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Iron based nanotherapeutics for ferroptosis-induced cancer therapy

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Abstract. – Traditional anti-cancer treatments are far from satisfactory. There is an urgent to combine new therapeutics with traditional treatments to improve anti-cancer effectiveness. Ferroptosis is a new type of iron dependent non-apoptotic cell death could still offer benefits to patients who failed in apoptosis and necroptosis induction treatment. Iron plays a vital role during ferroptosis induction. While iron is a double-edged sword in cancer treatment, tumor specific distribution of iron is especially important. Nanotechnology is an efficient way to help drugs targeting distribution. We intended to review the latest progress in ferroptosis and iron based nanotherapeutics. First, the relationship between ferroptosis and iron metabolism was reviewed briefly to demonstrate the central role of iron in ferroptosis induction. Second, the latest progress of iron-based nanotechnology was presented and discussed according to the different designs. Finally, the future expectations of iron based nanotherapeutics for ferroptosis were spotlighted.

Key Words:
Ferroptosis, Iron Metabolism, Nanotherapeutics, Cancer therapy.

Introduction

Traditional therapeutic approaches including surgery, chemotherapy and radiotherapy have limited progress for cancer treatment in recent years because of their inherent limitations and heterogeneity of tumors[1-3]. Research about the mechanism of cancer cell death is a promise way to find out effective therapeutic approaches. Triggering apoptotic cell death is an effective approach to kill cancer cells. However, the effectiveness of apoptosis induction is limited because of the acquired or intrinsic resistance of cancer cells to apoptosis[4-6]. Recently as new findings come out, various new types of cancer death were found, including necroptosis, ferroptosis, pyroptosis and parthanantos, etc.[7-9].

Ferroptosis is a new type of non-apoptotic cell death found and named by Dixon et al[10] in 2012, defined as an iron-catalyzed form of regulated necrosis that occurs through excessive peroxidation of polyunsaturated fatty acids (PUFAs)[11-13]. A growing number of studies indicate ferroptosis is a promised approach for cancer treatment because of the potent tumor suppressive ability[14] and propagation among cells in a wave-like manner, which exhibits a potent killing effect on neighboring cells[14-16]. Compared with other types of cell death influenced by caspases activity directly or indirectly, ferroptosis seems to have little known molecular cross-talk to other types of cancer death[17]. Ferroptosis has distinctive mitochondria morphology, biochemistry and gene expression compared with other forms of cell death, with increased membrane density and smaller size of mitochondria, normal level of intracellular ATP, and Gln-, CS-, and ACSF2-regulated lipid synthesis required for ferroptosis[10]. The emerging evidences up to now indicate patients could still get benefits from ferroptosis induction treatment who failed in apoptosis and necroptosis induction treatment[18]. As an iron dependent type of cell death, the activity of ferroptosis is mainly dependent on the amount of bioavailable ferrous iron (Fe^{2+})[19]. Iron based Fenton reaction plays a vital role in ferroptosis induction. Targeting iron supply and metabolism has been regarded as a promising strategy for ferroptosis induction.

Nanotherapy has obtained great advance in improving efficiency of therapeutic drugs. As the

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understanding of human physiology and pathology progresses, a lot of new types of functional nanoparticles (NPs) were synthesized to control drug bio-distribution, cell targeting, drug stability and drug release kinetics. As a new onset type of non-apoptotic cell death, many nanotherapeutic approaches has been applied to ferroptosis induction, mainly focused on iron supply and drug delivery (Figure 1). However, the effect of ferroptosis-driven NPs is far from satisfactory. In this review, we briefly described the relationship between ferroptosis and iron metabolism. The latest development of iron-based NPs applied in ferroptosis induction was reviewed, and finally we provide a future perspective on this emerging field.

Ferroptosis and Iron Metabolism

Ferroptosis is recognized as iron-catalyzed excessive peroxidation of PUFA-containing phospholipids, having a typical necrotic morphology, along with shrinking small mitochondira[10,20]. Iron is a double-edged sword in the cancer process. Excess Fe may cause the cumulation of reactive oxygen species (ROS), which may initiate tumor formation, growth and metastasis. However, Fe can also work as cancer defender to induce ferroptosis and synthesize Fe-regulatory proteins to display antitumor properties[21]. Iron is recognized as the sword to induce ferroptosis mainly through Fenton and Fenton-like reaction to initiate lipid peroxidation[22,23], though there are other manners to induce lipid peroxidation, for example, through the lipoxygenase family, which are non-heme iron-containing enzymes catalyze PUFAs into various lipid hydroperoxides[24]. While glutathione peroxidase 4 (GPX4)-associated pathways are considered as the shield to defense ferroptosis to protect membranes against peroxidation damage[25]. GPX4 is responsible for phospholipid hydroperoxides (PPHs) removing. Inhibiting GPX4 pathway to produce more PPHs is another strategy to trigger an iron-based catalytic reaction that eventually causes cell death though ferroptosis[26].

Iron (Fe^{2+}/Fe^{3+}) plays a vital role in ferroptosis induction, not only as catalyst to induce Fenton and Fenton-like reactions, but also decomposed PPHs to alkoxyl phospholipid radical[27]. Iron-based ferroptosis induction can be divided into three parts according to the different types of iron metabolism: iron loading based lipid peroxidation, iron oxidation based lipid peroxidation (Figure 2A) and labile iron pool (LIP)-based ferroptosis induction (Figure 2B).

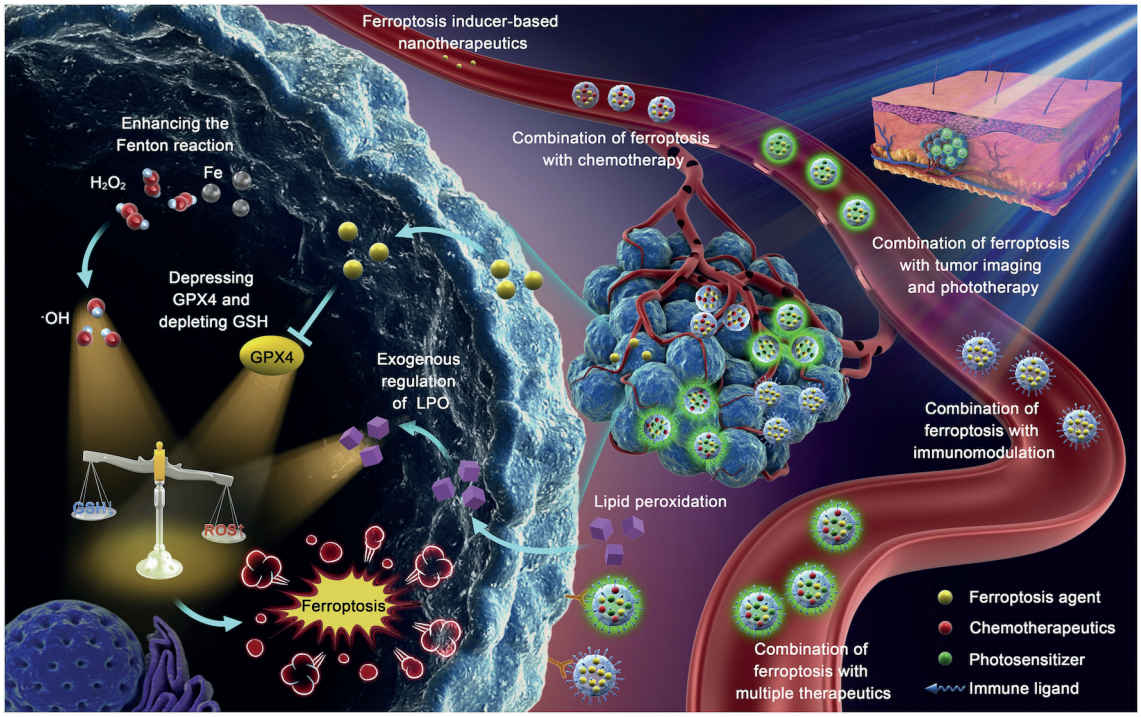

Figure 1. Schematic diagram of tumor cell ferroptosis and ferroptosis-driven nanotherapeutics. Reproduced with permission from (31917296).

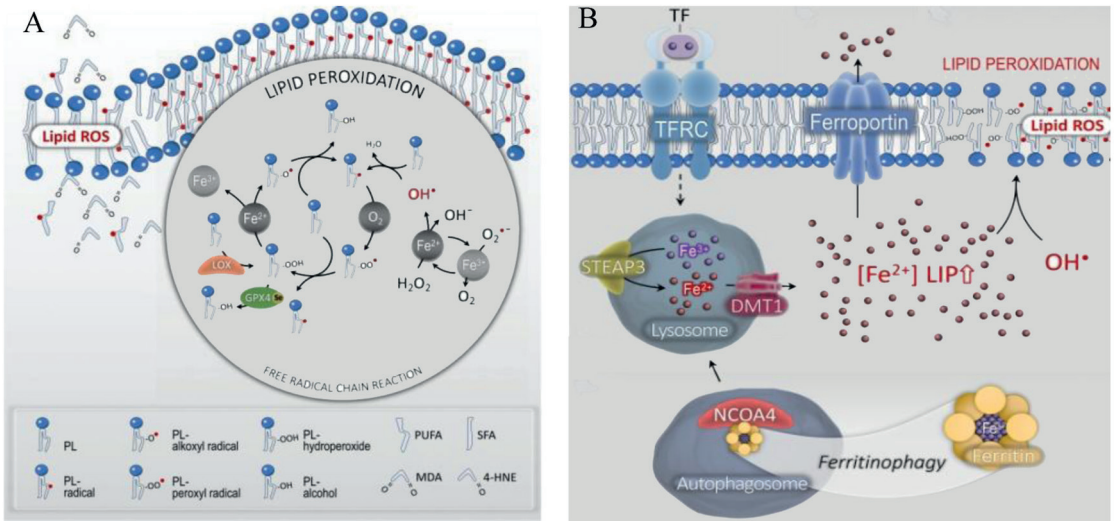

Figure 2. Lipid Peroxidation Process and Iron metabolism by increasing the levels of intacellular labile iron pool (LIP). (A) iron-based lipid peroxidation process, (B) LIP regulated by transferrin, TFRC and ferritinophagy. Reproduced with permission from (31105042).

Iron Loading Based Lipid Peroxidation

Iron loading based lipid peroxidation is defined as ROS inducing oxidation of PUFAs through a free radical-driven chain reaction catalyzed by iron externally supplied. Hydroxyl radical (•OH) is the main form of ROS to initiate lipid peroxidation, which produced by iron-catalyzed Fenton reaction consisting of a transition metal (Fe^{2+}/Fe^{3+}) and hydrogen peroxide (H_2O_2). As the auto-amplifying lipid radical chain reaction catalyzed by iron and oxygen process, the membrane of cancer cell will finally be destroyed because of overloaded ROS and lipid peroxidation. Apart from ferric ion (Fe^{2+}/Fe^{3+}), other types of Fe can also induce ferroptosis through Fenton reaction. Huo et al[28] demonstrated PEGylated sing-atom Fe-containing nanocatalysts could effectively trigger Fenton reaction to generate abundant toxic hydroxyl radicals under the acidic tumor microenvironment (TME). Huang et al[29] also considered zero-valent iron nanoparticles can induce ferroptosis by mitochondrial lipid peroxidation and GPX4 reduction in subcellular organelles . Iron chelation also has ability to catalyze Fenton reaction. Sagasser et al[30] demonstrated iron salophene complexes Chlorido[N,N'-disalicylidene-1,2-phenylenediamine] iron(III) complexes can generate lipid-based ROS and induce ferroptosis. Though Fe supplied externally is an effective strategy to induce ferroptosis of cancer cells, we should fist carefully evaluate the targeting capacity of Fe supplied. Excess Fe incorporated is associated with a lot of diseases including the development of cancer[31].

Iron Oxidation Based Lipid Peroxidation

Besides the concentration of Fe^{2+}/Fe^{3+}, the proportion of Fe^{2+}/Fe^{3+} is also very important in iron associated lipid peroxidation with or without enzyme catalyzing[13]. The amount of hydroxyl radicals produced by Fenton reaction is largely depend on the conversion rate of Fe^{2+} and Fe^{3+} which is indirectly associated with proportion of Fe^{2+}/Fe^{3+}. At the same time, in enzymatic lipid peroxidation, Fe^{2+} works as an important reductant to help lipoxygenase catalyze the deoxygenation of PUFAs and generate PPHs. To influence cancer cell ferroptosis by Iron oxidation is another important strategy for iron-based nanotechnology. Gaschler et al[27] mentioned $FINO_2$ has the ability to cause widespread lipid peroxidation through directly oxidizes iron. While iron oxidation has its intrinsic mechanism to induce ferroptosis, certain compounds to oxide Fe^{2+} into Fe^{3+} with little side-effect are hard to synthesize. Further studies about iron oxidation and ferroptosis are needed to carefully explain iron oxidation based lipid peroxidation.

LIP-Based Ferroptosis Induction

LIP indicates a metabolically active pool of chelatable and redox-active Fe^{2+} that represents a transient reservoir for Fe[32], which can directly catalyze Fenton reactions and induce ferroptosis. According to iron metabolism, non-heme Fe is absorbed by divalent metal transporter 1 (DMT1)[33] from dietary Fe and storaged as cytoplasmic ferritin[34] or delivered to the plasma by basolateral

transporter ferroportin (FPN1)[35,36]. In the plasma, Fe^{3+} binds to the protein transferrin (Tf) to form diferric Fe transferrin complex (Tf-$[Fe^{3+}]_2$) and assimilated into Endosome by receptor-mediated endocytosis (RME)[37]. Endosomal Fe^{3+} is reduced to Fe^{2+} by "Steap" protein family[38] and transported out of the endosome into LIP by DMT1[39]. LIP-based ferroptosis induction indicates increasing the LIP by modulate proteins mentioned above, for example, increased expression of heme oxygenase 1 (HMOX1) and transferrin and decreased expression of ferroportin[40, 41].

Iron-Based Nanotherapeutics For Ferroptosis Induction

Iron is the most abundant heavy metal in human, absorbed from food via DMT1[33]. Excess iron is considered the most common cause of oxidative stress and carcinogenesis, while there is no active metabolic pathway to release away from the body[42]. Though iron concentration is closely associated with ferroptosis induction as mentioned above, cancer cells can resist iron-induced oxidative stress in a wider range through genetic alteration compared with normal cells[43]. Nanotechnology has been widely used in tumor treatment because of unique functions supplied through rational design and synthesize. There are several aspects to evaluate the effectiveness of nanotechnology, including biocompatibility, physical characteristics and target distribution. As iron plays a vital role in ferroptosis induction, iron-based nanotechnology has been the most important approach to control tumor cell ferroptosis.

Biocompatibility

Polymers have been widely used in surface modification of nanomedicine either as a solubilizers, stabilizers, release-modifiers, bioavailability enhancers, carriers for drug payload, or to provide mechanical support as in bone scaffolds[44]. Among the US FDA-approved biodegradable polymers, polyethylene glycol (PEG) is always a benchmark and a polymer of choice because of its ability to increase the blood half-life, enhance aqueous solubility, protect against *in vivo* biological inactivation, and reduce immunogenicity[45]. PEG is also the most frequently used surface modification polymers in iron-based NPs. Zheng et al[46] indicates PEGylated FePt/MoS_2-folic acid (FA) NPs showed superior colloidal stability and better tumor cells recognition through FA receptor. Zhao et al[47] conjugated thiol-terminated polyethylene glycol (SH-PEG-NH_3) to yield a high affinity Au-S-bond to reduce the aggregation of NPs. Huo et al[28] PEGylated SAF NPs with DSPE-PEG-NH_2 via hydrophobic-hydrophobic interaction after violent sonication to improve physiochemical stability. Except PEG, there are also some other polymers as surface modification to give specific functions. Bao et al[48] synthesized an up-conversion nanoparticle and doxorubicin encapsulated in an oxidized starch-based gel nanoparticle, cross-linked by Fe^{3+} ions. Polyethylenimine and 2,3-dimethylmaleic anhydride (DMMA) were further decorated on the surface to offer a negatively charged surface after intravenous injection to prolong the circulation time and provide more opportunities to reach tumor site via enhanced permeability and retention effect (EPR) effect (Figure 3). Good biocompatibility and long circulating time are the basic requirements for NPs used *in vivo*. Ferroptosis-driven NPs is a quite young emerging field that researches about biocompatibility is mainly focused on PEGylated surface modification. Further studies about other biocompatible polymers, such as poly (L-lactic acid) (PLA), poly (D, L-lactic-co-glycolic acid) (PLGA) and polycaprolactone (PCL), are needed in future.

Physical Characteristics

In addition to PEGylation of nanoparticle surface, the size and shape of NPs are also important for blood half-life. So, NPs smaller than 6 nm will be excreted by the kidneys quickly[49]. While NPs larger than 200 nm will accumulated in the spleen and liver by MPS cells[50]. Blood vessels of tumors produced by angiogenesis are always poor and high fenestrations, which allow NPs to accumulate in the tumor during circulation. This phenomenon we termed EPR effect. The ideal diameter to produce long-circulating NPs is between 30 nm and 200 nm[51]. The sizes of iron-based NPs used in ferroptosis induction are all in this desirable range, ranging from 42 nm[52] to 198 nm[53]. Shapes of NPs is another important determinant of biodistribution *in vivo*. rod-shaped NPs obtained ten times longer circulation time than spherical-shaped NPs[54]. While up to now there are no other shapes except spherical shape used in iron-based NPs. Size shrinkable NPs triggered by tumor acidic PH, light or overexpression of MMPs in tumors are also a popular way to improve penetration and distributio[55]. Though there are no size shrinkable NPs used in iron-based nanotherapy, ferroptosis-driven nanomedicine synthesized depend on the size shrinkable principle seems to be a promised future.

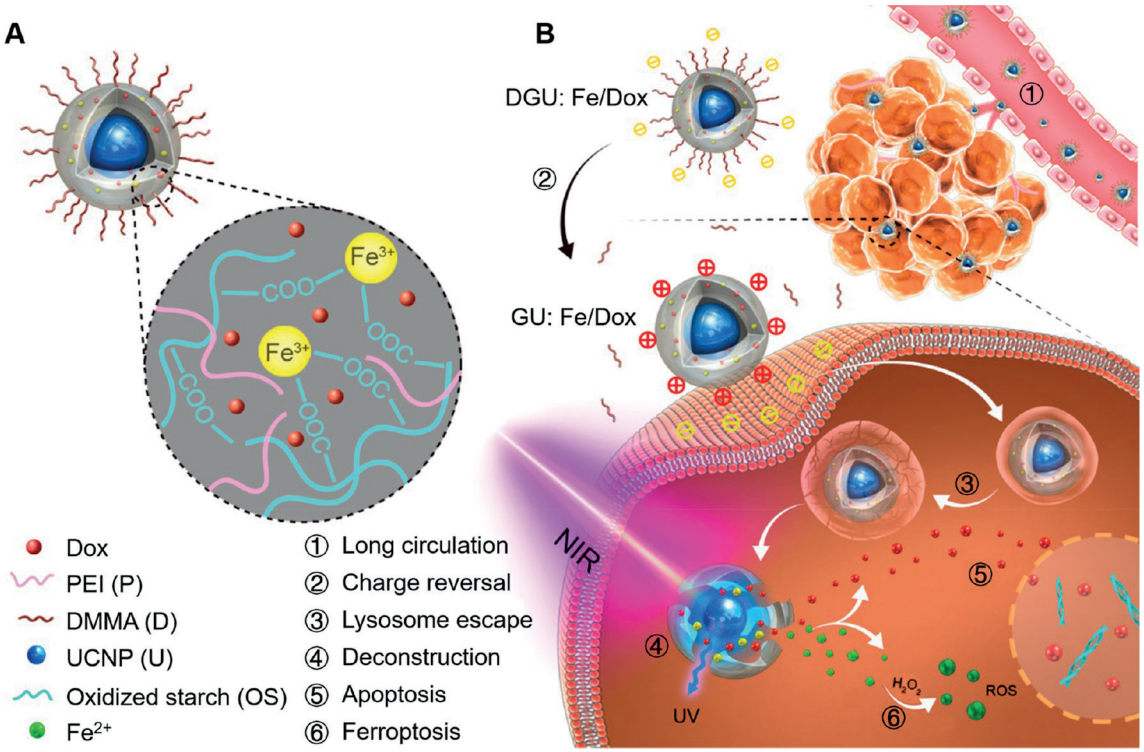

Figure 3. Schematic Illustration of Nanologan with Multiple Conversions and the Corresponding Anticancer Mechanism. Reproduced with permission from (30616348).

Target Dstribution

Site-specific drug delivery is still a great challenge for nanomedicine based tumor treatment because of high heterogeneity of solid tumor *in vivo*. Although there is a plenty of nanocarriers for delivering iron available, only few of them have tumor targeting function. These iron delivery nanomaterials thus typically exhibit nonspecific action and uncontrollable distribution, and may cause significant side-effects when applied *in vivo*, with delivery mainly through EPR effect[14,56,57]. To overcome the poor targeting function of iron containing nanocarriers, several technologies have been applied besides EPR effect. Fe^{2+}/Fe^{3+} is not only a good contrast for MRI which is always used in NPs tracing *in vivo*, but also a good magnetic targeting with MRI. Several studies have synthesized iron containing magnetic NPs accumulating in tumor sites under MRI guidance to induce ferroptosis[58-61]. Apart from the intrinsic magnetic characteristic of iron containing NPs, adding specific functional groups on the surface of NPs targeting tumor cells is another feasible approach. Several studies synthesized iron based NPs functionalized with the tumor-targeting moiety HS-PEG-FA to endow them with biocompatibility and targeting capacity[46,62]. According to different types of tumor cells, specific functional groups can also be used in tumor cell targeting NPs synthesize, for example endothelin-3 for melanoma[63] and intergrin $\alpha_v\beta_3$ for orthotopic glioblastoma[60] (Figure 4). Stimuli-sensitive NPs responded to external stimulation, such as light, temperature or magnetic field, or TME, such as hypoxia, enzyme or pH value, are another important style of tumor-targeting nanotechnology. Photothermal therapy (PTT) has obtained more and more attention in cancer treatment due to its non-invasion and superior therapeutic effect[64, 65]. At the same time, the Fenton reaction may be accelerated by the elevated local temperature due to the photothermal conversion, which may also result in enhanced ferroptosis by PTT[66]. Several studies have synthesized kinds of polymers to encapsulate iron-based NPs to absorb light in the near-infrared region to elevate local regional temperature, which makes them possible to enhance the therapeutic efficacy through the combination of ferroptosis[46,48,52,58,59,63,66-68]. Zhang et al[52] synthesized a novel nanoprobe consisting of upconversion luminescence nanoparticles as a core and coordinatively unsaturated Fe^{3+}-containing Fe^{3+}/gallic acid complex as a shell (Figure 5). Fe^{3+}in the nanoprobe can be released only in the tumor

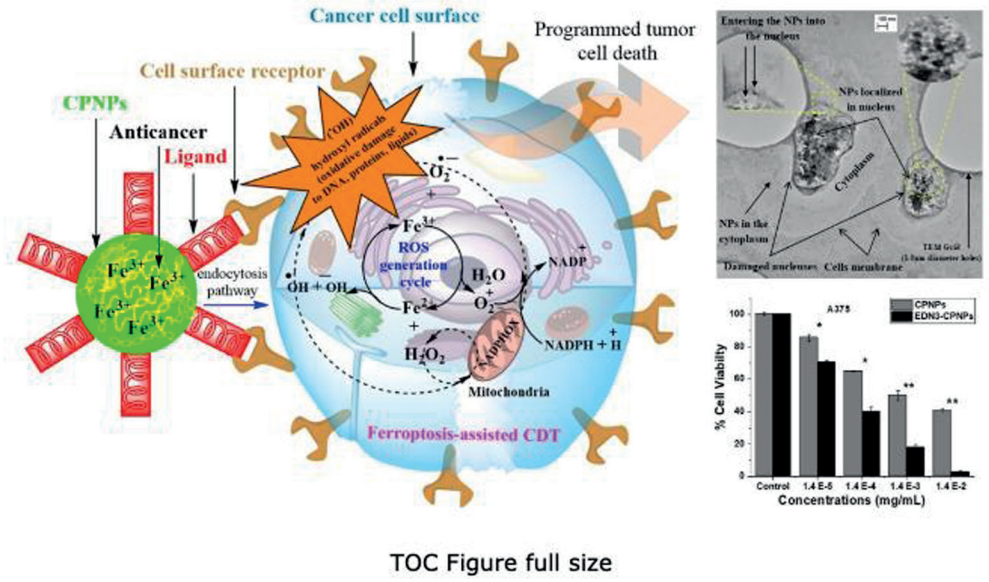

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Figure 4. Schematic illustration of Fe^{3+}contained EDN3-CPNPs targeting melanoma through overexpressed EDNRB receptor on the surface of cancer cells to induce ferroptosis. Reproduced with permission from (31613630).

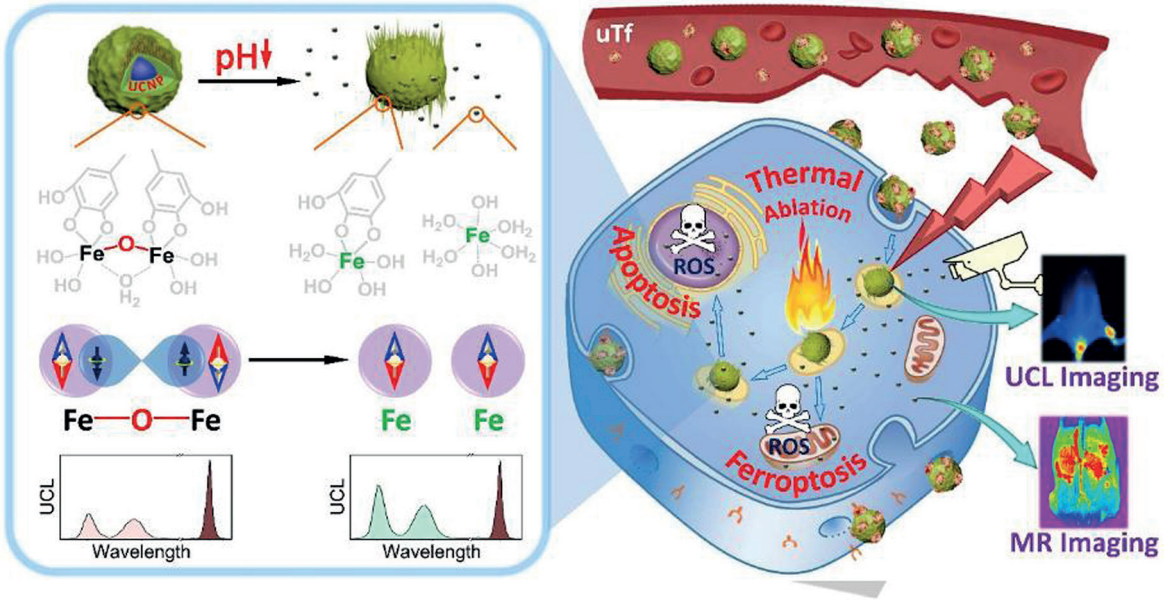

Figure 5. Schematic illustration to demonstrate the activable function of UCNP@GA-Fe(III) probe for MRI and its therapeutic function involving multiple pathways. Reproduced with permission from (31131511).

microenvironment in response to the lightly acidic pH value. In order to increase targeting capacity, two or more strategies will be adopted during NPs synthesize.

Combined Therapy

Chemotherapy and immunotherapy have gained more and more attention as non-surgical therapeutic approaches for cancer treatment[69,70]. While chemotherapy or immunotherapy alone has certain disadvantages which limited their therapeutic efficacy, including systemic toxicity with narrow therapeutic windows, poor tumor-targeting capacity, and quickly drug resistance[69,71]. Two or more types of cancer treatments combined is a promise strategy to get satisfactory therapeutic efficacy in cancer treatment. The multikinase inhibitor sorafenib is the only first-line drug for

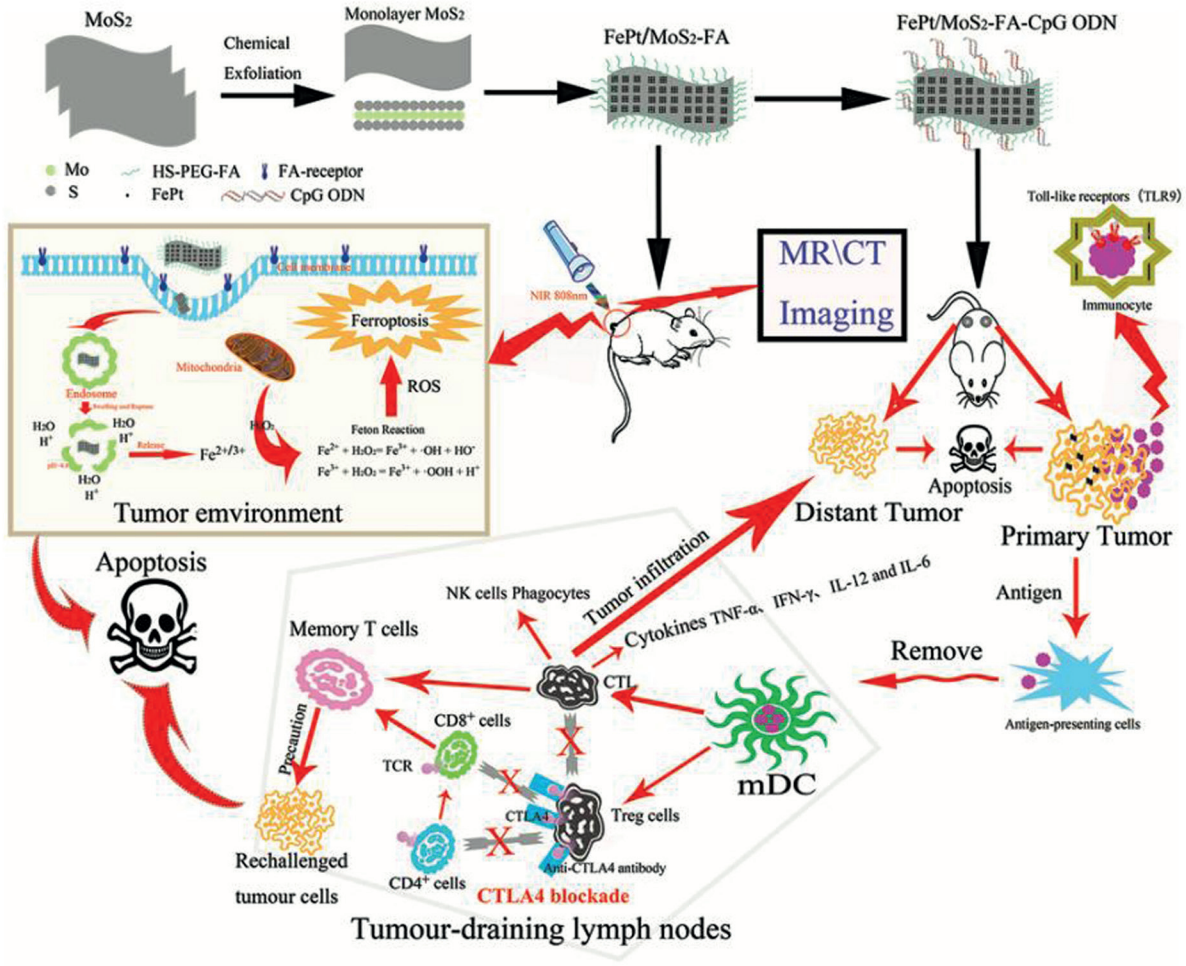

Figure 6. The fabrication process of FPMF nanocomposites and the mechanism of anti-tumor immune responses by FPMF@CpG ODN nanocomposites by combining chemotherapy, PTT and immunotherapy for anticancer therapeutic applications. Reproduced with permission from (31599915).

advanced HCC[72]. However, sorafenib can only contribute two months compared with placebo group in survival time because of quickly drug resistance appeared in patients. Some studies indicated sorafenib resistance has closely connection with ferroptosis inhibition. So sorafenib combined with ferroptosis induction seems to be a promise strategy to overcome sorafenib resistance. Sorafenib combined with iron-based NPs showed better tumor targeting capacity and ferroptosis-driven therapeutic effect compared sorafenib alone[58,59]. Even more, Zhang et al[46] synthesized multifunctional FePt/MoS_2-FA nanocomposites to eliminate primary tumors and prevent tumor relapses by combining chemotherapy, photothermal therapy and immunotherapy, and showed great promise for anticancer therapeutic applications (Figure 6).

Conclusions

Although great progresses have been obtained in cancer treatments recent years, the outcomes of various approaches for cancer cells are far from satisfactory. The most important reason is cancer cells quickly become resistant to treatments available. Ferroptosis is a new type of non-apoptotic cell death different from apoptosis and necroptosis. The emerging evidences up to now indicate patients could still get benefits from ferroptosis induction treatment who failed in apoptosis and necroptosis induction treatment, which gives us a chance to win the battle of cancer treatment. As an iron dependent type of cell death, iron plays a vital role in ferroptosis induction. While iron is a double-edged sword in cancer treatment, tumor specific distribution of iron is especially import-

ant. The appearing of nanotechnology in anticancer drug delivery offers the opportunity for tumor-specific iron supply. This review briefly described the relationship between iron metabolism and ferroptosis and emphases on the development of iron-based NPs used in ferroptosis induction and rational design of iron-based NPs.

Although iron-based NPs for ferroptosis induction has made great progress, as a newly emerging field, it still has many limitations. The modern nanomedicine has developed many advanced theories, such as size shrinkable drug delivery nanosystems and TME stimulation. While ferroptosis-driven NPs is still focus on simple encapsulation and external stimulation, which cannot get satisfactory results because of complicate situations of tumor *in vivo*. There is still a long way to go to develop desirable iron-based NPs for ferroptosis induction.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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