Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.jzus.zju.edu.cn; www.springerlink.com
E-mail: jzus@zju.edu.cn

Review:

Advances in low-frequency ultrasound combined with microbubbles in targeted tumor therapy*

Li-ying WANG†[1], Shu-sen ZHENG[2]

[1]*Department of Ultrasound, Shaoxing Second Hospital, Shaoxing 312000, China*

[2]*Key Laboratory of Combined Multi-organ Transplantation, Ministry of Health, Division of Hepatobiliary and Pancreatic Surgery, Department of Surgery, the First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, China*

†E-mail: drwang88@qq.com

Received Oct. 9, 2018; Revision accepted Feb. 12, 2019; Crosschecked Mar. 1, 2019

Abstract: The development of low-frequency ultrasound imaging technology and the improvement of ultrasound contrast agent production technology mean that they play an increasingly important role in tumor therapy. The interaction between ultrasound and microbubbles and their biological effects can transfer and release microbubbles carrying genes and drugs to target tissues, mediate the apoptosis of tumor cells, and block the embolization of tumor microvasculature. With the optimization of ultrasound parameters, the development of targeted microbubbles, and the emergence of various composite probes with both diagnostic and therapeutic functions, low-frequency ultrasound combined with microbubble contrast agents will bring new hope for clinical tumor treatment.

Key words: Low-frequency ultrasound; Microbubble; Gene; Drug; Cell death
https://doi.org/10.1631/jzus.B1800508 **CLC number:** R73-3

1 Introduction

Recently, with the development of ultrasonography, molecular biology, and microbubble contrast agents, the biological effects of low-frequency ultrasound combined with microbubbles have attracted wide attention for clinical treatment. Therefore, the application of low-frequency ultrasound combined with microbubbles in tumor has become a hot topic.

The cavitation effect varies inversely with ultrasound frequency (Lavon and Kost, 2004). Ueda et al. (2009) found that the increase in the relative cavitation values was dependent on the ultrasound frequency and the cavitation bubbles are more easily generated at a low frequency. Low-frequency ultrasound can be related to indirect actions, such as mechanical impact, due to the collapse of cavitation bubbles, resulting in disruption of the structures and forced convection (Tang et al., 2001). Findings obtained by Tezel et al. (2002) emphasized the importance of low frequencies (20–100 kHz), because the amplitude of broadband noise induced by the low-frequency transient cavitation correlated well with enhanced skin conductivity. Some studies revealed that the thresholds of cavitation effect increased as the ultrasonic frequency increased, and the duration of the rarefaction phase decreased because the pressure oscillation period decreased (Pétrier and Francony, 1997; Grieser et al., 2015; Nguyen et al., 2017). Consequently, it becomes more difficult for cavitation bubbles to occur. Hodnett and Zeqiri (2008) estimated the cavitation threshold by numerical analysis and reported the cavitation threshold at 25 to 82 kHz. Cavitation thresholds increased as the frequency increased.

* Project supported by the Health and Family Planning Commission of Zhejiang Province (No. 2017KY676), China
ORCID: Li-ying WANG, https://orcid.org/0000-0001-9325-869X

Ultrasound contrast agents were originally designed to increase the effectiveness of ultrasound imaging for diagnosis. Gramiak and Shah (1968) first reported the enhancement of cloud-like echoes in the aorta when the oscillating saline was injected into the heart through a left ventricular catheter. Since then, researchers have begun the study of microbubble ultrasound contrast agents.

Ultrasound microbubble contrast agents were originally an air- or oxygen-containing non-enveloped bubble in the early stage. This is large and unstable and cannot be injected into peripheral veins. Since the 1990s, a new type of contrast agent encapsulating albumin, lipid, and polymer or surfactant shell has been developed (Schutt et al., 2003). It has good stability and small volume, and can last for about 5 min in the blood. Since then, with breakthroughs in elasticity and stability, a new type of microbubble containing fluorocarbon gas encapsulated in membranes has appeared (Wu and Nyborg, 2008). This lasts for more than 15 min in the blood. Most new microbubble contrast agents are 2–8 μm in diameter, similar to that of red blood cells (Lin et al., 2016; Mulvana et al., 2017). After rapid injection from peripheral veins, they can enter the systemic circulation smoothly through the pulmonary circulation. With the rapid development of molecular imaging technology, nanoscale ultrasound contrast agents, such as nanoliposome contrast agent and nano-fluorocarbon emulsion, have emerged in recent years, which can not only pass through the blood vessel of a tumor, but also have good echo characteristics (Upadhyay and Dalvi, 2019). The continuous improvement of ultrasound microbubbles is the basis of low-frequency ultrasound combined with microbubbles in tumor therapy.

There are mainly three mechanisms of the effect of low-frequency ultrasound in a tumor: mechanical, thermal, and cavitation. The ultrasonic cavitation effect was first proposed in Rayleigh's famous research paper on the pressure produced by the collapse of a spherical cavity in a liquid in 1917 (Feng, 2004). This effect refers to the oscillation, expansion, contraction, and collapse of the tiny vesicular nucleus (cavitation nucleus) in the liquid when the ultrasonic wave propagates in the liquid, resulting in a tremendous shear force to break down the cell wall and plasma membrane around the cavitation nucleus. The tiny bubbles in the liquid are called cavitation nuclei, and the lowest sound pressure or intensity threshold required for the liquid to produce cavitation effects is called the cavitation threshold. Different liquids have different cavitation thresholds.

Ultrasound as a safe and non-invasive treatment method has been widely used in tumor therapy. For example, high-intensity focused ultrasound (HIFU), which relies on the thermal effect of ultrasound to cause coagulative necrosis of tumors, has been used in the ablation of malignant solid tumors. However, the local high temperature produced by HIFU is not easy to control or measure, the penetration ability is limited, and the intensity is high. There are also insecurity risks in local ablation such as radio frequency ablation, laser ablation, and cryoablation. However, the absorption coefficient of low-frequency ultrasound is small, the tissue damage is small, the thermal effect is weak, and the thermal effect and temperature rise are negligible. At the same time, tumor cells are more sensitive to low-frequency ultrasound than normal cells. Thus, low-frequency ultrasound has received a lot of attention and is widely used in clinical treatment, for such as inhibiting bacterial growth, repairing wounds, and thrombolysis in vitro, and treating vascular diseases (Shi et al., 2018; Wu et al., 2018).

The physical properties of ultrasound microbubble contrast agent make it an ideal cavitation nucleus. It can not only significantly increase the number of cavitation nuclei enhancing the cavitation effect of low-frequency ultrasound, but also greatly reduce the cavitation threshold. Zhao et al. (2015) reported that low-frequency ultrasound combined with microbubbles could increase the apoptosis index, mitochondrial depolarization, and cytochrome *c* release of K562 chronic myeloid leukemia cells. Cavitation could significantly induce mitochondrial dysfunction and apoptosis, and mitochondrial permeability transition pore opening.

Recently, it has been found that low-frequency ultrasound combined with microbubbles has potential for tumor therapy. It can improve cell membrane permeability, mediate gene transfection, increase intracellular drug concentration, embolize tumor trophoblastic vessels, open the tissue barrier, and so on, playing a targeted therapeutic role. It is safe, targeted, and non-invasive.

2 Mediating gene-targeted therapy as a vector for improving the transfection efficiency

Low-frequency ultrasound makes microbubbles break instantly. Shock waves produced by the cavitation effect will increase the permeability of local capillaries and adjacent tissue cell membranes. The extracellular macromolecule can enter cells, and the gene transfection and expression of DNA can be significantly improved. It is the basis of gene-targeted therapy with microbubbles (Wang et al., 2016).

Low-frequency ultrasound combined with microbubble-mediated targeted gene therapy can not only enhance the transfection and expression of naked plasmid DNA in cancer cells, but also improve the targeting of gene therapy and reduce systemic side effects. Different from common liposomes of viral vectors and non-viral vectors, microbubbles of gene vectors will not cause immune response and mutation of viral vectors. It is a new safe and efficient gene transfection technology (Qian et al., 2018).

Wu et al. (2016) reported that low-frequency ultrasound combined with microbubbles could significantly improve the transfection efficiency of small interfering RNA (siRNA) in SMMC-7721 hepatocellular carcinoma cells. Zhao et al. (2018) reported that low-frequency ultrasound combined with microbubbles increased the transfection efficiency of siRNA (4 times) and porphyrin uptake (8 times) in MCF-7 (a human breast cancer cell line, estrogen receptor positive (ER^+)), and inhibited ER^+ breast cancer cells more effectively, showing a good synergistic effect. Delalande et al. (2017) reported that low-frequency ultrasound combined with microbubbles could effectively transfer plasmid DNA (pDNA), mRNA, or siRNA.

Microbubbles can be bound to genes by directly mixing microbubbles with naked genes or gene vectors, and by wrapping genes inside microbubbles or binding them with microbubble shells. After microbubbles are prepared as gene vectors, they can be injected into the body through peripheral blood vessels or directly into the target area under the guidance of ultrasound. The microbubbles are destroyed by ultrasound irradiation and the genes bound to the inside or on the surface are released.

3 Mediating targeted tumor drug therapy, improving the efficacy of chemotherapy and the patient's tolerance to chemotherapy

Chemotherapy is one of the most important methods in the clinical treatment of tumors. It is important to enhance the sensitivity of chemotherapy drugs, improve the curative effect, reduce the dosage, and reduce the side effects of drugs. Many studies have attempted to reduce the toxic and side effects on normal cells while enhancing the antitumor effect of chemotherapeutic drugs in order to improve efficacy.

It has been found that low-frequency ultrasound combined with drug-carrying microbubbles can increase the concentration of chemotherapeutic drugs in tumor cells, increase efficacy, resist drug resistance of cells, and have a chemotherapeutic sensitization effect (Moon et al., 2015a; Ren et al., 2016).

Microbubbles containing chemotherapeutic drugs are injected intravenously into the body and irradiated with low-frequency ultrasound to target tissues. The microbubbles rupture, resulting in local capillary rupture and a widened endothelial gap. Thus, the drug carried by microbubbles is released to the target areas through the endothelial cell barrier, thereby increasing the local drug concentration. At the same time, low-frequency ultrasound leads to a temporary gap in the cell membrane, which increases the permeability. Drug molecules can then enter the cell. It results in increased drug concentration in tumor cells, enhances the antitumor effect of chemotherapeutic drugs on tumor cells, and improves treatment efficiency (Zhang et al., 2018). On the other hand, it reduces the dosage of drugs and side effects (Moon et al., 2015b). Low-frequency ultrasound combined with microbubbles with low-dose drugs can achieve the antitumor effect of high-dose drugs alone on tumor cells, increase the targeted utilization rate and cytotoxicity of drugs, improve the efficacy of chemotherapy, but also improve the patient's tolerance to chemotherapy. Wang (2015) found that low-frequency ultrasound combined with microbubbles increased the permeability of DU-145 cells to different molecular weight substances such as Calein, FD4, and FD70, and increased chemotherapeutic efficacy for human prostate cancer DU-145 cells and PC-3 cells.

Ren et al. (2013) reported that docetaxel-loaded microbubbles could significantly enhance the anti-tumor effect of paclitaxel under low-frequency ultrasound irradiation. Xiao et al. (2018) reported that low-frequency ultrasound combined with microbubbles could enhance chemotherapeutic drugs into tumor stroma by reducing interstitial pressure and thus improve the curative effect of tumor therapy. Mariglia et al. (2018) reported that low-frequency ultrasound combined with microbubbles could enhance the cytotoxicity of the nucleoside analogue gemcitabine in pancreatic cancer, improve the effect of chemotherapy, and prolong the survival time of patients with pancreatic cancer. Zhao et al. (2018) reported that low-frequency ultrasound combined with multifunctional FOXA1 porphyrin-loaded microbubbles significantly increased the local accumulation of porphyrin and siRNA by ultrasound-induced acoustic perforation, and had a good therapeutic effect on estrogen-dependent ER^+ breast cancer. Li et al. (2017) reported that 5-FU polylactic acid nanobubbles irradiated by low-frequency ultrasound could further improve drug targeting and effectively inhibit the growth of hepatocellular carcinoma in nude mice.

4 Inducing autophagy and apoptosis of tumor cells, inhibiting proliferation, invasion, and metastasis, and playing an anti-tumor role

Cell proliferation disorder and death inhibition are the main causes of tumorigenesis. Low-frequency ultrasound combined with microbubble contrast agent can significantly inhibit the proliferation and cloning of tumor cells through mechanical and cavitation effects. Morphological changes include various death modes, namely tumor cell necrosis, autophagy, and apoptosis.

Autophagy and apoptosis both cause cell death. Autophagy is considered to be a different programmed cell death mode from apoptosis, known as type II programmed cell death. Excessive autophagy can kill tumor cells (Shimizu et al., 2014; Lin and Baehrecke, 2015; Rebecca and Amaravadi, 2016). Induction of autophagy in tumor cells is a novel potential method for tumor therapy. Autophagy has three stages: formation of a bilayer membrane structure, formation of the autophage, and formation of autophagic lysosome and degradation of content (Zarzynska, 2014).

Apoptosis is known as type I programmed cell death. It is controlled by genes. In recent years, inducing apoptosis of tumor cells has become a novel method of tumor therapy (Lin and Baehrecke, 2015). Low-frequency ultrasound combined with microbubbles can induce tumor cell apoptosis, and inhibit tumor cell proliferation, invasion and metastasis (Shen et al., 2014; Rebecca and Amaravadi, 2016; Ren et al., 2016). It may be related to the cavitation effect (Shen et al., 2014). The modes of apoptosis include the endogenous and exogenous. Exogenous apoptosis directly activates the downstream Caspase effector molecule by binding apoptosis-stimulating factors to cell surface-related receptors. Endogenous apoptosis is a mitochondrial-activated apoptosis process regulated by the Bcl-2 protein family. It is reported that low-frequency ultrasound combined with microbubbles can promote apoptosis of prostate cancer cells, liver cancer cells, ovarian cancer cells, glioma cells, and human leukemia cells (Lagneaux et al., 2002; Bai et al., 2016; Gangeh et al., 2016), and reduce damage to normal tissues.

Hou et al. (2017) reported that low-frequency ultrasound combined with microbubbles can affect the hypoxic response of a tumor, inhibit tumor growth, and promote cell apoptosis. Shen YY et al. (2017) reported that low-frequency ultrasound combined with microbubbles enhanced delivery of paclitaxel liposomes treating nude mice bearing intracranial glioblastoma xenografts. Immunohistochemistry proved that low-frequency ultrasound combined with microbubbles could induce apoptosis and inhibit proliferation. Zhang et al. (2017) reported that low-frequency ultrasound combined with microbubbles could significantly inhibit the expression of surface antigen markers CD11c and CD83 of the prostate cancer cell, and inhibit the proliferation of tumor cells. Sadeghi-Naini et al. (2016) reported that low-frequency ultrasound combined with microbubbles could induce the death of human fibrosarcoma xenografts cells in mice with severe combined immunodeficiency. Ji et al. (2016) reported that when low-frequency ultrasound combined with exogenous microRNAs-133A-carried microbubbles was used to treat MCF-7 and MDA-MB-231 cells, the expression of microRNAs

was significantly raised. When the expression of epidermal growth factor receptor and the phosphorylation of Akt were significantly inhibited, the proliferation of breast cancer cells was inhibited and the survival rate increased. Wu et al. (2016) reported that low-frequency ultrasound combined with microbubbles increased the apoptosis of SMMC-7721 human hepatocellular carcinoma cells, and decreased the ability of migration and invasion significantly. Yang et al. (2016) reported that the group of low-frequency ultrasound combined with microbubbles was more effective than other groups in inhibiting tumor growth, promoting cell apoptosis, inhibiting cell proliferation and angiogenesis.

5 Mediating tumor microvascular embolization and anti-angiogenesis

The occurrence, development, invasion, and metastasis of solid tumors depend on tumor angiogenesis. Tumor angiogenesis is defined as when the tumor is larger than 1 mm^3 or has more than 1×10^7 cells, the microvascular network through endothelial cells begins to form new blood vessels to provide adequate oxygen and other nutrients for tumor cell proliferation. Tumor neovascularization lacks an elastic fiber layer with weak wall, incomplete basement membrane, and high permeability. Therefore, anti-angiogenesis and blocking tumor blood supply are new ways for tumor therapy.

However, at present, anti-angiogenesis is almost chemical or biological with drugs attacking tumor neovascularization, with many side effects and unsatisfactory therapeutic effects. Intravenous microbubbles can be used to enhance the cavitation effect of low-frequency ultrasound, which can, in a targeted manner, destroy the neovascularization of a tumor. Microbubbles can reduce the threshold of the ultrasonic cavitation effect, enhance the cavitation effect, damage the vascular wall, activate endogenous or exogenous coagulation, induce large areas of capillary embolism, and block the nutritional supply of tumor cells, leading to tumor cell death (Yang et al., 2015b). Shock waves produced by the cavitation effect not only increase the permeability of the cell membrane, but also rupture the microvessel and widen the gap between endothelial cells. At the same time, they also mediate the destruction of vascular endothelial layers, expose the vascular subendothelial layer, cause thrombosis in blood vessels, and block the blood supply of malignant tumor tissue (Shen ZY et al., 2017).

Shen et al. (2013, 2018) reported that in low-frequency ultrasound combined with a microbubble group, rabbit VX2 cancer cells were found with interstitial hemorrhage and thrombosis, vascular endothelial cell wall rupture, endothelial cell gap expansion, interstitial erythrocyte leakage, microvascular thrombosis, carotid artery elastic membrane separation, local blood vessel wall defects, and hole formation, and the surface of the ruptured area was rough and irregular. Low-frequency ultrasound combined with microbubbles has anti-angiogenic effects.

6 Enhancing anti-tumor immunity

Low-frequency ultrasound combined with microbubbles markedly inhibited expression of the general marker CD11c and the mature marker CD83, down-regulated VEGF expression, induced the high expression of the anti-tumor immune response biomarkers CD4 and CD8a in mice spleen, activated immunity, and promoted the synergistic effect of anti-tumor immunity. Low-frequency ultrasound combined with microbubbles could not only directly suppress prostate cancer cell evolution, but also promote activation of anti-tumor immunocytes in the VEGF-inhibited microenvironment (Zhang et al., 2017).

7 Synergistically enhancing other tumor therapies

Low-frequency ultrasound combined with microbubbles enhanced microvessel destruction of radiofrequency ablation in prostate cancer xenografts in nude mice. It produced larger volumes of ablation compared with treatment with radiofrequency ablation alone, and increased the apoptosis index and reduced the proliferation index in residual carcinoma cells induced by radiofrequency ablation (Yang et al., 2015a).

8 Prospects

Low-frequency ultrasound combined with microbubbles for tumor therapy is still in the experimental stage. There are several problems to be solved urgently.

8.1 Parameter optimization

The cavitation effect of low-frequency ultrasound is affected by many factors, such as ultrasonic frequency, intensity, irradiation time, and method, etc. (Nguyen et al., 2017). The ultrasonic parameters reported in the literatures are not completely consistent. Therefore, it is very important to optimize the ultrasonic irradiation parameters (including the optimal irradiation time, frequency, intensity, irradiation mode, ultrasonic instrument type, etc.) and the types, dosages, concentrations and injection modes of microbubbles by orthogonal experimental design. It is necessary to study the application value of different diseases and optimize the parameters for various applications.

In addition, different effects of low-frequency ultrasound are related to irradiation modes. The larger dosage and longer duration of ultrasound easily cause cavitation (Shriki, 2014). The cavitation threshold increases with the decrease of dose. The cavitation threshold of pulse waves is higher than that of continuous waves. The shorter the duration of the pulse, the higher the cavitation threshold. Intermittent emission of pulsed wave energy may reduce the impact of continuous radiation energy deposition on the surroundings and reduce the complications caused by the thermal effect.

8.2 Targeting and stability of microbubbles

Enhancing the binding force between microbubbles and ligands and the binding strength between ligands and receptors are the basis of targeted microbubbles. Improving the efficiency of microbubbles carrying drugs and genes, enhancing the stability of microbubbles in circulation, increasing targeting, prolonging the time of microbubbles staying in the region of interest, and effectively controlling the release time are urgent problems to be solved. For example, in the microcapsule shell, tissue specific antibodies or ligands are added to modify the composition and characteristics of the shells.

8.3 Safety

Low-frequency ultrasound combined with microbubbles can damage adjacent cells and tissues as well as tumor cells. It can cause hemolysis, microvascular leakage, capillary rupture, and troponin-T elevation. Therefore, it is necessary to further improve the safety of low-frequency ultrasound combined with microbubbles.

8.4 Probe development

The development of a composite probe which can be used for both ultrasound imaging and ultrasound therapy will provide great convenience for low-frequency ultrasound combined with microbubbles to mediate tumor targeted therapy.

9 Conclusions

In summary, low-frequency ultrasound combined with microbubbles is a promising method for tumor therapy. It has the advantages of safety, high efficiency, good targeting, and controllability, and is one of the hot topics in the field of ultrasound study. With the optimization of ultrasound parameters, the development of targeted microbubbles and the emergence of various composite probes with both diagnostic and therapeutic functions, low-frequency ultrasound combined with microbubbles will be widely used in basic and clinical research, bringing new hope for clinical tumor therapy with important application value.

Contributors

Li-ying WANG designed the research, wrote and edited the manuscript. Shu-sen ZHENG provided expertise advice and revised the draft. All authors read and approved the final manuscript.

Compliance with ethics guidelines

Li-ying WANG and Shu-sen ZHENG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Bai WK, Zhang W, Hu B, et al., 2016. Liposome-mediated transfection of wild-type P53 DNA into human prostate cancer cells is improved by low-frequency ultrasound

combined with microbubbles. *Oncol Lett*, 11(6):3829-3834. https://doi.org/10.3892/ol.2016.4477

Delalande A, Bastié C, Pigeon L, et al., 2017. Cationic gas-filled microbubbles for ultrasound-based nucleic acids delivery. *Biosci Rep*, 37(6):BSR20160619. https://doi.org/10.1042/BSR20160619

Feng R, 2004. Ultrasound cavitation and ultrasound medicine. *Chin J Ultrasonogr*, 13(1):63-65 (in Chinese).

Gangeh MJ, Hashim A, Giles A, et al., 2016. Computer aided prognosis for cell death categorization and prediction *in vivo* using quantitative ultrasound and machine learning techniques. *Med Phys*, 43(12):6439-6454. https://doi.org/10.1118/1.4967265

Gramiak R, Shah PM, 1968. Echocardiography of the aortic root. *Invest Radiol*, 3(5):356-366.

Grieser F, Choi PK, Enomoto N, et al., 2015. Sonochemistry and the Acoustic Bubble. Elsevier, Amsterdam, The Netherlands, p.41-83. https://doi.org/10.1016/C2013-0-18886-1

Hodnett M, Zeqiri B, 2008. Toward a reference ultrasonic cavitation vessel: Part 2: investigating the spatial variation and acoustic pressure threshold of inertial cavitation in a 25 kHz ultrasound field. *IEEE Trans Ultrason Ferroelectr Freq Control*, 55(8):1809-1822. https://doi.org/10.1109/TUFFC.2008.864

Hou R, Xu YJ, Lu QJ, et al., 2017. Effect of low-frequency low-intensity ultrasound with microbubbles on prostate cancer hypoxia. *Tumour Biol*, 39(10):1010428317719275. https://doi.org/10.1177/1010428317719275

Ji YL, Han Z, Shao LM, et al., 2016. Evaluation of in vivo antitumor effects of low-frequency ultrasound-mediated miRNA-133a microbubble delivery in breast cancer. *Cancer Med*, 5(9):2534-2543. https://doi.org/10.1002/cam4.840

Lagneaux L, de Meulenaer EC, Delforge A, et al., 2002. Ultrasonic low-energy treatment: a novel approach to induce apoptosis in human leukemic cells. *Exp Hematol*, 30(11):1293-1301. https://doi.org/10.1016/S0301-472X(02)00920-7

Lavon I, Kost J, 2004. Ultrasound and transdermal drug delivery. *Drug Discov Today*, 9(15):670-676. https://doi.org/10.1016/S1359-6446(04)03170-8

Li QY, Li HY, He CJ, et al., 2017. The use of 5-fluorouracil-loaded nanobubbles combined with low-frequency ultrasound to treat hepatocellular carcinoma in nude mice. *Eur J Med Res*, 22:48. https://doi.org/10.1186/s40001-017-0291-8

Lin HY, Chen JF, Chen CP, 2016. A novel technology: microfluidic devices for microbubble ultrasound contrast agent generation. *Med Biol Eng Comput*, 54(9):1317-1330. https://doi.org/10.1007/s11517-016-1475-z

Lin L, Baehrecke EH, 2015. Autophagy, cell death, and cancer. *Mol Cell Oncol*, 2(3):e985913. https://doi.org/10.4161/23723556.2014.985913

Mariglia J, Momin S, Coe IR, et al., 2018. Analysis of the cytotoxic effects of combined ultrasound, microbubble and nucleoside analog combinations on pancreatic cells *in vitro*. *Ultrasonics*, 89:110-117. https://doi.org/10.1016/j.ultras.2018.05.002

Moon H, Kang J, Sim C, et al., 2015a. Multifunctional theranostic contrast agent for photoacoustics- and ultrasound-based tumor diagnosis and ultrasound-stimulated local tumor therapy. *J Control Release*, 218:63-71. https://doi.org/10.1016/j.jconrel.2015.09.060

Moon H, Yoon C, Lee TW, et al., 2015b. Therapeutic ultrasound contrast agents for the enhancement of tumor diagnosis and tumor therapy. *J Biomed Nanotechnol*, 11(7):1183-1192. https://doi.org/10.1166/jbn.2015.2056

Mulvana H, Browning RJ, Luan Y, et al., 2017. Characterization of contrast agent microbubbles for ultrasound imaging and therapy research. *IEEE Trans Ultrason Ferroelectr Freq Control*, 64(1):232-251. https://doi.org/10.1109/TUFFC.2016.2613991

Nguyen TT, Asakura Y, Koda S, et al., 2017. Dependence of cavitation, chemical effect, and mechanical effect thresholds on ultrasonic frequency. *Ultrason Sonochem*, 39:301-306. https://doi.org/10.1016/j.ultsonch.2017.04.037

Pétrier C, Francony A, 1997. Ultrasonic waste-water treatment: incidence of ultrasonic frequency on the rate of phenol and carbon tetrachloride degradation. *Ultrason Sonochem*, 4(4):295-300. https://doi.org/10.1016/S1350-4177(97)00036-9

Qian LJ, Thapa B, Hong J, et al., 2018. The present and future role of ultrasound targeted microbubble destruction in preclinical studies of cardiac gene therapy. *J Thorac Dis*, 10(2):1099-1111. https://doi.org/10.21037/jtd.2018.01.101

Rebecca VW, Amaravadi RK, 2016. Emerging strategies to effectively target autophagy in cancer. *Oncogene*, 35(1):1-11. https://doi.org/10.1038/onc.2015.99

Ren ST, Liao YR, Kang XN, et al., 2013. The antitumor effect of a new docetaxel-loaded microbubble combined with low-frequency ultrasound *in vitro*: preparation and parameter analysis. *Pharm Res*, 30(6):1574-1585. https://doi.org/10.1007/s11095-013-0996-5

Ren ST, Shen S, He XY, et al., 2016. The effect of docetaxel-loaded micro-bubbles combined with low-frequency ultrasound in H22 hepatocellular carcinoma-bearing mice. *Ultrasound Med Biol*, 42(2):549-560. https://doi.org/10.1016/j.ultrasmedbio.2015.10.008

Sadeghi-Naini A, Stanisz M, Tadayyon H, et al., 2016. Low-frequency ultrasound radiosensitization and therapy response monitoring of tumors: an in vivo study. Proc 38th Annual Int Conf of the IEEE Engineering in Medicine and Biology Society, p.3227-3230. https://doi.org/10.1109/EMBC.2016.7591416

Schutt EG, Klein DH, Mattrey RM, et al., 2003. Injectable microbubbles as contrast agents for diagnostic ultrasound

imaging: the key role of perfluorochemicals. *Angew Chem Int Ed Engl*, 42(28):3218-3235. https://doi.org/10.1002/anie.200200550

Shen YY, Pi ZK, Yan F, et al., 2017. Enhanced delivery of paclitaxel liposomes using focused ultrasound with microbubbles for treating nude mice bearing intracranial glioblastoma xenografts. *Int J Nanomed*, 12:5613-5629. https://doi.org/10.2147/IJN.S136401

Shen ZY, Shen E, Zhang JZ, et al., 2013. Effects of low-frequency ultrasound and microbubbles on angiogenesis-associated proteins in subcutaneous tumors of nude mice. *Oncol Rep*, 30(2):842-850. https://doi.org/10.3892/or.2013.2492

Shen ZY, Shen E, Diao XH, et al., 2014. Inhibitory effects of subcutaneous tumors in nude mice mediated by low-frequency ultrasound and microbubbles. *Oncol Lett*, 7(5): 1385-1390. https://doi.org/10.3892/ol.2014.1934

Shen ZY, Liu C, Wu MF, et al., 2017. Spiral computed tomography evaluation of rabbit VX2 hepatic tumors treated with 20 kHz ultrasound and microbubbles. *Oncol Lett*, 14(3):3124-3130. https://doi.org/10.3892/ol.2017.6557

Shen ZY, Jiang YM, Zhou YF, et al., 2018. High-speed photographic observation of the sonication of a rabbit carotid artery filled with microbubbles by 20-kHz low frequency ultrasound. *Ultrason Sonochem*, 40:980-987. https://doi.org/10.1016/j.ultsonch.2017.09.015

Shi MM, Chen L, Wang YX, et al., 2018. Effect of low-frequency pulsed ultrasound on drug delivery, antibacterial efficacy, and bone cement degradation in vancomycin-loaded calcium phosphate cement. *Med Sci Monit*, 24: 797-802. https://doi.org/10.12659/MSM.908776

Shimizu S, Yoshida T, Tsujioka M, et al., 2014. Autophagic cell death and cancer. *Int J Mol Sci*, 15(2):3145-3153. https://doi.org/10.3390/ijms15023145

Shriki J, 2014. Ultrasound physics. *Crit Care Clin*, 30(1):1-24. https://doi.org/10.1016/j.ccc.2013.08.004

Tang H, Mitragotri S, Blankschtein D, et al., 2001. Theoretical description of transdermal transport of hydrophilic permeants: application to low-frequency sonophoresis. *J Pharm Sci*, 90(5):545-568. https://doi.org/10.1002/1520-6017(200105)90:5<545::AID-JPS1012>3.0.CO;2-H

Tezel A, Sens A, Mitragotri S, 2002. Investigations of the role of cavitation in low-frequency sonophoresis using acoustic spectroscopy. *J Pharm Sci*, 91(2):444-453. https://doi.org/10.1002/jps.10024

Ueda H, Mutoh M, Seki T, et al., 2009. Acoustic cavitation as an enhancing mechanism of low-frequency sonophoresis for transdermal drug delivery. *Biol Pharm Bull*, 32(5): 916-920. https://doi.org/10.1248/bpb.32.916

Upadhyay A, Dalvi SV, 2019. Microbubble formulations: synthesis, stability, modeling and biomedical applications. *Ultrasound Med Biol*, 45(2):301-343. https://doi.org/10.1016/j.ultrasmedbio.2018.09.022

Wang P, Yin TH, Li JG, et al., 2016. Ultrasound-responsive microbubbles for sonography-guided siRNA delivery. *Nanomedicine*, 12(4):1139-1149. https://doi.org/10.1016/j.nano.2015.12.361

Wang Y, 2015. Researches in Vitro About Mechanism of Increased Chemotherapeutic Effect of Prostate Cancer Cells by Low-frequency and Low-power Ultrasound Combined with Microbubble. PhD Dissemination, Shanghai Jiao Tong University, Shanghai, China (in Chinese).

Wu BL, Qiao Q, Han X, et al., 2016. Targeted nanobubbles in low-frequency ultrasound-mediated gene transfection and growth inhibition of hepatocellular carcinoma cells. *Tumour Biol*, 37(9):12113-12121. https://doi.org/10.1007/s13277-016-5082-2

Wu JR, Nyborg WL, 2008. Ultrasound, cavitation bubbles and their interaction with cells. *Adv Drug Deliv Rev*, 60(10): 1103-1116. https://doi.org/10.1016/j.addr.2008.03.009

Wu YQ, Liu XB, Qin ZZ, et al., 2018. Low-frequency ultrasound enhances chemotherapy sensitivity and induces autophagy in PTX-resistant PC-3 cells via the endoplasmic reticulum stress-mediated PI3K/Akt/mTOR signaling pathway. *Oncol Targets Ther*, 11:5621-5630. https://doi.org/10.2147/OTT.S176744

Xiao NN, Liu JH, Liao LL, et al., 2018. Ultrasound combined with microbubbles increase the delivery of doxorubicin by reducing the interstitial fluid pressure. *Ultrasound Quart*, in press. https://doi.org/10.1097/RUQ.0000000000000381

Yang Y, Bai WK, Chen YN, et al., 2015a. Low-frequency and low-intensity ultrasound-mediated microvessel disruption enhance the effects of radiofrequency ablation on prostate cancer xenografts in nude mice. *Mol Med Rep*, 12(5): 7517-7525. https://doi.org/10.3892/mmr.2015.4375

Yang Y, Bai WK, Chen YN, et al., 2015b. Optimization of low-frequency low-intensity ultrasound-mediated microvessel disruption on prostate cancer xenografts in nude mice using an orthogonal experimental design. *Oncol Lett*, 10(5):2999-3007. https://doi.org/10.3892/ol.2015.3716

Yang Y, Bai WK, Chen YN, et al., 2016. Low-frequency ultrasound-mediated microvessel disruption combined with docetaxel to treat prostate carcinoma xenografts in nude mice: a novel type of chemoembolization. *Oncol Lett*, 12(2):1011-1018. https://doi.org/10.3892/ol.2016.4703

Zarzynska JM, 2014. The importance of autophagy regulation in breast cancer development and treatment. *Biomed Res Int*, 2014:710345. https://doi.org/10.1155/2014/710345

Zhang L, Yin TH, Li B, et al., 2018. Size-modulable nanoprobe for high-performance ultrasound imaging and drug

delivery against cancer. *ACS Nano*, 12(4):3449-3460. https://doi.org/10.1021/acsnano.8b00076

Zhang W, Shou WD, Xu YJ, et al., 2017. Low-frequency ultrasound-induced VEGF suppression and synergy with dendritic cell-mediated anti-tumor immunity in murine prostate cancer cells *in vitro*. *Sci Rep*, 7:5778. https://doi.org/10.1038/s41598-017-06242-8

Zhao L, Feng Y, Shi AW, et al., 2015. Apoptosis induced by microbubble-assisted acoustic cavitation in k562 cells: the predominant role of the cyclosporin A-dependent mitochondrial permeability transition pore. *Ultrasound Med Biol*, 41(10):2755-2764. https://doi.org/10.1016/j.ultrasmedbio.2015.05.021

Zhao RR, Liang XL, Zhao B, et al., 2018. Ultrasound assisted gene and photodynamic synergistic therapy with multi-functional FOXA1-siRNA loaded porphyrin microbubbles for enhancing therapeutic efficacy for breast cancer. *Biomaterials*, 173:58-70. https://doi.org/10.1016/j.biomaterials.2018.04.054

中文概要

题　目：肿瘤靶向治疗中低频超声联合微泡造影剂的研究新进展

概　要：随着超声影像新技术的不断发展和超声造影剂制备技术的不断改进，低频超声和超声造影剂在肿瘤治疗中的作用日趋重要。利用超声波与微泡造影剂的相互作用及所产生的生物学效应，可实现微泡携的基因、药物等向靶目标组织的转移释放，介导肿瘤细胞的凋亡及肿瘤微血管的栓塞阻断等，从而起到靶向治疗的作用。随着超声波参数的优化和靶向微泡造影剂的研制，以及各种兼具诊治功能的复合型应用探头的面世，低频超声联合微泡造影剂介导肿瘤靶向治疗将为临床肿瘤的治疗带来新的希望。

关键词：低频超声；微泡；基因；药物；细胞死亡