Review Article

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

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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.

