Overview

Human ferritin for tumor detection and therapy

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Ferritin, a major iron storage protein found in most living organisms, is composed of a 24-subunit protein cage with a hollow interior cavity. Serum ferritin serves as a critical marker to detect total body iron status. However, recent research reveals a number of novel functions of ferritin besides iron storage; for example, a ferritin receptor, transferrin receptor 1 (TfR1), has been identified and serum ferritin levels are found to be elevated in tumors. A particular new finding is that magnetoferritin nanoparticles, biomimetically synthesized using H-chain ferritin to form a 24-subunit cage with an iron oxide core, possess intrinsic dual functionality, the protein shell specifically targeting tumors and the iron oxide core catalyzing peroxidase substrates to produce a color reaction allowing visualization of tumor tissues. Here we attempt to summarize current research on ferritin, particularly newly identified functions related to tumors, in order to address current challenges and highlight future directions. © 2013 Wiley Periodicals, Inc.

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INTRODUCTION

Ferritin was discovered in 1937 by Laufberger, who isolated it from horse spleen. It was subsequently found in many other organisms, including humans and other mammals, plants, fungi, and bacteria.[1–3] In spite of large variations in amino acid sequences from bacteria to humans, ferritins have essentially the same architecture.[4] The typical structure of ferritin is a 24-subunit spherical protein encapsulating an iron oxide core.[4] Mammalian ferritins are mainly present intracellularly in the cytosol, as well as in the nucleus and the mitochondria. Extracellular ferritins are found in fluids, such as serum as well as synovial and cerebrospinal fluids (CSF).[5] The cytosol ferritins play an important role in iron storage and detoxification, but the physiological function of secreted ferritin is still unclear. It has been shown that elevated serum ferritin levels are linked to inflammation, angiogenesis, and tumors,[3,5,6] and this is therefore considered a marker for these conditions.

With the emergence of nanotechnology, ferritin nanoparticle has been biomimetically synthesized using H-chain ferritin as a template,[7–12] which self-assembles to form a 24-subunit cage-like nanostructure, with an internal iron oxide core. This engineered ferritin, which has the same architecture as natural H-ferritin, is termed magnetoferritin. Because of the unique architecture, magnetoferritin provides an ideal nanoplatform for multifunctional loading to enhance the functionality of its surface (e.g., to target tumors) and metal cations can be encapsulated in the interior (e.g., contrast imaging probes). A recent breakthrough finding is the identification of a ferritin receptor, transferrin receptor 1 (TfR1).[13] It has also been demonstrated that magnetoferritin can be used to directly target and visualize tumors.[14] These findings bring us new insight to better understand the physiological functions of ferritin and allow its application as a powerful nanoplatform for cancer diagnosis and therapy.

FERRITIN: STRUCTURE AND FUNCTION

Members of the ferritin superfamily are spherical proteins composed of 24 subunits with an outer

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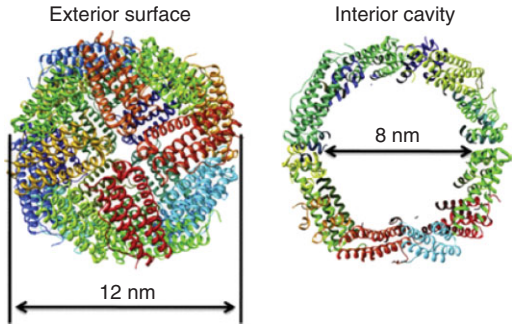

FIGURE 1 | Ribbon diagrams of exterior surface view and interior cavity of human heavy chain ferritin. (Reprinted with permission from Ref 18. Copyright 2010 Elsevier).

diameter of 12 nm and interior cavity diameter of 8 nm. The interior cavity can accommodate up to 4500 Fe(III) atoms as an iron mineral core, traditionally described as ferrihydrite[4] (Figure 1). Apoferritin refers to the iron-free form of the protein, and the iron-containing form is termed holoferritin or simply ferritin. The apoferritin shell is composed of 24 subunits, a mixture of ferritin H-chain and ferritin L-chain, arranged with fourfold, threefold, and twofold symmetry axes, of which the threefold axis form hydrophilic channels that allow transport of metal in and out of the protein cage.[2,4] The H-chain is named for its initial isolation from heart, whereas the L-chain is named for its initial isolation from liver.[15] In humans, the H-chain is also heavier with a molecular mass of 21 kDa, whereas the L-chain has a molecular mass of 19 kDa.[2,4] Therefore, the ferritin subunits are sometimes referred to as heavy (H) and light (L) ferritins, respectively.

The function of H-ferritin differs from L-ferritin. The H-chain is important for Fe(II) oxidation because it possesses the ferroxidase center which can catalyze the oxidation of Fe(II) to Fe(III), while the L-chain lacking this center assists in the iron core formation. This is the reason why L-ferritin, unlike H-ferritin, is iron poor. Moreover, the ratio of these two subunits in ferritin varies widely depending on tissue type, with H-chains predominant in the heart and L-chains predominant in the liver.[15] In addition, the H/L ratio can be modified in inflammation and other pathological conditions.

Besides their characteristic architecture, ferritins possess unique physical and chemical properties. Unlike most other proteins, which are sensitive to temperature and pH outside of the physiological range, ferritin is able to bear high temperatures up to 75°C for 10 min and is stable in various denaturants such as urea or guanidinium chloride. These unique features are owing to the fact that ferritin contains large numbers of salt bridges and hydrogen bonds formed between subunits.[4,16] An interesting recent finding is that the assembly of ferritin, despite its rigidness under physiological conditions, is pH-dependent.[17] The ferritin architecture can be broken down in an acidic environment and restored, almost completely, by returning the pH back to physiological conditions.[16–18] These unique properties make ferritin an ideal and powerful nanoplatform on which to construct multifunctional nanoparticles for imaging and delivery of drugs.

Natural human ferritin exists in both intracellular and extracellular compartments. In most tissues, ferritins are mainly present in the cytosol, nucleus, and mitochondria, and play a role in iron storage as well as iron homeostasis. Iron, a major trace element, is both potentially toxic and essential for life. Iron is an integral component of many proteins. The presence of free Fe^{2+}ions is lethal because they catalyze formation of reactive hydroxyl radicals in oxygenated tissues by the Fenton reaction, leading to damage of DNA, lipids, and proteins.[4,15] The balance between iron storage and utilization is maintained by regulation of intestinal absorption of the metal from the diet, along with the expression of iron transport and storage proteins, including ferritin, transferrin (Tf), and the transferrin receptor. In addition to iron storage, ferritins also play an important role in iron detoxification, by capturing and sequestering the intracellular labile iron pool. This protective ability of ferritin is based on the ferroxidase activity of the H-chain to catalyze highly toxic Fe(II) to less toxic Fe(III).[4] The ferritins in the nucleus and mitochondria protect DNA or mitochondria from iron toxicity and oxidative damage.[1] Recently, some studies have shown that cytosolic ferritins are elevated in malignant tissues, for example, cytosolic ferritin is expressed in mammary carcinomas at levels up to a 10-fold higher than in benign breast tissues,[19–21] indicating that cytosolic ferritin may be involved in tumor progression.

Serum ferritins are predominantly composed of L-chains, which have a low iron content. Although several studies have reported that serum ferritin might arise from the secretion of hepatocytes, macrophages, and Kupffer cells,[3,5] the source of serum ferritins is still an interesting issue to be addressed. In addition, the physiological functions of serum ferritin as well as the identity of the L-chain receptor are not yet clear. Nevertheless, a significant increase in serum ferritin levels has been confirmed to be related to pathological processes including inflammation, angiogenesis, and tumor formation, implying that serum ferritins are a potential biomarker for clinic diagnosis (discussed further below).[3–5]

THE FERRITIN RECEPTOR

As far back as the 1960s, several research groups reported that human ferritin could be selectively taken up by tumor cells.[22–24] Then, in the 1980s, Fargion et al. found that H-ferritin specifically binds to a protein of ~100 kDa molecular weight.[25] However, it was a long route to identification of the receptor for ferritin on human cells. It was not until 2010 that TfR1 was identified as the human H-ferritin receptor by Seaman's group using expression cloning,[15] following their earlier identification of the mouse H-ferritin receptor, TIM-2, in 2005.[26] They found that TfR1 binds specifically to H-ferritin with little or no binding to L-ferritin. After binding of H-ferritin to TfR1 on the cell surface, H-ferritin enters both endosomes and lysosomes. The demonstration that TfR1 can bind H-ferritin as well as Tf raises the possibility that this dual receptor function may coordinate the processing and use of iron by these iron-binding molecules. Soon after the identification of the H-ferritin receptor, our group reported that H-ferritins target TfR1 on both tumor cells and on tumor tissues from clinical samples.[14]

The TfR1 (also named CD71) is a type II transmembrane glycoprotein, which forms a homodimer on the surface of cells.[27] TfR1 was originally identified as the receptor for Tf. It is required for iron delivery from Tf to cells and the Tf-TfR1 regulated iron uptake pathway is the most important route for cellular iron uptake. In addition, TfR-1 is also involved in regulating cell growth.[28,29] It has been shown that the expression of TfR1 in proliferating cells, such as cancer cells, may be up to 100-fold higher than in normal cells.[27,30] This might be because of rapidly proliferating cells requiring more iron. The fact that TfR1 is overexpressed in a variety of malignancies and is efficiently internalized makes it an excellent target for tumor diagnosis and treatment.[30]

The strategy of targeting TfR1 for tumor imaging and therapy is summarized in Figure 2. Monoclonal antibodies to TfR1 and its natural ligand Tf have been successfully used to target malignant cells, either alone or carrying various cytotoxic or imaging agents.[30,31] For instance, Tf-conjugated diphtheria toxin, termed Tf-CRM107, has been applied in treatment of brain tumors and has been approved for phase III clinical trials.[32,33] Anti-TfR1 antibody-conjugated ricin A chain, termed 454A12-RTA, is currently in phase II clinical trials.[31]

In our laboratory, instead of using Tf and antibodies to TfR1,[30] we have recently demonstrated the use of H-ferritin nanoparticles as a new ligand, and successfully targeted TfR1 on different tumor tissues as well as using this as a visualization technique to distinguish between tumors and normal tissues. We screened 474 clinical samples and found that H-ferritin specifically binds to the nine most common solid tumors, including liver, lung, colon, cervical, ovarian, prostate, breast, and thymus cancers.[14] Furthermore, it has also been shown that TfR1 can be used as a prognosis indicator in breast cancer,[34] leukemia,[35,36] lung cancer,[37] and bladder cancer.[38]

FERRITIN AS A TUMOR MARKER

Secretary ferritins exist in serum, synovial, and CSF. Their physiological function and source are still unknown. However, since elevated serum ferritin is correlated with pathological processes, it is therefore considered as a useful clinical indicator. For instance, low concentration of serum ferritin indicates iron deficiency (e.g., anemia) and high serum ferritin indicates iron overload (e.g., hemochromatosis).[3,6] In addition, elevated serum ferritin is also found in inflammation, infection, and liver diseases.[1,3,6]

There is increasing evidence that serum ferritin levels are elevated in many malignancies and it has attracted widespread attention that serum ferritin can be used as a tumor biomarker. For instance, serum ferritin and tissue ferritin are both elevated in breast cancer patients.[3,6,39] Serum ferritin is also used as a biomarker for relapse of malignant disease. Matzner et al. reported that serum ferritin was markedly increased in all relapsed cases of acute leukemia.[40] In all cases, remission was associated with the normalization of serum ferritin levels.[40] These correlations suggest that serum ferritin may be useful in the initial clinical evaluation and in the assessment of response to therapy in patients with acute leukemia and malignant lymphoma.[40] Szymendera et al. and Volpino et al. found that measuring serum ferritin levels is a useful clinical indicator in patients with testicular germ-cell tumors[41] and lung cancer,[42] respectively. Moreover, it is found that melanoma cells can secrete ferritin, which contributes to the progression of melanoma.[43]

Besides serum levels, secretory ferritin levels in CSF are also found to increase during malignant infiltration of the central nervous system, and CSF ferritin has been studied for its ability to serve as a biomarker for the diagnosis of brain malignancies.[5,44] Intriguingly, exhaled ferritin has been reported as potential biomarker for lung cancer.[45]

The H/L ratio of serum ferritin also varies in pathological conditions.[46] Under normal physiological conditions, serum ferritin is predominantly composed of L-chains. However, in many malignant conditions, the ratio of H/L in serum ferritin is increased. A number of studies have shown that H-ferritins are highly expressed in tumorigenic cell

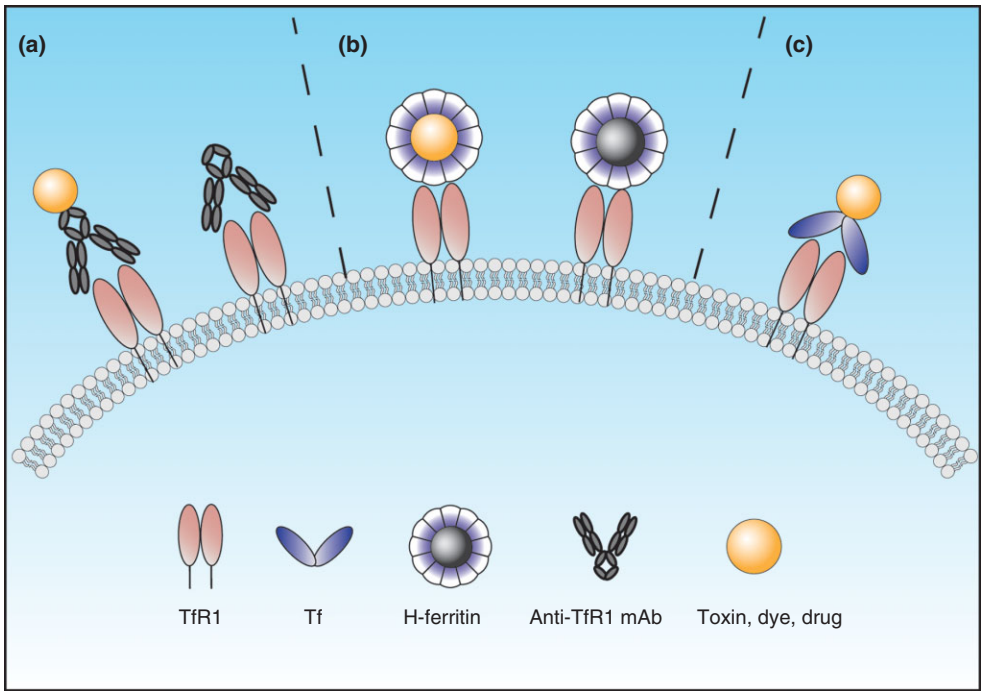

FIGURE 2 | Strategies for targeting transferrin receptor 1 (TfR1) in tumor therapy and diagnosis. These strategies can be achieved by employing (a) Anti-TfR1 monoclonal antibodies or conjugated-antibodies, (b) H-ferritin nanoparticles, and (c) conjugated Tf as carrier to deliver chemotherapeutic drugs, imaging dyes, and radionuclides to treat or detect tumors.

lines as well as in some malignant tissues from patients.[1,3,5,6] Although mechanisms underlying these changes are still unclear, these studies reveal that H-ferritin may play an important role in malignancy, and could be a potential biomarker for many kinds of cancers.

MAGNETOFERRITIN AGAINST TUMORS

Ferritins can be conveniently mineralized or synthesized to produce many kinds of nanoparticles without disruption of the integrity of protein shells.[47–51] By employing recombinant human H-ferritin as a template, Douglas's group biomimetically synthesized a type of magnetic nanoparticle in 2006.[8] This engineered ferritin molecule contains an iron core in the form of magnetite (Fe_3O_4), which is different from the natural ferritin iron core of ferrihydrite (Fe_2O_3).[8,11] Therefore, we refer here to the biomimetically synthesized ferritin as magnetoferritin.

Compared with other nanoparticles, magnetoferritin has the following advantages: (1) The nanoparticle size, as shown in Figure 3, is 12 nm and homogeneous, which is appropriate for tumor penetration and accumulation. It has been established that nanoparticles of between 11.2 and 14.6 nm in size can increase tumor accumulation.[52] (2) Magnetoferritin could be low toxicity or immune response when it is used for tumor imaging *in vivo*, because of its natural human protein shell. (3) The external protein shell of magnetoferritin can be genetically and/or chemically modified with tumor specific ligand for tumor targeting or with fluorescence agent for optical imaging in cancer diagnosis. (4) Dual-functional magnetoferritin allows use of the protein shell to target tumors and use of the internal core as an imaging probe to visualize tumors[9,53] On the basis of these features, two strategies for magnetoferritin targeting of tumors are discussed below.

Indirect Targeting of Magnetoferritin against Tumors

In general, engineered nanoparticles have been used to provide diagnostic, therapeutic, and prognostic information about the status of disease. Nanoparticles developed for these purposes are typically modified with targeting ligands, such as antibodies, peptides, or small molecules, to enhance their tumor targeting capability. For instance, Douglas's group modified magnetoferritin with RGD-4C that can specifically target tumor angiogenesis via binding to integrin molecules on vascular endothelium.[8,54] They found that the RGD-4C modified magnetoferritin could

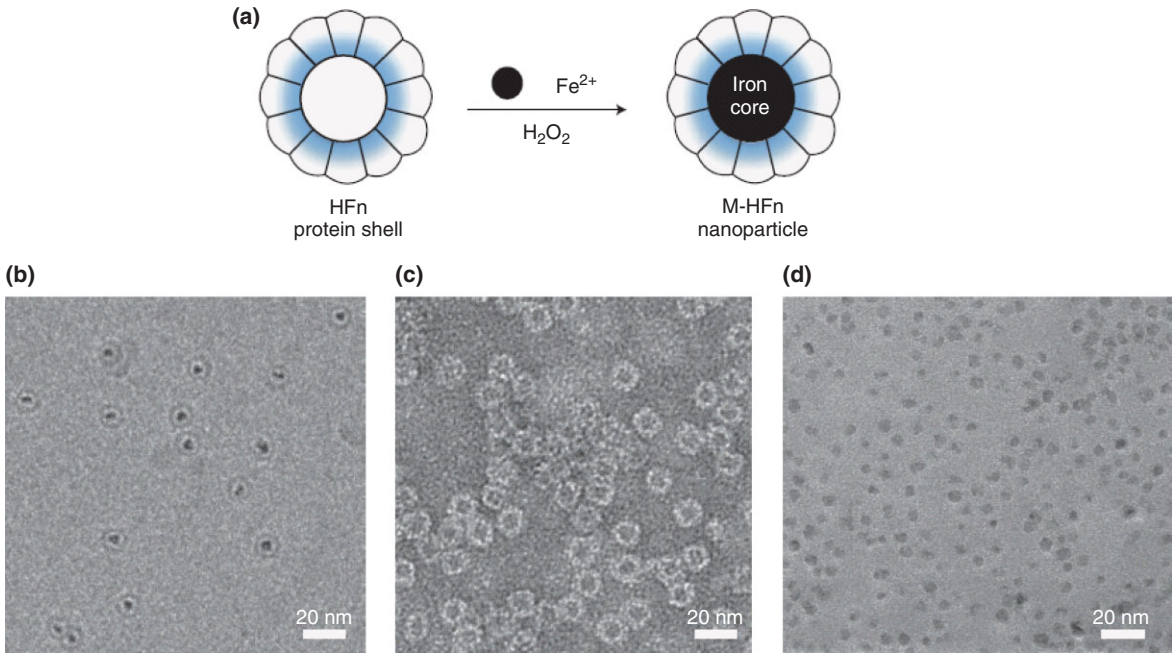

FIGURE 3 | The preparation of magnetoferritin nanoparticles (a) Schematic showing the preparation process. (b) Cryo transmission electron microscopy (TEM) image of magnetoferritin nanoparticles. (c,d) TEM images of H-ferritin protein shells (c) and iron oxide cores (d). H-ferritin protein shells were negatively stained with uranyl acetate for TEM observations and iron oxide cores in magnetoferritin were unstained. (Reprinted with permission from Ref 14 Copyright 2012 Nature Publishing Group).

bind to many types of tumor cells, including amelanotic melanoma, glioblastoma, and lung adenocarcinoma cells.[8,12,55] Another ligand, an matrix metalloproteinase (MMP) protease substrate, was also introduced onto the exterior surface of magnetoferritin, and could be cleaved by MMP enriched in the tumor area.[56] Actually, any ligand as long as it is able to target tumor markers such as melanocyte-stimulating hormone (MSH) and its receptor, or epidermal growth factor (EGF) and its receptor EGFR,[57,58] could be used for modification of magnetoferritin. An Fc-binding peptide was also genetically introduced into the exterior of ferritin and allowed the ferritin nanoparticles to target cancer cells.[59] In addition to use of single-ligand modified magnetoferritin, Chen's group recently generated chimeric ferritin nanocages for multiple function loading and multimodal imaging by combination of chemical modification and genetic engineering[55] to visualize tumors with high resolution and sensitivity (Figure 4).

Magnetoferritin nanoparticles consist of iron nanocrystals. The superparamagnetism of magnetoferritin makes it an ideal contrast agent for magnetic resonance imaging (MRI) for tumor diagnosis. MRI can provide high spatial resolution and functional anatomic and physiological information with simultaneous noninvasive imaging. Normally, use of magnetoferritin nanoparticles for imaging results in reduced signal intensity in T_2-weighted or T_2*-weighted MRI.[8,9] The tumor-bearing area displays weakened intensity in MRI when using ferritin nanoparticles as the contrast agent compared to MRI of normal tissue. But the negative contrast can also be due to artifacts. So, this negative change makes it difficult to acquire enough accurate information for tumor diagnosis.

Therefore, multimodality imaging is used, which combines MRI with other imaging modalities, such as fluorescence imaging or positron emission tomography (PET). On the basis of the special architecture of magnetoferritin nanoparticles, two modification strategies could allow performance of multimodal imaging in a single examination. One is introduction of functional groups onto the exterior surface of the nanoparticles by chemical or genetic modification of ferritin. For example, a fluorescent dye can be covalently conjugated onto H-ferritin,[8] and green fluorescent protein (GFP) could be fused into H-ferritin by genetic engineering.[12] However, fluorescence imaging may not obtain sufficient signal intensity during noninvasive detection, especially when tumors are located in deep tissues, because the light could not effectively penetrate the skin and deep tissues even using *in situ* exposure.[10]

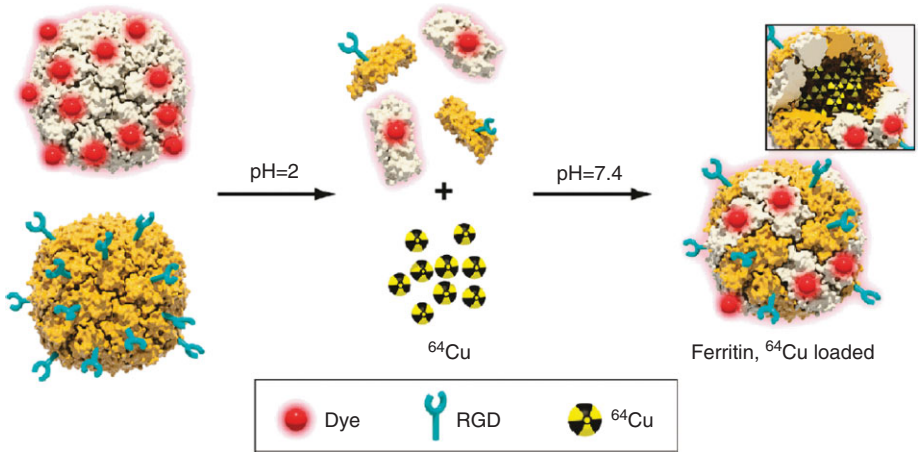

FIGURE 4 | Schematic illustration of multifunctional H-ferritin nanoparticles. RGD4C and Cy5.5 are introduced onto the surface via genetic and chemical means. ^{64}Cu radiolabeling via disassembly/reassembly of H-ferritin nanoparticles with pH control. (Reprinted with permission from Ref 55. Copyright 2011 American Chemical Society).

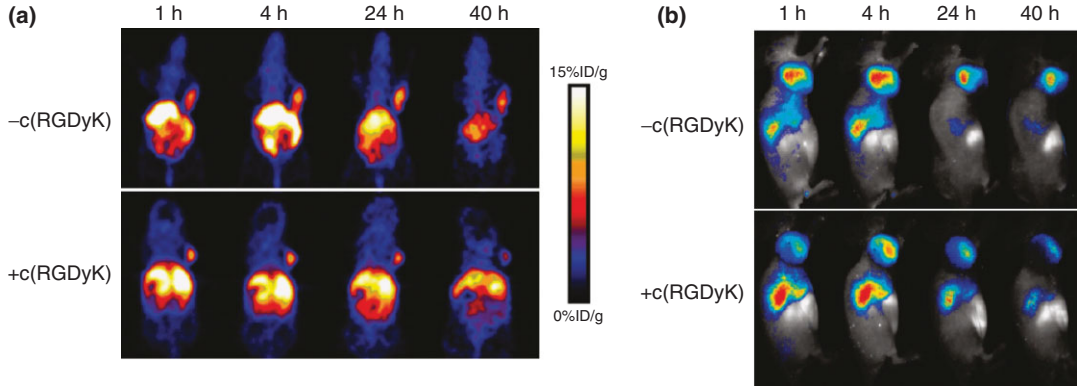

FIGURE 5 | *In vivo* (a) positron emission tomography (PET) and (b) near-infrared fluorescence (NIRF) images after the administration of ferritin probes. In the comparison group, a blocking dose of c(RGDyK) was injected 30 min prior to the ferritin probe administration. (Reprinted with permission from Ref 55. Copyright 2011 American Chemical Society).

Novel fluorescence imaging technology is needed to overcome this limitation. Chen's group have developed near-infrared fluorescence (NIRF) imaging by conjugation of Cy5.5 dye onto H-ferritin for *in vivo* imaging of tumor xenografted mice either with NIRF only or combining it with PET for multimodal imaging[55,56] (Figure 5). Another route is entrapping functional contrast agents into the core of the nanoparticles by regulating the disassembly/reassembly process under pH control. Gadolinium (Gd(III)) chelates could be loaded into the core of ferritin nanoparticles for *in vivo* tumor MRI with enhanced T1-weighted signal because of high r1 relaxivity.[60,61] Radioisotopes are another potential contrast agent that can be loaded into the core of the nanoparticles for multimodal tumor imaging. Chen's group loaded ^{64}Cu into the core of H-ferritin nanocages to carry out *in vivo* PET for tumor imaging by combining NIRF imaging in a single examination.[55] The generality of modification and functionalization makes ferritin nanoparticles a promising nanoplatform to achieve multimodal imaging in translational cancer diagnosis.

Because magnetoferritin nanoparticles are a biomimetic product, this means that natural ferritin nanoparticles exist in the human body. It is possible to directly use endogenous ferritin as the reporter to monitor disease progression *in vivo* with MRI rather than administrating foreign pre-made ferritin nanoparticles.[62,63] Kim et al. and Choia et al. have developed a model which allows tumor cells overexpressing H-ferritin to use ferritin as a reporter for MRI. This model could be used for *in vivo* tumor imaging and monitoring of tumor metastasis in lymph nodes.[64,65] The advantage of using ferritin as a reporter for MRI is that it does not require exogenous contrast agent to be delivered to the targeted tumor

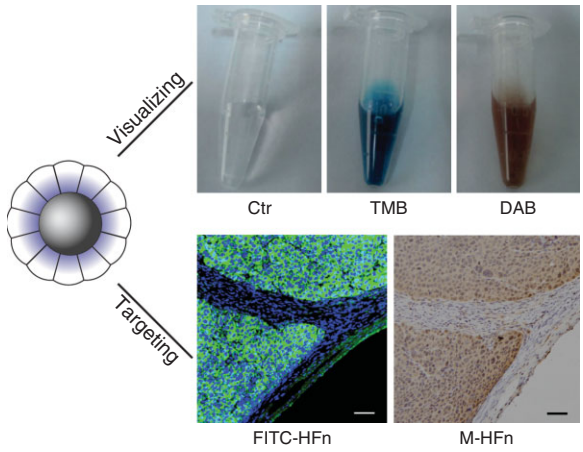

FIGURE 6 | Magnetoferritin nanoparticles with intrinsic dual functions, targeting tumor tissues without any modification, and giving a color signaling by its peroxidase-like activity. (Reprinted with permission from Ref 14. Copyright 2012 Nature Publishing Group).

area and makes it possible to carry out long term *in vivo* imaging. However, the level of ferritin expression during tumor development needs to be investigated. The magnetization of endogenous ferritin is much smaller and the r2 relaxivity is hundred times lower than that of magnetoferritin nanoparticle. Therefore high expression level of ferritin is needed to get enough contrast signal in MRI for tumor imaging.

As a powerful nanoplatform, ferritin nanoparticles not only show promising applications for cancer diagnosis and therapy, but also have great potential for other applications in the area of translational medicine. It is reported that human H-ferritin nanoparticles can be readily taken up by macrophages *in vitro* and provide strong T_2* MR contrast, which suggests that they could be used as an MRI contrast agent to assess inflammatory status such as atherosclerotic plaque progression/regression.[9,10,54] Ferritin nanoparticles have also been used in stem cell research. It is possible to track cell proliferation, differentiation, and migration in noninvasive real-time *in vivo* imaging. The nanoparticles formed by endogenous ferritin overexpressed in stem cells can be used as an effective contrast agent to track cells transplanted into the infarcted heart with noninvasive MRI.[66,67] It is reported that the receptors of L-ferritin are highly expressed in kidney.[68] Therefore, L-rich ferritin can be potentially developed as a contrast agent to image the kidney by far-red imaging.[47] Nie's group found that apoferritin (22 L-ferritin and 2 H-ferritin subunits) loaded with gold nanoclusters could be used for *in vivo* kidney imaging with far-red fluorescence technology.[47]

Direct Targeting of Magnetoferritins against Tumors

Recently, our group demonstrated the use of magnetoferritin as a dual-functional reagent, allowing simultaneous targeting and visualization of tumors. On the basis of this finding, we developed a new technique for tumor detection. As shown in Figure 6, H-ferritin, like anti-TfR1 antibody and Tf, can be used to directly target tumor cells via overexpressed TfR1. After verifying the interaction between H-ferritin and its receptor TfR1 in more than 474 clinical tissue specimens, we found that H-ferritin specifically recognizes nine types of tumor tissues, including liver, lung, colon, cervical, ovarian, prostate, breast, and thymus cancer tissues, but it does not bind to non-tumor tissues[14] (Figure 7). In addition, we found that magnetoferritin nanoparticles have intrinsic peroxidase-like activity.[14,53] They can catalyze peroxidase substrates to produce the same color reaction as peroxidase enzymes. For instance, magnetoferritin nanoparticles react with 3,3′,5,5′-Tetramethylbenzidine (TMB) to produce a blue color and 3,3′-Diaminobenzidine (DAB) to produce a brown precipitate. The peroxidase activity of the magnetoferritins comes from their mineral cores consisting of magnetite or maghemite. Compared with engineered magnetoferritin, the natural holoferritin with a core of hydrated iron oxide mineral ferrihydrite exhibits weaker peroxidase activity. However, apoferritin, without a mineral core, exhibited no peroxidase activity. The advantage of magnetoferritin makes it as an ideal dual-functional reagent for tumor detection, tumor imaging, and therapy.

On the basis of the above findings, we have developed a novel reagent, a single nanoparticle with dual-function, and established a new method to detect tumor tissues. Using the novel reagent and new technology, we screened 474 clinical specimens, including 247 clinical tumor tissue samples and as well as 227 non-tumor control samples, and found that magnetoferritin could distinguish cancer cells from normal ones in tissue specimens[14] (Figure 7).

Compared with conventional antibody-based immunohistological (IHC) methods, this new magnetoferritin-based method has the following advantages: (1) efficiency, with sensitivity of 98% and specificity of 95%, both of which are higher than traditional IHC methods; (2) ease of use, as the new technology uses one reagent and one step (Figure 8), instead of traditional IHC using primary antibody, secondary antibody, or enzyme-labeled third antibody with multiple steps between each incubation; (3) speed, as it takes less than 1 h, rather than 3–4 h

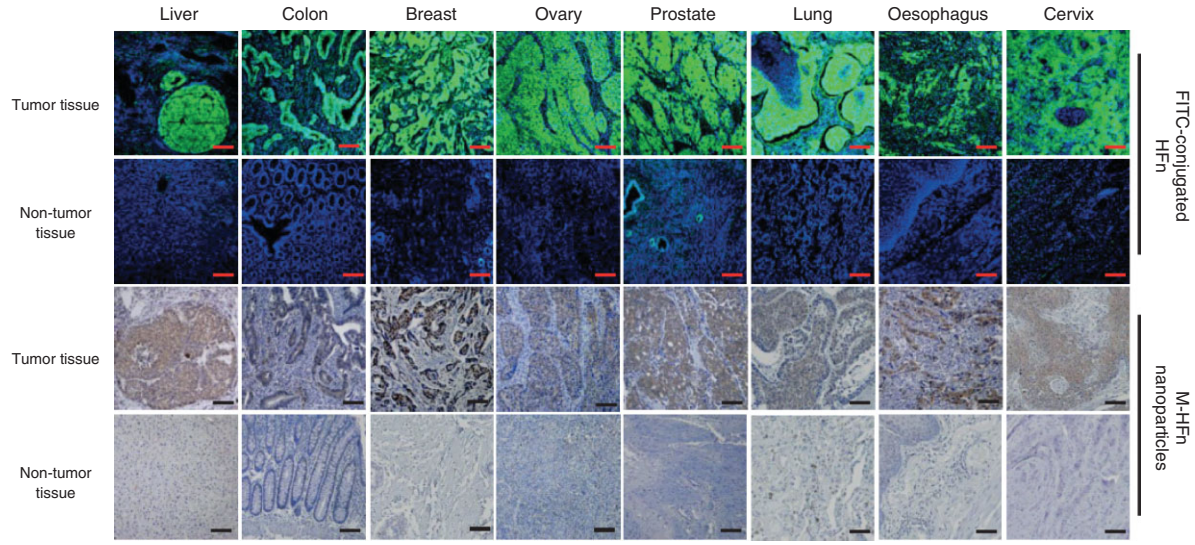

FIGURE 7 | Magnetoferritin nanoparticle detection in clinical specimens. (Reprinted with permission from Ref 14 Copyright 2012 Nature Publishing Group).

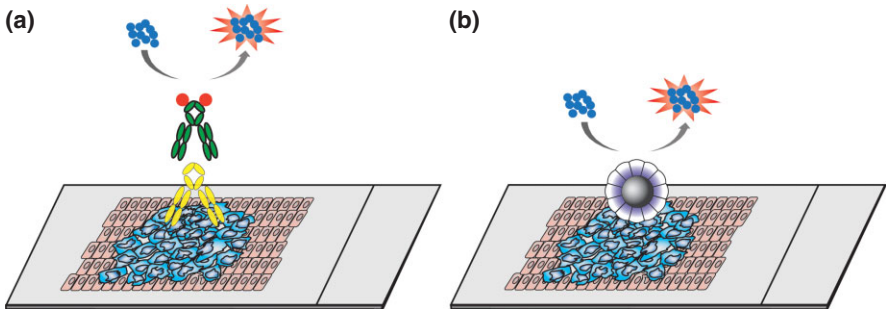

FIGURE 8 | Detecting tumor tissues by two methods: (a) antibody-based immunohistochemistry and (b) magnetoferritin-based immunostaining.

required for IHC methods; and (4) low cost and convenient production in *Escherichia coli*, avoiding the use of expensive antibodies. All these characteristics indicate that magnetoferritin as a novel reagent is a promising tool for disease diagnosis.

FERRITIN FOR DRUG DELIVERY

A few studies have begun to design ferritin nanoparticles as a carrier to deliver drugs for the purpose of therapy. Instead of a magnetite core, apoferritin nanoparticles can be used to encapsulate chemical drugs in the interior cavity by manipulating disassembly/reassembly of the nanoparticle under pH control.[69,70] For instance, using apoferritin-encapsulated doxorubicin (DOX), the maximum loading was up to 28 DOX per ferritin molecule and the drug-carrying ferritin maintained high stability. Another example is use of apoferritin to carry Cisplatin by a similar process, with each ferritin encapsulating around 11 cisplatin molecules. Functional studies showed that ferritin with entrapped cisplatin could induce apoptosis of gastric cancer cells.[71]

In addition to encapsulating chemical drugs in the core, the exterior surface is another platform to load protein drugs, such as antibodies, toxins, and peptides, by genetic engineering. It has been reported that antibody-modified ferritin can be used to target breast cancer,[59] which may have potential application in tumor therapy. Similarly, the single-chain variable fraction (scFv), which is the antigen-binding portion of the antibody, can be genetically fused with ferritin.[72] It will be interesting to use this nanoplatform to combine multimodal imaging and targeted drug delivery for cancer diagnosis and therapy.

CONCLUSIONS AND PROSPECTS

Ferritin has been investigated for more than 75 years. Recent studies have shown that ferritin, besides being essential for iron storage and homeostasis, is

involved in a wide range of physiological and pathological processes. Notably, serum ferritin and the ferritin receptor TfR1 have been identified as tumor biomarkers. Although numerous new findings have provided us with insights to better understand this protein, the novel and unexpected functions of ferritin continue to be revealed and further study of this interesting molecule is warranted. For example, the issue of the source of secretory ferritin and identification of human L-ferritin receptors; the mechanism and function of ferritin in its involvement in many pathological processes; and the exact binding site of the ferritin/TfR1 interaction are aspects that will be important to unravel. Also, dealing with the toxicity and immune response of the human body to magnetoferritin is important for its application in *in vivo* imaging and therapy. As magnetoferritin has been recognized as an ideal nanoplatform, further advances in its application for tumor diagnosis, imaging, and therapy are expected.

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