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Decoding the Molecular Magic of ACPS in Combating Metastatic Gastrointestinal Stromal Tumors

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ABSTRACT

Objective: This study aims to investigate the molecular mechanisms underlying the antimetastatic effects of *Akebia trifoliata* root (ACPS), a traditional Chinese herbal medicine, on gastrointestinal stromal tumors (GIST).

Methods: An integrated approach combining network pharmacology, GEO transcriptomic datasets, and molecular docking was employed to identify bioactive compounds in ACPS and their potential gene targets. Functional enrichment analyses were conducted, followed by in vitro experiments using GIST cell lines and profiling of the tumor immune microenvironment.

Results: Six bioactive compounds from ACPS were identified, targeting 34 key genes involved in cell-cycle regulation and immune responses. Among these, three lead compounds demonstrated strong binding affinities to four critical targets. In vitro assays confirmed that ACPS inhibited GIST cell proliferation, invasion, and cell-cycle progression. Additionally, seven immune-related targets were identified, suggesting ACPS may modulate the tumor immune microenvironment.

Conclusion: ACPS exhibits multitarget therapeutic potential against metastatic GIST by disrupting cell-cycle signaling and modulating immune responses within the tumor microenvironment (TME). These findings support further investigation into ACPS as a complementary or alternative therapeutic strategy for GIST.

1 | Introduction

Gastrointestinal stromal tumors (GIST), the most prevalent mesenchymal neoplasms of the digestive tract, possess a distinct capacity for malignant progression [1]. At present, complete surgical excision is still regarded as the optimal curative modality for GIST. These tumors exhibit inherent resistance to conventional radiotherapy and cytotoxic chemotherapy; nevertheless, imatinib is able to obstruct tyrosine-kinase autophosphorylation and downstream receptor signaling, thereby attaining clinical efficacy [2, 3]. Although operative intervention and molecularly targeted agents prolong survival, a subset of patients inevitably develop tumor recurrence, distant metastasis or acquired drug resistance.

Consequently, novel therapeutic entities that can overcome resistance and improve long-term prognosis are urgently required.

Akebia trifoliata root, derived from *Actinidia chinensis* Planch of the Actinidiaceae family, is a traditional Chinese medicinal herb [4]. Empirically, it has been widely prescribed against a variety of solid tumors, including gastric, esophageal, hepatic, and colorectal malignancies, and has shown pronounced activity toward cancers of the alimentary canal [5–7]. Corless et al. [8] reported that Trifoliata root extracts could retard gastric cancer cell progression. Gao et al. [9] demonstrated that flavonoid fractions from wild kiwifruit root suppress gastric-cancer proliferation, trigger apoptosis and induce G1-phase arrest. Chang and Case

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[10] further indicated that polysaccharides from kiwifruit root inhibit breast-cancer growth by replicating proliferation and provoking apoptotic death.

Contemporary pharmacological investigations have revealed that Trifoliata root exerts antineoplastic and immunoregulatory actions, scavenges reactive oxygen species and displays antiviral properties without appreciable adverse effects. To date, however, its potential impact on GIST remains poorly documented. Therefore, the present work integrated network-pharmacology docking with in vitro cellular assays to dissect how bioactive constituents of Trifoliata root influence tumor cells and immune components within the metastatic GIST microenvironment. By delineating the key active principles and their corresponding molecular targets, we aimed to furnish new insights for deeper mechanistic studies and eventual clinical translation.

2 | Methods

2.1 | Prediction of Potential Targets for the Identified ACPS Components

All bioactive ingredients of ACPS were retrieved from the TCMSP repository (<https://old.tcmsp-e.com/tcmsp.php>). After filtering (oral-bioavailability [OB] $\geq 30\%$ and drug-likeness [DL] ≥ 0.18), six compounds were advanced for downstream investigations. Target annotations for these compounds were downloaded, gene symbols were standardized through the UniProt portal (<https://www.uniprot.org/>), and redundant entries were discarded [11, 12].

2.2 | Identification of Metastatic GIST-Related Targets

GIST-linked genes were harvested from OMIM (<https://omim.org/>), DisGeNet (<https://www.disgenet.org/home/>), the Therapeutic Target Database (<https://db.idrblab.net/ttd/>), and GeneCards (<https://www.genecards.org/home>). GeneCards entries were retained only when relevance scores reached ≥ 10 . After removal of duplicates, the GIST gene pool was established. Expression profiles comparing primary and metastatic lesions (GSE136755, GSE17743, and GSE31802) were obtained from GEO; differential analysis highlighted genes significantly upregulated in metastases. These candidates were merged, deduplicated, and intersected with the GIST gene set to generate the final target list [13–17].

2.3 | Construction of the Protein–pPotein Interaction (PPI) Network

Shared targets derived from the above datasets were overlapped via the OE Biotech Cloud platform (https://cloud.oebiotech.com/#/bio/tools?cat_id%26tag_id). The resulting genes were imported into STRING v11.0 (<https://string-db.org>) to construct a PPI network using a medium-confidence threshold (score ≥ 0.400) [18, 19].

2.4 | Drug–Compound–Target–Disease (DCTD) Network

Overlapping targets, disease identifiers and the six ACPS compounds were loaded into Cytoscape 3.9.0. Topological properties

of DCTD nodes were computed to establish the DCTD network model [20].

2.5 | Functional and Pathway Enrichment Analyses

GO, KEGG, and Reactome enrichment of the selected targets were performed with R and Bioconductor; significance was defined as $p < 0.05$ and $q < 0.05$.

2.6 | Molecular Docking of Compound–Target Interactions

All 3-D structures of ACPS compounds were downloaded from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) and the Protein Data Bank (<https://www.rcsb.org>). Ligand and protein files were converted with OpenBabel, and docking was executed in AutoDock Tools v1.5.7. Poses were visualized in Discovery Studio 2021; binding energies ≤ -4.25 kcal mol⁻¹ were regarded as reliable [21–23].

2.7 | Cell Culture

The human GIST line A673 was maintained in DMEM; HMC-1.1 cells were cultured in IMDM plus 10% FBS (Gibco, USA). A human T cell was cultured in T cell medium (Pricella, China). Both lines were obtained from Pricella and MERCK, authenticated by DNA fingerprinting, and used within 2 months of resuscitation at 37°C with 5% CO₂.

2.8 | Antibodies and Reagent

Primary antibodies against Cyclin B1 (AF6168), Cyclin D1 (AF0931), Cyclin E1 (AF0144), p-AKT^{T308} (AF3262), AKT (AF6261), p-PI3K^{p85/p55} (AF3242), PI3K (AF6242), CD44 (BF9213), CD133 (AF5120), and GAPDH (AF7021) were employed. Quercetin was purchased from MCE (Shanghai, China).

2.9 | Colony Formation Assay

Cells were seeded in six-well plates (200 cells/well, triplicate) and cultured for 8 days. Colonies were washed with PBS, fixed with methanol (30 min), stained with crystal violet (30 min, room temperature), rinsed, imaged, and counted; statistical evaluation followed reference [24].

2.10 | MTT Assay

Viability was quantified via MTT. Cells were plated at 3×10^4 per well (96-well, triplicate), exposed to compounds for 24 h, then maintained with medium renewal every 48 h. MTT (0.2%, 50 μ L) was added for 30 min (37°C, 5% CO₂); absorbance at 560 nm was recorded and analyzed by correlation testing.

2.11 | Flow Cytometry

After 24 h drug exposure and a further 24 h culture, cells were harvested, washed, fixed in ice-cold 70% ethanol overnight, resuspended in PBS containing RNase A (0.2 mg mL⁻¹) and propidium iodide (10 μ M), incubated (30 min, 37°C, dark), and

analyzed on a FACSCalibur (Becton Dickinson); data were processed with correlation tests.

2.12 | Invasion Assay

Matrigel-coated inserts (8 μm, 24-well) were used. 5 × 10⁴ cells in serum-free medium were placed in the upper chamber; the lower chamber contained medium plus 10% FBS. After 22 h incubation, noninvading cells were removed, membranes were fixed and stained with crystal violet, and invading cells were counted in nine random 40x fields; means were calculated.

2.13 | RT-qPCR and Western Blot

RNA was extracted and reverse-transcribed following kit protocols; qPCR was performed with gene-specific primers. For Western blot, cells were lysed in RIPA buffer supplemented with PMSF and inhibitor cocktails. Proteins (equal loading) were resolved by SDS-PAGE, transferred to PVDF, blocked, incubated with primary antibodies (4°C, overnight) and HRP-conjugated secondaries, and visualized with an ECL system (Tanon 5200). Band intensities were quantified by densitometry, normalized to loading controls and evaluated via correlation methods.

2.14 | Ethical Statement

The investigation adhered to the Declaration of Helsinki. Bioinformatic analyses were approved by the Ethics Committee of Quzhou Affiliated Hospital, Wenzhou Medical University (Approval Number 2022-067).

2.15 | Statistical Analysis

Results are presented as mean ± SD from ≥3 independent experiments. Two-group comparisons used Student’s *t*-test; multiple groups were analyzed by one-way ANOVA. Kaplan–Meier curves were compared with the log-rank test; correlations were assessed with Spearman’s rank. Analyses were performed in SPSS v20 (IBM) and GraphPad Prism 8.0. Significance levels: **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001.

3 | Results

3.1 | Screening of Bioactive Constituents and Target Genes of ACPS

The TCMSP platform was first queried to capture ACPS ingredients displaying favorable OB and DL metrics, yielding six lead

monomers: β-sitosterol, sitosterol, aloe-emodin, (+)-catechin, ent-epicatechin, and quercetin. Compound names together with OB and DL values are supplied in Table 1. Two-dimensional structures retrieved from PubChem were forwarded to PharmMapper for target fishing; redundant hits were removed, leaving 216 unique ACPS-related genes. GeneCards returned 4286 GIST-relevant entries. Parallel analysis of GEO datasets (GSE136755, GSE17743, and GSE31802) comparing primary versus metastatic lesions produced 3491 differentially expressed genes after deduplication (Figure 1A, B). Overlay of both cohorts via the OE Biotech Cloud gave 1312 metastasis-associated candidates; intersecting these with the 216 ACPS targets produced 25 overlapping genes proposed as putative ACPS targets against metastatic GIST (Figure 1C).

3.2 | PPI Network Construction

The 25 common genes were imported into STRING to generate a PPI network; isolated nodes were deleted, retaining 25 vertices and 352 edges. Betweenness and degree centrality ranked AR, TGFβ1, CCND1, RB1, CASP9, NCOA2, PGR, MMP2, HSPB1, ACACA, FASN, BCL2, and RELA as hub proteins. These core nodes were carried forward for compound-target exploration (Figure 1D).

3.3 | DCTD Visualization

A Cytoscape 3.9.0 network was assembled to illustrate relationships among DCTD. Color- and shape-coded nodes denote entity type; edge number reflects node importance. The six ACPS compounds are connected with 34 distinct targets, affirming the polypharmacology of ACPS against metastatic GIST (Figure 2A, B).

3.4 | Functional Enrichment Profiling

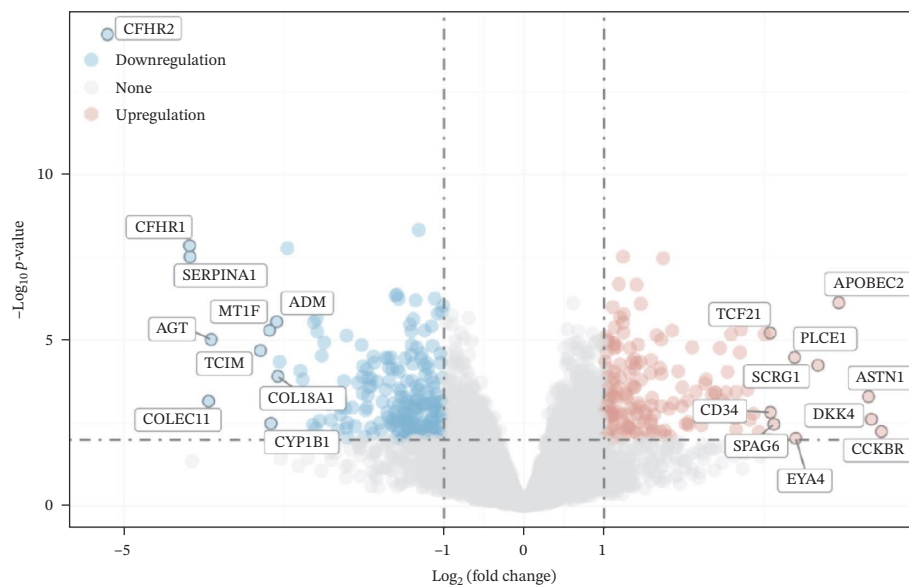
GO analysis (Bioconductor, R) showed that target genes participate predominantly in intracellular steroid-hormone-receptor signaling, cytoplasmic activities, and estrogen-response element binding (Figure 2C). KEGG and WikiPathway enrichment pointed chiefly to AGE-RAGE and NF-κB prosurvival cascades (Figure 2D, E), whereas Reactome highlighted ChREBP-regulated metabolic gene activation as a potential therapeutic mode of ACPS (Figure 2F).

3.5 | Molecular-Docking Validation

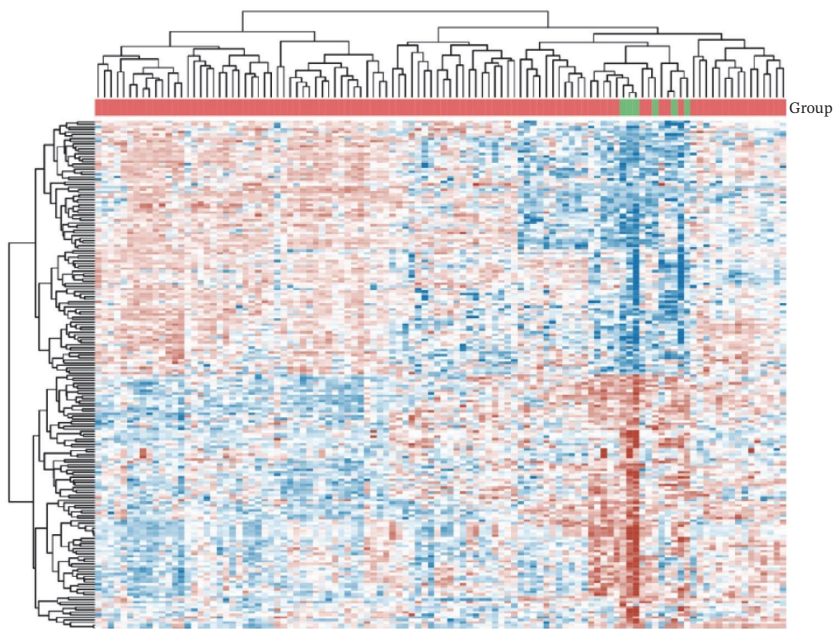
To corroborate network predictions, aloe-emodin, quercetin, and β-sitosterol were docked against four enriched targets—FASN,

TABLE 1 | Identification of six chemical components absorbed from the TCMSP.

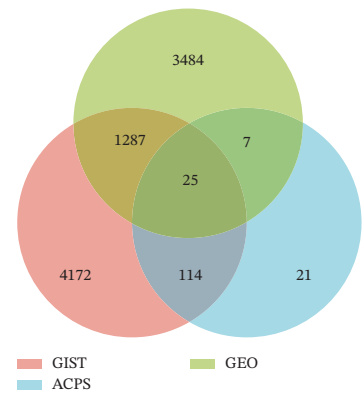
Mol ID	Molecule name	MW	AlogP	Hdon	Hacc	OB (%)	Caco-2	BBB	DL	FASA-	HL
MOL000358	Beta-sitosterol	414.79	8.08	1	1	36.91	1.32	0.99	0.75	0.23	5.36
MOL000359	Sitosterol	414.79	8.08	1	1	36.91	1.32	0.87	0.75	0.22	5.37
MOL000471	Aloe-emodin	270.25	1.67	3	5	83.38	−0.12	−1.07	0.24	0	31.49
MOL000492	(+)-catechin	290.29	1.92	5	6	54.83	−0.03	−0.73	0.24	0	0.61
MOL000073	ent-epicatechin	290.29	1.92	5	6	48.96	0.02	−0.64	0.24	0.34	0.63
MOL000098	Quercetin	302.25	1.5	5	7	46.43	0.05	−0.77	0.28	0.38	14.4



(A)



(B)



(C)

FIGURE 1 | (Continued)

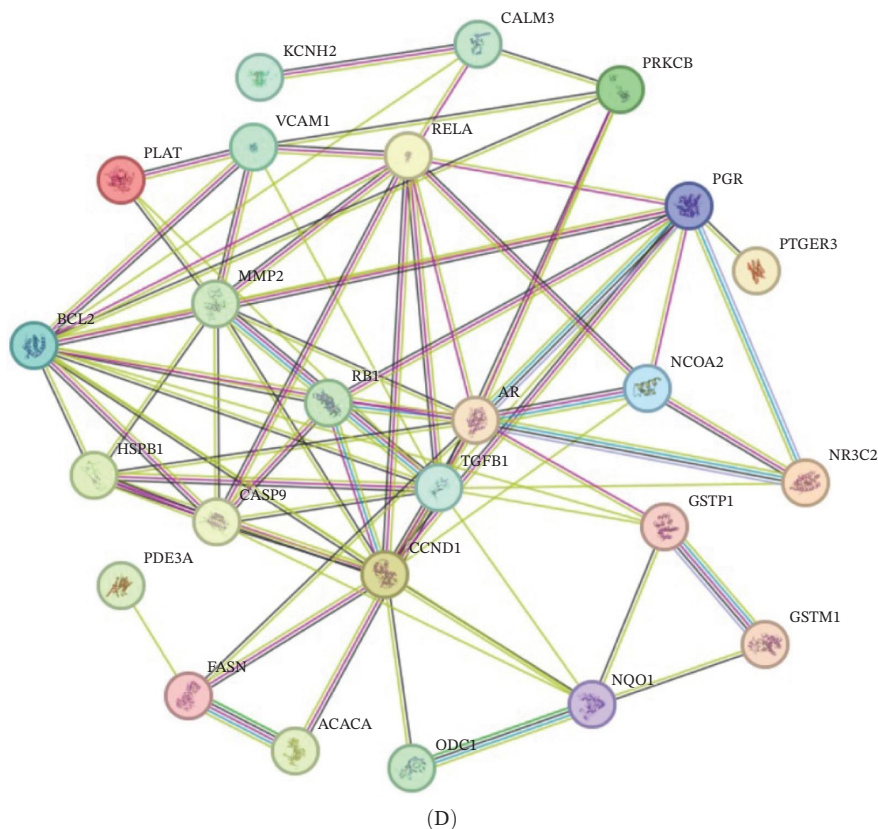


FIGURE 1 | Identification of ACPS targets in metastatic GIST. (A,B) Volcano plots and heatmaps display the differential analysis results of primary and metastatic GIST data downloaded from the GEO database, with plot A showing the distribution of highly expressed and lowly expressed genes and plot B showing the sequencing outcomes. (C) Intersection results of traditional Chinese medicine active component target genes and disease target genes. (D) protein–protein interaction (PPI) network. The red circle nodes represent core targets, and the orange circle nodes represent noncore targets.

ACACA, CCND1, and TGFB1—using AutoDock. Affinities ≤ -4.25 kcal mol⁻¹ were considered acceptable. All three ligands displayed strong binding to the selected proteins (Table 2); representative 3-D complexes are depicted in Figure 3A–E. Notably, quercetin shared high affinity with ACACA, TGFB1, and CCND1; the CCND1–quercetin pair exhibited the lowest free energy (-9.4 kcal mol⁻¹), stabilized by a hydrogen bond to Arg-183 plus hydrophobic contacts with Glu-180, Asn-493, Arg-176, and Asp-491 (Figure 3E). These *in silico* data suggest that the identified constituents may possess activity against metastatic GIST, although experimental confirmation is required.

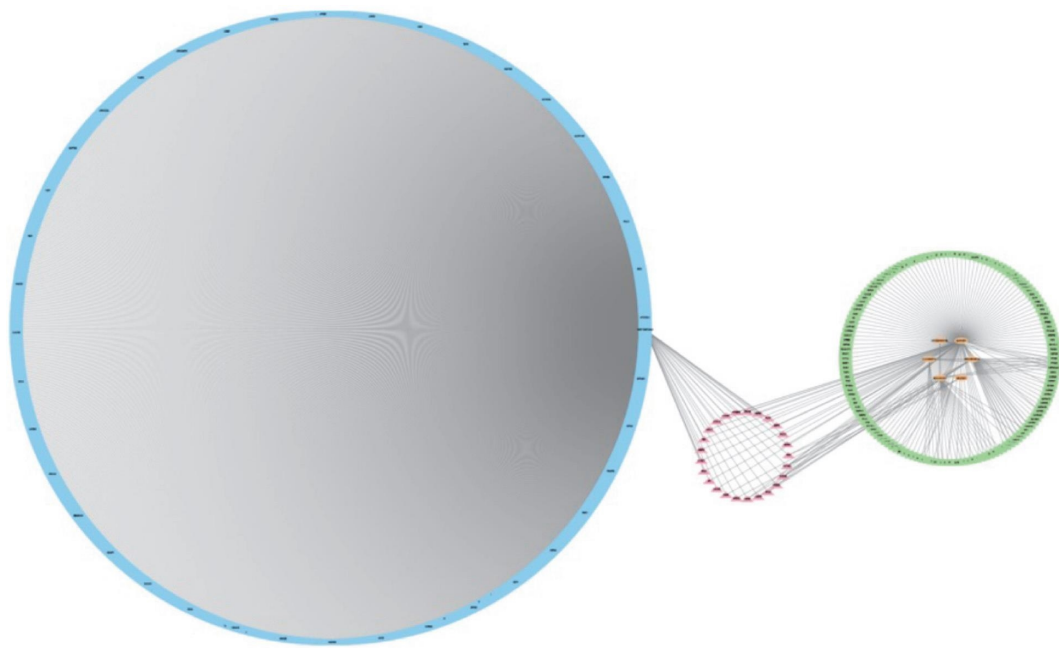
3.6 | In Vitro Evaluation of ACPS Activity in GIST Cells

Capitalizing on the predicted quercetin–CCND1 interaction, we assessed ACPS bioactivity in A673 and HMC-1.1 lines. Growth-curve, clonogenic, invasion, and cell-cycle assays were performed. Following 24 h ACPS exposure, proliferation of both lines declined markedly over the next 72 h (Figure 4A, B). Colony formation and Matrigel invasion were also significantly curtailed (Figure 4C–E), while flow-cytometric analysis revealed G0/G1 accumulation and concomitant S- and G2/M-phase reductions relative to controls (Figure 4F, G). Furthermore, to further validate the therapeutic target CCND1 of the ACPS drug, we

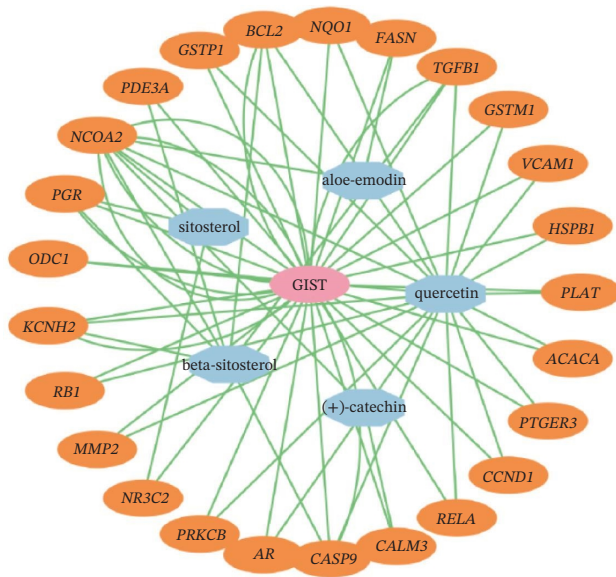
transfected the A673 and HMC-1.1 cell lines with a CCND1-over-expressing plasmid under the same drug treatment conditions and subsequently assessed the cell cycle. The results demonstrated a significant increase in the proliferative phase following CCND1 overexpression, indicating that CCND1 can partially reverse the therapeutic effects of the ACPS drug (Figure 4H). Collectively, the data indicate that ACPS attenuates proliferation, anchorage-independent growth, invasiveness, and cell-cycle progression in GIST cells, thereby slowing tumor kinetics.

3.7 | ACPS Blocks PI3K/AKT Signaling and Downregulates CCND1, Including Tumor Stem Capacity

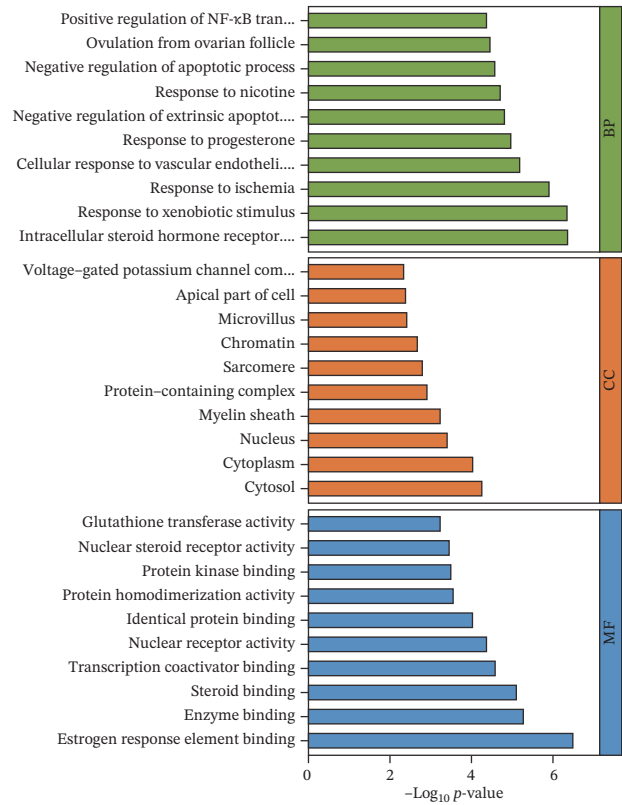
Building on phenotype observations, qRT-PCR and Western blot were employed to probe CCND1 and PI3K/AKT status. Quercetin markedly reduced CCND1 protein without altering Cyclin B1 or Cyclin E1 transcripts (Figure 5A–E). Consistent with literature reports of PI3K inhibition, phosphorylated PI3K and AKT levels declined after quercetin treatment (Figure 5F, G). Additionally, the protein expression levels of common stem cell markers CD44 and CD133 in GIST was significantly suppressed after treatment with quercetin (Figure 5H, I). These findings implicate suppression of the PI3K/AKT axis and CCND1 downregulation in ACPS-mediated restriction of GIST cells.



(A)



(B)



(C)

FIGURE 2 | (Continued)

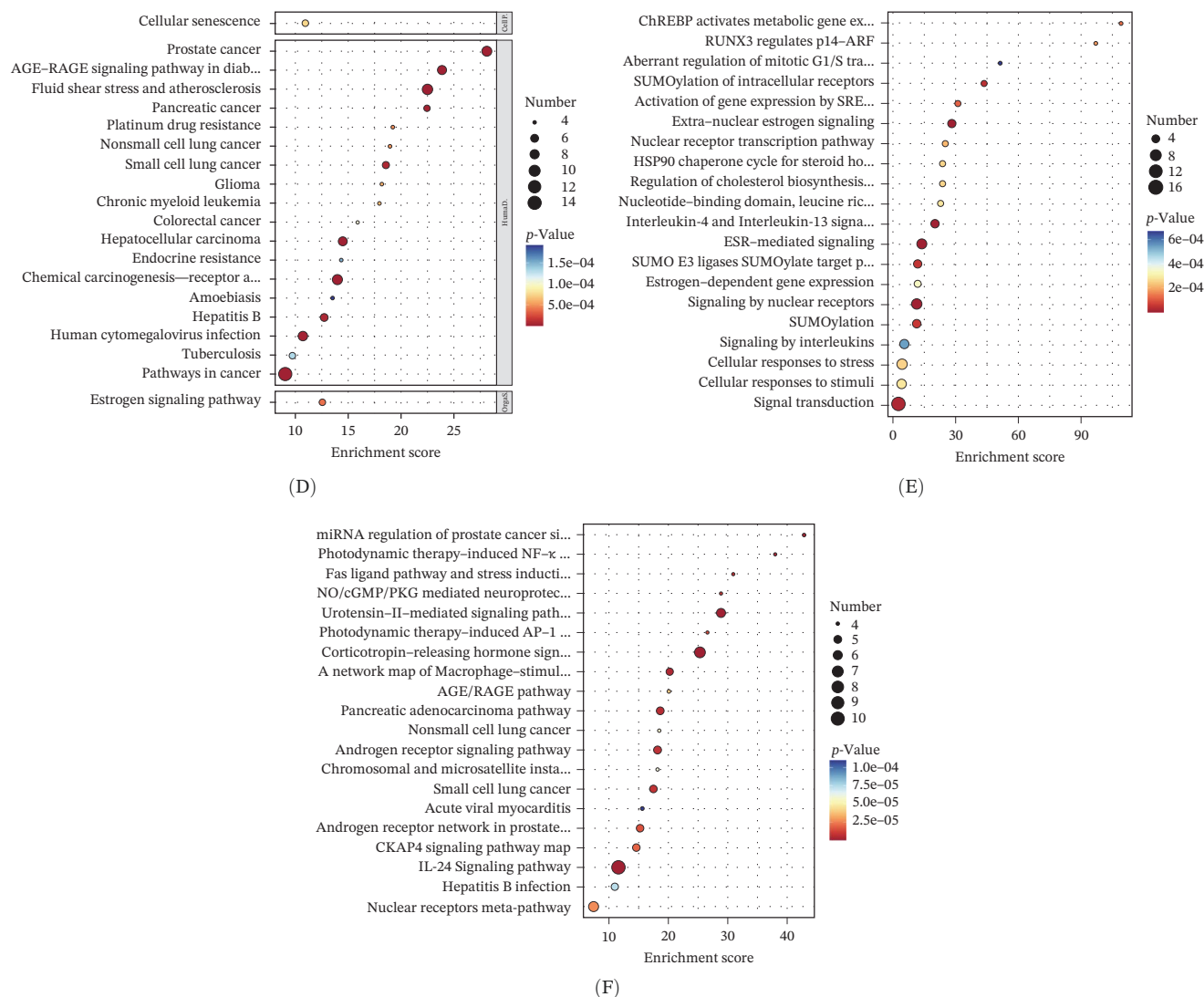


FIGURE 2 | DCTD network and enrichment analysis. (A,B) Topological network connection results of drugs, active compounds, intersecting genes, and diseases. (C) GO (Gene Ontology) enrichment analysis. (D) KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis. (E) Wiki enrichment analysis. (F) Reactome enrichment analysis.

TABLE 2 | Target molecule docking results for candidate active components.

Compound name	Affinity (kcal/mol)			
	ACACA	TGFB1	CCND1	FASN
Aloe-emodin	−4.6	−4.8	−4.5	−6.7
Quercetin	−7.5	−6.1	−9.4	−4.4
Beta-sitosterol	−4.3	−5.7	−4.8	−4.7

3.8 | ACPS Modulates the Metastatic GIST Immune Microenvironment

Growing evidence underscores the contribution of the tumor microenvironment (TME) to GIST progression. Having clarified tumor-cell-intrinsic actions, we explored ACPS targets within immune compartments. Single-cell RNA-seq data (GSE162115 and GSE254762) were downloaded from GEO; differential analysis yielded 145 genes. Intersection with ACPS targets via the OE Biotech Cloud identified eight overlapping genes as putative

immune modulators (Figure 6A). A STRING PPI network (seven nodes, 28 edges after culling isolates) was generated (Figure 6B). GO enrichment indicated involvement in integrated stress-response signaling, AP-1 complex assembly, and RNA-polymerase-II-specific DNA-binding activity (Figure 6C). KEGG and WikiPathway analyses highlighted IL-17, leishmaniasis, TNF, and quercetin-triggered NF-κB/AP-1 apoptosis pathways (Figure 6D, E), whereas Reactome pointed to the MyD88-dependent endosomal cascade as a probable immune-regulatory

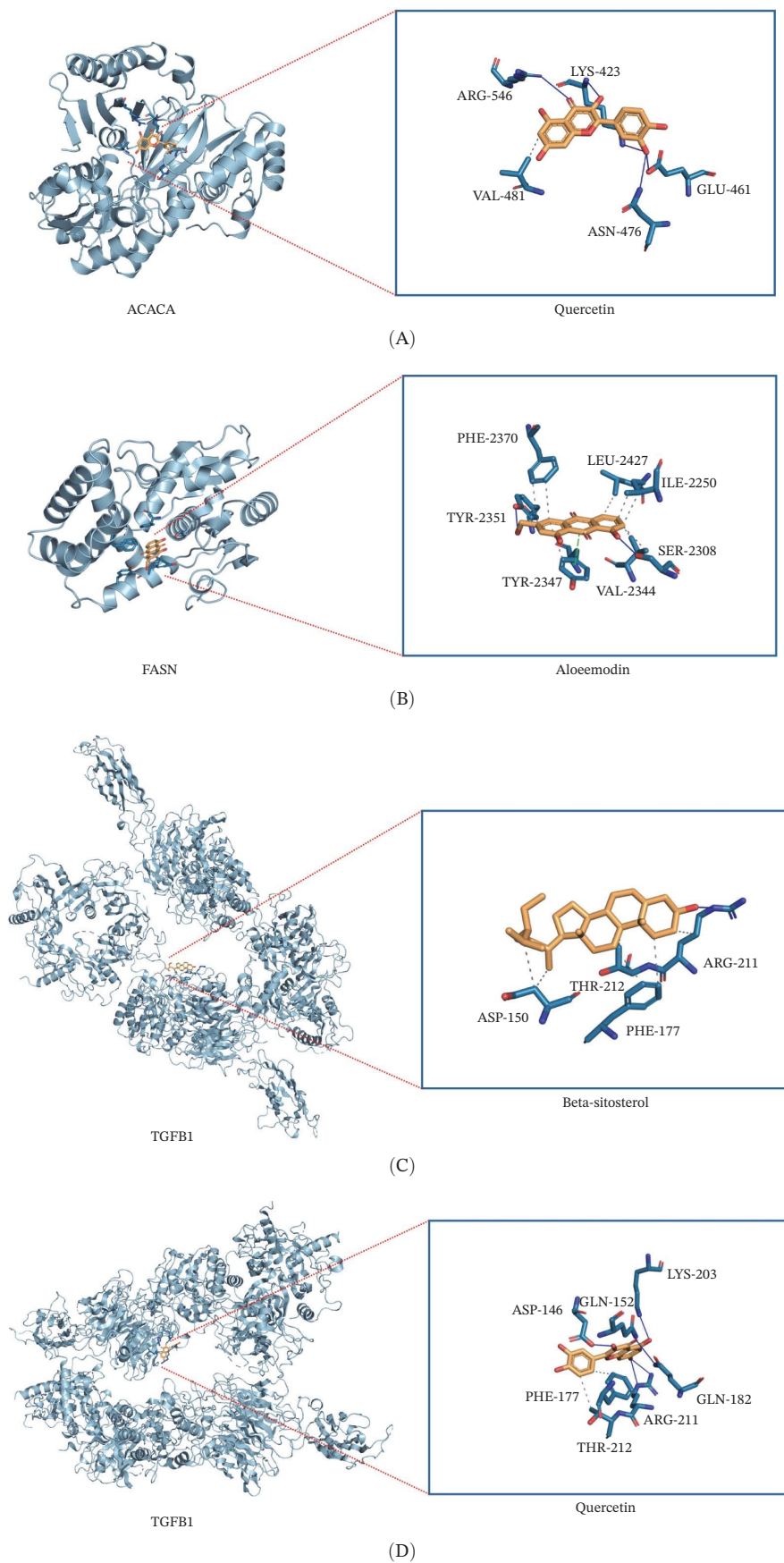


FIGURE 3 | (Continued)

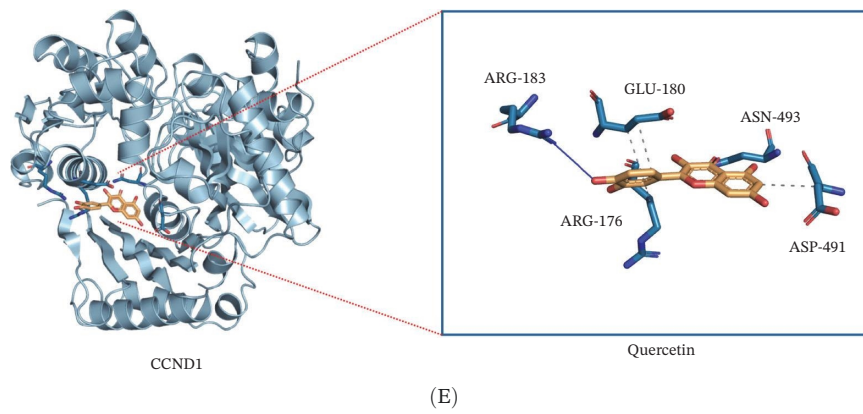


FIGURE 3 | 3D docking patterns of critical targets and specific active compounds. (A) Quercetin is binding to the ACACA crystal structure. (B) Aloeemodin binding to FASN crystal structure. (C) Beta-sitosterol binding to TGFB1 crystal structure. (D) Quercetin binding to TGFB1 crystal structure. (E) Quercetin is binding to the CCND1 crystal structure.

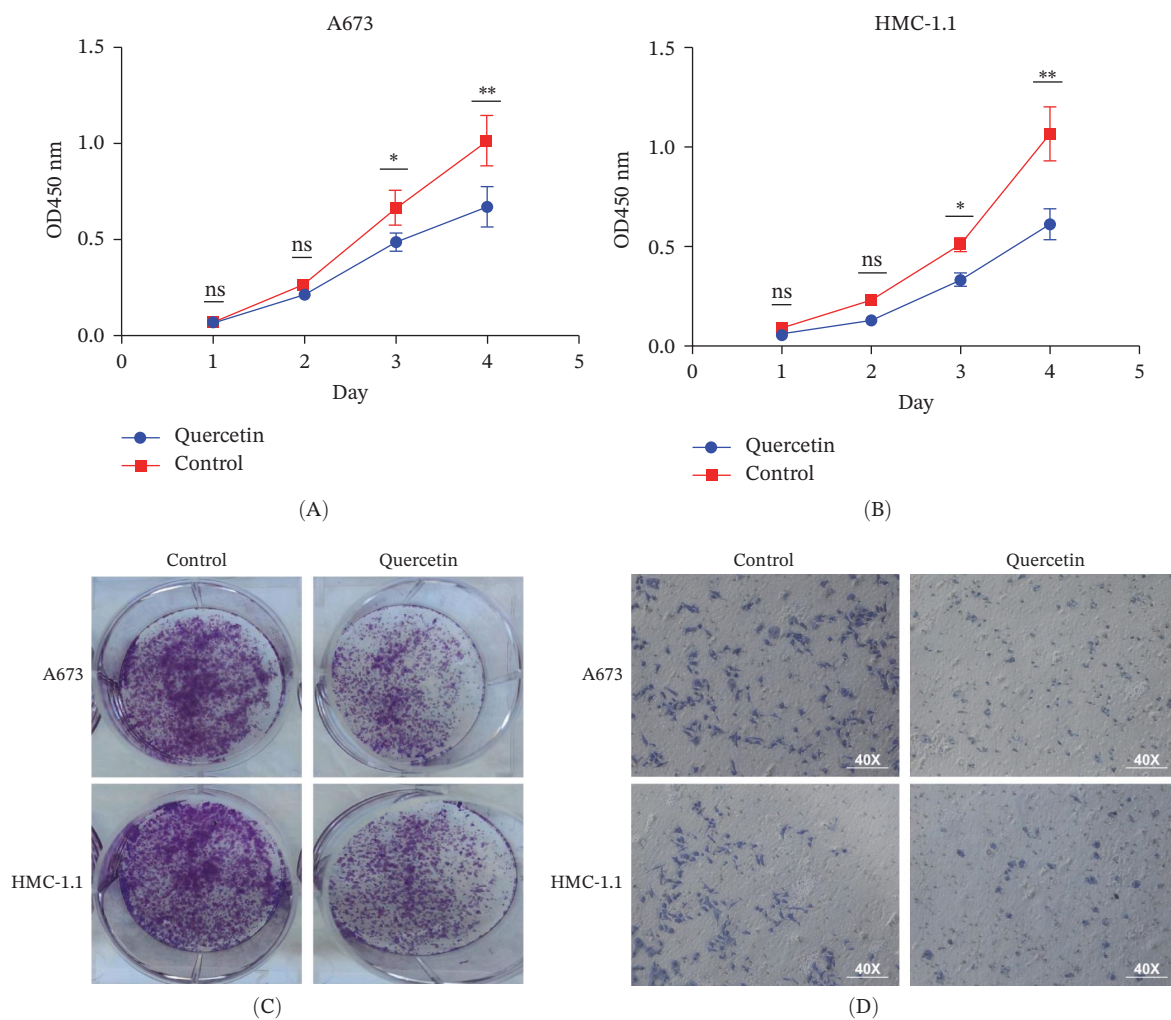


FIGURE 4 | (Continued)

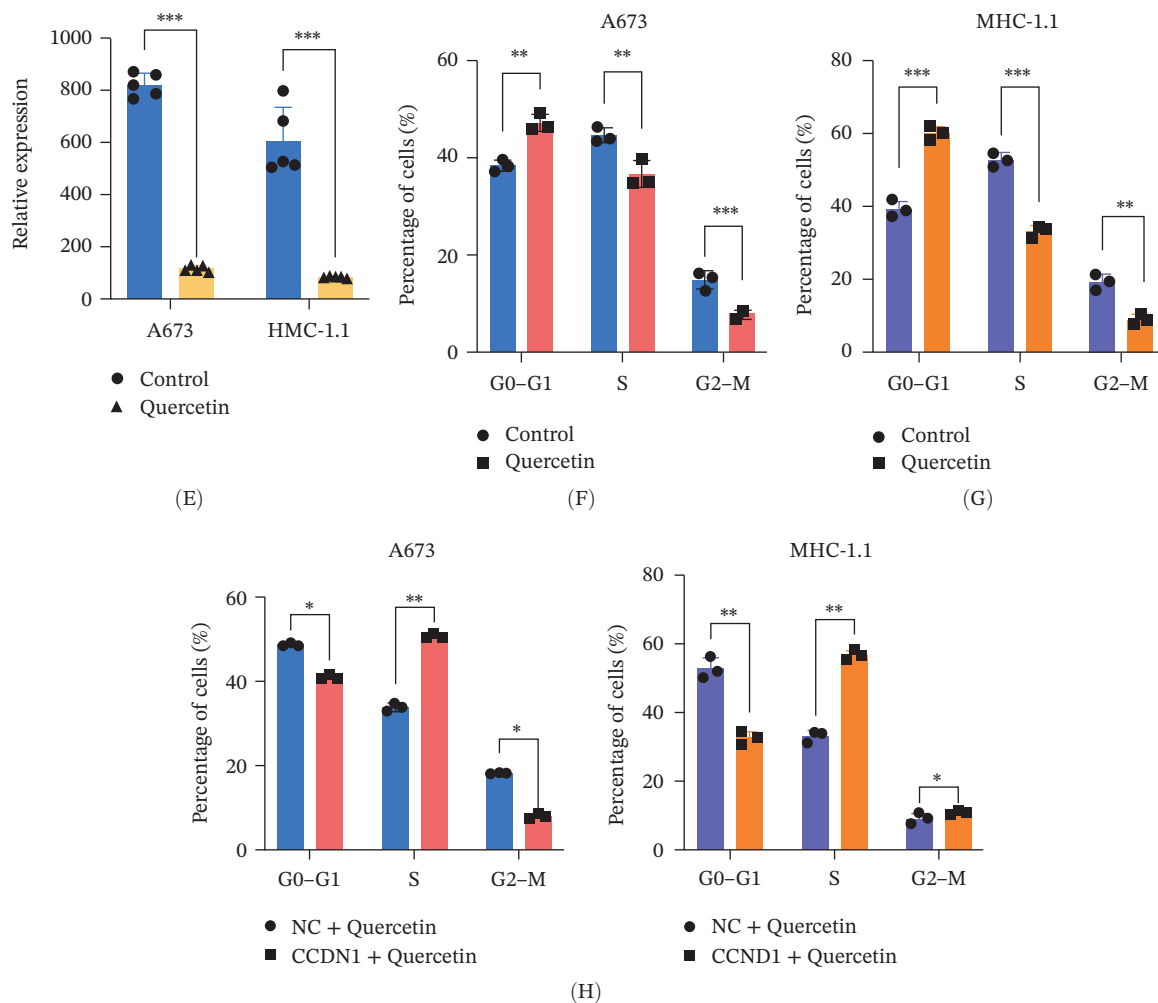


FIGURE 4 | ACPS Inhibits GIST cells in vitro via the PI3K/AKT and CCND1 pathways. (A,B) Proliferation rates of control and quercetin, A673, and HMC-1.1 cells treated as indicated were measured by MTT assay. (C) After 14 days, cells were stained with crystal violet and imaged to assess their colony formation ability. (D,E) Matrigel invasion chambers were used to measure the invasiveness of each group cell treated as indicated, then stained with crystal violet and photographed (Magnification, $\times 40$ for cells; scale bar, 100 μm). (F–H) The cell cycle distribution of each group cell was analyzed by flow cytometry.

mechanism (Figure 6F). A Cytoscape DCTPD network linked ACPS compounds to immune targets IFNG, GJA1, NFKBIA, FOS, IL1B, JUN, PDE3A, and NFE2L2 (Figure 6G), implying that ACPS may simultaneously restrain tumor cells and reshape immune functionality within the metastatic GIST niche. Finally, in vitro cell experiments also confirmed that under ACPS drug treatment conditions, the previously inhibited CD8+ T cell activity in the A673 and HMC-1.1 cell culture medium was significantly reversed and enhanced (Figure 6H).

4 | Discussion

GIST remain the commonest mesenchymal malignancy of the digestive tract, and their incidence has risen noticeably in recent years. Imatinib—a selective KIT inhibitor—has revolutionized first-line management, yet most patients experience progressive disease, frequently disseminated, within 2–3 years of starting therapy [25]. Motivated by this unmet need, we explored the antimetastatic potential and mechanistic basis of active constituents derived from the traditional Chinese medicinal plant *Akebia*

trifoliata (Radix Trachelospermi), aiming to provide a theoretical platform for future drug discovery and clinical application.

Teng-Li-Gen is traditionally valued for clearing heat, detoxifying, dispelling wind, and dampness and is increasingly recognized for antineoplastic properties. Its major bioactive components—sitosterol, β -sitosterol, quercetin, ent-epicatechin, (+)-catechin, and aloe-emodin—belong predominantly to the triterpenoid, flavonoid, and phenolic families [26]. Carboxylic triterpenoids from the herb have previously displayed pronounced cytotoxicity across several cancer cell lines (e.g., the ethanol extract of *Actinidia chinensis* radix and evodiamine modulating CCR7 in SW480 cells). In the present work, network pharmacology identified four high-priority metastatic-GIST targets—FASN, ACACA, CCND1, and TGFBI—that interact with these constituents; quercetin, in particular, exhibited robust docking affinity toward the cell-cycle regulator CCND1, underscoring its promise for further development [27].

Given the synergistic effects of multiple active components and multitarget mechanisms of ASPC, further comprehensive studies on its therapeutic mechanisms are needed in the future. Recent

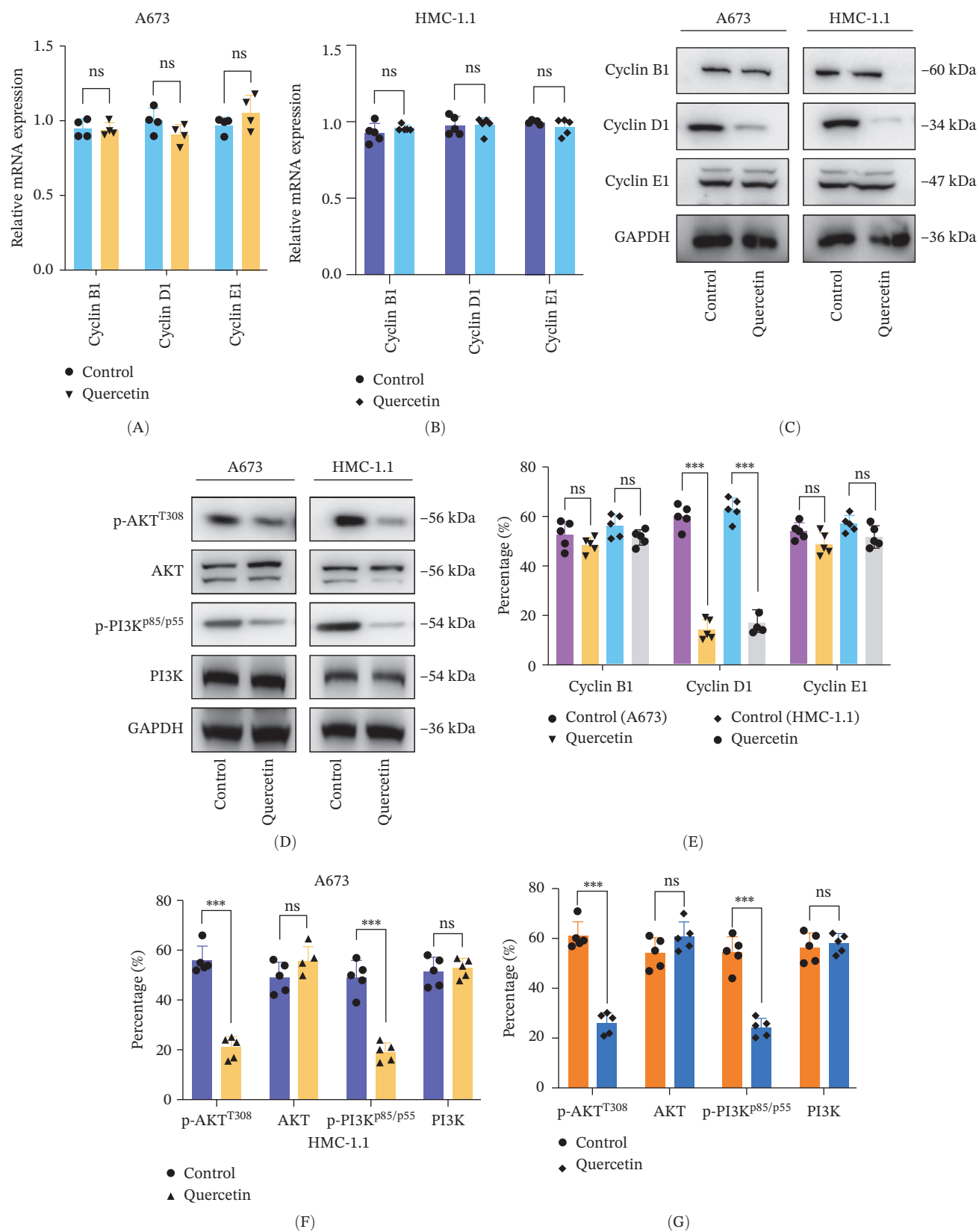


FIGURE 5 | (Continued)

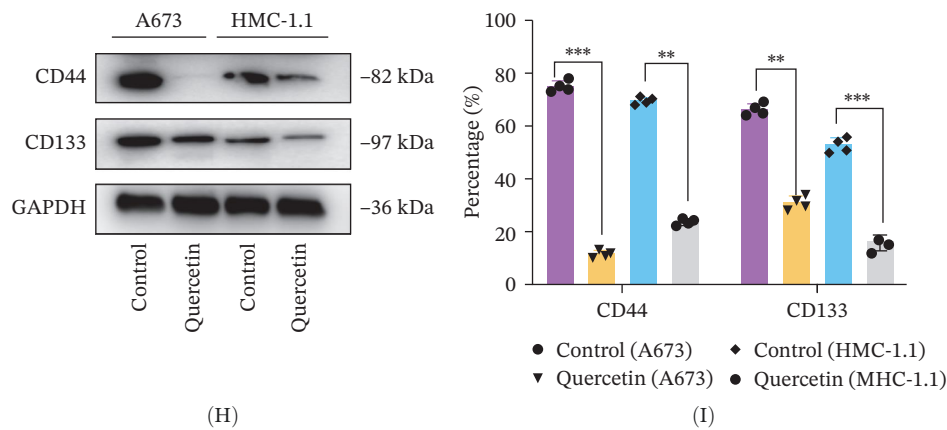


FIGURE 5 | ACPS inhibits GIST cells in vitro via the PI3K/AKT and CCDN1 pathways. (A,B). The Cyclin B1, D1, and E1 PCR results in A673 and HMC-1.1 cells were treated as indicated. (C,E) The western blot results of Cyclin B1, D1, and E1 in A673 and HMC-1.1 cells were treated as indicated. (D, F,G) The western blot results of p-AKT^{T308}, AKT, p-PI3K^{p85/p55}, and PI3K in A673 and HMC-1.1 cells treated as indicated. (H,I) The western blot results of CD44 and CD133 in A673 and HMC-1.1 cells treated as indicated. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ns, not significant.

studies have confirmed that specific active components of traditional Chinese medicine (TCM) can achieve synergistic anti-tumor effects by targeting multiple key cancer pathways [24]. For instance, AD-1, a ginsenoside derivative, has been shown to inhibit proliferation and migration and induce apoptosis in colorectal cancer (CRC) cells. Through network pharmacology analysis and experimental validation, AD-1 shares 39 potential targets with CRC, significantly enriching the PI3K-Akt signaling pathway. Further mechanistic studies indicate that AD-1 not only affects cell growth signals via the PI3K/AKT/mTOR pathway but also interferes with tumor cell glycolytic metabolism, thereby inhibiting malignant progression [28]. Additionally, traditional TCM formulas such as Tianjihuang in the treatment of hepatocellular carcinoma (HCC) have shown that Tianjihuang contains seven active components acting on 207 targets, with 77 related to HCC. Core targets such as AKT1 and STAT3 exert positive effects on HCC through the synergistic action of multiple components, targets, and pathways [29]. Therefore, for TCM therapeutic research, comprehensive application of network pharmacology and multiomics techniques is necessary to reveal the overall regulatory network of these complex formulas.

Cyclin D1 serves as a pivotal positive regulator of G1/S transition, and its amplification or overexpression accelerates proliferation, enhances DNA repair and facilitates invasion in multiple cancers [30, 31]. We observed that ACPS exposure markedly reduced Cyclin D1 protein abundance in A673 and HMC-1.1 GIST cells, implying that interruption of cell-cycle progression contributes to its antiproliferative effect.

Phosphatidylinositol 3-kinase (PI3K) orchestrates phosphoinositide signaling, chiefly by activating protein kinase B (Akt), thereby governing proliferation, differentiation and survival [32, 33]. Vitiello et al. [34] documented pronounced loss of PTEN—a negative PI3K/Akt modulator—in sunitinib-resistant GIST and showed that PTEN restoration diminished Akt phosphorylation and resensitized cells to sunitinib. Our data reveal that ACPS lowers phosphorylated and total levels of PI3K and Akt in A673 and HMC-1.1 cells, suggesting that PI3K/Akt blockade is an additional mechanism through which ACPS constrains GIST growth.

Significant progress has also been made in GIST drug resistance and tumor stemness research. Some studies indicate that GIST resistance is not solely the result of tyrosine kinase mutations but

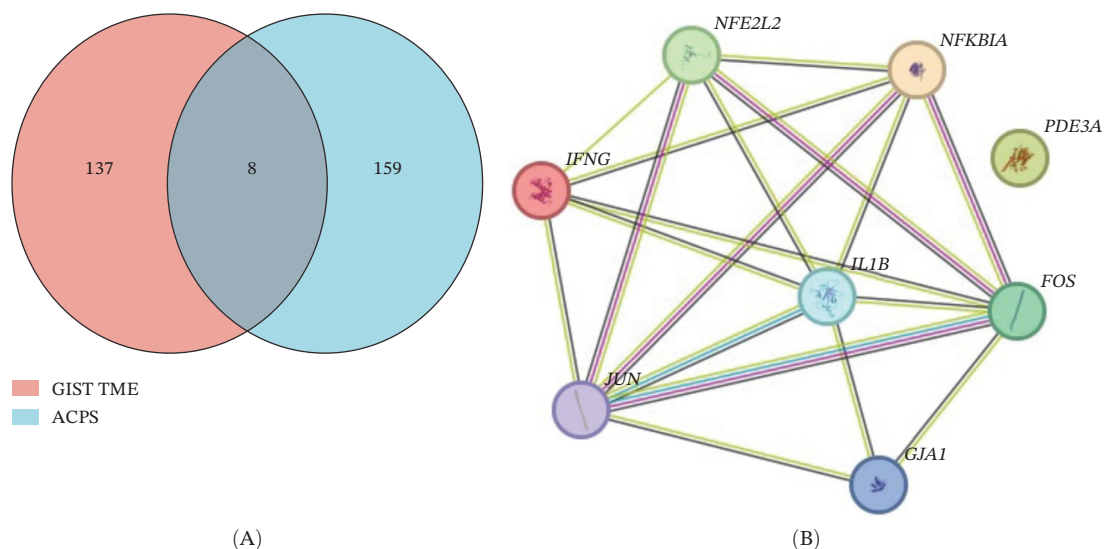


FIGURE 6 | (Continued)

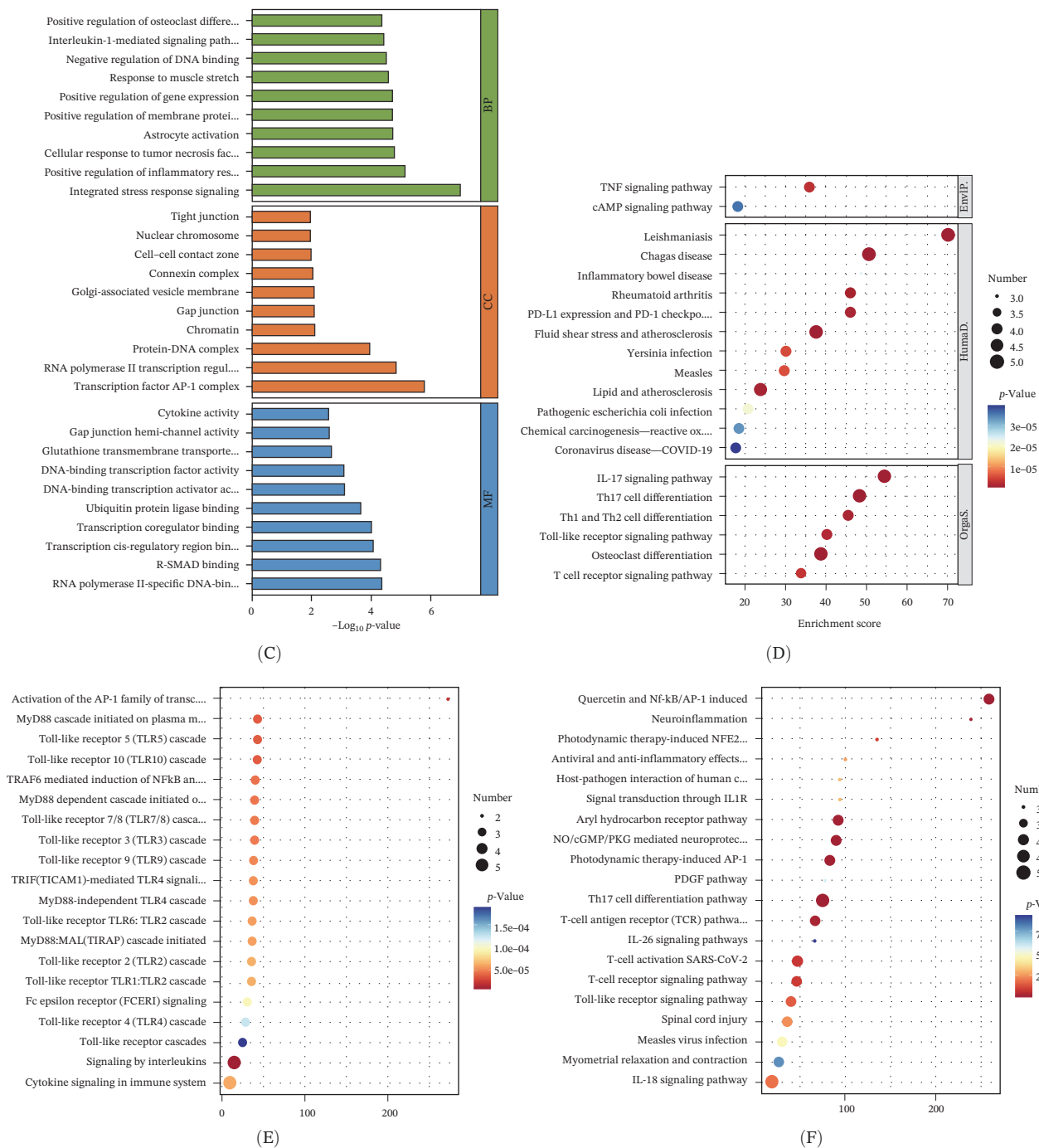


FIGURE 6 | (Continued)

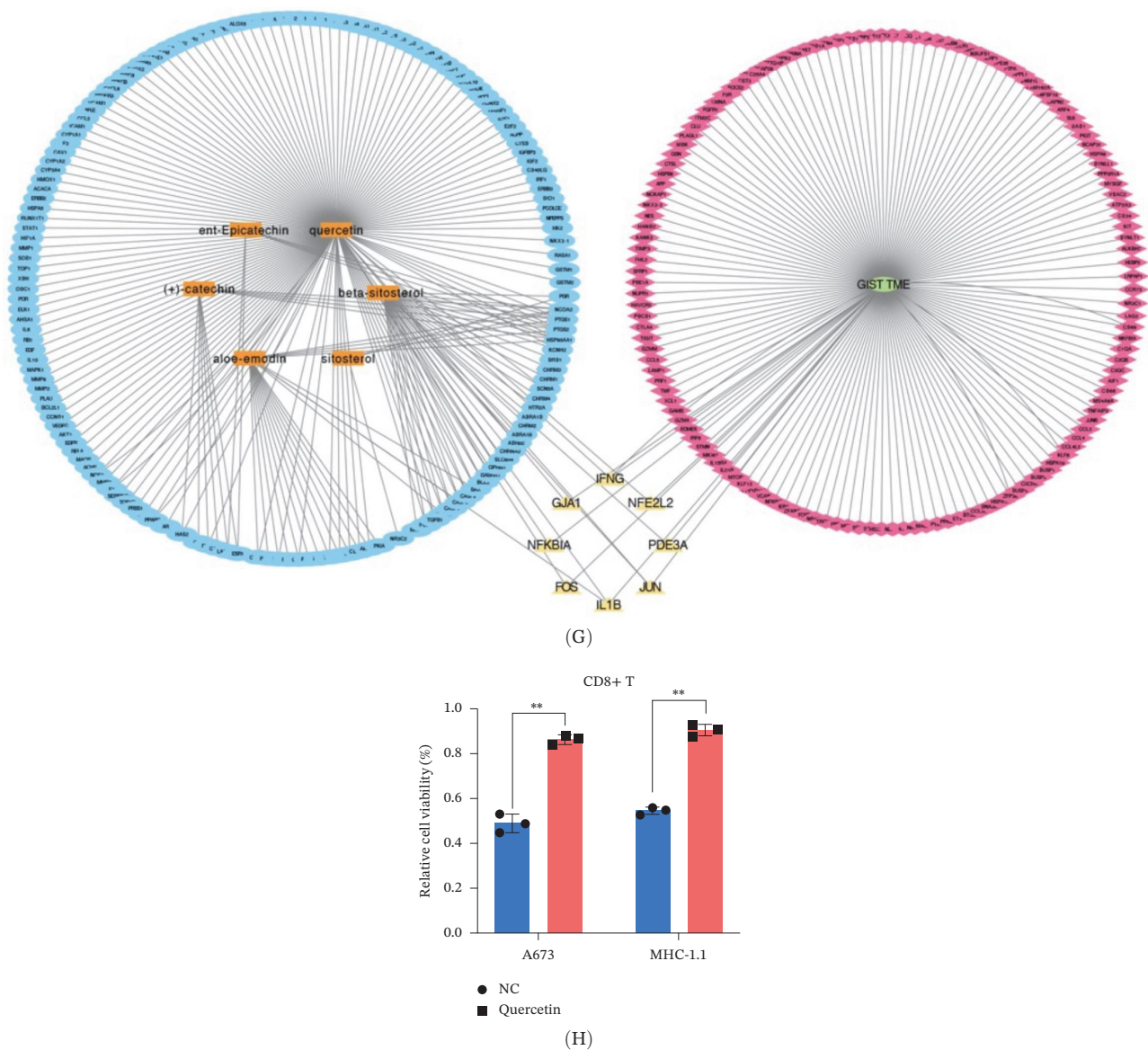


FIGURE 6 | The Inhibitory Effect of ACPS on the Tumor Microenvironment of Metastatic GIST. (A) Intersection results of metastatic GIST target genes and ACPS active component target genes. (B) PPI (Protein-Protein Interaction) network of intersection results. The red circle nodes represent core targets, and the orange circle nodes represent noncore targets. (C) GO (Gene Ontology) enrichment analysis. (D) KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis. (E) Wiki enrichment analysis. (F) Reactome enrichment analysis. (G) Topological network connection results of drugs, active compounds, intersecting genes, and diseases. In A673 and HMC-1.1 cells treated as indicated. In A673 and HMC-1.1 cells treated as indicated. (H) Proliferation rates of CD8+ T cells treated as indicated were measured by MTT assay. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ns, not significant.

also involves TME remodeling. The gastrointestinal microbiota (GM) system, by regulating TME, significantly affects tumor stem cell (CSC) characteristics, leading to increased tumor invasion, metastasis, and chemotherapy resistance [35]. In immune microenvironment regulation, CD33, as a key marker of myeloid-derived suppressor cells (MDSCs), has become a research focus. CD33+ MDSCs may participate in maintaining GIST drug resistance and protecting stemness cells by suppressing anti-tumor immune responses [36]. Additionally, CD144 is considered an important molecule in regulating tumor angiogenesis and stemness phenotypes. CD144 expression is usually associated with endothelial cell activation and vascular remodeling, which may provide a niche for GIST stem cells, promoting their survival and drug resistance [37]. Therefore, CD144 is not only a marker of angiogenesis but may also indirectly affect GIST drug resistance by regulating the TME. Our

current study also shows that ACPS drugs can inhibit the protein expression levels of CD33 and CD144 to a certain extent, suggesting that ACPS may exert its effects by inhibiting the stemness of GIST, thereby resisting metastasis or drug resistance.

Immune effector cells are indispensable for tumor clearance and for augmenting immunotherapeutic efficacy. Macrophages constitute ~87.7% of the immune infiltrate in GIST tissue [38], and single-cell studies have resolved multiple subclusters of NK, dendritic and endothelial cells within these tumors [39]. Network pharmacology docking in this study highlighted eight ACPS targets embedded in the GIST microenvironment—IFNG, GJA1, NFKBIA, FOS, IL1B, JUN, PDE3A, and NFE2L2. These molecules offer new leverage points for dissecting ACPS action and raise the question of whether front-line agents such as imatinib might also modulate the same microenvironmental hubs.

On the other hand, when compound preparations transition from preclinical translation to clinical application, the fundamental bottleneck of quality standardization and control must be addressed. Recent studies have shown that traditional single-index quality control methods can no longer meet the requirements of multicomponent compounds, and a comprehensive evaluation system combining multi-fingerprint mapping and network pharmacology verification must be established for standardization [40]. Specifically, research of fingerprint maps based on high-throughput chromatography–mass spectrometry (HPLC–MS) has been established, combined with chemometric models such as principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA), to strictly monitor the content, proportion, and batch-to-batch consistency of key active components, ensuring each batch of preparation has consistent chemical composition spectra and biological activity. Simultaneously, the verification of targeted network pharmacology could be incorporated into the scope of quality control, not only detecting chemical components but also verifying whether these components can synergistically regulate key signaling pathways, thereby might establish a biological function quality evaluation standard for future clinical application [41].

Although this study integrated multiple bioinformatics analyses with experimental validation, two limitations remain: first, the issue of binding energy in molecular docking. This study employed AutoDock molecular docking to validate predicted compound–target interactions, revealing that quercetin exhibits strong binding affinity to cyclin D1 (CCND1) with a binding free energy of -9.4 kcal/mol. The quercetin–CCND1 complex is primarily stabilized by hydrogen bonds between the phenolic hydroxyl groups of quercetin and the Arg-183 residue of CCND1, along with extensive hydrophobic contacts and van der Waals forces involving Glu-180, Asn-493, Arg-176, and Asp-491. We hypothesize that this binding interferes with CCND1 function by inducing local conformational changes that disrupt its interaction with CDK4/6 or by sterically hindering its functional regions, thereby inhibiting the CCND1–CDK4/6 complex and causing G1 phase cell cycle arrest, which aligns with our experimental observation of ACPS-induced G0/G1 arrest in GIST cells. While binding energy alone is only a preliminary indicator, future studies will incorporate molecular dynamics simulations and site-directed mutagenesis to rigorously validate the stability of this binding mode and confirm the essential role of key residues such as Arg-183 in quercetin’s inhibition of CCND1 function.

The second issue of target priority selection. Our selection of CCND1 and the PI3K/AKT pathway for experimental validation was based on both mechanistic and biological rationale: mechanistically, network pharmacology, and PPI network analysis identified CCND1 as a core hub gene with the lowest quercetin binding energy (-9.4 kcal/mol), while KEGG enrichment highlighted the PI3K–Akt pathway; biologically, CCND1 is a critical G1/S cell cycle regulator whose dysregulation drives tumor proliferation, and PI3K/AKT is a central oncogenic axis in GIST linked to progression and drug resistance, giving them high translational priority. In contrast, while metabolic enzymes FASN and ACACA were predicted targets, their specific role in GIST is less established than the PI3K/AKT–CCND1 axis, leading us to prioritize limited resources on the most robust targets, though we acknowledge these metabolic pathways warrant future investigation.

5 | Conclusion

In summary, by integrating network–pharmacology docking with in vitro phenotypic assays, we have charted the direct targets of ACPS within tumor cells and its regulatory nodes inside the GIST immune microenvironment, offering fresh leads for next-generation drug design and translational application. Nevertheless, the absence of in vivo or clinical corroboration remains an inherent limitation of the present work, and further validation in animal models and patients is indispensable to advance ACPS-based GIST therapeutics.

Author Contributions

Jun Qian designed the study. Jun-Hua Yu performed experiments. Lu-Lu Cao performed data analyses. Jian-ping Fan wrote the manuscript. Ji-yun Zhu reviewed the manuscript and supervised this study.

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Disclosure

All authors have approved and agreed to submit the final version of the manuscript for publication in this journal.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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