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Polysaccharides: Natural candidates for targeting immunometabolic regulation in hepatocellular carcinoma (Review)

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Abstract. Hepatocellular carcinoma (HCC) is a leading cause of cancer-related morbidity and mortality worldwide. HCC pathogenesis is predominantly associated with chronic liver disease, driven by a vicious cycle of persistent hepatic injury, chronic inflammation and aberrant hepatocyte regeneration. Within this complex pathological landscape, remodeling of the tumor immune microenvironment (TIME), metabolic dysregulation and gut-liver axis imbalance collectively contribute to HCC initiation and progression. Given the limited efficacy of conventional therapeutic modalities against such multifaceted pathologies, there is an urgent need to explore novel strategies that are safe, effective and capable of multi-target regulation. Natural polysaccharides, a class of bioactive macromolecules, have emerged as promising candidates for prevention and treatment. Polysaccharides not only directly suppress malignant phenotypes in HCC cells by modulating cell cycle progression, inducing apoptosis, and inhibiting invasion and metastasis, but also exert potent immunomodulatory effects. Specifically, they activate key immune effectors, including cytotoxic T lymphocytes, dendritic cells, natural killer cells and tumor-associated macrophages, thereby reversing immunosuppression and reshaping the TIME. Furthermore, polysaccharides can modulate the gut microbiota and its metabolites, enrich beneficial bacterial taxa, and reinforce intestinal barrier integrity. These actions subsequently influence intrahepatic immune responses and inflammatory status, achieving systemic intervention via the 'gut-liver-immune' axis. The present review provides a systematic overview of the multifaceted mechanisms by which polysaccharides combat HCC, including direct antitumor effects, modulation of the TIME and regulation of the gut microbiota. By synthesizing current evidence, the present review aims to offer critical perspectives to advance the mechanistic understanding and facilitate the clinical translation of polysaccharides for HCC treatment.

Contents

1. Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths worldwide (1). Pathogenesis is

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Abbreviations: HCC, hepatocellular carcinoma; TIME, tumor immune microenvironment; APS, *Astragalus* polysaccharide; TACE, transarterial chemoembolization; ICI, immune checkpoint inhibitor; LMF, low-molecular-weight fucoidan; DCR, disease control rate; SCFA, short-chain fatty acid; DC, dendritic cell; TAM, tumor-associated macrophage; NK, natural killer; EMT, epithelial-mesenchymal transition; ER, endoplasmic reticulum; ROS, reactive oxygen species; HIF-1α, hypoxia-inducible factor-1α; PRDX1, peroxiredoxin 1; TLR4, toll-like receptor 4; MyD88, myeloid differentiation primary response 88; FXR, farnesoid X receptor; BA, bile acid

Key words: polysaccharides, HCC, TIME, gut-liver axis, immunomodulation, gut microbiota, combination therapy

predominantly associated with chronic liver disease, driven by a vicious cycle of persistent hepatic injury, chronic inflammation and aberrant hepatocyte regeneration (2,3). The disease is characterized by rapid progression and poor prognosis, posing challenges for clinical management. Current treatment primarily involves surgical resection, often combined with adjuvant drug therapy (4). However, surgery entails substantial risks and the recurrence rate within one year post-surgery is as high as 50% (5), severely limiting long-term outcomes. Therefore, the development of safer and more effective therapeutic strategies is an urgent priority in HCC research.

Tumors can evade immune recognition and elimination primarily by secreting various immunosuppressive factors that dampen host antitumor immune responses, thereby achieving immune escape (6,7) (Fig. 1A). Accumulating evidence indicates that, beyond the intrinsic biological properties of tumor cells, the tumor immune microenvironment (TIME) serves a pivotal role in regulating the initiation, progression and recurrence of HCC (8,9). Notably, the liver and gut are anatomically and functionally interconnected through the portal venous and biliary systems, forming the 'gut-liver axis'. The portal vein transports gut-derived microbial metabolites to the liver, whereas bile and certain liver-synthesized immune factors are returned to the intestine via the biliary tract (10), establishing a bidirectional communication network. This intimate crosstalk suggests that dysbiosis or functional alterations in the gut microbiota influence hepatic immunity and inflammation. Consequently, modulating the gut microbiota and its metabolic activities represents a novel and promising therapeutic strategy for HCC intervention (11,12).

Natural polysaccharides have attracted widespread attention (13-15) due to their antitumor activity. Following delivery to the liver via the portal vein, these polysaccharides not only directly target HCC cells by modulating multiple key signaling pathways, thereby inhibiting proliferation, migration and invasion, as well as inducing apoptosis (16), but also exert indirect antitumor effects by remodeling the TIME and enhancing immune surveillance (17). The fraction of polysaccharides that remains unabsorbed in the gastrointestinal tract can interact with the gut microbiota, modulate microbial composition and metabolic activity, improve intestinal barrier integrity, and activate specific immune or metabolic pathways, such as the IL-6/Janus kinase (JAK)1/STAT3 signaling axis, thereby generating systemic and distal anti-HCC effects (18,19).

The search strategy was as follows: PubMed (https://pubmed.ncbi.nlm.nih.gov/), Web of Science (https://www.webofscience.com/), China National Knowledge Infrastructure (CNKI, https://www.cnki.net/) and Wanfang Data Knowledge Service Platform (http://www.wanfangdata.com.cn/) were among the major English and Chinese databases in which a thorough literature search was carried out. The search covered the period from database inception to October 2025. The literature search was conducted using a combination of keywords, primarily focusing on 'polysaccharides', 'HCC' and 'clinical research'. More specific terms, such as 'gut-liver axis', 'clinical trials' and 'mechanism', were also incorporated to refine the search. Studies involving treatment were eligible for inclusion if they were conducted on patients with pathologically or radiologically confirmed HCC, or in relevant HCC cellular and animal models, and investigated either a single purified natural polysaccharide or a formulation primarily composed of natural polysaccharides. The exclusion criteria included the following: i) Studies that used complex formulas (rather than single polysaccharide components) or chemically synthesized drugs as the primary intervention; ii) studies that focused only on extraction technology without pharmacodynamic evaluation; and iii) studies involving polysaccharides that did not assess HCC prevention or treatment.

In summary, the present article provides a systematic review of current research advances regarding natural polysaccharides for the treatment of HCC.

2. Polysaccharide-mediated modulation of tumor cell behavior

Upon reaching the liver, polysaccharides derived from plants and fungi can suppress the initiation and progression of HCC through multi-target, multi-pathway mechanisms. These compounds exert antitumor effects by inhibiting tumor cell proliferation, migration and invasion, as well as by inducing programmed cell death through specific molecular pathways.

The 4°C-extracted *Grifola frondosa* polysaccharide (GFG-4) suppresses HepG2 cell proliferation by engaging the membrane-bound Fas/Fas ligand death receptor system and activating the downstream caspase-8/-3 pathway (13). In the intrinsic (mitochondrial) apoptotic pathway, the acid-soluble polysaccharide from *Grifola frondosa* upregulates the expression of the pro-apoptotic protein Bax, thereby inducing the opening of the mitochondrial permeability transition pore. This facilitates the release of cytochrome *c* (cyto-c) from the mitochondria into the cytosol, resulting in sequential activation of caspase-9 and caspase-3. The ensuing caspase cascade drives apoptosis in HepG2 cells (14) (Fig. 1C).

In addition to modulating classical apoptotic pathways, polysaccharides inhibit the progression of HCC through multiple mechanisms, including cell cycle regulation, suppression of epithelial-mesenchymal transition (EMT), induction of endoplasmic reticulum (ER) stress and modulation of redox homeostasis. Regarding oxidative stress, polysaccharides from *Ulva lactuca* and *Lactarius deliciosus* increase intracellular reactive oxygen species (ROS) levels, leading to DNA damage and dissipation of the mitochondrial membrane potential (20,21). These effects result in the inhibition of HepG2/H22 cell proliferation and promotion of apoptosis (20,21). In the context of cell cycle arrest, *Polygonatum sibiricum* polysaccharides induce G_1 phase arrest in HepG2 cells, thereby reducing the proportion of cells progressing to the S phase (22). By contrast, daylily polysaccharide triggers G_2/M phase arrest by inhibiting the Wnt/β-catenin signaling pathway, ultimately suppressing tumor cell proliferation and promoting apoptosis (23) (Fig. 1C). The *in vitro* and *in vivo* anti-HCC effects of polysaccharides are summarized in Table SI (13,14,21-49).

Numerous polysaccharides have been shown to exhibit anti-migratory and anti-invasive effects, and thus, to inhibit tumor metastasis. *Codonopsis pilosula* polysaccharide suppresses EMT, reduces cancer stemness and impairs intercellular adhesion by inhibiting cyclin-dependent kinase 1 activity and modulating the pyruvate dehydrogenase kinase 1/β-catenin signaling pathway, thereby markedly attenuating

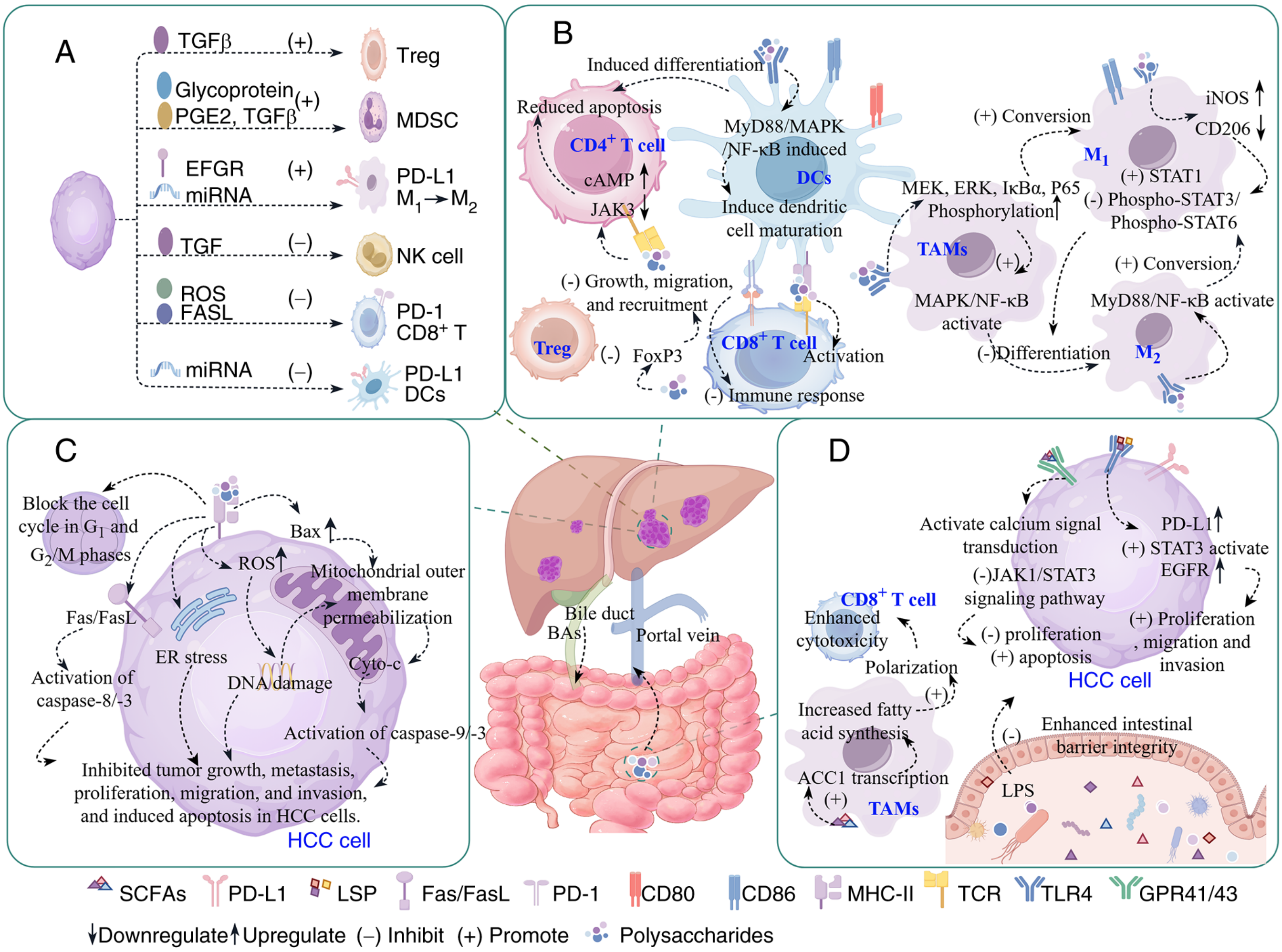

Figure 1. Overview of the molecular and cellular mechanisms by which polysaccharides exert anti-HCC activity. (A) Pro-tumorigenic cellular response in HCC. (B) Polysaccharide-mediated modulation of tumor cell behavior. (C) Polysaccharide modulation of the tumor immune microenvironment. (D) Regulation of gut microbiota and their metabolites by polysaccharides. Materials provided by FigDraw2.0 (https://www.figdraw.com/). ACC1, acetyl-CoA carboxylase 1; BAs, bile acids; Cyto-c, cytochrome *c*; DC, dendritic cell; ER, endoplasmic reticulum; FASL, Fas and its ligand; GPR41/43, G protein-coupled receptor 41/43; HCC, hepatocellular carcinoma; iNOS, inducible nitric oxide synthase; JAK, Janus kinase; LPS, lipopolysaccharide; MDSC, myeloid-derived suppressor cells; MHC-II, major histocompatibility complex class II; miRNA, microRNA; MyD88, myeloid differentiation primary response 88; NK, natural killer; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; PGE2, prostaglandin E2; ROS, reactive oxygen species; SCFA, short-chain fatty acid; TAM, tumor-associated macrophage; TCR, T cell receptor; TLR4, toll-like receptor 4; Treg, regulatory T cell.

malignant phenotypic traits in Huh-7 HCC cells (39). Similarly, basil polysaccharide targets hypoxia-inducible factor-1α (HIF-1α), alleviates tumor hypoxia and reverses EMT by downregulating mesenchymal markers (such as β-catenin, N-cadherin and vimentin) and upregulating epithelial markers [such as E-cadherin, vacuole membrane protein 1 and zonula occludens-1 (ZO-1)], leading to cytoskeletal reorganization and suppression of HCC cell invasion and metastasis (33). Furthermore, *Astragalus* polysaccharide (APS) enhances ER stress and promotes apoptosis in HCC cells by reducing protein O-GlcNAcylation and increasing O-GlcNAcase expression (50).

However, it is imperative to acknowledge that the antitumor efficacy of polysaccharides is strictly structure-dependent, and not all polysaccharide fractions exhibit bioactivity. Variations in molecular weight, glycosidic linkages and branching degrees can lead to distinct biological outcomes. For instance, polysaccharide fractions with extremely low molecular weights may fail to effectively recognize cell surface receptors, such as toll-like receptors (TLRs) or scavenger receptors, resulting in a loss of immunomodulatory or pro-apoptotic activity (51). Similarly, specific glycosidic bond configurations (for example, α-vs. β-linkages) or the absence of essential functional groups (such as sulfate or carboxyl groups) can render a polysaccharide biologically inert against HCC cells (52). This structural specificity suggests that the 'negative' findings often reported in polysaccharide research may not indicate a failure of the therapeutic class *per se*, but rather reflect the critical necessity of precise structural features for bioactivity.

Overall, while the structural heterogeneity of polysaccharides dictates their specific molecular targets and efficacy, those possessing optimal structural features consistently exhibit potent bioactivity against HCC, including suppression of cell proliferation, induction of apoptosis, induces cell cycle arrest, inhibition of metastasis and remodeling of the tumor microenvironment. These pleiotropic effects highlight the synergistic potential of polysaccharides as multicomponent, multi-target and multi-pathway therapeutic interventions.

3. Polysaccharide modulation of the TIME

Polysaccharide interactions with adaptive immune cells. The T cells within the TIME primarily comprise CD8$^+$ cells, CD4$^+$ cells and regulatory T cells (Tregs) (53). CD8$^+$ T cells exert cytotoxic effects by recognizing tumor-specific antigens presented by major histocompatibility complex class I (MHC-I) molecules on antigen-presenting cells (APCs). By contrast, CD4$^+$ T cells recognize antigenic peptides presented by MHC class II (MHC-II) molecules, thereby coordinating and modulating adaptive immune responses to enhance antitumor immunity. Tregs, which express high levels of the transcription factor forkhead box protein P3 (FoxP3), mediate immunosuppressive functions by inhibiting the activation and proliferation of effector T cells, thereby promoting tumor immune evasion (54,55) (Fig. 1B). Notably, polysaccharides can be internalized by APCs via endocytic pathways and subsequently degraded into low-molecular-weight carbohydrates via intracellular nitric oxide (NO)-dependent processes. These processed fragments are then loaded onto MHC-II molecules and presented to the T-cell receptor, leading to the activation of antigen-specific CD4$^+$ T-cell responses (56).

CD4$^+$CD25$^+$ Tregs have been shown to serve a pivotal role in suppressing CD8$^+$ T-cell activity within the tumor margin regions of HCC (15). APS counteracts this immunosuppressive effect by downregulating FoxP3 mRNA expression in the local TIME, thereby inhibiting the proliferation and expansion of CD4$^+$CD25$^+$ Tregs (Fig. 1B). Additionally, APS disrupts the C-X-C motif chemokine receptor 4 (CXCR4)/C-X-C motif chemokine ligand 12 signaling axis, blocking stromal-derived factor-1-mediated recruitment of Tregs into the HCC microenvironment, thus reducing their infiltration and immunosuppressive activity (57). Similarly, *Ganoderma lucidum* spore polysaccharide (GLSP) suppresses HCC growth in tumor-bearing mice by upregulating microRNA (miR)-125b expression. This leads to inhibition of the Notch1 signaling pathway and reduced FoxP3 expression, thereby alleviating Treg-mediated suppression of effector T cell proliferation (58).

In the context of immune checkpoint regulation, engagement of programmed death-1 (PD-1) on T cells with its ligand programmed death-ligand 1 (PD-L1), which is expressed on tumor or immune cells, results in T cell exhaustion and impaired effector function (59). In HCC, IFN-γ secreted by activated immune cells induces acetylation of myocyte enhancer factor 2D, leading to the upregulation of PD-L1 expression and subsequent attenuation of CD8$^+$ T cell-mediated antitumor immunity (60). APS mitigates this immunosuppressive mechanism by modulating the miR-133a-3p/moesin signaling axis, which enhances the infiltration of CD4$^+$ and CD8$^+$ T cells into tumor tissues (17,61). Furthermore, APS promotes the generation and long-term persistence of CD122-positive C-X-C motif chemokine receptor 3-positive PD-1-negative memory-like T cells, and enhances their migration to tumor sites, boosting the cytotoxic activity of CD8$^+$ chimeric antigen receptor T cells against HCC (62).

In addition to modulating Treg cells and immune checkpoints, other polysaccharides enhance antitumor immunity through alternative mechanisms. Dandelion polysaccharides increase the proportion of CD4$^+$ T cells in the spleen and improve T-cell infiltration into tumor tissues. In H22 tumor-bearing mice, these immunomodulatory actions mediated by T-cell homeostasis exert antitumor activity by inhibiting IL-6-activated JAK/STAT signaling and modulating hepcidin expression (63,64). Salvianolic acid polysaccharides ameliorate CD4$^+$ T cell apoptosis and serum cytokine dysregulation induced by H22 tumor engraftment. Salvianolic acid polysaccharides reduce intracellular cyclic adenosine monophosphate levels in CD4$^+$ T cells, downregulate JAK3 protein expression, promote STAT5 phosphorylation and facilitate transcriptional activation of downstream anti-apoptotic genes. Collectively, these effects enhance the cytotoxic function of natural killer (NK) cells and CD8$^+$ T cells (65).

Despite the potential of polysaccharides in enhancing T-cell infiltration and cytotoxicity, a critical challenge lies in the potential induction of T-cell exhaustion and immune tolerance. The tumor microenvironment is characterized by persistent antigen exposure, and while polysaccharides amplify T-cell activation, this sustained hyperstimulation may drive CD8$^+$ T cells into a state of terminal exhaustion, marked by the upregulation of multiple immune checkpoints [such as PD-1, T cell immunoglobulin- and mucin-domain-containing 3 (TIM-3) and lymphocyte-activation gene 3 (LAG-3)] and loss of effector function (66). Additionally, the liver's complex immunosuppressive network, characterized by the recruitment of regulatory T cells and myeloid-derived suppressor cells, may counteract the initial stimulatory effects of polysaccharides, leading to a state of adaptive immune resistance (67). This suggests that monotherapy with immunostimulatory polysaccharides may face limitations due to the development of compensatory inhibitory mechanisms, highlighting the necessity of combination strategies or structural optimization to maximize therapeutic windows.

Polysaccharide interactions with innate immune cells

Dendritic cells (DCs). DCs serve as central sentinel cells in immune surveillance within the TIME and serve a pivotal role in initiating adaptive immune responses against pathogens and malignant cells, particularly HCC cells. This is achieved by presenting antigenic peptides via MHC-I and MHC-II molecules to CD4$^+$ and CD8$^+$ T cells, respectively (68). Notably, DC-derived exosomes also express MHC-I, MHC-II, and co-stimulatory molecules such as CD80 and CD86, and possess potent antigen-presenting capacity. These exosomes can effectively activate both naïve and memory T cells, making them promising candidates for eliciting specific antitumor immunity (69,70).

Studies have shown that polysaccharides can bind specifically to TLR4 on DCs, triggering downstream signaling cascades that promote DC maturation and functional activation. This enhances DC-T cell interactions and critically contributes to anti-HCC immune responses (71). Notably, polysaccharide components isolated from *Portulaca oleracea* and *Carica papaya* leaves have been demonstrated to trigger the TLR4/myeloid differentiation primary response 88 (MyD88)/MAPK/NF-κB signaling cascade. In this pathway, the toll/interleukin-1 receptor/resistance protein (TIR) domain of the MyD88 adaptor protein engages with the TIR domain of TLR4, facilitating the recruitment and activation of downstream signaling intermediates and kinases. This cascade culminates in the nuclear translocation of the NF-κB transcription factor, which binds to specific promoter sequences to upregulate the expression of maturation-associated genes, thereby driving the phenotypic and functional maturation of DCs (72,73).

Polysaccharides from *Alhagi sparsifolia* and APS upregulate the expression of costimulatory molecules (CD80 and CD86), MHC-I and MHC-II in DCs and their subsets. Concurrently, they enhance the secretion of key pro-inflammatory cytokines, including IL-12, TNF-α and IL-6 (74,75). These changes markedly improve the capacity of DCs for antigen uptake, processing and presentation, enabling them to efficiently drive the differentiation of naïve T cells into T helper 1 (Th1) cells and enhance the cytotoxic activity of $CD8^+$ T cells, ultimately resulting in suppression of tumor growth (73). Pretreatment with TAK-242, a selective TLR4 inhibitor, attenuates polysaccharide-induced upregulation of DC maturation markers and impairs Th1-polarized immune responses. This pharmacological blockade confirms the central role of TLR4 in mediating the immunomodulatory effects of p-SGP (the purified acidic polysaccharide from *Sarcandra glabra*) (76) (Fig. 1B).

Beyond direct T-cell activation, the immunostimulatory effects of polysaccharides on DCs initiate broader immune cascade responses. Mature DCs function as central hubs in the immune network, promoting the activation and recruitment of NK cells and macrophages, thereby orchestrating a multi-layered, synergistic antitumor defense within the tumor microenvironment (77).

Macrophages. Tumor-associated macrophages (TAMs) are among the most abundant immune cell populations in the TIME and exhibit high plasticity, enabling their polarization into two functionally distinct phenotypes: Pro-inflammatory, antitumor M1 macrophages and anti-inflammatory, pro-tumorigenic M2 macrophages (78). Accumulating evidence indicates that tumor cells actively suppress M1 polarization and promote the transition of TAMs toward the M2 phenotype through multiple mechanisms (79,80). This shift fosters an immunosuppressive microenvironment that facilitates tumor growth, invasion and metastasis (81,82). Polysaccharides have emerged as promising immunomodulatory agents capable of reversing TAM polarization imbalance. These natural compounds not only enhance the phagocytic capacity and antigen-presenting function of macrophages, but also precisely regulate their polarization at the molecular level, promoting M1 activation while inhibiting M2 differentiation, thereby exerting potent anti-HCC effects (83-85).

Mechanistic investigations have demonstrated that polysaccharides exert their effects by activating the TLR4/MAPK/MyD88/NF-κB signaling cascade. This leads to the upregulation of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) and phosphorylation of critical downstream effectors, including MEK, ERK, IκBα and p65. Simultaneously, the expression of M2-associated markers, including IL-10 and arginase-1 (Arg-1), is downregulated. These molecular events culminate in the phenotypic reprogramming of M2-polarized TAMs into an M1-like state (85-87).

TLR4 serves as a central receptor mediating the immunomodulatory effects of polysaccharides on TAMs. It has been reported that in tumor-bearing mouse models with genetic deletion of TLR4 or MyD88, APS fails to induce the production of inflammatory mediators such as NO, TNF-α, IL-1β and IL-6, and the activation of TNF receptor associated factor 6 and NF-κB is impaired (83). These findings underscore the essential role of TLR4-dependent signaling in the antitumor activity of polysaccharides (Fig. 1B).

In addition to activating the TLR4 signaling pathway, polysaccharides shift the polarization balance of TAMs by upregulating M1 markers (including CD86 and inducible nitric oxide synthase) and pro-inflammatory cytokines, while downregulating M2 markers (such as CD206 and Arg-1) and anti-inflammatory factors. This phenotypic reprogramming is associated with enhanced activation of STAT1 and suppression of STAT3 and STAT6 phosphorylation, effectively blocking the transcriptional program that drives M2 polarization (84,85,88).

In summary, polysaccharides not only enhance the phagocytic and antigen-presenting functions of TAMs but also orchestrate their polarization through coordinated modulation of multiple signaling pathways. By promoting M1 repolarization and counteracting M2-mediated immunosuppression, they facilitate the activation of effector immune cells, including T cells and DCs, ultimately contributing to the formation of a robust antitumor immune network. These properties highlight the multitarget, high-efficacy potential of polysaccharides as immunomodulatory agents in HCC therapy.

However, the potent immunomodulatory capacity of polysaccharides also presents a double-edged sword, necessitating careful consideration of the risk of immune hyperactivation and cytokine storms. Given that TLR4 signaling serves as the primary mechanism for polysaccharide recognition, excessive or uncontrolled stimulation of this pathway can lead to the overproduction of pro-inflammatory mediators, such as TNF-α, IL-1β and IL-6 (89). While these cytokines are essential for antitumor responses, their systemic elevation may trigger a 'cytokine storm', leading to collateral tissue damage and endotoxin-like toxicity. Furthermore, the dynamic balance between M1 and M2 macrophage polarization is delicate; while polysaccharides aim to repolarize TAMs towards the M1 phenotype, persistent and excessive M1 activation may paradoxically contribute to chronic inflammation (90) and immune exhaustion (91) within the TIME, potentially undermining long-term therapeutic efficacy.

NK cells. NK cells have emerged as promising candidates for cancer immunotherapy. As key effector cells of the innate immune system, NK cells serve a pivotal role in antitumor immune surveillance (92,93). Accumulating evidence indicates that various polysaccharides can effectively enhance NK cell function and antitumor activity. For instance, *in vivo* studies have demonstrated that *Salvia miltiorrhiza* polysaccharide, *Panax notoginseng* neutral polysaccharide and *Angelica sinensis* polysaccharide (ASP) increase NK cell cytotoxicity, boost splenic lymphocyte activity, elevate the levels of critical cytokines such as IL-2 and TNF-α, and modulate peripheral blood lymphocyte subsets, collectively contributing to tumor suppression and exerting anti-HCC effects (65,94,95). Despite these findings, the molecular mechanisms by which polysaccharides specifically regulate NK cells in HCC remain poorly understood, and systematic investigations in this area are lacking.

NK cell function is frequently impaired in patients with HCC, which is a phenomenon linked to both immunosenescence and an immunosuppressive tumor microenvironment (96,97). Notably, macrophage migration inhibitory factor interacts with its receptor CXCR4 to upregulate immunoglobulin-like transcript 2 (ILT2) in NK cells. Elevated ILT2 expression is strongly associated with diminished NK cell effector functions, including reduced cytotoxicity and antibody-dependent cellular cytotoxicity. Thus, ILT2 serves not only as a phenotypic marker of exhausted NK cells in HCC but also as a promising therapeutic target; blockade of the ILT2 pathway may restore NK cell antitumor activity (98). Advances in research on engineered NK (eNK) cells have further highlighted their therapeutic potential. Functionally enhanced eNK cells exhibit high surface expression of antitumor molecules such as TNF-related apoptosis-inducing ligand, CD226 and CD16, along with increased intracellular levels of perforin, granzyme B, TNF-α and IFN-γ. These eNK cells exhibit potent cytotoxic activity against multiple HCC cell lines, including HepG2, HuH7 and SNU-423 cells (99), highlighting the feasibility of harnessing NK cells for HCC immunotherapy.

Growing evidence highlights the critical role of microbiota in regulating NK cell function and antitumor immunity (100). Although the influence of gut microbiota-derived metabolites on NK cells is increasingly recognized, the contribution of intratumoral bacteria to NK cell-mediated responses remains unclear (101). For example, *Streptococcus* species within the HCC tumor microenvironment activate the NF-κB pathway, leading to upregulation of peroxiredoxin 1 (PRDX1) expression. This induces excessive lactate accumulation, which suppresses $CD8^+$ T-cell cytotoxicity, and upregulates immune checkpoint molecules TIM-3 and LAG-3 (102). *In vivo* experiments have demonstrated that *Streptococcus* colonization reduced the efficacy of PD-1 blockade therapy by 43%, whereas inhibition of PRDX1 reversed this resistance (102). These findings suggest that PRDX1 is a central node in bacteria-driven metabolic reprogramming and a key mediator of HCC immune evasion.

Notably, NK cells in the HCC microenvironment are often functionally impaired or susceptible to cell death (101). By contrast, *Bifidobacterium parabrevis* exerts protective effects. *B. parabrevis* promotes lipolysis to generate acetyl-CoA, thereby inhibiting NK cell ferroptosis, and prolonging cell survival and effector functions (103). Furthermore, *B. parabrevis* drives NK cell differentiation toward an adaptive, highly cytotoxic phenotype characterized by elevated heat shock protein expression, while suppressing terminal exhaustion, enhancing anti-HCC activity (103).

In summary, NK cells serve an indispensable role in the immune response against HCC. As natural immunomodulators, polysaccharides exhibit the potential to activate NK cells, and further have the unique capacity to modulate both gut and intratumoral microbiota (104). Future research should prioritize the elucidation of synergistic mechanisms within the multidimensional polysaccharide-microbiota-NK cell interaction network. Specifically, investigations should focus on how this axis reshapes the TIME, reverses NK cell exhaustion and potentiates antitumor immunity. Such efforts will provide novel insights for the development of precise immunotherapeutic strategies targeting HCC via NK cell-mediated mechanisms.

4. Regulation of gut microbiota and their metabolites by polysaccharides

Characteristics of the gut microbiota in patients with HCC. The gut microbiota serves a pivotal role in the initiation, progression, postoperative recovery and recurrence of HCC. Accumulating evidence indicates that both patients with HCC and murine models exhibit dysbiosis compared with healthy individuals (11,105,106). Specifically, there is a marked reduction in the relative abundance of beneficial bacterial genera capable of producing short-chain fatty acids (SCFAs), including *Lactobacillus*, *Bifidobacterium*, *Bacteroides*, *Faecalibacterium*, *Ruminococcus* and *Fusobacterium*, along with enrichment of pro-inflammatory lipopolysaccharide (LPS)-producing pathogens (106). This microbial imbalance compromises intestinal barrier integrity, increases gut permeability and promotes a 'leaky gut' state, facilitating the translocation of microbial products such as LPS into the portal circulation (107). Once in the liver, these metabolites exacerbate hepatic inflammation and injury, thereby contributing to HCC development (11,106).

Alterations in specific gut microbial taxa are closely associated with clinical outcomes in HCC. Depletion of *Lactobacillus*, *Bacteroides* and *Bifidobacterium* is associated with an increased risk of early tumor recurrence and delayed postoperative recovery (108,109). Notably, exogenous probiotic supplementation has been shown to reshape the gut microbiome, increase hepatic C-X-C motif chemokine receptor 6-positive NK T cells and reduce TAM infiltration, effectively suppressing progression from non-alcoholic steatohepatitis (NASH) to HCC (110). These findings suggest that modulation of the gut microbiota may represent a promising strategy for HCC prevention and intervention.

LPS is a key mediator of the gut-derived influence on HCC progression and acts through multiple molecular pathways. First, LPS activates TLR4 in the liver, reinforcing its own expression via a lin-28 RNA binding posttranscriptional regulator A/let-7g-mediated positive feedback loop, which subsequently triggers the NF-κB signaling pathway and drives the release of pro-inflammatory cytokines, promoting tumor progression (111,112). Second, LPS enhances the stability and expression of GNAS mRNA through N^6-methyladenosine (m^6A) RNA methylation, leading to accumulation of the G protein α subunit. This suppresses the inhibitory effect of the long non-coding RNA TPTEP1 on STAT3, resulting in sustained STAT3 activation and accelerated HCC cell proliferation and invasiveness (113). Furthermore, we found that the gut microbiota dysbiosis was associated with upregulated EGFR expression in HCC tissues (Fig. 1D), which may subsequently enhance the proliferation, migration and invasion of tumor cells (114). LPS also promotes tumor immune evasion through epigenetic regulation. LPS upregulates the methyltransferase METTL14, increasing m^6A methylation of lncRNA MIR155HG. This modified transcript is stabilized by the reader protein ELAV like RNA binding protein 1 and subsequently modulates the miR-223/STAT1 signaling axis, leading to increased PD-L1 expression. As a result, T-cell function is suppressed, facilitating immune escape in HCC (115).

Substantial evidence has demonstrated that polysaccharides exert protective and therapeutic effects against HCC by modulating gut microbiota composition and restoring intestinal barrier function. For example, oral administration of GLSP or *Echinacea purpurea* polysaccharides (EPP) enriches beneficial taxa, such as members of the Muribaculaceae family, *Coprococcus*, *Clostridium*, *Roseburia*, *Bifidobacterium*, *Lactobacillus* and *Bacteroides*, and elevates levels of SCFAs, including acetate, propionate, butyrate and D-lactate, in HCC-bearing mice relative to the model group (116,117). These metabolites promote intestinal epithelial cell proliferation, enhance the expression of tight junction proteins (such as occludin, claudin-1 and ZO-1) and alleviate oxidative stress, collectively reinforcing the integrity of the intestinal mucosal barrier (118,119). By reducing systemic LPS translocation, polysaccharides suppress aberrant activation of the TLR4/NF-κB signaling pathway, downregulate inflammatory cytokines (such as IL-6 and TNF-α) and inhibit migration-associated factors such as MMP-2. Consequently, they attenuate liver injury, inhibit HCC cell proliferation and induce apoptosis (118,119).

Collectively, these findings indicate that the gut microbiota and its metabolites act as ‘distal drivers’ in the pathogenesis of HCC. Polysaccharides exert systemic, multi-target regulation by reshaping microbial homeostasis, reinforcing the intestinal barrier and blocking inflammatory signaling cascades. This mechanism offers a novel perspective for the prevention and treatment of HCC.

Promotion of SCFA production. In patients with HCC and corresponding animal models, dysbiosis of the gut microbiota is frequently associated with reduced levels of SCFAs (11,106,120). Growing evidence indicates that the anti-HCC effects of polysaccharides are closely associated with their ability to modulate both the composition of the gut microbiota and its metabolic output. For example, administration of EPP, GFG-4 and GLSP alters colonic concentrations of acetate, propionate, butyrate and D-lactate in HCC-bearing mice compared with model controls. Concurrently, these treatments upregulate butyrate metabolism-related pathways in tumor cells (118,119,121). These metabolic changes not only reflect selective remodeling of gut microbial communities by polysaccharides but also suggest their potential to indirectly suppress HCC initiation and progression through enhanced SCFA production and distribution.

Among SCFAs, acetate exerts multiple antitumor effects upon reaching the liver. Acetate promotes histone acetylation in the promoter region of acetyl-CoA carboxylase 1 (ACC1), a key enzyme in fatty acid synthesis, thereby enhancing its transcriptional activity. This increases *de novo* lipogenesis, which modulates TAM polarization and enhances CD8$^+$ T cell-mediated immune responses, ultimately suppressing HCC growth (109) (Fig. 1D). Additionally, acetate inhibits histone deacetylase activity, leading to increased acetylation of the transcription factor Sox13 at lysine 30. This post-translational modification reduces Sox13 protein stability and suppresses secretion of the pro-inflammatory cytokine IL-17A by group 3 innate lymphoid cells, thereby alleviating chronic inflammation in the tumor microenvironment (120). Furthermore, acetate binds to the G protein-coupled receptor 43 on hepatocytes, inhibiting activation of the IL-6/JAK1/STAT3 signaling pathway. This suppression blocks proliferation and induces apoptosis of non-alcoholic fatty liver disease (NAFLD)-associated HCC cells, effectively impeding NAFLD-HCC progression (18) (Fig. 1D).

Butyrate also exhibits potent anti-HCC activity. Butyrate triggers intracellular calcium dyshomeostasis and robust ROS accumulation by activating calcium signaling pathways, leading to mitochondrial dysfunction. This cascade inhibits the proliferative and metastatic capacities of HCC cells (122). In contrast, Valerate, another SCFA, delays malignant transformation from NAFLD to HCC by enhancing intestinal barrier integrity and binding to G protein-coupled receptor 41/43 on hepatocytes, which in turn suppresses activation of Rho-GTPase signaling (19).

Collectively, SCFA depletion due to gut microbiota dysbiosis is a critical pathophysiological event in HCC development. Polysaccharides can restore metabolic and immunological homeostasis at the host-microbiota interface by selectively modulating gut microbial composition and function, particularly through promotion of SCFA production, thereby exerting systemic anti-HCC effects. Notably, this regulatory effect is highly structure-dependent; variations in monosaccharide composition, glycosidic linkage type and molecular conformation result in distinct microbial modulation profiles among different polysaccharides (123). Thus, deciphering the molecular interactions between polysaccharides and the gut microbiota clarifies their anti-HCC mechanisms and paves the way for novel therapeutic strategies based on the ‘gut-liver axis’.

Regulation of bile acid (BA) metabolism. BAs are amphipathic metabolites synthesized from cholesterol in the liver. As important signaling molecules, they regulate key physiological processes, such as BA homeostasis, lipid and glucose metabolism, and inflammatory responses, primarily through the activation of the nuclear receptor farnesoid X receptor (FXR) and G protein-coupled BA receptor 1 (124). A study has shown that FXR-deficient mice exhibited increased susceptibility to HCC when exposed to chemical carcinogens or metabolic liver disease, with a higher tumor burden than controls (125). These findings highlight the critical protective role of FXR in suppressing HCC development and progression.

A complex bidirectional crosstalk exists between the gut microbiota and BAs, jointly maintaining the dynamic equilibrium of the ‘gut-liver axis’. During HCC development, gut dysbiosis disrupts this balance, leading to dysregulated BA metabolism that promotes hepatic inflammation, fibrosis and malignant transformation (126,127). For example, secondary BAs such as deoxycholic acid (DOCA) and lithocholic acid are cytotoxic, and can induce DNA damage, oxidative stress and mitochondrial dysfunction. These compounds also modulate the infiltration and function of immune cells within the TIME, thereby facilitating HCC progression (128). Alterations in microbial taxa involved in BA metabolism, such as members of the phylum Bacteroidetes and the genus *Lactobacillus*, are associated with disease progression in NASH-related HCC, changes in serum BA profiles and severity of liver injury (129). Notably, certain commensal bacteria, including specific strains of *Lactobacillus* and *Bifidobacterium*, express bile salt hydrolases, enabling them to deconjugate primary BAs into their free forms. This limits their conversion into more carcinogenic secondary BAs by other gut microbes, potentially reducing disease risk (130). Thus, understanding the interactive network between BAs and the gut microbiota is essential for elucidating the pathogenesis of HCC.

Polysaccharides have emerged as promising modulators of gut microbiota composition and BA metabolism due to their prebiotic-like properties and metabolic regulatory functions (131). Crude fucoidan derived from *Sargassum,* which exhibits a strong BA-binding capacity and reduces intracellular total cholesterol levels in HepG2 cells without compromising viability, holds antitumor potential via modulation of the cholesterol-BA metabolic axis (132). Polysaccharides from *Gardenia jasminoides* (Gardenia polysaccharide) and *Artemisia capillaris* (Yinchenhao polysaccharide) have exhibited protective effects in cholestatic liver injury models. Gardenia polysaccharides upregulate hepatic FXR expression and enrich butyrate-producing bacteria such as *Roseburia* and *Faecalibacterium*. This enhances FXR activation by microbially derived SCFAs, reverses BA homeostasis imbalance, suppresses TLR4/NF-κB signaling, reduces pro-inflammatory cytokine expression (including TNF-α and IL-6 expression) and alleviates hepatic inflammation (133,134). The *Artemisia capillaris Thunb.* polysaccharide promotes nuclear translocation of nuclear factor erythroid 2-related factor 2 (Nrf2) and upregulates BA efflux transporters [for example, bile salt export pump and multidrug resistance-associated protein 2 (MRP2)] and detoxifying enzymes (for example, UDP-glucuronosyltransferases and sulfotransferases), thereby improving BA excretion. Concurrently, *Artemisia capillaris Thunb.* polysaccharide increases the abundance of SCFA-producing bacteria, further potentiating the Nrf2-mediated antioxidant pathway and mitigating oxidative stress-induced hepatocyte injury (135).

Polysaccharides have been widely recognized to modulate immune responses in the tumor microenvironment by suppressing polarization of M2-type TAMs and promoting their shift toward a pro-inflammatory (M1-like) phenotype (136). Specifically, studies on Huaier polysaccharide (HP) have demonstrated that it effectively induces this phenotypic transition, enhancing the capacity of macrophages to secrete pro-inflammatory cytokines, thereby strengthening antitumor immunity (137). Notably, antibiotic-mediated depletion of the gut microbiota attenuated the antitumor efficacy of HP, accompanied by reduced expression of key pro-inflammatory factors such as TNF-α and IL-6. Further analysis suggested that chenodeoxycholic acid, a secondary BA produced by gut microbial metabolism, may act as a critical mediator of the immunomodulatory effects of HP (137).

In summary, the dynamic interplay between the gut microbiota and BAs is crucial for hepatic homeostasis (126,127). Dysregulation of BA metabolism not only directly increases HCC risk (125,128) but also establishes a vicious cycle by reshaping gut microbial composition, thereby exacerbating liver pathology (126,127,129). Polysaccharides exert multi-target, multi-pathway regulation of the gut-liver axis, modulating BA synthesis and signaling while restoring the microbial balance (131-137), offering unique therapeutic advantages in HCC prevention and treatment. Future research should focus on deciphering the molecular mechanisms of the polysaccharide-microbiota-BA axis to pave the way for precise interventions and clinical translation.

5. Development and utilization of hepatocyte-targeted polysaccharides

Polysaccharide-based targeted formulations. Polysaccharide-based liver-targeted drug delivery systems have emerged as promising candidates for cancer therapy (138). Polysaccharides exhibit potent immunomodulatory activities, are capable of activating various immune cells and reshape the TIME. However, their clinical translation is limited by unfavorable pharmacokinetic properties, including high molecular weight, low bioavailability and rapid systemic clearance (139).

Selenium (Se), an essential trace element, serves critical roles in antioxidant defense, DNA repair and the regulation of apoptosis, and is closely associated with the development and progression of multiple cancer types, including HCC (140). Emerging evidence suggests that conjugating polysaccharides with Se to form polysaccharide-Se nanoparticles (PS-SeNPs) not only overcomes the limitations of the individual components but also generates synergistic antitumor effects (140). This approach represents a promising strategy for developing novel, multifunctional anticancer agents.

Studies have shown that PS-SeNPs induce HCC apoptosis by co-activating mitochondrial and death receptor pathways. Specifically, *Polyporus umbellatus* polysaccharide-SeNPs and fermented *Lactarius deliciosus* polysaccharide-SeNPs were found to upregulate the Bax/Bcl-2 ratio and activate the cyto-c/caspase-8/9/3 cascade (20,141).

In addition, certain PS-SeNPs inhibit HCC cell proliferation by modulating cell cycle progression. For example, *Marsdenia tenacissima* polysaccharide (MTP70)-SeNPs, composed of a heteropolysaccharide MTP70 isolated from the stem of *Marsdenia tenacissima* (Roxb.) Wight et Arn., simultaneously activate the Bax/Bcl-2/caspase apoptotic pathway and the p21/Akt/cyclin A2 cell cycle regulatory axis, inducing S-phase cell cycle arrest, and effectively suppressing tumor cell proliferation, invasion and metastasis (142). Similarly, APS-SeNPs induce morphological changes in HepG2 cells via the mitochondrial pathway and cause S-phase arrest, exerting pro-apoptotic effects (143).

In addition to direct cytotoxicity, PS-SeNPs exhibit immunomodulatory properties and can actively reshape the TIME. Certain polysaccharide-based nanocomposites selectively target immune cells, particularly macrophages, promoting their polarization toward the antitumor M1 phenotype. For instance, *Pholiota adiposa* polysaccharide (PAP)-based gold NPs enhance macrophage secretion of NO, TNF-α and IL-12p70, thereby augmenting immunomodulatory and antitumor responses (144). By contrast, PAP-SeNPs exhibit dual targeting capabilities, accumulating in both tumor tissues and key immune organs. These NPs enter macrophages via lysosome-dependent phagocytosis and activate the TLR4/MyD88/NF-κB signaling pathway in a time-dependent manner. This drives repolarization of immunosuppressive M2 macrophages toward the pro-inflammatory M1 phenotype, initiating robust antitumor immunity. Furthermore, PAP-SeNPs increase the proportion of peripheral $CD3^+CD4^+$

and $CD3^+CD8^+$ T cells, further amplifying the anti-HCC immune response (145). Additionally, selenium nanoparticles synthesized from *Fructus corni* acidic polysaccharide-3 (FCP-3-SeNPs), prepared from an acidic polysaccharide extracted from *Fructus corni* (*Cornus officinalis*), exhibit strong immunomodulatory activity. They enhance NO, TNF-α and IL-12p70 production by macrophages, increase the $CD4^+$/$CD8^+$ T-cell ratio in peripheral blood, and indirectly induce HCC cell apoptosis (146). An *in vivo* study has demonstrated that FCP-3-SeNPs reduce tumor volume, with superior efficacy compared with FCP-3 alone, indicating that the Se nanoformulation markedly enhances the antitumor activity of the polysaccharides (146).

In summary, polysaccharide-Se nanocomposites combat HCC via multiple mechanisms, including direct induction of tumor cell apoptosis, cell cycle arrest and modulation of the TIME, underscoring their therapeutic potential. Future research should prioritize optimization of the targeting efficiency and stability of these nanocarriers, clarify their *in vivo* pharmacokinetics and underlying molecular mechanisms, and accelerate their clinical translation.

Polysaccharides as nanocarriers for drug delivery. Natural polysaccharides have emerged as promising materials for the design of nanoscale drug delivery systems due to their abundant natural sources, excellent biocompatibility and multivalent functional groups that allow diverse chemical modifications (147). In the field of cancer diagnosis and therapy, polysaccharides offer notable advantages, including high stability, low systemic toxicity, and the ability to be functionally engineered for targeted delivery and combination therapies (148). These features enhance the therapeutic efficacy of anticancer agents while minimizing off-target side effects.

Certain polysaccharides exhibit intrinsic liver-targeting properties that make them ideal candidates for hepatic drug delivery. For instance, polysaccharide fractions isolated from vinegar-baked Radix Bupleuri (VBRB) exhibit marked liver-specific accumulation (149). A study has shown that VBRB polysaccharides enhance both the relative uptake efficiency and the relative targeting efficiency of drugs in the liver. This improved hepatic targeting is associated with upregulation of hepatocyte nuclear factor 4α and organic cation transporter 1, coupled with downregulation of MRP2, collectively promoting favorable drug metabolism and transport (149). Furthermore, a novel VBRB-derived polysaccharide (VRP), VRP3-4, can self-assemble into stable NPs in aqueous solution and specifically target breast cancer resistance proteins (BCRP), such as ATP-binding cassette sub-family G member 2, as well as MRP2. By suppressing the expression of these efflux transporters, VRP3-4 enhances the inhibitory effect of methotrexate against HCC cells, exhibiting strong potential as a chemosensitizing agent (150). These findings highlight the role of VBRB polysaccharides as functional carriers for overcoming drug resistance in HCC therapy.

ASP, a representative plant-derived polysaccharide, exhibits excellent water solubility, biocompatibility and inherent liver-targeting capabilities. By conjugating hydrophobic moieties such as DOCA or ursodeoxycholic acid (UDCA) to ASP, amphiphilic conjugates (ASP-DOCA and ASP-UDCA) can be synthesized. These conjugates spontaneously self-assemble into NPs in aqueous media. When loaded with therapeutic agents such as oridonin (ORI) or doxorubicin (DOX), they form drug delivery nanosystems, including ORI/ASP-DOCA NPs, DOX/ASP-DOCA NPs and ORI/ASP-UDCA NPs. *In vivo* studies have demonstrated that these nanotherapeutics are efficiently internalized by hepatocytes via asialoglycoprotein receptor (ASGPR)-mediated endocytosis, resulting in enhanced cellular uptake and cytotoxicity (147,151,152). Compared with free drugs, these formulations exhibit superior ASGPR-dependent tumor targeting. Notably, ORI/ASP-DOCA and DOX/ASP-DOCA NPs exhibit markedly improved antitumor efficacy compared with free ORI and DOX, respectively (147,151,152).

Another study developed a redox-responsive amphiphilic polymer, ASP-disulfide-berberine (BBR), by conjugating ASP with BBR through a disulfide linker and further encapsulating the mitochondria-targeting agent honokiol (HNK) to construct ASP-BBR-PM@HNK NPs. This system was efficiently internalized by HepG2 cells, enhancing hepatic delivery of HNK and its functional impact on HCC mitochondria (153). Additionally, a biomimetic nanoplatform, glycyrrhetinic acid-APS-disulfide-curcumin-Cur@RBCm NPs, has been fabricated by coating erythrocyte membranes onto glycyrrhetinic acid-APS-disulfide-curcumin micelles. This design enabled prolonged circulation and enhanced tumor accumulation *in vivo* (154). Compared with hyaluronic acid-based nanocarriers, ASP-based systems exhibit superior antitumor efficacy and targeting specificity in HCC models (154), underscoring the unique advantages of ASP in constructing high-performance liver-targeted nanotherapeutics.

Scrophularia ningpoensis polysaccharide (SNP) has been conjugated with UDCA to form SNP-UDCA, which was used to encapsulate bufalin (BF), yielding BF/SNP-UDCA NPs. *In vitro* uptake assays revealed time-dependent internalization of these NPs in HepG2 cells, which was inhibited by pre-incubation with free SNP, suggesting that cellular uptake was mediated by specific recognition of SNP. This finding supports receptor-mediated targeting (155). For instance, a recent study demonstrated that polysaccharide-based nanoplatforms not only enhance drug accumulation in HCC tissues and improve tumor suppression but also actively modulate the tumor microenvironment. Specifically, that study showed these systems promote the repolarization of TAMs from the pro-tumorigenic M2 phenotype to the antitumor M1 phenotype and effectively recruit NK cells (156).

In conclusion, natural polysaccharide-based nanocarriers have considerable potential for the precise treatment of HCC. Their native liver-targeting properties, tunable structures and potential for multifunctional designs warrant further investigation.

Current challenges and limitations of polysaccharide-based nanotherapeutics. Despite the promising advances in polysaccharide-based nanotherapeutics for HCC, significant challenges and limitations remain to be addressed before clinical translation can be realized. A primary concern lies in the pharmacokinetic instability and potential immunotoxicity of these formulations. While polysaccharides exhibit intrinsic liver targeting, their high molecular weight and complex structures often lead to rapid clearance by the reticuloendothelial system, resulting in short circulation half-lives (157). Furthermore, although generally considered biocompatible, the potent immunostimulatory effects of polysaccharides, mediated primarily through TLR4, pose a risk of unintended systemic inflammation or immune overactivation when administered in NP form, necessitating rigorous evaluation of their immunotoxicological profiles (158).

Another critical limitation is the lack of standardization and scalability in the preparation of polysaccharide nanocarriers. Natural polysaccharides are inherently heterogeneous; their molecular weight, branching degree and monosaccharide composition can vary depending on the source, extraction method and season. This batch-to-batch variability poses a major hurdle for Good Manufacturing Practice compliance and reproducible drug loading (159). Additionally, the stability of self-assembled NPs is highly sensitive to environmental conditions (for example, pH and ionic strength). A number of polysaccharide-based formulations exhibit limited stability in the acidic environment of the stomach or the high-salt environment of the bloodstream, leading to premature drug leakage and off-target toxicity (160).

While receptor-mediated targeting (for example, via ASGPR) is a hallmark of these systems, the targeting efficiency remains suboptimal. The ‘protein corona’ formed upon contact with blood plasma can mask the targeting ligands on the NP surface, reducing their specific uptake by hepatocytes (161). Furthermore, the enhanced permeability and retention effect in human HCC tumors is often less pronounced than that in murine xenograft models, suggesting that passive targeting strategies alone may be insufficient for deep tumor penetration in clinical settings (162). Addressing these limitations through structural engineering, surface modification and rigorous preclinical validation will be crucial for the future development of polysaccharide-based nanomedicines.

Considering the challenges posed by the protein corona and other biopharmaceutical limitations of monotherapy, there is a compelling rationale to explore combinatorial strategies. Combining polysaccharide-based nanotherapeutics with conventional chemotherapeutic or targeted agents may offer a synergistic approach to overcome these delivery barriers and enhance therapeutic efficacy (163).

6. Polysaccharides in combination therapy

Although targeted therapy and conventional chemotherapy remain central to the clinical management of HCC, their long-term efficacy is often limited by declining therapeutic response, emergence of acquired resistance and significant systemic toxicity (4,5). These challenges severely compromise treatment outcomes and patient survival. Polysaccharides have attracted growing interest due to their excellent biocompatibility, low toxicity, immunomodulatory properties (57,61) and potential to enhance the efficacy of conventional anticancer therapies. As a class of bioactive macromolecules, polysaccharides are increasingly recognized as promising adjuvants for optimizing HCC treatment, offering strategies to overcome drug resistance, reduce adverse effects and improve therapeutic outcomes. In addition to their use as components of targeted drug delivery systems or nanocarriers, research has increasingly focused on combining polysaccharides with established antitumor agents, including targeted and chemotherapeutic drugs, to achieve synergistic effects (107).

Polysaccharides can enhance the sensitivity of HCC cells to targeted agents by modulating key signaling pathways. For example, fucoidan restores sensitivity to sorafenib in sorafenib-resistant HepG2-SR cells. Mechanistically, fucoidan directly interacts with EGFR on the cell membrane, inhibiting its redistribution into lipid raft microdomains and thereby suppressing downstream pro-survival signaling. Notably, this synergistic effect is markedly diminished when EGFR is blocked by a neutralizing antibody or silenced via gene knockdown (164), confirming that EGFR is a critical mediator of this combinatorial action. *In vivo*, co-administration of fucoidan and sorafenib leads to expanded regions of tumor apoptosis and necrosis, reduces the expression of the proliferation marker Ki67 and the angiogenesis marker CD34, and increases activation of executioner caspase-3 (165). These findings highlight that the combination not only enhances antitumor activity but also induces multidimensional pathological improvements.

Polysaccharides have exhibited strong chemosensitizing and protective effects in combination with chemotherapy. Cisplatin-induced cell death is impaired in apoptotic peptidase activating factor 1 (Apaf1)-knockout HepG2 cells, highlighting the essential role of Apaf1 in mediating chemosensitivity. When combined with cisplatin, lentinan upregulates Apaf1 transcription, induces mitochondrial membrane depolarization and restores the apoptotic capacity. Lentinan reduces the effective dose of cisplatin required for cytotoxicity (166) while preserving its efficacy and minimizing the risk of toxicity and resistance. Similarly, *Ulva lactuca* polysaccharides enhance the antitumor effects of 5-fluorouracil by downregulating serpin family H member 1, thereby suppressing collagen secretion and disrupting extracellular matrix deposition. This inhibits tumor cell migration and invasion, while concurrently alleviating chemotherapy-induced oxidative stress (167). APS acts synergistically with DOX by inhibiting O-GlcNAcylation, which exacerbates ER stress and activates apoptotic pathways, ultimately promoting DOX-induced apoptosis in HCC cells (50). Additionally, tramete polysaccharides enhance the sensitivity of HCC cells and xenograft models to oxaliplatin by modulating the miR-224-5p/ATP binding cassette subfamily B member 1/P-glycoprotein (P-gp) axis, resulting in reduced expression of the drug efflux pump P-gp and improved intracellular drug retention (168).

The synergistic potential of polysaccharides extends beyond conventional drugs. For instance, asparagus polysaccharide combined with HIF-1α RNA interference synergistically suppresses proliferation, migration and invasion in SK-Hep1 and Hep-3B HCC cell lines. This combination co-inhibits the PI3K/AKT and MAPK signaling pathways, and effectively reduces tumor angiogenesis (169), demonstrating the promise of multimodal combinatorial strategies in HCC therapy.

In summary, polysaccharides not only exert synergistic antitumor effects by modulating the tumor microenvironment, enhancing drug sensitivity and reversing resistance mechanisms, but also effectively mitigate the toxic side effects associated with chemotherapy and targeted therapy (164-167). These multifaceted properties make polysaccharides promising adjuvant interventions for the comprehensive treatment of HCC, offering novel insights and experimental foundations for optimizing existing therapeutic regimens.

7. Current clinical evidence and gaps

The existing literature (170) and database searches indicate that clinical research on natural polysaccharides for HCC treatment lags behind that on modern antineoplastic agents, such as immune checkpoint inhibitors (ICIs), resulting in a fragile evidence base. This disparity is primarily attributed to a longstanding structural imbalance characterized by intense basic research but limited clinical translation, manifesting as a distinct 'inverted pyramid' pattern.

Basic research is robust, as evidenced by extensive *in vitro* and *in vivo* studies on representative polysaccharides such as lentinan, *Ganoderma lucidum* and *Astragalus* polysaccharides. These studies have provided profound insights into molecular mechanisms, including immunomodulation, apoptosis induction, metastasis suppression, and regulation of the gut-liver axis (38,58,166).

However, high-quality clinical evidence remains limited. There is a critical lack of high-level evidence-based medicine studies, specifically phase III, multicenter, randomized, double-blind controlled trials, dedicated to treating HCC with single purified polysaccharides. Currently, polysaccharide-based agents are predominantly used as adjunctive therapies in combination with transarterial chemoembolization (TACE), chemotherapy or targeted/immune therapies. Furthermore, existing applications largely rely on compound formulations or crude extracts rather than structurally defined, quality-controlled monomeric polysaccharides (171). This limitation hinders precise elucidation of their mechanisms of action and standardized evaluation of their efficacy.

Nevertheless, emerging clinical evidence suggests the value of polysaccharide-based combination therapies. For instance, a recent prospective study by Zou *et al* (170) evaluated the efficacy of low-molecular-weight fucoidan (LMF), a sulfated polysaccharide derived from brown algae, in combination with TACE for HCC treatment. The results demonstrated that, compared with the control group, the LMF combination group achieved a significantly higher disease control rate (DCR; 95.24 vs. 80.00%; P=0.035) and a markedly lower disease progression rate (4.76 vs. 20.00%). Regarding safety and hepatic function preservation, the LMF group exhibited superior outcomes, with improved liver function retention (P=0.029) and a higher proportion of patients with Child-Pugh Class A status (80.95 vs. 62.50%) (170). These findings demonstrate that LMF not only enhances tumor control but also effectively mitigates TACE-associated hepatotoxicity, thereby extending the therapeutic window for patients while maintaining a favorable safety profile.

In conclusion, although clinical research on natural polysaccharides remains insufficient, preliminary evidence underscores their unique advantages in improving prognosis and achieving synergistic effects with reduced toxicity (170). There is an urgent need for large-scale, rigorously designed clinical trials to further validate their clinical value and facilitate their translation into standardized therapeutic regimens.

8. Challenges and limitations

While the specific engineering challenges associated with polysaccharide-based nanocarriers are discussed earlier in the manuscript, the broader translation of natural polysaccharides into clinical oncology faces even more fundamental bottlenecks. These challenges span across the entire drug development pipeline, from the elucidation of chemical structures to regulatory approval. These macro-level limitations spanning chemistry, biology and translational medicine are categorized and described subsequently.

Complexity of the material basis and challenges in structure-activity relationship (SAR) elucidation. Unlike chemically synthesized small-molecule drugs, natural polysaccharides are inherently heterogeneous mixtures characterized by high polydispersity, and microheterogeneity in molecular weight distribution, degree of branching and monosaccharide composition (51,52). Their biological activity depends strongly on fine structural features ranging from primary to higher-order structures (including glycosidic bond configuration, branching positions and triple-helix spatial conformation), rendering establishment of clear SARs difficult (172). Although analytical techniques such as nuclear magnetic resonance and mass spectrometry have advanced considerably, they still face limitations in resolving precise sequence connectivity and three-dimensional conformations, hindering identification of key 'active fragments' or 'pharmacophores' (173). Furthermore, polysaccharide structures are highly sensitive to extraction, purification and storage conditions, and even minor structural perturbations (for example, debranching, degradation or conformational transitions) can trigger drastic fluctuations in biological activity, further complicating SAR modeling (174). While the characterization of primary structures has reached relative maturity for some polysaccharides, research into higher-order structures, dynamic SARs and rational structural modification remains in its infancy.

Biopharmaceutical deficiencies: Dual barriers of low intestinal permeability and enzymatic instability. Natural polysaccharides generally possess physicochemical properties such as high molecular weight, high viscosity and complex branched architectures, which hinder their ability to cross the intestinal epithelial barrier into systemic circulation (139). Following oral administration, they are prone to enzymatic degradation or exhibit extremely low bioavailability, severely limiting *in vivo* efficacy (175). Although strategies such as nanocarrier encapsulation, liposomal wrapping and chemical modifications (for example, sulfation and acetylation) can improve absorption profiles, these processes often modify native conformations, potentially reducing intrinsic activity or introducing unforeseen toxicological risks (140). Balancing improved bioavailability with preservation of native bioactive structures remains a key challenge.

Source heterogeneity and lack of standardized production protocols. The quality of natural polysaccharides is influenced by multiple factors related to raw material sources (for example, geographical origin, harvest season, cultivation patterns and processing methods) and extraction parameters, resulting in significant batch-to-batch variability (176). Polysaccharides are highly sensitive to temperature, pH and extraction duration. Traditional water extraction followed by alcohol precipitation may lead to structural degradation or residual impurities (including proteins and nucleic acids). Modern assisted extraction techniques, such as ultrasound- or microwave-assisted methods, may induce glycosidic bond cleavage and shifts in molecular weight distribution if not carefully controlled (177). The absence of internationally recognized standard operating procedures for specific polysaccharides hinders cross-study comparability, reproducibility and industrial translation.

Incomplete safety evaluation systems and potential risks. Although polysaccharides are generally regarded as low-toxicity agents, current safety evaluations are largely limited to acute toxicity and short-term studies. There is a notable lack of systematic data on long-term chronic toxicity, reproductive toxicity and carcinogenicity, particularly at high doses or with prolonged administration (178). As macromolecules, certain structurally modified polysaccharides or crude extracts containing protein impurities may exhibit immunogenicity, potentially triggering allergic reactions or neutralizing antibody production. Additionally, incomplete removal of residual solvents, heavy metals, pesticides and endotoxins during extraction poses significant safety hazards (179). Polysaccharides of animal origin require rigorous evaluation of viral inactivation and prion contamination. Furthermore, data on pharmacokinetic drug-drug interactions between polysaccharides and other antineoplastic agents remain limited (40).

Obstacles in clinical translation and regulatory adaptation. A substantial gap exists between laboratory research and clinical application. Clinical trial design is challenging due to the lack of well-defined pharmacokinetic profiles and specific analytes for quantification, complicating compliance with U.S. Food and Drug Administration/European Medicines Agency standards (170). Additionally, the unique taste and viscosity of polysaccharides complicate placebo matching, increasing the risk of unblinding. Indication positioning remains ambiguous: Polysaccharides typically exhibit 'multi-target, weak-effect' profiles, making them more suitable for chronic management or adjuvant therapy, which contrasts with the 'single-target, strong-effect' paradigm favored in drug approval systems (180). Furthermore, limitations in administration routes persist. Low oral bioavailability necessitates injectable formulations for some compounds, increasing production complexity and clinical risks (181). Finally, global regulatory frameworks differ markedly. In China, polysaccharides may be classified as novel traditional Chinese medicine (TCM) drugs, health food ingredients or food additives, each with distinct requirements. In Europe and the US, they are often categorized as dietary supplements, complicating drug approval unless comprehensive chemistry, manufacturing and control data are provided (182). Current pharmacopoeial standards largely rely on total sugar content assays (such as the phenol-sulfuric acid method), which cannot distinguish active components from impurities and lack robust fingerprinting methods (183). Although policies supporting TCM innovation have been introduced in China, specific regulatory guidelines governing polysaccharide-based macromolecular drugs (for example, guidance on methods for evaluating bioequivalence) remain underdeveloped, limiting industrial investment and clinical translation.

Limited research perspective: Lack of systemic immune network analyses. Most studies have focused on changes in isolated immune cell populations, such as TAMs, DCs, T cells and myeloid-derived suppressor cells, in terms of quantity, phenotype or single signaling pathways (57,74,80). However, they often neglect the dynamic crosstalk among diverse immune cells within the TIME. Given the multitarget nature of polysaccharides, future research should shift from 'single-cell effects' to 'immune network modulation', aiming to construct comprehensive maps of systemic immune responses using advanced tools such as single-cell sequencing and spatial transcriptomics.

Unclear receptor recognition mechanisms and insufficient targeting studies. Polysaccharides initiate downstream signaling by binding to pattern recognition receptors on immune cells, such as TLRs, C-type lectin receptors and NOD-like receptors (51). However, most studies have focused on terminal signaling events rather than the molecular details of polysaccharide-receptor interactions, including binding specificity, kinetics and conformational dynamics. Elucidating the identities of key receptors and their functional roles is crucial for understanding how polysaccharides precisely regulate immune cell fate and for enabling rational drug design.

Incomplete understanding of gut-liver axis regulation. The gut microbiota serves a critical role in HCC pathogenesis. Dysbiosis can promote LPS translocation, impair APC functions (including the function of DCs and TAMs), and drive hepatic inflammation and tumorigenesis (107,113). Emerging evidence indicates that polysaccharides enrich beneficial bacteria (such as *Lactobacillus* and *Bifidobacterium*), suppress pathogenic species, maintain gut homeostasis and generate metabolites such as SCFA. These metabolites can activate hepatic immune cells (including $CD8^+$ T cells and DCs), thereby reshaping the TIME (118,119,121). Therefore, investigating the 'polysaccharide-gut microbiota-metabolite-immune cell' axis represents a pivotal direction for uncovering the systemic antitumor mechanisms of these compounds.

Scarcity of high-quality clinical evidence. Although preclinical data from *in vitro* and animal studies are abundant, high-level clinical evidence, particularly from multicenter, randomized, double-blind, placebo-controlled trials, is lacking (22-30). Most existing clinical studies are limited to the Chinese population, which restricts their generalizability and international acceptance (170). International multicenter trials are required to validate the efficacy and safety across diverse patient cohorts.

Slow pharmacological kinetics: A mismatch with aggressive tumor phenotypes. Polysaccharides exert their effects primarily through indirect immunomodulatory mechanisms, resulting in a relatively slow onset of therapeutic action. Therefore, they are not ideal as monotherapies for patients

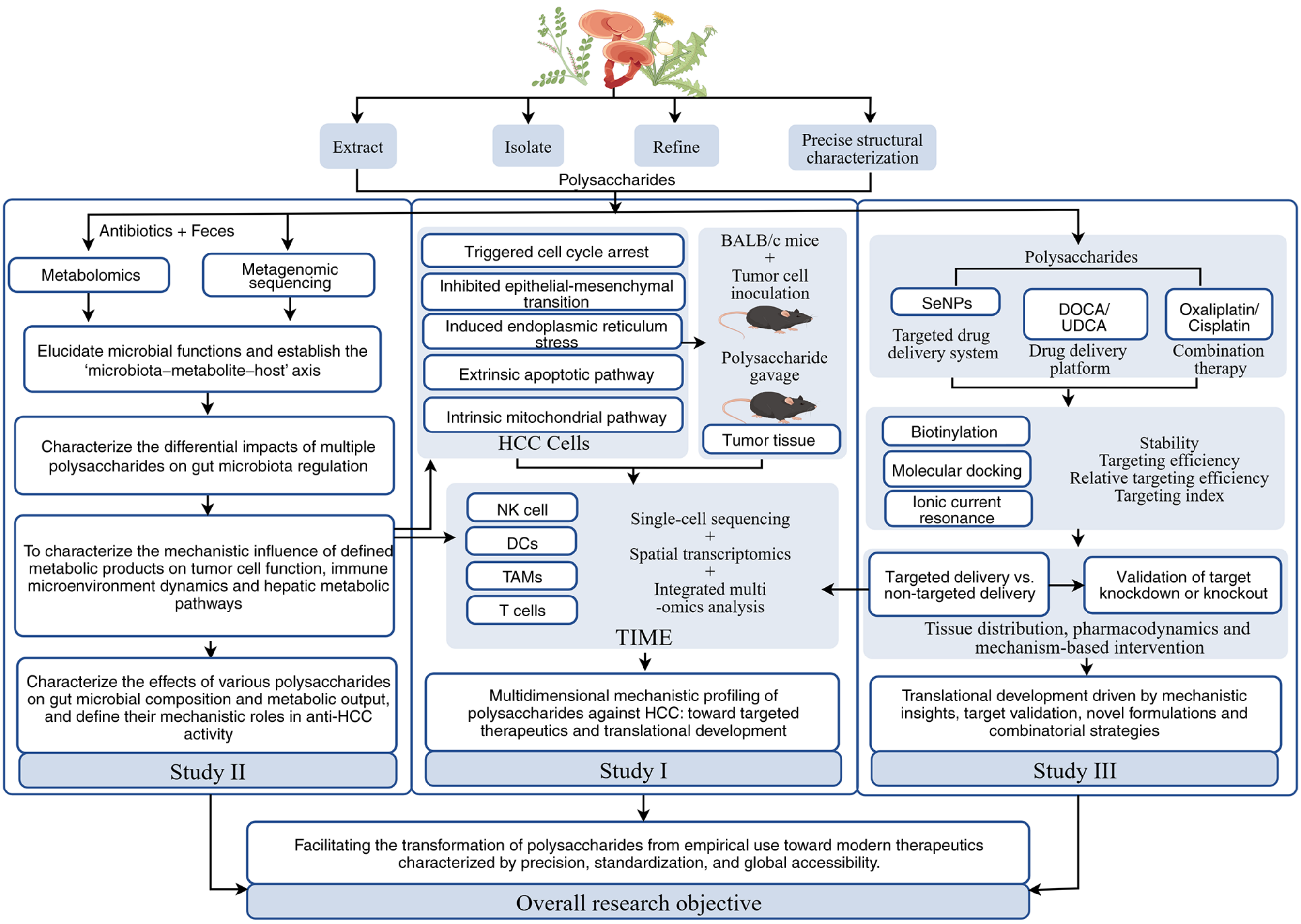

Figure 2. Strategic framework for the development and investigation of anti-HCC therapies using polysaccharides. Materials provided by FigDraw2.0 (https://www.figdraw.com/). DC, dendritic cell; DOCA, deoxycholic acid; HCC, hepatocellular carcinoma; NK, natural killer; SeNP, selenium nanoparticle; TAM, tumor-associated macrophage; TIME, tumor immune microenvironment; UDCA, ursodeoxycholic acid.

with advanced, rapidly progressing HCC. Instead, they are better suited for early intervention, postoperative adjuvant therapy and combination regimens with conventional treatments (164,166,167).

9. Future directions

The development of natural polysaccharide therapeutics is currently at a critical juncture, transitioning from the empirical application of crude extracts to modern precision medicine characterized by well-defined structures and controllable quality. To overcome existing bottlenecks and accelerate modernization and global integration of polysaccharides for HCC treatment, multidisciplinary convergence is imperative. Fig. 2 presents the overall design framework.

Multi-omics integration for deciphering systemic regulatory networks. To overcome limitations of traditional 'single-pathway' studies, this strategy (Fig. 2, Study I) aims to provide a comprehensive elucidation of the mechanisms by which polysaccharides remodel the TIME. This involves collecting HCC tissue and blood samples before and after polysaccharide treatment for multidimensional sequencing, including transcriptomics, proteomics, metabolomics and epigenomics. Advanced bioinformatics tools [such as MOFA+ (184) and iCluster (185)] can be used to integrate these datasets and construct a dynamic interaction network linking polysaccharides, metabolites, immune factors and signaling pathways. By directly correlating polysaccharide-induced metabolic shifts (for example, increased SCFAs) with immune cell gene expression profiles (such as IFN-γ pathway activation), key hub nodes can be identified (60,109). This approach avoids the 'blind men and an elephant' trial-and-error paradigm, facilitating a paradigm shift from isolated mechanistic discovery to a systems-level understanding.

Microbiomics to decode indirect gut-liver axis-mediated effects. This direction (Fig. 2, Study II) aims to unravel the 'black box' of oral polysaccharide administration, specifically determining whether efficacy is mediated through a cascade of 'oral ingestion-gut microbiota modulation-hepatic delivery of active metabolites'. Metagenomic sequencing can provide strain-level resolution and functional gene profiling (186). Causality can be validated using germ-free mouse models and fecal microbiota transplantation. Stable isotope tracing technologies can further enable real-time tracking of polysaccharide-derived metabolites (such as butyrate) as they are generated via microbiota-mediated conversion and transported to the liver to modulate immune responses (187). Distinguishing between 'direct systemic absorption' and 'indirect microbiota-mediated mechanisms' will inform optimization of administration routes (oral vs. injection) and formulation design.

Single-cell and spatial multiomics for mapping intercellular communication. This approach (Fig. 2, Study I) aims to characterize, at single-cell resolution, how polysaccharides reshape immune cell states and intercellular interactions. Combined application of single-cell RNA sequencing and single-cell T/B cell receptor sequencing enables analysis of clonal expansion and reversal of exhaustion in tumor-infiltrating lymphocytes. Coupled with spatial transcriptomics, this allows *in situ* mapping of $CD8^+$ T-cell infiltration trajectories into the tumor core and their spatial proximity to tumor and stromal cells following polysaccharide treatment (188). Computational tools such as CellChat (189) and CellPhoneDB (190) can be used to predict and validate enhanced ligand-receptor interactions. This strategy aims to precisely lock onto the specific 'effector cell' subsets targeted by polysaccharides, providing high-resolution evidence for developing targeted combination therapies.

Artificial intelligence (AI)-enabled rational drug design and structural. To address structural complexity and unclear SARs, this strategy *optimization* (Fig. 2, Study I) aims to transition from 'empirical extraction' to 'precision design'. A structured database encompassing primary structures (monosaccharide composition and glycosidic linkages), higher-order conformations (helical conformation and branching degree) and biological activities (immunostimulatory potency and receptor affinity) can be developed. Machine learning models (such as graph neural networks or transformers) can predict the immunological activity of novel polysaccharides. AI-driven molecular docking can identify key structural fragments with high affinity for pattern recognition receptors (such as TLR4), guiding rational chemical modification (including precise control of sulfation degrees and molecular weight tailoring) and synthesis of optimized derivatives (191).

Synergistic combinations with ICIs to overcome resistance. This strategy leverages the immunomodulatory properties of polysaccharides to reverse resistance to ICIs. Based on multiomics and single-cell data, polysaccharides may enhance tumor immunogenicity (for example, upregulating MHC-I expression or reducing immunosuppressive signals) (188). The efficacy of combination therapies (such as polysaccharide + anti-PD-1) should be evaluated in humanized HCC models, focusing on long-term immune memory. Early-phase clinical trials (phase Ib/II) using endpoints such as objective response rate and DCR can rapidly validate these synergistic effects, accelerating the bench-to-bedside translation.

Development of advanced nanodelivery systems and alternative administration routes. This strategy (Fig. 2, Study III) addresses challenges such as low oral bioavailability and poor targeting. Approaches include: i) Constructing NPs via amphiphilic modification or self-assembly with hydrophobic drugs/metal ions (such as Se and Zn) to enhance stability and absorption (140); ii) using liposomes or PEGylated micelles to prolong circulation and improve tumor accumulation via the enhanced permeability and retention effect (162); and iii) incorporating targeting ligands (such as galactose for ASGPR receptor targeting on hepatocytes and RGD peptides for tumor vasculature) and designing chemical bonds sensitive to the HCC microenvironment (low pH, high glutathione and specific enzymes such as hyaluronidase that are overexpressed or exhibit increased activity) to achieve smart, site-specific release (192). Alternative administration routes, such as TACE, intraperitoneal hyperthermic perfusion or transdermal systems, may further enhance therapeutic efficacy by bypassing first-pass metabolism.

Through integration of these strategies, research can transition from exploratory approaches to precision-driven development. By leveraging multiomics technologies for target identification, AI-assisted approaches for rational drug design and combination immunotherapy for clinical transition, this integrated framework provides a promising pathway to accelerate the development and global adoption of polysaccharide-based therapies for HCC.

10. Conclusion

In conclusion, natural polysaccharides possess unique advantages in the comprehensive management of HCC due to their multi-target regulation, low toxicity and potent immunomodulatory properties. They are particularly well suited as adjuncts to surgery, locoregional therapies, targeted therapy or immunotherapy, offering benefits in enhancing efficacy, improving quality of life and preventing recurrence (57,58,164,193). Nevertheless, their clinical translation faces significant challenges, including mechanistic ambiguity, lack of standardization, suboptimal formulations and insufficient high-quality clinical evidence. Future efforts should focus on deeper mechanistic exploration, precise structural characterization, formulation innovation and robust clinical validation to drive the transition of polysaccharides from empirical applications to precision medicine and global clinical adoption. By combining traditional insights with modern biomedical advancements, natural polysaccharides offer a promising pathway for developing novel HCC immunotherapies and personalized treatment strategies.

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Availability of data and materials

Not applicable.

Authors' contributions

CW was involved in conceptualization, data curation (the systematic screening, organization, and extraction of relevant literature retrieved from electronic databases), and prepared the original draft. JD was involved in supervision and project administration, and reviewed and edited the manuscript. XZ acquired funding, was involved in project administration and supervision, and reviewed and edited the manuscript. Data authentication is not applicable. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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