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Rationalized landscape on protein-based cancer nanomedicine: Recent progress and challenges

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ABSTRACT

The clinical advancement of protein-based nanomedicine has revolutionized medical professionals' perspectives on cancer therapy. Protein-based nanoparticles have been exploited as attractive vehicles for cancer nanomedicine due to their unique properties derived from naturally biomacromolecules with superior biocompatibility and pharmaceutical features. Furthermore, the successful translation of Abraxane™ (paclitaxel-based albumin nanoparticles) into clinical application opened a new avenue for protein-based cancer nanomedicine. In this mini-review article, we demonstrate the rational design and recent progress of protein-based nanoparticles along with their applications in cancer diagnosis and therapy from recent literature. The current challenges and hurdles that hinder clinical application of protein-based nanoparticles are highlighted. Finally, future perspectives for translating protein-based nanoparticles into clinic are identified.

1. Introduction

Nanomedicine exploited the application of nanotechnology to deliver diagnostic and therapeutic agents for imaging and therapeutic purposes via nanocarriers with a size range of 1–1000 nm (Heshmati Aghda et al., 2022). These nanocarriers have been extensively explored to provide efficient tools for diagnostic and therapeutic applications, especially for cancer imaging and targeted therapy, reasonably due to their small size, easy modification and functionalization, enhanced permeation and retention (EPR) effect, superior cellular uptake, as well as good biocompatibility (Du et al., 2020). In particular, various proteins have recently been exploited as a naturally occurring nanocarriers for a variety of medical purposes, involving cancer nanomedicine, with non-immunogenic, biocompatible, and biodegradable characteristics (Iqbal et al., 2021; Bellini et al., 2020). To date, several approaches have been utilized to fabricate protein nanocarriers including non-covalent interaction, covalent protein conjugates, desolvation, self-assembly, emulsification as well as in situ biomineralization in protein cavities (Wu et al., 2020; Türkeş and Sağ Açıkel, 2024). For a range of analytical and medical applications, different proteins have typically been used to encapsulate or conjugate with cytotoxic pharmaceuticals, radioactive agents, nuclear or photoactive compounds, and functional ligands. Additionally, certain proteins are currently used as hollow nanocage nanoreactors, allowing for the simple and well-regulated synthesis of therapeutic or diagnostic agents inside the nanocages via the biomineralization process (Iqbal et al., 2021; Wang et al., 2023). In this mini-review article, we present an overview of protein nanoparticles that are constructed through these approaches for cancer diagnosis and therapy. Representative examples are highlighted, and ourview on the recent progressive landscape, current obstacles and impeding possibilities are alsohighlighted.

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2. Protein nanocarriers

In recent years, many attempts have been made to develop effective nanocarriers for cancer therapy. Among a variety of functional nanomaterials, many protein-based nanocarriers have been considered as promising drug vehicles with enhanced anticancer activity and minimal adverse side effect, owing to their advantages including easy fabrication in mild condition, high affinity to bind various drugs, and considerable tumor-targeting (Iqbal et al., 2021).

Serum albumin is a very bountiful endogenous physiological molecule playing an indispensable role in the regulation of physiological osmotic pressure and carrying various endogenous substances (Van de Sande and Cosyns, 2020). Human serum albumin (HSA) is comprised of 585 amino acids assembled as a single polypeptide chain and bearing 66.5 kDa molecular weight. Each HSA molecule has two specified drug-binding domians or sites, namely domain I or warfarin intrinsic region and domain II or benzodiazepine intrinsic region. In addition to these two specified drug-binding domains, HSA molecule exhibited other drug binding strategies including electrostatic interaction as well as hydrophobic interaction (Iqbal et al., 2021; Wang et al., 2023). Various kinds of cytotoxic agents (Zhang et al., 2018; Motevalli et al., 2019), anti-inflammatory agents (Czub and Handing, 2020; Spada et al., 2021) hypoglycemic agents (Lu et al., 2020; Jose et al., 2015), carbohydrates (Wang et al., 2024) and other drugs (Mickaela Martinez et al., 2022; Patel et al., 2022)might have high affinity to bind to albumin, thereby improving their pharmacokinetics and therapeutic efficiencies with reduced adverse side effects. Usually, therapeutic drug molecules interact with albumin either through electrostatic, hydrophobic interaction or specific binding domain to generate albumin-drug conjugates or nanoparticles with nanometers size (Hassanin and Elzoghby, 2020; Pal et al., 2023). In the past two decades, a number of achievements in protein-based nanoparticles have been approved by the U.S. Food and Drug Administration (FDA) for advanced cancer therapy and diabetes treatment, with many more trials ongoing for active clinical investigation (Table 1). For instance, a clinically approved protein-based anticancer nanomedicine (Abraxane) was constructed by assembling paclitaxel (PTX) HSA nanocarriers (ca. 130 nm) via hydrophobic interactions with improved anticancer efficiency and reduced chances of side effects (Kundranda and Niu, 2015).

In addition, cisplatin derivative was also incorporated in albumin to improve its therapeutic efficiency through their interaction with albumin. Further, fatty acid was decorated to cisplatin to regulate the non-covalent interaction with HSA by controlling its chain length, resulting in the formation of albumin-platinum (IV) prodrug with superb intracellular delivery, DNA-specific injury, prolonged blood circulation, and subsequent potent anticancer activity (Zheng et al., 2014; Chen et al., 2023a). Distinctly, the interaction of anticancer compounds with albumin might play a crucial role in regulating their anticancer efficacy. Therefore, with better distribution in blood and tumor penetration, albumin may act as an excellent drug carrier for effective drug delivery.

The natural cavity of proteins is also utilized to deliver versatile functional molecules for cancer therapy. For example, ferritin is one important iron storing protein, which is made up of 24 monomers and has a naturally occurring hollowed nanocage featuring an 8 nm internal cavity and an exterior diameter of 12 nm, has also been applied for efficient drug delivery (Han et al., 2014; Lee et al., 2022). H-ferritin nanoparticles caging doxorubicin (DOX) (14.2 nm) were fabricated by enclosing the apertures with DOX of H-ferritin nanocages (Liang et al., 2014). Briefly, H-ferritin was disassembled in the solution of urea, followed by addition of DOX. Then, DOX was encapsulated in the hollow cages during the reassembling process via dialysis against gradient concentrations of urea buffer. These nanoparticles exhibited a prolonged blood circulation as compared to free DOX, and were able to specifically target to TfR1-overexpressed tumor cells, thus generating a substantially

Table 1
Protein-based nanomedicine in clinic and clinical trails.

Drug name	Molecular type	Clinical trial identifiers	Indication	Company	Status
Albiglutide	GLP-1 albumin conjugate	NCT01357889	Diabetes	GlaxoSmithKline	Approved
Nanocoll®	^{99m}Tc labeled micro-aggregated human albumin	NCT00929032	SPECT imaging	Nycomed Amersham	Approved
Abraxane® (ABI-007)	Nanoparticle albumin-bound paclitaxel	NCT01307891 NCT02027428 NCT00732836	Breast/Lung/Prostate cancer	Celgene Corporation	Approved
Ontak	Denileukin diftitox	NCT00880360	Ovarian/Peritoneal cancer	Eisai Inc.	Approved
Levemir	Insulin detemir	NCT00655044	Diabetes mellitus	Novo Nordisk	Approved
Liraglutide	Fatty acid peptide Conjugate	NCT01795248	Diabetes mellitus	Novo Nordisk	Approved
Adynovate	Protein polymer Conjugate	NCT04158934	Hemophilia	Baxalta (Shire)	Approved
Plegridy	Protein polymer Conjugate	NCT02230969	Multiple sclerosis	Biogen	Approved
Fyarro (ABI-009)	Nanoparticle albumin-bound rapamycin	NCT00635284	Solid tumors	Celgene Corporation	Approved
ELZONRIS	Interleukin-3 truncated diphtheria toxin	NCT 02113982	Blastic plasmacytoid dendritic cell neoplasm	Stemline Therapeutics	Approved
BESPONSA	Monoclonal antibody-calicheamicin conjugate	NCT01564784	Lymphoblastic leukemia	Pfizer	Approved
LUMOXITI	Moxetumomab pasudotox	NCT 01829711	Refractory hairy cell leukemia	AstraZeneca Pharmaceuticals LP	Approved
ABI-008	Nanoparticle albumin-bound docetaxel	NCT00477529	Prostate cancer	Celgene Corporation	Phase I/II
ABI-010	Nanoparticle albumin-bound 17-AAG	NCT00820768	Solid tumors	Celgene Corporation	Phase I
ABI-011	Nanoparticle albumin binding thiocolchicinedimer	NCT01163071	Solid tumor	Celgene Corporation	Phase I
BTP-114	Albumin-binding Cisplatin	NCT02950064	Pancreatic/Ovarian/Breast/Prostatic neoplasms	Placon Therapeutics	Phase I
Tf-CRM107	CRM107	NCT00052624	Malignant brain tumor	Xenova Biomedix	Phase II
MTX-HSA	Methotrexate albumin conjugate	EORTC 30951 EORTC 30947	Metastatic renal cell carcinoma	Klinge Pharma	Phase II
INNO-206	Albumin-binding prodrug of doxorubicin	NCT01337505	Malignant solid tumor	CytRx, Inc	Phase III
CALAA-01	Transferrin- siRNA Nanocomplex	NCT00689065	Solid tumor	Calando Pharmaceuticals	Phase I
MBP-426	Transferrin-oxaliplatin nanoparticles	NCT00964080	Advanced/ metastatic solid tumors	Mebiopharm Co., Ltd	Phase II

extended median survival period along with reduced toxicity. Apparently, ferritin can act as a promising nanocarrier for cancer therapy. In another example, IR 820 exhibited an enhanced photothermal effect upon 808 nm light exposure after being loaded into ferritin nanocages due to the red-shifted absorbance from 600 nm to 800 nm, thus leading to photoacoustic (PA) imaging and simultaneous tumor ablation. Thus, this type of ferritin-based nanocarriers canact as a promising vehicle with considerable targeting capability for cancer therapy.

Transferrin (Tf) is an iron binding large serum protein, with a molecular weight of about 79 KDa comprise of single polypeptide chain of 679 amino acids stabilized by disulfide bonds and exhibiting long blood circulation half-life of ~8 days (Iqbal et al., 2021). For more than 30 years, Tf protein has been widely studied as nanocarrier for target-specific delivery of cancer therapeutic or diagnostic moieties or both therapeutic and diagnostic (theranostics), as it is biodegradable, biocompatible and non-immunogenic in nature (Neves et al., 2021). For example, a smart theranostic nanoprobe, transferrin–indocyanine green NPs (Tf-ICG-NPs) was fabricated by a green and facile method for imaging-guided PTT of tumor cells (Zhang et al., 2016a). The fabricated Tf-ICG NPs have excellent water solubility, consistent particle diameter, stable colloidal structure, and remarkable in vivo and in vitro theranostic targeting effect. Furthermore, Tf modeled copper nanoclusters - doxorubicin (Dox) nanoparticles (Tf-Cu NCs Dox NPs) were fabricated for biological imaging as well as targeting tumor cells over-expressing TfRs (Goswami et al., 2018). In this nanodrug, Cu-NCs act as bioimaging probe to evaluate the intracellular Dox and its release from nanoparticles based on forster resonance energy transfer (FRET) after internalization as shown in Fig. 1. Additionally, the synergistic anticancer effect is also achieved as the nanodrug (Tf-Cu NCs Dox NPs) contains Cu-NCs and Dox**.** Thus, this type of Tf-based nanocarriers can act as a promising vehicle with considerable targeting capability for cancer therapy.

In addition, some other proteins can also be exploited to construct nanocarriers including plant-derived viral capsids (Aljabali et al., 2021), cowpea chlorotic mottle virus (Shukla et al., 2020), tobacco mosaic virus (Lumata et al., 2021), as well as small heat shock protein cages (Chen et al., 2021), casein (Zahariev and Draganova, 2023), elastin-like polypeptide (van Strien et al., 2023), gliadin (Voci et al., 2021), zein (corn protein) (Liu et al., 2023), soy/whey protein (Liu et al., 2022), collagen (Lo and Fauzi, 2021), and gelatin (Song et al., 2019). For instance, various virus capsid/envelop proteins have been employed as drug vehicles that possess unique characteristics such as uniform size, considerable specificity and superior delivery efficiency, thus improving the pharmacokinetics and biodistribution behaviors of imaging agents (Allen et al., 2005), photosensitizers (Suci et al., 2007) and anticancer compounds (Franke et al., 2018) for improving diagnostic imaging or therapeutic efficacies.

3. Protein-drug nano-conjugates

Generally, proteins are rich in free -COOH and -NH2 groups, which can be covalently conjugated or linked with functional molecules including target ligands, bioimaging probes, and cytotoxic agents (Liu and Chen, 2016; Gharbavi et al., 2023). This covalent conjugation strategy often displays specific characteristics, for example, decreased drug's leakage risk in physiological circulating system, high loading efficiency, along with unaffected biocompatibility and biodegradability (Liu et al., 2020; Wall et al., 2019). Thus, this strategy might also act as an alternative regimen in order to develop protein-drug conjugate nanocarriers. Generally, malleable compounds such as bioimaging probes, cytotoxic agents, cancer diagnostic therapeutic agents, as well as other medicinal compounds may be approached for conjugation with protein molecules such as albumin (Table 2).

As a biomacromolecule with ~6.6 nm in diameter, albumin is utilized as a single-molecule nanoparticle to be functionalized with imaging agents or therapeutic drugs, showing great advantages such as

Table 2
Representative example of protein drug nano-conjugates and nano-complexes.

Protein drug nano-conjugates		
Agent type	Examples	Ref. No.
Imaging agents	^{68}Ga-labeled HSA	(Brunner et al., 2012)
	^{68}F-labeled HSA	(Chang et al., 2005)
	^{111}In-labeled HSA	(Palmowski et al., 2013)
	Gd-labeled HSA	(Ogan et al., 1987)
Therapeutic agents	Doxorubicin HSA conjugates	(Chen et al., 2023b)
	Doxorubicin prodrug	(Yousefpour et al., 2019)
	Pt(II) prodrugs	(Paul et al., 2024)
	Camptothecin prodrug	(Cheng et al., 2021)
	Methotrexate	(Faghfoori et al., 2020)
Theranostic agents	CysCOOH HSA conjugate	(Rong et al., 2015)
	Ce6 HSA conjugate	(Zhang et al., 2020b)
	Cyanine albumin conjugate	(Tang et al., 2018)
Imaging agents	SQ-BSA complex	(Jiang et al., 2023)
Therapeutic agents	DOX-HSA prodrug	(Zhang et al., 2020a)
	ICG@holo-Tf NAs	(Zhu et al., 2017)
	SQ-BSA complex	(Gao et al., 2014)
Theranostic agents	PTX/ICG-HSA complex	(Chen et al., 2015b)
	HSA@IR780/DTX	(Lian et al., 2017)

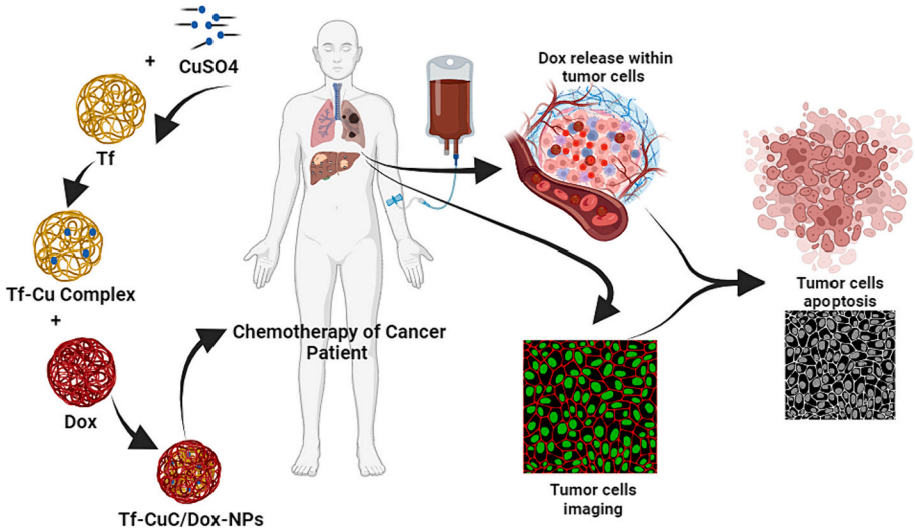

Fig. 1. Tf-Cu/Dox nanoclustures for bioimaging, targeted delivery and synergistic in vitro and in vivo therapeutic activity.

extended plasma residence time, superior biocompatibility, as well as preferable tumor targeting (Ghadi et al., 2023; Kuche et al., 2023). For instance, a conjugate designed for NIR fluorescence and photoacoustic multidimensional imaging was synthesized when a near-infrared (NIR) cyanine dye, which had elevated fluorescence intensity was covalently coupled to the -NH_2 groups of lysine residues in HSA using the EDC/NHS process (Fig. 2). Pursuant to the EPR impact, the albumin-dye combo also showed increased tumor penetration, which led to complete eradication of the tumor when exposed to laser light (Rong et al., 2015).

Additionally, the pharmacokinetics of drugs could be optimized using albumin-drug conjugates. Employing albumin nanocarriers, the plasma mean residence time of cytotoxin was considerably extended from 0.183 to 17.4 h, demonstrating a 22-fold improvement in their bioavailability (Simon et al., 2013). Additionally, radionuclide-labeled albumin, such as ^{68}Ga-albumin, has been investigated as a circulation pooling positron emission tomography (PET) tracer for measuring the left ventricle's function in rodents, demonstrating an ideal fit with magnetic resonance imaging (MRI) utilized in clinical settings (Brunner et al., 2012). Consequently, covalent coupling allows imaging agents and therapeutic medications to make use of albumin's distinctive characteristics, thus showing heightened stability, prolonged blood circulation time and subsequent improved diagnosis and therapeutic performances.

β-casein nanoparticles conjugated with chitosan were used to deliver a Pt complex (anticancer agent), whose cytotoxicity was studied in the HCT 116 cell line. The uptake, as well as the cytotoxicity, were enhanced upon encapsulation within the nanoparticles as compared to when it is free (Razmi et al., 2013). Among the protein-drug nanoconjugates for cancer therapeutics, antibody-drug conjugates (ADCs) are one of the most promising classes. ADCs for cancer therapy are composed of monoclonal antibodies (mAbs) allied with a cytotoxic agent through specific linker, increasing the distribution window and directing the drug to specific antigen for specified mAbs, making possible the targeted drug delivery to tumor microenvironment (TME) (Weiner, 2015; Dumontet et al., 2023; Chau et al., 2019). The type of cytotoxic drug, mAbs specificity, and the allied linker types, and their interactions with TME greatly influence the efficacy of ADCs. The effectiveness of ADCs in treating a variety of solid cancers and hematological malignancies encourages the emergence of ADCs. The projection of ADC market was approximately four billion US dollars in 2023, and is expected to increase significantly by 2030 (Tsuchikama and An, 2018). Target expression, ADC in vivo characteristics, drug transport parameters, and the pharmacologic profile of therapeutic action in preclinical investigations and later clinical trials must all be thoroughly understood for the effective synthesis of ADCs (Shim, 2020; Tsuchikama and An, 2018). To prevent the onset of refractory medical conditions, several clinical trials are now comparing ADCs with other medications such as checkpoint inhibitors, tyrosine kinase inhibitors, and traditional chemotherapy strategies. Multiple ADCs have shown remarkable efficacy against cancers that do not respond to medication, leading to authorizations in a wide range of indications (Table 3); Nevertheless, a number of obstacles prevent ADCs from being used more widely, such as toxicities, inadequate predictive biomarkers, unclear clinical value when used in conjunction with standard therapies, and poorly understood drug resistance pathways (Walsh and Walsh, 2022; Shi et al., 2022; Hafeez and Parakh, 2020).

Chemotherapies utilizing monoclonal antibodies (mAbs) first became available in the course of the 1970s. mAbs have the potential to target tumor cells, minimize non-specific toxicities, change their signaling patterns toward a therapeutic result, or trigger an immune response against the tumor cell by precisely binding an antigen on a malignant cell (Hafeez and Parakh, 2020; Drago et al., 2021; Tsuchikama and An, 2018).The US Food and Drug Administration (FDA) has approved about thirty mAbs for use in cancer indications thus far. A concept of focused delivery first proposed more than a century ago by German chemist Paul Erlich, who developed the magic bullet theory, referring to a chemical that selectively attacked microbes, prompted to the development of mAb-based targeted treatments and immunotherapies, including a potent class of biopharmaceuticals known as ADCs (Walsh and Walsh, 2022).

ADCs in particular have great promise for treating solid tumors and hematological malignancies, and more research is needed to determine the best ways to reduce toxicities and increase effectiveness (Alley et al., 2010). Molecular imaging advances can significantly impact ADC development in preclinical research as well as clinical trials by guiding patient selection and improving results (Deonarain et al., 2015). Notwithstanding their promise, further research on the biochemical, immunological, pharmacological, and molecular aspects of ADCs is

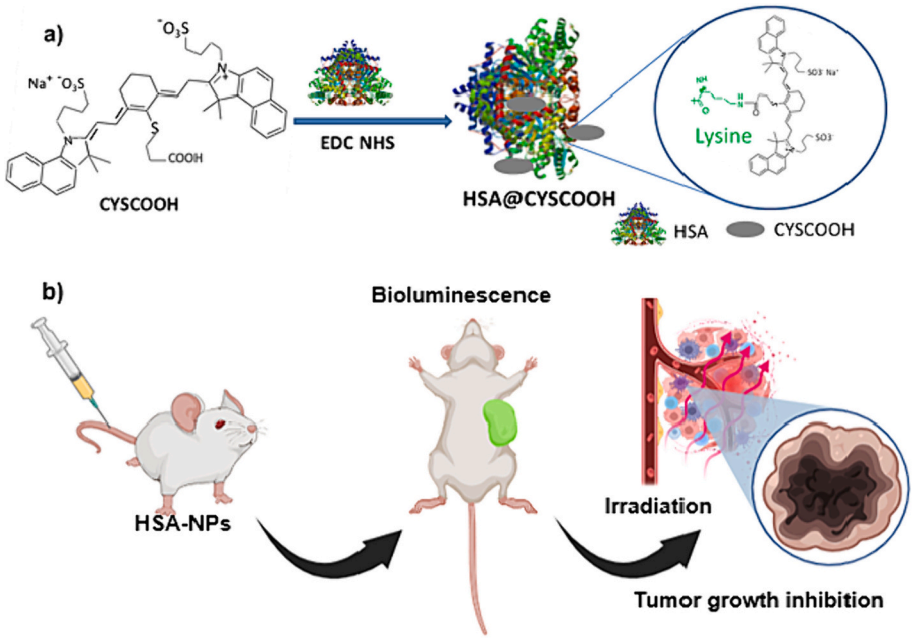

Fig. 2. (a) Synthetic procedure for the generation of HSA@CySCOOH through the conjugation of NIR cyanine dye and HSA. (b) Treatment with HSA-NPs followed by irradiation results in significant tumor growth inhibition.

Table 3
Representative examples of antibody-drug conjugates in clinic.

ADCs	Indications	Cytotoxic drug	mAbs	Specific-antigen	Status
Belantamab mafodotin-blmf	Refractory multiple myeloma	Monomethyl auristatin F	IgG1	BCMA	Approved
Enfortumab vedotin-ejfv	Advanced-stage urothelial carcinoma	Monomethyl auristatin E	IgG1	Nectin 4	Approved
Gemtuzumab ozogamicin	Refractory acute myeloid leukemia	Ozogamicin	IgG4	CD33	Approved
Sacituzumab govitecan-hziy	Advanced stage triple-negative breast cancer	SN-38	IgG1	TROP2	Approved
Polatuzumab vedotin-piiq	Relapse Diffuse large B cell lymphoma	Monomethyl auristatin E	IgG1	CD79b	Approved
Fam-trastuzumab deruxtecan-nxki	Advanced stage breast cancer ($HER2^+$)	DXd (DX8951 derivative)	IgG1	HER2	Approved
Inotuzumab ozogamicin	Refractory/B cell acute lymphoblastic leukemia	Ozogamicin	IgG4	CD22	Approved
Ado-trastuzumab emtansine (T-DM1)	Metastatic breast cancer ($HER2^+$)	DM1 (Maytonsinoid)	IgG1	HER2	Approved
Brentuximab vedotin	Hodgkin's lymphoma, Primary/systemic cutaneous anaplastic large cell lymphoma	Monomethyl auristatin E	IgG1	ErbB2, CD30	Approved

needed to improve their design and development. For the production of effective ADCs, linker chemistry and antibody-payload conjugation techniques are just as vital as the selection of target antigens and payloads (Beck et al., 2017). Specifically, the instability of the linker and the product's heterogeneity (wide dispersion of DARs) frequently have a negative effect on the therapeutic window and ADC efficacy, which frequently makes it difficult or impossible to optimize for medical use and ultimately results in clinical trial failure. Presently, efforts are focused on creating new stable linkers (with or without a payload release mechanism) and site-specific conjugation techniques that allow homogenous ADCs to be constructed in order to address these issues. Future research in this area will yield more understanding and advanced methods from the perspectives of medicinal chemistry and pharmacology, which will result in novel cancer treatments.

4. Protein-drug nano-complexes

Proteins such as albumin comprises hydrophobic packets or binding sites facilitating its non-covalent interaction with small drug molecules and organic dyes, forming albumin-based nanocomplexes for efficient delivery. Albumin interacts with small drug molecules or organic dyes via hydrophobic interaction or hydrogen bonding and formulates stable nanocomplex in the absence of exogenous toxic crosslinkers. For instance, Squaraine (SQ), an organic fluorophore selectively binds to hydrophobic pockets of BSA via hydrophobic interaction or H- interactions with intensified fluorescence up to 80-fold (Gao et al., 2014). As-prepared supra-molecular additives of BSA and SQ (SQ@BSA) served as an effective bioimaging probe and PTT agent in vivo. Additionally, the conjugate of SQ@BSA with folic acid (SQ@BSA-FA) shows excellent tumor targeting. Similarly, albumin-based Abraxane-like nanoparticles were also developed to enhance the therapeutic efficiency of PTX via

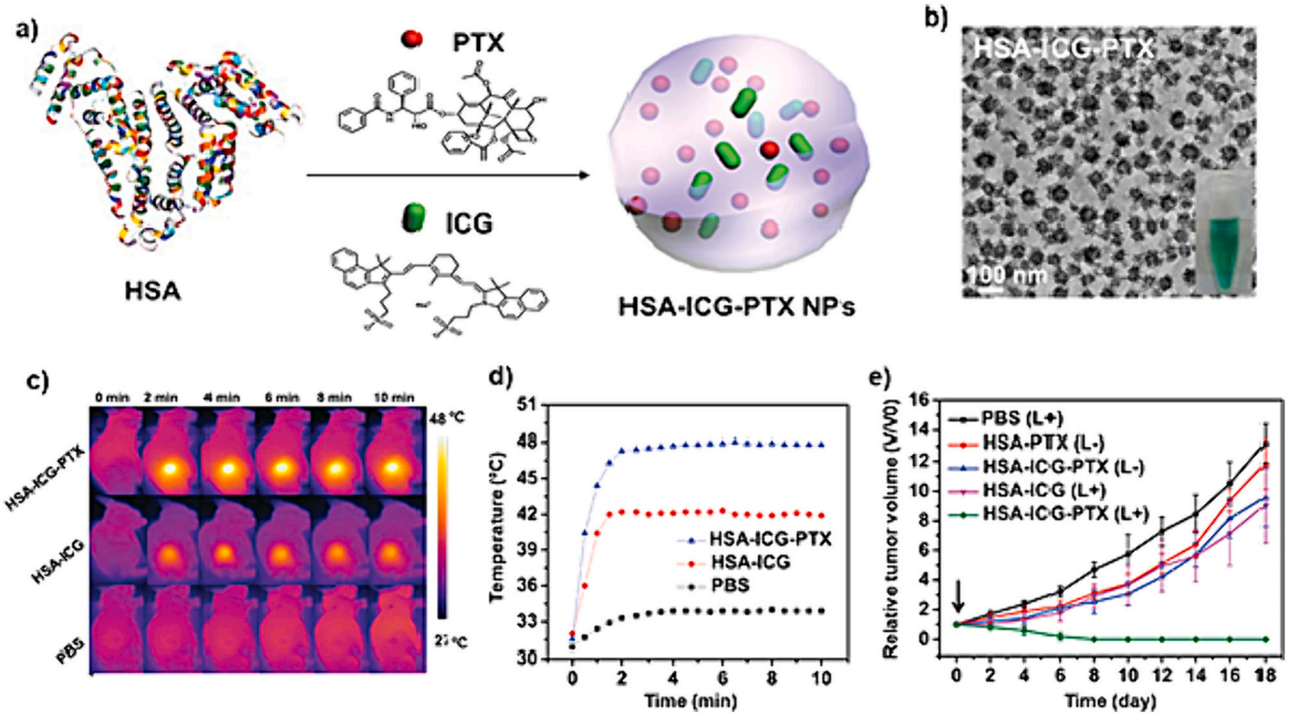

Fig. 3. (a) Synthesis scheme of HSA–ICG–PTX (b) TEM micrograph of HSA–ICG–PTX. (c) IR thermal micrographs of 4 T1 tumor-bearing nude mice treated with HSA–ICG, HSA–ICG–PTX, or PBS irradiated with 808 nm laser (0.4 W cm^{-2}) (d) The temperature changes tumor tissue based on IR thermal tumor imaging in c. (e) The tumor growth curves of mice after treatments ($n = 5$). Reproduced with permission (Chen et al., 2015b).

self-assembled albumin with indocyanine green (ICG) and PTX for dual-model tumor treatment via non-covalent or hydrophobic interaction (Fig. 3) (Chen et al., 2015b). The hybrid nanocomplex of chemotherapeutic and photothermal agent could not only cause eradication of subcutaneous solid tumors, but also significantly inhibited cancer metastasis.

Another multifunctional albumin-based theranostic nanoplatform payload with IR780 (NRI dye) and docetaxel (DTX) was constructed for imaging-guided PDT /PTT with chemotherapy for castration-resistant prostate cancer treatment (Lian et al., 2017). In the nanosystem (HSA@IR780/DTX), hydrophobic drugs DTX and IR780, induces the self-assembly of albumin proteins. The NPs preferentially accumulated in the tumor tissue via EPR effect showing strong fluorescent signal in tumor and further tumor cells were completely destroyed after irradiation with NRI laser (combined therapy, (HSA@IR780/DTX + NRI laser) compared to monotherapy (HSA@IR780/DTX). The result demonstrated that HSA@IR780/DTX delivered an excellent imaging-guided synergistic therapeutic effect of the combined PTT and chemotherapy. In another study, self-assembled indocyanine green loaded holo-transferrin nanoassemblies (ICG@holo-Tf NAs) were fabricated by a single step method for PA and fluorescence (FL) dual-modal tumor imaging and PTT of malignant brain cancer (Zhu et al., 2017). In this nanosystem, ICG, an FDA approved NIR, dye can be efficiently integrated via electrostatic contact and hydrogen bonding into Tf nanocages. The obtained ICG@holo-Tf NAs showed good biocompatibility, active targeting of tumors and notable imaging-guided PTT efficiency for subcutaneous as well as orthotopic brain tumors.

Cisplatin was reacted with gelatin to form a protein-platinate complex. The complex was further de-solvated and cross-linked to formulate the nanoparticles which were surface decorated with con-A. The cisplatin loaded and concanavalin-A decorated gelatin nanoparticles preferentially reach to the tumorous site by enhanced permeability and retention effect because of leaky vasculature in the tumor microenvironment (Vaghasiya et al., 2021). As with animal protein, herbal protein-based nanomedicines as anticancer drug delivery platforms such as soy protein, zein, and so forth, has become popular for drug delivery due to their minimal toxicity, easy accessibility, surface shift with the availability and chemical stability of unbound functional groups. For example, the nanocomplexes of soy protein isolates with curcumin (Cur) can enhance the solubility, stability, and bioaccessibility of natural anticancer agent Cur (Chen et al., 2015a). Recently, zein-chondroitin sulfate nanocomplex with Cur was developed by anti-solvent co-precipitation technique to protect and controlthe release of anticancer drug Cur (Shi et al., 2024). The zein-chondroitin sulfate nanocomplex had high encapsulation efficacy (94.7%) and drug loading capacity (3.8%) of curcumin, and further releases curcumin in a controlled and sustained manner. The nanocomplexes of Cur with proteins provided a base for the development and application of the hydrophobic bioactive compounds in cancer therapy.

5. Protein nanoreactor

Various proteins including HSA, bovine serum albumin (BSA), ferritin, and ovalbumin utilized their metal binding sites or domains such as N-terminal amine, Cys residues, and other metal binding domains or sites for transportation of metal ions (Fe^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}) in particular physiological processes (Bal et al., 2013). Particularly, some proteins with their flexible molecular chains and excessive charges, may expand in an acidic or basic environment, exhibiting hollow nanocage shape. These proteins can therefore be used as hollow-cavity single-molecule nanoreactors, where metal ion coupling to protein binding sites can lead to reduction/precipitation processes, and subsequently the nucleation and development of metal nanocrystals or nanoclusters. Generally, this process is considered as biomineralization mechanism in protein nanocages, that permits them to serve as a nanoreactor template for the controlled production of inorganic nanocrystals with several nanometer dimensions for bioimaging or medical use (Iqbal et al., 2021; Li et al., 2020). This technique offers multiple advantages, including easy and repeatable production, accurate size control and monodisperse size distribution, favored enhanced cellular intake, extended residence time in plasma, and significant tumor targeting ability.

5.1. Albumin nanoreactor

Albumin nanoreactor provides a confined space for reaction inside the cavity for nanoparticle synthesis via reduction or precipitation or both reduction and precipitation simultaneously (Table 4). The albumin molecule could trap the Au(III) ions inside the cavity via biomineralization process, which can then cause metal ions to develop into ion complexes and eventual nanoclusters within albumin nanocages under mild condition (Xie et al., 2009; Tkachenko et al., 2003). After decreasing the pH down to about 12, the decrease in the potential of BSA monomers was triggered, enabling the entrapped Au(III) ions to gradually reduce into Au nanoclusters for localized cell imaging. The study implies the fact that BSA may be rapidly and effectively employed as a nanoreactor for producing nanoclusters as small as a few nanometers, which enabling bloodstream visualization. Similar reduction reaction was induced within albumin nanoreactor to synthesize ultra-small radioactive Cu nanoclusters with radiation stability for orthotopic lung cancer imaging and therapy (Gao et al., 2015). However, such ultra-small nanocluster usually suffers from poor tumor targeting capacity.

Arsenotherapy of arsenic trioxide (ATO) has achieved remarkable attention to get treatment benefits for solid tumors as well as promyelocytic leukemia. The arsenic-manganese nanoprobes inside the albumin nanocage (As/Mn-NHs) are reported for high spatial MRI and ATO synergistic therapy of TNBC. Herein, self-activated As/Mn-NHs were prepared within the hollow albumin nanocage using biomineralization method for smart bioimaging and ATO synergistic therapy. The lysosomal and glutathione reduced (GSH) ablation resulting in self-activated release of Mn^{2+} and H_3AsO_3 (ATO) along with conversion of GSH to GSSH that results in glutathione depletion. The MnO_2-induced depletion of GSH resulted in prompt release of Mn^{2+} and H_3AsO_3, so both pH reduction responsive and GSH depletion favors the production of MRI manganese probe and ATO for synergistic treatment of solid as well as metastatic tumors. These nanohybrids/albumin provided simultaneous ATO therapy across multiple apoptotic routes for powerful suppression of percutaneous and orthotopic breast cancer models, as well as in vivo

Table 4
Representative examples of nanoparticles synthesized using albumin nanoreactor.

Reaction type	Applications	Examples	Ref. No.
Reduction	Imaging/PDT/PTT	Pt nanodots	(Tang et al., 2018)
	PET	[^{64}Cu]Cu clusters	(Gao et al., 2015)
	PTT/PDT	Te nanodots	(Yang et al., 2017b)
Precipitation	PDT/Chemo-immunotherapy	CuCH NCs	(Li et al., 2023b)
	NIR-II PA/Fluorescent imaging	Ag_2S NPs	(Yang et al., 2017c)
	MR imaging	Gd_2O_3 NPs	(Zhou et al., 2017)
	CT/PA imaging/PTT	Bi_2S_3 NPs	(Wang et al., 2016a)
		PtS	(Li et al., 2023a)
Dual precipitations	PA/MR imaging/PTT	CuS/Gd_2O_3 hybrid NPs	(Wen et al., 2017)
Reduction/ Precipitation	MR/PTT/PDT	Mn:CuSe@BSA	(Dehvari et al., 2019)

high-resolution T1-weighted MRI images for identifying tumor boundaries in diverse tumor models. At subcutaneous 4 T1 tumor model, As/Mn-NHs demonstrated the highest tumor-to-normal tissue (T/N) contrasting ratio of 205% and tumor growth suppression by 88%. When used with immediate thermal treatment, these tiny hybrids also produce optimal synergistic anticancer activity against both primary and metastatic breast cancers. This biomineralized strategy provided an excellent high spatial MRI approach for tumor detection and suppression by ATO treatment (Zhai et al., 2022). Iron-oxide nanoparticles (IONPs) are favored for molecular magnetic resonance (MR) imaging due to their extended plasma residence time, minimal toxicity, and inherent magnetization. A great deal of work has been put into investigating IONPs as T2-weighted MR contrast molecules because of their strong propensity to produce a long-range magnetic field that interferes with diagnosis. Simultaneously induced nanoprecipitation and polymerization, biomineralized iron oxide–polydopamine hybrid nanodots (IO/PDA-NDs) were synthesized employing albumin as the nanocage, which enabled an upsurge of T1-weighted resolution as well as PT therapeutic potential (Wang et al., 2021). The hybrid nanodots' strong PT emission efficiency and tumor-homing ability may lead to the radical elimination of tumors. This biomineralization approach discloses successful clinical slant for detection and therapy of solid tumors.

Transition metal ions like copper, cobalt, nickel, and silver have high binding affinity for protein;s nanocarriers with semiconductance can be fabricated within the albumin nanocage (Yang et al., 2016a; Yang et al., 2017d; Zhang et al., 2016b). In this regard, numerous functional groups in amino acid residues, such as -COOH and -SH groups at basic environments, were combined with metal ions and albumin in aqueous environment to produce albumin-ion complexes. Then, nucleation was proceeded via precipitation interaction between the metal ions and S^{2-} within albumin nanocage (Fig. 4). Remarkably, the development of metallic sulfide tiny crystals within nanocages could be efficiently regulated by the proportion of metallic ions to S^{2-}ions, reaction mixture temperature, and reaction time. This makes it possible for accurately tune of the particle size of nanocrystals, like Ag_2S, PtS, and CuS, to optimize their physical and chemical characteristics, including boosted NIR absorbance values and photothermal effect for strong hyperthermia at tumor sites (Yang et al., 2016a; Yang et al., 2017d). Given its customizable physicochemical properties, this method has been introduced to synthesize nanostructures by controlled precipitation inside albumin nanoreactor, that can provide effective CT scans, PA scans, and PTT tumor eradication. Additionally, albumin surface can be further modified with imaging or therapeutics photothermal Cypate, NIR fluorescence dye (Cy7.5), or radionuclide (99mTc) for significantly improved cancer imaging and treatment (Yang et al., 2016a; Wang et al., 2016a; Mao et al., 2016). Overall, albumin nanocage acts as unique and versatile framework for the synthesis of sulfide semiconductor nanostructure for cancer diagnostics and treatments.

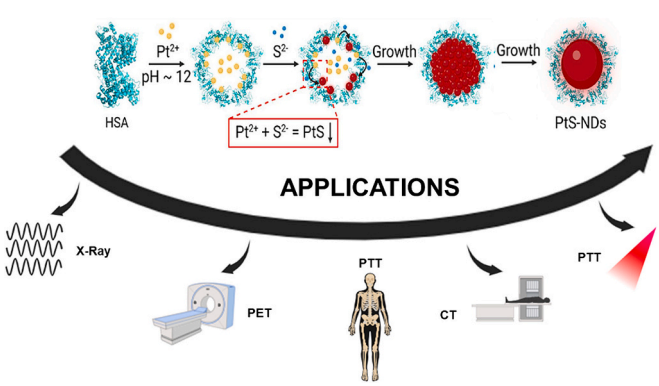

Fig. 4. Synthesis of PtS nanodots (PtS-NDs) via precipitation reaction using albumin template strategy, and their therapeutic applications in cancer theranostics (Li et al., 2023a).

Albumin nanoreactor can also be assimilated with inorganic molecules in addition to the covalent coupling to strengthen the diagnostics and therapeutic applications for cancers (Zhou et al., 2017). Particularly, albumin nanoreactor can allow the production of two simultaneous nanocomplexes (e.g., Gd_2O_3/CuS) in a single step nanoprecipitation method for cancer theranostics applications (Wen et al., 2017; Yang et al., 2016b). A single albumin nanoreactor may be used to carry out two distinct processes (precipitation and reduction), concurrently to create a Gd_2O_3/Au hybridized nanocomplex for NIR fluorescence and MR imaging of tumor (Sun et al., 2013). As gadolinium is spatially confined in the small area of the albumin cage, the Gd/Cu molar proportion in hybrid nanocarriers may be adjusted to enhance their relaxivity (Bridot et al., 2007; Ananta et al., 2010). It indicates that the albumin nanoreactor can facilitate two or more reactions in a single step which may be further tailored to enhance their physicochemical properties.

Furthermore, the inside cavity of a nanoreactor might play a vital role in the regulation of particle's shape and morphology. Similarly, copper (II) carbonate hydroxide nanocrystals (CuCH-NCs) synthesized within albumin cavity showed prominent results for chemo-immuno therapy against TNBC. Tellurium (Te) nanoprobes normally pursue *p*-type semi-conductance (Lee et al., 2013; Liu et al., 2012; Wu and Yan, 2013; Liu et al., 2010b), which limits their possible biomedical applications. Utilizing the constrained spaces within the albumin nanoreactor cavity, zero dimensional Te nanodots were effectively produced for PT synergistic cancer treatment (Yang et al., 2017a). In an environment of the reducing agents NaBH4, TeO_3^{2-} entrapped in the hollowed albumin nanocages was reduced to generate pure Te nanocrystals, culminating in the generation of ultra-small Te nanodots (Te-NDs, 5.9 nm) within albumin. Te-NDs' electrons may be driven from the valence band to the conduction spectrum when being exposed to NIR light. This can result in a variety of reactive oxygen species (ROS), including dismutated OH and O^{2-}, as well as a photothermal effect via non-radiative relaxation. Te-NDs in particular have a favorable tumor retention and simple renal excretion, thereby providing combined effects between PTT and PDT to completely ablate the tumor. Therefore, in a size/morphology-controllable way, this albumin nanogenerator may be efficiently used to produce multifunctional inorganic nanocrystals with cancer-targeting potential for theranostic applications.

5.2. Ferritin nanoreactor

In the early 1990s, the iron-storage protein ferritin was first developed as a supramolecular protein nanoreactor to form inorganic nanoparticles (Meldrum et al., 1991). Like zeolites, phospholipid vesicles, and reverse micelles (Mann, 2009) ferritin with nanoscale muffled sphere-shaped cavities of approximately 8–9 nm internal diameter, act as a reaction cage, in which the biomineralization can be utilized to synthesize various functional nanoparticles.(Meldrum et al., 1992; Sun et al., 2000; Ueno et al., 2004; Kudr et al., 2015). To date, ferritin has been utilized as the nanoreactor to synthesize manganese oxide nanoparticles (Meldrum et al., 1995; Geninatti Crich et al., 2012), cadmium-sulfide quantum dots (Wong and Mann, 1996), uranyl-oxide nanocarriers (Meldrum et al., 1991), cobalt-platinum nanoparticles (Warne et al., 2000), and magnetic-mineral magnetite (Chasteen and Harrison, 1999). PbSAq quantum dots were successfully fabricated via the nanoprecipitation of $PbAc_2$ and Na_2S within muffled cavity of ferritin for NIR-fluorescent imaging (Hennequin et al., 2008). Parallel approach was also applied to fabricate biodegradable Au and Pt nanoclusters using ferritin as a nanoreactor (Sun et al., 2011; Fan et al., 2011). The generated Au-Ft complex exhibited comparatively higher fluorescence because of coupling-interactions between paired Au clusters, and also exhibited specific targeting capacity and good tissue penetration for enhanced fluorescent imaging.

Additionally, magnetic nanoparticles synthesized using this ferritin nanoreactor can be applied as MRI contrast agents for tumor imaging.

Mn(II) was loaded into the nanocage of apoferritin, and further oxidized at pH 9, followed by the formation of Mn(III) oxyhydroxide nanoparticles with enhanced *T1*-weighted MRI capability in vivo (Meldrum et al., 1995, Geninatti Crich et al., 2012). Iron-oxide nanoparticles have also been synthesized in the hollow cage of recombinant human ferritin to achieve magnetoferritin nanoparticles for visualizing tumors via peroxidase activity of iron oxide (Fan et al., 2012; Gao et al., 2007). In order to distinguish malignant cells from healthy cells with the means of color response, the iron-oxide core can accelerate the oxidation of peroxidase targets in the presence of hydrogen peroxide, showing a great potential for rapid, low-cost, and universal tumor diagnosis. Hence, ferritin as an efficient nanoreactor might be a promising tool to generate various inorganic imaging agents with TfR1-mediated targeting capability.

In a recent study, copper sulfide semiconducting nanocomplexes were prepared within ferritin nanocages via a biomimetic process, resulting in the formation of CuS nanoparticles with assimilation of ^{64}Cu for PET/photoacoustic (PA) dual imaging. Meanwhile, these nanoparticles also resulted in the effective photothermal tumor ablation owing to their ideal tumor targeting capacity and good photothermal conversion (Fig. 5) (Wang et al., 2016b). Cobalt-doped ferritin-magnetic nanocarriers were also developed for tumor hyperthermia treatment under flashing magnetic field. Controlled doping of the magnetic core with 5% Co(II) was sufficient to generate preferable hyperthermic efficiency, showing a diverse antitumor efficiency when exposed to the flashing magnetic field (Fantechi et al., 2014). Rationally, the ferritin nanoreactor is also capable of fabricating multifunctional nanocarriers for highly effectual theranostics applications.

In spite of the above stated developments, protein nanoparticles still face various challenges in clinical translation. For example, the existing protein-based drug delivery system mainly bestows anticancer drugs with capabilities to inhibit cancer cells growth with abridged systemic toxic effects with extended survival rate, whereas the therapeutic effectiveness might greatly be hindered by limited targeting productivity. Furthermore, uncontrollable chemical/ surface property and particle size present another challenge in terms of incapacitating the biological barriers, profound tumor penetration, enhanced cell interaction and intracellular translocation. Hence, the fabrication of protein nanoparticles with tunable properties and high therapeutic efficacy in cancer nanomedicine with improved performance is still highly desired.

6. Actively targeted protein-based cancer nanomedicines

Since after the FDA approval of recombinant insulin (protein therapeutics) for diabetes treatment in 1982, protein therapeutics have gained substantial interest and developed in considerable numbers. So far, protein-based therapeutics have played a remarkable roles in medical field, amid which the protein-based targeted therapeutics with imaging and antitumor activities have been widely investigated for cancer treatment (Fig. 6) (Zhang et al., 2021). Some proteins are very potent to tumor cells at very low concentration with IC_{50} values in nanomole/l (nM) range. For example, endogenous mammalian protein granzyme B (GrB) plays a vital role in natural killer cells and cytotoxic T lymphocytes inducing the apoptosis of cancer cells in the human body. So, the assistance of pore-forming protein (perforin) is important for GrB to show its therapeutic effects, as GrB cannot enter cell cytosols. GrB exhibited IC50 value (1–20 nM) depending on the delivery system, however, free GrB showed very low cytotoxicity at a very high concentration of about 40 nM (Gu et al., 2021). In addition to deficient cell entry, protein drugs are further associated with potential

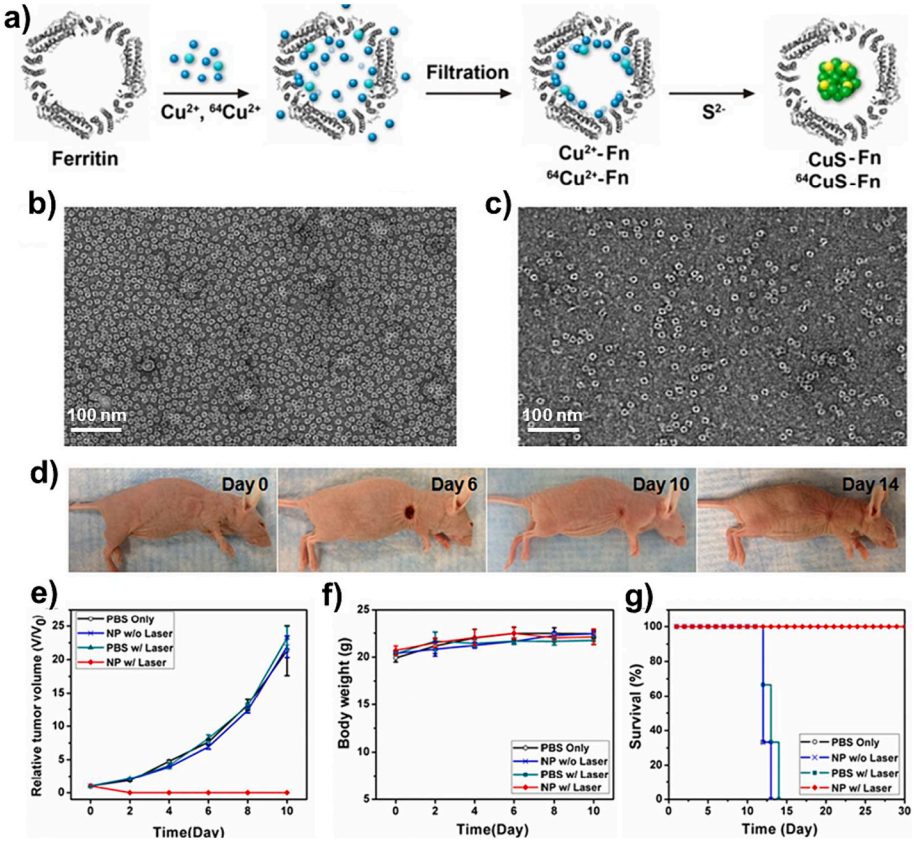

Fig. 5. (a) The synthesis scheme of CuS-Fn NCs by biomineralization method (b) Representative TEM micrograph of uranyl acetate (1%) stained iron free ferritin (c) TEM micrograph of uranyl acetate (1%) stained CuS-Fn NCs. A black dark CuS core visible inside the ferritin cage. (d) Representative images of U87MG tumor bearing nude mice at different days after treatment. (e) Relative tumor volume during treatment period (f) Average body weight during treatment period (g) Mice survival curve during and after treatment period. Reproduced with permission (Wang et al., 2016b).

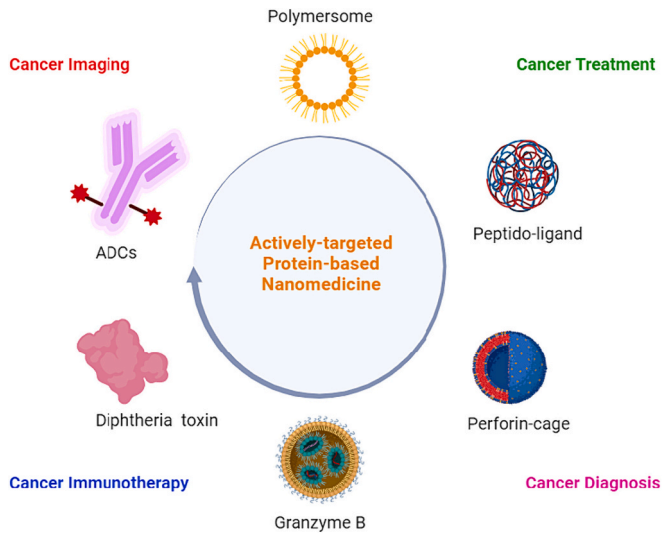

Fig. 6. Representative exameples of actively-targeted protein nanomedicine for cancer diagnosis and therapy.

immunogenicity and rapid degradation in vivo. Moreover, the high potency of protein drugs could bring about acute cytotoxic effects if delivered to the healthy cells other than cancer cells.

Interestingly, with the advances in the field drug delivery, nanotechnology, and polymer chemistry, along with the deep understanding of tumor microenvironment, have pioneered strategies for the development of protein-based therapeutics in targeted cancer therapy. For instance, chimeric polymersomes (CPs) loaded proteins in the hydrophilic compartment separated via a thick hydrophobic membrane from the outside environment, which not only efficaciously inhibit the protein leakage but also circumvent the protein degradation (Liu et al., 2010a). CPs with a polyelectrolyte as inner shell could load notable amounts of proteins with different sizes at high efficiencies (up to 100%). Prostate-specific membrane antigen and sigma receptor targeting and pH-sensitive CPs efficiently transport to and release GrB at prostate cancer cells and H460 lung cancer cells with IC50 values of 1.6 nM and 6.25 nM, respectively (Li et al., 2015; Lu et al., 2015). GrB-encapsulated disulfide-crosslinked CPs conjugated with lung cell-specific penetrating peptide and anisamide effectively inhibited the growth of orthotopic A549-Luc lung and subcutaneous H460 lung tumor at 2.88 nmol GrB/kg and 1.56 nmol GrB/kg, respectively, without inducing noticeable side effects (Yang et al., 2018; Yang et al., 2017e). Besides solid tumors, GrB encapsulated hyaluronic acid-functionalized CPs exhibited high therapeutic potency (IC50 = 8.1 nM) to CD44-positive LP1 human MM cells and effective inhibition of both orthotopic and subcutaneous LP1 tumors in mice and significantly improving the survival rates and reducing bone loss (Zhong et al., 2020). Interestingly, ApoE and angiopep-2 peptide-conjugated disulfide-crosslinked CPs were found to efficiently deliver Sap across the BBB in vivo and accumulate in the U-87 MG orthotopic glioblastoma, leading to influential tumor suppression and prolonged survival benefits.

Diphtheria toxin (CRM107) conjugated with Tf for targeting malignant brain tumors. CRM107 is a highly potent bacterial mutant toxin protein that kilsl the cancer cells intracellularly via blocking eukaryotic protein synthesis pathway, while lacking the native toxing binding capacity. CRM107-Tf conjugate selectively transports CRM107 to Tf receptor (TfR1) expressed cancer cells such as GBM cells (Laske et al., 1997). CRM107-Tf conjugate showed high affinity toward TfR1-overexpressing tumor cells at very concentrations (picomolar). Furthermore, clinical studies demonstrated that injection of Tf-CRM107 via direct interstitial perfusion can instigate up to 60% tumor shrinkage in refractory malignant brain tumor patients with minimal toxicities. However, the Tf-CRM107 (con1.0 μg/mL) can cause peritumoral toxicity via regional perfusion which could be circumvent by reducing the concentrations of Tf-CRM107. The phase II clincal studies demonstrated that 35% of patients showed complete or limited tumor response after treatment with Tf-CRM107 (Weaver and Laske, 2003).

7. Conclusions and future perspectives

Current review reflects the rational design and recent developments of protein-based nanostructures in the applications of tumor diagnosis and therapy. Tremendous efforts have been made to fabricate various protein nanoparticles encapsulating chemotherapeutic drugs, organic fluorescent dyes, and photosensitizers, inorganic nanodots, as well as therapeutic biomacromolecules. In recent years, single-molecule protein nanoreactors are being investigated with the goal of producing well-defined tiny crystals with few nanometers in an easy, gentle, and controlled method, that possess immense capability for controlling their cell-uptake, pharmacokinetics parameters, and tumor penetration for cancer imaging and treatment. These strategies reinforce many protein nanocarrier and conjugate strategies that are typically used to deliver a variety of anticancer drugs.

Recently, the development of protein-based nanodrug carriers is an emerging field, however several key challenges still need to be addressed. Firstly, complicated prepartion processes are usually employed for the fabrication of protein-based nanocarriers. Generally, a major challenge in translation of nanomedicines for real-time clinical application involve large scale industrial production, validation, batch-to-batch stability and controllability of both chemical and physical properties. The clinical translation of nanomedicine always requires the optimization of nanoformulations and development strategies, so it might be hard to fabricate protein-based nanomedicine with reproducible manner if there are complicated manufacturing processes. Furthermore, the multi-step fabrication makes it tough to preserve the structural integrity of protein molecules for exemplary performance such as extended blood residence and precise receptor binding. For instance, the binding affinity of transferrin nanoparticles to the receptors is compromised the large-scale production. Hence, further optimization of preparation strategies is therefore necessitated to maintain the structural integrity of proteins and enhance the delivery performance.

The second problem arises from the inept observance of safety-related standards. First off, it can be challenging to keep natural protein excipients at a high standard. Since these natural proteins are usually extracted from human blood, there are several issues to be considered, such as the potential risk of spreading infections, structural variations in proteins, and restricted availability. Recombinant DNA technology could be utilized to overcome this obstacle by producing suitable drug carrier proteins. More importantly, the design of versatile amino acid sequences can yield innovative functional proteins for advantageous drug loading and targeted specific delivery. Furthermore, the development of complex protein nanoparticles with numerous components could addressed various commercial and regulatory issues.

Although several protein nanoreactors were designed for chemotherapy and diagnostics of various tumors, many scientific and engineering issues still need to be addressed. It is necessary to put additional work into developing protein nanoreators for their diagnostic or therapeutic applications. The exploration of highly efficient protein nanoreactors is very crucial for precise cancer nanomedicine including cancer-targeted proteins, antibodies, and viral capsids, but a major challenge still remains in tuning the growth of inorganic or organic nanocrystals in hollow nanocages and maintaining their biological activities in biomineralization process. Moreover, protein-based nanoreactors are commonly used for the synthesis of inorganic nanodots as a novel biomineralization strategy that enables organic chemotherapeutic compounds to grow and nucleate in nanocages are urgently needed for the wide range of chemotherapeutic compound delivery to tumors targets. It is possible that organic molecules in protein nanoreactors might

develop into multi-nanometer drug nanocrystals via precipitation or electrostatic interaction.

Thirdly, various complex biological barriers such as opsonization, the mononuclear phagocyte system, low cellular internalization, endolysosomal compartments escape, efflux pumps, and high intratumoral pressure severely influenced the efficacy of protein nanomedicine by compromising their ability to target specific sites and thus have limited anticancer effects. Therefore, to get past these obstacles, protein-based nanoparticles with sophisticated designs are desperately needed.

In short, it's a great inspiration to encourage the fabrication of protein-based formulations of imaging agents and chemotherapeutic compounds using protein nanocarriers, such as protein-nanoconjugates and nanocomplexes, as evidenced by their preclinical performances. Moreover, protein nanoreactors also show a great promise in the development of protein formulations for cancer-targeted imaging or therapeutics in the future. Besides, quality control of protein nanoparticles actually needed to be addressed in reproducible and large-scale production owing to the flexible protein structures and limited characterization techniques. Nevertheless, we believe that the protein nanoparticles highlighted in this mini-review article provide a valuable paradigm to significantly improve the applicability and performances for efficient cancer nanomedicine.

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Declaration of competing interest

The authors report there are no competing interests to declare.

Data availability

Data will be made available on request.

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