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Contributed Mini Review

Research progress on hydrogel-based drug therapy in melanoma immunotherapy

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Melanoma is one of the most aggressive skin tumors, and conventional treatment modalities are not effective in treating advanced melanoma. Although immunotherapy is an effective treatment for melanoma, it has disadvantages, such as a poor response rate and serious systemic immune-related toxic side effects. The main solution to this problem is the use of biological materials such as hydrogels to reduce these side effects and amplify the immune killing effect against tumor cells. Hydrogels have great advantages as local slow-release drug carriers, including the ability to deliver antitumor drugs directly to the tumor site, enhance the local drug concentration in tumor tissue, reduce systemic drug distribution and exhibit good degradability. Despite these advantages, there has been limited research on the application of hydrogels in melanoma treatment. Therefore, this article provides a comprehensive review of the potential application of hydrogels in melanoma immunotherapy. Hydrogels can serve as carriers for sustained drug delivery, enabling the targeted and localized delivery of drugs with minimal systemic side effects. This approach has the potential to improve the efficacy of immunotherapy for melanoma. Thus, the use of hydrogels as drug delivery vehicles for melanoma immunotherapy has great potential and warrants further exploration. [BMB Reports 2024; 57(2): 71-78]

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INTRODUCTION

Melanoma is a tumor usually caused by the malignant transformation of melanocytes, referred to as malignant melanoma (1, 2), which occurs at the junction of the epidermis and dermis and accounts for approximately 3% of all tumors. Melanoma is called "the cancer that rises with the sun" and has the characteristics of high malignancy, poor prognosis, fast local growth and a high metastasis rate. It has become one of the most troublesome tumors. In recent years, with the increasing incidence of melanoma, an increasing number of people have become fearful of melanoma (3). The development and malignancy of melanoma, including the mutation of normal cells, the growth of malignant tumors and even tumor metastasis, are easily affected by the physiological state and the external environment (4, 5). Therefore, there is an urgent need to develop effective strategies to eradicate melanoma and prevent tumor growth (6).

With the development of immunotherapy, especially the application of immune checkpoint inhibitors, significant progress has been made in the treatment of melanoma, significantly prolonging the survival of melanoma patients (7, 8). Immunotherapy is involved in almost all stages of melanoma treatment and plays important roles in postoperative adjuvant therapy and in advanced first-line treatment (9, 10). Immunotherapy for malignant melanoma mainly activates the body's immune system to eliminate tumor cells using the body's own defenses. Compared with chemotherapy and radiotherapy, immunotherapy can harness the host's immune system, kill tumor cells, and inhibit tumor growth and recurrence in the long term (11, 12). However, immunotherapy requires repeated high-dose injections, and there are many challenges, such as large individual differences in treatment response and low efficiency of the immune response (13). To overcome the above shortcomings, there is an urgent need to develop new immunotherapy methods, new delivery materials and delivery routes.

In recent years, hydrogels have attracted attention as carriers for local and sustained drug delivery in tumors due to their unique advantages for orthotopic tumor drug delivery (14),

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which can simultaneously deliver multiple anticancer drugs at the tumor site, maintain relatively high drug concentrations and reduce systemic toxic side effects (15). Hydrogels are well suited for immunotherapy because they allow the localized and sustained release of drugs, thereby minimizing systemic toxicity and maximizing effectiveness. In preclinical studies, hydrogel delivery systems have been shown to have higher efficacy than injected immunotherapeutic agents alone, and they can also prevent systemic toxicity.

DRUG DELIVERY AND RELEASE OF HYDROGELS

Hydrogels are polymeric networks that are formed through the chemical or physical crosslinking of hydrophilic polymer chains. These networks can absorb large amounts of water without dissolving, resulting in substantial swelling with the retention of their initial three-dimensional structure. Hydrogels are characterized by their high water content and exhibit softness and pliability akin to those of biological tissues, resulting in exceptional biocompatibility. Hydrogels can transport diverse biological substances and are therefore used extensively in drug administration. As carriers for sustained drug release, hydrogels protect the damaged area from adverse external stimuli, keep the damaged area moist, and improve drug utilization (16-19). Hydrogels are scaffolds formed by a network of cross-linked polymer units with high water content ($>$ 90%), in which forms a three-dimensional structure similar to the extracellular matrix of natural tissues (20). The skeleton contains numerous hydrophilic groups that make the hydrogel capable of absorbing and storing up to 90% of its mass in water (21). The water storage capacity of hydrogels is related to the content of hydrophilic groups, and hydrogels exhibit good swelling and flexibility (22). The aggregated form of the hydrogel is not a pure solid state or a completely liquid form. Its unique physical structure confers good biological and physical functions, including biocompatibility, water solubility, degradability, porosity, and sensitivity to various stimuli (23-26). It can be loaded with therapeutic drugs, and moreover, it can be degraded gradually over a period.

Research on drug delivery is of great importance for efficient disease treatment (27). Hydrogels are important drug delivery platforms because they can release drugs at a designated rate in a local area or can perform responsive on-demand drug release through various functional modifications (28). The multiple interactions between hydrogels and encapsulated drugs ensure that the release of various drugs can be controlled temporally and spatially (29). The advantages of hydrogels as drug delivery materials include 1) tunable physicochemical properties; 2) controllable biodegradability; and 3) prevention of failure caused by drug degradation.

The drug delivery process is influenced by the inherent characteristics of the hydrogel, including its macrostructure, its microstructure (specifically porosity), and the extent of cross-linking within the polymer network, which collectively determine the mesh aperture. The mesh size, in turn, governs the diffusion of the drug within the hydrogel, as it regulates the spatial interaction between the drug and the mesh (30). Consequently, augmenting the polymer concentration or cross-linking agent concentration serves as a viable means to diminish the mesh size of the hydrogel. The extent of cross-linking in the hydrogel can be modulated through alterations in the cross-linking agent's type, dosage, and reaction conditions. This cross-linking degree is directly linked to the hydrogel's stability and the length of the diffusion pathway for drug molecules, thereby influencing the rate at which drugs are released (either accelerated or delayed). Manipulating the gaps can further impact the drug release rate, either increasing or decelerating it. Moreover, distinct polymers exhibit diverse physicochemical properties and biocompatibility profiles. Drug adsorption, diffusion, and release in hydrogels can be achieved through the careful selection of polymers. Furthermore, the manipulation of the copolymer's molecular weight, composition, and proportion can significantly reduce the gelation time, thereby enhancing the sol-gel transition. By adjusting the cross-linking degree, voids, and polymers of hydrogels, we can effectively regulate the interaction between drugs and hydrogels.

A drug with a smaller size relative to the mesh can rapidly diffuse through the hydrogel, resulting in a shorter drug release time. Drug release is significantly slowed as the size of the drug approaches the mesh size (31). When the drug is larger than the grid size, the drug is physically encapsulated in the mesh. As a biomedical material delivery system, hydrogels are commonly used to deliver drugs smaller than 15 nm, such as small molecules or proteins. In general, the release of drug-loaded hydrogels relies mainly on diffusion. The controlled release of drugs can be achieved by controlling the degradation, swelling, and mechanical deformation of the hydrogel mesh. With a suitable release mechanism, the hydrogel can maintain a high local drug concentration for a long time. Controlled drug delivery systems can overcome the shortcomings of traditional drug delivery (32).

RESEARCH PROGRESS ON HYDROGELS IN MELANOMA IMMUNOTHERAPY

Antitumor immunotherapeutic drugs have shown great promise in cancer treatment, but these therapeutic drugs are easily cleared from the blood circulation, resulting in ineffective delivery; they cannot be retained in the tumor tissue for a long time; and they cannot eliminate the tumor, which hinders their application in tumor immunotherapy. The hydrogel can improve drug delivery by serving as a drug carrier, loading therapeutic drugs into its interior and releasing them, ultimately improving the effect of immunotherapy. Hydrogels loaded with drugs can control the drug release rate and can perform sustained drug release at the tumor site to maximize drug utilization. To date, hydrogel-loaded therapeutic drugs have been widely used in the immunotherapy of tumors. Compared with other tumors,

melanoma is a superficial tumor, so hydrogels are more suitable for melanoma treatment. Hydrogels have many advantages in the treatment of melanoma, including good local therapeutic effects, high feasibility of drug delivery and immunotherapy, and controllable drug release rates. These advantages make hydrogels a promising method for the treatment of melanoma. In this section, we will summarize the application of hydrogels in melanoma immunotherapy.

Classification of hydrogels

There are several types of hydrogels made with different raw materials, cross-linking methods, and properties (33). Hydrogels can be classified in a variety of ways according to different classification criteria (Table 1). According to the sensitivity of external stimuli, hydrogels can be divided into traditional hydrogels and stimulus-responsive hydrogels. Traditional hydrogels are not sensitive to external factors, including changes in temperature and pH. Stimulus-responsive hydrogels can be classified into pH-responsive, temperature-responsive, photoresponsive and magnetic field-responsive hydrogels (34-36). Stimulus-responsive hydrogels are very sensitive to small external changes and stimuli. After stimulation, the physical or chemical properties of the material will change, causing changes in the hydrogel. Based on this property, hydrogels can be used in various fields.

According to the difference in material preparation, hydrogels can be divided instead into natural polymer and synthetic polymer hydrogels. Common natural polymeric materials include chitosan, sodium alginate, and hyaluronic acid. Common synthetic polymer materials include polyethylene glycol, polyvinyl alcohol, and polystyrene. Natural polymeric materials are derived from natural sources and have good biocompatibility and environmental sensitivity. Moreover, they are rich in sources, low in cost, poor in stability and easily degraded (37). Synthetic polymer hydrogels are formed by crosslinking tens of thousands to millions of polymer materials from single-molecule raw materials by chemical synthesis, and they have high stability, elasticity and plasticity (38). However, some synthetic polymer materials have poor biocompatibility (39).

To date, different forms of hydrogels have been used to improve the therapeutic effect of melanoma. By designing the chemical structure of the hydrogel network, controlled drug release by pH, temperature or external stimuli can be achieved, which can increase the efficiency and improve the therapeutic effect (40). Currently, hydrogels are used alone or as carriers of cells and drugs and are injected directly into the desired site via a syringe (41-43). As a carrier for sustained drug release, hydrogels can protect the damaged area from adverse external stimuli, keep the damaged area moist and improve the utilization rate of drugs.

Application of hydrogels in melanoma immunotherapy

Melanoma is categorized as an immune malignancy, characterized by a higher incidence in individuals with low immune system and immunodeficiency disorders (44-46). Due to the strong immunogenicity of melanoma, immunotherapy is the most promising treatment method (47). However, there are still many problems, such as delayed antitumor effects and disease progression after immunotherapy, which pose many challenges to immunotherapy (48, 49). Therefore, exploring new strategies and optimizing design schemes to enhance the efficacy of immunotherapy is an important direction in melanoma immunotherapy research (50).

In recent years, hydrogels as delivery carriers have become a new research focus in immunotherapy. Hydrogels can allow the precise positioning and continuous release of drugs, minimize systemic toxicity and improve the effectiveness of immunotherapy (51). With deepening research in the field of hydrogels, researchers have combined the characteristics and advantages of hydrogels with clinical needs to design and develop hydrogels equipped with nucleic acid drugs and other drugs for melanoma immunotherapy (52).

HYDROGELS LOADED WITH NUCLEIC ACID DRUGS FOR MELANOMA IMMUNOTHERAPY

Nucleic acid drugs are susceptible to liver clearance and nuclease degradation, so suitable delivery vectors are needed. Hydrogels have great potential in the medical field as an effective tool for nucleic acid drug delivery (53). Due to the advantages of hydrogels, such as good biocompatibility, injectivity, efficient nucleic acid drug loading capacity, good mechanical effectiveness and local localization control release (54), researchers are committed to loading nucleic acid drugs into

Table 1. Classification of common hydrogels

Source	Natural hydrogels	Collagen, chitosan, hyaluronic acid, gelatin
	Synthetic hydrogels	Polyethylene glycol (PEG), N-isopropyl acrylamide (PNIPAM), poloxamer
Crosslinking method	Physically crosslinked	Hydrogen bonding, ionic interactions, hydrophobic interaction
	Chemically crosslinked	Glutaraldehyde, epichlorohydrin, adipic dihydrazide and polyaldehydes
Response to environmental stimuli	Environmentally sensitive hydrogels	Temperature-sensitive
		Electric field-sensitive
		pH-sensitive
		Light-sensitive

hydrogels to achieve systemic or local delivery, preventing adverse effects on other tissues and improving efficacy *in vivo*.

Recently, researchers have engineered hydrogels to exert precise spatial and temporal control over the release of RNA therapies, thereby minimizing systemic toxicity and improving the efficacy *in vivo*. RNA-based therapies have shown great promise. Lu and his team (55) designed a versatile injectable hydrogel system for the treatment of melanoma. A calcium-phosphorus shell was constructed on the surface of dopamine nanoparticles through biosimulated mineralization to achieve efficient loading of siRNA. Then, the dopamine nanoparticles loaded with siRNA were suspended in alginate solution to form injectable hydrogels under acidic conditions in the tumor microenvironment. The results showed that the hydrogel system could inhibit the growth and metastasis of melanoma, indicating the potential for clinical application and bringing new hope for the treatment of melanoma.

mRNA vaccines are promising candidates for cancer immunotherapy (56, 57), but their action period *in vivo* is limited, and their cost performance is high, so it is necessary to construct an effective vector for vaccine delivery or storage (58). Hydrogel with good biocompatibility is an excellent carrier for vaccine loading. For example, by optimizing the hydrogel formula, a physical barrier can be constructed to extend the release cycle of the vaccine (59). Alternatively, injectable hydrogels, gel films and other forms suitable for different application scenarios can be designed. Currently, hydrogels loaded with vaccines are one of the important research directions in the field of tumor immunotherapy. Yin and colleagues (60) designed a non-chemically bonded hydrogel containing graphene oxide (RO) and low-molecular-weight polyethylenimide (LPEI) to deliver mRNA vaccines carrying immune-stimulating adjuvants. The hydrogel can steadily release the vaccine (including mRNA and adjuvant), protect the mRNA from degradation, and successfully migrate to the lymph nodes to function stably for at least 30 days. Compared with mouse models of melanoma injected with free adjuvant and hydrogel-free mRNA, mouse models of melanoma that received only hydrogel loaded with the mRNA vaccine had significantly smaller tumors, and more CD8+ T cells entered the tumor tissue. In addition, the hydrogel-delivered mRNA vaccine successfully induced high levels of ovalbumin-specific antibodies, which not only inhibited tumor growth but also prevented the recurrence or metastasis of melanoma. In general, graphene oxide polyethyleneimine hydrogels that deliver mRNA and adjuvant for cancer immunotherapy can achieve lasting effects and are expected to become an effective cancer mRNA vaccine delivery platform.

During the drug administration process, the rapid enzymatic degradation and fragmentation of DNA may lead to limited vector expression, resulting in poor efficacy. Therefore, it is necessary to explore new DNA delivery strategies. Hydrogels have the potential to load large amounts of DNA and achieve extended retention at the injection site, increasing the possibility of sustained drug expression and thus enhancing the immune response. Scott H. Medina and colleagues (61) found that a cationic self-assembled peptide hydrogel was able to encapsulate and deliver plasmid DNA (TA) encoding a fusion protein consisting of the melanospecific tumor antigen gp100 and the adjuvant HMGN1. The HLT2 gel loaded with DNA (TA) was implanted into mice, causing acute inflammation. The presence of multinucleated cells was observed, followed by the infiltration of macrophages. These cell infiltrates help process the encased DNA, promote increased lymphocyte proliferation and produce an immune-enhancing response mediated by CD4+/IFNγ+ on Th1-expressing cells, supplemented by the formation of GP100-specific antibodies.

The above studies show that loading nucleic acid drugs into hydrogels can effectively achieve controlled drug release and improve the immunotherapy response, which is an important development direction in melanoma immunotherapy. Although there are few studies on hydrogels carrying nucleic acid drugs, this innovative method can solve the limitations of traditional immunotherapy and has great development potential.

HYDROGELS ARE LOADED WITH OTHER DRUGS FOR MELANOMA IMMUNOTHERAPY

In the gastrointestinal physiological environment, peptides and other substances are easily degraded but not easily absorbed, and various obstacles lead to very low bioavailability of oral peptides. To overcome the obstacles of oral dosage forms of polypeptide drugs, researchers have adopted direct or indirect coping strategies (62). The latest study found that hydrogels can both extend the storage period of a peptide drug and extend the residence time of the drug in a specific area. Li and colleagues (63) discovered and optimized the PD-1/PD-L1 blocking peptide OPBP-1 and loaded it into a TMC-based hydrogel oral drug delivery system, thereby maximizing the oral bioavailability of the peptide drug, effectively inhibiting melanoma growth, and enhancing the infiltration and function of CD8+ T cells.

Monoclonal antibodies that block the immune checkpoint PD-1/PD-L1 have shown unprecedented clinical efficacy against many cancers (64, 65). However, many drugs have short retention times and low bioavailability in the body (66). Studies have shown that the unique properties of hydrogels can keep the loaded substances active for a long time while ensuring the long-term release of drugs in the body. Li and colleagues (67) used an alginate saline gel system to locally deliver celecoxib and PD-1, which synergistically enhanced the presence of CD4+ interferon (IFN)-γ+ and CD8+IFN-γ+ T cells both within tumors and in the immune system, providing effective cancer treatment. In addition, Yu and colleagues (68) delivered polypeptide hydrogel-loaded anti-PD-L1 by intratumoral injection into a C57BL/6 mouse tumor-bearing model of B16F10 melanoma, and the hydrogel-loaded antibodies showed a longer retention time (> 7 days) in B16F10 tumors than free antibody solution alone (~3 days). Hydrogels containing anti-PD-L1 antibodies

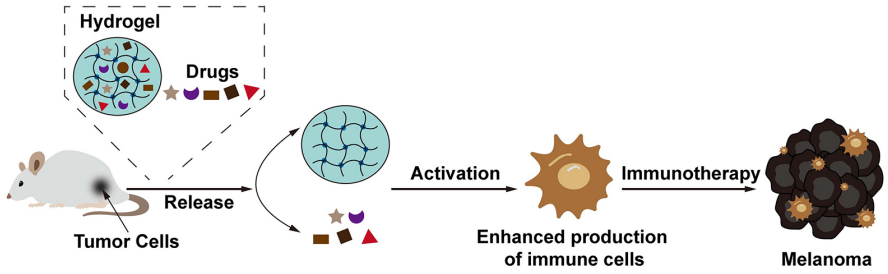

Fig. 1. Immunotherapy with hydrogels in melanoma.

showed significantly higher tumor suppression efficacy than the anti-PD-L1 antibody/D-1MT mixture. In addition, the mice treated with the anti-PD-L1-loaded hydrogel achieved a stronger antitumor immune response, suggesting that CD8+ T cells with the ability to kill have a higher rate of tumor accumulation.

Studies have shown that the sudden release of large amounts of drugs often causes adverse reactions. To block the side effects of burst release, we utilized injectable, biodegradable, and *in situ* formable hydrogels to effectively and uniformly encapsulate the drug. Hydrogels can control and delay the release rate of the drug and increase bioavailability by providing a local environment that allows the drug to continue to work. Wang and colleagues (69) prepared injectable monomethoxy PEG-B-poly (L-valine) (mPEG-b-PVal) hydrogels for the local delivery of tumor cell lysate (TCL) and poly (I:C), the latter of which is a TLR3 agonist. As a tumor antigen, TCL can costimulate the recruitment, activation and maturation of host DCs at the tumor site with poly (I:C). The hydrogel significantly prolonged antigen persistence at the injection site. In tumor-bearing mice treated with a melanoma model, the hydrogel treatment system promoted the production of CD8+ T cells in the tumor-infiltrating T cells of draining lymph nodes and showed a strong antitumor effect.

The above studies show that hydrogels, as delivery carriers, are conducive to the slow release of drugs in the body, improve the utilization rate of drugs, effectively cause the immune system to kill tumor tissue, improve the efficacy of immunotherapy, and have great potential for the treatment of melanoma (Fig. 1).

CONCLUSIONS

Melanoma immunotherapy faces problems such as poor specificity and insufficient delivery ability (70-72). However, with the rapid development of biotechnology, melanoma immunotherapy methods are constantly improving (73-76). In recent years, the combined application of immunotherapy and biomaterials has overcome the clinical barriers that limit cancer immunotherapy (77, 78), and an increasing number of studies have been conducted on the use of hydrogels to improve the efficacy of antitumor immunotherapy for melanoma (79, 80). This paper reviews the extensive application of hydrogels as drug carriers in melanoma immunotherapy. Hydrogels can be loaded with nucleic acids, cytokines, peptides, antibodies and other types of drugs and can achieve sustained drug release at the tumor site, improve drug utilization and thus improve the effectiveness of melanoma immunotherapy (81-83). The hydrogel loaded with nucleic acid drugs effectively controls drug release and prevents the degradation of nucleic acid, thereby improving the effect of melanoma immunotherapy. Hydrogels loaded with polypeptides, monoclonal antibodies, etc., can also increase the retention time of drugs in the body and effectively cause the immune system to kill tumor tissue, thereby improving the efficacy of melanoma immunotherapy. The use of hydrogels to carry various drugs is thus highly suitable for the current development of melanoma immunotherapy and has broad application value (84-88).

In summary, research on hydrogel-based immunotherapy for melanoma is advancing rapidly, and drug delivery technology is constantly improving (89, 90), but there are still obstacles to translating the materials from the experimental stage to clinical application. First, biological materials are foreign substances to human organisms, and acute or chronic inflammation may occur in the body in response to the degradation of biological substances. Therefore, the safety and efficacy of these biomaterials need to be further evaluated. Second, the mechanical strength of the hydrogels needs to be more consistent and stable. Therefore, the mechanical properties of hydrogels should be rigorously evaluated (91). It is believed that with deepening research on hydrogels and the continued improvement of immunotherapy, hydrogels will become a potential drug carrier, leading to new breakthroughs in melanoma immunotherapy and promoting further development (92, 93).

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

AUTHOR CONTRIBUTIONS

HW, ZYQ, and QY wrote and revised the article. LGD and PLX conceived the idea of the article. LMM, XXY and SGG collated the data and searched the relevant literature. LF, HQ and GY conceived the study, designed the study and revised the manuscript. All the authors have read and approved the final manuscript.

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