

Recent advances and pathological mechanisms in photodynamic and sonodynamic therapy in the treatment of bone tumors (Review)

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Abstract. Given the recent advances that have been made with photodynamic therapy (PDT) combined with sonodynamic therapy (SDT) (PDT/SDT; also known as SPDT), the application of this combination therapy in the clinic has provided another major breakthrough in the medical field, especially with regard to the treatment of deep tumors. Concerning its application in the treatment of bone tumors, numerous pathological mechanisms have been taken advantage of to overcome the barrier of tissue hypoxia, and SPDT is expected to achieve radical effects, with high penetration depth and low aggressiveness. In the present review, it is comprehensively shown how, according to the histoanatomy of bone tumors, PDT and SDT target cells in a coordinated manner, affecting such processes as necrotizing apoptosis, pyroptosis, autophagy and ferroptosis on the macroscopic level, and crucially, thrombosis at the vascular level, which leads to the triggering of immunogenic cell death in local and distant locations. Additionally, PDT and SDT have been shown to have roles in: i) degrading the extracellular matrix; ii) influencing the receptor activator of nuclear factor- κ B (RANK)/RANK ligand signaling pathway; iii) disrupting the equilibrium between glutathione peroxidase 4 and reactive oxygen species (ROS); and iv) destroying the microscopic structure of the bone tumor. Upon PDT/SDT

stimulation, several mechanisms act in concert to ensure that the targeted bone tumor is eliminated. Furthermore, widely distributed ROS have been revealed to promote osteoclast formation and osteogenic mineralization through the regulation of macrophages, processes that greatly improve the effects of postoperative repair. Finally, the developmental prospects of bone tumor engineering in the future are discussed in the present review.

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1. Introduction

Bone tumors either occur in the bone or originate from various tissue components of bone, irrespective of whether they are primary malignant or metastatic bone tumors, thereby seriously affecting the quality of life of patients. Traditional surgical methods are either incapable of reconstructing the function of tumor-bearing limbs or can only achieve this with great difficulty; moreover, these traditional techniques are associated with numerous complications, all having the effect of bringing great pain to patients (1,2). The method of photodynamic therapy (PDT) mainly comprises irradiation with near-infrared light (NIR) to stimulate the photosensitizer (PS) enriched in the tumor site, which has the effect of shifting energy to the circumambient ground-state oxygen molecules, thereby producing reactive oxygen species (ROS) such as $\text{OH}^\cdot$, $^1\text{O}_2$, $\text{O}_2^\cdot$, etc.; these are then responsible for producing cytotoxic singlet oxygen ($^1\text{O}_2$) and oxidizing biological macromolecules such as lipids, proteins and DNA in the bone tumor, causing the death of the tumor cells (3-5). However, one disadvantage

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of PDT is that the radiation depth of the common excitation light sources is <1 cm, and within the physiological safety limits the concentration of H_2O_2 is low, which leads to the poor toxicity of ROS, therefore, this technique is not ideal for use in deep bone tumors. Considering the particularity of the location of the bone tumor and the complexity of the tumor microenvironment (TME), the degree of response to tumor therapies continues to present a huge challenge. Due to the high penetrative ability of ultrasound (US), sonodynamic therapy (SDT), however, is able to make up for the deficiencies of PDT. The toxicity mechanisms that are instigated through the application of SDT to kill cells mainly include US cavitation and heating, ROS oxidative stress mechanisms, mechanical stress damage, apoptosis, and the comprehensive interactions that occur among the different mechanisms (6,7). Among the mechanisms, US cavitation refers to the high shear stress and strong shock waves caused by US, which enhance the physical damage to the cell membrane (CM) of the bone tumor, eventually leading to mechanical injury and necrosis of tumor cells. SDT, as a novel non-invasive method of bone tumor therapy, was initially proposed by Yumita *et al* (8) in 1989. US is applied to penetrate biological tissues; focused US can specifically be applied as a non-invasive technique to focus sound energy on deep tissues, which can lead to the activation of certain sound-sensitive drugs (such as hemoporphyrins), thereby producing antitumor effects. US mainly attacks the subcellular organelles of tumor cells, including mitochondria, rough endoplasmic reticulum and smooth endoplasmic reticulum, to disrupt the cellular metabolic processes. The underlying mechanism of SDT in tumor microvessels comprises destruction of the endothelial cells of tumor-nourishing vessels, thereby releasing thromboxane, and the resultant formation of thrombus causes a disruption of the local microcirculation in tumor tissue, which further leads to ischemic necrosis of the diseased tissue. SDT also elicits a substantial effect on body immunity. After the aforementioned process has occurred, in terms of tumor cell necrosis, the body is capable of producing a large number of antigens that stimulate antitumor immunity (9-11).

In addition, the technique of SDT is associated with a number of additional advantages. First, the method is non-invasive. Making use of the good penetrative ability of US, it is best to use the therapeutic head of the corresponding body surface position as the focus. Secondly, the method is targeted. The acoustic sensitizer has the 'targeting' characteristic of high concentration aggregation in tumor cells and tumor neovascularization endothelial cells, which results in the serious damage that is caused being restricted to the lesion area. Thirdly, the method has a high degree of accuracy. Sound sensitizers can be selectively concentrated in tumor cells, and accurately located with the assistance of imaging prior to treatment. Fourthly, the technique is safe. With the exception of preventable photosensitive reactions, sound sensitizers exert no effects on hematopoiesis, immunity or organ function. The fifth advantage is efficiency. SDT uses a sonochemical reaction to stimulate the immune function of the body when destroying the diseased tissue, which greatly improves the effective rate of treatment and reduces the recurrence rate. Sixthly, the method is repeatable. Patients will not develop drug resistance, and therefore they may be treated

repeatedly. Finally, and essentially, the technique has the advantage of synergistic therapy. For patients who experience tumor sequelae following surgical resection, SDT is capable of eliminating the remaining tumor cells more thoroughly, thereby reducing tumor recurrence, and in this manner, radiotherapy and chemotherapy are combined (12,13). It has been demonstrated that the sensitizer has both photodynamic and sonodynamic anticancer effects. SPDT circumvents the typical disadvantages of conventional methods (i.e., surgery, chemotherapy and radiotherapy), among which are drug resistance, limitations of the anatomical site, huge trauma and numerous side effects. However, two disadvantages of SPDT are that the majority of the sensitizers are toxic to the skin and that they readily accumulate in the solution, which results in the disappearance of 1O_2 and poor tumor-targeting specificity. Therefore, there is a demand for the generation of improved sensitizers that lack these defects (14-16). Compared with PDT limited penetration depth of light source and SDT palliative treatment strategy, SPDT as a new type of highly selective and non-invasive treatment, it utilizes smaller dose of PS and exert stronger cytotoxicity through releasing more ROS to destroy the cell membrane, DNA and protein by NIR or US radiation. SPDT not only has a strong ability to actively target tumors, but also greatly enhances the accumulation of drugs and regulates the release of sensitizers (14,17). For a single PDT or SDT, it is a great challenge to deliver sensitizers and targeted chemotherapeutic drugs to the same tumor cell, therefore, single PDT or SDT exhibit numerous shortcomings, such as sensitizer accumulation, enrichment selection and endogenous hypoxia (18,19). The rapid rise of nanotechnology, provides an emerging approach to solve the problem of drug resistance, as nanotechnology can improve the sensitivity of tumors by inhibiting drug resistance-related proteins. In addition, the drug delivery system based on nanotechnology breaks through the biological barrier and provides spatial and temporal control of drug release (20-22). Nanotechnology artificially activates and promotes drug internalization. US exposure also triggers the release of PSs and the synergistic effect of the two strongly affects the vascular system of the tumor.

2. Pathological mechanism of PDT/SDT combination therapy (or SPDT) in bone tumors

The implementation of SPDT is generally based on metabolic and inherent characteristics of bone tumors as the targets. The cellular process of ferroptosis, the extracellular matrix (ECM), the receptor activator of nuclear factor- κ B (RANK)/RANK ligand (RANKL) signaling pathway and combined autophagy inhibitors are all features that may be targeted via the technique (Fig. 1). For example, to illustrate this concept in greater detail, SPDT leads to the production of ROS, which accumulate in the mitochondria; this not only induces activation of the iron death pathway marked by glutathione (GSH) and promotes the production of a high concentration of the lipid peroxidation product 4-hydroxynonenal (4-HNE) on the surface of tumor cells, but it also obliterates RANKL/RANK-mediated bone resorption in the bone tumor, which effectively inhibits the occurrence and metastasis of bone tumors (23-25). Furthermore, via US cavitation-excited mechanical stress, the tumor may be effectively attacked, and

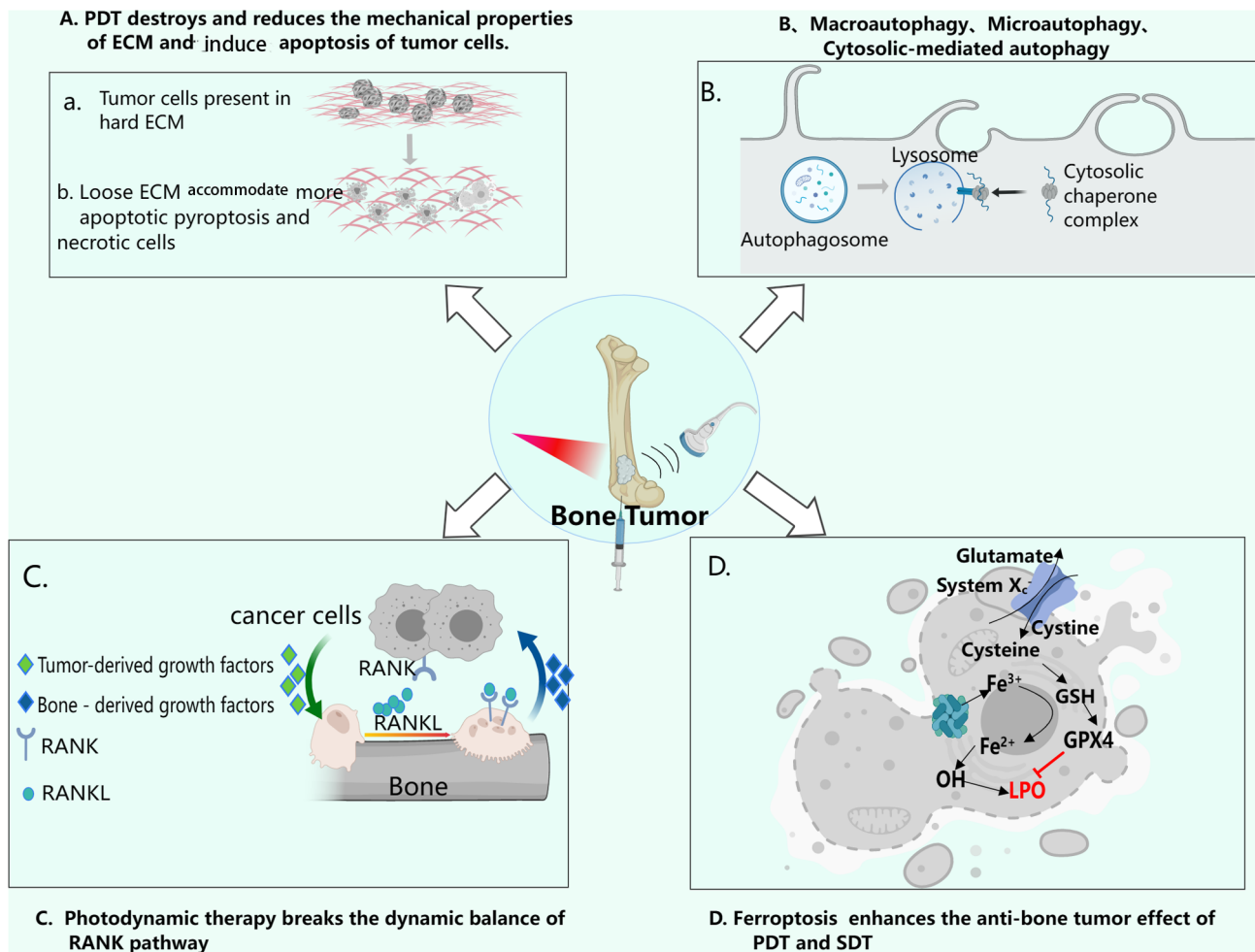


Figure 1. Multimode therapy based on PDT/SDT is cytotoxic to bone tumor cells. With the exception of the classical pathway used to induce apoptosis and necrosis of cancer cells, (A) releasing the hardness of the ECM, among them, (A-a) PDT/SDT destroys hard ECM, down-regulates the mechanical force of tumor cells, (A-b) increases the sensitivity of treatment and inhibits tumor metastasis. (B) Combining autophagy inhibitor therapy, (C) inhibiting the RANK/RANKL pathway and (D) focusing on ferroptosis, all provide new directions for potential development strategies. PDT/SDT, photodynamic therapy combined with sonodynamic therapy; RANK, receptor activator of nuclear factor- κ B; RANKL, RANK ligand; ECM, extracellular matrix.

its metastasis obstructed through either breaking the hard structure of the ECM or pushing targeted nanomaterials into the soft ECM (26,27).

Targeting iron death therapy affords a very promising avenue for treatment, although it still has certain shortcomings. The physiological process of ECM degradation is also accompanied by the deposition of different tumor-specific types of ECM, resulting in an increase in the density and hardness of the ECM. The chemical composition and mechanical function of the ECM changes with the degree of injury, and the induced cascade reactions subsequently result in alterations to a large number of immune processes (28,29). In addition, implementing a targeted therapeutic strategy for RANK/RANKL may also lead to the occurrence of immune side effects, due to its indiscriminate inhibition of soluble RANKL and membrane-bound RANKL (30). In the next section, osteosarcoma (OS) is investigated, as it has the highest incidence and is the most widely studied in the field of bone tumors.

PDT/SDT are aimed at ECM degradation in bone tumors. The remarkable feature of OS is the upregulation of osteoclast activity, which leads to an increase in bone resorption.

Phenotype is determined by the interaction between genes and the environment. Cells and the surrounding ECM transmit and interact with each other through physical or biochemical signals. Especially in bone tumors, the physical forces mainly involved are rigidity, matrix orientation and cell distance (31-35). Cells will preferentially migrate to areas with a hard matrix. When there is a physical force acting on the cell, this traverses the membrane and is converted into biochemical signals after its perception, affecting the function of molecular signaling pathways (Fig. 1A). The destruction of the ECM by nano-purine-based PDT/SDT, however, reduces the mechanical force on tumor cells, which causes a marked increase in the sensitivity of the treatment and the suppression of metastasis. The sound-sensitive nanosonosensitizer FePO₂@HC provides rich oxygen content for the degradation of deep ECM, expanding its permeability, whereas PSs reduce the number of cancer-associated fibroblasts, cells that shape the normal flow of tumor blood vessels (35,36). Yang *et al* (37) discovered that circular nanostructure PA-Apt-CHO-polyethylene glycol, which achieves an efficient imaging and PDT effect *in vivo*, is able to effectively overcome the obstacle of the ECM biological microenvironment in the cells, target the bone tumor cells, and

achieve good clinical transformation. The pre-existing ECM is repaired via its replacement with ECM possessing bone-like properties ('bony ECM') via the activation of osteoblasts, which go straight to the soft tissue and traverse the bone cortex and endosteum (38). However, highly malignant OS is associated with bone resorption and 'volcanic vent-like' bone boundaries, which, mainly through the dissolution of the pore or central hollow area, expand and raise the periosteum, thereby destroying the bone boundary at the same time. The underlying genetic basis of bone tumors lies in the mutation of the proto-oncogene Ras and tumor suppressor genes pRB and p53, which leads to the abnormal functioning of several signaling pathways, especially the RANK/RANKL, Hippo and Wnt pathways (39-42). Glycogen synthase kinase-3 β and AKT act upstream of Wnt/ β -catenin, and phosphorylation of glycogen synthase promotes downstream activation of the pathway, targeting the corresponding genes and biological effects.

Sound sensitizers/PSs associated with the autophagy depressor. When tumor cells are confronted with different stress stimuli, the process of autophagy maintains the tumor cells in their active and living condition through recovering damaged organelles and disordered folding proteins. Autophagy therefore offers a stabilizing, regulatory mechanism in cells. Through the process of autophagy, certain damaged organelles and dysfunctional proteins, or other components, are degraded in lysosomes, thereby participating in the cyclic remodeling of intracellular structures and functional macromolecules. In general, PDT/SDT is blocked by inefficient production of ROS and protective autophagy activation (43,44). Therefore, the cascade nanoreactor based on a sensitizer combined with an autophagy inhibitor is widely used to enhance the antitumor efficacy. Among them, the sensitizer can load a variety of nano-enzymes to catalyze the formation of ROS (Fig. 1B). The process of autophagy can be divided into three types. First, macroautophagy, which is the most common type of autophagy, is the type of autophagy through which spontaneous lipid bilayer autophagosomes enfold small molecules or damaged organelles, which are then transported to the lysosomes for degradation (45). Chen *et al* reported results suggesting that the PS pyropheophorbide- α methyl ester (MPPa) is able to exert similar effects (46). Secondly, microautophagy refers to the process through which the lysosome itself is actively phagocytized via the invagination of the membrane without the formation of autophagosomes; in this manner, the substrate is enfolded and then degraded (18). A combination of the hydrophobic PS zinc phthalocyanine/bovine serum albumin with autophagy inhibitors has been revealed to enhance the extent of PDT/SDT via restricting the expression of programmed death-ligand 1 (PD-L1) in bone tumors (47). Thirdly, there is molecular chaperone-mediated autophagy, a process through which the target protein first binds to the molecular chaperone to form a complex, and the receptor on the lysosome membrane subsequently specifically recognizes the complex, which is then degraded. This process occurs commonly in mammalian cells (48). Since autophagy induced by PDT/SDT greatly weakens the killing effect, the synergistic targeting of autophagy inhibitors presents a novel strategy worthy of further exploration. Nanoparticles (NPs) themselves can also be used as compounds that influence autophagy, which have the

effect of regulating intracellular oxidative stress or changing the expression levels of autophagy-associated genes through the dual regulation of lysosome internal physical and chemical properties and surface functionalization. L(D)-PAV-gold NPs mainly produce ROS and activate the lysosome, leading to autophagy and death. SDT is dependent on US and sound sensitizers present in the tumor tissue, and at a specific frequency and intensity, after radiation of the deep tissue of the tumor site for a certain period of time, the processes of apoptosis and necrosis are induced through inflexible stress and biochemical reactions, leading to the successful destruction of the bone tumor, depending on the penetration depth and the targeting characteristics (49).

PDT/SDT activates the RANK/RANKL signaling pathway to disperse the bone tumor. RANK and RANKL exist in osteoblasts, which serve to promote bone resorption via activating osteoclast signaling. According to a number of previously published studies (50,51), the gene expression levels of RANKL and osteoprotegerin (OPG) are markedly increased following PDT treatment of periodontitis (52). Although there are numerous types of osteolytic bone tumors, if the RANKL/RANK pathway is blocked in the early stage and the bone resorption that is caused by the tumor is inhibited, the growth of bone tumors can be effectively prevented (Fig. 1C). Lai *et al* (51) demonstrated that the efficacy of PDT against malignant cancer is closely associated with the expression levels of RANKL/Yes-associated protein (YAP). Franco *et al* (53) further showed that there is a reduction in the process of osteoclastogenesis. Although research in this area is only in its infancy, the potential of exploiting this system for therapeutic interventions is promising. Romagnoli *et al* (54) revealed that the level of OPG influences the redox coupling of GSH/glutathione disulfide (GSSG), ultimately affecting the formation of osteoclasts, whereas a negative correlation existed in the ratio of OPG to GSH. In particular, a physiological decrease in the GSH/GSSG ratio was identified to promote the formation of osteoclasts during the final stage of differentiation.

The Hippo/YAP and transcriptional coactivator with PDZ-binding motif (TAZ) signaling pathways have been demonstrated to regulate organ size and to maintain the blockade on the processes of homeostasis and regeneration (55,56). If the expression is maladjusted and these signaling pathways are disrupted, the cancer cells are able to repair themselves and proliferate widely. Verteporfin-PDT binds to YAP/TEAD protein, which leads to the subsequent inhibition of the Hippo pathway that drives the progression of sarcoma; very impressive results have been achieved in inhibiting cancer employing this dual action mechanism (57). After entering the nucleus, TEAD transcription factor binds to YAP and TAZ, which promotes the binding of bromodomain-containing protein 4 (BRD4) and RNA polymerase II to histones 3 and 4, thereby activating their biological function. It is worth mentioning that the binding of BRD4 with acetylated RELA (also known as nuclear factor NF- κ B p65 subunit) can also accelerate the osteolytic progression of the RANK/RANKL pathway. Zhan *et al* (58) showed that mevalonate is able to activate the RhoA/YAP or RhoA/ROCK2/LIMK2/YAP signaling pathways, which, in turn, promote the resistance of

bone tumors to PDT therapy. As a PS of PDT, Vitipofen not only inhibits the upregulation of the Hippo signaling pathway, but it also binds to YAP/TEAD protein in the nucleus, which has the effect of fully inhibiting bone tumor formation (59,60).

PDT/SDT cooperates with ferroptosis to reinforce bone tumor treatment. In the different stages of cancer progression, GSH has been revealed to exert a dual effect. During the initial stage of bone tumorigenesis, intracellular GSH degrades carcinogens and protects DNA from oxidative damage, which prevents the cells from becoming cancerous at the outset. Upon aggravation of the malignancy of the bone tumor, however, cancer cells produce vast amounts of ROS to meet the needs of tumor metabolism, and concomitantly, the expression of GSH is markedly upregulated to counteract the imbalance of intracellular homeostasis; therefore, a large intracellular concentration of GSH serves to protect against the oxidative damage of cancer cells (61). Consequently, targeting GSH is an attractive developmental strategy for the purposes of enhancing the degree of oxidation, and to improve the therapeutic effects (Fig. 1D). From the perspective of GSH synthesis, approaches to accomplish this aim include suppressing the antiporter system Xc⁻, and inhibiting γ -glutamyl transpeptidase, γ -glutamylcysteine ligase or glutathione synthetase. From the perspective of GSH consumption, it may be necessary to stimulate GSH oxidation and to promote GSH outflow. Among these strategies, they are most commonly applied in the field of bone tumor therapy using the techniques of PDT and SDT.

Based on the balance between GSH and ROS, identification of the breakthrough point of PDT/SDT in the elimination of bone tumor. Ferroptosis is a regulatory form of necrosis that is mediated by iron catalysis and an excessive oxidation of polyunsaturated fatty acids. In PDT, Fe²⁺ produces an excessive amount of hydroxyl-free radicals, which promotes the production of lipid peroxides. In addition, the level of glutathione peroxidase 4 (GPX4), which regulates the level of lipid peroxides, is significantly down-regulated. Upon exposure to hypoxia in the TME over a long period of time, GSH, as a ROS scavenging system, becomes significantly upregulated (62-64). With the application of SDT technology and the increased level of oxidative stress, the regulation of the GSH antioxidative system becomes weakened, which thereby disrupts the redox system; at the same time, GSH is oxidized to GSSG, which is its final oxidative form (Fig. 2) (65,66). Moreover, Mn-MOF-guided SDT exerts antitumor immune effects through increasing the activity of CD8⁺ T cells and mature dendritic cells (DCs) to promote an environment of immunosuppression. In breast cancer, with treatment with post-transplant cyclophosphamide upon US or NIR stimulation, SDT/PDT led to a marked downregulation of GSH and GPX4, resulting in cancer ferroptosis (67-70). Moreover, with GSH as the target, the administration of polydopamine-methylene blue (PDA-MB) eliminated the endogenous ROS scavenging system, thereby enhancing the effect of phototherapy and effectively inhibiting tumor growth both *in vivo* and *in vitro* (71). There are four ferroptosis markers triggered by ROS in bone tumors: GPX4, dihydroorotate dehydrogenase (DHODH), ferroptosis suppressor-protein 1 (FSP1) and GTP cyclohydrolase 1 (GCH1).

GPX4 maintains its inherent characteristics through the stabilization of GSH and the activation of cystine transport system Xc⁻, which contains two subunits: SLC7A11 and SLC3A2. Furthermore, additional regulatory systems have been identified to exist in the cytoplasm of tumors, such as the FSP1/CoQ10 and GCH1/BH4 systems (72). This indicates that the GSH-GPX4 system fulfills an important role in PDT-induced cell death. Its downstream compound is 4-HNE, i.e., one of the products of lipid peroxidation, and a positive correlation was identified to exist between the iron level and the 4-HNE content. Accumulated 4-HNE is a typical lipid peroxidation product that mainly attacks proteins, DNA and membrane lipids, also promoting oxidative stress damage in the hippocampus (62,73). The antioxidant system compound DHODH mediates a protective effect on ferroptosis via converting ubiquinone into ubiquinol, and acyl-CoA synthetase long-chain family member 4 (ACSL4), adenosine monophosphate-activated protein kinase (AMPK)-ACC2 and NF2-YAP are further signaling pathways that act through controlling PUFA metabolism and the proportion of phospholipid components in cells to resist the oxidative damage of CMs, thereby inhibiting the occurrence of iron death (74-76).

There are two main methods that are employed to kill bone tumors. One is to increase the number of ROS via inducing DNA damage and protein oxidation disorder; the other is to increase the consumption of GSH by amplifying the oxidative pressure and improving the cancer treatment level (61). In bone tumors, PDT/SDT produces the requisite ROS, which combine with the PINK1/Parkin signaling pathway in mitochondria to activate autophagy synergistically (77). In parallel, ROS activate the PI3K/AKT/mTOR signaling pathway in generating endoplasmic reticulum (ER) stress (Fig. 3). As dysfunctional mitochondria are engulfed via the process of autophagy, tumor cells are thereby assisted to adapt to external stimuli, including oxidative stress damage. Therefore, the implementation of a combined therapy with autophagy inhibitors can significantly improve the efficiency of killing bone tumors, not only through the enhancement of oxidative damage, but also through the promotion of apoptosis (48,78). Among them, under specific US conditions, cavitation can be divided into several stages, i.e., nucleation, growth, internal bursting and collapse. According to its native characteristics, cavitation can be roughly divided into two types: Stable cavitation and inertial cavitation (79). When the microbubble has stable cavitation, its collapse produces the effect of the massaging the cell, which destroys the integrity of the plasma membrane through the Vera operation. Experiencing more intense inertial cavitation, microbubbles can cause membrane perforation and cytoskeletal rupture. Strong US waves and high stress finally cause the cell to undergo necrosis. US piercing produces an extreme temperature, and releases pressure, which will lead to a variety of biological effects, including increasing the level of ROS in the cytoplasm, enhancing CM permeability, and eliciting CM potential depolarization and hyperpolarization. Hydroxyapatite NPs are employed in bone tissue regeneration by taking advantage of the implosive power of cavitation bubbles, and this is effective as the energy of the implosion pushes NPs onto the surface of damaged bone tissue (80). Following PDT/SDT treatment, histopathological investigations have provided clear evidence of bubble clouds

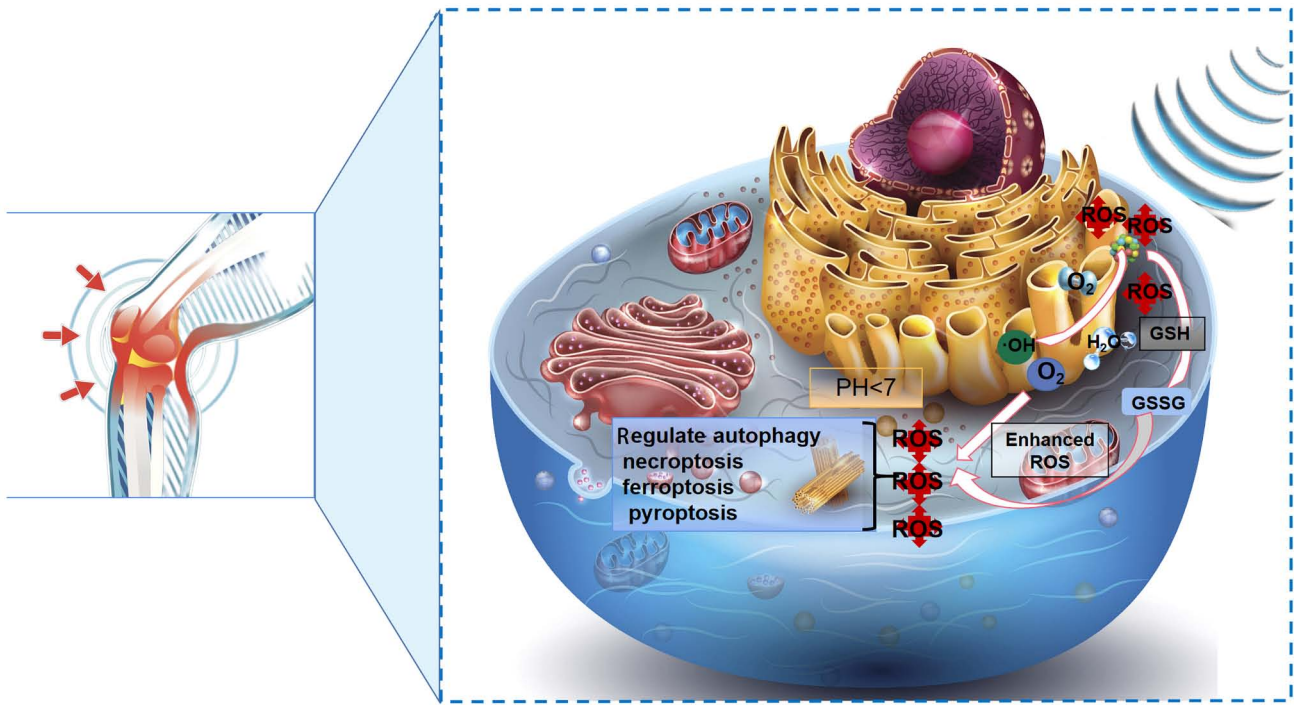


Figure 2. SDT therapy strongly reverses the acidity, hypoxia and hypermetabolic microenvironment of bone tumors, which creates a large number of ROS, as well as restoring GSH to GSSG. The enhanced ROS induce cancer cells to undergo ferroptosis, pyroptosis, and necroptosis. SDT, sonodynamic therapy; ROS, reactive oxygen species; GSH, glutathione; GSSG, glutathione disulfide.

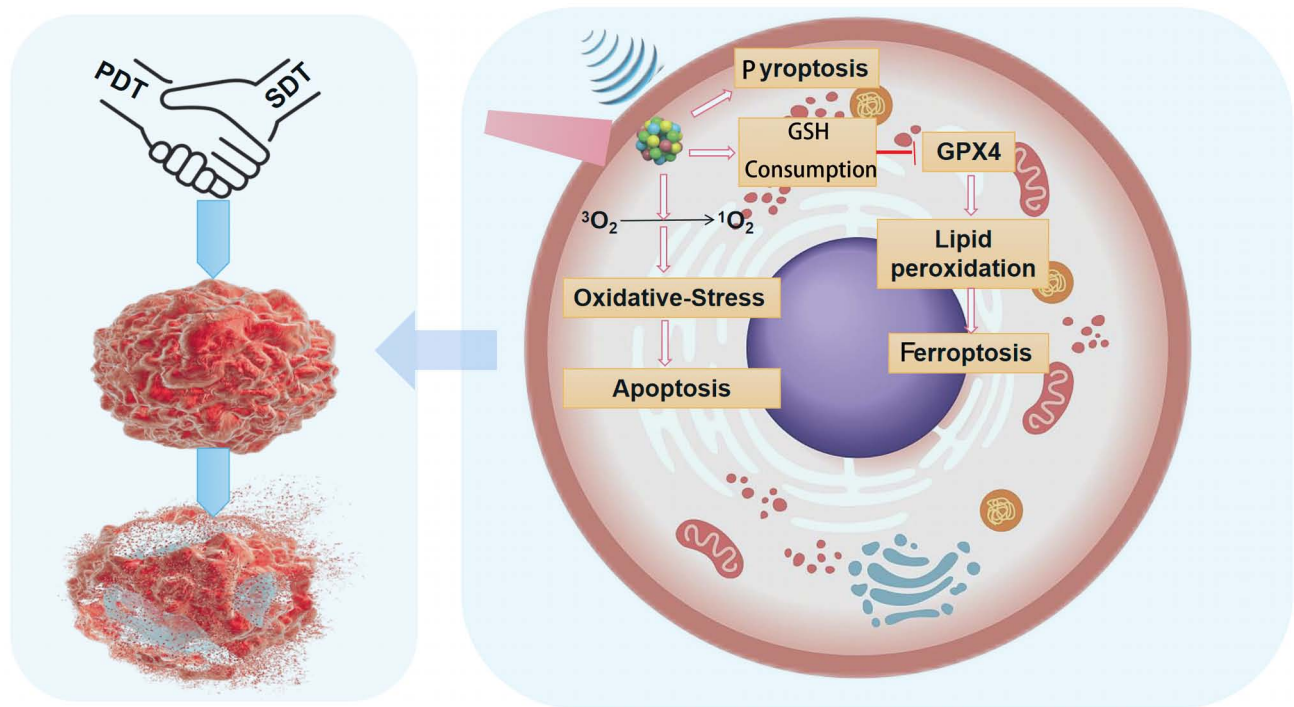


Figure 3. PDT/SDT therapy is able to effectively provide a balance of ferroptosis and oxidative stress in bone tumors. PDT/SDT, photodynamic therapy combined with sonodynamic therapy; GSH, glutathione; GPX4, glutathione peroxidase 4.

and tissue ablation in the affected area, thereby demonstrating the feasibility of using this technique for the treatment of bone tumors (81). Targeting mitochondria and suppressing hypoxia-inducible factor-1 α are essential for improving the PDT/SDT effect (82,83).

In addition, downstream inhibitory effects in various signaling pathways were also detected in cells treated by sound pores, including cell cycle arrest, morphological inhibition, apoptosis and cell necrosis. The use of SPDT significantly enhanced the therapeutic effects. It was determined that, after

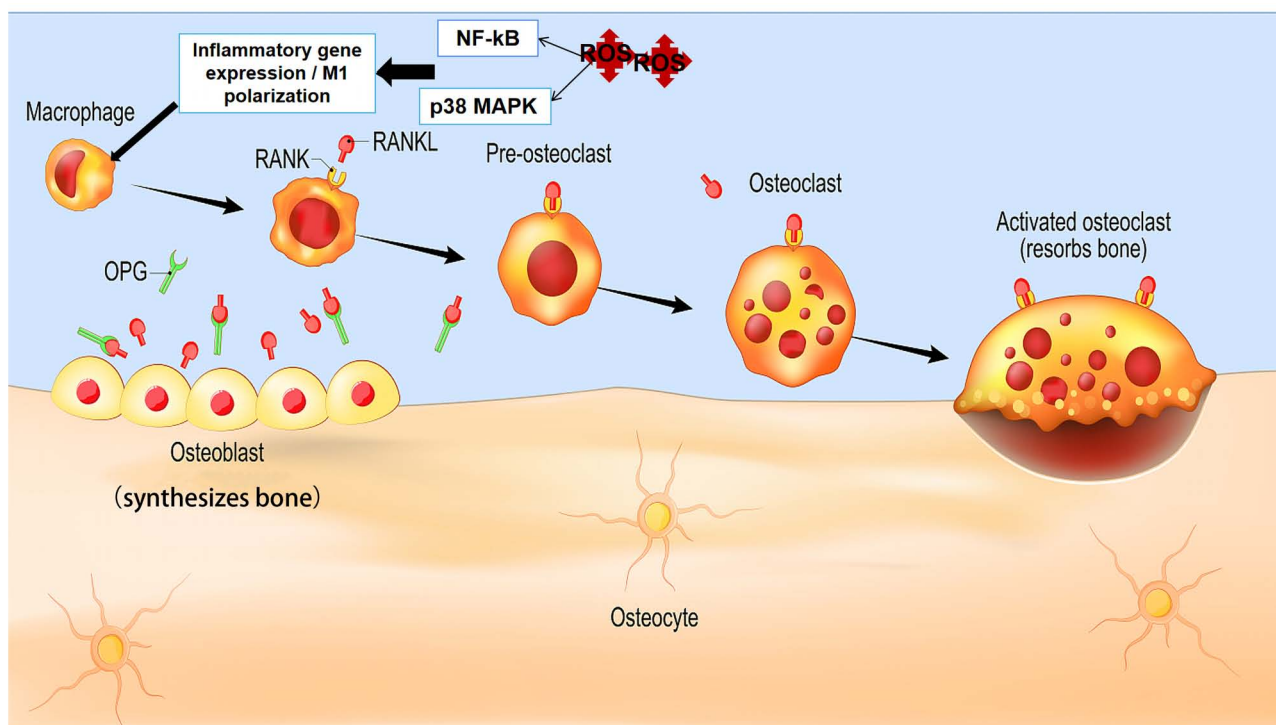


Figure 4. Crosstalk between elevated ROS levels and the bone tumor microenvironment. Excessive ROS triggers the polarization of macrophages to the M1 state through activating the NF- κ B and p38 MAPK signaling pathways, resulting in the secretion of inflammatory cytokines, which induces a chronic inflammation state in tissues, thereby promoting the formation of osteoclasts, which greatly enhance the mineralization of the extracellular matrix and assists the osteogenic effects of photodynamic therapy combined with sonodynamic therapy in the treatment of bone tumors. ROS, reactive oxygen species; OPG, osteoprotegerin; RANK, receptor activator of nuclear factor- κ B; RANKL, RANK ligand.

implementing titanium dioxide-induced SPDT, the expression level of malondialdehyde was significantly increased, whereas the levels of superoxide dismutase, catalase and GSH were downregulated (84).

In osteolytic OS, bone tumor cells have been demonstrated to secrete osteoclast active factors, which activate osteoclasts. Once the balance between the osteoblasts and osteoclasts is broken, excited osteoclasts secrete acid and lysozymes, which cause degradation of the bone matrix, leading to pain via eventual bone resorption for patients (85,86). The degree of destruction of bone tissue, especially through the process of progressive osteolysis, is closely associated with the severity and frequency of breakthrough pain. The RANK/RANKL pathway is mainly responsible for the genesis of osteoclasts, in which the upregulation of osteoclast activity and an increase in bone resorption are mainly based on the imbalance of RANK, RANKL and OPG (Fig. 4). The RANK/RANKL/OPG pathway is particularly important in terms of stimulating bone resorption in osteoclasts.

It is well known that acetylation, methylation and the associated epigenetic enzymes are able to disrupt the molecular pathways of normal bone tumors (87,88). Among them, the genetic modification of methylation is widely reported to be mediated by the methyltransferase, EZH2-mediated H3K27me3. Photodynamic regulators have been shown to inhibit H3K27 methylation by inhibiting the epigenetic regulatory factor EZH2, which stimulates tumor cells to express MHC class I molecules and release a large amount of C-X-C motif chemokine ligand 10, which ultimately enhances the immunosuppressive tumor microenvironment of the tumor

cells, and promotes the capability of T cells to recognize tumors (89,90).

In order to increase the level of ROS to boost the therapeutic effects of tumor treatment, Liu *et al* (91) confirmed that, after mPEG₂₀₀₀-P(HDI-DN)₂₀ had entered the 143B cells, OS cells were inhibited through promoting the accumulation of ROS in the presence of GSH.

SPDT enhances bone tumor immunotherapy. When the application of PDT combined with SDT is successful in terms of destroying the bone tumor, not only are the processes of apoptosis and necrosis involved, but an immunogenic reaction is also mediated. For example, treatment of the bone tumor with black phosphorus (BP) (with its phosphorus element in the BP), which targets the nucleic acid and cell bilayer membrane skeleton, upon being radiated by NIR and US, leads to the 'double killing' of the targeted cancer cells; to be specific, the process not only induces tumoral structural damage through heat, ROS and shock waves, but an anticancer immune stress response is also stimulated that originates from the damaged tumor *in situ* tissue, and this process is termed photoimmunotherapy (92). Bone tumor cells immediately endocytose BP and undergo oxidative stress (at the same time increasing the level of energy metabolism), whereupon BP is converted into PO_4^{3-} ; this is accompanied by a massive induction of apoptosis. At higher temperatures, necrosis is triggered, which both stimulates the outflow of tumor-associated antigens and promotes the maturation of follicular DCs; this process greatly enhances the CD_8^+ T cell-mediated cytotoxic killing response, which

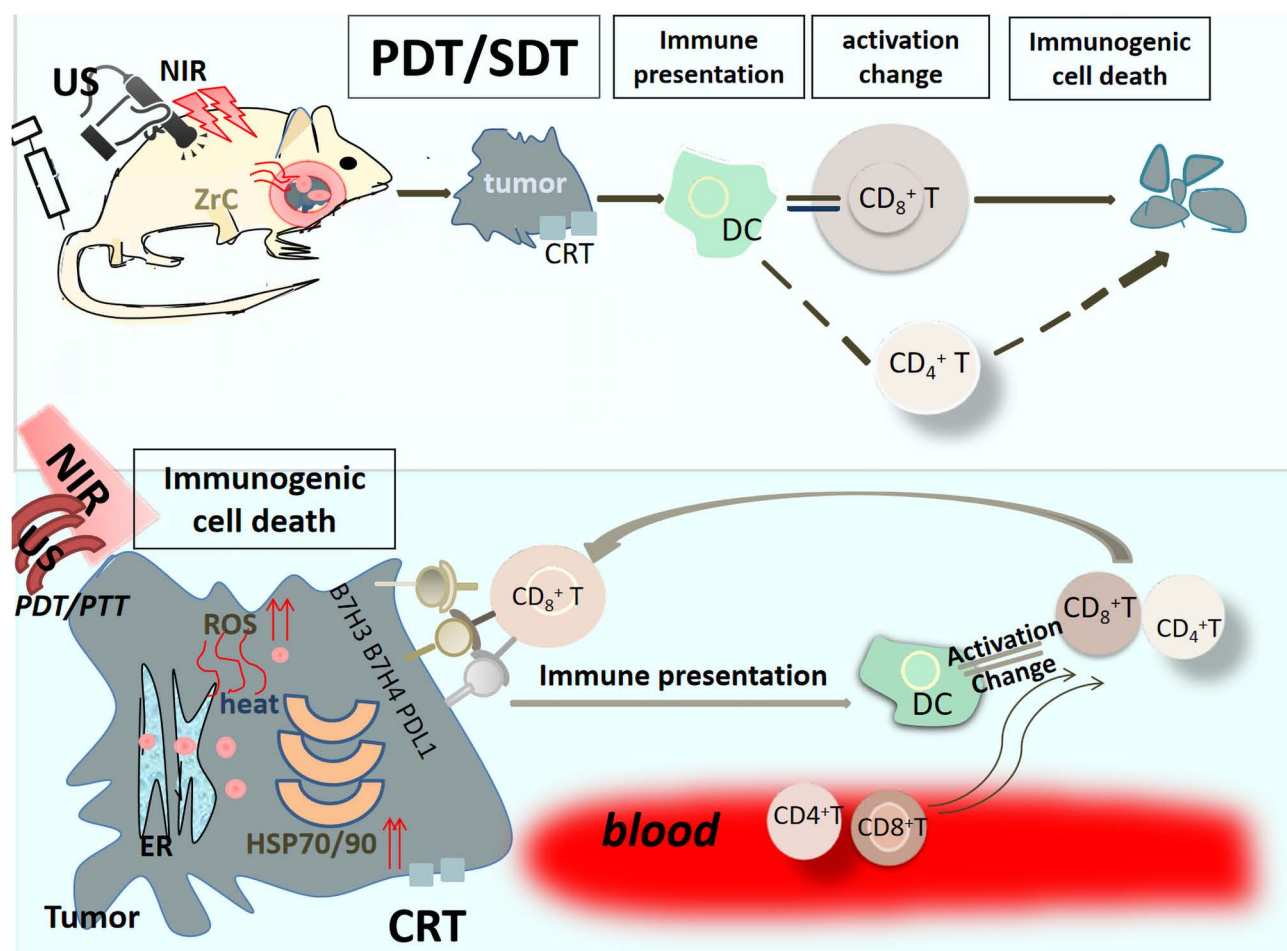


Figure 5. PDT/SDT destroys bone tumor cells and induces the immunogenic cell death response *in situ*. PDT/SDT, photodynamic therapy combined with sonodynamic therapy; DC, dendritic cell; NIR, near-infrared light; US, ultrasound; CRT, calreticulin; PTT, photothermal therapy.

is termed 'immunogenic cell death'. Moreover, CD_8^+ T cells are able to specifically recognize and eliminate the B7 family ligand/receptor molecules B7-H3, B7-H4 and PD-L1, which are expressed by tumor cells (Fig. 5). In addition, the infiltration of immune cells, such as natural killer cells, lymphocytes and DCs, is effectively reversed through relieving the damage-associated molecular patterns, which includes calreticulin, the release of ATP, the secretion of high mobility group box 1 and heat-shock proteins (93). 'Cold' tumors are thereby converted into 'hot' tumors, and the body can spontaneously form an immune system response to target the tumor.

3. Research on direction-targeted materials of bone tumor based on PDT

As the number of publications has grown, the therapeutic materials of PDT for bone tumors may be currently divided into the categories presented in Table I. MPPa is a second-generation PS which is used in the treatment of OS, and is has been successfully shown to induce the apoptosis of MG-63 cells (94). In addition, mTHPC (95), hiporfin-PDT (96), aloe-emodin (97) and PS M007 (98) have been used in conjunction with PDT to counteract the multiplication of human OS cells in seconds to restore the

function of DCs via upregulating HSP70 (99). The first generation (which has now been superseded) utilized metal NPs, for example, gold (100), silver and Pt (101). The second generation comprised carbon materials, including the preparation of PDT nanomaterials through the combination of graphene oxide (GO) and PEG (102). These have attractive photophysical and photochemical properties, although their disadvantages are poor solvability and high crystallinity, which serve as limiting factors in terms of their loading into most drug delivery systems (DDSSs), thereby hindering further drug development. PEG-d to carry ZnPc, thereby achieving π - π interaction (103). In addition, the constituent PEG-PMAN/ZnPc NPs (PPZ) was revealed in physical experiments *in vivo* to markedly increase the production of ROS in OS cells, resulting in mitochondrial damage and arresting the cell cycle at the G_2/M checkpoint (103). Drug-mediated bone tumor PDT and photothermal therapy (PTT) mainly comprise 5-aminolevulinic acid (5-ALA) and synchronous hyperthermia (104).

At present, one limitation of available DDSSs is the difficulty in efficiently transmitting the photothermal agents to the source of the tumor. The surface of modified silicon dioxide NP (SLN) has been demonstrated to be adaptable by the CM to construct a platform (CM/SLN) that is able to target homogenous 143B cells. Moreover, Zhang *et al* (105)

Table I. Representative materials targeting therapy for bone tumors and their associated mechanisms of PDT.

PDT nanomaterials	PDT photosensitizer	Types of bone tumors treated	Associated underlying mechanism that is affected by the treatment	(Refs.)
-	MPPa	MG-63 osteosarcoma cells	Mitochondrial apoptosis pathway induces apoptosis, whereas the ROS-JNK signaling pathway participates in autophagy induced by MPPa-PDT	(79)
-	5,10,15,20-Tetrakis (methahydroxyphenyl)	Human 143B cells	Tumor cell-targeting mechanism that causes antivascular effect and stimulates the immune system	(80)
Carbon materials: The combination of GO and PEG	-	HOS cells	Attenuates the M2 polarization of macrophages that is induced by IL-4, regulating its antitumor ability	(87)
-	PPZ nanoparticles	Osteosarcoma cells	Mitochondrial damage and cell cycle arrest at the G2/M checkpoint	(88)

PDT, photodynamic therapy; Ref, references; MPPa, pyropheophorbide- α methyl ester; GO, graphene oxide; IL-4, interleukin 4; PPZ, PEG-PMAN/ZnPc.

used indocyanine green at a PTT-level dose in conjunction with CM/SLN. PDT is also becoming increasingly popular in the adjuvant therapy of bone tumors, such as treatment with cisplatin (106).

4. Current status of SDT-based treatment of bone tumors

SDT has promising prospects in terms of treating cancer. Compared with organic nanomaterials, inorganic materials have the characteristics of stronger skin sensitivity, faster pharmacokinetics, a higher stability and a faster conversion rate. At the same time, however, the low conversion efficiency and biosafety challenges of SDT should also be taken into account (107,108). The effects of SDT on the growth of implantable bone tumors has also been widely studied. 5-ALA-mediated SDT caused a marked attenuation of the bone tumor volume and the vitality of UMR-106 cells, although ROS generation was promoted (109). The experimental results obtained confirmed that the expression of Bcl-2 was downregulated. By contrast, the expression levels of Bax, p53 and caspase-3 were markedly increased, demonstrating that ALA-SDT was able to act on the associated mitochondrial ROS-mediated apoptotic pathway of OS cells. A number of other studies have produced similar results, including a study of hematoporphyrin monomethyl ether-sonodynamic therapy on MG-63 OS cells (94). Geng *et al* (110) designed W-doped TiO₂ (W-TiO₂) nanometer material for the treatment of OS. Oxygen and Ti-vacancies were introduced into W-TiO₂ nanorods to enhance their sonodynamic properties, and W6+ restored the endogenous GSH to GSSG. Following a similar principle (and mechanism), other studies on Cu(II) NS sonosensitizers containing porphyrins, Cu²⁺, and PEG, have yielded similar results. In summary, fundamentally changing the redox microenvironment of the tumor has a great effect in terms of promoting the curative effect on the bone tumor (111).

5. Conclusion

PDT produces ROS, which eliminate superficial tumor tissue according to the toxic oxidation level. In SDT, when the acoustic sensitizer releases energy, this forms cavitation bubbles around the cancer cells. After a period of time, the energy produced by the collapse of cavitation bubbles causes sound, light and heat damage to the cancer tissue via mechanical stress. The efficient combination of these can bring about the complete destruction of primary and metastatic bone tumors. A large number of studies have demonstrated that targeting GSH consumption significantly promotes ROS-based therapies (i.e., those containing PDT, SDT, CDT, ferroptosis, etc.). The present review analyzed the sources, metabolism and associated functions of GSH in detail. Due to the special anatomical structure of bone tumors, PDT/SDT combination therapy has been identified to have an indispensable role in terms of targeting the ECM and the RANK/RANKL signaling pathway. In addition, discussions are still ongoing regarding the different mechanisms of action. In terms of the work of the authors of the present review, their research is focused on PS/sound sensitizers in the obliteration and clinical transformation optimization of bone tumors.

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Authors' contributions

YH, DZ, XY reviewed the literature and wrote the first draft. CG revised and interpreted the related information. MW and JB contributed to the conception and design of the manuscript. GQ and HM supervised the manuscript and provided critical revisions. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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