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Application of Targeted Ultrasound Contrast Agent in Ovarian Cancer

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Abstract: *Epithelial ovarian cancer (EOC) is one of the major malignant tumors that seriously threaten women's health. Due to the lack of effective screening tools and vague symptoms, more than 70% of ovarian cancer patients are not diagnosed until the advanced stage, so it is difficult to treat them. Ultrasound targeted microbubble destruction (UTMD) technology shows great advantages in the delivery of clinical therapeutic materials, and can significantly improve the therapeutic effect. Therefore, the application of UTMD in the treatment of ovarian cancer has attracted the attention of excellent scholars at home and abroad. This article reviews targeted ultrasound contrast agents and their application strategies in the treatment of ovarian cancer.*

Keywords: *ovarian cancer, contrast agents, targeted contrast agent, microbubble, ultrasound targeted microbubble*

1. Application of Ultrasound Contrast Agent

In 1942, Shampo published the first paper on using ultrasound to obtain images of brain tumors[1,2]. Ultrasound has the advantages of non-invasive, non-radiation, low cost, high spatio-temporal resolution and penetration of soft tissue, so it is one of the main means of medical image diagnosis and image-guided treatment. Because the contrast medium is easy to distinguish biological tissue by changing the image contrast, it is suitable for diagnostic applications[3]. The size of contrast media particles is a limiting factor in the diagnosis and treatment of many extravascular diseases. Smaller-scale nanobubbles can enter the extravascular chamber of the tumor and can be used for imaging and treatment closer to cancer cells. Carly[4] directly demonstrated the passive and active complete extravasation of nano-bubbles in tumor tissue for the first time, and was the first to directly capture the acute vascular effect caused by ultrasound stimulation of nano-bubbles. This study is an important step in releasing the potential of nano-bubbles and expanding ultrasound-based applications. Gas vesicles (GVs) are nanoscale gas-filled protein structures expressed in some cyanobacteria bacteria as a mechanism for regulating cell buoyancy in water environment[5]. In recent years, GVs has been reported as a new nano-scale contrast agent. GVs is a naturally stable nanostructure encoded by genes, and it is also the first ultrasound biomolecule contrast agent for contrast enhancement applications[6].

2. The Rise of Targeted Ultrasound Contrast Agents

In order to transport and control drug delivery while actively targeting tumors, people began to modify the contrast medium, so as to explore the therapeutic potential. Microbubbles[7] can be modified with antibodies or ligands that bind to disease-related markers expressed on endothelial cells or target cells to achieve better targeting efficiency. During tumor angiogenesis, various molecular markers are overexpressed on tumor vascular endothelial cells, such as vascular endothelial growth factor receptor type 2 (VEGFR-2), αvβ3 integrin or cell membrane glycoprotein (endoglin)[8].In addition, inflammatory markers such as E-and P-selectin, anti-intercellular adhesion molecule-1 (ICAM-1) and anti-vascular cell adhesion molecule-1 (VCAM-1) can also be targeted by microbubbles to quantify inflammation[9]. Other molecular targets include prostate specific membrane antigen (PSMA) and thymocyte differentiation antigen 1 (Thy1)[10]. In order to meet the needs of practical applications, more and more researchers have shifted their focus to double or triple targeting microbubbles to improve binding efficiency[11].

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3. Research Progress and Challenges in the Treatment of Ovarian Cancer

Ovarian cancer is a kind of malignant tumor with the characteristics of occult growth, frequent metastasis and rapid drug resistance. its high mortality is due to its late diagnosis and recurrence[12].Patients often develop drug resistance within a few years after receiving traditional chemotherapy, so immunotherapy has become a new revolution in the treatment of ovarian cancer. The metabolic disorder of tumor cells and the characteristics of evading immune destruction are common problems in immunotherapy at present. So far, the clinical activity of single use of immune checkpoint inhibitors is still very limited[13]. Preclinical data suggest that the addition of poly (ADP-ribose) polymerase inhibitor (PARPI) may create a more immunogenic tumor environment and make cancer sensitive to checkpoint blocking therapy[14]. PD-L1 inhibitors have also been shown to enhance the anti-tumor effect of PARPI by restoring anti-tumor immunity and show moderate clinical activity in recurrent ovarian cancer. Therefore, to evaluate the efficacy of immune checkpoint blocking combined with PARPI in clinical trials of ovarian cancer is a potential therapeutic strategy[15]. At the same time, LampertEJ et al.[16] also proposed that VEGF/VEGFR pathway can counteract the immunostimulatory effect of PARPI and become a target to improve the efficacy of PARPI and anti-PD-L1 combined therapy. Therefore, blocking the VEGF/VEGFR pathway can further regulate the immunosuppressive environment of ovarian cancer. Therefore, the combination of immunotherapy with chemotherapy, radiotherapy, antiangiogenic drugs and PARPI is essential[13].

4. Targeted Ultrasound Contrast Agent in the Diagnosis and Treatment of Ovarian Cancer

The five-year survival rate of patients with ovarian cancer is low, so the early qualitative and localized diagnosis of ovarian cancer is the key to improve the survival rate. CA125, also known as mucin 16 (MUC16), has become a reliable biomarker for screening epithelial ovarian cancer[17]. Gao Yong et al. [18] found that after surface functionalization of nanobubbles with CA-125 antibody, the aggregation rate of CA125 positive OVCAR-3 tumors was significantly higher than that of CA-125 negative SKOV-3 tumors, and the ultrasound peak signal intensity was higher and the elution rate was lower. Compared with non-targeted nano-bubbles, targeted nano-bubbles also showed more tumor retention and longer echoes. Therefore, ultrasonic molecular imaging using CA125 antibodies coupled with nano-bubbles may help to improve the diagnosis of epithelial ovarian tumors. In addition, some studies[19] have proved that there is a certain correlation between targeted contrast-enhanced ultrasound imaging parameters and neovascularization density of ovarian cancer xenografts in nude mice, and this correlation is more significant in the early stage of ovarian cancer， thus it provides a new method, new idea and new basis for the diagnosis of early ovarian cancer.

4.1 Application of targeted ultrasound contrast agent in the treatment of ovarian cancer-- drug delivery

Drug resistance is a thorny problem in the treatment of ovarian cancer in the world[20].The standard postoperative chemotherapy for ovarian cancer is paclitaxel (PTX), a natural antimitotic drug that has been shown to be effective in many epithelial ovarian cancers [21]. However, due to the emergence of drug resistance, the recurrence rate is often high[17]. Studies[22] have shown that inhibition of autophagy under certain conditions can inhibit cancer drug resistance. Fan et al.[23] treated with low intensity focused ultrasound combined with microbubbles (LIFU+MB) combined with PTX. Through CCK8 (cell counting kit-8) and flow cytometry, it was found that the proliferation of drug-resistant cells was significantly inhibited and the rate of apoptosis increased. Transmission electron microscopy (TEM) was used to observe the drug-resistant cells treated with LIFU+MB. It was found that autophagy was significantly inhibited. Therefore, the combination of LIFU+MB+ chemotherapeutic drugs may be an innovative way to improve drug resistance, which can effectively reduce the drug dose and minimize the side effects of conventional chemotherapy. Methotrexate (MTX) is similar to folic acid (FA) in structure and can target folate receptor (FR). Methotrexate is one of the most widely used cancer chemotherapy drugs[24]. Marziyeh et al.[25] constructed methotrexate (MTX) coupled multifunctional nanoparticles (NPs) using chitosan (CS) as carrier for targeted drug delivery of erlotinib (ETB). The drug release spectrum showed that the release of drug molecules increased at pH=5.5 and 37 ℃. Cell uptake confirmed the significantly higher internalization of MTX-coupled nanoparticles, indicating that MTX molecules have a targeting effect in vivo. MTT cytotoxicity test and flow cytometry apoptosis test showed that MTX-CSC@MNPs containing ETB had more toxic effect on cells than free drugs. Therefore, MTXCSC@MNPs is considered to be an effective intelligent

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nano-carrier for targeted treatment of FR-positive solid tumors.

4.2 Targeted Ultrasound Contrast Agents in the Treatment of Ovarian Cancer - Gene Delivery

Standard treatment options such as cytoreductive surgery followed by adjuvant chemotherapy are ineffective in the long-term treatment of ovarian cancer. Therefore, in recent years, researchers have explored new treatment strategies, such as gene therapy[26]. Gene therapy can be delivered to cancer cells by viral or non-viral methods. However viral vectors carry a potential risk of insertional mutations and interference with the response[27]. Non-viral gene delivery systems are relatively safe and easier to apply, but they have low transgenic efficiency and transient gene expression rates[28]. Ultrasound targeted microbubble destruction technique can produce transient pores in cell membrane, stimulate cell membrane permeability and significantly improve gene transfection rate[29]. Gene therapy mainly suppresses the expression of oncogenes or replaces the activated tumor suppressor genes with their wild-type copies. Luteinizing hormone-releasing hormone (LHRH) is often used as a target because it is expressed in 70% of ovarian cancer cell lines but not in most healthy human organs[30]. Due to the instability of natural LHRH in vivo, LHRH analogues (LHRHa) with higher biological activity have been synthesized to target LHRH receptors[31]. In various gene therapy schemes for ovarian cancer, p53 gene mutation exists in nearly 60% of advanced ovarian cancer, which makes it possible to target wild-type p53 (wtp53) tumor suppressor gene for clinical treatment. Zhang Shufang et al.[32] combined lipid microbubbles with LHRHa, then mixed microbubbles with pEGFP-N1-wtp53 plasmid and applied ultrasonic pulse to deliver wtp53 gene to cancer cells, which provided a new and attractive scheme for gene therapy of ovarian cancer. Survivin is a new member of the apoptosis inhibitor protein inhibitor (IAP) family, which is involved in the occurrence of cell resistance. It is selectively expressed in embryonic tissues and most types of tumors, but not in normal adult tissues, thus providing an attractive target for anticancer therapy[33]. Some scholars[34] successfully mixed LHRHa modified microbubbles with shRNA recombinant expression plasmid (pshRNASurvivin) targeting Survivin gene, and targeted shRNA-Survivin into ovarian cancer A2780/DDP cells mediated by UTMD, thus playing a selective role in apoptosis of ovarian cancer cells. It also provides a novel method for gene therapy of ovarian cancer. As another new member of IAP family, Livin is highly expressed in many kinds of tumor cells[35]. Therefore, ultrasound targeted destruction of Livin microbubbles[36] is also an effective method to inhibit proliferation and induce apoptosis of ovarian cancer cells. Meanwhile, suicide gene therapy is one of the most attractive techniques. The most commonly used suicide gene method uses herpes simplex virus thymidine kinase (HSV-TK), which converts nucleoside analogues such as ganciclovir (GCV) into monophosphorylated molecules and then from cellular enzymes to triphosphorylated form. These triphosphate molecules are bound to the slender DNA, resulting in premature chain termination and cell death[37]. Zhou Xianlong et al.[38] found that HSV-TK+ microbubble + ultrasound irradiation group had the highest transfection rate and tumor inhibition effect of HSV-TK gene, that is, UTMD-mediated HSK-TK/GCV system could significantly improve the anti-tumor effect of HSV-TK and the survival rate of mice.

5. Problems and Prospects

Ultrasonic wave combined with appropriate target delivery system has been widely used. Although UTMD for the treatment of ovarian cancer has made great progress in delivering therapeutic materials, there are still many difficulties to be solved in clinical practice. First, microbubbles as foreign bodies may cause unnecessary immune systems in the body. Secondly, it is not enough to modify microbubbles with targeted ligands. Ideally, it is necessary to understand the mechanism of ultrasound controlling the fixed-point release of therapeutic materials. In addition, the size of microbubbles should be considered. Larger microbubbles have poor vascular permeability, so it is difficult to use them for extravascular ultrasonic molecular imaging and targeted delivery of drugs or genes to tumor parenchyma cells. Nanomedicine has a great influence in the field of drug / gene delivery and imaging, but it lacks the contrast of ultrasonic imaging because of its small size. Therefore, the development of contrast media that can combine with tumor cells through tumor blood vessels, carry a sufficient amount of bioactive molecules and significantly enhance ultrasound imaging is a key step in cancer treatment. We hope that with the further study of targeted contrast agents, these problems can be solved.

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