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Review article

Protein nanoparticles as drug delivery systems for cancer theranostics

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ABSTRACT

Protein-based nanoparticles have garnered significant attention in theranostic applications due to their superior biocompatibility, exceptional biodegradability and ease of functionality. Compared to other nanocarriers, protein-based nanoparticles offer additional advantages, including biofunctionality and precise molecular recognition abilities, which make them highly effective in navigating complex biological environments. Moreover, proteins can serve as powerful tools with self-assembling structures and reagents that enhance cell penetration. And their derivation from abundant renewable sources and ability to degrade into harmless amino acids further enhance their suitability for biomedical applications. However, protein-based nanoparticles have so far not realized their full potential. In this review, we summarize recent advances in the use of protein nanoparticles in tumor diagnosis and treatment and outline typical methods for preparing protein nanoparticles. The review of protein nanoparticles may provide useful new insights into the development of biomaterial fabrication.

1. Introduction

The optimal cancer treatment seeks to precisely deliver suitable therapeutic agents to specific targets and facilitate effective localized drug release with minimal side effects on the rest of the body [1–3]. However, the development of such optimal treatments faces many challenges. These include the poor aqueous solubility of many chemotherapeutic drugs, which complicates absorption and bioavailability after administrations. Additionally, chemotherapeutics often cause severe systemic side effects, primarily due to their low specific distributions to tumor tissues. Furthermore, cytokines and antibody therapies, while promising, have their limitations such as instability and short blood circulation time, which reduces their therapeutic efficacy and necessitates more frequent or higher dosages. These challenges collectively pose significant obstacles in the advancements of cancer treatments.

Recent efforts towards overcoming these challenges have led to the vigorous development and broad applications of various nanobiomaterials as drug delivery systems due to their advantages of high bioavailability, mitigating side effects, controlled release, and specific targeting delivery [4]. Moreover, long blood circulation of nanocarriers increases the chance of accumulating at the targeted tumor site [5]. Liposome, a widely used nanoparticle featured with good biocompatibility, multiple design possibilities and easy targeting to a particular tissue, has been developed as a promising drug delivery vehicle for decades [6,7]. However, its inherent issues, including uncompetitive loading capacity, fast release of hydrophilic drugs in blood, and harsh storage requirements, restrict further applications [8]. Apart from lipids, other biofunctional materials, including inorganic metals, organic polymers, and proteins, are under slow development as clinic-available drug carriers [9]. Recently, protein-based nanoparticles synthesized with natural protein cages and engineered peptides have drawn lots of attention in drug delivery area. Protein-based nanoparticles are composed of subunits derived from a single type of proteins or a group of different proteins, providing abundant moieties ready for chemical modifications with drug-binding, imaging or targeting entities [10,11]. The protein nanoparticles have shown the advantages of outstanding biocompatibility [12], high loading capacity [13,14], easy functional modifications [15], and excellent biodegradability [16,17], making them highly suitable for developing into a druggable drug delivery system for the theranostics of diseases. In addition, the protein-based size effect can prolong its blood circulation time and significantly

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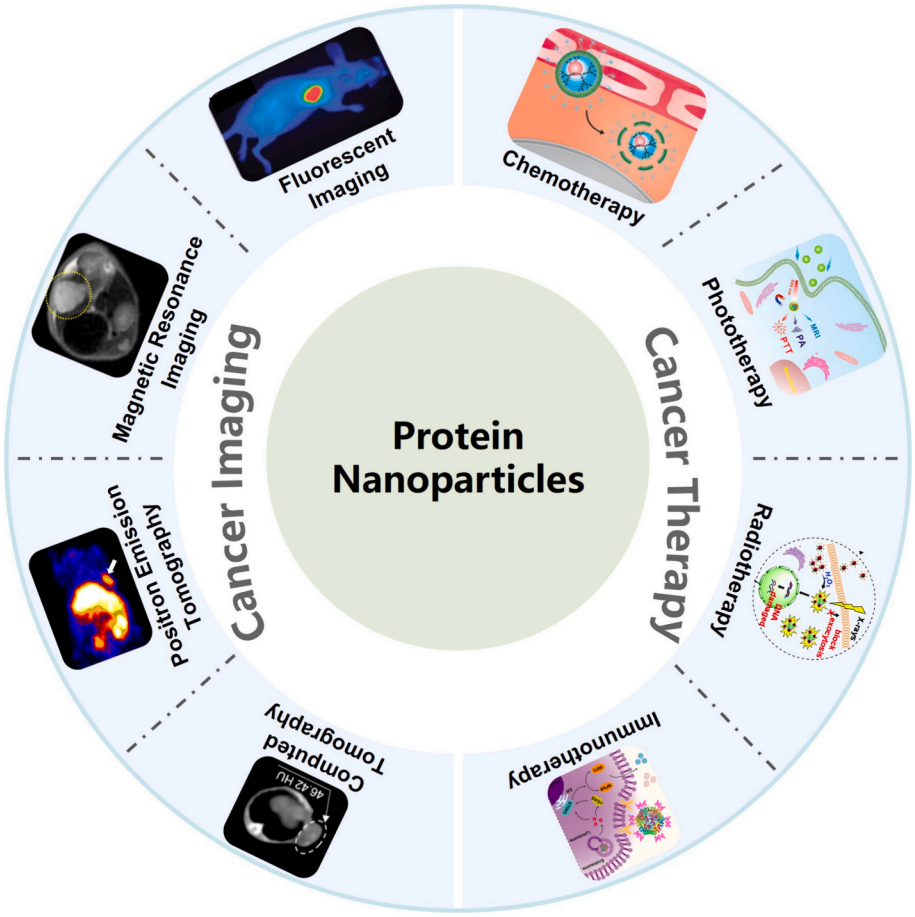

Fig. 1. The schematic illustration of protein nanoparticles for cancer imaging and therapy.

$$\mathbf{TrpH} \xrightarrow{\mathrm{UV}} \mathbf{TrpH}^{\cdot+} + \mathbf{e}^{-}$$

$$\mathbf{RSSR} + \mathbf{e}^{-} \rightarrow (\mathbf{RSSR})^{\cdot-}$$

$$(\mathbf{RSSR})^{\cdot-} \rightarrow \mathbf{RS}^{\cdot} + \mathbf{RS}^{-}$$

Formula 1. Tryptophan mediated photo-reduction of disulfide bonds in proteins.

reduce the renal filtration rate, further improving the bioavailability [18]. Moreover, protein-based carriers can regulate the clearance and tissue distribution of nanodrugs. Thus, the drug delivery selectivity can be further improved [5].

Moreover, with the rapid development of nanotechnology, various proteins have been studied to prepare protein-based nanoparticles, such as albumin, silk protein, gliadin and legumin. For example, albumin plays an indispensable role in regulating the osmotic pressure and delivering nutrients to tissues and cells [19]. Silk protein has been used to drive intracellular nucleic acid delivery [20]. Ovalbumin can be used as a tumor antigen model to recapitulate the different *in vivo* expansion dynamics of overtly metastatic ovalbumin-specific OT-II TCRtg $CD4^+$ T cells and OT-I TCRtg $CD8^+$ T cells. Moreover, ovalbumin-specific OT-II TCRtg $CD4^+$ T cells were able to eradicate ovalbumin-expressing major histocompatibility complex-deficient HCmel12 Jak1-KO tumors, whereas ovalbumin-specific OT-I TCRtg $CD8^+$ T cells were ineffective [21]. Recently, protein therapeutics have emerged as the dominant therapeutic modalities compared to other biologics, with over 50 approved monoclonal antibody (mAb)-based therapeutics and over 500 mAb-based therapies in clinical development. Of the recently approved biologics, greater than 90% were mAb-based drugs. This review will present the recent advances of protein-based nanoparticles and provide a detailed summary of the main synthesis techniques and their application as drug delivery systems for tumor imaging and therapy (Fig. 1). (See Formula 1.)

2. The methods for synthesis of protein nanoparticles

Many physicochemical techniques, including sonochemistry, thermal decomposition, colloidal method, microemulsion and hydrothermal method [[22,23]], have been extensively developed to prepare protein nanoparticles for drug delivery. Besides, cross-linking or surface modified with functional molecules are also involved in the preparation of protein-based nanoparticles [[24,25]].

2.1. Synthesis of protein nanoparticles with coacervation or equivalent desolvation

A traditional approach in preparing protein nanoparticles is the coacervation or equivalent desolvation process under relatively mild conditions. The preparation process starts with the generation of colloidal systems by extracting protein solvents into the antisolvent phase [26,27]. The following phase separation process causes the solid colloidal phases to be scattered in the phase consisting of the incipient solvent and anti-solvent that dissolve the proteins, which should be subsequently miscible [28–30]. The solution pH before desolvation is the main factor in determining the size and yield of the formed protein nanoparticles.

Lauane Nunes et al. prepared adriamycin doxorubicin (DOX)-loaded

Table 1
The comparisons of protein-nanoparticles preparing methods.

Method	Coacervation	Solvent extraction or emulsion process	Polyelectrolyte complexation	Salt precipitation	Heat denaturation
Advantages	relatively mild conditions	high encapsulation efficiencies	high encapsulation efficiencies	synthesis method is simple	equipped with functional moieties
Disadvantages	easily promote aggregation	uncontrollable particle size	strong pH influence	loss of bioactivity; conformation changing; higher heterogeneity	uncontrollable particle size; loss of bioactivity

bovine serum albumin (BSA) nanoparticles by a modified desolvation technique and modified their surface with hyaluronic acid (HA) that can specifically recognize the CD44 receptor overexpressed in metastatic breast cancer cells. The experimental results showed that Dox-loaded BSA HA nanoparticles accumulated the most in mice tumors *in vivo* and had a higher tumor suppression effect [31]. In addition, the group investigated the stability and *in vitro* bioaccessibility of luteolin in different aqueous-oil systems, and prepared whey isolate protein nanoparticles by desolvation, which were used as the internal aqueous phase, revealing the importance of structural design in improving the stability and bioaccessibility of bioactive compounds [32]. Boram Son et al. prepared spherical human serum albumin (HSA) nanoparticles loaded with basic fibroblast growth factor (bFGF) (HSA-bFGF NP) by a desolvation and cross-linking process. HSA-bFGF NP can be used not only as a delivery vehicle but also as a protein stabilizer to promote wound healing and tissue regeneration [33].

2.2. Synthesis of protein nanoparticles with electrospray

Electrospray based on the emitter-mediated high solution voltage was developed as a novel technology for the synthesis of protein nanoparticles. It is widely used to prepare a particular group of protein nanoparticles, including gliadin and elastin-like polypeptides [34,35]. The protein nanoparticles are formed by emitting atomized droplets and their sizes can be well controlled. The chemical drugs and nucleic acid agents are easy to be carried into the protein nanoparticles by using this electrospray method. Elastin-like peptide nanoparticles for drug delivery were prepared by electrospray technique by Wu et al [36]. The elastin-like peptide nanoparticles and drugs were dissolved in organic solvents and the experimental results showed significant effects on particle diameter, polydispersity and surface charge. Maryam Asadi et al. prepared curcumin-walnut protein nanoparticles by electrospraying technique using walnut protein isolate as a carrier, which increased the solubility of curcumin and is an effective way to improve its bioavailability [37].

Although the proposal of preparing protein-based nanoparticles has been extensively optimized, the factors of introducing toxic chemicals and failure to control particle size precisely still limited their application in the biomedical fields (Table 1).

2.3. Protein cages-based nanoparticles as drug delivery system

Artificially and symmetrically constructed with three interfaces-interior, exterior, and intra-subunit, protein cages represent a multifunctional protein structure that can be designed by chemical and genetic modifications with specific biomedical functions, such as immunoassay and drug delivery. A reverse metal-templated interface redesign method to convert a natural protein-protein interface into a selectively responding metal ion was proposed by Harrison et al. [38] In this way, the ferritin protein cage coupled by copper metal was formed, the process of which imitate the ferritin isolation under physiological conditions.

Protein cage is a type of cage-like structure consisting of the self-assembling subunits of a single or mixture of proteins, the void of which can be loaded with drugs. The drug-encapsulated protein cages can selectively deliver the cargos to specific cells [39–41]. The protein cages usually have union cage size and load a certain number of drugs, which holds great potential in clinics [42]. Naturally derived ferritin- or apoferritin-protein cages are physiologically stable biocompatible structures that are used for drug delivery [43–46]. At low pH conditions, the subunits of ferritin/ apoferritin could dissociate and then reassemble into a shell structure accompanied with the gradual increase of pH upon to neutral or basic. This dissociation-reassembly process facilitates loading of small molecule drugs and biomarkers into the protein cages [47,48]. Moreover, the drug-loaded magnetoferritin nanoparticles have been applicated as a tumor targeting and imaging platform [49–51].

2.4. Photo-synthesis of protein nanoparticles

Given the complex behavior of biomolecules the synthesis of protein nanoparticles is conventionally processed under harsh conditions and may cause undesired genetic mutations of the protein sequences. In addition, it is not easy to control and generate uniform particles through the self-assembly process of proteins under the above conditions. These shortages are the critical bottlelack for the application of protein nanoparticles as drug delivery carriers. Recently, the ultraviolet (UV) illumination has been reported to break the disulfide bonds of protein molecules [52]. The tryptophan residues neighboring disulfide bonds have been proposed as the mediators of the photo-reduction process. The number of free thiols in cutinase was raised by UV illumination [53,54]. In addition, Hanssens et al. observed a similar photo-reduction phenomenon in goat α-lactalbumin [55]. A comprehensive study on the mechanism of lysozyme unfolding under UV illumination was conducted by Xie et al. [56,57], and the authors proposed that the proteins could only form nanoparticles through self-assembly by UV illumination. Moreover, alteration of the illumination time or intensity of UV light could precisely control the size and shape of the formed protein nanoparticles. Thus, the synthesis of protein nanoparticles by UV irradiation has a bright prospect.

2.4.1. UV illumination-mediated disruption of disulfide bonds

The protein molecule performs its biological function while keeping its natural conformation under physiological conditions. However, protein molecule undergoes a conformational unfolding process under certain stressful conditions, including extreme pH values, high concentrations of denaturants and high temperatures, leading to a certain degree of sacrifice for their biological functions [58–60]. In addition, unfolded proteins assemble themselves into aggregates or amyloid-like fibers, which have been reported to be toxic to the nervous system and are closely associated with some neurodegenerative diseases [61–63].

Therefore, studying the mechanism of protein unfolding and self-assembly is very important to understand protein bio-functions and explore the pathogenesis of neurodegenerative diseases. Generally, gene mutations or harsh conditions are the main factors inducing the self-assembly of protein molecules into aggregates [64,65]. The proteins that have their secondary structures converting into β-sheet can quickly self-assemble into amyloid fibrils [66–68]. Moreover, the application of denaturants and reducing agents may increase the synthesizing steps and raise the potential biological toxicity of the protein nanoparticles system. The reductive cleavage of the disulfide bonds, which are essential in stabilizing the molecular conformations of proteins, may

lead to the unfolding of proteins and further assembly of them into nanoparticles [69,70]. In most natural protein molecules, however, disulfide bonds are usually located in deep intramolecular structures, where the disulfide bonds cannot be disrupted unless under the condition containing of high concentrations of denaturants and reducing agents.

Recently, it was reported that a certain of physical method, ultraviolet illumination could break the disulfide bonds in proteins under the physiological environment [71–73]. Zhao et al. investigated the cleavage efficiency of disulfides by UV light and identified the oxidation products formed by UV light under anaerobic and aerobic conditions in systems with protein backbones composed of combinations of cystine-free and tryptophan or tyrosine [71]. The photo-reduction was mediated by the disulfide bonds-surrounded tryptophan, an aromatic amino acid, which has a strong absorption effect on ultraviolet light and could transfer the electron, thereby breaking the nearby disulfide bonds (Formula 1). Cystine (RSSR) may undergo a direct electron transfer reaction with solvated electrons generated from the photoionization reaction of tryptophan or with the triplet excited state of tryptophan to produce a free thiol anion (RS^-) *via* disulfide electron adduct ($RSSR^{\cdot-}$) cleavage [72,73]. Free thiols can also be formed by the breaking of disulfide bonds and protonation of the initial disulfide anion [73]. Similar to the way tryptophan breaks disulfide bonds, tyrosine can be excited by UV light thereby inducing breakage of nearby disulfide bonds [74].

2.4.2. Photo-reduction synthesis of protein nanoparticles

Recently, the mechanism of 'photo-reduction self-assembly to form protein-based nanoparticle' theory was proposed based on the internal structure changes of the protein molecules induced by photo-illumination [57,69,75]. In particular, the photo-reduced protein molecules can assemble themselves into nanoscale aggregates relying on the hydrophobic interaction between different molecules. In addition, the self-assembly efficiency was increased rapidly due to the newly formed intermolecular disulfide bonds leading to the formation of protein nanoparticles. The adjustment of UV light intensity and illumination time allowed size controllable for the protein nanoparticles [76]. A recent report of our group showed that some proteins could self-assemble into amyloid fibers or granular by altering the ultraviolet illumination dose under physiological conditions [70].

The anti-cancer drug-loaded protein nanoparticles showing efficient tumor targeting and significant therapeutic effects have been synthesized using this photo-reduction synthesis of protein nanoparticles theory [64,65]. In addition, based on the works of photo-synthesis protein aggregates, a one-step preparing approach was developed by us in synthesis of protein nanoparticles loading of anticancer drugs under UV illumination, which could be conducted under conditions free of any toxic organic solvents or chemical denaturants [57]. These protein-nanoparticles showed controlled drug release under acidic and reducing conditions, while limited-release under physiological conditions, which was suitable to be drug delivery system for cancer therapy. *In vivo* evaluation showed that the RGD-conjugated protein nanoparticles elicited excellent tumor growth suppression. The simple method of photo-synthesis of protein nanoparticles can be applied to prepare a variety of protein-based nanoparticles with disulfide bonds, although some potential problems, including the possible drug decomposition caused by UV illumination and the low loading capacity, should be solved.

2.4.3. Self-assembly of lysosome to form aggregates

It was reported that through altering the UV illumination dose, the size of the photo-synthesized chicken egg white lysozyme aggregates could be increased from nanoscale to microscale [70]. Based on the results of size and morphology measured by dynamic light analysis and atomic force microscopy, the produced free thiol groups analysized by mass spectrometry and the circular dichroism, it was proposed that the light-induced synthesis protein aggregates depends on hydrophobic interactions and the formation of intermolecular disulfides. In particular, the disruption of disulfide bridges induced by light irradiation leads to partial unfolding of the protein. Moreover, the hydrophobic interactions between these unfolded protein molecules forced them to self-assemble into granules, which is a slow kinetic process since the partially unfolded proteins still have a certain level of natural conformation and may block forming new intermolecular disulfide bonds. Thus, the granular nanoparticle was formed by the protein aggregates rather than the amyloid fibrils consisting of β-sheet secondary structures. However, the granule particles are unstable and easy to form larger aggregates due to the further hydrophobic interactions and the newly formed intermolecules disulfide bridges.

Protein aggregation is commonly associated with a variety of neurodegenerative diseases such as Alzheimer's disease and Parkinson's diseases [77–80]. Thus, it is imperative to develop new strategies that can detect and inhibit protein aggregation. Chai et al. investigated a cationic polystyrene derivative polyphenylenevinylene with trimethylammonium (PPV-NMe_3^+) for the detection and inhibition of UV irradiation-induced hen egg white lysozyme (HEWL) aggregation [81]. They found that the aggregation process of HEWL was effectively suppressed due to the competitive hydrophobic interactions between HEWL and PPV-NMe^{3+} dominating the self-assembly of HEWL and the electrostatic repulsion between neighboring PPV-NMe^{3+}-coated HEWL aggregates.

2.4.4. Self-assembly of bovine ribonuclease a (RNase a) triggered by tyrosine mediated photo-reduction of disulfide bonds

It was reported that disulfide bonds in RNase A, a protein that has no tryptophan but six tyrosine, could also be disrupted under UV illumination [82,83]. The disruption of disulfide bonds induced the partial unfolding of RNase A, subsequently assembling themselves into small granular aggregates. However, these aggregates are quite stable and grow slowly along with the increase of UV illumination dose. This is quite different from the rapid self-assembly of nanoparticles for those tryptophan-containing proteins and thus controllable in the synthesis of biomaterials due to the slow growing process [82]. This work opened new horizons for us on the usage of light energy in biophysical area and the synthesis approach of biomaterials in drug delivery area.

2.4.5. Light-induced synthesis of drug-loaded protein nanoparticles

The protein-inorganic nanoparticles, typically formed *via* the co-assembly, have been extensively studied to develop functional materials in various fields, including drug delivery, biosensing and biocatalysis [84,85]. The simple photo-induced self-assembly approach could be used to synthesize protein-inorganic hybrid nanoparticles. The photo-synthesized drug-loaded protein nanoparticles have a large number of free thiol groups on their surface, which have been regarded as a viable platform for the conjugation of functional molecules [86,87].

Notably, the functional theragnostic hybrid nanoparticles were formulated with empty hydrophobic quantum dots (QDs) nanoparticles and paclitaxel (PTX), a hydrophobic anticancer small molecule drug, where the disulfide bond rich proteins of lysozyme treated by photo performed as the host materials [57]. The detailed process of protein nanoparticle synthesis and functional modifications with targeting ligands is illustrated in the following. After the formation of drug-carrying nanoparticles by co-assembly, the sulfhydryl groups on the surface of the nanoparticles can be easily modified by a variety of ligands without the addition of any new chemical reagents. Moreover, the further conjugation of cyclic RGD (Arginine-Glycine-Aspartic acid) peptide (cRGD)-PEG-modified functional polymers on the particle surface enabled this protein-inorganic nano-vehicle to target tumors in systemic delivery actively [57]. The RGD peptides have been intensely reported as an active and effective tumor-targeting ligand that can interact with the integrin $\alpha_v\beta_3$ expressed on tumor cells [88–92]. The RGD-conjugated nanoparticle showed more effective tumor accumulation and efficient tumor growth suppression than unmodified ones. This multifunctional

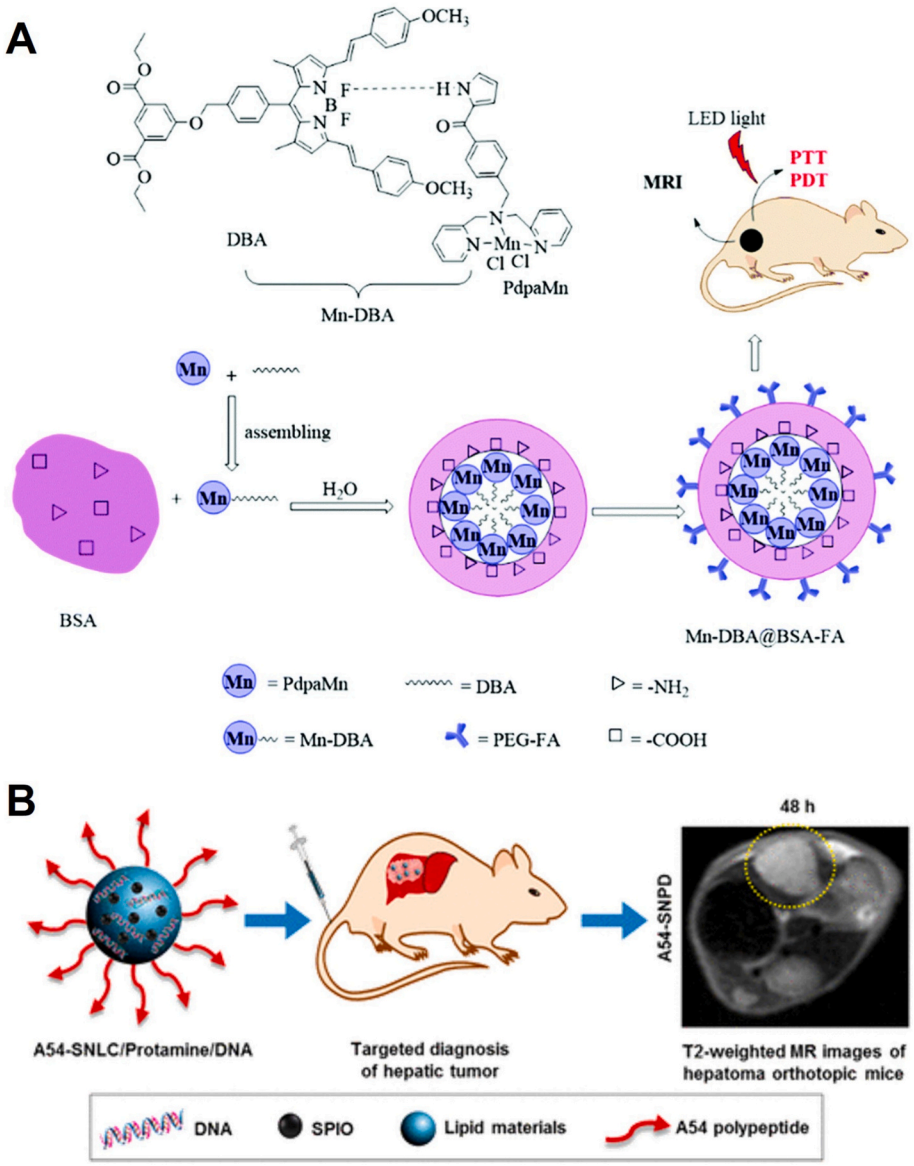

Fig. 2. Protein nanoparticles for MRI. (A) Schematic representation of the preparation of Mn-DBA@BSA-FA nanoparticles [104] Copyright 2017 Royal Society of Chemistry. (B)A54-SNLC/protamine/DNA-guided T_2-weighted magnetic resonance imaging of hepatic tumors [105] Copyright 2017 American Chemical Society.

drug carrier effectively accumulates and inhibits tumor growth at the tumor site. Moreover, the protein-inorganic hybrid nanoparticles showed excellent dual-sensitive ability to acidic and reductive conditions, which could be suitable for controlled release of drugs in tumor tissues. This photo-synthesis of protein nanoparticles method could be applied to prepare a variety of protein-based drug delivery systems with the proteins containing aromatic amino acids-surrounding disulfide bonds, whereas proteins without disulfide bonds cannot be synthesized as nanoparticles by this method [69]. The general approach involved the hybrid nanoparticle synthesis provided new insights in developing the protein-inorganic nanoparticles formed by the photo-induced assembly as a multi-functional delivery system with broad biomedical applications in many diseases.

Herein, a multi-functional protein-based nanocarrier of chemotherapy was prepared for effective tumor targeting, imaging, and treatments by using this light-induced assembly approach. UV irradiation-mediated disulfide bond disruption and exposure of the hydrophobic structural domains of proteins led to the preparation of nanoparticles *via* hydrophobic interactions between QDs and PTX, followed by conjugation of tumor-targeting cRGD peptides with newly formed free thiols on the surface of the nanoparticles. The unique advantage of this synthesis approach is represented by the non-existence of any chemical denaturants or harsh conditions. The hydrophobic interactions in this approach could be used to formulate protein-inorganic nanoparticles with other hydrophobic inorganic materials. The above results indicated that the universal self-assembly approach might provide helpful insights in the development of biomaterial fabrication methods.

3. Protein nanoparticles for cancer imaging

3.1. Fluorescent imaging

In vivo fluorescence imaging allows for non-invasive quantitative detection of subcutaneous tumors in mice models. Compared with bioluminescence imaging, fluorescence imaging *in vivo* has a faster detection time, and does not require substrate injection, thereby reducing the overall detection cost [93,94]. Protein nanoparticles can carry fluorescent materials for *in vivo* fluorescence imaging. Qin et al. reported TPE-TPA-DCM nanoparticles with or without BSA carrier. The

resulting compound TPE-TPA-DCM has stronger emission in the aggregate state. Interestingly, compared with TPE-TPA-DCM nanoparticles, BSA-carried nanoparticles exhibited enhanced cancer cell uptake and tumor-targeting ability due to the enhanced permeability and retention (EPR) effect, further demonstrating the great potential of BSA-carried nanoparticles prepared with aggregation induced luminescence activity as *in vitro* and *in vivo* far-red/near-infrared fluorescent probes [95]. mCardinal is a far-red fluorescent protein that combines strong fluorescence and high light stability. Kim et al. combined the mCardinal FPs gene with hepatitis B virus capsid (HBVC) to form bilayer mC-DL-HBVC nanoparticles with enhanced fluorescence and photostability. Compared with the conventional fluorescent dye Cy5.5, mC-DL-HBVC effectively detects tumors *in vivo* in mice and rarely accumulates in the liver [96]. Zayed et al. designed a multifunctional mannose-coupled BSA-QD nanoplatform for the co-delivery of pemetrexed and resveratrol to achieve fluorescence imaging of breast cancer cells [97]. This study provides a new therapeutic platform for co-delivery of mannose-modified albumin QD nanocomplexes of pemetrexed and resveratrol to breast cancer cells.

In one study, An et al. developed a fluorescent protein nanoparticle scaffold containing a small ultra-red fluorescent protein for fluorescence imaging [98]. The fluorescent nanoparticles stabilize in the blood after intravenous injection and can perform noninvasive tumor imaging in mice. Bellini et al. coupled a linker of disulfide to the surface of nanoparticles for the release of fluorescein in response to glutathione stimulation in cancer cells [99]. This functionalization strategy not only facilitates rapid detection of the nanoparticles once they enter the cytoplasm of the tumor cells, but also does not interfere with the encapsulation and release of the drug, thus enabling the co-addition of the drug and bioluminescent probe into a single tumor-targeting nanosystem to monitor the efficiency of nanomedicine delivery to cancer cells. Due to the advantages of deep tissue penetration and high spatial and temporal resolution, near-infrared (NIR)-II window (1000–1700 nm) imaging agents have received much attention in biomedical applications. Dou et al. developed a NIR-II fluorescent developer consisting of dual-peptide (glypican-3 and signal-regulatory protein α)-functionalized HSA particles and successfully used it for *in vivo* targeted imaging of hepatocellular carcinoma. Notably, the introduction of the signal-regulatory protein α-binding peptide mimics the immune checkpoint effect and greatly improves the imaging accuracy of the system [100].

3.2. Magnetic resonance imaging (MRI)

MRI is a safe and reliable screening method that provides timely feedback of three-dimensional topographic data and information about diseased tissue [101,102]. Protein nanoparticles are typically imaged for MRI by carrying magnetic nanoparticles or MRI contrast agents. Wang et al. used a biomimetic synthesis method to combine BSA with gadolinium (Gd) nanoparticles, which were then iodinated. The iodinated BSA-Gd (I-BSA-Gd) nanoparticles were obtained with good biocompatibility, a strong X-ray attenuation coefficient, and good MRI ability [103]. The prepared long-circulating I-BSA-Gd nanoparticles multimodal imaging probes can accumulate in tumor *via* EPR effect and have potential applications in image-guided drug delivery and surgery. In addition, hypoxia promotes tumor angiogenesis, invasive growth and distant metastasis, thereby increasing tumor malignancy. Lu et al. constructed a light-dependent attenuator for hypoxic environments by assembling photoluminescent Mn (II) nano-assemblies with BSA and modifying them with poly(ethylene glycol) folic acid (PEG-FA) for precise MRI and phototherapy of hypoxic cancers [104] (Fig. 2A). The assembly selectively penetrates and accumulates in tumor tissue with a clear T_1 MRI signal. Currently, the early diagnosis of liver cancer has a great demand for hypersensitivity contrast agents. Lu et al. designed a novel A54-SNLC/protamine/DNA ternary nanoparticle by using A54 peptide functionalized superparamagnetic iron oxide and protamine as cationic medium [105] (Fig. 2B). It was used as T_2-weighted MRI contrast agent for liver tumor imaging, which greatly improved the sensitivity and specificity of liver cancer diagnosis.

Chen et al. prepared BSA-Gd complex by biomineralization method, and coupled it with MoS_2 nanosheets by amide bond to prepare MoS_2-Gd-BSA nanoparticles. The biomineralized BSA-Gd complex on the surface of the hybrid nanosheets can make it have a relatively high r_1 relaxation rate, so that MoS_2-Gd-BSA is suitable for T_1-weighted MR and PA dual-modality imaging-guided tumor photothermal therapy [106]. Gd-based compounds are one of the commonly used MRI contrast agents in clinical practice. However, safety issues related to the toxicity of free ion forms have promoted the development of a new generation of metal-free contrast agents. Lock et al. transformed the FDA-approved anticancer drug pemetrexed into a molecular gel with intrinsic chemical exchange saturation transfer MRI signals through a supramolecular strategy. It was finally demonstrated that pemetrexed peptide nanofiber hydrogels could be used for non-invasive monitoring of its *in vivo* distribution and drug release by chemical exchange saturation transfer MRI signals [107]. This construction of metal-free MRI contrast agents using high-potential supramolecular materials provides a safer way to achieve image-guided drug delivery.

3.3. Positron emission tomography (PET)

PET is a new imaging technique for clinical applications. The commonly used nuclides that emit positrons are fluorine-18 (^{18}F), oxygen-15 (^{15}O), nitrogen-13 (^{13}N), and carbon-11 (^{11}C), which can accurately reflect the physiological functions and pathological conditions of the body due to the fact that they are the elements that make up the structure of the body and have a short half-life, and can be repeated dynamically [108,109]. Stangl et al. developed a novel PET tracer, TPP-PEG_{24}-DFO[^{89}Zr], that specifically recognizes Hsp70 membranes on tumor cells by modifying the tumor cell penetration probe TPP on the surface of nanoparticles. The prepared TPP-PEG_{24}-DFO[^{89}Zr] has good stability *in vivo* [110].

The radionuclide copper-64 is widely used to bind to biomolecules such as antibodies for PET imaging. For example, Xie et al. prepared nanoconjugates by modifying dopamine on the surface of iron (IO) oxide nanoparticles, and then encapsulated them into HSA matrix (clinical drug carrier) [111]. They then investigated the *in vivo* behavior of HSA-IO nanoparticles by PET/NIR fluorescence/MRI tri-modal imaging. Aanei et al. developed a targeted nano-imaging platform based on bacteriophage MS2. The MS2 shell not only has biodegradability, but also is coupled with specific antibodies through outer surface receptors. The outer surface of the MS2 shell is endowed with cell-specific targeting ability, and then labeled with radionuclide ^{64}Cu for *in vivo* drug delivery and PET imaging [112]. Skovsgaard et al. prepared the Tz-Az-NOTA conjugate by affinity-guided coupling, and then prepared the Tz-SCN-NOTA conjugate by the conventional method of isothiocyanate derivatives of NOTA [113]. [^{64}Cu]Cu-Tz-Az-NOTA and [^{64}Cu]Cu-Tz-SCN-NOTA were obtained by labeling the two conjugates with ^{64}Cu, respectively. After injection of [^{64}Cu]Cu-Tz-Az-NOTA and [^{64}Cu]Cu-TzSCN-NOTA into SK-OV-3 tumor-bearing mice for 40 h, PET imaging showed tumor uptake and localization.

3.4. Computed tomography (CT)

CT imaging has become one of the most widely used noninvasive clinical imaging modes due to its high efficiency, low cost and easy accessibility [114,115]. Moreover, CT imaging can accurately display human tissues and organs, which greatly promotes the development of diagnostic techniques.

Sasidharan et al. first proposed the synthesis of albumin nanoparticles *via* the polycationic amino acid poly-L-arginine (PLA) [116]. This strategy not only provides the required cationic groups on the surface of the nanoparticles, but also avoids the use of glutaraldehyde as cross-linkers to form stable nanoparticles. Moreover, gold

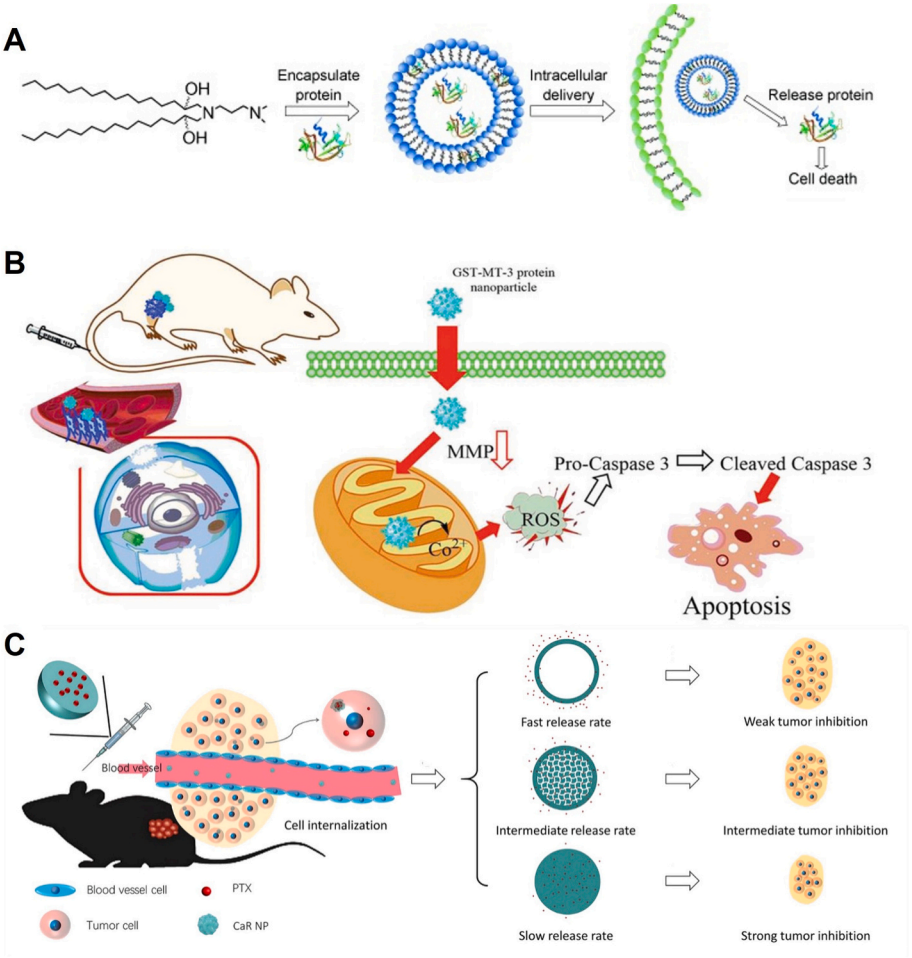

Fig. 3. Protein nanoparticles as drug delivery systems. (A) Combinatorially designed lipid-like nanoparticles deliver cytotoxic proteins inside cells for treat cancer [120] Copyright 2014 Wiley-VCH Verlag. (B) Schematic representation of the *in vivo* anti-tumor mechanism of novel GST-MT-3 protein nanoparticles [12] Copyright 2018 Wiley-VCH Verlag. (C) Carving all-natural protein nanospheres with tunable internal topology and drug release properties [124] Copyright 2023 Elsevier.

nanostructures (BGNs) formed by PLA-coated albumin (PLA-Alb) nanoparticles have high cell-friendliness and blood compatibility, and are used as CT contrast agents for KB cells for the first time. After 24 h of digestion of BGNs-treated KB cells, KB cells uptake about 80% of BGNs. Compared with untreated cells, BGNs-treated cells showed higher contrast, indicating higher gold uptake. Using BSA as a biological template, Chu et al. successfully synthesized a novel and stable ultra-small CT imaging contrast agent (Au-Ag@BSA) [117]. Moreover, this contrast agent has adjustable performance, low cytotoxicity, and can be targeted to lysosomes. To further evaluate the CT imaging properties of the alloy materials, they compared the X-ray attenuation behavior of component-dependent gold and silver alloy nanoparticles with iodohexanol. The experimental results showed that the X-ray attenuation coefficient of Au—Ag alloy nanoparticles was optimized when the Au/Ag ratio is 3:2.

Zhang et al. synthesized for the first time a novel ferritin nanotherapeutic agent loaded with the anticancer drug Dox in its hollow interior and bismuth sulfide nanoradiosensitizer grown on the outer shell layer, which can be used for CT imaging-guided tumor combination therapy [118]. *In vivo* and *in vitro* CT imaging experiments showed that Dox@AFBS nanoparticles had excellent CT imaging performance. In addition, Dox@AFBS could be efficiently enriched in tumors due to the EPR effect.

4. Protein nanoparticles for cancer therapy

The introduction of proteins into nanomaterial-based disease therapies provides more options and possibilities for their application in biology. The first protein nanoparticle approved by the FDA as a clinical drug carrier in cancer therapy was albumin in combination with paclitaxel (Abraxane®) for the treatment of metastatic breast cancer [119]. The FDA approved sirolimus albumin-conjugated nanoparticles for intravenous infusion for the treatment of adult patients with locally advanced unresectable or metastatic malignant perivascular epithelioid cell tumor. Moreover, sirolimus albumin-conjugated nanoparticles is currently the first and only drug approved for the treatment of advanced malignant perivascular epithelioid cell tumor in adults. In this part, we focus on the application of protein nanoparticles in biotherapeutics, including tumor chemotherapy, phototherapy, radiotherapy and immunotherapy.

4.1. Chemotherapy

Wang et al. designed a novel protein delivery platform by combining cationic lipid-based materials and reversible chemical protein engineering methods [120]. Binding of two cytotoxic proteins, RNase A and saponin, to lipids effectively delivers the proteins to cancer cells and inhibits cell proliferation (Fig. 3A). In a mouse breast cancer model, a representative lipidoid (EC16–1)/saponin nanoparticles could aggregate at the tumor site and inhibit tumor growth. Accumulation of EC16–1/

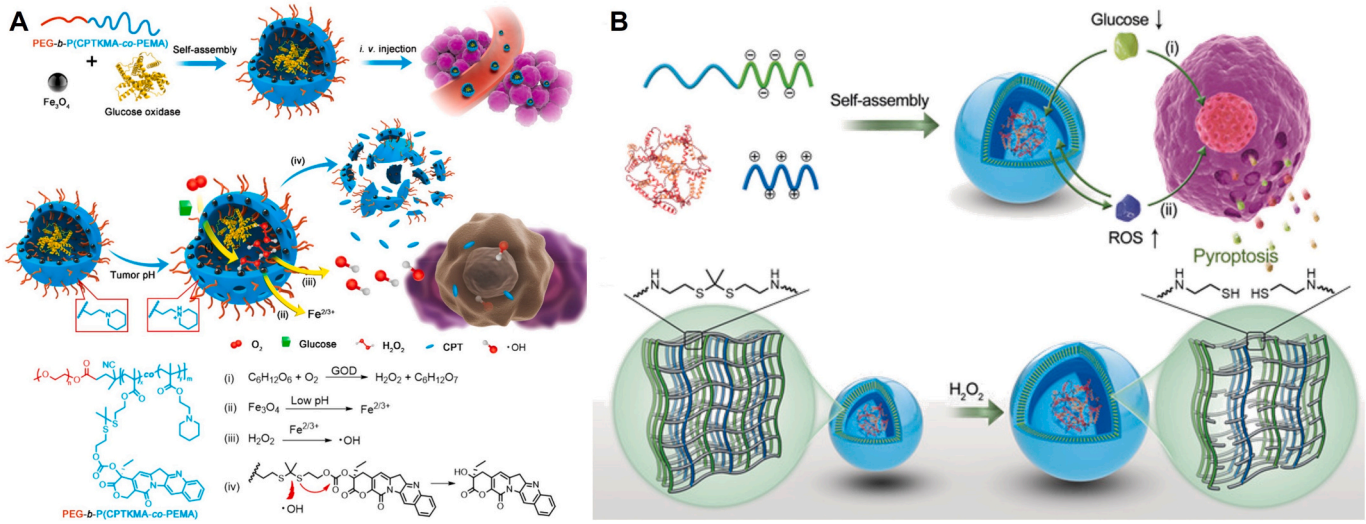

Fig. 4. Glucose oxidase-based protein biologics for tumor therapy. (A) Schematic illustration of polymersome nanoreactors for cooperative cancer therapy [127] Copyright 2019 American Chemical Society. (B) Schematic illustration of the ROS-responsive glucose oxidase-loaded therapeutic nanoreactor [128] Copyright 2020 Wiley-VCH Verlag.

saponin nano-formulations at the tumor site may be attributed to the enhanced permeability and retention effect of leaky vascular structures of the tumor tissue. The study shows that co-developed lipids can serve as an efficient platform for protein therapeutic delivery. Zhu et al. synthesized a new self-assembled metal protein nanoparticle from metallothionein-3 (MT-3) and glutathione (GST), naming it GST-MT-3. To the authors' knowledge, GST-MT-3 is the first protein nanoparticles that could target tumor cell mitochondria [12]. The authors suggest that glutathione tag fusion may be responsible for the mitochondrial targeting effect, as glutathione itself has a strong co-localization effect. Nanoparticles chelate cobalt ions [GST-MT-3(Co^{2+})], which induces reactive oxygen species (ROS) production and decreases mitochondrial membrane potential, leading to antitumor activity *in vivo*. In addition, GST-MT-3(Co^{2+}) and covalently bound paclitaxel synergistically could inhibit tumors and prolonge the survival time of mice (Fig. 3B).

Proteins are less likely to be cleared by macrophages, making proteins a promising base material for the development of drug carriers with high blood circulation efficiency [121–123]. Zhang et al. reported a simple nanoscale carving method to synthesize monodisperse protein nanoparticles with controlled internal topology and release profiles (Fig. 3C) [124]. Diffusion of eugenol containing plant-derived PTX into the hydrophobic core of nanoparticles, followed by removal of eugenol from the core by dialysis, led to sculpting of the nanoparticle interior. As the casein to rice protein amount ratio increases, this process produces all-natural nanoparticles with PTX loaded in their full, half-full, or solid cores. These nanoparticles are effectively absorbed by breast cancer cells and kill the cancer cells 删除(interleukins). This work opens a new avenue for the use of diffusion-mediated nanoscale carving to produce biomaterials with controllable internal topologies associated with drug release profiles.

Protein biologics including antibodies, enzymes, Cas9 proteins, *etc.* Chen et al. developed pH-sensitive polymeric nanocarriers with modifiable endosomal escape potency for selectively reaching the cytoplasm of specific cancer cells with dysregulated endosomal/lysosomal acidification. By loading the nanocarriers with an anti-c-MYC antibody directed against the nuclear pore complex, the expression of c-MYC in mouse colon adenocarcinoma CT26 tumors was significantly inhibited, resulting in potent inhibition of tumor growth [125]. This study informs the design of nanocarriers for pH response to enable cell-specific intracellular delivery of biomolecule therapeutics.

Anraku et al. prepared enzyme-loaded polyion complex vesicles as nanoreactors by a simple protein loading method. The β-galactosidase-loaded complex vesicles could selectively accumulate in mouse tumor tissues. The results suggested that polymer vesicles are excellent carriers for enzymes and enzyme-loaded complex vesicles are promising nanoreactors for activating prodrugs in targeted tissues for enzyme/prodrug therapies and enzyme replacement therapies [126]. Ke et al. designed and synthesized polymeric nanoreactors with tumor acid-responsive membrane permeability *via* tumor pH-sensitive block copolymers, ultrasmall iron oxide nanoparticles, and glucose oxidase (Fig. 4A). Through the permeability of the tumor acidic response membrane, a cascade reaction including the consumption of glucose to produce hydrogen peroxide, the accelerated release of iron ions, the Fenton reaction between hydrogen peroxide and iron ions to produce hydroxyl radicals, and the hydroxyl radicals-triggered rapid release of the parent drug can be specifically activated to achieve synergistic oncology therapy including starvation therapy, chemodynamic therapy, and chemotherapy, and thus can efficiently suppress the tumor inhibition [127]. Li et al. used glucose oxidase as a carrier to load ROS-responsive polyionic hybrid vesicles consisting of poly([2-[[1-[(2-aminoethyl)thio]-1-methylethyl]thio]ethyl]-α,β-aspartamide) as a polycationic segment and PEG-*b*-poly(α,β-aspartic acid) as a polyanionic segment (Fig. 4B). The prepared self-enhanced catalytic glucose oxidation by reactive oxygen species-responsive nanoreactors could protect glucose oxidase for cell-killing function through oxidative stress induction and glucose starvation. Moreover, to the authors' knowledge, this is the first self-enhanced catalyzed nanoreactor that induces immunogenic cellular focal death [128]. Hammel et al. utilized erythrocyte-encapsulated asparaginase in a phase IIb clinical trial for advanced pancreatic and triple-negative breast cancer. The combination of asparaginase and chemotherapy significantly prolonged overall and progression-free survival in the entire asparagine synthetase population, with an average 40% reduction in the risk of death over time, compared with chemotherapy alone. To the authors' knowledge, this study represents the largest patient population treated with gemcitabine alone after fluoropyrimidine-based chemotherapy. Thus, the results of this study are consistent with those of the study of nanoliposomal irinotecan, and with the results of the study after gemcitabine FOLFIRINOX [129].

Protein therapies offer promising prospects for the treatment of a variety of important diseases. He et al. developed a novel protein delivery platform based on the self-assembly of multi-armed amphiphilic cyclodextrins. Cell-targeting moieties were designed onto the surface of the nanocarriers to enhance their endocytosis. The results showed that the AS1411 aptamer-modified cyclodextrins/saponin therapeutic

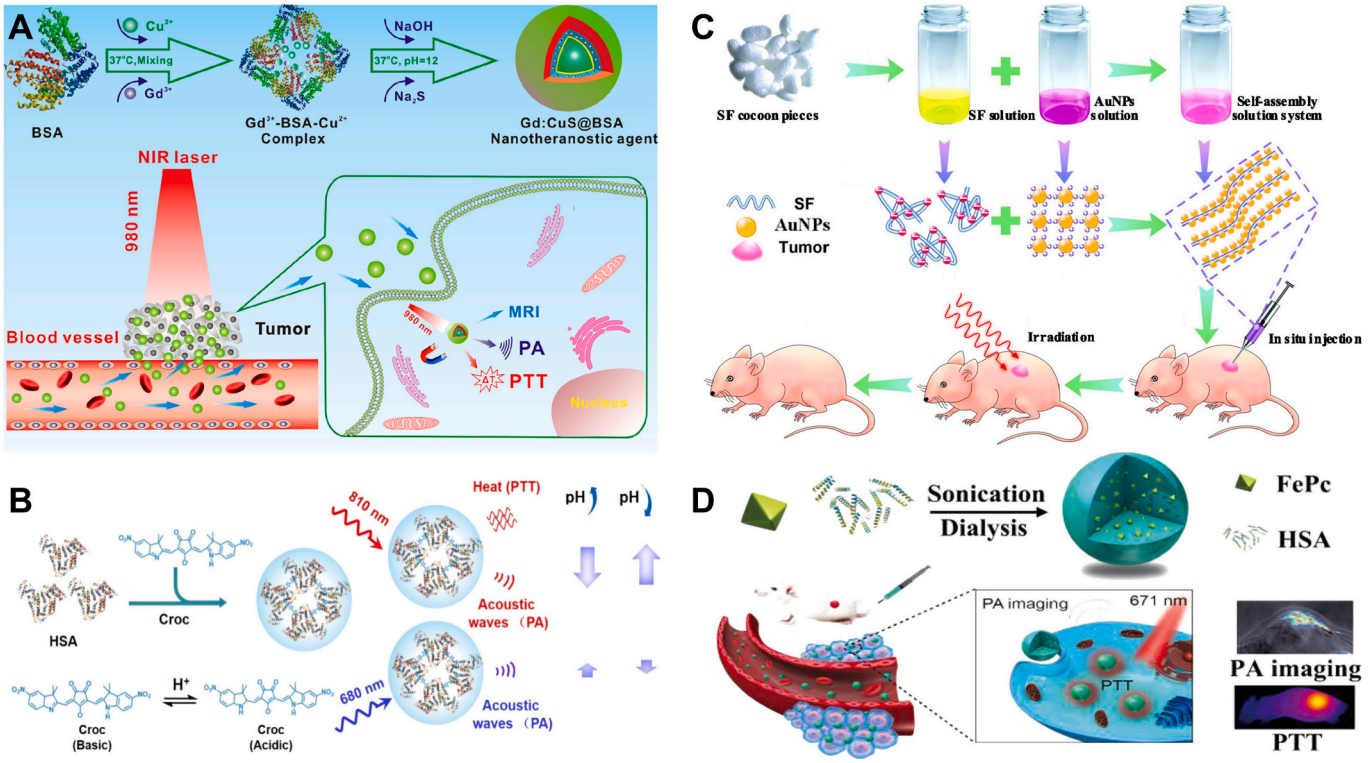

Fig. 5. Protein-loaded nanoparticles for phototherapy. (A) Schematic illustration of Gd:CuS@BSA nanomedicine for tumor photothermal therapy guided by *in vivo* PA/MR imaging [137] Copyright 2016 American Chemical Society. (B) Schematic representation of the synthesis of HSA-Croc and its application in PTT [130] Copyright 2019 Wiley-VCH Verlag. (C) Schematic illustration of Au NPs assembled on SF fiber templates to form Au NPs/SF nanofibers and their application in tumor photothermal therapy [131] Copyright 2022 Royal Society of Chemistry. (D) Schematic representation of iron phthalocyanine albumin assemblies for photoacoustic imaging-mediated *in vivo* tumor PTT [143] Copyright 2017 American Chemical Society.

protein nanoparticles could selectively accumulate at the tumor site and had a significant inhibitory effect on tumor growth in the MDA-MB-231 tumor mouse model. In addition, folate-targeted cyclodextrins nanocarriers could deliver Cas9 protein and siRNA into Hela cells, leading to specific gene deletion and tumor growth inhibition in Hela-loaded mice. Targeted multi-armed amphiphilic cyclodextrin nanocarriers were used for intracellular protein delivery, thus improving the effectiveness of protein therapy [130].

4.2. Phototherapy

Phototherapy is a promising technique for cancer treatment with the advantages of minimally invasiveness, high efficacy and low side effects [131–133]. Photothermal therapy (PTT) and photodynamic therapy (PDT) are the two main modalities of phototherapy. PTT converts light energy through photothermal agents under light exposure into thermal energy through photothermal agents, leading to thermal ablation of cancer cells [134–136]. Protein nanoparticles can be assembled with photothermal agents for *in vivo* photothermal therapy. Yang et al. synthesized nanotherapeutic agents (Gd:CuS@BSA) with good biocompatibility by a biomimetic strategy using BSA as a biotemplate [137]. The prepared Gd:CuS@BSA nanoparticles increased the temperature of the tumor region by about 21 °C (30–51 °C) under NIR laser irradiation, which was sufficient to ablate the tumor (Fig. 5A). Chen et al. utilized pH-responsive croconine (Croc) dye with near-infrared absorption to effectively adsorb onto HSA *via* hydrophobicity and induced albumin self-assembly to form albumin dye nanoparticles (HSA-Croc) [138]. HSA-Croc nanoparticles were injected intravenously to efficiently enter tumors. With decreasing pH, Croc absorption at 810 nm increased, and the absorption at 680 nm decreases, allowing real-time detection of intra-tumor pH by dual-wavelength proportional photoacoustic imaging, thus revealing a dramatic decreased in pH inside the core of a large tumor (Fig. 5B). In addition, HSA-Croc had a more uniform heating distribution within the tumor than conventional pH-insensitive photothermal agents. Therefore, effective photothermal ablation of large tumors can be achieved using the pH-responsive photothermal agent HSA-Croc.

Gold nanoparticles (Au NPs) are a promising photothermal agent for cancer therapy. Spherical Au NPs absorb weakly in the NIR window of tissue penetration, which leads to their low photothermal efficiency and limits their therapeutic effect. Wang et al. found that fibrous nanostructures assembled from spherical Au NPs could redshift the optical absorption to the NIR due to the template effect of silk fibroin (SF), thus improving their photothermal efficiency in the NIR window [139]. Due to electrostatic interaction, Au NPs on SF nanofiber templates could be assembled into nanofibers. The morphology of the synthesized Au NPs/SF nanofibers was controlled by SF concentration and incubation time. Both *in vivo* and *ex vivo* experiments showed that Au NPs/SF nanofibers could kill tumor cells and inhibit tumor growth more effectively than Au NPs (Fig. 5C).

Porphyrin phthalocyanine photosensitizers have the advantages of high tumor-selective uptake rate and absorption wavelengths located in the red light region with strong ability to penetrate tissues, and are widely used in tumor therapy [140–142]. Jia et al. prepared human serum albumin-iron (II) phthalocyanine FePc nanoparticles (HSA-FePc) for photoacoustic (PA) imaging-guided *in vivo* tumor PTT (Fig. 5D) [143]. The prepared HSA-FePc nanoparticles were characterized by high stability, high NIR absorption efficiency, and high photothermal conversion efficiency up to 44.4%. To the authors' knowledge, this is the first use of FePc for PA imaging-guided tumor PTT.

Although proteins have been used in the biomimetic synthesis of various nanostructures, the most fundamental intrinsic properties of proteins have been largely ignored. Yang et al. [144] synthesized ultrasmall gadolinium (Gd) nanodots based on horseradish peroxidase by

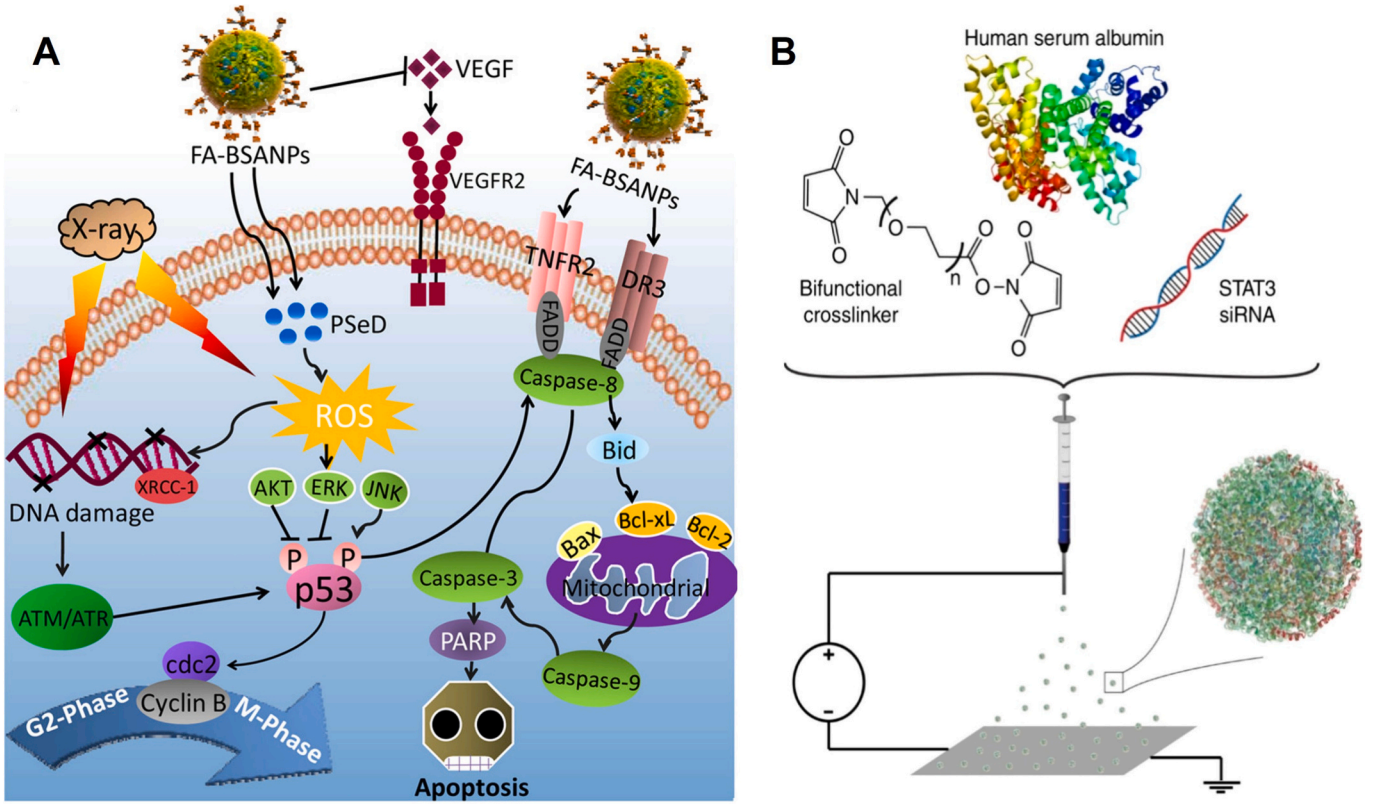

Fig. 6. Protein-loaded nanoparticles for radiotherapy. (A) Schematic diagram of tumor treatment with FA-BSA nanoparticles in combination with X-rays [157] Copyright 2014 American Chemical Society. (B) Schematic illustration of STAT3i SP nanoparticles [161] Copyright 2020 Springer Nature.

a bionic synthesis method and then loaded their substrate 2,2-biazobis (3-ethyl-benzothiazole-6-sulfonic acid) diammonium salt, which showed the retention of HRP enzyme activity by about 70%. They exploited the highly retained enzyme catalytic activity of $Gd@HRP^{ABTS}$ to catalytically oxidize ABTS to ABTS· + in the presence of endogenous H_2O_2 from tumors, thus enabling tumor-selective catalytic photoacoustic imaging and PTT. Yu et al. designed a cascade nanoreactor through a glucose consumption-induced thermal sensitization strategy [145]. The nanoreactor utilized zeolite imidazolate framework-8 to encapsulate the near NIR-II photothermal reagent Bi—Au in a glucose oxidase protein polyphenolic structure. Glucose oxidase-induced glucose degradation and the enzyme-like activity of the photothermal material constitute a closed-loop cascade reaction. This study improves tumor therapeutic efficacy by modulating the metabolic pathways of tumor cells and provides a promising strategy for combination therapy of tumors.

PDT generates ROS through photosensitizers, such as mono-linear oxygen ($^1O_2$), superoxide radicals ($O_2^{\cdot-}$) and hydroxyl radicals (·OH), which induce phototoxicity and kill cancer cells [146–148]. Chlorin e6 (Ce6), with its remarkable high-sensitivity activity and its ability to be internalized by receptor-expressing cells, has become the subject of much attention in the study of new photodynamic cancer drugs. Adimoolam et al. reported a protein nanoparticle photosensitizer delivery system that does not involve any chemical modification [149]. The Ce6-Lf nanoparticles were prepared by the water-in-oil emulsion method using lactoferrin as a matrix and Ce6 photosensitizer. Notably, Ce6-Lf nanoparticles can specifically release Ce6 at low pH, which was advantageous in the acidic environment of tumors and endosomes. Minimalist multi-functional platform for the safe delivery of therapeutic agents to tumor sites in universities and colleges are urgently desired in nanomedicine. Yang et al. synthesized a multifunctional platform based on $SF@MnO_2$ nanoparticles using SF as a reducing agent and template. Then loaded with photosensitizer indocyanine green (ICG) and chemotherapeutic drug DOX, $SF@MnO_2$/ICG/DOX nanocomplexes were prepared. It was demonstrated that $SF@MnO_2$/ICG/DOX nanoparticles could significantly improve tumor suppression with minimal systemic toxicity or adverse effects by combined PTT/PDT/chemotherapy [150].

The problem of limited light penetration and constant oxygen consumption results in poor photodynamic therapy. Wang et al. constructed a novel anoxia-responsive protein-based nanoreactor by covalently cross-linking the photosensitizer Ce6 coupled to BSA and ferritin using the hypoxia-responsive unit azobenzene and encapsulating the sorafenib within a protein shell, which provides an unparalleled opportunity for efficient PDT and synergistic therapy with ferritin deposition [151]. Liang et al. constructed a metal polyphenol network based on epigallocatechin gallate (EGCG) using BSA as a template to encapsulate the photosensitizers Ce6 and Fe^{3+} using Fe-EGCG network. Under 660 nm laser irradiation, this nanoreactor induced more ROS production, thereby promoting tumor cell apoptosis. This highly efficient and low-toxicity nanoreactor is considered to be a viable postoperative adjuvant therapy [152].

Zinc phthalocyanine (ZnPc) is used as a second-generation photosensitizer for phototherapy because its light absorption emission curve is in the phototherapeutic wavelength region. In addition, ZnPc and its derivatives are effective in generating ROS [153]. Wang et al. designed a caspase-3 ferritin-activated probe (FABP/ZnPc) for delivering the photosensitizer ZnPc and monitoring its apoptosis during PDT [154]. *In vitro* and *in vivo* experiments have also demonstrated that FABP/ZnPc can effectively destroy tumor tissue by laser irradiation.

4.3. Radiotherapy

Radiotherapy is the most commonly used treatment in cancer therapy, which kills cancer cells with high-energy radiation. A controlled dose of fixed-focus radiation therapy can maximize tumor elimination

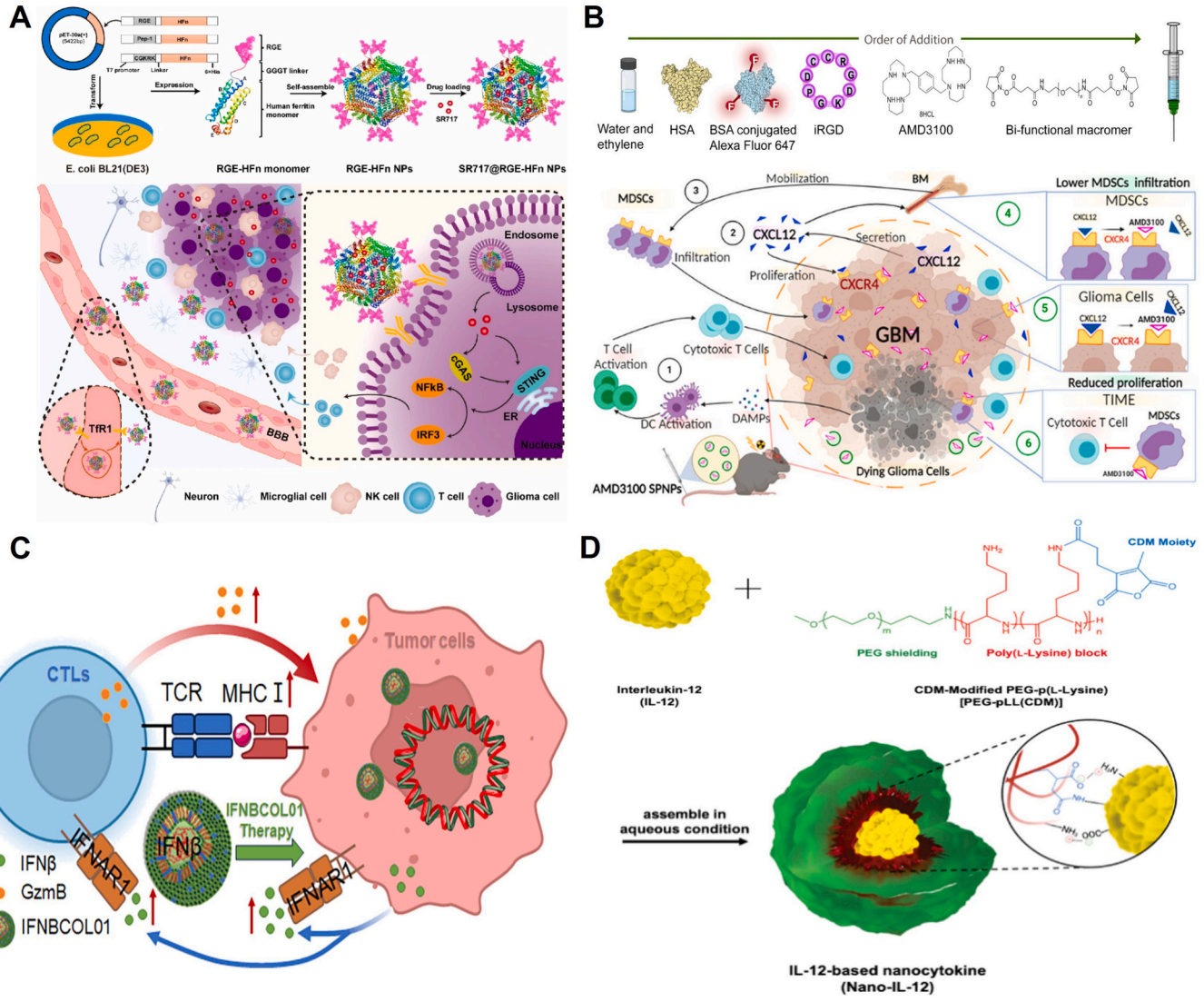

Fig. 7. (A) Schematic representation of SR717@RGE-HFn nanoparticles with dual targeting strategy for effective anti-tumor immune response [167] Copyright 2022 Elsevier. (B) Schematic illustration of systemic delivery of iRGD peptide protein nanoparticles-assisted CXCR4-CXCL12 signaling inhibitor for glioma immunotherapy [168] Copyright 2022 American Chemical Society. (C) Schematic diagram of delivery of interferon β-encoding plasmid to restore interferon β expression to enhance anti-tumor immunity in colon cancer [171] Copyright 2024 American Chemical Society. (D) Formation and structure of interleukin 12-based nanocytokines [172] Copyright 2023 Wiley-VCH Verlag.

and reduce side effects [155,156]. The introduction of radiation therapy sensitizers can effectively improve the local treatment effect.

To overcome the emergence of radioresistance in long-term radiotherapy, Huang et al. prepared cancer-targeting nanosystems using biocompatible BSA nanoparticles as carriers for organic selenocompound and FA as targeting ligands [157]. Under X-ray irradiation, FA-BSA nanoparticles could synergistically enhance intracellular ROS production through nanoparticle interfacial effects. This tumor-targeted drug delivery nanosystem can be used to overcome radiation resistance in cancer (Fig. 6A). Ding et al. first reported protein sulfenic acid reactive gold nanoparticles as an effective radiosensitizer for enhanced X-ray computed CT imaging and radiotherapy of tumors *in vivo* [158].

Glioblastoma (GBM) is the most common primary intracranial malignant tumor in adults. In the treatment of glioblastoma diseases, drugs must be transmitted to the brain parenchyma through the blood-brain barrier (BBB) to maximize the efficacy [159,160]. Inspired by the ability of natural proteins and viral particles to cross BBB, Gregory et al. designed synthetic protein nanoparticles (SPNP) based on HSA and cell penetrating internalized RGD (iRGD) peptide polymerization [161]. The introduction of tumor-targeting tissue-penetrating iRGD peptide enables SPNP to penetrate the BBB and distribute throughout the tumor volume, allowing efficient delivery of siRNAs for targeting STAT3 without invasive surgical procedures. A second tumor was induced again in the contralateral hemisphere, and without additional therapeutic intervention, all of these mice reached the second long-term survival time point (Fig. 6B).

4.4. Immunotherapy

Ovalbumin (OVA) is a protein antigen that is widely used in tumor immunotherapy to trigger cellular and humoral immune responses [162–164]. Habibi et al. developed an engineering approach for reactive electrospray-based protein nanoparticles. The generated protein nanoparticles. Consisted of polymerized OVA, where individual OVA molecules were chemically linked by PEG units [165]. Precision-designed OVA protein nanoparticles. Improve the overall antitumor response compared to lysosomal antigens. In a mouse melanoma model, smaller (200 nm) protein nanoparticles. (PEG/OVA ratio of 10%) improved

survival in mice with advanced melanoma. Cheng et al. constructed a new dual-antigen delivery system by genetically recombining the model antigen $OVA_{257–264}$ and the melanoma-specific antigen gp100 into an HBc-based VLP [166]. Hybrid VLPs vaccine significantly induces antigen-specific anti-tumor immunity and inhibits tumor growth and metastasis.

Ferritin can cross the blood-brain barrier by binding to transferrin receptor 1, which is overexpressed on blood-brain barrier endothelial cells. Wang et al. developed ferritin nanoparticles for targeted delivery of small molecule immunomodulators to enhance immunotherapy for glioma *in situ* [167]. They genetically engineered a fusion protein nanoparticle platform loaded with a STING agonist (SR717), which is able to target gliomas after crossing the brain barrier, providing a promising therapeutic idea for glioma treatment (Fig. 7A). CXC motif chemokine ligand 12/CXC motif chemokine receptor 4 (CXCL12/CXCR4) is one of the signaling pathways for the treatment of GBM. The poor pharmacokinetic properties and low bioavailability of CXCR4 antagonists (AMD3100) have hampered their clinical use. Based on this, Mahmoud S. Alghamri et al. synthesized protein nanoparticles by loading the CXCR4 inhibitor AMD3100 for immunotherapy of GBM [168]. The prepared protein nanoparticles triggered anti-GBM adaptive immune responses by inducing immunogenic death and reprogramming the immunosuppressive microenvironment (Fig. 7B).

Cytokines are key proteins for signaling in the tumor microenvironment with pleiotropic effects [169,170]. In terms of anti-tumor, interferon can directly inhibit the growth of tumor cells, and interleukins such as interleukin 2, interleukin 12, and interleukin 15 can enhance the cytotoxic activity of lymphocytes or myeloid cells and thus inhibit cell growth. Type I interferons play a critical role in host cancer immune surveillance, but their expression is often compromised in the tumor microenvironment. Yang et al. delivered interferon β-encoding plasmids to tumor sites *via* cationic lipid nanoparticles, which could effectively restore interferon β expression to inhibit tumor immune escape (Fig. 7C). The experimental results showed that the delivery of interferon β-encoding plasmid DNA by lipid nanoparticles could enhance tumor immunogenicity and T-cell effector function, and thus inhibit the growth of colon tumors *in vivo* [171]. Interleukin 12 can activate immune checkpoint inhibitors against cold tumors by building strong anti-tumor immunity. However, its toxicity and the systemic induction of counteracting immunosuppressive signals hinder translation. Chen et al. developed response-stimulating nanocytokines by encapsulating natural interleukin 12 in biocompatible polymers to achieve spatiotemporal control of its activity (Fig. 7D). The interleukin 12-based nanocytokines released fully active interleukin 12 upon sensing the acidic pH within the tumor, ensuring precise stimulation of intratumor immunity, leading to satisfactory therapeutic effects by synergizing with checkpoint blockade in primary and metastatic cold tumor models. Moreover, since the polymers used to assemble interleukin 12-based nanocytokines can be designed to encapsulate a wide range of proteins with different molecular weights and surface charges, the system holds the promise of broader biomedical applications [172].

5. Conclusion and outlook

Protein-based biomaterials, either natural proteins or designed peptides, showed great potential for drug delivery. The high loading efficiency and excellent targeting effect facilitates protein nanoparticles to be excellent drug delivery system. Therefore, we summarize the several common methods for the preparation of protein nanoparticles and their recent advances in cancer imaging and therapy.

Protein nanoparticles can carry drugs by electrostatic adsorption and other ways to directly or indirectly target tumors and achieve stimulus-responsive release of drugs. In addition, protein nanoparticles can be combined with inorganic nanoparticles, photothermal agents and photosensitizers to achieve radiation therapy, photothermal therapy and photodynamic therapy of tumors. Some virus-like nanoparticles can also

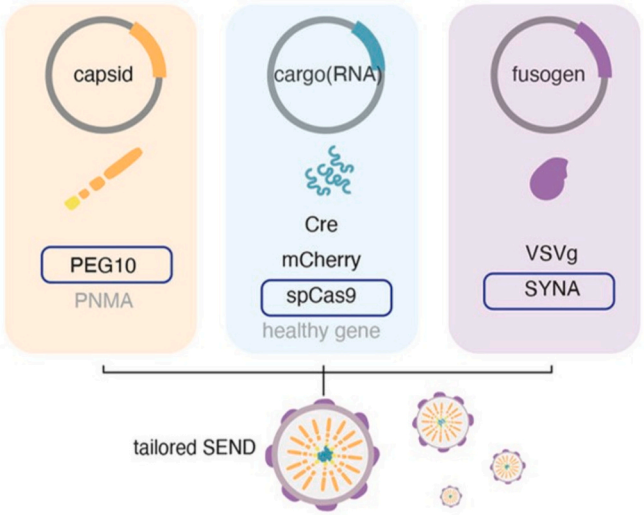

Fig. 8. SEND is a modular delivery platform that combines endogenous Gag homologs, cargo mRNAs, and fusion proteins that can be tailored for specific environments [173] Copyright 2021 American Association for the Advancement of Science.

be used to achieve tumor immunotherapy using their own immunogenicity. Meanwhile, protein nanoparticles can not only be combined with fluorescent dyes for *in vivo* fluorescence imaging, but also be combined with clinical contrast agents or make use of their own characteristics for MRI, PET imaging and CT imaging of tumors. Future research should determine whether these materials and preparation methods are economically feasible in large-scale production and whether protein nanoparticles can safely and effectively deliver therapeutic agents to target sites. In addition, the mechanism of protein nanoparticle-mediated translocation is unclear. With in-depth research on the biosafety and biocompatibility of protein nanoparticles, future protein nanoparticles will have higher safety and lower immunogenicity. By optimizing the preparation process and surface modification methods, protein nanoparticles will be able to be better compatible with organisms and reduce the occurrence of side effects and adverse reactions. Moreover, protein nanoparticles can be prepared and modified in a variety of ways, allowing personalized treatment plans to be tailored to patients' specific conditions and needs. In the future, through in-depth study of the molecular characteristics of tumor cells and individual differences, protein nanoparticles will be able to achieve more precise and personalized treatment, and improve the therapeutic effect and quality of life of patients.

The biomedical community has been developing powerful molecular therapies, but delivering them to cells in a precise and effective manner is challenging. Segel et al. [173] have developed a platform for delivering molecular drugs to cells. The platform, called selective endogenous eNcapsidation for cellular delivery (SEND), can be programmed to encapsulate and deliver different RNA cargoes. SEND utilizes the mouse and human retrovirus-like protein PEG10 to package, secrete and deliver specific RNAs, greatly expanding the applications of nucleic acid therapy and it may cause less of an immune response than other delivery methods (Fig. 8). This a new way to make protein nanoparticle for mRNA delivery. Although it is not for cancer treatment, but still showing potential for future applications.

Machine learning is a subfield of artificial intelligence that enables scientists, clinicians and patients to address some of these challenges. In recent years many scientists have begun to explore machine learning based methods to study medical images for early diagnosis as well as to develop new treatments for diseases [174,175]. Compared with traditional methods, machine learning can also design protein molecules more accurately and quickly, reducing the length of time it takes to

design a protein from “months” to “seconds”. Schissel et al. combined machine learning and other methods to design an unnatural miniprotein with a cell-penetrating effect for the transport of antisense morpholino ring oligonucleotides [176]. Combining machine learning approaches beneficial for designing tissue- or cell-targeted functional protein nanoparticles with low non-specificity. In conclusion, with the development of proteomics, molecular biology and recombinant technology, protein-based delivery systems have a broad application prospect.

Declaration of Interest Statement.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Yue Hua: Writing – original draft, Software, Methodology, Investigation. **Zibo Qin:** Writing – original draft, Software. **Lin Gao:** Validation. **Mei Zhou:** Validation. **Yonger Xue:** Writing – review & editing, Supervision. **Yue Li:** Writing – review & editing, Supervision. **Jinbing Xie:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Data availability

No data was used for the research described in the article.

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