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Research Progress and Clinical Translation Prospects of Polysaccharide-Based Drugs in Anti-Hepatocellular Carcinoma Therapy

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Abstract

Hepatocellular carcinoma (HCC), a highly lethal malignancy worldwide, faces significant clinical treatment challenges due to tumor heterogeneity, multidrug resistance, and therapeutic limitations. There is an urgent need for the development of novel, highly effective, and low-toxicity therapeutic agents [1]. Polysaccharides, natural macromolecular compounds widely found in plants, fungi, and marine organisms, possess advantages such as good biocompatibility, multi-target characteristics, and low toxicity, making them a significant focus in anti-HCC drug development [2]. This article systematically reviews the anti-HCC mechanisms of polysaccharide-based drugs, including direct inhibition of tumor cell proliferation, induction of apoptosis, cell cycle arrest, suppression of invasion and metastasis, as well as indirect pathways such as regulating the tumor immune microenvironment and reversing chemotherapy resistance. It elaborates on the anti-HCC activity and structural characteristics of representative polysaccharides from sources like Astragalus, Bupleurum, and Phyllanthus emblica, analyzing the regulatory role of structure-activity relationships on their bioactivity. Furthermore, it summarizes the research progress in applying polysaccharides in nano-targeted delivery systems for HCC. Key challenges hindering the clinical translation of polysaccharide-based drugs, including low bioavailability, insufficiently elucidated mechanisms, and lack of unified quality control standards, are analyzed. Finally, future research directions and clinical application prospects are discussed, aiming to provide a theoretical reference and practical basis for fundamental research and industrial development of polysaccharide-based anti-HCC drugs.

Keywords

Polysaccharides; Hepatocellular carcinoma; Mechanism of action; Immune regulation; Structure-activity relationship; Nano-delivery system; Clinical translation

1.Introduction

Hepatocellular carcinoma (HCC) accounts for 75%-85% of primary liver cancer cases and is one of the malignant tumors with persistently high incidence and mortality rates worldwide [3]. According to 2022 global cancer statistics from the World Health Organization's International Agency for Research on Cancer, there were approximately 905,000 new cases and 830,000 deaths from liver cancer annually, ranking it as the third leading cause of cancer

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death [4]. Asia, particularly China, bears over 50% of the global liver cancer burden, driven by high prevalence rates of risk factors such as hepatitis B virus infection, aflatoxin exposure, and liver cirrhosis [4]. With population aging and lifestyle changes, epidemiological data project that new global liver cancer cases will increase to 1.392 million by 2040 [5], presenting severe challenges for clinical prevention and treatment.

Current clinical treatment strategies for liver cancer encompass surgical resection, liver transplantation, local ablation, transarterial chemoembolization, radiotherapy, and systemic drug therapy [6]. However, due to its insidious onset and rapid progression, most patients are diagnosed at intermediate or advanced stages, missing the opportunity for curative treatment. For these patients, systemic chemotherapy and molecular targeted therapy are primary options. Nevertheless, the clinical efficacy of existing drugs is limited by factors such as tumor heterogeneity, the development of multidrug resistance (MDR), and severe adverse effects [7]. While molecular targeted drugs like sorafenib and lenvatinib can prolong patient survival to some extent, their objective response rates are limited, and resistance often develops. Immune checkpoint inhibitors represent a breakthrough but are only effective in a subset of patients [8]. Therefore, developing novel anti-HCC drugs combining high efficacy, low toxicity, and multi-target effects is a core task in HCC basic research and clinical treatment.

Polysaccharides are macromolecular polymers formed by linking ten or more monosaccharides via glycosidic bonds. Widely distributed in plants, fungi, algae, and animal tissues, they are not only crucial structural components of organisms but also play key regulatory roles in life processes such as cell recognition, signal transduction, and immune response [2]. Over the past two decades, advancements in isolation, purification, and structural analysis techniques have led to breakthroughs in understanding the pharmacological activities of polysaccharides. Research has confirmed that polysaccharides from various sources possess bioactivities including anti-tumor, immunomodulatory, antioxidant, anti-inflammatory, and hepatoprotective effects [9]. Compared with traditional chemotherapeutic drugs, polysaccharide-based drugs offer significant advantages such as wide availability, relatively low preparation costs, low toxicity, fewer adverse reactions, multi-target actions, and the ability to modulate the tumor microenvironment, positioning them as important candidates for anti-HCC drug development [10].

This article systematically reviews the research progress on polysaccharide-based drugs against HCC. It elucidates the molecular basis of their anti-HCC effects from both direct and indirect mechanisms, summarizes the anti-HCC activities and structural features of characteristic polysaccharides from different sources, analyzes the influence of structure-activity relationships on their bioactivity, discusses their application value in HCC nano-targeted delivery systems, and addresses current research challenges while proposing future directions. The aim is to provide theoretical support for the in-depth investigation and clinical translation of polysaccharide-based anti-HCC drugs.

2. Mechanisms of Anti-HCC Action of Polysaccharides

Polysaccharides exhibit prominent multi-target and multi-pathway characteristics in their anti-HCC effects. They can directly act on HCC cells, inhibiting their malignant biological behaviors, and indirectly exert anti-tumor effects by modulating the host immune system and improving the tumor microenvironment [2]. In recent years, with the rapid development of molecular biology techniques, research on the molecular mechanisms of polysaccharides against HCC has deepened, progressively clarifying their targets and signaling pathways [9].

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2.1 Direct Inhibition of Tumor Cell Proliferation and Induction of Apoptosis

Apoptosis is a crucial physiological mechanism for eliminating abnormally proliferating cells and a core pathway through which anti-tumor drugs exert their effects [11]. Polysaccharides can induce apoptosis in HCC cells by activating both the intrinsic mitochondrial pathway and the extrinsic death receptor pathway. In the intrinsic mitochondrial pathway, polysaccharides upregulate the pro-apoptotic protein Bax, downregulate the anti-apoptotic protein Bcl-2, disrupting the Bax/Bcl-2 balance. This leads to decreased mitochondrial membrane potential and increased permeability, prompting the release of cytochrome c into the cytoplasm, which subsequently activates the caspase-9 and caspase-3 cascade, ultimately triggering HCC cell apoptosis. In the extrinsic death receptor pathway, polysaccharides can upregulate the expression of death receptors such as Fas and TNFR, activating caspase-8 and thereby initiating downstream apoptotic signaling, inducing HCC cell apoptosis [4].

LHP, a polysaccharide extracted from the fungus Lactarius hatsudake Tanaka (belonging to the Russulaceae family), exhibits significant anti-HCC activity in both in vitro and in vivo experiments. Mechanistic studies indicate that LHP can upregulate p53 and p21 expression, inhibiting cyclin-dependent kinase 4 activity and arresting the HCC cell cycle at the G0/G1 phase, thus directly suppressing proliferation [5]. Concurrently, LHP modulates the Bax/Bcl-2 balance, promoting caspase-3 activation and inducing tumor cell apoptosis. Immunohistochemical analysis confirmed that xenograft tumor tissues treated with LHP showed significant nuclear pyknosis and a markedly reduced tumor cell proliferation index [5].

Cell cycle regulation is another important molecular mechanism by which polysaccharides inhibit HCC cell proliferation [11]. Various polysaccharides can induce HCC cell cycle arrest at the G1/S or G2/M phase by regulating the expression of cell cycle-related proteins. Specifically, polysaccharides can upregulate cyclin-dependent kinase inhibitors like p21 and p27, or downregulate positive regulators such as cyclins and CDKs. This prevents HCC cells from successfully passing through cell cycle checkpoints, blocking DNA replication and mitosis, thereby inhibiting malignant proliferation [4].

2.2 Inhibition of Tumor Cell Invasion and Metastasis

Tumor invasion and metastasis are primary causes of treatment failure and mortality in HCC patients. These processes involve multiple key steps, including extracellular matrix (ECM) degradation, enhanced cell migration, and tumor angiogenesis. Polysaccharides can inhibit HCC cell invasion and metastasis by regulating these steps through various pathways [9].

First, polysaccharides can downregulate the expression and activity of matrix metalloproteinases (MMPs). MMPs are proteolytic enzymes capable of degrading the ECM, with MMP-2 and MMP-9 playing central roles in HCC cell invasion and metastasis. Polysaccharides can significantly downregulate MMP-2 and MMP-9 expression by inhibiting MAPK/ERK or NF-κB signaling pathways, reducing the ability of HCC cells to degrade the basement membrane, thereby suppressing their invasive capacity [4].

Second, polysaccharides can reverse the epithelial-mesenchymal transition (EMT) process in HCC cells [12]. EMT is a critical step for HCC cells to acquire migratory and invasive capabilities, characterized by downregulation of the epithelial marker E-cadherin and upregulation of mesenchymal markers N-cadherin and vimentin. Astragalus polysaccharides have been shown to effectively reverse EMT in HCC cells by inhibiting the TGF-β/Smad signaling pathway, significantly reducing their migration and invasion abilities [4,5].

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Furthermore, polysaccharides can indirectly limit HCC growth and metastasis by inhibiting tumor angiogenesis [9]. Angiogenesis is fundamental for tumors to obtain nutrients, oxygen, and facilitate distant metastasis. Some polysaccharides can downregulate the expression of vascular endothelial growth factor (VEGF) or directly inhibit the proliferation and migration of vascular endothelial cells, reducing neovascularization within tumor tissue, thereby blocking the nutritional supply and metastatic routes for HCC [3].

2.3 Immunomodulatory Effects

Immunomodulation is a core mechanism underlying the anti-HCC effects of polysaccharides. Unlike traditional chemotherapeutic drugs that directly kill tumor cells, polysaccharides primarily exert indirect anti-tumor effects by activating the innate and adaptive immune systems, enhancing the host's immune surveillance and clearance capabilities against tumors [10]. This mode of action allows polysaccharides to be used not only alone for anti-HCC effects but also in combination with chemotherapy, radiotherapy, or immunotherapy to achieve synergistic effects, enhancing efficacy while reducing toxicity [2].

2.3.1 Regulation of Macrophage Polarization

Macrophages are the most abundant immune cells in the tumor microenvironment, exhibiting high plasticity and heterogeneity. Under the influence of tumor microenvironment signals, they can polarize into two functionally distinct phenotypes: M1 and M2 [13]. M1 macrophages possess pro-inflammatory and anti-tumor activities, secreting pro-inflammatory cytokines like IL-12 and TNF-α to initiate Th1-type immune responses. In contrast, M2 macrophages display anti-inflammatory and pro-tumor activities, secreting immunosuppressive cytokines such as IL-10 and TGF-β, which inhibit effector T cell function and promote tumor growth, invasion, and angiogenesis. Tumor-associated macrophages in HCC tissues often exhibit an M2-polarized phenotype, closely associated with poor patient prognosis [13].

Recent studies have confirmed that polysaccharides can remodel the tumor immune microenvironment by regulating macrophage polarization. Notably, PEP-1, a pectic polysaccharide isolated and purified from the fruit of Phyllanthus emblica, displays unique pharmacological activity [6]. PEP-1 is a homogalacturonan with a molecular weight of 156.8 kDa, its core repeating unit being galacturonic acid linked by α-1,4 glycosidic bonds [6]. Research shows that PEP-1 can significantly induce the polarization of M2 macrophages towards the M1 phenotype in vitro. The mechanism involves activating NF-κB and MAPK signaling pathways, promoting the release of pro-inflammatory factors like IL-6 and TNF-α, while downregulating the expression of M2 macrophage markers Arg-1 and CD206 [6]. Conditioned medium from M2 macrophages pretreated with PEP-1 dose-dependently induced apoptosis in HCC cells without significant toxicity to normal hepatocytes. In tumor-bearing mouse models, orally administered PEP-1 was absorbed via the gastrointestinal tract, targeted the tumor microenvironment, and bound to tumor-associated macrophages. This significantly increased the proportion of M1 macrophages while decreasing M2 macrophages, transforming the immunosuppressive "cold" tumor microenvironment into an immune-activated "hot" microenvironment, ultimately leading to significant inhibition of HCC xenograft growth [6]. This study not only elucidated the structure-activity relationship of PEP-1 but also validated the feasibility of oral natural polysaccharides exerting anti-HCC immunotherapy by targeting and regulating macrophages [6].

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2.3.2 Enhancement of Natural Killer Cell and T Cell Function

Polysaccharides can significantly enhance the anti-tumor functions of natural killer (NK) cells and T lymphocytes, further activating the body's anti-tumor immune response [10]. NK cells are crucial components of the innate immune system, capable of directly recognizing and killing tumor cells without prior antigen sensitization. Various fungal polysaccharides can enhance the cytotoxic activity of NK cells, promoting the secretion of effector molecules like perforin and granzymes, thereby improving their efficiency in killing HCC cells [5].

Specific immune responses mediated by T lymphocytes play a central role in anti-tumor immunity. Astragalus polysaccharides can promote the maturation of dendritic cells, enhance their antigen-presenting capacity, subsequently activate naive T cells, and induce the generation of cytotoxic T lymphocytes (CTLs) [5]. Additionally, Astragalus polysaccharides can modulate the function of regulatory T cells (Tregs). Tregs, a subset of T cells with immunosuppressive functions, accumulate abundantly in the HCC microenvironment and promote tumor immune escape by inhibiting effector T cell function. Astragalus polysaccharides can suppress the immunosuppressive function of Tregs by downregulating the expression of the transcription factor Foxp3, relieving their inhibition on effector T cells, and thereby enhancing the body's anti-HCC immune response [4].

2.3.3 Regulation of the Cytokine Network

The regulatory effects of polysaccharides on immune cells are partly achieved through modulation of the cytokine network. Cytokines serve as crucial messengers for information transfer between immune cells, and the balance of this network determines the direction and intensity of immune responses [13]. Various polysaccharides can positively regulate anti-tumor immune responses by controlling cytokine secretion from immune cells: on one hand, they promote the secretion of Th1-type pro-tumor immunity cytokines such as IL-2, IL-12, IFN-γ, and TNF-α; on the other hand, they inhibit the production of Th2-type or immunosuppressive cytokines like IL-4, IL-10, and TGF-β, thereby steering the body's immune response towards an anti-tumor direction [5].

2.4 Reversal of Chemotherapy Resistance

Multidrug resistance (MDR) is a major cause of chemotherapy failure in HCC. Its mechanisms are complex, involving factors such as overexpression of drug efflux pumps, alterations in drug targets, enhanced DNA damage repair, and blocked apoptotic pathways. Among these, drug efflux mediated by the ATP-binding cassette (ABC) transporter superfamily is a primary MDR mechanism [7]. P-glycoprotein (P-gp), multidrug resistance-associated protein 2 (MRP2), and breast cancer resistance protein (BCRP) are the most extensively studied efflux pumps. They utilize energy from ATP hydrolysis to actively pump intracellular chemotherapeutic drugs out of the cell, reducing the effective intracellular drug concentration and ultimately leading to chemotherapy resistance [1]. Bupleurum (Radix Bupleuri) is commonly used in traditional Chinese medicine as a meridian-guiding herb. Vinegar-baking enhances its effects in soothing the liver and guiding drugs into the liver meridian. Recent studies have found that polysaccharide components extracted from vinegar-baked Bupleurum possess significant chemosensitizing effects [1]. A novel pectic polysaccharide isolated from vinegar-baked Bupleurum, named VRP3-4, has a molecular weight of 14.38 kDa. It comprises galacturonic acid, arabinose, rhamnose, and galactose in a molar ratio of 76.89:11.51:7.18:4.42 [1,6]. Its backbone consists of 1,4-linked α-GalpA, with some 1,3,4-linked α-GalpA and Rhap also involved. Side chains are 1,5-linked α-Araf attached at the O-3 position of GalpA. Notably, this

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polysaccharide can self-assemble into nanoparticles in solution [1,6]. Functional studies show that VRP3-4 significantly enhances the anti-proliferative effect of methotrexate on HCC cells, increasing its efficacy by 59.37% and boosting the intracellular uptake of methotrexate by 1.90-fold [1,6]. In vivo experiments further confirmed that VRP3-4 significantly enhances the tumor inhibitory effect of methotrexate. Mechanistic investigations revealed that VRP3-4 directly binds to BCRP and MRP2, downregulating their expression and inhibiting drug efflux pump function, thereby reversing chemotherapy resistance in HCC cells [1,6]. This discovery not only reveals the potential of vinegar-baked Bupleurum polysaccharides as liver-targeting materials to sensitize chemotherapy but also provides experimental evidence for the modern scientific interpretation of the traditional Chinese meridian-guiding theory [1].

Furthermore, polysaccharides can influence drug transporter expression by modulating upstream regulatory molecules like nuclear receptors. Hepatocyte nuclear factor 4α (HNF4α) is a key transcription factor regulating the expression of various drug-metabolizing enzymes and transporters in the liver. Vinegar-baked Bupleurum polysaccharides can modulate the expression of MRP2 and organic cation transporter 1 (OCT1) by regulating HNF4α, promoting the selective distribution of chemotherapeutic drugs in the liver and increasing local drug concentration [6].

3. Anti-HCC Activity of Polysaccharides from Different Sources

Polysaccharides are derived from a wide range of sources. Due to differences in their structural characteristics, their anti-HCC activities and mechanisms also vary [2]. Currently, plant polysaccharides, fungal polysaccharides, and marine-derived polysaccharides are the main research directions for anti-HCC polysaccharides. Among these, the anti-HCC activities of plant polysaccharides like those from Astragalus, Bupleurum, and Phyllanthus emblica, as well as fungal polysaccharides like those from Lactarius mushrooms, have been relatively well-studied, providing a rich pool of candidate materials for anti-HCC drug development [9].

3.1 Astragalus Polysaccharides

Astragalus (Astragalus membranaceus or A. mongholicus) root is a classic tonic herb in traditional Chinese medicine, known for its effects in replenishing Qi (vital energy) and consolidating the exterior [5]. Astragalus polysaccharides (APS) are the most extensively studied and widely applied active components of Astragalus. They are acidic heteropolysaccharides primarily composed of glucose, galactose, arabinose, and rhamnose [5].

The anti-HCC mechanisms of APS have been systematically elucidated. At the cellular level, APS significantly inhibits the proliferation of various HCC cell lines, including HepG2, Hep3B, and Huh7, and induces their apoptosis [4]. In H22 HCC-bearing mouse models, continuous administration of APS (100, 200, 400 mg/kg) for 15 days dose-dependently inhibited xenograft tumor growth, with the high-dose group achieving a tumor inhibition rate of 59.01% [5]. The anti-HCC effects of APS are mediated through the regulation of multiple signaling pathways, including PI3K/AKT/mTOR, Wnt/β-catenin, JAK/STAT, and TGF-β/Smad pathways [4,5]. By modulating these pathways, APS simultaneously achieves multiple effects: inhibiting HCC cell proliferation, inducing apoptosis, reversing EMT, and regulating the function of immune cells within the tumor microenvironment [4].

Beyond direct anti-tumor effects, APS exhibits significant efficacy-enhancing and toxicity-reducing effects when combined with chemotherapeutic drugs [8]. For instance, combining APS with sorafenib synergistically inhibits HCC cell proliferation and enhances sorafenib's

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pro-apoptotic effects. In HCC patients undergoing transarterial chemoembolization, adjuvant therapy with APS can improve immune function, alleviate treatment-related adverse reactions, and enhance patients' quality of life [4].

3.2 Bupleurum Polysaccharides

Bupleurum (Radix Bupleuri) root is a commonly used herb in traditional Chinese medicine for treating exterior disorders. It has functions such as dispersing heat, soothing the liver, resolving depression, and elevating Yang Qi. Vinegar-baking significantly enhances its ability to guide drugs into the liver [6]. Research on Bupleurum polysaccharides has made significant progress recently, revealing their unique advantages in chemosensitization and liver-targeted drug delivery, establishing them as important material basis for Bupleurum's meridian-guiding effect [1].

In addition to the aforementioned VRP3-4, the total polysaccharides from vinegar-baked Bupleurum also exhibit significant liver-targeting regulatory effects [6]. They can promote the selective distribution of oxymatrine in the liver, enhancing its activity against hepatitis B surface antigen [6]. This mechanism is related to the regulation of HNF4α and downstream drug transporter expression by vinegar-baked Bupleurum polysaccharides. Furthermore, co-administration with vinegar-baked Bupleurum polysaccharides significantly increases the accumulation of hydroxycamptothecin-loaded nanomicelles in the liver [6]. These findings suggest that Bupleurum polysaccharides hold promise for development as novel auxiliary materials for liver-targeted drug delivery, offering new carrier options for targeted HCC therapy [1].

3.3 Phyllanthus emblica Polysaccharides

Phyllanthus emblica (also known as Emblica officinalis or Amla) fruit is a traditional medicinal and edible resource in China, known for its effects in clearing heat, cooling blood, and aiding digestion [6]. Research on P. emblica polysaccharides is relatively limited, but recent studies have uncovered their unique anti-HCC immunomodulatory activity, opening new avenues for developing the medicinal value of P. emblica and polysaccharide-based immunomodulators [6].

PEP-1, a pectic polysaccharide isolated from P. emblica fruit, is the first P. emblica polysaccharide confirmed to exert anti-HCC effects by regulating the polarization of tumor-associated macrophages [6]. The significance of this study is threefold: first, it reveals that the anti-HCC effect of P. emblica polysaccharides relies on immune modulation rather than direct tumor cell killing; second, it confirms that natural polysaccharides can be absorbed orally via the gastrointestinal tract and target the tumor microenvironment, providing experimental evidence for the development of oral polysaccharide formulations; third, it offers a novel polysaccharide-based intervention strategy to convert the immunosuppressive "cold" tumor microenvironment into an immune-activated "hot" microenvironment, laying a foundation for developing polysaccharide-based tumor immunotherapeutic agents [6].

3.4 Fungal Polysaccharides

Fungal polysaccharides represent one of the most active research areas in anti-tumor polysaccharide studies. Polysaccharides from sources like Lentinan (from Lentinula edodes), Ganoderan (from Ganoderma lucidum), and Polysaccharopeptide (from Coriolus versicolor) are already widely used clinically as adjuncts in cancer therapy [10]. In recent years, new

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fungal polysaccharide resources continue to be discovered, and research on their anti-HCC activity and mechanisms deepens, expanding the potential clinical applications of fungal polysaccharides [5].

LHP, a polysaccharide extracted from the fruiting bodies of the fungus Lactarius hatsudake Tanaka, demonstrates significant anti-HCC activity in both in vitro and in vivo experiments without notable toxicity [5]. In H22 tumor-bearing mouse models, LHP significantly inhibited xenograft tumor growth. Histopathological and immunohistochemical analyses revealed increased nuclear pyknosis in tumor cells and significantly upregulated expression of p53, p21, and caspase-3 following LHP treatment. Cellular experiments confirmed that LHP induces apoptosis and cell cycle arrest in HCC cells by regulating Akt, NF-κB, and MAPK signaling pathways [5].

Furthermore, classic fungal polysaccharides from sources like Ganoderma lucidum (reishi), Cordyceps species, Poria cocos, and Polyporus umbellatus have also been confirmed to possess anti-HCC activity [10]. For example, Ganoderma polysaccharides enhance anti-tumor immunity by activating NK cells and CTLs. Cordyceps polysaccharides can induce HCC cell apoptosis and inhibit tumor angiogenesis. Poria polysaccharides can reverse MDR in HCC cells, enhancing sensitivity to chemotherapeutic agents. These studies provide a solid scientific basis for the application of fungal polysaccharides in comprehensive HCC treatment [10].

3.5 Marine-Derived Polysaccharides

The unique marine environment endows marine organisms with metabolites featuring novel structures and diverse bioactivities, making them important resources for new drug discovery [14]. Due to their unique structural features and promising bioactivities, marine-derived polysaccharides have become a significant research direction for anti-HCC polysaccharides. Among them, seaweed polysaccharides and chitosan are the most intensively studied [14].

Seaweed polysaccharides are the most extensively studied marine polysaccharides. Fucoidan (from brown algae), laminarin, and agarooligosaccharides have all been shown to possess significant anti-HCC activity. Their mechanisms involve inhibiting HCC cell proliferation, inducing apoptosis, suppressing invasion and metastasis, and modulating immune functions [14]. Chitosan is the deacetylated product of chitin, primarily derived from the shells of crustaceans like shrimp and crabs. It possesses good biocompatibility, biodegradability, and low toxicity. Beyond its own intrinsic anti-HCC activity, chitosan is rich in functional groups such as hydroxyl and amino groups along its molecular chain. This makes it widely used in constructing nano-drug delivery systems for HCC, representing an important form of marine polysaccharide application in HCC therapy [15].

4.Structure-Activity Relationship and Structural Modification of Polysaccharides

The biological activity of polysaccharides is closely related to their chemical structure. Elucidating the structure-activity relationship (SAR) of polysaccharides is a crucial theoretical foundation for screening highly active polysaccharides and conducting structural optimization [2]. Polysaccharide structure includes primary structure (involving molecular weight, monosaccharide composition, glycosidic bond configuration, degree of branching, etc.) and higher-order structure (spatial conformation). Both jointly regulate the biological activity of polysaccharides [9]. Furthermore, chemical modification of natural polysaccharides

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can effectively improve their physicochemical properties and bioactivities, providing an effective means for developing highly active polysaccharide derivatives [15].

4.1 Influence of Molecular Weight on Activity

Molecular weight is an important structural factor affecting the anti-HCC activity of polysaccharides. The anti-tumor activity of polysaccharides typically exhibits a certain molecular weight dependence, but higher molecular weight does not always correlate with stronger activity [2]. Polysaccharides with excessively high molecular weight have poor solubility and difficulty penetrating biological membrane barriers, leading to reduced bioavailability. Conversely, polysaccharides with very low molecular weight may fail to form the spatial conformation necessary for exerting biological activity, thereby losing or diminishing their anti-HCC efficacy [9]. The optimal active molecular weight range varies significantly among polysaccharides from different sources. This variation is closely related to their structural characteristics, targets, and mechanisms of action. For instance, P. emblica polysaccharide PEP-1 has a molecular weight of 156.8 kDa, while vinegar-baked Bupleurum polysaccharide VRP3-4 has a molecular weight of 14.38 kDa. Both exhibit significant anti-HCC activity, suggesting that polysaccharides with different structures can achieve their bioactivity through different optimal molecular weights [1,6].

4.2 Monosaccharide Composition and Glycosidic Bond Configuration

Monosaccharide composition and glycosidic linkage patterns are core elements of polysaccharide primary structure, directly influencing their spatial conformation and biological activity. They form the structural basis for polysaccharides to exert anti-HCC effects [2]. The core repeating unit of P. emblica pectic polysaccharide PEP-1 is galacturonic acid linked by α-1,4 glycosidic bonds. This structure is crucial for its activation of NF-κB and MAPK signaling pathways and regulation of macrophage polarization [6]. The backbone of vinegar-baked Bupleurum polysaccharide VRP3-4 consists of 1,4-linked α-GalpA, with side chains of 1,5-linked α-Araf. This unique monosaccharide composition and glycosidic bond configuration endow it with the ability to self-assemble into nanoparticles and specifically bind to BCRP and MRP2 [1,6]. These studies indicate that specific monosaccharide compositions and glycosidic bond configurations are necessary conditions for polysaccharides to exert their anti-HCC activity [9].

4.3 Degree of Branching and Higher-Order Structure

The degree of branching and higher-order structure of polysaccharides play more complex roles in regulating anti-HCC activity [2]. Appropriate branched structures facilitate the recognition and binding of polysaccharides to receptors on target cell surfaces, thereby initiating downstream signaling pathways and exerting biological activity. Excessively high or low branching degrees can affect the binding efficiency of polysaccharides to receptors, reducing their bioactivity [9]. Higher-order structures of polysaccharides, including spatial conformations such as triple helices, spheres, or rods, are essential for their biological activity. Only upon forming specific higher-order structures can polysaccharides specifically bind to receptors and activate relevant signaling pathways [15]. Vinegar-baked Bupleurum polysaccharide VRP3-4 can self-assemble in solution to form a nanoparticle higher-order structure [1,6]. This characteristic allows it to exert direct chemosensitizing effects while also serving as a natural nanomaterial for encapsulating and delivering hydrophobic anti-tumor drugs, thus combining polysaccharide activity with carrier functionality [1].

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4.4 Enhancing Activity through Chemical Modification

Chemical modification of natural polysaccharides is an effective strategy to improve their physicochemical properties and enhance their anti-HCC activity [15]. Commonly used chemical modification methods include sulfation, carboxymethylation, acetylation, and phosphorylation. These modifications can alter the charge distribution, spatial conformation, water solubility, and other physicochemical properties of polysaccharides, thereby enhancing their binding ability to target receptors and increasing biological activity [2]. Sulfation introduces negatively charged sulfate groups into the polysaccharide molecule, altering its charge distribution and enhancing receptor binding; the anti-HCC activity of various polysaccharides is significantly improved after sulfation [9]. Carboxymethylation can significantly improve the water solubility of polysaccharides, increasing their bioavailability and addressing the poor solubility issue of some natural polysaccharides [15]. Acetylation can alter the hydrophobicity and flexibility of polysaccharides, enhancing their interaction with biological membranes and improving cellular uptake efficiency [2].

5. Application of Polysaccharides in Nano-Targeted Delivery Systems for HCC

Nano-drug delivery systems can effectively improve the pharmacokinetic behavior of traditional anti-tumor drugs, enhance their selective distribution in tumor tissues, and reduce systemic toxicity. They represent a crucial development direction for targeted HCC therapy [15]. Polysaccharides offer advantages such as wide availability, low cost, excellent biocompatibility and biodegradability, abundant functional groups on their molecular chains, and inherent targeting capabilities of some polysaccharides. These attributes make them ideal materials for constructing nano-targeted delivery systems for HCC. In recent years, significant progress has been made in research on polysaccharide-based nano-targeted delivery systems [3,15].

5.1 Advantages of Polysaccharides as Nanocarriers

The advantages of polysaccharides as carriers for HCC nano-targeted delivery systems are fivefold [3]: First, they are widely available with relatively low preparation costs, facilitating large-scale production and clinical translation. Second, they possess excellent biocompatibility and biodegradability; their metabolic products are monosaccharides or oligosaccharides, which are non-toxic to the body, reducing the biosafety risks associated with nanocarriers. Third, polysaccharide chains are rich in reactive functional groups like hydroxyl, carboxyl, and amino groups, facilitating drug conjugation and targeting ligand modification through chemical reactions, providing a basis for functionalizing nanocarriers [15]. Fourth, some polysaccharides possess inherent targeting recognition capabilities, enabling active targeting of specific receptors on HCC cells or within the tumor microenvironment, thereby enhancing the tumor-targeting efficiency of nanocarriers [1]. Fifth, certain polysaccharides themselves possess anti-HCC activity and can serve as "active carriers" to construct nano-delivery systems, achieving both drug loading and therapeutic functions, resulting in synergistic anti-HCC effects [3].

5.2 Strategies for Constructing Polysaccharide Nanoparticles

Current methods for constructing polysaccharide nanoparticles primarily include self-assembly, cross-linking, and polyelectrolyte complexation. Different methods are suitable for polysaccharides with varying structural features and can produce polysaccharide

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nanoparticles with different physicochemical properties [15]. Self-assembly utilizes the amphiphilic nature of polysaccharide molecules to spontaneously form core-shell structured nanoparticles in aqueous solution. This method is simple, operates under mild conditions, and requires minimal chemical reagents. Vinegar-baked Bupleurum polysaccharide VRP3-4 can form nanoparticles via self-assembly [1,6] and can be directly used for encapsulating hydrophobic anti-tumor drugs, serving as a typical example of natural polysaccharide self-assembly for nanoparticle construction [1]. Cross-linking uses chemical or ionic cross-linkers to interconnect polysaccharide molecules, forming nanoparticles with a three-dimensional network structure. This method allows effective control over nanoparticle size, drug loading capacity, and release characteristics [15]. Polyelectrolyte complexation relies on electrostatic interactions between oppositely charged polysaccharides, or between polysaccharides and other oppositely charged polymers, to form stable nanocomplexes. This method requires no chemical cross-linkers, offers high biosafety, and is suitable for constructing drug-loaded nano-delivery systems [3].

5.3 Active Targeting Modification Strategies

To further enhance the liver-targeting ability of polysaccharide-based nanocarriers for HCC, active targeting modifications can be applied. By coupling targeting ligands onto the surface of polysaccharide nanoparticles, the nanocarriers can specifically recognize receptors overexpressed on HCC cells, enabling active targeted drug delivery [15]. Ligands currently used for liver-targeting modification of polysaccharide nanocarriers include galactose, N-acetylgalactosamine, folic acid, transferrin, and peptides [3]. Hepatocytes highly express the asialoglycoprotein receptor (ASGPR), which specifically recognizes galactose and N-acetylgalactosamine. Therefore, galactosylation is a classic strategy for achieving liver-targeted delivery of polysaccharide nanocarriers; coupling galactose ligands onto the nanoparticle surface significantly enhances their accumulation in liver and HCC tissues [6]. Additionally, the CD44 receptor is overexpressed on HCC cells, and hyaluronic acid specifically binds to CD44. Hence, hyaluronic acid-modified polysaccharide nanoparticles can achieve active targeting recognition of HCC cells, improving drug tumor selectivity [15].

6. Challenges and Prospects

Although significant progress has been made in research on polysaccharide-based drugs against HCC, with deepening understanding of their mechanisms, SAR, and nano-delivery systems, several critical issues remain to be resolved before transitioning from laboratory research to clinical application [2]. Conducting in-depth research to address these issues is the core task for promoting the clinical translation of polysaccharide-based anti-HCC drugs. Concurrently, integrating multidisciplinary technologies and exploring new research directions and application strategies will pave new paths for the development of polysaccharide-based drugs [9].

6.1 Enhancing Bioavailability

As macromolecular compounds, the low bioavailability of polysaccharides is a major bottleneck limiting their clinical application [8]. Upon oral administration, polysaccharides are susceptible to degradation by the gastrointestinal pH environment and digestive enzymes, and they struggle to cross the intestinal epithelial cell membrane, resulting in low oral absorption rates. Although injection can improve bioavailability, it faces issues like poor patient compliance and limited administration routes[2]. Improving the bioavailability of polysaccharides, especially oral bioavailability, is a key challenge in developing

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polysaccharide-based anti-HCC drugs [15]. Nanotechnology applications can effectively improve the physicochemical properties of polysaccharides, enhancing their cellular uptake efficiency and in vivo circulatory stability [3]. Co-administration with absorption enhancers can increase the permeability of the intestinal epithelial membrane, promoting intestinal absorption of polysaccharides [9]. Structural modifications of polysaccharides, such as carboxymethylation or sulfation, can improve their water solubility and resistance to enzymatic degradation, enhancing their in vivo pharmacokinetic behavior [15]. Recent studies confirming that P. emblica polysaccharide PEP-1 can be absorbed orally and reach the tumor microenvironment [6] provide a positive signal for developing oral polysaccharide formulations. Future research should focus on screening and modifying polysaccharides with high oral bioavailability [2].

6.2 In-Depth Elucidation of Mechanisms

Current research on the anti-HCC mechanisms of polysaccharides often focuses on describing phenomena at the cellular and molecular levels, such as effects on tumor cell proliferation/apoptosis and regulation of related signaling pathways. However, deeper mechanistic understanding remains insufficient, including the direct binding mode of polysaccharides to target proteins, conformational changes upon binding, global regulation of signal transduction networks, as well as the metabolic processes and target tissue distribution patterns of polysaccharides in vivo [4]. Pull-down assays confirming that vinegar-baked Bupleurum polysaccharide VRP3-4 directly binds to BCRP and MRP2 [1,6] provide excellent examples for elucidating the molecular mechanisms of polysaccharides [1]. Future research should integrate multidisciplinary techniques such as structural biology, bioinformatics, and omics to investigate the interactions between polysaccharides and target proteins, identifying direct targets and binding sites [11]. Techniques like in vivo imaging and isotope tracing should be utilized to elucidate the absorption, distribution, metabolism, and excretion patterns of polysaccharides in vivo [9]. From a systems biology perspective, the regulatory effects of polysaccharides on the tumor microenvironment and the body's overall signaling network should be analyzed to comprehensively and deeply explain the molecular mechanisms of anti-HCC polysaccharides, providing theoretical guidance for structural optimization and activity screening [2].

6.3 Establishment of Quality Standards and Clinical Translation

The complex structure of polysaccharides, whose biological activity is influenced by various factors including raw material source, extraction process, purification methods, and storage conditions, leads to significant batch-to-batch and source-to-source variations in structure and activity, making quality control challenging [10]. Establishing scientific and quantifiable quality control standards for polysaccharides is a critical step for their clinical translation [2]. Future efforts should focus on establishing multi-index quality control systems for polysaccharides, including not only conventional parameters like content, molecular weight, and monosaccharide composition but also incorporating biological activity indicators to achieve dual quality control of structure and activity [15]. Developing standardized extraction, isolation, and purification processes is necessary to achieve large-scale and standardized production of polysaccharide products [9]. Furthermore, current research on anti-HCC polysaccharides is predominantly at the preclinical level; high-quality clinical studies are relatively scarce, and unified clinical application protocols have not yet been established [8]. Future efforts should strengthen clinical research on polysaccharide-based drugs, conducting large-sample, multi-center, randomized controlled trials to determine their clinical efficacy, optimal dosing regimens, safety profiles, and combination strategies in HCC treatment [7].

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Simultaneously, strengthening industry-academia-research collaboration is needed to promote the industrial development and clinical translation of polysaccharide-based drugs, enabling laboratory research findings to be genuinely applied in the clinical management of HCC [2].

6.4 Exploration of Combination Treatment Strategies

Based on the multi-target, multi-pathway characteristics of polysaccharides in combating HCC, combination therapy represents an important direction for realizing their clinical potential [8]. Polysaccharides can be combined with chemotherapeutic drugs to achieve synergistic effects of enhancing efficacy and reducing toxicity by reversing MDR, improving tumor targeting of chemotherapeutics, and alleviating their adverse effects, as exemplified by vinegar-baked Bupleurum polysaccharide VRP3-4 combined with methotrexate [1,6]. They can be combined with molecular targeted drugs to improve their efficacy by modulating the tumor microenvironment and enhancing cellular uptake of targeted agents [7]. They can be combined with immune checkpoint inhibitors to enhance the efficacy of these inhibitors by converting the "cold" tumor microenvironment into a "hot" one, thereby boosting the body's anti-tumor immune response[13]. Additionally, polysaccharide-based nano-delivery systems enable the co-delivery of multiple agents, such as chemotherapeutics, immunomodulators, and nucleic acid drugs, potentially improving HCC treatment outcomes through synergistic multi-drug, multi-target actions [3,15]. Future research should delve into the synergistic mechanisms of polysaccharides with different treatment modalities to screen optimal combination regimens, providing new strategies for comprehensive HCC treatment [2].

7. Conclusion

Polysaccharides, as a class of widely available natural macromolecular compounds, exhibit promising application prospects in the field of HCC treatment due to their significant advantages of high efficacy, low toxicity, and multi-target actions [2]. In recent years, with advancements in separation/purification techniques, structural analysis methods, and molecular biology technologies, research on the anti-HCC mechanisms of polysaccharides has deepened. They can directly inhibit HCC cell proliferation, induce apoptosis, arrest the cell cycle, and suppress invasion/metastasis, while also exerting anti-HCC effects indirectly by modulating the tumor immune microenvironment and reversing chemotherapy resistance [9]. Characteristic polysaccharides from different sources, such as those from Astragalus, Bupleurum, and Phyllanthus emblica, each possess unique features. Studies on their structure-activity relationships provide theoretical guidance for screening highly active polysaccharides and optimizing their structures [2]. The application of polysaccharides in nano-targeted delivery systems for HCC not only improves their own bioavailability but also provides novel carriers for targeted delivery of HCC drugs, expanding the administration routes and therapeutic modalities for polysaccharides [3,15].

Although current research on polysaccharide-based anti-HCC drugs still faces challenges such as low bioavailability, insufficiently elucidated mechanisms, lack of unified quality control standards, and limited clinical studies [8], these issues are expected to be gradually resolved through in-depth interdisciplinary research integrating structural biology, nanotechnology, systems biology, translational medicine, and other fields with polysaccharide research [2]. In the future, through the screening of highly active polysaccharides, structural optimization and modification of polysaccharides, construction of polysaccharide-based nano-targeted delivery systems, and exploration of combination treatment strategies, polysaccharide-based drugs are expected to become an important component of comprehensive HCC treatment. They may

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offer new therapeutic options for HCC patients and drive advancements in the field of HCC therapy [9].

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