Review Article

Induction of the apoptosis of cancer cell by sonodynamic therapy: a review

Wen-Kun Bai[1,2], E Shen[1], Bing Hu[1]

[1]Department of Ultrasound In Medicine, Shanghai Jiaotong University Affiliated 6th People's Hospital, Shanghai Institute of Ultrasound in Medicine, Shanghai 200233, China; [2]Department of Ultrasound in Medicine, Shandong University Affiliated Qian Fo Shan Hospital, Jinan 250014, China
Corresponding to: Bing Hu. Department of Ultrasound In Medicine, Shanghai Jiaotong University Affiliated 6th People's Hospital, Shanghai Institute of Ultrasound in Medicine, Yishan Road 600, Shanghai 200233, China. Email: bing-hu021@hotmail.com.

Abstract: Ultrasound can be used not only in examination, but also in therapy, especially in the therapy of cancer. Sonodynamic therapy is an experimental cancer therapy method which uses ultrasound to enhance the cytotoxic effects of agents known as sonosensitizers. It has been tested *in vitro* and *in vivo*. The ultrasound could penetrate the tissue and cell under some of conditions which directly changes cell membrane permeability, thereby allowing the delivery of exogenous molecules into the cells in some degree. Ultrasound could inhibit the proliferation or induce the apoptosis of cancer cells *in vitro* or *in vivo*. Recent researches indicated low-frequency and low-intensity ultrasound could induce cell apoptosis, which could be strengthened by sonodynamic sensitivity, microbubbles, chemotherapeutic drugs and so on. Most kinds of ultrasound suppressed the proliferation of cancer cells through inducing the apoptosis of cancer cells. The mechanism of apoptosis is not clear. In this review, we will focus on and discuss the mechanisms of the induction of cancer cell apoptosis by ultrasound.

Key Words: Sonodynamic therapy; apoptosis; cancer; mechanism

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Introduction

Ultrasound is widely used for soft tissue imaging because of its perceived safety, noninvasiveness and low cost. It is also used in therapy, which has shown effect on the suppression of bacteria proliferation, the improvement of the therapeutic effect of the drug, and in thrombolysis *in vitro* and so on (1-3). This effect of ultrasound can be strengthened by microbubbles (4,5). Microbubble is a blood contrast medium, and it can not permeate outside blood vessels. For this reason, microbubbles can be used in the ultrasound examination to observe the blood stream information of organs, and large or small vessels. The diameter of the common microbubbles is 2-6 μm, which is similar to that of the red cell. After jet injection from the peripheral vein, and getting into the body, microbubbles can pass pulmonary circulation and go into circulation system, also can strengthen the imaging of the organ (6,7). Cell permeabilization using microbubbles and ultrasound has the potential of delivering molecules into the cytoplasm. The collapsing microbubbles and cavitation bubbles created by this collapse can generate impulsive pressures that cause transient membrane permeability, allowing exogenous molecules to enter the cells. Collapsed microbubbles or cavitation bubbles generated by collapsed microbubbles induce impulsive pressures such as liquid jets and shock waves, and these pressures affect the neighboring cells. The shock wave propagation distance from the center of a cavitation bubble that has the potential to damage the cell membrane is considerably larger than the maximum radius of the cavitation bubble (8). Several generations of the microbubble agents have also been developed. Early microbubbles contained an air core and were stabilized by a coating of albumin, starting with Albunex[R]. Agents

with a fluorinated gas core were then developed, including Optison™ with a protein shell and perfluoropropane gas core and Definity® with a phospholipid shell and perfluoropropane core. Microbubbles are typically manufactured by mechanical agitation although microfluidic methods to engineer precise size distributions are in development (9). Cancer cells are more susceptible than normal cells to sonodynamic therapy (SDT) (10,11), which serves as the experimental foundation for the application of SDT to the treatment of cancer. Recently, SDT has been widely used in the therapy of cancer and has shown the effect of mediating apoptosis in many experimental systems *in vitro* or *in vivo*, but the detailed mechanism of this process is unclear. Moreover, the effect of ultrasound-induced apoptosis could be enhanced by porphyrin, anticancer drugs and other chemical compounds. The synergistic effect between SDT and other chemical compounds is referred to as sonodynamic therapy. In this review, we will discuss the mechanism of the induction of cancer cell apoptosis by SDT.

Mechanism

Blood vessels of cancer were influenced by SDT

Angiogenesis, the process by which the existing vascular network expands to form new blood vessels, is required for the growth of solid tumors (12). Angiogenesis, the development of new blood vessels from the endothelium of a pre-existing vasculature, is a critical process required by most solid tumors to support their growth and metastasis. Therefore, anti-angiogenic therapy has been demonstrated to be an attractive strategy for cancer treatment. SDT could influence the vascular to induce cancer cell apoptosis *in vivo* (13). SDT combined with microbubbles also has effect on the blood vessels of cancer. Because the microbubbles are compressible, they alternately contract and expand in the acoustic field, a phenomenon referred to as cavitation. The low peak negative acoustic pressures are usually less than 0.2 MPa. As a result, microbubbles usually grow and shrink rhythmically and symmetrically around their equilibrium size, which is a phenomenon known as stable cavitation. However, under higher acoustic pressures, typically greater than 0.60 MPa, the expansion and contraction of microbubbles usually become unequal and markedly exaggerated, leading to vessel destruction. This activity is termed inertial cavitation, which induced the improvement of cell membrane permeability and angiorrhexis of small vessels (14). When microbubbles are irradiated by ultrasound, they may induce the destruction of vessels and vascular endothelium, causing thrombopoiesis in the vessels. It blocked the blood supply of the malignant tumor to induce cancer apoptosis (15). Other researches had found that SDT can facilitate anti-angiogenic gene delivery and inhibit prostate tumor growth *in vitro* and *in vivo* (16,17). Since glucose, oxygen, and other requirements are not evenly delivered through the tumor vasculature, the blood vessels develop and harbor hypoxic regions, the cells undergo oxidative stress and the vessels fail to mature, inducing the apoptosis of cancer cells (18).

SDT induced cancer cell apoptosis through the influence of genes correlating with apoptosis

Modulating the expression of key molecular components of the apoptotic processes comprising cell death is an attractive antineoplastic approach. In some experiments, it was found that SDT could influence the gene expression to induce apoptosis. In a study, human myelomonocytic lymphoma cell line U937 cells were exposed to the frequency of 1.0 MHz with 100 Hz pulse repetition frequency ultrasound. After that, cell viability, apoptosis and gene expression were analyzed. This study showed that SDT could induce apoptosis, and down-regulate 193 genes and up-regulate 201 genes. For down-regulated genes, the significant genetic network was associated with cellular growth and proliferation, gene expression, or cellular development. For up-regulated genes, the significant genetic network was associated with cellular movement, cell morphology, and cell death. The present results indicate that SDT affect the expression of many genes and will provide novel insight into the bio-molecular mechanisms of SDT in therapeutic application for cancer therapy (19).

SDT could also improve gene transfection to treat cancer and induce cancer cell apoptosis. Survivin, a member of the mammalian inhibitor of apoptosis protein (IAP) family, possesses multiple functions, including apoptosis inhibition, proliferation, tumorigenesis, and cell cycle promotion (20). In all *in vitro* and *in vivo* experiments, it was found that ultrasound with microbubbles could improve survivin gene transfection, and could induce more of the apoptosis than that of the control group (21,22). Silencing of survivin gene expression with short hairpin RNA (shRNA) could be facilitated by this non-viral technique, and lead to significant cell apoptosis. This novel method for RNA interference represents a powerful and promising non-viral technology that can be used in tumor gene therapy and research.

SDT can influence the genes relating to apoptosis. There are two main apoptotic pathways: the extrinsic (receptor-mediated) and the intrinsic (mitochondria-mediated). The intrinsic pathway of apoptosis may be triggered by both internal and external stimuli, including many mediators, which either promote or inhibit the process (23). The most representative regulators of the mitochondria-mediated pathway are P53, an inducer of apoptosis, and Bcl-2, a molecule with the opposite function (24-26). In a study, it was found that P53 and Bcl-2 were involved in ultrasound-induced apoptosis. Apoptosis and G_1 arrest were induced primarily in $P53^+$ cells, while $P53^-$ cells showed less apoptosis but exhibited G_2 arrest. Likewise, the proliferation of cancer cells was much more strongly inhibited in $P53^+$ than in $P53^-$ cells (27), and Bcl-2 was shown to respond to ultrasound irradiation (28).

SDT could influence signal pathway of cancer cells

Cell signal pathway plays an important role in the apoptosis of cancer cells. The mitochondria-caspase signaling pathway was activated in the SDT-induced apoptosis of cancer cells, and ultrasound promotes the expression of pro-apoptotic proteins such as Bax and caspase-3 in cancer cells (29). In another study, the significant reduction in sonodynamically induced apoptosis, nitroxide generation, and caspase-3 activation by histidine suggested that active species such as singlet oxygen are important in the sonodynamic induction of apoptosis (30). Apoptosis induction has been reported to occur through a partial mediation of a Ca^{2+} dependent pathway. Calcium also has effect in the SDT-induced cancer cell apoptosis. SDT can influence the cell ion pathway to induce apoptosis. Intracellular Ca^{2+} levels can vary depending on the bubble activity (31), which may be related to cell membrane damage and the amount of time required to repair this damage. Furthermore, some sonicated cells retain high levels of Ca^{2+} long after ultrasound exposure, which indicates a complete loss of cell membrane eintegrity (32). Ca^{2+} can improve the cell apoptosis level (33).

Drug chemotherapy could enhance SDT-induced cancer cell apoptosis

The drug chemotherapy is a very important method in the therapy of cancer (34). We constantly study how to enhance the sensitivity of the drug to reduce the therapeutic dose of the drug, and reduce the toxic and side effects of the chemotherapeutic drugs. Ultrasound increases the membrane permeability without causing complete cell destruction which provides the experimental foundation for the enhancement of the drug osmosis of SDT in the treatment of cancer (35). In an *in vitro* study, MCF-7 cells were treated with 5-FU and sonicated at the frequency of 3.0 MHz and intensity of 3.0 W/cm^2 for 1 min in the presence of Optison. Immediately after the treatment, cell death was mostly dependent on Optison, however, 24 h after treatment, cell death was more dependent on 5-FU. Ultrasound duty cycle increased cell death associated with either Optison or 5-FU. Furthermore, the study showed that the treatment with 5-FU and ultrasound increases the levels of Bax and $P27^{kip1}$ proteins (36). These studies not only showed that ultrasound can enhance the chemotherapeutic effect *in vitro* but also studied its mechanism.

The other *in vitro* and *in vivo* studies also showed that SDT could enhance the chemotherapeutic effect of drugs by inducing cancer cell apoptosis (37,38). These findings showed that ultrasound exposure was a promising technique for cancer chemotherapy.

SDT could reverse chemo-drug resistance of cancer cells

Experimental and clinical investigations demonstrated that the chemotherapy-induced toxic effect and the development of drug resistance are the main barriers to successful therapy (39,40). Investigators hope to overcome drug resistance, whilst maintaining or even improving the therapeutic effects. Ultrasound exposure could make drug-resistant cancer cells more sensitive to anticancer drugs, which is a noninvasive physical approach for the induction of chemo-drug resistance reversal in cancer cells. A study showed the reverse effect of ultrasound on multidrug resistance (MDR) in cancer cells. They investigated the mechanisms of therapeutic ultrasound as a physical approach to overcoming MDR in a multidrug-resistant human hepatocarcinoma cell line ($HepG_2$/ADM). Their results demonstrated that ultrasound could reverse the chemo-drug resistance. Using reverse transcription-polymerase chain reaction (RT-PCR) technique, they found that ultrasound could significantly down-regulate the expression of P-glycoprotein (P-gp) and multidrug resistance-associated protein (MRP) at the mRNA level in $HepG_2$/ADM cells (41). The study not only confirmed that the SDT could reverse the chemo-drug resistance of cancer cells but also found its mechanism. The study provided the experimental foundation for the clinical application of SDT combining with drugs to treat drug-

resistant cancers.

Microbubbles could enhance the apoptosis-inducing effect of SDT on cancer cells together with drugs

One recent approach targeting solid tumors is the application of microbubbles, which loaded with chemotherapeutic drugs. The advanced drug carriers could be safely administered to the patients by intravenous infusion, and would circulate through the entire vasculature. The drug load could be locally released by ultrasound-targeted microbubble destruction. In addition, tumors could be precisely localized by diagnostic ultrasound since microbubbles act as contrast agents (42). Microbubbles combined with ultrasound could release drugs in specific positions to save the drugs and reduce the toxic and side effects (43).

Summary

SDT can induce the apoptosis of cancer cells, and permeabilize the cell membrane directly, thereby allowing the delivery of exogenous molecules into the cells (44). It could also make bio-effects through physical methods, which gives us a new method to treat cancers.

Future direction

SDT has been widely used in cancer therapy, and has got curative effect. Microbubbles combined with ultrasound have showed superiority in cancer therapy. Especially, microbubbles together with drugs or genes can even cure cancers. However, the mechanisms of that are not yet clear, and in the future, it still needs to be further studied. The best concentration of microbubbles and the frequency of the ultrasound in the treatment also need to be explored. Therefore, better microbubbles that can cure cancer together with drugs or genes are required. Furthermore, they must be stable and exhibit high performance in the delivery of the drugs or genes. The mechanisms of SDT-induced apoptosis in cancer cells *in vitro* also need to be studied, whether it occurs *in vivo* or not. We think that in the future SDT will be used more effectively in cancer treatment.

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