80205_Auto_Edited-check.docx

WORD COUNT	4364	TIME SUBMITTED	14-DEC-2022 05:44PM
		PAPER ID	93985427

Name of Journal: *World Journal of Gastroenterology*

Manuscript NO: 80205

Manuscript Type: MINIREVIEWS

Nanomedicine-based multimodal therapies: Recent progress and perspectives in colon cancer

He YC *et al*. Nanomedicine-based multimodal therapies in colon cancer

Abstract

Colon cancer has attracted much attention due to its annually increasing incidence. Conventional chemotherapeutic drugs are unsatisfactory in clinical application because of their lack of targeting and severe toxic side effects. In the past decade, nanomedicines with multimodal therapeutic strategies have shown potential for colon cancer because of their enhanced permeability and retention, high accumulation at tumor sites, co-loading with different drugs, and combination of various therapies. This review summarizes the advanced research on various nanomedicine-based therapeutic strategies including chemotherapy, radiotherapy, phototherapy (photothermal therapy and photodynamic therapy), chemodynamic therapy, gas therapy and immunotherapy. Additionally, the therapeutic mechanisms, limitations, improvements and future of the above therapies are discussed.

Key Words: Colon cancer; Nanomedicine; Drug permeability; Drug retention; Multimodal therapies; Therapeutic mechanism

He YC, Hao ZN, Li Z, Gao DW. Nanomedicine-based multimodal therapies: Recent progress and perspectives in colon cancer. *World J Gastroenterol* 2022; In press

Core Tip: Nanomedicine has exhibited great potential in the colon cancer therapy over the past decades. In this review, we summarize the advances in research on various nanomedicine-based therapeutic strategies including chemotherapy, radiotherapy, phototherapy (photothermal therapy and photodynamic therapy), chemodynamic therapy, gas therapy and immunotherapy. Additionally, the therapeutic mechanism, limitations and improvement in these therapies are also introduced. The challenges and future prospect of the nanomedicine-based multimodal therapies for colon cancer are discussed.

INTRODUCTION

Colon cancer is one of the most intractable gastrointestinal diseases with increasing incidence worldwide[1,2]. For the past few years, human lifestyles and diets have changed markedly with the rapid development of the global economy, which further increases the risk of colon cancer. According to the global cancer statistics, the incidence and mortality of colon cancer were 6.1% and 5.8% in 2018[3], which ranks fourth and fifth among all cancers, respectively. The characteristics of colon cancer are mainly reflected in rapid energy metabolism and proliferation that enhance tumor invasion and metastasis. Therefore, colon cancer has become one of the major unresolved problems in medicine[4,5]. Conventional small molecule chemotherapeutic drugs (such as paclitaxel, doxorubicin and camptothecin) are unsatisfactory because of their lack of targeting, solubility and severe toxic side effects. Thus, there is an urgent need to develop novel and efficient therapeutic strategies for colon cancer. In the past decade, the emergence of nanomedicine has shown potential in cancer therapy. Compared with traditional chemotherapeutic drugs, nanomedicine has better tumor targeting because the vascular gaps in tumor tissue are wider than those of normal tissue, so that nanomedicine can penetrate tumor tissue through these vascular gaps but not into normal tissue. Because of the lack of lymphatic reflux in the tumor region, nanomedicine can remain in the tumor tissue, and this mechanism of nanomedicine-based tumor targeting is called the enhanced permeability and retention (EPR) effect[6]. Additionally, various nanoscale drug delivery systems can load the chemotherapeutic drugs to enhance their solubility, which improves their utilization. Finally, nanomedicine is able to combine multimodal therapies to enhance the antitumor effect. Above all, nanomedicine has shown numerous advantages and potential for multimodal therapy of colon cancer.

In this review, we summarize recent progress of nanomedicine-based multimodal colon cancer therapy. Firstly, we introduce all types of organic and inorganic nanomedicine and explore their drug loading, drug release and tumor targeting. Moreover, the biosafety of nanomedicine is also discussed. Then, we introduce various therapeutic strategies for colon cancer including chemotherapy, phototherapy [photothermal therapy (PTT) and photodynamic therapy (PDT)], radiotherapy, gas

therapy, chemodynamic therapy (CDT) and immunotherapy (Figure 1). The therapeutic mechanisms of these approaches are also discussed. Among them, nano drug delivery systems (NDDSs) are widely used to improve the therapeutic effect due to its characteristics of improving the water solubility of chemotherapy drugs, prolonging the blood circulation time, targeted drug delivery, few side effects, and reversing multi-drug resistance. PDT is a new treatment for colon cancer that uses specific wavelengths of light to excite photosensitizers. In the excited state, the photosensitizers transfer energy or electrons to the surrounding oxygen, thus producing singlet oxygen and killing cancer cells. Radiation therapy can cause DNA strand break of tumor cells under X-ray irradiation, and produce high cytotoxic free radicals to damage colon tumor cells. Compared with other reactive oxygen species (ROS) therapies, CDT has stronger *in situ* catalytic ROS generation, higher tumor specificity and deeper tissue penetration, and does not require additional stimulation, providing a new idea for the future treatment of colon cancer. Gas therapy can enhance drug release, when used with chemotherapy and synergistic therapy with other therapies, it can improve therapeutic effects, but its application in colon cancer requires extensive studies. Immunotherapy has been widely used in the treatment of colon cancer. The immunogenicity of tumor cells is activated by means of photothermal and ROS, and immunoadjuvant is used to reduce the immunosuppression in the tumor microenvironment and enhance the immune effect. These strategies provide new insights into the clinical treatment of colon cancer. Finally, the main limitations and challenges in the development of nanomedicine for colon cancer are addressed, and future research directions proposed. It is believed that nanomedicine-based multimodal therapy will play an important role in colon cancer.

MULTIMODAL THERAPIES FOR COLON CANCER

Chemotherapy

Chemotherapy is the core method in current cancer treatment, and various drugs such as 5-fluorouracil (5-FU), platinum drugs, irinotecan and epirubicin, are widely used[7-11]. However, there are still some problems in conventional chemotherapy: (1) Free small-

molecule drugs have a limited half-life *in vivo* and lack of tumor targeting, leading to severe side effect; (2) Poor aqueous solubility of drugs limits their clinical effect; (3) Dense solid tumor tissue hinders drug delivery, resulting in insufficient drug dose in tumor tissue; and (4) Tumor microenvironment, such as hypoxia, low pH and high H_2O_2 concentration, leads to multidrug resistance. To improve the therapeutic effect of chemotherapy, NDDSs have received extensive attention because of their properties such as improving the aqueous solubility of drugs, prolonging the blood circulation time of, achieving targeted delivery to tumors, and few side effects. Various NDDSs have been designed to enhance tumor targeting and aqueous solubility of drugs, leading to improved therapeutic effect[12-15].

Most drugs exhibit poor aqueous solubility and low bioavailability. To solve this problem, Chen *et al*[16] adopted a cucurbituril-based supramolecular chemical strategy to improve the aqueous solubility and long-term circulation of the drugs for enhancing the therapeutic effect of oxaliplatin on colon cancer. Chen *et al*[17] prepared fisetin micelles using monomethyl poly (ethylene glycol)-poly(ε-caprolactone) copolymers. Compared with free fisetin, the micelles exhibited excellent aqueous solubility and cytotoxicity. Additionally, Xiao *et al*[18] used the intermolecular noncovalent interaction of curcumin and irinotecan to self-assemble into nanoparticles, which enhanced the aqueous solubility of curcumin, reduced the side effects of irinotecan, and showed better targeting and therapeutic effect. To prolong the blood circulation of drugs, Jiang *et al*[19] designed OxPt/SN38 nanoparticles to hitchhike on low-density lipoprotein (LDL) particles and accumulate at the tumor site through LDL-receptor-mediated endocytosis, which showed excellent antitumor efficacy in murine tumor models. Liu *et al*[20] developed an active targeting strategy to specifically combine with glucose-regulated protein 78 overexpressed on the surface of colon cancer cells with PEGylated WL8 peptide, which enhanced the enrichment of doxorubicin in the tumor region.

Inflammation is an important reason for promoting tumor proliferation, invasion, metastasis and drug resistance. Therefore, anti-inflammatory drugs such as aspirin and dexamethasone can improve the therapeutic effect of antitumor drugs[21,22]. Natural

products such as curcumin and fisetin, which show good anti-inflammatory and antitumor properties, have also been widely used as chemotherapeutic drugs[23-26]. Wang *et al*[27] found that the anti-inflammatory drug dexamethasone significantly enhanced the antitumor activity of carboplatin and gemcitabine and increased their accumulation in tumors, providing a basis for dexamethasone as a chemosensitizer. Ma *et al*[28] developed a pH- and redox-responsive peptide-dexamethasone conjugate (L-SS-DEX) that reduces inflammation and modulates the tumor microenvironment for an effective antitumor effect.

Multidrug resistance is another reason for the failure of chemotherapy. The multidrug-resistance-related proteins such as P-glycoprotein (P-gp) of tumor cells results in significant drug excretion[29,30]. Currently, some NDDSs have been designed to co-deliver P-gp inhibitors or microRNAs to suppress multidrug resistance and enhance the drug sensitivity of tumor cells[31,32]. Sivak *et al*[33] overcame multidrug resistance by simultaneously delivering doxorubicin and the P-gp inhibitor (reversin 121) into cancer cells. The neurokinin-1 receptor antagonists inhibited expression of P-gp to enhance the chemotherapy effect[34].

Studies have shown that the development of colon cancer is closely related to the gut microbiota, which is involved in regulating the sensitivity of tumor cells to chemotherapy. As a Gram-negative anaerobic bacterium, *Fusobacterium nucleatum* (*F. nucleatum*) is enriched in colon cancer patients, adheres to the intestinal mucosa, and invades epithelial cells to induce carcinogenesis. It can combine with E-cadherin on the surface of colon cancer cells to form a tumor immunosuppressive microenvironment, promote tumor proliferation, and enhance drug resistance of colon cancer cells[35-38]. Therefore, inhibiting the activity of *F. nucleatum* is important for enhancing the efficacy of colon cancer chemotherapy. Lauric acid has a specific inhibitory effect on *F. nucleatum*. Yan *et al*[39] used polyglycidyl ether as a nanodrug carrier, introduced the antibacterial agent lauric acid and oxaliplatin through esterification, selectively inhibited the biological activity of *F. nucleatum*, and improved the resistance of colon cancer cells to oxaliplatin. The antibiotic metronidazole and the chemotherapy drug 5-

FU were mixed into the metal polyphenol network coated mesoporous silica nanoparticles (MSN), and then added with carboxymethyl cellulose to obtain anti-colorectal cancer gel to eliminate *F. nucleatum* in colon cancer and inhibit the drug resistance, and proliferation and metastasis of colon cancer cells[40].

Phototherapy

Phototherapy is an emerging strategy to kill tumor cells by stimulating photosensitizers under light irradiation. In recent years, phototherapy, as a noninvasive treatment, has attracted widespread attention because of its specificity, low toxicity for normal tissues, and excellent antitumor effect. PTT and PDT are two common methods in colon cancer treatment[41-44]. PTT utilizes photosensitizer accumulated in tumor tissue to convert light energy into heat for killing tumor cells under light irradiation (generally near-infrared, NIR), which shows spatiotemporal controllability, high selectivity, and low cost. Recently, NDDSs have been designed to delivery photothermal agents for enhancing tumor targeting. For example, Ren *et al*[45] designed CT26 cell membrane-coated Bi nanoparticles, which had good long-term circulation and tumor homologous targeting ability *in vivo* compared with Bi nanoparticles. In addition, it is reported that epidermal growth factor receptor (EGFR) is abundantly expressed on the surface of some colorectal cancer cells. Shih *et al*[46] combined cetuximab (EGFR inhibitor) with the organic NIR dye IR780 to target colon cancer cells with high EGFR expression for PTT. Excessive H_2S (0.3-3.4 mmol/L) produced by colon cancer cells can promote the proliferation of colon cancer cells and angiogenesis in the tumor area[47,48]. Biocompatible iron oxide nanospindles have been developed, which can efficiently remove endogenous H_2S gas in colon tumor tissues and inhibit tumor growth, and generate FeS *in situ* for magnetic resonance imaging (MRI) and PTT under NIR irradiation[49-51].

PDT is a new method for colon cancer that utilizes light of a specific wavelength to excite a photosensitizer, and the photosensitizer in the excited state transfers energy or electrons to the surrounding oxygen, thereby producing singlet oxygen to kill cancer

cells[52]. Various NDDSs have been designed to deliver PDT-based photosensitizers to colon tumors. By adjusting the size of the NDDSs and modifying with hydrophilic groups, the photosensitizers can be passively targeted to the tumor area through the EPR effect. Besides the EPR effect, biomimetic membrane or tumor-specific affinity ligands-modified NDDSs have also been extensively studied for tumor targeting. Xie *et al*[53] designed a translocator protein (TSPO)-targeted photosensitizer (IR700DX-6T) for tumor targeting of photosensitizers *via* combination with overexpressed TSPO in colon cancer cells. Additionally, because of the high expression of EGFR in colon cancer cells, EGFR antibody has been used to target delivery of the photosensitizer IR700, which effectively eradicated colon cancer cells[54]. Traditional photosensitizers have high fluorescence quantum yields in dilute solutions, which lead to weaker fluorescence in the aggregated state. Aggregation of photosensitizers during delivery can lead to reduced ROS yields, so it is crucial to develop novel nanocarriers that efficiently load photosensitizers and prevent their aggregation. Covalent organic frameworks, as a class of organic polymers, have attracted much attention because of their excellent biocompatibility and biodegradability. Gan *et al*[55] showed enhanced phototherapeutic effects by adsorbing the NIR dye indocyanine green (ICG) onto the covalent organic framework *via* π–π interaction to prevent its aggregation. In addition to this, aggregation-induced emission luminescence agents have been used to enhance PDT because the agents exhibit enhanced fluorescence emission in the aggregated state[56]. Hypoxia is one of the main reasons for the poor effect of PDT. Thus, researchers have developed a variety of oxygen generators such as hemoglobin, MnO_2, and perfluorocarbon, to increase oxygen in the tumor to enhance the effect of PDT[57-59]. For example, He *et al*[60] designed gold nanocages coated with MnO_2 and hyaluronic acid (HA) for tumor targeting, and MnO_2 was designed to react with the overexpressed H_2O_2 in the tumor to relieve tumor hypoxia and enhance the effect of gold nanocage-based PDT.

Radiotherapy

Radiotherapy is a local cancer treatment that is widely applied in clinical therapy. The mechanism of action of radiotherapy is to cause DNA strand breaks in tumor cells and generate highly cytotoxic free radicals under X-ray irradiation to damage tumor cells[61-65]. Radiosensitizers are usually used to boost the effect of radiotherapy against colon cancer[66]. 7-Dehydrocholesterol is utilized as a radiosensitizer, which can react with ROS to promote lipid peroxidation, double-strand breaks, and mitochondrial damage in cancer cells, enhancing the radiotherapeutic effect[67]. As we know from the mechanism of action of radiotherapy, tumor hypoxia limits the efficacy of radiotherapy; thus, relief of hypoxia by nanomedicine can improve the therapeutic effect. MnO_2 can react with excess H_2O_2 in the tumor to generate oxygen, which can relieve the hypoxic microenvironment, eliminate tumor resistance to radiotherapy, and reshape the immunosuppressive microenvironment. Zhang *et al*[68] designed bovine-serum-albumin-coated MnO_2 as a radiosensitizer. MnO_2 can decompose excess H_2O_2 in the tumor into oxygen to relieve tumor hypoxia and convert tumor-promoting M2 tumor-associated macrophages into antitumor M1-type macrophages to reshape the immunosuppressive microenvironment and eliminate tumor resistance to radiotherapy. In addition, perfluorocarbon is a good oxygen carrier that can be used to delivery oxygen to tumors and reverse hypoxia, leading to enhancement of radiotherapy[69].

CDT

CDT is a promising therapeutic strategy that utilizes endogenously overexpressed H_2O_2 in tumors to generate toxic hydroxyl radicals (•OH) through Fenton/Fenton-like reactions catalyzed by metals (Fe^{2+}, Cu^{+}, Mn^{2+}, Mo^{4+}, W^{4+}, Ti^{3+} *etc.*)[70-73]. Compared with other ROS therapies, CDT has the advantages of stronger *in situ* catalytic ROS generation, tumor specificity, and deep tissue penetration, which does not require additional stimulation. However, the effect of CDT is still limited by its high dependence on tumor endogenous H_2O_2 concentration (10-100 μM) and slow ion release from inorganic nanoparticles[74,75]. The problem of low levels of H_2O_2 in tumor tissue can be solved by directly loading H_2O_2 or encapsulating H_2O_2-producing drugs

such as glucose oxidase and calcium peroxide. However, nanocarriers directly encapsulating exogenous H_2O_2 have the risk of leakage causing damage to normal tissues. Therefore, new strategies are urgently needed to address the challenges associated with CDT. Su *et al*[76] used a microfluidic method to prepare a nanogel (DOX@Mn-Alg) composed of alginate (Alg), Mn^{2+} and doxorubicin as an ideal CDT/chemotherapy synergistic therapeutic nanoplatform. Because doxorubicin can activate NADP oxidases to convert oxygen to $\bullet O_2^-$ and then superoxide dismutase further catalyzes $\bullet O_2^-$ to generate endogenous H_2O_2 *via* a disproportionation reaction. Subsequently, the elevated H_2O_2 can be converted into a sufficient amount of •OH through a Mn^{2+}-mediated Fenton-like reaction. Ultimately, DOX@Mn-Alg can rationally combine doxorubicin chemotherapy with Mn^{2+}-mediated CDT and immunotherapy for synergistic cancer treatment. Chen *et al*[77] selected Pd nanoparticles as the CDT reagent, and showed that the ultra-small Pd nanozyme as the core had high catalytic activity and pH selectivity. Under acidic conditions, it exhibited peroxidase activity to produce OH and $^1O_2$, while under neutral conditions, it promoted the decomposition of H_2O_2 to produce O_2 through catalase activity. In terms of biological activity, the bidirectional anisotropic nanocluster not only directly inhibited tumor cells through ROS production, but also induced H_2O_2 production in CT26 cells, which enhanced the therapeutic effect. The nanoparticles inhibited tumor growth in CT26 mice, and improved tumor hypoxia and enhanced the therapeutic effect.

The intracellular glutathione in tumor cells can eliminate the oxidative activity of •OH through powerful reducing activity. Lin *et al*[78] devised a strategy to enhance CDT by inhibiting expression of glutathione in tumors and remodeling the reductive state of the tumor microenvironment, indicating that inhibition of glutathione can improve the effect of CDT. Wang *et al*[79] reported a degradable $MnSiO_3$ nanosystem for CDT/chemical synergistic therapy. First, $MnSiO_3$ nanoparticles were synthesized, and then the surface-initiated living radical polymerization of monomer of SN38 and oligo (ethylene glycol) methacrylate was conducted to obtain the product of CAMNSN@PSN38. Nanoparticles delivered to tumor tissues were gradually

biodegraded by glutathione over time, during which SN38 and Mn^{2+} were gradually released. The released SN38 showed a favorable chemotherapeutic effect and increased accumulation of H_2O_2. The interaction of CAMNSN@PSN38 with glutathione depleted glutathione in tumor tissues and led to Mn^{2+} release for CDT and MRI-guided therapy. CAMNSN@PSN38 had a good inhibitory effect on colon tumor growth and assisted MRI-guided imaging through ROS accumulation *in vivo*. Unlike other tumor types, colon tumor shows high expression of H_2S (0.3-3.4 mmol/L), whose reductive activity is stronger than that of glutathione[80,81]. Therefore, in the treatment of colon cancer, the effect of CDT is also limited by endogenous H_2S. Liu *et al*[82] constructed $CuFe_2O_4$ nanoparticles to explore the potential of endogenous H_2S depletion to enhance CDT for colon cancer. $CuFe_2O_4$ nanoparticles remodel endogenous H_2S in colon cancer and enhance the Fenton or Fenton-like reaction of Cu(I) and Fe(II) by a photothermal effect to generate more •OH. The results suggest that $CuFe_2O_4$ nanoparticles effectively enhance the effect of CDT by depleting H_2S. In addition, H_2S-responsive therapeutic nanoplatforms have been designed. Xiao *et al*[18] synthesized a copper-based metal-organic framework named HKUST-1 as a smart therapeutic platform. PTT and CDT were activated in the presence of H_2S in colon cancer cells. H_2S-triggered nanosystems can minimize side effects on surrounding normal tissues and precisely inhibit colon cancer growth. Above all, CDT shows potential for colon cancer treatment.

Gas therapy

As an emerging treatment method, gas therapy has attracted research interest in recent years[83-86]. Gas therapy refers to use of H_2S[87], NO[88], and CO *etc.* to kill tumor cells[89]. Liu *et al*[90] designed a nanoplatform (PEG/SCNPs@DMSN-SNO-g-C_3N_4) to release NO under X-ray irradiation, and then NO reacted with superoxide anions to generate $ONOO^-$ toxic free radicals, leading to apoptosis through mitochondrial damage. NO has been proven to activate innate and adaptive responses of the immune system against tumors. Previous *in vivo* results showed that all NO-treated colon tumor-bearing (CT26 model) mice were resistant to secondary CT26 cell inoculation. Nonsteroidal anti-

inflammatory drugs (NSAIDs) are prototypical anticancer agents. NO and H_2S are gaseous mediators with physiological relevance and NSAIDs that possess an H_2S- and NO-releasing moiety have shown beneficial effects. Chattopadhyay *et al*[91] synthesized and characterized a new class of anti-inflammatory NO- and H_2S -releasing compounds. This induced apoptosis, inhibited cell proliferation, and reduced colon tumor growth in a mouse xenograft model. Zhang *et al*[92] designed gas-generating MSNs, which can load ammonium bicarbonate and doxorubicin in the pores, and ICG coated on a polydopamine layer and modified with RGD peptides on the outer surface [M(ABC)-DOX@PDA-ICG-PEG-RGD] for triggering drug release and targeted chemotherapeutic photothermal combination treatment. At high temperature and low pH, the encapsulated ammonium bicarbonate can effectively generate CO_2. The CO_2 can damage the polydopamine layer and accelerate the release of doxorubicin. The results proved the excellent antitumor effect of gas therapy and chemotherapy, as well as good biosafety. Therefore, the gas therapy showed potential for colon cancer therapy.

Immunotherapy

Immunotherapy exhibits potential against colon cancer because it relies on the autoimmune system to attack malignant tumors. Immunotherapy for colon cancer is mainly divided into the following categories: (1) Activation of tumor immunogenicity; (2) Relief of tumor microenvironment immunosuppression; (3) Design of antitumor neoantigen vaccines and novel immune adjuvants; and (4) Design of therapeutic strategies using macrophages as target cells. However, only a subset of cancer patients responds to current immunotherapies because of the low immunogenicity of tumor cells and the immunosuppressive tumor microenvironment. Therefore, new strategies are needed to activate tumor immunogenicity and relieve the immunosuppression of the tumor microenvironment to improve the effect of immunotherapy. Fan *et al*[93] reported pH-responsive core-shell nanoparticles (HCLO NPs) for co-delivery of oxaliplatin intermediate and cytosine-guanine-containing oligodeoxynucleotide (CpG) for colon cancer treatment, and the oxaliplatin intermediate intratumoral injection

induced *in situ* antigen production *via* immunogenic cell death. Subsequently, CpG enhanced antigen presentation and promoted production of cytotoxic T lymphocytes (CTLs). The results indicated that the HCLO NPs enhanced the toxicity of oxaliplatin intermediate for CT26 cells and upregulated expression of calreticulin, which exhibited significant immunity and antitumor effect. Hu *et al*[94] integrated HA, pheophorbide A heterodimer and NLG919 into a supramolecular nanocomposite, which generated ROS under NIR laser irradiation to kill tumor cells, stimulated antitumor immunogenicity, and enhanced intratumoral infiltration of CTLs. The immunosuppressive tumor microenvironment was reversed by NLG919-mediated inhibition of indoleamine 2,3-dioxygenase 1. The results showed that this strategy could effectively kill CT26 colon tumors. Ding *et al*[95] designed liposome-encapsulating phosphatidylinositol 3-kinase γ inhibitor IPI-549 and photosensitizer Ce6 for immunotherapy of colon cancer. When the liposomes were internalized into CT26 cells, ROS were generated under laser irradiation, causing immunogenic tumor cell death. IPI-549 transported by liposomes promoted apoptosis of myeloid-derived suppressor cells and reduced the immunosuppressive activity of $CD8^+$ T cells to inhibit growth of CT26 tumors. Checkpoint inhibitors, such as antibodies that block the programmed cell death protein 1/programmed cell death ligand 1 (PD-1/PD-L1) pathway, are among the most promising immunotherapies for metastatic cancer. However, the responses rates remain low. To solve this problem, Yu *et al*[96] developed nanoparticles with PD-L1 blocking ability, which integrated PTT, antitumor immunity, and PD-1/PD-L1 blockade to enhance antitumor efficacy. In the mouse CT26 bilateral tumor model, intravenously injected nanoparticles accumulated at the tumor site and mediated a strong photothermal effect, eliminated the primary tumor by inducing immunogenic cell death, and elicited strong antitumor immunity. Growth of untreated distant tumors was inhibited by the synergistic effect of systemic antitumor immune activation and PD-L1 blockade. This strategy provided a promising approach for the treatment of metastatic cancer.

The reported immunoadjuvants have many limitations, such as poor cellular uptake and biocompatibility, excessive particle size, single function, and unsatisfactory therapeutic effect. Ding *et al*[97] prepared mesoporous silica-coated upconversion nanoparticles (UCMSs) and used them as a novel immune adjuvant. UCMSs had significant loading of the photosensitizer merocyanine 540, chicken ovalbumin and tumor cell fragments. The UCMSs exhibited the best synergistic immune enhancement under 980 nm NIR irradiation, with the strongest Th1 and Th2 immune responses, and the highest frequencies of $CD4^+$, $CD8^+$ and effector memory T cells. In addition, nanovaccine UCMSs inhibited tumor growth more effectively and improved survival of tumor-bearing mice compared with PDT or immunotherapy alone, indicating that UCMSs have higher immunotherapeutic efficacy and clinical potential. As a new tumor vaccine based on zymosan shell particles[98], GP-Neoantigen can stimulate the body to generate a strong antigen-specific $CD8^+$ T cell immune response and an immune response to a variety of neoantigen peptides, and thereby be used for effective tumor treatment. The vaccine induced strong specific $CD8^+$ T cell immune responses and humoral immune responses *in vivo*, which also showed strong tumor growth inhibitory activity in the CT26 colon cancer model. Binding to toll-like receptor agonists PolyI:C and CpG 2395 enhanced the antitumor effect and achieved complete tumor clearance. These results provide broad possibilities for further clinical promotion and personalized vaccine therapy.

M2 macrophages are polarized by stimulatory factors in the tumor microenvironment and promote tumor growth. They are involved in limiting T cell function, tumor angiogenesis, and tumor invasion and metastasis. Increasing the ratio of M1/M2 macrophages in the tumor microenvironment is a promising cancer immunotherapy strategy. An erythrocyte membrane nanoparticle encapsulating *Porphyromonas gingivalis* can modulate the ratio of M1/M2 macrophages for cancer immunotherapy[99], which nanoparticles inhibited the growth of primary and secondary tumors of CT26 colon cancer under the action of laser and anti-PD-1. Immunotherapy based on nanomedicine has been widely used in cell and animal models, and has shown good anti-tumor

efficacy. It is expected to become one of the most potential therapeutic means in cancer treatment.

CONCLUSION

Several advanced nanomedicine applications have been developed for colon cancer therapy, which overcome the poor tumor targeting and efficacy of conventional drugs. This review presents various organic- and inorganic-based nanomedicines applied in colon cancer therapy using CT26 cells as the tumor model. We introduced the mechanism of nanomedicine-based therapeutic strategies including chemotherapy, phototherapy (PTT and PDT), radiotherapy, gas therapy, CDT and immunotherapy. These multimodal therapeutic strategies based on nanomedicine against colon cancer have shown excellent antitumor effect and potential.

Although the nanomedicine-based multimodal therapies have shown a superior effect against colon cancer, several limitations need to be overcome in future development. The first limitation is the unsatisfactory tumor penetration of nanomedicine. Drug delivery *in vivo* includes circulation, accumulation, penetration, internalization and release. Poor tumor penetration has become a long-standing problem for the development of nanomedicine, which leads to the survival of tumor stem cells in deep tumor sites. The reason is the serious hinders of dense extracellular matrix and elevated tumor interstitial pressure. Thus, there is an urgent need to develop novel strategies to enhance tumor penetration of nanomedicine. The second limitation is obstruction of various therapies by the tumor microenvironment. For example, tumor hypoxia limits oxygen-dependent therapy such as PDT and radiotherapy. Additionally, M2 tumor-associated macrophages constructs the tumor immunosuppression environment, which limits the effect of immunotherapy. Not only that, the immune checkpoint protein on the tumor cell inhibits the recognition and combination of cytotoxic T cells. Therefore, reversing the adverse effects of the tumor microenvironment is the key to improving the therapeutic effect of nanomedicine. It is

expected that nanomedicine-based multimodal therapeutic strategies will have potential for clinical translation into colon cancer therapy.

80205_Auto_Edited-check.docx

ORIGINALITY REPORT

9%
SIMILARITY INDEX

PRIMARY SOURCES

1	Xu Chen, Zhi Jia, Yayu Wen, Yuqin Huang, Xiaoyu Yuan, Yutong Chen, Yanan Liu, Jie Liu. "Bidirectional anisotropic palladium nanozymes reprogram macrophages to enhance collaborative chemodynamic therapy of colorectal cancer", Acta Biomaterialia, 2022 Crossref	68 words — 1%
2	Binbin Ding, Shuai Shao, Chang Yu, Bo Teng, Meifang Wang, Ziyong Cheng, Ka-Leung Wong, Ping'an Ma, Jun Lin. "Large-Pore Mesoporous-Silica-Coated Upconversion Nanoparticles as Multifunctional Immunoadjuvants with Ultrahigh Photosensitizer and Antigen Loading Efficiency for Improved Cancer Photodynamic Immunotherapy", Advanced Materials, 2018 Crossref	46 words — 1%
3	Xiao Liu, Yilan Jin, Tingting Liu, Shengju Yang, Mengxue Zhou, Weiqi Wang, Haijun Yu. "Iron-Based Theranostic Nanoplatform for Improving Chemodynamic Therapy of Cancer", ACS Biomaterials Science & Engineering, 2020 Crossref	38 words — 1%
4	Hila Confino, Shay Yarkoni, Matan Goldshtein, Elya Dekel et al. "Abstract PO072: Nitric oxide tumor ablation stimulates an anti-tumor immune response in mice", Cancer Immunology Research, 2021 Crossref	37 words — 1%

5	www.ncbi.nlm.nih.gov Internet	36 words — 1%
6	Yunjian Yu, Jie Li, Boyi Song, Zhuang Ma et al. "Polymeric PD-L1 blockade nanoparticles for cancer photothermal-immunotherapy", Biomaterials, 2021 Crossref	35 words — 1%
7	Miao Su, Yueqiang Zhu, Junbin Chen, Beibei Zhang, Chunyang Sun, Meiwan Chen, Xianzhu Yang. "Microfluidic Synthesis of Manganese-Alginate Nanogels with Self-Supplying H2O2 Capability for Synergistic Chemo/Chemodynamic Therapy and Boosting Anticancer Immunity", Chemical Engineering Journal, 2022 Crossref	32 words — 1%
8	www.x-mol.com Internet	32 words — 1%
9	Zhe Jing, Shuqing Wang, Keyuan Xu, Qian Tang, Wenjing Li, Wei Zheng, Haobo Shi, Kailing Su, Yanting Liu, Zhangyong Hong. "A Potent Micron Neoantigen Tumor Vaccine GP‐Neoantigen Induces Robust Antitumor Activity in Multiple Tumor Models", Advanced Science, 2022 Crossref	26 words — 1%
10	www.science.gov Internet	16 words — < 1%
11	Chattopadhyay, M.. "NOSH-aspirin (NBS-1120), a novel nitric oxide- and hydrogen sulfide-releasing hybrid is a potent inhibitor of colon cancer cell growth in vitro and in a xenograft mouse model", Biochemical and Biophysical Research Communications, 20120316 Crossref	15 words — < 1%

12 Yite Li, Junli Zhou, Lei Wang, Zhigang Xie. "Endogenous Hydrogen Sulfide-Triggered MOF-Based Nanoenzyme for Synergic Cancer Therapy", ACS Applied Materials & Interfaces, 2020
Crossref
14 words — < 1%

13 Donglin Liu, Mengjun Liu, Ying Wan, Xuesu Zhou, Shiping Yang, Lu An, Gang Huang, Qiwei Tian. "Remodeling endogenous H2S microenvironment in colon cancer to enhance chemodynamic therapy", Chemical Engineering Journal, 2021
Crossref
12 words — < 1%

14 www.pubfacts.com
Internet
12 words — < 1%

EXCLUDE QUOTES	ON	EXCLUDE SOURCES	< 12 WORDS
EXCLUDE BIBLIOGRAPHY	ON	EXCLUDE MATCHES	< 12 WORDS