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New Progress in Ultrasound Molecular Imaging

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Abstract: *With the development of molecular imaging, ultrasound imaging is no longer a means to diagnose diseases, but to the molecular level of accurate medical treatment. The tool of ultrasonic molecular imaging is ultrasound contrast medium. in order to meet the diversified clinical needs, ultrasound contrast agent has developed from the first generation and the second generation to multimodal contrast agent. Targeted ultrasound contrast agent can carry corresponding biomarker factors for localization, imaging and treatment of lesions, which is an important development direction to achieve accurate ultrasound diagnosis and treatment. In this paper, the research progress of ultrasonic molecular imaging technology is reviewed.*

Keywords: *molecular imaging, ultrasound, targeted contrast agent*

1. Introduction

With the development of molecular imaging technology, imaging is no longer a physical science that simply deals with imaging accuracy and accuracy, but chemical imaging based on the sensitivity and specificity of imaging diagnosis based on molecular probes [1]. Ultrasound molecular imaging （USMI) is based on conventional ultrasound imaging combined with contrast-enhanced ultrasound (CEUS), using targeted ultrasound contrast agent to accurately locate the lesions to achieve the purpose of diagnosis or treatment [2]. By using targeted functionalized ultrasound microbubbles to form ligands with high affinity for molecular markers of specific disease processes, this will contribute to non-invasive molecular imaging and enable the diagnosis and treatment of diseases at the molecular level [3].

2. The types of ultrasound contrast agents

2.1 Micron ultrasound microbubbles and nanometer ultrasound microbubbles

The first generation of ultrasound contrast agents are air-containing vesicles, which burst soon after injection into the blood vessels, which limits the effect of ultrasound development. The microbubble size of the first generation of microbubble contrast agent is in the order of microns, and the size is generally 1-8μm, which is about the same size as red blood cells, while the endothelial gap of tumor neovascularization is about 380~780 nm, so micron-sized ultrasound microbubble contrast agent is particularly suitable for blood pool imaging of vascular target molecules.However,micron-sized microbubbles cannot seep out and accumulate in the space around the blood vessels, so tumor vascular imaging cannot be achieved through the tumor vascular space. The size of micron-sized contrast agent prevents it from obtaining more precise molecular imaging effect [7] [8] [9] [10] [11].

The second generation contrast medium contains inert gases (such as octafluoropropane, sulfur hexafluoride) and its shell is made of proteins, polymers or phospholipids. This thin shell reduces surface tension, stabilizes the gas core and prevents rapid dissolution [4] [5] [6]. At present, the commonly used ultrasound contrast agent is micron microbubbles, which is similar in size to the diameter of red blood cells, so it is used as blood pool contrast agent. With the development of ultrasound contrast agents, smaller nano-bubbles can exudate from the circulatory system into the tumor parenchyma, which can effectively enhance the microbubble permeability and maintain a high retention effect [12] [13].

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2.2 Phase-transformed ultrasound contrast agent

By cooling or applying mechanical pressure, low boiling point gases (such as octafluorobutane and decafluorodibutane) are converted into liquid phases to obtain nano-liquid contrast agents in the submicron size range, which is called phase change contrast agent (PCCAs)[14]. Phase change ultrasound contrast agent provides a new idea for the transformation of contrast agent in micron and nanometer scale. Zhou Yang et al developed a magnetothermal phase change nanoparticle contrast agent (PFH-HIONS) which encapsulates liquid fluorocarbon perfluorohexane (PFH) in nano-hollow iron spheres (HIONS),it can enhance ultrasound, photoacoustic and magnetic resonance imaging in multiple modes, providing a new and efficient research platform for the integration of diagnosis and treatment based on molecular imaging. After photoacoustic, magnetic resonance and magnetically heated phase transition in vitro, ultrasonic imaging was performed in vitro. It is proved by software analysis that the nanoparticle contrast agent can enhance multi-mode development and has good magnetic heating properties [15]. This liquid nano-drop contrast agent can effectively overcome various defects of ultrasound microbubbles, such as poor stability, large particle size, and inability to pass through the endothelial space of tumor blood vessels. Other advantages are small particle size, not easy to be affected by chemicals, safe degradation products, long retention time in the body, and enhanced ultrasound development through liquid phase change.David et al have studied the ability of self-made phase change nano-ultrasound contrast agent to detect insulitis in type 1 diabetes mellitus (T1D). In vivo experiments have confirmed that high contrast signals can also be observed before the detection of insulin autoantibodies in mice. The self-made phase change nano-contrast agent ultrasound imaging can detect islet inflammation before the onset of diabetes, which is helpful to monitor disease progress, guide and evaluate the preventive intervention of T1D [16].

2.3 Multimodal ultrasound contrast agent

The single imaging method of traditional ultrasound contrast agent still has some limitations, and it is difficult to meet the requirements of accuracy, specificity and targeting at the same time [17], which promotes the development of multimodal ultrasound contrast agent. Multimodal molecular imaging combines two or more detection techniques to obtain more information in diagnosis, treatment and monitoring [18] [19] [20] [21]. The researchers made multimodal contrast media by changing the material of the contrast agent's shell and internal fillers. Hu Chencheng et al constructed polylactic acid-glycolic acid copolymer (PLGA) as carrier, SDF-1 as ligand, indocyanine green (ICG) and liquid fluorocarbon (PFH) as core nanoparticle contrast agent. Photoacoustic imaging of rabbit tongue cancer model tumor with lymph node metastasis was performed by photoacoustic imaging equipment. The results showed that it could enhance the photoacoustic and ultrasonic imaging effect of cervical lymph node metastasis of rabbit tongue cancer [22]. Protein materials are good shell materials for the preparation of multimodal contrast agents because of the stability of soft shell and drug loading of hard shell. Barmin et al made gold nanoparticles coated with bovine serum albumin and added photodynamic dyes (zinc phthalocyanine and indocyanine green) to evaluate them in vitro by fluorescence tomography, photoacoustic microscope and medical ultrasound equipment. The results show that the multimodal contrast agent can be used in fluorescence (FL), photoacoustic imaging (PA) and ultrasonic imaging (US) [23].

3. Application of ultrasonic molecular imaging technology in disease diagnosis and treatment

3.1 Atherosclerotic disease

Inflammation and thrombosis in atherosclerosis are related to the interaction between endothelial cells and platelets. At the site of vascular injury, the adhesion between endothelial cells and platelets is mediated by von Willebrand factor (vWF) through the vWF-A1 domain and platelet receptor glycoprotein Ib α (GPIB α)[24]. Some researchers have used this special role to prepare a targeted contrast agent that attaches the vWF-A1 domain to the microbubble shell. In vitro experiments, it was found that the imaging signal degree of targeted microbubbles within a certain range of shear stress was related to the progression of the disease, which proved that molecular imaging with this targeted contrast agent could not only detect activated platelets on vascular endothelium. It can also indicate the severity of atherosclerotic lesions [25]. Atherosclerotic plaques are composed of lipids (including oxidized low density lipoprotein and cholesterol crystals) and immune cells (including macrophages). Regulating cholesterol metabolism can prevent and treat atherosclerotic plaques [26]. Therefore, Sourabh et al. synthesized an ultrasound responsive cyclodextrin nanoparticles (CDNPs) for multimode imaging and treatment of atherosclerosis. The nanoparticles were prepared from n-butyl cyanoacrylate (BCA) and loaded with 2-hydroxypropyl-β-cyclodextrin (CD) and indocyanine green (ICG) / IR780 dyes.[27]. Animal experiments have confirmed that CDNPs can be used not only for in vivo multimodal ultrasound and optical imaging, but also for the treatment of atherosclerotic plate: promoting the regression of atherosclerosis and reducing plasma cholesterol and VLDL/LDL- cholesterol levels.

3.2 Tumor diseases

The clinical efficacy of commonly used antineoplastic drugs is not ideal, and some studies have shown that it may be related to the special tumor microenvironment, so combined therapy for tumor cells and tumor microenvironment has become the trend of tumor therapy [28] [29].Neovascularization is an important sign of tumorigenesis. Tumor vessels provide oxygen and nutrients for tumors. Tumor invasion and metastasis depend on the development of tumor vessels [30]. The researcher locates the corresponding targets on the tumor vessels to realize the diagnosis of tumor location [31] and tumor targeted therapy [32]. Kinase insertion domain receptor KDR is one of the key regulators of tumor neovascularization. Some researchers also used ultrasound microbubbles modified by kinase insertion domain receptor (KDR) as a clinical study. 40 patients included in the analysis confirmed that targeted ultrasound contrast agents could show strong targeted signals in breast tumor lesions, and the KDR expressed in tumor immunohistochemistry matched well with ultrasound image signals [33]. Many researchers explore the potential targets of tumor therapy by carrying tumor-related genes on ultrasonic microbubbles [34] [35]. However, ultrasound molecular contrast agents carrying drugs or genes achieve tumor treatment by activating or inhibiting specific targets on tumor cells. On the basis of ultrasonic molecular imaging, ultrasound-mediated microbubble destruction (UTMD) is used to break the microbubbles carrying drugs or genes, and targeted drug delivery to tumor cells can achieve accurate treatment of tumor cells [36] [37]. Using the strong absorbency of human hemoglobin at 532nm, Luo Shuilian et al prepared Herceptin targeting doxorubicin / Indian ink multi-functional nano-bubble ultrasound contrast agent. The results showed that the in vitro targeting rate (71.64 ± 9.32)%, the signal intensity of contrast-enhanced ultrasound (60.33 ± 4.51) dB, the imaging intensity of photoacoustic signal (0.8567 ± 0.0950) a.u. and the tumor inhibition rate (62.93 ± 6.96)% in the targeted group were significantly higher than those in the non targeted group and the control group [38]. Xu et al mixed ultrasound microbubbles with recombinant plasmids carrying shRNA-Livin gene and transfected them into human ovarian cancer cells (OVCA-433 cells). After UTMD, ultrasound-targeted microbubbles-mediated shRNA-Livin could significantly reduce the survival rate of OVCA-433 cells and promote tumor cell apoptosis [39]. Liu et al encapsulated two kinds of silent RNA [salt-induced kinase 2 (SIK2siRNA) and antisense microRNA21 (AntimiR21)] in nanoparticle contrast medium (FALPHNPs), and observed the accumulation of the nanocomplex in the transplanted tumor of ovarian cancer model mice under ultrasound monitoring. It was confirmed that the co-delivery of ultrasound microbubble-mediated SIK2siRNA and AntimiR21 into folate-lipid-PLGA hybrid polymer nanoparticles could significantly improve the sensitivity of ovarian cancer to paclitaxel (PTX) [40].

4. Summary

The development of molecular imaging technology promotes the iterative updating of ultrasound contrast agents. Targeted ultrasound contrast agents link molecular imaging, molecular biology, pharmacy, oncology and other disciplines together to promote the development of accurate diagnosis and treatment. However, at present, the research of ultrasonic molecular imaging technology is still in the animal experimental stage, many studies are limited to xenogeneic subcutaneous tumors, and the in situ or homologous model has not yet been realized, and the real clinical application still needs to be tested by long-term practice. It is believed that the great research value and broad application potential of ultrasonic molecular imaging technology can make accurate diagnosis and treatment possible.

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