


Gold Nanoparticles as Radiosensitizers in Cancer Radiotherapy

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Yao Chen^{1,*}

Juan Yang^{1,*}

Shaozhi Fu¹ 

Jingbo Wu^{1,2}

¹Department of Oncology, The Affiliated Hospital of Southwest Medical University, Luzhou, Sichuan Province, People's Republic of China; ²Nuclear Medicine and Molecular Imaging Key Laboratory of Sichuan Province, Luzhou, Sichuan Province, People's Republic of China

*These authors contributed equally to this work

Abstract: The rapid development of nanotechnology offers a variety of potential therapeutic strategies for cancer treatment. High atomic element nanomaterials are often utilized as radiosensitizers due to their unique photoelectric decay characteristics. Among them, gold nanoparticles (GNPs) are one of the most widely investigated and are considered to be an ideal radiosensitizers for radiotherapy due to their high X-ray absorption and unique physicochemical properties. Over the last few decades, multi-disciplinary studies have focused on the design and optimization of GNPs to achieve greater dosing capability and higher therapeutic effects and highlight potential mechanisms for radiosensitization of GNPs. Although the radiosensitizing potential of GNPs has been widely recognized, its clinical translation still faces many challenges. This review analyses the different roles of GNPs as radiosensitizers in cancer radiotherapy and summarizes recent advances. In addition, the underlying mechanisms of GNP radiosensitization, including physical, chemical and biological mechanisms are discussed, which may provide new directions for the optimization and clinical transformation of next-generation GNPs.

Keywords: gold nanoparticles, cancer radiotherapy, radiosensitization, mechanisms

Introduction

Cancers are a major threat to human health and quality of life. Radiotherapy, including external beam radiotherapy and internal radioisotope therapy, plays a significant role in the treatment of early and terminal solid tumors, as well as metastatic tumors and regional lymph nodes. This type of treatment relies on cellular damage caused when biological tissues are exposed to ionizing radiation.¹⁻³ The high energy radiation beam (external or internal implanted source of radiation) is delivered to tumors for the destruction of intracellular components or stem cells that induces tumor death. This course of treatment is effective in more than half of cancer patients.^{1,4-7} However, this treatment still has some limitations due to toxic side effects such as dose heterogeneity, local discomfort, and long-term exposure to healthy tissues.^{4,8} There is strong evidence to suggest that with the development of tumor cell radio-resistance, higher radiation doses may be required for treatment, which may cause damage or even death of normal cells and tissues.⁸

Numerous innovative therapeutic methods have emerged in radiotherapy during the last few decades, such as intensity-modulated radiation therapy, improved computer-assisted inverse treatment planning, image guidance, stereotactic radiation therapy, and particle therapy, that aim to achieve more efficient and accurate dose delivery to the targeted organs and tissues.^{4,7,9-11} However, increasing the maximum dose

Correspondence: Shaozhi Fu; Jingbo Wu
Department of Oncology, The Affiliated Hospital of Southwest Medical University, Luzhou, Sichuan 646000, People's Republic of China
Tel/Fax +86 8303165696
Email shaozhifu513@163.com; wjb6147@163.com

accumulation in tumor tissues while also looking to reduce the damage to normal tissues has always been a great challenge in radiotherapy. Different strategies have been proposed to balance treatment outcomes and side effects, such as reversing the radiation resistance of tumor tissues, enhancing the radiation tolerance of normal tissues, increasing the radio-sensitization of tumor tissues and limiting the deposition of radiation dose in tumor volume.² Hence, radiosensitizers, defined as chemical or biological compounds that enhance the effective dose of radiotherapy in cancer cells, have gained widespread attention.^{11–13} In particular, high atomic element (*Z*) nanomaterials, such as bismuth (*Z*=83),¹⁴ gold (*Z*=79),^{15–17} tungsten (*Z*=74),^{18,19} tantalum (*Z*=73),²⁰ hafnium (*Z*=72),²¹ and silver (*Z*=47),²² are commonly used as dose enhancers for radiotherapy, due to their strong attenuation of photons, and ability to increase the deposition of radiation. These elements are also called “nanoenhancers” and they have much higher mass-energy absorption coefficients than soft tissues.^{4,23} For keV photon energies, the absorption advantage can even increase ~100 times.¹¹ Among the various nanomaterials investigated for radiotherapeutic applications, gold nanoparticles (GNPs) have long been considered as a potential tool for the diagnosis and treatment of multiple cancers.^{1–3,5,13,24–28}

As emerging tumor radiosensitizers, GNPs are widely studied based on the following advantages:

1. They have strong photoelectric absorption coefficients because of the high *Z* number;
2. They are inert materials with excellent biocompatibility and low biological toxicity when compared with traditional agents (such as cisplatin, nitroimidazole, cyclooxygenase-2 inhibitor and iodinated DNA-targeting agents);
3. They have a high surface area to volume ratio that allows drugs and other therapeutic agents (eg, peptides, proteins, antibodies, small molecules) to attach to their surface for targeted treatment and combination therapy of tumors;¹¹
4. Owing to their enhanced permeability and retention (EPR) effect and low systemic clearance, GNPs preferentially deposit at tumor sites. They have low permeability to normal capillaries and blood vessels in different tissues such as heart, lung, and skin;^{8,11,16}
5. As an imaging contrast agent, they can be used in disease diagnosis as well as biological imaging;^{9,24,29,30}
6. They have a well-controlled size distribution and GNPs have unique chemical, electrical, and optical properties in the range of 1–150 nm,^{3,26,31}

A number of unique GNP formats are currently undergoing preclinical development for various therapeutic and diagnostic applications – including radiotherapy, disease diagnosis, bioimaging (eg, computed tomography, photoacoustic imaging), therapeutic agent delivery (eg, drugs, genes, RNAs), biosensing and other therapies (eg, photothermal therapy, photodynamic therapy).^{24,32–40} In addition, many different parameters of GNPs such as particle size, shape, surface chemistry, concentration, biological distribution and localization may influence their effectiveness in radiotherapy.^{39,41–46} Therefore, several efforts have been made in the past few decades to continuously improve the different impact parameters of GNPs and achieve high radiation efficiency.

This review summarizes the role and applications of GNPs under various types of ionizing radiation including γ -rays, X-rays and proton therapy. We also discuss the potential mechanisms of radiosensitization to provide a theoretical basis for the future development of nanoparticle-assisted radiotherapy.

The Forms of GNPs as Radiosensitizers

Numerous reports have demonstrated that the use of nano-platforms to selectively deliver therapeutic agents to tumor tissues not only enhances the bioavailability of cytotoxic agents but also minimizes potential toxicity to healthy tissues. This strategy is also applicable to tumor radiotherapy, limiting treatment radiation doses to tumor tissues as much as possible. The radiosensitization potential of GNPs has been supported and confirmed by various experimental data (Tables 1 and 2). Additionally, studies have suggested that the biodistribution of GNPs is one of the most important factors affecting radiation efficiency. Assuming that GNPs have preferentially distributed in tumor tissue and when it follows the same energy absorption mode as that of surrounding healthy tissues, they produce high local ionization in tumor tissues that shortens treatment time and reduces radiation doses. As such, the radiation doses absorbed by healthy tissues are lowered and adverse reactions and side effects caused by radiotherapy are also decreased. However, the size, shape, surface chemistry, and surface modification of GNPs have different effects on its biodistribution, explaining the different radiotherapy outcomes reported.^{2,5,11,24,47–50} Based

Table 1 In vitro Studies on Radiosensitization of GNPs

Study [Ref]	Particle Size	Surface Modifier	Concentration	Cell Line	Radiation Source	Outcomes/DEF
Chithrani et al ⁶⁴	50nm	Citrate	1nM	Hela	105kVp	1.66
					220kVp	1.43
					660keV	1.18
					6MVp	1.17
Chang et al ²⁵	13nm	Citrate	10nM	BI6F10	6 MeV	Significantly reduced survival fraction
Liu et al ⁵⁹	14.8nm	Citrate	1.5–15µg/mL	Hela	50kVp	1.14–2.88
Butterworth et al ¹⁹⁷	1.9nm	Thiol	100µg/mL	AGO-1552B	160kVp	1.97
				MCF-7		1.09
				MDA-MB-231		1.11
				PC-3		1.02
				T98G		1.91
Coulter et al ⁵⁰	1.9nm	Thiol	12µM	MDA-MB-231	160kVp	1.41
Wang et al ¹¹⁴	16nm	Glucose	20nM	MDA-MB-231	6MV	1.49
	49nm					1.86
Soleymanifard et al ¹¹⁵	16nm	Glucose	100µM	QU-DB MCF-7	100kVp 6MV	Increased inhibition of cell proliferation
Joh et al ⁵⁴	12nm	PEG	1mM	U251	150kVp	1.3
Zhang et al ¹⁶	4.8nm	PEG	0.05mM	Hela	662keV (¹³⁷ Cs)	1.41
	12.1nm					1.65
	27.3nm					1.58
	46.6nm					1.42
Khoshgard et al ⁹⁸	52nm	Folate	50µM	Hela	120–250kVp	1.64

Abbreviations: GNPs, gold nanoparticles; DEF, dose enhancement factor; PEG, polyethylene glycol.

on these potential advantages, various strategies have been modeled in simulations or demonstrated in experiments to explore the interaction of GNPs with radiation. Researchers are continuously working on these properties of GNPs to improve radiation effects.

Undecorated GNPs

The unique advantages of GNPs have motivated researchers to apply them to tumor radiotherapy. A pioneering study by Hainfeld et al⁵¹ found that EMT-6 mammary xenograft tumors in mice were completely eliminated in 30 days after intravenous injection of 1.9 nm GNPs and exposure to 250 kVp X-rays, while a high radiosensitization effect of GNPs was

observed. This study provided the first strong experimental evidence for in vivo radiosensitization effects of GNPs. The team further verified the radiosensitization effects of GNPs at different radiation doses and in different tumor models.^{52,53} As radiosensitizers, GNPs have been applied to various tumors – including brain tumors,⁵⁴ breast cancer,⁵⁵ melanoma,^{25,56} colon cancer,^{57,58} cervix carcinoma⁵⁹ and lymphoma.⁶⁰

Due to the increased permeability of tumor blood vessels, nanomaterials can be preferentially delivered to tumor tissue through the EPR effect. Recent intracorporal studies have shown that small-sized GNPs (< 6 nm) are cleared through renal excretion within minutes, and that larger nanoparticles are captured by the reticuloendothelial system

Table 2 In vivo Studies on Radiosensitization of GNPs

Study [Ref]	Particle Size	Surface Modifier	Concentration	Tumor Model	Radiation Source	Effects
Hainfeld et al ⁵¹	1.9nm	Thiol	1.35g Au/kg 2.7g Au/kg	EMT-6	250kVp 26Gy	50% long-term survival at 1.35 g Au/kg; 86% long-term survival at 2.7 g Au/kg;
Hainfeld et al ⁵²	1.9nm	Thiol	1.9g Au/kg	SCCVII	68keV, 42Gy 157keV, 50.6Gy	Increased in tumor volume doubling time; increased long-term survival rate;
Hainfeld et al ⁵³	1.9nm	Thiol	4g Au/kg	Tu-2449	100kVp,30Gy	50% long-term tumor-free survival;
Chang et al ²⁵	13nm	Citrate	200nM	B16F10	6MeV e ⁻	Significant delay in tumor growth;
Duo et al ²⁴	13.2nm	PEG	60nM/kg	Hela	6MeV,6Gy	Significant effects on inhibiting tumor growth;
Joh et al ⁵⁴	12nm	PEG	1.25g Au/kg	U251	175kVp,20Gy	Median survival time prolonged;
Liu et al ⁸⁹	8nm 50nm	BSA	4mg Au/kg	H22	6MV,5Gy	Tumor growth is inhibited, DEF is 1.93 and 2.02, respectively;
Koonce et al ²¹⁴	27nm	TNF- α	250 μ g/kg	SCCVII 4T1	150kVp,20Gy or 12Gy \times 3	2-fold or 5.3 times delay in tumor growth.

Abbreviations: BSA, bovine serum albumin; GNPs, gold nanoparticles; PEG, polyethylene glycol; TNF- α , tumor necrosis factor- α ; DEF, dose enhancement factor.

(RES).^{9,42,61} However, GNPs (< 100 nm) effectively accumulate in tumor tissues by taking advantage of the EPR effect; for instance, GNPs < 50 nm can easily pass through the cell membrane, and particles < 20 nm can pass through the vascular endothelium.^{16,62,63} Chithrani et al⁶⁴ verified the irradiation enhancement effect of 50 nm GNPs in lower- (~100–220kVp) and higher- (6 MVp) energy photons by utilizing nanoparticles ranging in size from 14–74nm. They showed that GNPs up to 50 nm in diameter have the highest radiosensitization enhancement factor (REF) (1.43 at 220kVp) when compared with GNPs of 14 and 74 nm sizes (1.20 and 1.26, respectively). In addition, they pointed out that the kinetics and saturation concentrations also rely on the physical shape of the nanoparticles. It has been reported that spherical GNPs are more easily internalized by HeLa cells than rod-like particles of similar size.^{42,43} For spherical GNPs, the cell uptake rate is significantly increased by ~375% - 500%. The in vitro cell uptake heterogeneity may be due to differences in particle curvature, which affects their interaction with cell membranes.^{9,45,65}

It is now widely accepted that small-sized spherical GNPs show significant advantages in tumor localization and penetration. They are more likely to penetrate into the tumor stroma and tumor microenvironment, and thus are more effectively internalized by nonphagocytic cells.^{41,66} However, it is well known that citrate-coated GNPs have a high zeta potential at physiological pH and that the

particles easily aggregate.⁶⁷ Additionally, an insufficient blood circulation time of GNPs and unavoidable phagocytosis by the RES will cause limited bioavailability. Therefore, surface modification or functionalization should be kept in mind in the design of GNPs to expand their range of applications and reduce side effects.

Multi-Functional GNPs

Given the limitations of the first generation of nanoparticles, such as the challenges of in vivo delivery,⁹ the design of second- and third-generation nanomaterials primarily focuses on surface modifications. The modifications could increase the absorption of nanoparticles, enable stealth and targeting effects, allow greater accumulation in the target volume and achieve a more precise controlled biodistribution.^{44,68} Overall, functionalized GNPs exhibit a wider range of application advantages (Figure 1):⁸

1. Increase blood circulation time and uptake into tumor cells, as well as modulate clearance, aimed at inhibiting tumor invasion and metastasis;
2. Selective targeting of sites for drug delivery, reduce toxicity, evade surveillance and clearance by the immune system, and inhibit multi-drug resistance (MDR);
3. Offer coupling sites for biomarkers for disease diagnosis and efficacy prediction;

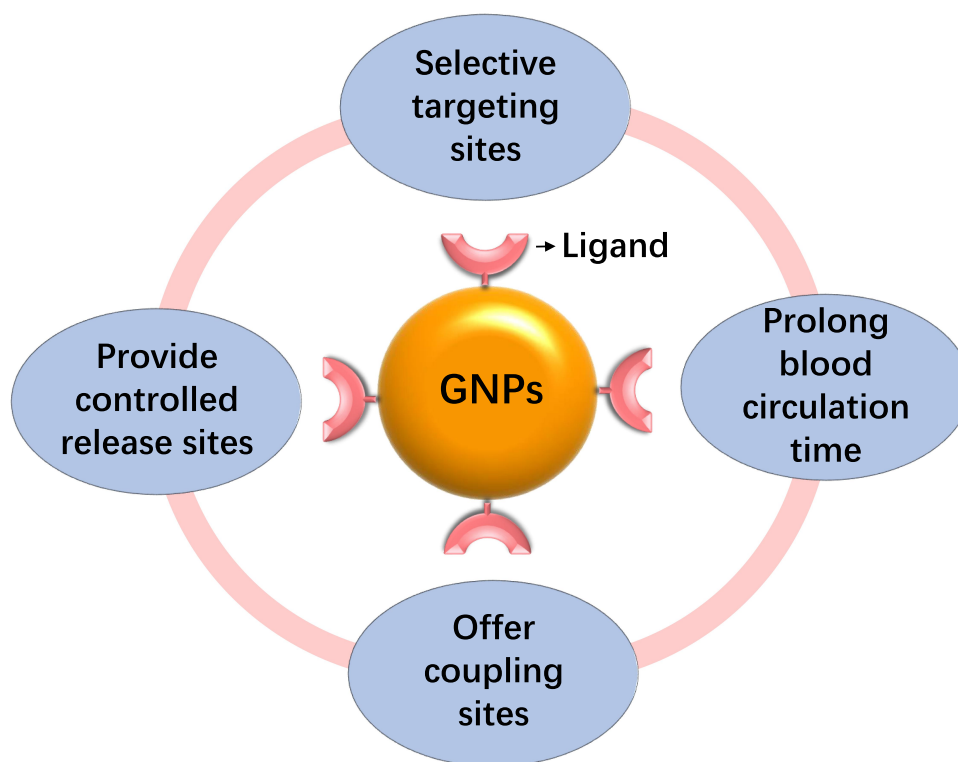


Figure 1 The application advantages of functionalized GNPs.

Notes: Prolong blood circulation time and increase the uptake of tumor cells; selective targeting sites for drug delivery, reducing toxicity, evading clearance of the RES and inhibiting multi-drug resistance (MDR); offer coupling sites for biomarkers for disease diagnosis and efficacy prediction; provide controlled release sites for drugs, reduce the side effects of drugs.

Abbreviations: GNPs, gold nanoparticles; MDR, multi-drug resistance; RES, reticuloendothelial system.

4. Provide controlled release sites for drugs and reduce the side effects.

Based on the available data and after optimizing key features, GNPs will be more appropriate for improving the efficacy of radiotherapy. Two types of targeting strategies are employed to ensure adequate concentrations of GNPs in tumor cells: passive targeting and active targeting. In passive targeting, one utilizes the higher endocytic uptake of cancer cells and vascular leakage around the tumors, which allows for the higher accumulation of nanoparticles in tumor tissues. For active targeting, GNPs are coated with specific molecules which then target receptors on cancer cells, similar to antibodies.

Passive-Targeting

Continuous improvements to surface functions of GNPs were made to achieve better biocompatibility, longer circulation time, lower cytotoxicity, and higher tumor uptake. However, due to the lack of tumor-targeting ligands, whether GNPs reach tumor tissues mainly depends on the EPR effect (Figure 2). One could design different

surface chemistry and surface functions on GNPs, so they could exert more significant biological functions in vitro and in vivo, and achieve superior radiosensitization effects in radiotherapy. Commonly used surface modifiers include polyethylene glycol, chitosan, cytotoxic drugs, proteins, and radioactive elements.

Polyethylene Glycol-Modified GNPs

Polyethylene glycol (PEG) is a widely used surface modifier. PEGylation can be defined as the “hide” of nanoparticles by modifying their surface with a PEG layer. This may reduce particle surveillance and identification by the RES, effectively extending the blood circulation time.^{16,43,69–74} PEGylation can be accomplished by covalent linking; the covalent bond encapsulates or adsorbs the PEG chain on to the surface of the nanoparticles.⁷⁵

The use of non-immunogenic and biocompatible hetero-bifunctional PEG molecules stabilizes the core size of the GNPs and also regulates the surface charge of the nanoparticles which affects their biodistribution. Kumar et al⁴⁴ demonstrated the low cytotoxicity and biocompatibility of PEGylated GNPs (pGNPs) in Hela cells. When coupled with

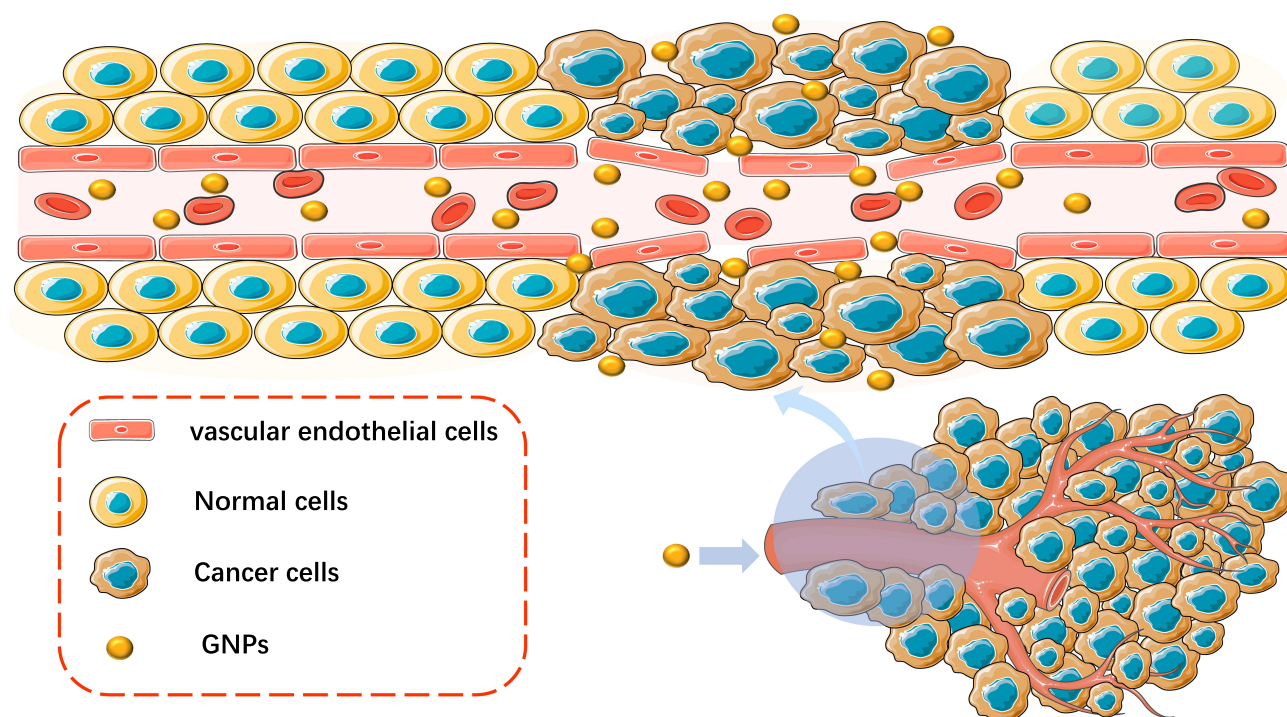


Figure 2 Passive targeting for GNPs.

Notes: The wide fenestrations of tumor vascular endothelial, as well as its poor structural integrity, high permeability and impaired lymphatic drainage, allowing the accumulation or passive targeting of GNPs in or around the tumor tissues, which is known as EPR effect.

Abbreviations: EPR, enhanced permeability and retention; GNPs, gold nanoparticles.

in vitro X-ray irradiation, cell death after treatment with different concentrations of pGNPs increased by 1.3–2.8 times compared to controlled group cells (without pGNPs). In another study, Yasui et al⁶⁹ reported that PEGylated nanogels containing GNPs combined with 220 kVp X-ray irradiation radiosensitized murine squamous cell carcinoma SCCVII and Chinese hamster lung fibroblasts V79. The mechanism of radiosensitization may be due to the increased apoptosis and impaired DNA repair capacity by overexpressing endoplasmic reticulum stress-related proteins. Another in vitro study showed that PEGylated GNPs enhanced cellular uptake in B16F10 murine melanoma cells and caused radiosensitization under irradiation with 6MeV.⁷⁶ By analyzing the cell survival curves plotted by fitting the linear-quadratic model, PEG-GNPs could achieve a maximum dose enhancement factor (DEF) of 1.22 and a maximum sensitization enhancement ratio (SER) value of 1.21 at a concentration of 30 μ M.

These results provide evidence for the clinical application of GNPs as theranostic agents in radiotherapy. In vivo, a majority of PEGylated nanoparticles aggregate around the blood vessels, but cellular uptake is limited. Hence, it is necessary to improve the surface of GNPs to preferentially sensitize tumors to irradiation.

Chitosan-Modified GNPs

Chitosan is a pseudonatural cationic polymer with unique properties, used in wide-ranging applications such as flocculants for protein recovery, and depollution. It also has favorable biocompatibility, biodegradability, sensitivity to chemical modifications, and modulates drug release; these pharmaceutical characteristics are applicable to certain areas of medicine.^{77–80} Fathy et al⁷⁹ explored a novel treatment based on the chitosan structure, to effectively achieve the simultaneous loading of chemotherapy drugs (doxorubicin, DOX) and GNPs as radiosensitizers. In combination with external irradiation, DOX acted as a broad-spectrum anticancer drug, while chitosan-capped GNPs (CS-GNPs) increased the sensitivity of tumor cells to radiation, thus achieving combination chemotherapy and radiotherapy (chemo-radiotherapy). In chemo-radiotherapy, intravenous chemotherapy drugs regulate systemic metastasis and exert synergistic anti-tumor effects with localized radiotherapy.⁸¹ The study was supported by a large number of randomized clinical trials; this combination of the two methods may solve the issue of sublethal damage repair and hypoxia-related radioactive resistance.^{82,83} Although this study confirmed that CS-GNPs-DOX combined with radiotherapy was a promising strategy, additional in vivo experiments

have not revealed more complex biological mechanisms that can be translated into clinical trials.

Bovine Serum Albumin-Modified GNPs

The advantages of bovine serum albumin (BSA) include biocompatibility, biosafety, excellent biodegradability, and flexible surface modification; all these make BSA a common surface modifier.^{84–86} BSA-modified nanoparticles can be temporarily “invisible” in the blood circulation, which prolongs the blood circulation time and allows nanoparticles to exert a better therapeutic effect. In addition, BSA-modified GNPs have been extensively studied in various fields such as biosensing, bioimaging, drug carriers, gene carriers, and cancer therapy.^{85–88}

To achieve better targeting, Liu et al⁸⁹ used BSA as a biological template to synthesize different sized particles of BSA-protected GNPs (8, 50 and 187 nm). They used these nanomaterials as radiosensitizers to examine their radiosensitization effects on H22 hepatocarcinoma-bearing mice. Their results have shown that BSA-GNPs efficiently aggregate in the cytoplasm and that there is no obvious cytotoxicity to HeLa, HepG2, and HeCat cells. In vivo experiments indicate that there is no apparent physiological injury to tumor-bearing mice after intravenous injection of BSA-GNPs. In the case of combined radiotherapy, 8 and 50 nm BSA-GNPs were found to induce apoptosis and inhibit tumor growth by up-regulating the expression of caspase-3 and Bax protein and down-regulating the expression of Bcl-2 protein, to obtain enhancement factors of 1.93 and 2.02, respectively. Therefore, BSA-templated GNPs are potential radiosensitizers for the radiotherapy of hepatocarcinoma.

Cytotoxic Drugs Conjugated GNPs

The narrow therapeutic range of many chemotherapeutic drugs is mainly due to their systemic toxicity. Cisplatin, for example, plays an important role in tumor chemotherapy.^{90,91} It has the ability to cross-link DNA, change the structure, and activate a variety of signal transduction pathways to induce apoptosis. However, its clinical application is limited by its significant systemic toxicity (ototoxicity, nephrotoxicity, and neurotoxicity). Therefore, different methods have been tried to reduce systemic exposure and side effects. Setua et al⁹² demonstrated that GNP+ RT-mediated monotherapy was not effective for all patients. To overcome this obstacle, they grafted cisplatin on to the surface of gold nanoparticles (GNP-Pt) and evaluated the multimodal

chemical-radiotherapy potential of the nanospheres in three glioblastoma multiforme (GBM) cell lines. Their results showed that similar inhibition effects were observed in the growth curves of all three patient-derived cell lines under brachytherapy (¹³⁷Cs as irradiation source), indicating that GNP-Pt-mediated synergistic chemical-radiotherapy may effectively eliminate the therapeutic resistance in GBM cells. Recent studies have confirmed the potential of this therapeutic modality.^{93,94}

In addition, as a multi-functional nanoplatform, hollow GNPs (HGNPs) loaded with doxorubicin (DOX) has been used in combination chemotherapy, radiotherapy, thermotherapy, and CT imaging. Park et al⁹⁵ evaluated the anti-tumor effects of DOX-HGNPs when combined with NIR laser irradiation and radiation. Compared to the control tumors, the combination strategy caused a 4.3-fold increase in tumor-growth delay and a 6.8-fold reduction in tumor weight.

Although surface modifications can improve the stability of GNPs and decrease the problem of aggregation and flocculation, the GNPs lack preferential concentration in the tumor areas that effect the efficacy of radiotherapy. This type of passive approach does not utilize specific units to target cancer cells. The passive-targeting ability of surface coatings depends on several factors such as the size of the nanoparticle core or the length and surface density of the capping molecules, which are major limitations of these methods.² Radiation damage caused by the non-specific biodistribution of radiosensitizers or radioisotopes are additional reasons that limit its application. Thus, increasing specific tumor accumulation of GNPs and enhancing the therapeutic efficiency of radiotherapy is still a challenge.

Active Targeting

It is well known that the radiosensitization of GNPs depends on their cellular uptake and localization. Studies have shown that areas highly radiosensitive to GNPs are located within or near the nucleus. Such nuclear-localized GNPs can be achieved by active targeting. In general, active targeting works primarily through the interaction of targeting ligands with receptors overexpressed on the surface of cancer cells, rather than simply relying on EPR effects.^{96,97} Active-targeting agents can be divided into three main types based on their target substrates: cell surface carbohydrates (carbohydrate targeting), cellular antigens for antibody (antibody targeting), and cell surface receptors (receptor targeting) (Figure 3). Currently, the targeting ligands used

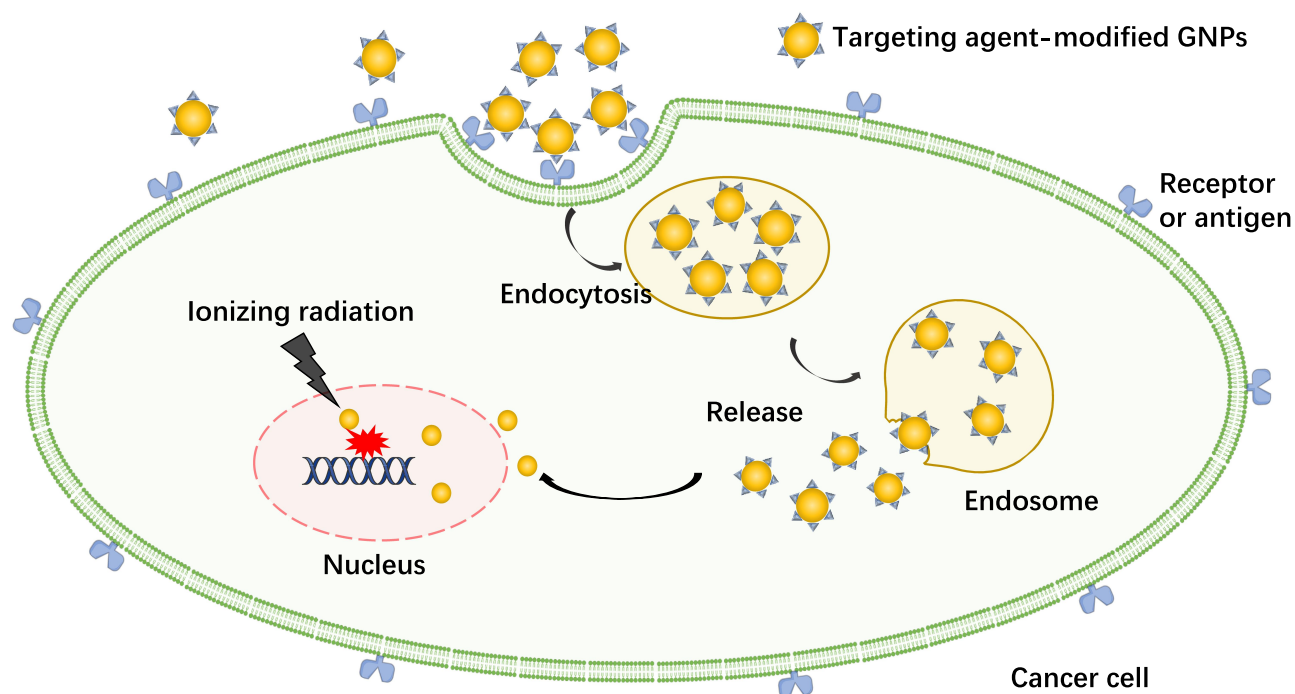


Figure 3 Active targeting for GNPs.

Notes: Overexpression of antibodies or surface-bound antigens on the surface of tumor cells or tumor blood vessels provides an effective pathway for uptake of nanomedicine, namely receptor-mediated endocytosis. Targeting agent-modified GNPs can specifically bind to certain cancer cells through this effect, is denominated as active targeting of GNPs.

Abbreviation: GNPs, gold nanoparticles.

include proteins, aptamers, and small molecules such as peptides, monoclonal antibodies, hormones, glucose molecules, and carbohydrates (Table 3). Based on molecular recognition processes, these ligands deliver therapeutic agents to pathological sites or through biological barriers.

Folate-Modified GNPs

Folate (folic acid, FA) is essential for the biosynthesis of purines and pyrimidines in DNA synthesis pathways, acting as one of the promising targeting ligands for cancer treatments. Folate is suitable as a targeting agent due to its stability, non-immunogenicity, specificity for cancer cells, and simple conjugation chemistry.^{98,99} Some rapidly dividing cancer cells upregulate levels of folate receptors to satisfy the increased demand of folate for DNA synthesis and rapid growth, which is the basis of folate targeting strategies. Previous attempts have shown that head and neck, cervix and ovarian tumors are positive models for folate targeting studies. Conversely, bladder, pancreas and liver tumors can be considered as negative models.⁹⁹ Overall, one can take advantage of the active-targeting property of folate and combine it with nanotechnology platforms to discover novel treatment strategies for cancer.

Khoshgard et al⁹⁸ compared the internalization of folate-conjugated GNPs and non-folate-conjugated (PEGylated) GNPs in HeLa cells, and also focused on enhanced cell damage following orthovoltage X-rays (-120–250 kVp) and megavoltage γ -rays (Co-60) exposure. They found that folate-conjugated GNPs had higher internalization ability in HeLa cells, as well as higher cancer cell death rates and DEF under different irradiation energies when compared to PEGylated-GNPs. Therefore, they highlighted that GNPs could effectively enhance cell lethality under orthovoltage X-ray photons and that folate nanoconjugates increase the selectivity of this lethal effect. In another study, Kefayat et al¹⁰⁰ prepared folate and BSA decorated gold nanoclusters (FA-AuNCs) and investigated their radiosensitivity effects on C6 glioma tumors in radiotherapy. ICP-OES was used to assess FA-AuNCs targeting efficiency and they showed that its accumulation in C6 cells was 2.5 times higher than that in normal cells, demonstrating excellent targeting ability. Furthermore, in tumor-bearing mice, the concentration of FA-GNPs localized in brain tumors was significantly higher than in surrounding normal tissues, and the mice had increased total survival time compared to the control group. The

Table 3 Summary of Active Targeting and Targeting Approaches of Surface-Modified GNPs

Surface Modifier	Targeting Receptor	Cell or Tumor Model	Reference
Folate	Folate receptor	Hela C6	Samadian et al ⁹⁹ Kefayat et al ¹⁰⁰
Trastuzumab	HER2	MDA-MB-361 BT474 SK-BR-3	Cai et al ¹⁰³
Panitumumab	EGFR	MDA-MB-468 MDA-MB-231 MCF-7	Yook et al ¹⁰⁴ Yook et al ¹⁰⁵
EGF	EGFR	MDA-MB-468 MCF-7	Song et al ¹⁰⁹
Glucose	GLUT receptors	THP-1	Hu et al ¹¹⁶
GAL	Asialoglycoprotein receptor	HepG2	Zhu et al ¹¹⁸
RGD	Transmembrane heterodimeric $\alpha v \beta 3$ integrin receptor	MDA-MB-231	Yang and Chithrani ¹²⁵
CPPs	The plasma membrane and membrane-associated proteoglycans	LS 180	Zhang et al ¹³⁰
NLSs	The NPC	Hela	Yang et al ¹³¹
NUAP	Nucleolin	A431 FaDu	Zhang et al ¹⁴¹
pHLIP	Acidic pH of TME	A549	Antosh et al ¹⁵¹
Salmonella typhi Ty21a	Tumor hypoxic regions	CT-26	Kefayat et al ¹⁶²

Abbreviations: EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; GAL, β -d-galactose; GLUT, glucose transporter; GNPs, gold nanoparticles; HER2, human epidermis growth factor receptor 2; NLSs, nuclear localization sequences; NPC, nuclear pore complex; NUAP, nucleolin aptamer; pHLIP, pH low-insertion peptide; TME, tumor microenvironment.

DEF of FA-AuNCs was 1.6 when irradiated with a single dose of 6 Gy. These studies indicate that folate-coated GNPs may improve radiotherapy efficacy.

Antibody-Coated GNPs

Human epidermis growth factor receptor 2 (HER2), an important predictor of breast cancer, is overexpressed in 20–30% of breast cancers and is a predictor of poor prognosis. As the first targeted drug in breast cancer treatment, trastuzumab may selectively bind to the extracellular structure of HER2 and destroy the dimer of the receptor via down-regulating HER2/nue. This induces endocytosis and degrades cell membranes through the signal transduction of downstream PI3K pathway to play a therapeutic role.^{101,102} In order to improve the therapeutic effects of locally advanced breast cancer, Cai et al¹⁰³ developed a novel radiation treatment termed “radiation nanomedicine”. This treatment consisted of trastuzumab-conjugated GNPs and then labelled it with ¹⁷⁷Lu (Trastuzumab-GNPs-¹⁷⁷Lu), a radionuclide emitting β -

particles [$E_{\beta\max}=0.5\text{MeV}$; $t_{1/2}=6.7\text{days}$] with a maximum range of 2 mm. The binding and internalization of this polymer have been observed in multiple HER2-positive breast cancer cell lines (SK-BR-3, BT474, MDA-MB-361). It was found that the internalization of GNPs increased in these three cell lines after incubation with trastuzumab-GNP-¹⁷⁷Lu and that there was negligible uptake of GNPs after incubation with non-targeted GNPs (GNP-¹⁷⁷Lu). The radioligand binding assay revealed that trastuzumab-GNP-¹⁷⁷Lu may be multivalently bound to HER2. Increased internalization of trastuzumab-GNP-¹⁷⁷Lu in the cytoplasm was supposed to deposit greater radiation doses, which showed increased apoptosis and more DNA double-strand breaks (DSBs). Further, the antitumor properties and normal tissue toxicity of trastuzumab-GNP-¹⁷⁷Lu and GNP-¹⁷⁷Lu were compared in MDA-MB-361 mice. Compared to treatment with GNP-¹⁷⁷Lu or untreated mice, trastuzumab-GNP-¹⁷⁷Lu significantly inhibited tumor growth and no toxic reactions were observed in normal tissues over a 16-day

observation period. These results are similar to the results of previous reports which demonstrated that panitumumab-GNP-¹⁷⁷Lu deposited more radiation dose in epidermal growth factor receptor (EGFR) positive breast cancer cells when compared to GNP-¹⁷⁷Lu.^{104,105} The radiosensitizing effect of HER2 antibody-decorated GNPs was also confirmed in an ovarian cancer cell line (SK-OV3).¹⁰⁶

Similarly, Popovtzer et al¹⁰⁷ used cetuximab to target EGFR, allowing GNPs to selectively target head and neck tumors and significantly increase the radiation dose absorbed in tumor tissues. When combined with radiotherapy (25 Gy, 6 MV, 1.4 Gy/min), a significant tumor-growth delay in tumor-bearing mice was observed. Further analysis indicated that there was no significant toxicity or side effects in any of the experimental mice. Inspired by GNPs and ¹⁰⁸In (a radionuclide commonly used for SPECT imaging), Song et al¹⁰⁹ used epidermal growth factor (EGF) as a target ligand, resulting in the effective nuclear localization of ¹⁰⁸In-radiolabelling of GNPs in breast cancer cells with high expression of EGFR. Such a distribution would increase the radiation toxicity of ¹⁰⁸In-EGF-GNPs when combined with external irradiation, as the electron pair emitted around perinuclear and the DNA radiation dose are both increased. They expect the ¹⁰⁸In-labelled EGF-GNPs nano-system to be a new tool for the treatment of EGFR-positive cancers.

Glucose Molecules Conjugated GNPs

Glucose is the main source of cellular metabolic energy. The rapid growth and proliferation of cancer cells depend on specific proteins and glucose. As such, glucose transporter (GLUT) receptors on the surface of cancer cells enable the internalization of a greater number of glucose molecules than normal cells^{108,110,111} to meet the increased energy demands of cancer cells. GNPs can covalently conjugate to various biomolecules via thiol groups, that allows effective coupling with glucose molecules to achieve better internalization of GNPs. The targeting ability of glucose-coated GNPs has been demonstrated in various solid tumors (eg, breast, lung, and ovarian cancers).^{112–115}

In a study by Hu et al,¹¹⁶ THP-1 cancer cells were suspended to simulate cancer stem cells and cancer metastasis and the adherent MCF-7 cancer cells mimicked solid tumors. In this system, the Glu-PEG-GNPs could successfully target cancer cells. The use of starvation media for an appropriate time could aid Glu-PEG-GNPs uptake in cancer cells. Based on the

radiosensitization of GNPs under megavoltage energy irradiation, they also assessed the therapeutic effects of Glu-PEG-GNPs combined with radiotherapy on both MCF-7 and THP-1 cells. The results showed that Glu-PEG-GNPs exhibit enhanced tumor-killing ability at 6 or 9Gy irradiation when compared to that by X-ray irradiation alone or GNPs treatment alone. This finding may provide a new treatment strategy for cancer stem cells and cancer metastasis. Similarly, as a hepatocyte-specific receptor, asialoglycoprotein receptor (ASGPR) is over-expressed on the sinusoidal surface of hepatocytes and mediates the uptake and endocytosis of galactose- or N-acetylgalactosamine-terminating glycoproteins by hepatocytes.^{117,118} Zhu et al¹¹⁸ used β -D-galactose (GAL) as a homing agent to design ASGPR-targeted GNPs to enhance the cytotoxicity of radiation therapy. This composite (GAL-PEG-GNPs) could be more rapidly and efficiently taken up by ASGPR over-expressing HepG2 cells and could avoid the rapid phagocytosis of RES. Compared to bare GNPs and radiation alone, HepG2 cells treated with GAL-PEG-GNPs showed significant DNA DSBs and apoptosis after exposure to 6MeV X-rays irradiation and achieved better radiosensitization effects.

Polypeptide-Capped GNPs

Polypeptides are one of the most commonly used targeted modifiers. RGD (Arg-Gly-Asp) is a polypeptide polymer composed of arginine, glycine, and aspartate and constitutes the core structure for cell surface receptor recognition. The oligopeptide RGD has a high affinity for the transmembrane heterodimeric α β 3 integrin receptor, which is highly overexpressed on the activated neonatal endothelium and has been used as a tumor vascular targeting ligand since the 1980s.^{119–122} Additionally, tumor neovascularization has been widely investigated as one of the important targets for both chemotherapy and radiotherapy. Clonal cell dysfunction triggered by radiation may be due to microvascular endothelial injury.^{123,124} In a study by Kunjachan et al,¹²³ GNPs were modified by the addition of RGD to enhance intracellular retention of NPs and served to deposit more radiation dose in tumor tissues. Under the radiation exposure by image guidance, more vascular damage could be directly induced to improve the radiation effect, thereby enhancing the tumor-killing effect indirectly. Another study by Yang and colleagues¹²⁵ confirmed the clinical potential of this innovative therapy. They used RGD-modified GNPs in combination with the

chemotherapeutic drug cisplatin (also with? Radiosensitizers) and demonstrated the therapeutic effect of this combination therapy on MDA-MB-231 cells in the context of MV radiation. Even at low concentrations, the survival rate of cells treated with GNP-RGD:Cis and radiation decreased significantly, at levels much lower than the cells treated with cisplatin and radiation alone. Hence, they predicted that this GNP-mediated chemoradiation would be integrated into cancer treatment in the near future.

Cell-penetrating peptides (CPPs) are another type of short peptides that can cross cell membranes and deliver various biologically active molecules, usually consisting of ~7–30 amino acids.^{126–129} It is widely believed that macropinocytosis, clathrin-mediated endocytosis and caveolae/lipid raft mediated-endocytosis are the main routes for most CPPs and CPP-cargo complexes to enter cells. They have been used to transport various therapeutic agents into cells such as plasmid DNA, siRNA, proteins, and other nanoparticles.^{128,129} Octaarginine peptide (R8), an arginine-dependent cationic CPP, has been shown to promote drug release. Zhang et al¹³⁰ utilized R8 and PEG to functionalize the surfaces of GNPs, increase cellular uptake, improve the stability of the NPs, and prevent particle aggregation. Subsequently, they then used the colorectal cancer cell line LS180 as a model system to verify the radiosensitization effect of R8-modified GNPs on in vitro megavoltage radiotherapy. In this study, R8 as a transmembrane vector could result in efficient internalization and cellular uptake of GNP-PEG-R8. When combined with 6 MV X-rays, the cells incubated with GNP-PEG-R8 showed a significant decrease in surviving fraction, increased apoptosis, and ROS levels.

Nuclear Targeting GNPs

As a vital target of cancer radiotherapy, the nucleus has attracted wide interest. Recent studies demonstrated that therapeutic effects enhanced when nanoparticles targeted the nucleus.^{122,131–133} However, previous studies have shown that NPs enter cells through the endo-lyso pathway. In this pathway, nanoparticles are trapped either in endosomes or lysosomes and are unable to enter the cytoplasm and nucleus of cells. Nuclear localization sequences (NLSs) are specific amino acid sequences that can transport the macromolecules across the nuclear pore complex (NPC). NLS modification on the surface of nanoparticles results in the binding of NPs to nuclear transport molecules (such as importin) through NLS peptides and enter via the NPC into the nucleus. One study published in

2014¹³¹ showed that GNPs modified with nuclear targeting peptides can target cell membranes and enter nuclei of cancer cells to significantly enhance the killing effect of cancer cells in radiotherapy. The authors functionalized GNPs with multiple nuclear targeting and non-targeting peptides. Cancer cells were exposed to 2 Gy of radiation dose (radiation energy: 220 kVp) after incubation with different GNPs. It was observed that cell deaths (when incubated with targeted GNPs) increased 4-fold when compared to control cells (without GNPs), and increased 3.5-fold when compared to cells cultured with non-targeted GNPs. An increase in intracellular GNPs led to more DNA DSBs and cell death.

Nucleolin aptamer is a G-quadruplex oligonucleotide with a high affinity for nucleolin and low immunogenicity. It can transfer molecules between the nucleus and the cytoplasm in cells and cytoplasm of nuclide overexpression, and it can also act as a receptor for targeting tumor cells.^{134–137} Additionally, STAT3 (a key regulator of the EGFR-STAT3-BclXL signalling pathway), is abnormally activated in most head and neck cancers (HNC).^{138–140} A STAT3 decoy (STAT3d) can be used to reduce STAT3 activation, inhibit cell proliferation and survival, and improve therapeutic resistance in HNC. However, its use in clinical treatment is limited because of a lack of stability and specific targeting. Recently, Zhang et al¹⁴¹ developed an innovative dual treatment strategy. In the study, they synthesized GNP-NUAP-STAT3d for the combined treatment of radiation-resistant HNC. Among them, GNPs served as a delivery vehicle for STAT3d and simultaneously as the radiosensitizers while the nucleolin aptamer (NUAP) is key to specifically target the nucleolin. They demonstrated that the construct was efficiently internalized in cells and that the combination of single-dose 4 Gy irradiation resulted in potent inhibition of A431, FADU cell proliferation, and greater radiosensitization when compared to cetuximab (an anti-EGFR humanized antibody).

Tumor Microenvironment Targeted GNPs

Hypoxia and acidic pH, common features in the tumor microenvironment (TME), reduce the sensitivity of tumor cells to anticancer drugs and the reactivity to free radicals, causing hurdles in cancer therapy.^{142–146} Hence, several features of TME can be used to design active-targeting methods.^{143,144,147,148} For example, the acidic pH of TME effectively internalizes pH-sensitive nanocarriers into cancer cells and releases their therapeutic payload.^{149,150} Given the acidic pH of TME, Antosh et al¹⁵¹ modified

1.4 nm GNPs with pH-sensitive tumor-targeting agents (pH low-insertion peptide, pHLIP) to bind them to the plasma membrane lipid bilayer of cancer cells, and compared cell viability under different treatments (pHLIP-GNPs, GNPs alone, without GNPs). With 250kVP X-rays radiation, the results showed that actively targeted pHLIP-GNPs significantly reduced cell viability.

Oxygen is recognized as a radiosensitizer. However, the centers of solid tumors are often anoxic and the hypoxia-induced microenvironment of the tumor weakens the generation of ROS and oxygen-fixation reaction, resulting in poor response to radiotherapy.^{145,152–155} In addition, hypoxia-induced factor-1 is activated under hypoxia, leading to the increased expression of genes associated with angiogenesis, invasion, and metastasis of tumor cells, and promotes tumor chemo- and radio-resistance.^{156–158} Hypoxia-specific radiosensitizers or non-invasive imaging techniques for therapy of guided hypoxic regions have been used to overcome radiotherapy resistance in tumor hypoxic regions. GNPs combined with radiotherapy have been demonstrated to enhance the antitumor effect of radiotherapy to hypoxic tumors.¹⁵⁹ Despite these advances, poor delivery to hypoxic regions remains an obstacle. Interestingly, the hypoxic regions may serve as an ideal habitat for many anaerobic bacteria. Recently, bacteria have been widely applied to cancer therapy because of the selective targeting of hypoxic or anoxic regions which activates the immune response.^{142,160,161}

Inspired by these intrinsic advantages, Kefayat et al¹⁶² utilized attenuated strains of *Salmonella* Typhi Ty21a (a motile facultative anaerobic organism) as radiosensitizers and smart carriers to deliver GNPs to hypoxic tumor regions. In this study, flow cytometry and ICP-OES were used to investigate the uptake of 7 different modified GNPs (Citrate-GNPs, Gelatin-GNPs, BSA-GNPs, FA-GNPs, Glutamine-citrate-GNPs, Glut-BSA-GNPs, Glu-BSA-GNPs) by *Salmonella typhimurium* Ty21a. Out of these, FA-GNPs are the best choice for preparing the Golden Bacteria (GB). The GB can transmit more GNPs to the central anoxic areas of the tumor, which will ameliorate the radiation resistance deep inside the tumors. Furthermore, bacteria-mediated hypoxia-specific delivery of GNPs will benefit tumor radiotherapy and also contribute to the photothermal therapy (GNPs could also serve as photothermal sensitizing materials).¹⁴²

In vivo Biodistribution of GNPs

The reticuloendothelial system (RES) is an indispensable barrier of the body, that includes the liver, spleen, lymph

nodes, etc., in which the resident mononuclear macrophage system, through phagocytosis, can eliminate foreign bodies, bacteria, aging and mutated cells, as well as nanoparticles similar in size to bacteria and viruses. Evidence suggests that the biodistribution of GNPs plays an essential role in affecting the radiotherapy therapeutic response. Crucial factors such as the size, shape, charge, and surface functionalization of GNPs determine their circulation time *in vivo* and their accumulation in tumor tissues.

Prior studies indicate that surface functionalization of GNPs affects their blood circulation time and clearance rate *in vivo*. For instance, Cho et al¹⁶³ investigated the biodistribution of PEG encapsulated GNPs in tumor-bearing mice. In their findings, nanoparticles (4 and 13 nm) were distributed in organs such as liver and spleen within 7 days after intravenous injection, and their concentration reached a peak in these RES organs at 7 days. While 100 nm PEG-GNPs were distributed to RES organs and peaked within 30 min after intravenous injection and maintained high levels for 6 months. Consistent with this finding, several studies confirmed that longer PEG chain lengths helped prolong the blood circulation time of NPs.⁴⁹ To investigate the accumulation of nanoparticles in the tumor, Chou et al¹⁶⁴ injected fluorescent-labeled PEG-GNPs into tumor-bearing mice and the tumor tissue was H&E stained 24h after injection. The results showed that nanoparticles accumulated more in areas with low cell density, open blood vessels and low level of extracellular matrix, and less in areas within dense extracellular matrix and collapsed blood vessels. Moreover, various types of targeting agents such as small molecules, peptides, antibodies and nucleic acids have been used to modify nanoparticles to achieve more tumor accumulation and increase the retention time of nanoparticles in tumors.

Apart from that, size and surface charge of GNPs also affect their *in vivo* distribution and thus radiosensitization.¹⁶⁵ As discussed earlier, smaller size (< 6 nm) nanoparticles are usually eliminated within minutes from systemic circulation through renal excretion after intravenous administration, while larger nanoparticles accumulate in the RES, which leads to a decrease in tumor accumulation. Huang et al⁴¹ have showed that the tiopronin-coated GNPs with core sizes of 2 nm and 6 nm significantly increased perfusion throughout the tumor, while 15 nm nanoparticles tended to accumulate in the blood vessels around the tumor. Furthermore, studies have shown that positively charged GNPs have higher cellular uptake than negatively charged or neutral charged

GNPs, which may be due to electrostatic interactions with negatively charged cell membranes.¹⁶⁶

Therefore, nanoparticles should be designed to ensure higher tumor uptake, lower RES phagocytosis, longer blood circulation times as possible, so as to provide greater therapeutic effect within the range of acceptable biotoxicity.

The Potential Mechanisms of Radiosensitization

The use of radiosensitizers may increase the local dose and overcome the heterogeneity of response within hypoxia and rapid proliferative areas of tumors, thereby improving the contrast between tumors and normal tissues for additional therapeutic benefits.^{167,168} Regarding GNPs, experimental evidence from several *in vitro* and few *in vivo* investigations have supported the potential of GNPs as radiosensitizing agents. Radiosensitization by GNPs was initially identified as stem from initiating physical dose enhancement due to the strong photoelectric absorption of Au. However, many reports have indicated that this sensitization was also attributed to subsequent chemical and biological enhancement. These three parts are auxiliary to each other and together affect the interaction of GNPs with radiation. Although the

precise mechanisms for GNPs-induced dose enhancement have not been fully elucidated, several bold conjectures have been proposed. These mechanisms will be discussed in different sections (from three different phases) to clarify the radiation effects on biological systems (Figure 4).

Physical Dose Enhancement

The basic principle of GNPs as radiosensitizers is based on the difference of mass-energy absorption coefficients between them and soft tissues.^{169,170} The Compton effect or the photoelectric effect is the main mechanism by which photons lose energy. In the Compton effect, scattering occurs when the incident photon collides with weakly bound electrons; some energy transfers from the energetic photons to the electrons, thereby ejecting the electrons from the atom. Since these photons retain most of the energy after collisions, they tend to decelerate and have longer ranges in cases where the Compton effect dominates, which results in a very sparse distribution of ionization events. For the photoelectric effect, the incident photons are absorbed by bound electrons, and the energized electrons in the inner shell are excited to be ejected. The vacancies present in the K, L or

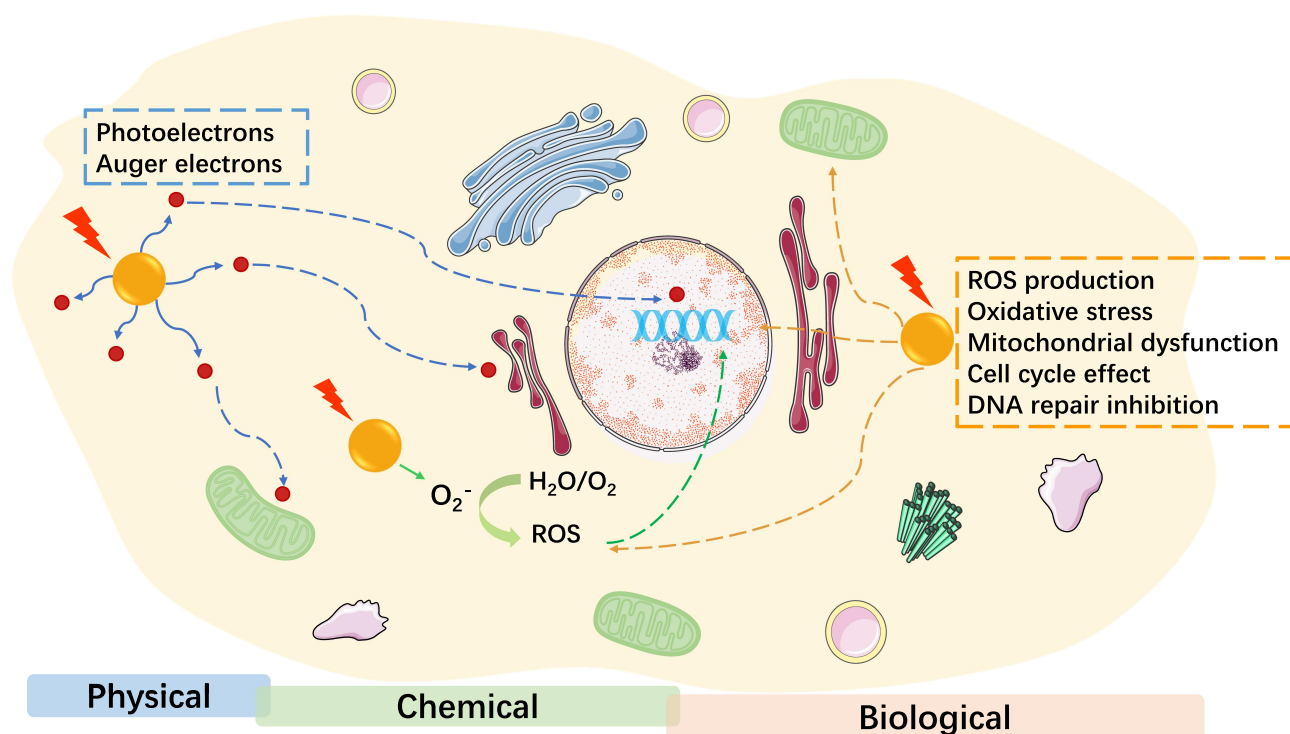


Figure 4 The potential mechanisms of GNP radiosensitization.

Notes: The potential mechanisms of GNP radiosensitization, which could be summarized as three parts: 1) physical dose enhancement, including the Compton effect and the photoelectric effect; 2) chemical contributions based on the chemical sensitization of DNA to radiation-induced damage as well as the increased generation and catalysis of radicals; 3) biological phase could be divided into ROS production, oxidative stress, mitochondrial dysfunction, cell-cycle effect, DNA repair inhibition and other biological mechanisms (such as autophagy and ER stress).

Abbreviations: ER, endoplasmic reticulum; GNPs, gold nanoparticles; ROS, reactive oxygen species.

M layers of the gold atom are usually filled by the Auger effect.^{4,169,171,172} When the outer shell layer electrons fail to fill the vacancy, the lower energy photons (fluorescence) and a cascade of secondary electrons (such as Auger electrons) are released. These low energy electrons have a range of several microns, which may lead to highly localized ionization events.¹⁷¹

The photoelectric effect is strongly dependent on the photon energy and the binding energy of the electrons in the atom. Considering the conservation of momentum, the absorption of photons in the photoelectric effect can only occur in the presence of the atomic nucleus. The X-ray cross section refers to the probability that the material interacts with radiation, depending on its atomic number. For the photoelectric effect, the X-ray cross section is approximately between Z^3 and $Z^{5.3,11,169,171}$. Consequently, by utilizing the difference in atomic number between Au ($Z=79$) and soft tissue (mainly composed of low atomic number organic materials), GNPs can provide more unit mass energy and increase the local radiation dose deposited in the target volume (tumor tissues), leading to radiosensitization.

Chemical Contributions

The “chemical mechanisms” of GNP radiosensitization are primarily involved in radical reactions and/or through inducing activation of “open” chromatin structures, which makes DNA more vulnerable to radiation-induced damage.^{3,169} Based on the subcellular localization of GNPs, two possible chemical mechanisms were proposed: 1) the chemical sensitization of DNA to radiation-induced damage; 2) the activation surface of GNPs increases the generation and catalysis of radicals. The former mechanism requires nuclear localization of GNPs to allow binding to DNA; however, in most studies, GNPs are usually limited to endo/lysosomal of the cytoplasm. Both low energy electrons (LEEs) with energies below the ionization threshold ($<10\text{eV}$) and secondary electrons play an important role in radiosensitization.^{169,173} Studies have shown that although the interaction between the LEEs and GNPs fails to produce secondary electrons, they may cause damage to a large number of DNA. This may be attributed to the formation of transient negative ions induced by LEEs, which weaken the hydrogen bond in DNA and produce chemical enhancement. In addition, this kind of chemical sensitivity also depends on the size and charge of the GNPs.^{3,173} Therefore, it is necessary to design GNPs that are localized to the nucleus and bind

to DNA, making full use of this chemical sensitization mechanism.

Concerning the second chemical mechanism of radiosensitization, the electronically active surfaces of GNPs have been proved to catalyze various chemical reactions^{3,174,175} (particularly small GNPs $<5\text{nm}$ with large surface areas exhibiting great catalytic activity) mediating the transfer of electrons from surface-bound donor groups to O_2 , thus generating free radicals. Such small-sized and high-curvature nanoparticles destroy the highly ordered crystal structure of bulk Au, and the alteration in the electron configuration of surface atoms leads to the production of free radicals on the reaction surface of GNPs.³ Alternately, the catalytic effect of GNPs also promotes the transfer of electrons and the production of ROS via the interaction between activated surface and molecular oxygen, and the catalytic performance would be further enhanced when combined with X-ray radiation. The increased ROS is closely related to the emission of photons and Auger electrons of GNPs, as well as the secondary radiolysis of water, resulting in indirect damage to DNA, proteins, and lipid membranes via oxidation, which initiates apoptosis/death.^{169,176–178}

From these data, it can be concluded that GNPs enhance and fix radiation-induced cell damage by catalyzing radical reactions and increasing the production of ROS. In addition, the elevated intracellular ROS levels may also have biological consequences through oxidative stress; this will be described in the next section.

Biological Phase

Monte Carlo simulation, as a theoretical calculation method, is often used to evaluate the dose distribution and dose enhancement ratio (DER) of GNPs.^{179,180} However, based solely on physical dose enhancement, the radiosensitization of GNPs is significant at KV energy levels, but not at MV levels.¹⁷⁹ Interestingly, the radiation dose enhancement ratio calculated using the biological model of MV radiation is significantly higher than the predicted theoretical Monte Carlo simulation values. The differences between theoretical predictions and experimental data reveal the existence of biological enhancement.^{181–184} The following three key biological pathways of radiosensitization are widely recognized: 1) ROS production, oxidative stress, and mitochondrial dysfunction; 2) cell-cycle effect; 3) DNA repair inhibition. Other possible biological mechanisms such as autophagy and endoplasmic reticulum (ER) stress have also been proposed.⁴

ROS Production, Oxidative Stress and Mitochondrial Dysfunction

One of the main mechanisms of radiation-induced cell death is the interaction of free radicals and ROS produced by water radiolysis with various cellular biomolecules, resulting in apoptosis/death.^{3,171,172,185} ROS, including superoxide anion radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH), may cause cell damage directly by interacting with biomolecules including cellular DNA or cause apoptosis or necrosis of cells indirectly through the oxidation of lipids, proteins, and DNA, as well as mitochondrial dysfunction. As described above, GNPs with different sizes, shapes, and surface functions can induce ROS formation effectively; the subsequent oxidative stress can damage cells via the interaction between critical targets or reducing substance.

Additionally, Po_2 in the tumor plays a crucial role in the efficacy of radiotherapy, and the hypoxic tumor micro-environment decreases the response of tumor cells to ionizing radiation.¹⁸⁶ Mitochondria are considered as the “energy powerhouse of cells” and amplifiers for ROS production. It is one of the vital targets that cannot be ignored in the process of radiosensitization.^{187–189} Oxidative stress-induced mitochondrial dysfunction, including mitochondrial DNA (mtDNA) damage, mobilization of cytochrome C and other biological effects, may cause apoptosis/-death.^{190,191} In addition, it has been suggested that inhibition of proteins involved in maintaining cellular oxidative homeostasis (such as thioredoxin reductase TrxR1) may also lead to GNPs-triggered oxidative stress.¹⁹² The precise mechanism of GNPs-induced oxidative stress is not well understood and further studies are needed to explore it; currently, it is considered to occur through mitochondrial dysfunction as a result of the high intracellular ROS levels.

Cell-Cycle Effect

The cell cycle affects the sensitivity of cells to ionizing radiation, and ionizing radiation exposure can delay mammalian cell-cycle progression by inducing G1 or G2 phase arrest. It is well known that cells show different radiosensitivities at different stages of the cell cycle, cells in late S-phase have the strongest radio-resistance and those in the G2/M phase are most sensitive.^{3,4,193} Activation of cell-cycle checkpoints, as one of the pathways for DNA damage following ionizing radiation exposure, maintains genomic integrity via repair of radiation-induced cell damage or prevention of cell division.¹⁹⁴ It has been reported that

GNPs can also change cell-cycle distribution and increase the accumulation of G2-/M phase cells, thus realizing radiosensitization.¹⁹⁵ The influence of GNPs on cell cycle depends on the physicochemical properties of nanoparticles and the cell line. However, some studies have also pointed out that GNPs have no significant effect on cell-cycle distribution.^{186,196,197} Therefore, further studies are necessary to elucidate the effects of GNPs on the cell cycle.

DNA Repair Inhibition

Radiotherapy can elicit a variety of DNA damage, including single-strand breaks (SSBs), double-strand breaks (DSBs), DNA-protein cross-links, and DNA base modifications. Among them, DSBs are associated with clonogenic cell killing and are the primary lethal type of radiation-induced damage. If the failure of DNA DSBs repair affects genomic stability, it results in cell death in a variety of ways. DNA damage repair is evaluated by comet assays, Western blotting, and immunostaining. Phosphorylated histone variant γ -H2AX and p53-binding protein 1 (53BP1) are considered to be the earliest sensitive markers.^{4,198,199} Dynamic monitoring of γ -H2AX and 53BP1 foci impacts GNPs on DNA repair after radiation exposure; this was confirmed in many experiments. Therefore, DNA repair inhibition seems to be another momentous biological mechanism of GNP radiosensitization. However, other studies have suggested that GNPs have no influence on DNA repair kinetics.¹⁸³ There is currently no consensus in the literature on the specific role of GNPs in the process of DNA damage repair. This requires further investigation to determine whether GNPs are involved in DNA repair inhibition.

Other Biological Mechanisms

In addition to the biological mechanisms described above, autophagy and ER stress are considered to be other possible biological mechanisms of radiation enhancement. Autophagy plays a major role in maintaining intracellular homeostasis, which promotes nutrient recycling through lysosome degradation of damaged and dysfunctional organelles, proteins, and other cellular components.^{200–203} The inhibition of autophagy in tumor cells induces anti-tumor effects,²⁰⁴ this autophagy response has positive and negative regulatory abilities to promote tumor development and is one of the radioprotective mechanisms of tumor cells.

ER stress derived from exogenous/endogenous damages resulting in impaired protein folding re-establishes ER homeostasis through activation of unfolded protein response

(UPR). If ER stress continues to develop, cell functions often worsen, leading to cell death.^{205–208} Metal-based nanoparticles such as GNPs, AgNPs, and ZnO-NPs have been reported to cause accumulation and aggregation of misfolded proteins, inducing ER stress and activation of UPR.^{209–212} As a potential treatment, ER stress may become a new target in the radiosensitization process.

Furthermore, GNPs have also been shown to directly regulate cell activity, function and behavior, or react with thiols (such as GSH) to decrease cellular defences against oxidative stress and promote persistent cell damage, thus producing synergistic effects with ionizing radiation, finally leading to apoptosis, necrosis, mitotic catastrophe, senescence and other adverse outcomes.²¹³

Preclinical Studies of GNPs

Overall, the unique physicochemical and biological properties of GNPs make them the new focus of cancer therapy. The diversity of design and function of GNPs can be developed in numerous ways aimed at enhancing radiotherapy through radiation dose enhancement. Although preclinical studies have demonstrated the possible applications of GNPs, the FDA has not yet approved their clinical use. To date, several GNPs-based nanomaterials have been analyzed in clinical research for cancer treatment:

1. phase 0 and 1 trials have been conducted on pegylated recombinant human tumor necrosis factor (rhTNF)-coated GNPs (CYT-6091)²¹⁴ for the treatment of primary, advanced and metastatic solid tumors (clinicaltrials.gov, NCT00436410, NCT00356980);
2. pegylated silica core-Au shell nanoparticles (AuroShell[®]) have been used for photothermal therapy of the head and neck and lung cancers (clinicaltrials.gov, NCT00848042, NCT01679470);
3. in an early Phase 1 trial, Bcl2-L12 targeted NU-0129 GNPs (the trial drug consisted of nucleic acids arranged on the surface of spherical gold nanoparticles) has been used in the treatment of patients with recurrent glioblastoma multiforme or gliosarcoma (clinicaltrials.gov, NCT03020017);
4. gold nanoparticles-based Nano-Ayurvedic drugs: Nano Swarna Bhasma (NSB), approved by the Indian government regulatory authority of AYUSH (DNA_SPN_B001_17), has been used in female patients with pathologically confirmed invasive breast cancer stage IIIA or IIIB and showed acceptable safety and excellent efficacy.²¹⁵

These studies provide strong evidence for the clinical potential of GNPs. However, as mentioned by the National Cancer Institute (NCI)-Radiation Therapy Oncology Group in translational program strategic guidelines for the early-stage development of radiosensitizers, the clinical transformation of any radiosensitizer is rarely a seamless transition directly from the bench to the bedside.²¹⁶ Therefore, the clinical applications of GNPs require further exploration.

Since preclinical data and clinical trials in the field of GNPs-mediated radiation sensitization are currently sparse, we would like to summarize and discuss the current situation and obstacles of this treatment here. One of the key factors to be considered for the smooth conversion of GNPs is their design and selection. The physicochemical properties, biodistribution, pharmacokinetics and molecular mechanisms of GNPs should conform to the practical clinical considerations. Secondly, the safety and long-term toxicity associated with retention of GNPs entering the human body cannot be ignored, although gold is relatively non-toxic and biocompatible. As with other pharmaceutical radiosensitizers, all GNP formulations used for clinical evaluation need to be rigorously tested and combined with other drugs and/or treatments to ensure their safety in vivo. Since GNPs-mediated radiation sensitization has been shown to achieve better sensitization effects at keV energy, and radiation types commonly used in clinical settings are megavoltage radiation, charged particles, or brachytherapy, further optimization of radiotherapy techniques may be necessary to achieve optimal radiotherapy efficacy. Another challenge to the clinical translation of GNPs-mediated radiation sensitization is the choice of the target population. Most patients could benefit from pre-operative adjuvant radiotherapy and postoperative radiotherapy, however, due to the limitation of normal tissue availability, the local dose escalation is not feasible. At present, there are no exact criteria to screen the study population, and there is a lack of appropriate biomarkers or clinical predictors to evaluate whether the study population can benefit from GNPs-mediated radiation sensitization.⁴⁹ Therefore, further preclinical studies are necessary to achieve optimum clinical translation.

Conclusion and Outlook

The main optimization of radiation therapy is to reduce the side effects of radiotherapy and increase the survival of

healthy tissues. The use of radiosensitizers may help to achieve this goal. As a potential radiosensitizer, GNPs have been widely recognized in experimental and theoretical studies. From the basic knowledge of the photoelectric effect and other related effects, the combination of GNPs and radiotherapy may significantly improve treatment efficacy. In this review, we first briefly summarize the radiosensitization effects of GNPs with different surface modifications in cancer radiotherapy, describe the in vivo distribution of GNPs, and explain in detail their radiosensitization mechanisms. Then, we collected the data of pre-clinical and clinical studies on GNPs in recent years and discussed the difficulties of its clinical transformation. Overall, GNPs could act as radiosensitizers in cancer radiotherapy which may open new opportunities for the development and progress of cancer radiotherapy, and improve the clinical efficacy of radiotherapy in various cancers.

With regards to the transformation of GNPs-enhanced radiotherapy into commercial clinical practice, although many in vitro and in vivo studies have shown promising results, numerous challenges such as physicochemical properties, drug metabolism, and pharmacokinetic (DMPK) screening, safety concerns, in vivo efficacy, biocompatibility and stability, preparation costs and immunogenic issues remain. The mechanisms of action at the molecular and cellular levels including the potential impact on cellular signaling are yet to be fully studied, which would limit its clinical transformation. In order to solve these problems, it is necessary to further optimize GNPs and elucidate the precise mechanisms of radiosensitization to achieve better therapeutic effects, accelerating the translation of GNPs into clinical practice and reach the patients in need.

Abbreviations

GNPs, gold nanoparticles; Z, atomic number; EPR, enhanced permeation and retention; RES, reticuloendothelial system; REF, radiosensitization enhancement factor; MDR, multi-drug resistance; PEG, polyethylene glycol; DEF, dose enhancement factor; DOX, doxorubicin; BSA, bovine serum albumin; GBM, glioblastoma multiforme; HGNNPs, hollow GNPs; FA, folic acid; HER2, human epidermis growth factor receptor 2; DSBs, double-strand breaks; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; GLUT, glucose transporter; ASGPR, asialoglycoprotein receptor; GAL, β -D-galactose; RGD, Arg-Gly-Asp; CPPs, cell-penetrating peptides; NLSs, nuclear localization sequences; NPC, nuclear pore

complex; HNC, head and neck cancers; STAT3d, STAT3 decoy; NUAP, nucleolin aptamer; TME, tumor microenvironment; pHLIP, pH low-insertion peptide; GB, golden bacteria; LEEs, low energy electrons; ER, endoplasmic reticulum; mtDNA, mitochondrial DNA; SSBs, single-strand breaks; UPR, unfolded protein response; TNF- α , tumor necrosis factor- α ; ROS, reactive oxygen species.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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