] Moreover, GIT1 participates in epithelial–mesenchymal transition and promotes the invasion of oral squamous cell carcinoma.[14] Interestingly, this protein is involved in a number of varied cellular processes, including enhancing neurite and spine maturation,[15] mediating vascular intima and pulmonary vasculature development,[16] as well as cell migration and adhesion.[17] While the overexpression of GIT1 has been shown to regulate chondrocyte proliferation and apoptosis via integrin-β1, it also increases autophagy via disruption of the Beclin-1 and Bcl-2 interaction in osteoclast. Mechanistically, GIT1 achieves these outcomes by altering ERK1/2, AKT, NF-κB, and Notch expression, and accelerating lung cancer cell migration and metastasis via Rac1/Cdc42 signal, which further validates its participation in cancer occurrence and development.[18–21] A previous study found that the suppression of GIT1 inhibits breast cancer cell invasion and metastasis via the upregulation of miR-149.[22] Recently, a report demonstrated that GIT1 is reduced in ER(–) breast cancer when compared to ER(+) cancer, and that higher GIT1 expression implied a better prognosis in ER(–) breast cancer patients.[11] Thus, GIT1 appears to have distinct functions in the growth and migration of breast cancer cells. However, its roles and mechanisms in pan-cancer demand further investigations.

Herein, we investigated various cancers for GIT1 expression and patient survival data. To elucidate the mechanisms of GIT1 and the associated proteins, we performed Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis and Gene Set Enrichment Analysis (GSEA). Furthermore, we evaluated the association between GIT1 levels and immune infiltration. Finally, single-cell sequencing results were assessed to examine the GIT1 expression in cells in related tumors.

Objectives

This study aimed to measure the expression of GIT1 in various cancers and the association between GIT1 levels and immune infiltration.

Materials and methods

Pattern of GIT1 expression based on the pan-cancer study

The GIT1 level patterns in cancer and corresponding samples were obtained using ONCOMINE (http://www.oncomine.org/resource/login.html) and TIMER2.0 (http://timer.comp-genomics.org/). For ONCOMINE, the parameters were set as p = 0.001, fold change: 2.0 and gene ranking: top 10%. The GIT1 level patterns in different cancer stages were acquired using the "Stage plots" module of GEPIA2 (http://gepia2.cancer-pku.cn/#index).

Survival and prognosis

Both overall survival (OS) and disease-free survival (DFS) results were obtained through the GEPIA website.[22] High and low GIT1 expression groups were established based on the median level of GIT1. The association between GIT1 levels and pan-cancer survival outcome was detected using the log-rank test. Furthermore, Cox regression examining GIT1 levels and the clinical variables was used to detect the effects of GIT1 on the prognostic value of liver hepatocellular carcinoma (LIHC) patients. Calibration curves and the concordance index (C-index) were evaluated by comparing predicted probabilities with the observed events.

GIT1-associated functional enrichment

Proteins interacting with GIT1 were analyzed using the STRING tool (http://string.embl.de/)[23] under the setting of no more than 100 interactors and low confidence (0.150) to obtain the potent GIT1-binding proteins. Furthermore, the top 100 genes demonstrating an expression profile similar to that of GIT1 in various cancers were analyzed with the GEPIA2 tool. Then, Gene Ontology (GO) and KEGG pathway enrichment analyses were performed using proteins interacting with GIT1, together with the top 100 genes, using the DAVID software. A p-value of <0.01 was considered statistically significant.

Immune infiltration

The relationship between GIT1 expression, immune infiltration and cancer-associated fibroblasts (CAFs) was analyzed with TIMER[24] using Spearman's correlation based on the ranked values. The p-values and partial correlation values were measured employing the purity-adjusted Spearman's rank correlation test, and data were visualized with heat maps and scatter plots. Furthermore, the relationship between GIT1 levels and various tumor immune subtypes was investigated through the TISDB tool (http://cis.hku.hk/TISIDB/index), and the distribution of the 6 immune subtypes was determined. The TISDB is an online tool for cross-linking studies of tumors and immunity, which contains data from PubMed, The Cancer Genome Atlas (TCGA) and other public databases.[25,26]

Single-cell sequencing results

The distinct functional states of various cancer cells at single-cell level,[27] and the association of GIT1 levels and pan-cancer functional status were obtained through the "correlation plot" module of CancerSEA (http://biocc.hrbmu.edu.cn/CancerSEA).[28] The threshold for the association between GIT1 and cancer functional states was set as a correlation strength >0.3 and a p-value <0.05.

GSEA

The GSEA is a method to demonstrate that the expression of a given gene set is overrepresented. The GSEA was employed to evaluate distinct functions among the high- and low-risk score subgroups, using the hallmark gene set h.all.v7.0.symbols.gmt. Gene sets with |normalized enrichment score (NES)| > 1, nominal (NOM) $p < 0.01$ and false discovery rate (FDR) $q < 0.25$ were considered significant.

Statistical analyses

To assess the different levels of GIT1 in normal and pan-cancer samples, we used Wilcoxon rank-sum test. Cancer patient survival was detected with the Kaplan–Meier curve, and Spearman's rank correlation coefficient was used to measure the correlation between the 2 groups. The statistical analysis was performed using R software v. 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria) and the 'edgeR' package. A value of $p < 0.05$ was considered statistically significant.

Results

Abnormal expression of GIT1 in different cancers

The GIT1 expression patterns were evaluated in pan-cancer through TIMER2.0, which includes data about gene expression patterns in normal and pan-cancer samples. We found that GIT1 expression levels were significantly upregulated in various cancers, including LIHC and lung adenocarcinoma (LUAD), among others (Fig. 1A).

Next, we used GEPIA2 to investigate the correlation between GIT1 levels and clinical stage. An association between GIT1 levels and clinical stage for glioblastoma multiforme, head and neck squamous cell carcinoma, kidney chromophobe (KICH), LIHC, lung squamous cell carcinoma, and others was found (Fig. 1B). Collectively, these data indicate that GIT1 expression is upregulated in pan-cancer and that GIT1 can be a promotor of pan-cancers.

Based on the above data, it became evident that GIT1 is involved in both pan-cancer and LIHC development and can thus serve as a potential biomarker.

Relationship between GIT1 levels and patient prognosis

To study the correlation between GIT1 levels and patient prognosis, we used GEPIA2 to conduct a survival investigation. The obtained data showed that the overexpression of GIT1 was indicative of poor OS in patients with LIHC ($p = 0.002$), skin cutaneous melanoma (SKCM) ($p = 0.026$) and uterine corpus endometrial carcinoma (UCEC) ($p = 0.006$). Conversely, better OS was found in patients with kidney renal clear cell carcinoma (KIRC) ($p = 0.011$) and glioma ($p < 0.001$) (Fig. 2A). Furthermore, the overexpression of GIT1 was associated with poor DFS in patients with LIHC and UCEC, and improved DFS in those with KIRC, SKCM and glioma (Fig. 2B). These data indicate that there is a close association of GIT1 overexpression with poor survival outcomes in some types of cancers, including LIHC.

Protein–protein interaction and enrichment pathway analyses

Unfortunately, the mechanism underlying GIT1-mediated oncogenesis remains unknown. To examine the protein–protein interaction (PPI) network and enrichment signal of GIT1, proteins that bind GIT1 were obtained from the STRING database, and the database was verified using the experimental setup. Eleven proteins were found to interact with GIT1, namely ADRBK1, ARHGEF6, ARHGEF7, CAMK4, ERC2, LPXN, PAK1, PAK2, PPFIA1, PTK2, and PXN (Fig. 3A).

Then, the top 100 proteins that closely interacted with GIT1 were found using GEPIA2, with PXN found to be common to both methods. Furthermore, GO and KEGG pathway enrichment analyses indicated that the above genes were involved in several cellular processes, including regulation of GTPase activity, microtubule polymerization/depolymerization, protein kinase activator activity, and Ras GTPase binding, among others (Fig. 3B,C). In addition, KEGG data revealed that GIT1 participated

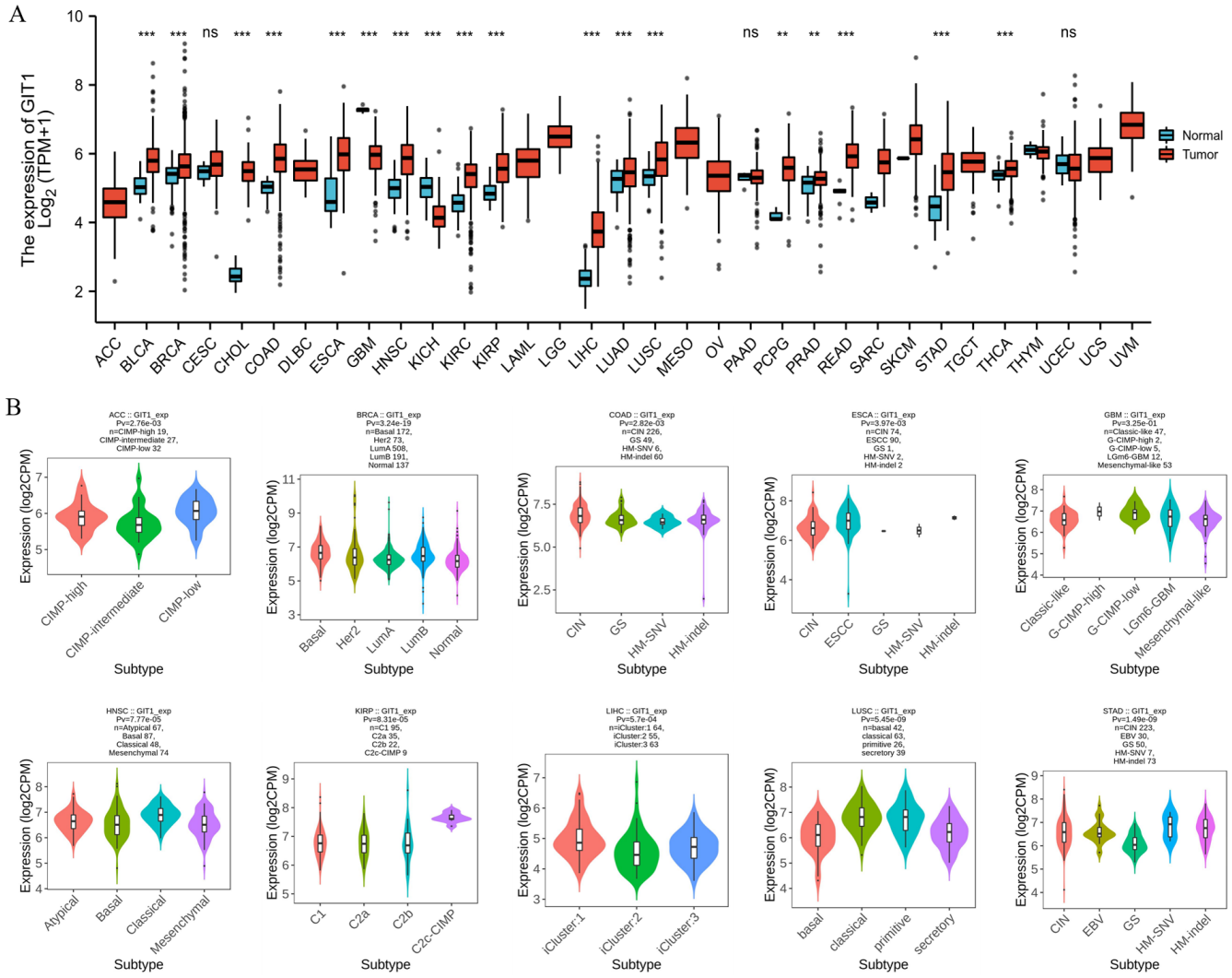

Fig. 1. Abnormal G-protein-coupled receptor kinase-interacting protein-1 (GIT1) expression patterns in various cancers. A. GIT1 levels in various cancers were presented as box plots using The Cancer Genome Atlas (TCGA) database via TIMER using R v. 3.6.3 software (Wilcoxon rank-sum test). Data in the box plot are shown as the median. The box and whisker plots were used to gain an in-depth understanding of the GIT1 level patterns in pan-cancer; B. Analysis of GIT1 levels in different clinical stages of various cancers using GEPIA2, according to TCGA data

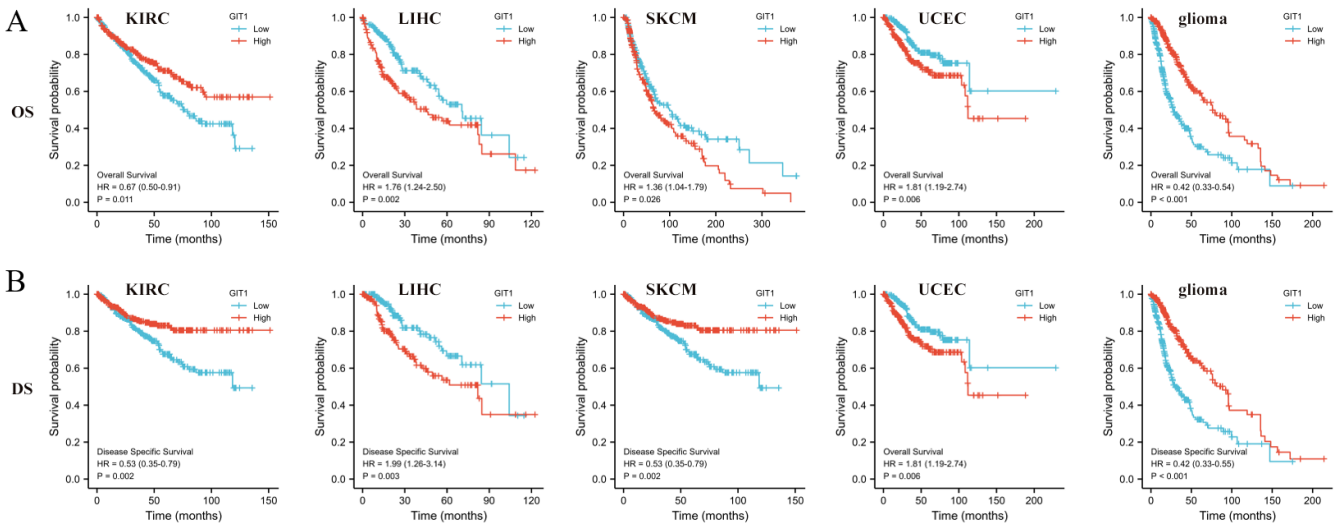

Fig. 2. Prognostic value of G-protein-coupled receptor kinase-interacting protein-1 (GIT1) expression in different tumors based on The Cancer Genome Atlas (TCGA) database. Correlations of GIT1 expression and overall survival (OS) (A) and disease-free survival (DFS) (B) were analyzed using the Kaplan–Meier plotter via GEPIA. The median expression of GIT1 was used to separate the high and low GIT1 expression groups

high – high-GIT1 expression group; low – low-GIT1 expression group; KIRC – kidney renal clear cell carcinoma; LIHC – liver hepatocellular carcinoma; SKCM – skin cutaneous melanoma; UCEC – uterine corpus endometrial carcinoma.

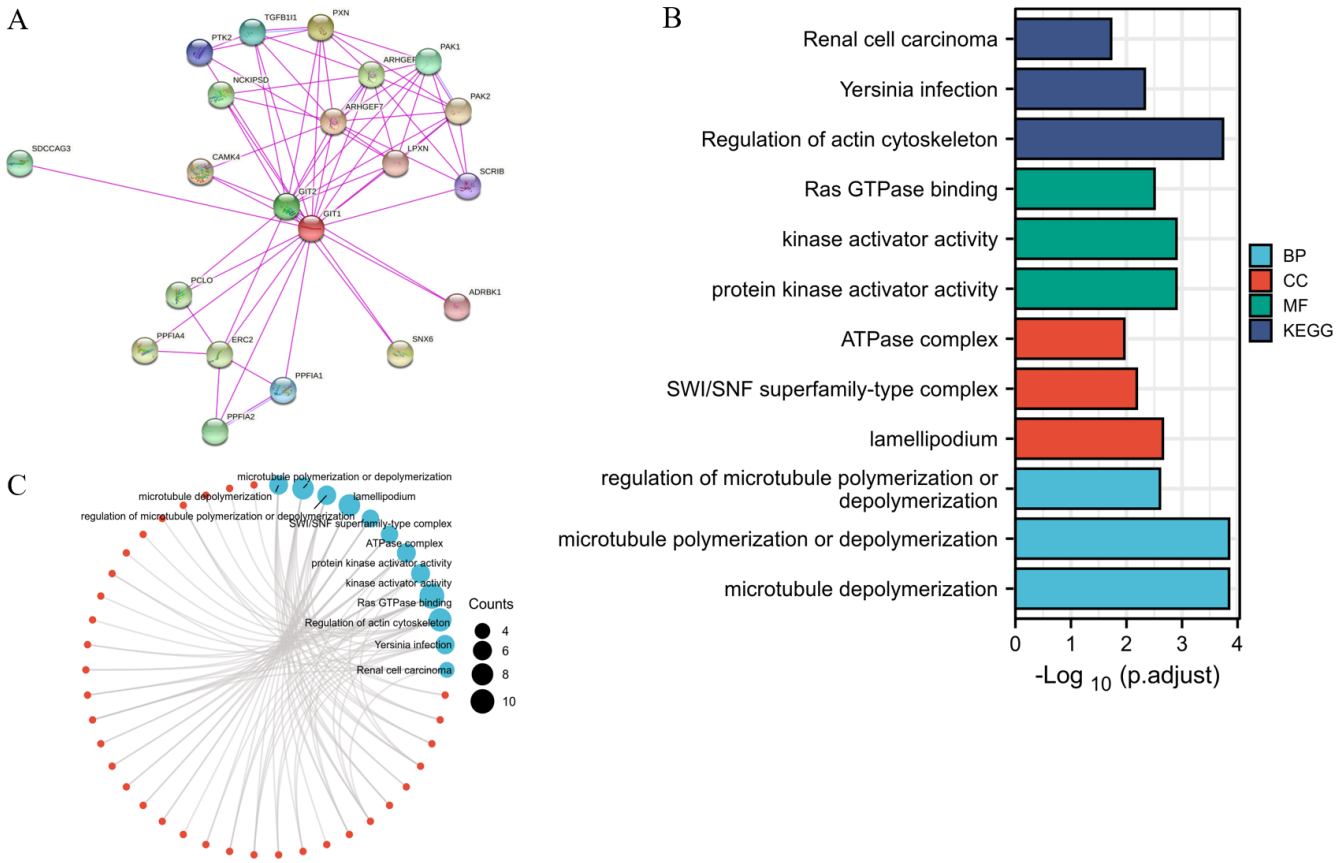

Fig. 3. Protein–protein interaction (PPI) and enrichment signal pathway study of G-protein-coupled receptor kinase-interacting protein-1 (GIT1). A. The combining genes of GIT1 were measured using the STRING website by setting the parameter of "no more than 100 interactors" via the STRING tool; B,C. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis based on GIT1-combining proteins and cooperating genes

MF – molecular function; bp – biological process; CC – cell component; SWI/SNF – yeast mating-type switching/sucrose non-fermenting.

in tumorigenesis of renal cell carcinoma, regulation of the actin cytoskeleton, focal adhesion, and other signaling pathways (Fig. 3B,C). Thus, these data found that GIT1 together with its closely interacting partner proteins correlated with focal adhesion and regulation of the actin cytoskeleton, which implied an increased complexity of the GIT1-mediated signal network.

Relationship between GIT1 levels and tumor microenvironment

To explain the effect of GIT1 expression on the immune microenvironment, TIMER was applied to study the association between GIT1 levels and tumor microenvironment (TME) characteristics in various cancers. We found that GIT1 levels were correlated with CAFs in adrenocortical carcinoma (ACC), cervical squamous cell carcinoma (CESC) and LIHC (Fig. 4A).

To further explore the relationship between GIT1 levels and CAFs, we examined biomarkers of CAF levels in different cancers and found that GIT1 expression was associated with the C1–C6 immune subtypes (Fig. 4B). Interestingly, the GIT1 levels were also connected with those immune subtypes in LIHC.

GIT1 expression pattern at the single-cell level and its relationship with biological functions

We validated GIT1 expression at the single-cell level across pan-cancers and determined its association with biological functions. The GIT1 levels were found to be positively correlated with acute lymphocytic leukemia (ALL), LUAD and ovarian serous cystadenocarcinoma (OV) apoptosis. Specifically, GIT1 expression was correlated with the LUAD cell cycle and retinoblastoma DNA damage (Fig. 5A).

There was an association between GIT1 levels and proliferation, epithelial–mesenchymal transition and metastasis in ALL (Fig. 5B). Moreover, t-distributed stochastic neighbor embedding (t-SNE) diagrams revealed GIT1 expression patterns in single cells in ALL, colorectal cancer (CRC), LUAD, and glioma (Fig. 5C). Collectively, these data suggest that GIT1 participates in mediating cancer development.

Cox regression study

A nomogram was developed for internal validation, and a predictive model was prepared (Fig. 6A). We found

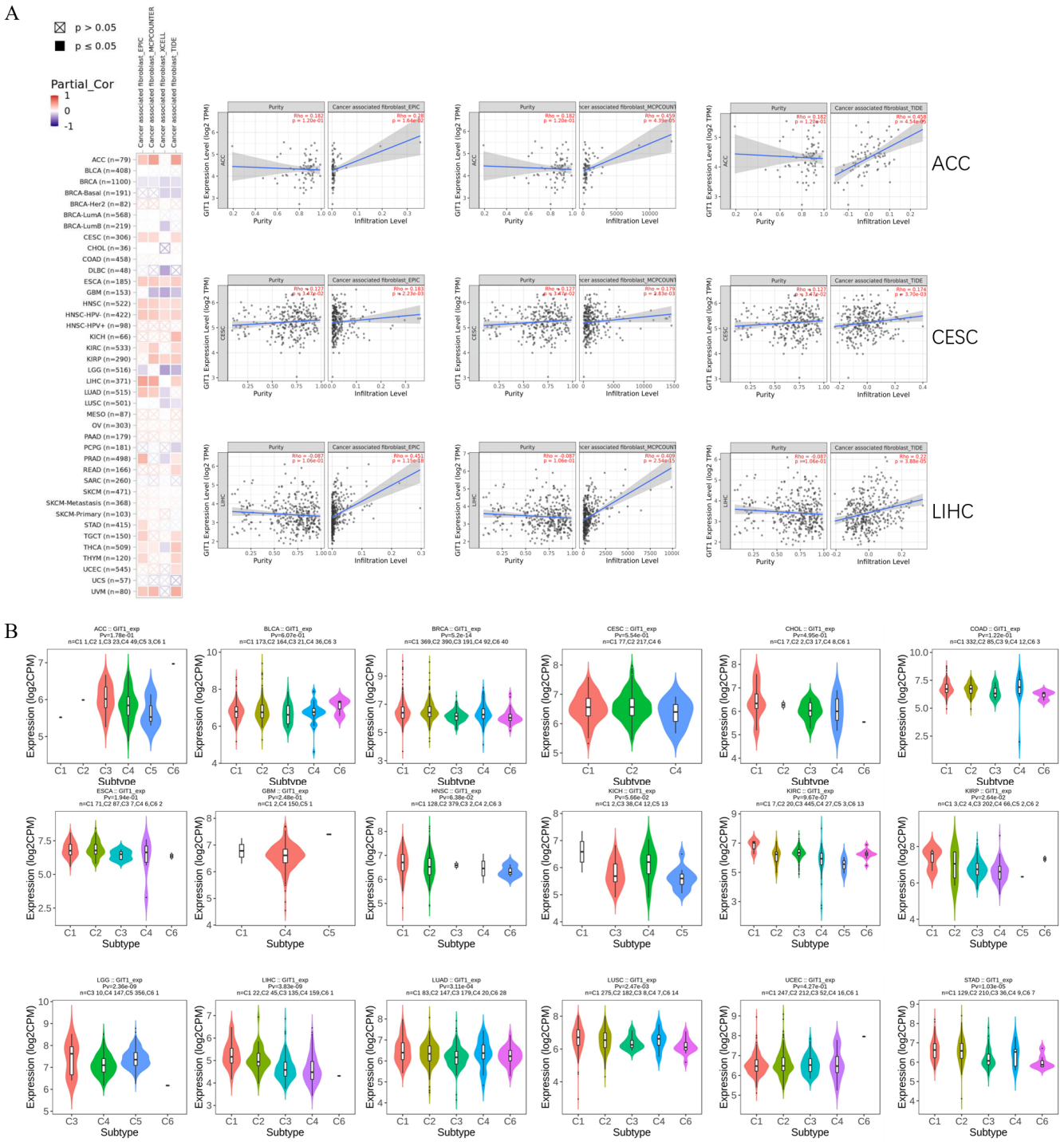

Fig. 4. The association between G-protein-coupled receptor kinase-interacting protein-1 (GIT1) expression and cancer-associated fibroblasts (CAFs). A. Different algorithms (EPIC, MCPCOUNTER, XCELL, and TIDE) were applied to confirm any potential correlation. The association between GIT1 levels and CAFs was obtained from TIMER. The p-values and the correlation values were acquired using the partial Spearman's correlation test with the "purity adjustment" option; B. GIT1 expression in various cancer immune subtypes was obtained from TISDB

ACC – adrenocortical carcinoma; LIHC – liver hepatocellular carcinoma; CESC – cervical squamous cell carcinoma.

that the C-index of the nomogram was 0.669 (95% confidence interval (95% CI): 0.637–0.701), and the calibration curve displayed the nomogram's desirable prediction for 1–5-year clinical consequences (Fig. 6B). Altogether, these data indicate that GIT1 may be a potent biomarker for LIHC.

GSEA

The GSEA revealed that the INFLAMMATORY_RESPONSE pathway and IL2_STAT5_SIGNALING were the most enriched in LIHC (Fig. 6C,D). Taken together, obtained data revealed that GIT1 expression was associated with specific gene signatures of these key pathways in LIHC.

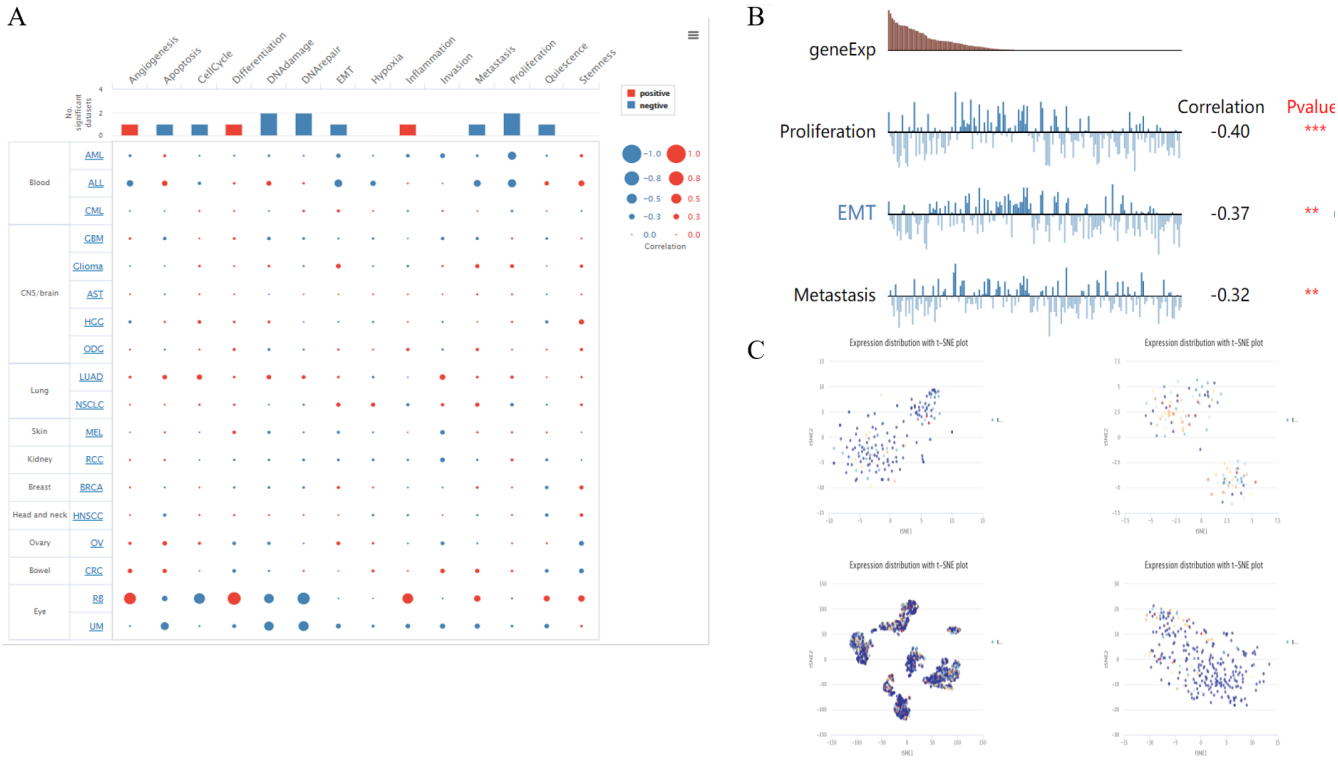

Fig. 5. Level of G-protein-coupled receptor kinase-interacting protein-1 (GIT1) in single-cell data and the association of GIT1 with cancer function. A. The association of GIT1 levels and pan-cancer function was shown using the CancerSEA database. Red plots indicated a positive association, whereas blue plots indicated a negative correlation; B. The association between GIT1 level and the different functions obtained from CancerSEA; C. T-distributed stochastic neighbor embedding (t-SNE) diagrams highlighted GIT1 levels in single cancer cells

EMT – epithelial–mesenchymal transition.

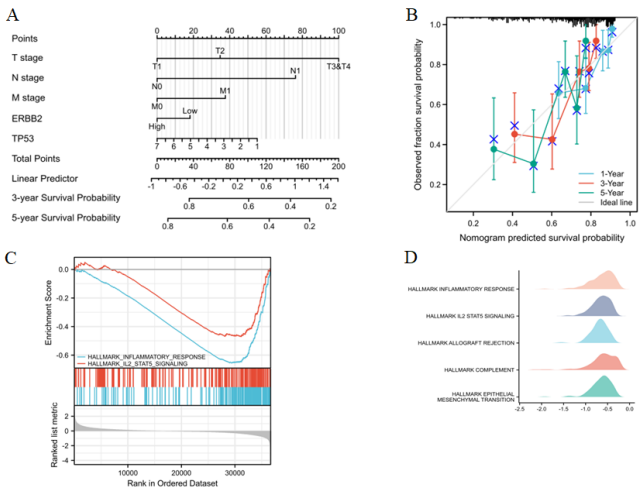

Fig. 6. Formation and verification of a nomogram for liver hepatocellular carcinoma (LIHC) based on G-protein-coupled receptor kinase-interacting protein-1 (GIT1) levels and the Gene Set Enrichment Analysis (GSEA) data. A. Nomogram for calculating the possibility of 1–5-year overall survival (OS) in LIHC; B. Calibration plots confirming the effectiveness of nomograms for OS in LIHC patients. Calibration curve for the OS nomogram model; C,D. The GSEA results presented the relevant enrichment signal. Gene sets with |NES| > 1, NOM p < 0.01 and FDR q < 0.25 were regarded as significant

NES – normalized enrichment score; NOM – nominal; FDR – false discovery rate.

Discussion

Previous studies have reported a close relationship between aberrant gene expression and the development of pan-cancers. Investigations into pan-cancer provide deep insights into the molecular mechanism underlying different malignancies and are useful to identify new therapeutic markers for cancer treatment.[29] Therefore, we investigated the expression and predictive significance of GIT1 in different tumors.

Firstly, we found that GIT1 is aberrantly expressed in different cancers, including LIHC. To further examine the prognostic value of GIT1, a Kaplan–Meier survival study was performed, which revealed an association between high GIT1 levels and the poor outcomes associated with pan-cancers, including LIHC. Thus, we found that the overexpression of GIT1 could be an independent indicator of poor prognosis in patients with LIHC and other cancers. Moreover, Cox regression analysis verified that GIT1 overexpression may be a risk factor for LIHC. Thus, our data suggested that GIT1 is a pro-oncogene in pan-cancers.

Recently, Chen et al. described a prognostic model for OS which included age and other factors for pan-cancer based on GIT1 expression.[30] In accordance with that model, we developed a prognostic nomogram model including clinical stage and GIT1 levels, which may increase

the accuracy of classifying high-risk cases. This model further assessed the relationship between clinical features and GIT1 levels in cases of LIHC, and demonstrated that increased GIT1 levels were associated with the clinical stage. The results revealed that GIT1 could act as a potent biomarker for different cancers, especially LIHC.

Additionally, the enrichment analyses revealed that GIT1 may impact cancer development through the regulation of focal adhesions and the actin cytoskeleton, together with their associated pathways. Chen et al. have shown that these signals have a key role in the development of pan-cancers.[31]

The TME has been shown to promote crosstalk between cancer cells and other cell types. In fact, CAFs have been reported to have a functional role in stimulating tumorigenesis. Thus, the signature of pan-CAF is associated with poor survival in cancer. Interestingly, other studies have suggested that CAFs inhibit cancer development, which implies that they have an antitumor effect.[32–34] Our results indicated an association between GIT1 levels and CAFs in different cancers, and therefore we believe that GIT1 mediates the development of pan-cancers. However, the molecular mechanism regarding how GIT1 modulates CAFs warrants further investigation. The well-defined immune subtype in various cancers could improve the effectiveness of targeted immune treatment. We found that GIT1 is aberrantly expressed in various immune subtypes of pan-cancer, which potentially makes it an important target in immune therapies aimed at various cancers.

Considering the complex nature of cancer cells, the utilization of single-cell transcriptomic data is a valuable method of examining various types of cancers. To elucidate the effect of GIT1 on pan-cancer progression, the CancerSEA website was used. The GIT1 expression was found to be positively associated with ALL, LUAD and OV apoptosis, and specifically positively associated with the LUAD cell cycle. Furthermore, an association was found between GIT1 levels and cell proliferation, epithelial–mesenchymal transition, and metastasis in ALL. However, the mechanism underlying GIT1 in pan-cancer warrants further investigation.

Finally, GSEA results indicated that GIT1 was associated with the inflammatory response pathway and IL2/STAT5 signaling in LIHC. These signals have been shown to be actively involved in the development of pan-cancers, including LIHC.

Recently, advances in the prediction abilities of computational biology began to offer new understanding of biomarkers and non-coding RNAs connected to pan-cancers, including ceRNA network prediction. A previous report presented data highlighting that GIT1 was involved in the ceRNA network, and, consequently, more research is necessary to investigate the role of GIT1 in ceRNA interaction.

Limitations

Our findings were mostly obtained from online tools, and more results based on clinical cases are needed to further authenticate our findings. Furthermore, in vitro and in vivo analyses should be performed to confirm the role of GIT in LIHC progression.

Conclusions

We comprehensively investigated the effects of GIT1 on various cancers. Our findings revealed that GIT1 was overexpressed in different cancers, including LIHC, which was in turn associated with a poor prognosis. Furthermore, GIT1 was shown to mediate pan-cancer development, namely LIHC progression, through the regulation of focal adhesion and the actin cytoskeleton, inflammatory response pathways, and IL2/STAT5 signaling. Further studies are needed to elucidate the molecular mechanisms underlying GIT1, which appears valuable for cancer-targeted therapy.

Availability of data and materials

The datasets generated and/or analyzed in this study are available in the TCGA database (https://portal.gdc.cancer.gov/).

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References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209–249. doi:10.3322/caac.21660
2. Ferlay J, Colombet M, Soerjomataram I, et al. Cancer statistics for the year 2020: An overview. *Int J Cancer*. 2021;149(4):778–789. doi:10.1002/ijc.33588
3. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. *CA Cancer J Clin*. 2021;71(1):7–33. doi:10.3322/caac.21654
4. Chen J, Liang H, Lu J, He Y, Lai R. Probiotics improve postoperative adaptive immunity in colorectal cancer patients: A systematic review and meta-analysis. *Nutr Cancer*. 2022;74(8):2975–2982. doi:10.1080/01635581.2022.2056619
5. Hong R, Sun H, Li D, et al. A review of biosensors for detecting tumor markers in breast cancer. *Life (Basel)*. 2022;12(3):342. doi:10.3390/life12030342
6. Zhang X, Yang Z, An Y, et al. Clinical benefits of PD-1/PD-L1 inhibitors in patients with metastatic colorectal cancer: A systematic review and meta-analysis. *World J Surg Onc*. 2022;20(1):93. doi:10.1186/s12957-022-02549-7
7. Zhang N, Cai W, Ma Y, Yin G, Nagel D, Berk B. GIT1 is a novel MEK1-ERK1/2 scaffold that localizes to focal adhesions. *Cell Biol Int*. 2009;34(1):41–47. doi:10.1042/CBI20090016

8. Segura I, Essmann CL, Weinges S, Acker-Palmer A. Grb4 and GIT1 transduce ephrinB reverse signals modulating spine morphogenesis and synapse formation. *Nat Neurosci*. 2007;10(3):301–310. doi:10.1038/nn1858
9. Li L, Tang P, Zhou Z, et al. GIT1 regulates angiogenic factor secretion in bone marrow mesenchymal stem cells via NF-κB/Notch signalling to promote angiogenesis. *Cell Prolif*. 2019;52(6):e12689. doi:10.1111/cpr.12689
10. Kim H, Kim JI, Kim H, Kim JW, Kim BN. Interaction effects of *GIT1* and *DRD4* gene variants on continuous performance test variables in patients with ADHD. *Brain Behav*. 2017;7(9):e00785. doi:10.1002/brb3.785
11. Zhang S, Miyakawa A, Wickström M, et al. GIT1 protects against breast cancer growth through negative regulation of Notch. *Nat Commun*. 2022;13(1):1537. doi:10.1038/s41467-022-28631-y
12. Wang G, Bai X, Jiang G, et al. GIT1 overexpression promotes epithelial-mesenchymal transition and predicts poor prognosis in hepatocellular carcinoma. *Bioengineered*. 2021;12(1):30–43. doi:10.1080/21655979.2020.1855914
13. Chen J, Yang P, Yang J, Wen Z, Zhang B, Zheng X. GIT1 is a novel prognostic biomarker and facilitates tumor progression via activating ERK/MMP9 signaling in hepatocellular carcinoma. *Onco Targets Ther*. 2015;8:3731–3742. doi:10.2147/OTT.S96715
14. Huang WC, Chan SH, Jang TH, et al. miRNA-491-5p and GIT1 serve as modulators and biomarkers for oral squamous cell carcinoma invasion and metastasis. *Cancer Res*. 2014;74(3):751–764. doi:10.1158/0008-5472.CAN-13-1297
15. Hong ST, Mah W. A critical role of GIT1 in vertebrate and invertebrate brain development. *Exp Neurobiol*. 2015;24(1):8–16. doi:10.5607/en.2015.24.1.8
16. Pang J, Hoefen R, Pryhuber GS, et al. G-protein-coupled receptor kinase interacting protein-1 is required for pulmonary vascular development. *Circulation*. 2009;119(11):1524–1532. doi:10.1161/CIRCULATIONAHA.108.823997
17. Zhu J, Zhou Q, Xia Y, et al. GIT/PIX condensates are modular and ideal for distinct compartmentalized cell signaling. *Mol Cell*. 2020;79(5):782.e6–796.e6. doi:10.1016/j.molcel.2020.07.004
18. Xu R, Xu R, Wang Y, Wang W, Jiang L, Gong S. G-protein-coupled receptor kinase-interacting protein 1 (GIT1) promotes head and neck squamous cell carcinoma metastases via activating the PI3K/AKT/mTOR signal pathway. *Comput Math Methods Med*. 2022;2022:6881932. doi:10.1155/2022/6881932
19. Ye X, Chen X. miR-149-5p inhibits cell proliferation and invasion through targeting GIT1 in medullary thyroid carcinoma. *Oncol Lett*. 2019;17(1):372–378. doi:10.3892/ol.2018.9628
20. Jiao Y, Zhang L, Li J, He Y, Zhang X, Li J. Exosomal miR-122-5p inhibits tumorigenicity of gastric cancer by downregulating *GIT1*. *Int J Biol Markers*. 2021;36(1):36–46. doi:10.1177/1724600821990677
21. Chang JS, Su CY, Yu WH, et al. GIT1 promotes lung cancer cell metastasis through modulating Rac1/Cdc42 activity and is associated with poor prognosis. *Oncotarget*. 2015;6(34):36278–36291. doi:10.18632/oncotarget.5531
22. Yang L, Zhang W, Li M, et al. Evaluation of the prognostic relevance of differential claudin gene expression highlights claudin-4 as being suppressed by TGFbeta1 inhibitor in colorectal cancer. *Front Genet*. 2022;13:783016. doi:10.3389/fgene.2022.783016
23. Ukey S, Ramteke A, Choudhury C, Purohit P, Sharma P. Differential expression of zinc-dependent HDAC subtypes and their involvement in unique pathways associated with carcinogenesis. *Asian Pac J Cancer Prev*. 2022;23(3):877–883. doi:10.31557/APJCP.2022.23.3.877
24. Guo Y, Shang A, Wang S, Wang M. Multidimensional analysis of CHMP family members in hepatocellular carcinoma. *Int J Gen Med*. 2022;15:2877–2894. doi:10.2147/IJGM.S350228
25. Wang Y, Zhang H. FAT10 is a prognostic biomarker and correlated with immune infiltrates in skin cutaneous melanoma. *Front Mol Biosci*. 2022;9:805887. doi:10.3389/fmolb.2022.805887
26. Wu H, Duan Y, Gong S, Zhu Q, Liu X, Liu Z. An integrative pan-cancer analysis of kinesin family member C1 (KIFC1) in human tumors. *Biomedicines*. 2022;10(3):637. doi:10.3390/biomedicines10030637
27. Wang Y, Song M, Gao B. EF-hand domain-containing protein D2 (EFHD2) correlates with immune infiltration and predicts the prognosis of patients: A pan-cancer analysis. *Comput Math Methods Med*. 2022;2022:4878378. doi:10.1155/2022/4878378
28. Ma J, Jin Y, Gong B, Li L, Zhao Q. Pan-cancer analysis of necroptosis-related gene signature for the identification of prognosis and immune significance. *Discov Onc*. 2022;13(1):17. doi:10.1007/s12672-022-00477-2
29. Liu Y, Lin L, Zou R, Wen C, Wang Z, Lin F. MSC-derived exosomes promote proliferation and inhibit apoptosis of chondrocytes via lncRNA-KLF3-AS1/miR-206/GIT1 axis in osteoarthritis. *Cell Cycle*. 2018;17(21–22):2411–2422. doi:10.1080/15384101.2018.1526603
30. Chen J, Yang P, Yang J, Wen Z, Zhang B, Zheng X. GIT1 is a novel prognostic biomarker and facilitates tumor progression via activating ERK/MMP9 signaling in hepatocellular carcinoma. *Onco Targets Ther*. 2015;8:3731–3742. doi:10.2147/OTT.S96715
31. Chen S, Hourwitz MJ, Campanello L, Fourkas JT, Losert W, Parent CA. Actin cytoskeleton and focal adhesions regulate the biased migration of breast cancer cells on nanoscale asymmetric sawteeth. *ACS Nano*. 2019;13(2):1454–1468. doi:10.1021/acsnano.8b07140
32. Mizutani Y, Kobayashi H, Iida T, et al. Meflin-positive cancer-associated fibroblasts inhibit pancreatic carcinogenesis. *Cancer Res*. 2019;79(20):5367-5381. doi:10.1158/0008-5472.CAN-19-0454
33. Ishibashi Y, Mochizuki S, Horiuchi K, et al. Periostin derived from cancer-associated fibroblasts promotes esophageal squamous cell carcinoma progression via ADAM17 activation. *Biochim Biophys Acta Mol Basis Dis*. 2023;1869(5):166669. doi:10.1016/j.bbadis.2023.166669
34. Dong J, Wang F, Gao X, et al. Integrated analysis of genome-wide DNA methylation and cancer-associated fibroblasts identified prognostic biomarkers and immune checkpoint blockade in lower grade gliomas. *Front Oncol*. 2023;12:977251. doi:10.3389/fonc.2022.977251