Recent advances of nano-drug delivery system in oral squamous cell carcinoma treatment

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Abstract. Oral squamous cell carcinoma (OSCC) is one of the main causes of death in oral diseases. With the development of nano-technology, great progress has been made in the diagnosis and treatment of tumors in nano-medicine. Being able to carry drugs, nucleic acids, contrast agents, and so on, nano-medical technology can effectively reduce the toxicity of nano-carried materials while playing a drug role. Nano-drug delivery system can help diagnosis and treatment of OSCC. In the chemotherapy of OSCC, nano-drug delivery system can significantly increase the anti-tumor activity of drugs in drug-resistant strains resistant to chemotherapy and can achieve good curative effect. We summarized the research progress of nano-drug delivery system in OSCC by reviewing relevant literature, so as to provide some reference for relevant clinicians.

Key Words:
 Oral squamous cell carcinoma, Nanometer, Drug delivery system, Targeted therapy.

Introduction

Oral cancer is a malignant tumor of the oral cavity and oropharynx, and oral squamous cell carcinoma (OSCC) is the most common pathological type. According to reports[1-4], the number of new patients with oral cancer in the United States reached 51,540 in 2018, with the number of deaths reaching 10,030. At present, the incidence rate of oral cancer ranks 6th among malignant tumors in the world[1-4].

Studies[5] have found that smoking and drinking are the main risk factors for oral squamous cell carcinoma. The occurrence of oral squamous cell carcinoma is a multi-step process, which involves changes in various genes and cells[6]. Scholars[7,8] have found that local lymphatic metastasis and distant metastasis in the late stage of the disease are important factors for poor prognosis of oral squamous cell carcinoma.

In addition, the treatment of oral squamous cell carcinoma includes early treatment and late treatment. The early treatment mainly covers surgery treatment and radiotherapy, and the late treatment usually involves chemotherapy and/or radiotherapy[4]. Chemotherapy may bring some adverse reactions for the patient and result in multi-drug resistance of oral squamous cell carcinoma, which indicates poor prognosis[9-11]. However, the application of nano-drug delivery system can effectively overcome the limitations of chemotherapy, reduce the toxicity of drugs, and thus increase the effectiveness of anti-tumor agents[10]. In addition, nano-drug delivery system has better drug accumulation effect and better drug delivery capacity in tumor treatment. Compared with traditional chemotherapy, nano-delivered drugs show better anti-tumor efficacy, longer blood cycle time, and better drug solubility. Currently, the nano-drug delivery system has been clinically approved for treatment of ovarian cancer, metastatic breast cancer, etc[12,13].

At present, the nano-drug delivery system is still in continuous updating. The second generation of nano-drug delivery system, by increasing active targeting components and enhancing the release of carried drugs, has achieved better tumor targeting effects[14]. The third generation of nano-drug delivery system consists of multi-stage nano-carriers, and its components include the mesoporous silicon particles in the first stage and the drug delivery system embedded in the nano-pores in the second stage, which promotes the interaction between the mesoporous silicon particles and endothelial cells, and enables the drug delivery system in the second stage to directly enter the tumor stroma to achieve better anti-tumor efficacy[15].

Nanotechnology

Nanometer is a unit of length, and one billionth of a meter is 1 nanometer. Medical nanometer materials can be divided in two groups, nanoparticles, and nanosolids. Nanotechnology refers to a technology that studies the structure and physical characteristics of materials with a particle size <100 nm. Currently, the combination of nanotechnology and medical technology has been widely explored and used, and it provides a valuable strategy for treatment of many clinical diseases, especially treatment of human tumors.

Nano-Drug Delivery System

Structurally, there are evident differences between tumor and normal tissue. Capillary of tumor tissue has porous structure and poor integrity compared with normal tissue. Thus, lipid particles and macromolecules leak from the capillary and cannot flow back into blood, which leads to the retention of these molecules in tumor tissue and exerts some biological effects upon their intake by tumor cell. This effect is called the enhanced permeability and retention effect (EPR)[16-18]. The nano-drug delivery system utilizes EPR to produce a drug carrier transport system within the nanometer size range, which can also be used for targeted therapy of nano-drug carriers. Nano-drug delivery system can increase the number and circulating time of drugs in blood, and the carried drugs can selectively accumulate in tumor tissues to achieve the best anti-tumor efficacy[19].

Compared with traditional drug carriers, nano-drug carriers have the following advantages: 1) they increase the stability of drugs. The drug obtains a physical barrier under the encapsulation of the nano-drug delivery system, which has a certain protective effect on the drug and can reduce the damage and degradation of the drug by enzymes. 2) They increase the bioavailability of drugs. Nano-drug delivery system can increase the permeability of drugs on biomembranes such as blood brain barrier, thus increasing the bioavailability of drugs. It can also improve the solubility of some or insoluble macromolecular drugs. Another advantage of nano-drug delivery system is that it can aggregate drugs in tumor tissues. Nano-drug delivery system has small particle size, large surface area, so it can carry many more drugs and increase the binding time between drugs and tumor tissues, thus increasing the absorption rate of drugs and their bioavailability. 3) They increase the targeting of drugs. The nano-drug delivery system can control the distribution of drugs in the body and avoid damages caused by drug leakage. Modification of nano-carrier materials can change the drug loading, kinetic characteristics, and biocompatibility. In the process of tumor occurrence and development, there are no intact blood vessel walls, most of which are foraminous gaps, and these empty gaps are nanoscale. Therefore, nano-carrier drugs can reach the inside of tumor tissues rather than normal tissue and achieve better anti-tumor efficacy[20].

Classification of Nano-Drug Delivery Systems

Targeted therapy with nano-drug carriers refers to the targeted action of therapeutic drugs or agents on target organs, target cells or molecular by taking nanoparticles with diameters between 1 nm-1000 nm as carriers. Since the diameter of the particles is at the nanometer level, the particles obtain many special properties, mainly including large specific surface area, high surface reactivity, strong adsorption capacity, etc. These unique properties make it easy for nanoparticles to carry drugs or agents through encapsulation, adsorption, or chemical group connection. Moreover, we can realize targeted therapy according to the groups or substances with target site identification carried on the surface of nanoparticles to be used, or by changing the charge or space conformation on the surface of nanoparticles. The classification of nano-drug delivery system is shown in Figure 1.

Passive Targeting

Based on the enhanced permeability and EPR in tumor tissue, passive targeting enables drugs to reach specific tumor sites through modification and adjustment of nano-carriers, thus prolonging drug distribution time and retention time. Passive targeting can also exert better anti-tumor effect by reducing drug-resistance in tumor tissues.

Passive targeting functions mainly act through the small diameter of nanoparticles. In normal tissues, vascular endothelium is arranged orderly, tightly, and completely, and macromolecules and lipid particles do not pass easily through the vascular wall. However, in tumor tissue, there is a higher neovascular permeability, and the na-
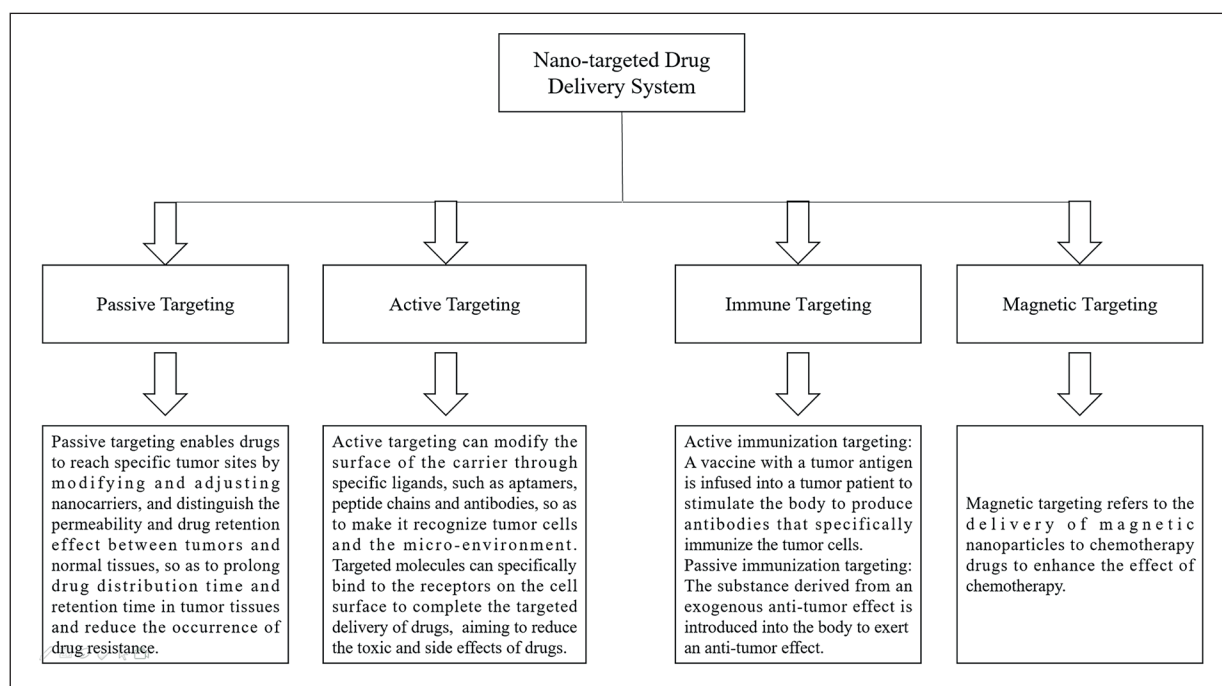
Figure 1. Classification of Nano-Drug Delivery Systems Sk etch Map. We have shown more clearly the brief introduction of various drug delivery methods and methods of nano drug delivery system, which can be simply divided into: Passive Targeting, Active Targeting, Immune Targeting, Magnetic Targetin.

no-carriers can easily pass through the vascular wall and reach the tumor tissue. Passive targeted drug delivery system mainly refers to the use of physical and chemical characteristics such as surface hydrophobicity or hydrophilicity of nanoparticles, electrostatic effect, magnetic force effect, nanoparticle diameter size, and surface pH value to achieve targeted therapy effect[21].

Active Targeting

For active targeting, the surface of the carrier is usually modified by specific ligands, including aptamers, peptide chains, and antibodies to identify special receptors in tumor cells and their microenvironment through ligand-receptor interaction. Targeting molecules combine specifically with receptors on the tumor cell surface to realize targeted delivering of drugs and thus achieve enhanced anti-tumor effects and cause less drug toxicity and side effects to normal tissues[22].

Immune Targeting

Immune targeting is divided into active immunity and passive immunity. At present, the research infuses vaccines with tumor antigens into tumor patients to stimulate the body to produce antibodies that can specifically immunize tumor cells. Passive immunity refers to the introduction

of foreign substances with anti-tumor effect into the body to exert anti-tumor effect. Xiao et al[23] prepared polymeric nanoparticles by nano-drug delivery system, and encapsulated it with tumor cell membrane, which shows good stability and has tumor antigenicity due to its similarity to the original tumor cells. Under the action of immune sensitizer, dendritic cells can stimulate the proliferation of T cells and increase the identification of tumor antigenicity, therefore enhancing T cells-mediated anti-tumor effect.

Magnetic Targeting

It refers to deliver magnetic nanoparticles with chemotherapy drugs to tumor cells to increase chemotherapy effect. Ferric oxide (Fe_3O_4) particles have superparamagnetism and can well locate tumors. The specific MENs prepared by Oliveira et al[24] deliver paclitaxel to reach tumor cells and have anti-tumor effect but no damage to surrounding normal cells.

Targeting Sites of OSCC with Nano-Drug Delivery System

Within tumor microenvironment, there are many components for targeting treatment with nano-drug delivery system. According to differ-

ent action positions of the targeted system, the application of the targeted drug delivery system in oral squamous cell carcinoma can be divided into targeted tumor vessels, interstitial fluid and extracellular matrix, targeted tumor matrix cells, targeted tumor cells, related dendritic synaptic cells, and targeted tumor stem cells. In clinical practice, these targets are designed according to the characteristics of tumors, and drugs are delivered through nano-drug delivery systems to achieve the goal of targeted therapy. The content contained in this link is presented through images (Figure 2).

Targeting Blood Vessels of Oral Squamous Cell Carcinoma

Targeting the blood vessels in tumor tissue is the main strategy for treatment of oral squamous cell carcinoma, and the growth of blood vessels is essential for nutrition intake, invasion, and metastasis of tumor. Many growth factors contribute to the formation of new blood vessels, including fibroblast growth factor (FGF), VEGF family, platelet-derived growth factor (PDGF) family, and angiogenin (ANG), etc. Therefore, in targeted treatment of oral squamous cell carcinoma, better anti-tumor efficacy can be achieved through

specific anti-tumor angiogenesis. Nanoparticles in the nano-drug delivery system enter the tissue of oral squamous cell carcinoma. The endothelial cell layer internalizes the nanoparticles to exert better efficacy. Gu et al[25] prepared polyethylene glycol-polylactic acid (PEG-PLA) nanoparticles decorated with EDB target peptide APT EDB to carry paclitaxel (PTX) by fibronectin variable shear fragment (extra domain B, EDB), which can target tumor blood vessels and tumor cells. Compared with nanoparticles not modified by EDB, the nano-drug delivery system has better effect on tumor treatment.

Targeting Intercellular Fluid and Extracellular Matrix in Oral Squamous Cell Carcinoma

Extracellular matrix is a complex of many large proteins secreted from the cells into extracellular space and forms a special structure which can permit or limit transportation of certain substances. Studies have shown that dense extracellular matrix can slow down the infiltration and diffusion of macromolecules. For example, fibrous collagen can limit the movement of particles with a particle size greater than 50 nm[26]. If the intercellular fluid and extracellular matrix are
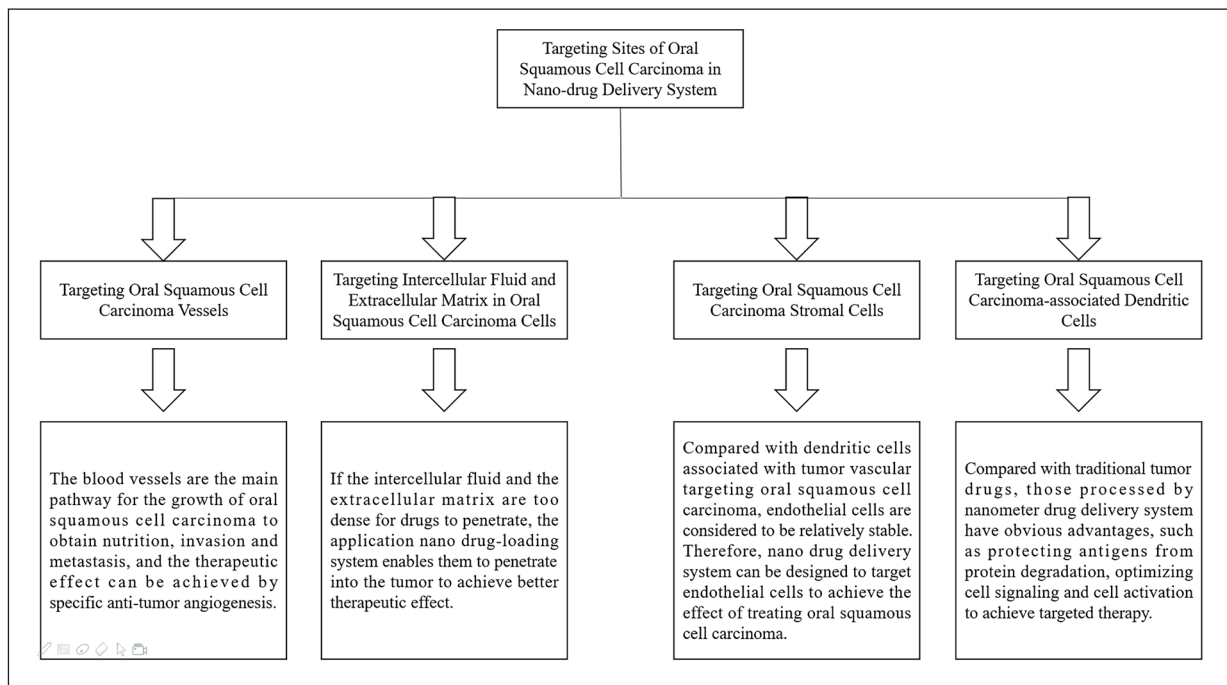

Figure 2. Targeting Sites of OSCC with Nano-Drug Deliver y System. We show the application of nano targeted drug delivery in OSCC more clearly in the figure, The targeted drug delivery applications are as follows: Targeting Oral Squamous Cell Carcinoma Vessels, Targeting Intercellular Fluid and Extracellular Matrix in Oral Squamous Cell Carcinoma Cells, Targeting Oral Squamous Cell Carcinoma Stromal Cells, Targeting Oral Squamous Cell Carcinoma-associated Dendritic Cells.

too dense for drugs to penetrate, the nano-drug delivery system can penetrate into the tumor to achieve better efficacy.

Wang et al[27] prepared a pH-responsive P (LE-IA-MEG) hydrogel by heat-initiated polymerization using the macromonomer polyethylene glycol monomethyl ether-polylactic acid-itaconic acid (PLE-AC). As one molecule of itaconic acid has two carboxyl groups, P (LE-IA-MEG) hydrogel shows good pH response performance, and can easily infiltrate tumor tissue, and achieve better anti-tumor efficacy.

Targeting Stromal Cells in Oral Squamous Cell Carcinoma

Stromal cells in oral squamous cell carcinoma can support tumor growth through cell adhesion or paracrine of relating cytokines or growth factor. Current studies believe that vascular growth factor (VGF), endothelial cells, inflammatory cells, etc., in oral squamous cell carcinoma are potential targets for treatment of oral squamous cell carcinoma. Compared with tumor vessels, endothelial cells are considered to be relatively stable, so the nano-drug delivery system can be designed to target endothelial cells to achieve the effect of treating oral squamous cell carcinoma. At the same time, it was found that the mannose receptor was highly expressed on the surface of macrophages in oral squamous cell carcinoma. Therefore, the nano-drug delivery system could achieve diagnostic and treatment efficacy against tumor cell by targeting the molecules on macrophage imaging[28]. Previous studies have found that macrophages are considered to be immune cells attacking tumors, but M2-type macrophages have recently been found to be involved in tumor angiogenesis, and M1 cells have also been found to have anti-tumor characteristics. The current research focuses on how to adjust the ratio of M1/M2 to improve the treatment of oral squamous cell carcinoma.

Targeted Dendritic Cells Related to Oral Squamous Cell Carcinoma

With the deterioration of oral squamous cell carcinoma, tumor stroma will produce immunosuppressive microenvironment, resulting in tumor immune tolerance[29]. At present, studies[30] have found that the main factors helping tumor immune escape are programmed death receptor and ligand (PD-1/PD-L1) and cytotoxic T lymphocyte associated antigen (CTL-4). Therefore, current drugs achieve the purpose of treating tumor by recognizing this receptor. Compared with traditional tumor drugs, this drug treated by nano-drug delivery system has evident advantages. They can protect antigen from protein degradation, optimize signal transmission between cells, and activate cells to achieve the goal of targeted therapy. Among them, tumor-associated dendritic cells (TADC) are currently considered as the most promising tumor cells, which may stimulate the host immune system to produce a specific anti-tumor immune response or induce cytotoxic T cells to kill tumor cells. Compared with normal dendritic cells, the Toll receptor (TLR) stimulates TADCs and is less responsive to TLR stimulation. Luo et al[31] prepared a kind of micelles through the nano drug-loading system, which can effectively improve the maturation and differentiation of TADCs to achieve anti-tumor effects.

Application of Nano-Drug Delivery System in Oral Squamous Cell Carcinoma

In oral squamous cell carcinoma, nano-drug delivery system can load chemotherapy drugs and has chemoprevention potential, including inhibition of tumor cells, prevention, and reversal of malignant tumor precursor cells, etc[32]. Flavonoids and stilbenes are two representatives in natural plant compounds for tumor treatment. Experimental studies have found that flavonoids and stilbenes have good anti-oral tumor activity[32], but their solubility is poor, and bioavailability is low, so their clinical anti-tumor effect is also reduced[33]. Since the emergence of nano-drug delivery system, this problem can be well solved. The chemotherapy prevention of nano-drug delivery system is well discussed clinically. Based on the development of nanomedicine, nano-drug delivery system has more advantages in the treatment of tumors, but the efficacy of nano-drug delivery system on advanced oral squamous cell carcinoma needs more evidence[34]. Adverse reactions of chemotherapeutic drugs and drug resistance of tumors limit the efficacy of chemotherapy, so many scholars have carried out *in vitro* experiments on nano-drug delivery systems, carrying some anti-tumor drugs, including cisplatin, 5-fluorouracil, etc. The drugs currently applied are integrated and presented through images (Figure 3).

The 5-Fluorouracil

Zhao et al[35] synthesized the 5-fluorouracil-loaded self-assembled nucleotide nanosystem, and found that the drug delivering nanosystem showed better anti-oral squamous cell carcino-
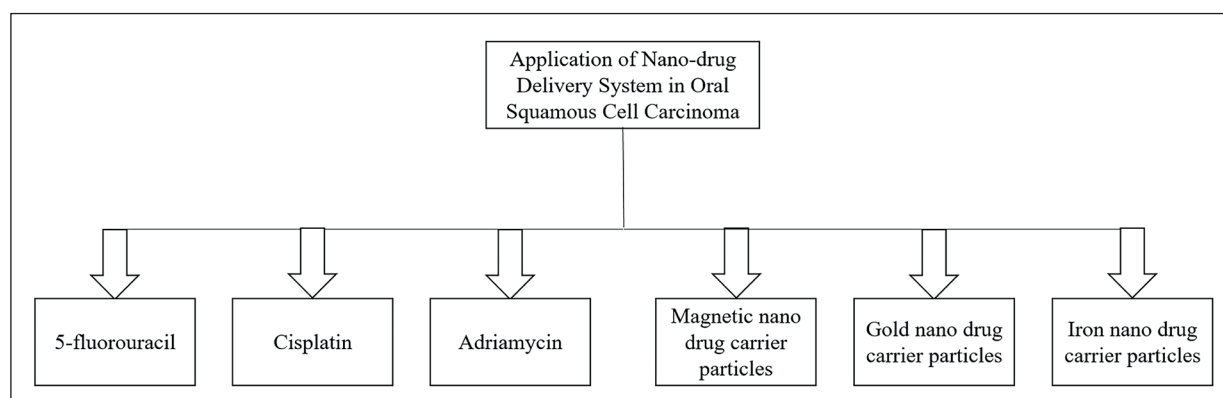
Figure 3. Application of Nano-drug Delivery Systemin Oral Squamous Cell Carcinoma. At present, the targeted therapeutic materials that have been used in clinic are asfollows: 5-fluorouracil, Cisplatin, Adriamycin, Magnetic nano drug carrier particles, Gold nano drug carrier particles, Iron nano drug carrier particles.

ma effect *in vitro* compared with the nanosystem without 5-fluorouracil. The researches in a subcutaneously transplanted oral squamous cell carcinoma model, also showed that experimental group demonstrated better anti-tumor effect and lower toxicity to normal cells 3 weeks after injecting 5-fluorouracil with nano-drug delivery system when compared with control.

Cisplatin

Wang et al[36] designed a targeted cisplatin nano-drug delivery system based on the tripeptide motif arrangement of epidermal growth factor binding domain. It was found that the cisplatin delivery system has better targeted anti-tumor cell effect, lower cytotoxicity to normal cells, and results in more tumor cell apoptosis. Furthermore, compared with cisplatin nano-drug delivery system, the polymer micelles of cisplatin encapsulated by the nano-drug delivery system have better treatment efficacy on human oral squamous cancer cell lines and lower toxic effect on normal cells. The cisplatin packaged in nano-drug delivery system showed lower degree of renal injury in the xenograft animal model of OSCC and the drug can markedly reduce lymph transfer rate in treated animals[37].

Doxorubicin[38,39,40]

Doxorubicin is a powerful chemotherapeutic drug, but its clinical application is limited due to its cardiac toxicity. Marcazzan et al[38] have developed a liposome doxorubicin using nano-drug delivery system, which can reduce adverse drug reactions and cytotoxicity. In adjuvant chemotherapy of oral squamous cell carcinoma, doxorubicin can improve the anti-tumor effect on primary tumors but is ineffective for distant metastatic tumors[38].

Magnetic Nano-Drug-Loaded Particles

Magnetic nano-drug-loaded particles have also been used as thermal media at present. They are useful theranostic agents for OSCC due to their nano-size and potential to improve their characteristics by surface modification with antibodies, drugs, and MRI contrast agents[38]. In addition, magnetic nanoparticles can induce apoptosis of local cells, which may be due to their ability to convert non-ionizing electromagnetic radiation from therapeutic light sources into heat energy related[41].

Gold Nano-Drug Delivery Particles

Currently, some scholars have explored the photothermal ablation effect of gold nano-shells in oral squamous cell carcinoma. At the same time, they have also found several drug delivery particles similar to gold nano-shells, which are expected to become promising drug carriers and photothermal agents[41]. Colloidal gold nanoparticles were obtained by reducing Au (gold) and reacted to the exposure of near infrared light by converting the gold nanoparticles into heat. Some scholars have explored the effect of gold nanoparticles and laser treatment on oral squamous cell carcinoma in an animal model, and the gold nanoparticles were injected directly into tumor tissues. The results showed that compared with control group (treated with laser only), the combination of gold nanoparticles and laser significantly inhibited tumor growth and proliferation[41].

Iron Nano-Drug Delivery Particles

Iron nano-drug delivery particles have also been explored by many scholars. A study showed that iron-core nanoparticles with a gold shell (Fe@Au) can delay iron oxidation and improve its magnetism. The Fe@Au nanoparticles exert OSCC toxicity through mitochondrial mediated autophagy, while normal oral cells are not affected[38]. Some scholars have used photothermal therapy to treat refractory oral squamous cell carcinoma and added gold-coated silicon core nano-drug delivery particles. The efficacy needs to be further tracked and is still in the clinical trial stage (NCT00848042). Melancon et al[42] developed a gold nano-shell with superparamagnetic iron oxide silicon core (SPIO@AuNPs) and combined with cetuximab, a C225 monoclonal antibody. It was found that compared with non-targeted nanoparticles, the combination of cetuximab significantly promoted binding of SPIO@ AuNPs with the tumor cells overexpression of epidermal growth factor receptor. Further research found that the targeted drug group had the lowest cell survival rate[38,43].

Other Nanotechnology-Based Treatment Systems

In addition to drug delivery system for oral squamous cell carcinoma, nanomedicine can carry out gene therapy based on lipid nano-drug delivery particles. Currently, there are reports of gene therapy using non-viral vectors and DNA and siRNA for oral squamous cell carcinoma[44]. In *in vivo* and *in vitro* experiments, it was found that relevant tumor-specific promoters were packaged by relevant nanotechnology to achieve gene therapy by influencing expression of tumor necrosis factor-related apoptosis ligand genes. The comparison revealed that the nano-carrier preparation (not packed by nanotechnology) in the control group failed to well inhibit tumor cell proliferation and promote apoptosis. After packaging HIF1α (hypoxia inducible factor) or vascular endothelial factor A siRNA with nanotechnology, it could effectively inhibit tumor cell proliferation, neovascularization, and promote tumor cell apoptosis[38].-

Therefore, the application of nanotechnology in gene therapy has also brought valuable results and further research is necessary.

Conclusions

At present, there are still great challenges in the treatment of oral squamous cell carcinoma. As a new type of anti-malignant tumor drug system, nano-drug carriers have excellent advantages in increasing drug stability and targeting, improving drug bioavailability, protecting drug molecules, and so on. Therefore, it has broad application prospects.

Nano-drug delivery technology has achieved good treatment efficacy in chemotherapy prevention, chemotherapy, development of targeted magnetic nanoparticles, and gene therapy of oral squamous cell carcinoma. At present, there are many kinds of nano-drug delivery systems, but there are still some problems to solve. Therefore, it is still necessary to continuously improve the nano-drug delivery technology to develop and obtain more and better new materials for the clinical treatment of tumors. With the continuous deepening of research and the continuous development of nanotechnology, nanomedicine will play a better and greater role in the treatment of oral squamous cell carcinoma.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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