Tumor therapy based on self-assembling peptides nanotechnology

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Abstract

Self-assembling peptides display great potential in biological and medical applications, especially tumor therapy with noninvasive ways. The rapid expansion of self-assembling peptides is almost on account of their favorable biocompatibility, tumor microenvironment responsiveness, multivalency, and structural versatility. With taking full advantage of self-assembling peptide peculiarities, peptide-based nanomaterials are applied for delivery carriers, chemotherapy, immunotherapy, and noninvasive tumor treatments including photothermal therapy (PTT), photodynamic therapy (PDT), and sonodynamic therapy (SDT). In addition, immunogenic cell death of tumor cells originating from PDT and SDT, and the necrosis of tumor cells due to PTT can elicit some degree of immune responses. Therefore, synergistic therapy has served as a more effective and powerful strategy for tumor therapy. Importantly, self-assembling peptides are capable of subtly making combination of various treatments. This review outlines biomedical applications of nanomaterials based on self-assembling peptides with emphasizing variegated treatment methods.

KEYWORDS
nanoengineering, peptide, self-assembly, treatment methods, tumor therapy

1 | INTRODUCTION

Peptides are amino acid chains composed of approximately <50 amino acids, which are easy to synthesize, even design to mimic the self-assembly property of proteins. Peptides possess excellent advantages of chemical versatility, high biocompatibility, and biological recognition capacities. Moreover, small peptides have the capacity of translocating cell membranes and cannot arouse immune response. However, free peptides are unstable and rapidly eliminated due to enzymatic degradation during the blood circulation in the body, and exhibit off-target effect simultaneously. Therefore, self-assembly method has been developed as an elegant nanotechnology for manipulating the peptides to construct stable and multifunctional nanomaterials, specifically applied for tumor therapy in recent years.
Peptides can form stable nanomaterials with various morphologies through self-assembly driven by noncovalent interactions, such as hydrophobic, hydrogen bonding, π-π stacking, and electrostatic interactions. Meanwhile, peptides are subject to changes in ionic strength, cosolvents, pH, and temperature. These factors make it possible to flexibly control the peptide self-assembly for formation of diverse nanostructures. The resulting peptide nanomaterials not only prolong the circulation in body but also enhance the accumulation at the lesion site with obvious therapeutic effect.

It is well-known that photothermal therapy (PTT), photodynamic therapy (PDT), and sonodynamic therapy (SDT) as minimally invasive methods, and immunotherapy considered as the most promising treatment are applied for tumor therapy field. These therapeutic modalities extremely depend on the use of active drugs that usually need to be designed for delivering into tumor. Self-assembling peptides show remarkable potential for nanoeengineering the drugs and thus enhancing the therapeutic efficacy. For example, small peptides with several amino acids were employed to design and assemble peptide-based nanomaterials with encapsulation of photosensitizers (or sonosensitizers) and metal ions for PDT, SDT, and PTT against malignant tumors. Peptides with antigen activity were selected to form self-assembling nanovaccines for immunotherapy. However, monotherapy is not powerful enough to some extent in tumor therapy. Recently, it has been found that immunogenic death of tumor cells originating from PDT and SDT, the necrosis and apoptosis of tumor cells due to PTT can elicit some degree of immune responses and attract more macrophages. Consider that tumor immunotherapy possesses remarkable perdurability advantage to eliminate tumor cells via regulating the autogenous immune system compared with traditional therapeutic methods, the synergistic therapy of PDT, SDT, and PTT combining with immunotherapy was further employed for tumor therapy. Additionally, novel peptide-based molecular probes and multifunctional nanomaterials were used as different biomarkers and applied for tumor imaging during the therapy. Therefore, it is enormously potential for nanomaterials based on self-assembling peptides to effectively enhance therapeutic efficacy on tumors via maximizing immunotherapy and noninvasive treatments.

Nowadays, self-assembling peptides are emerging as effective biomaterials for tumor therapy. To achieve more primary therapeutic effect, antigen peptides and other functional peptides are adopted to self-assemble for construction of nanomaterials, which achieves both targeted therapy and breakthrough therapeutic effect combined with different treatment methods. This review summarizes self-assembling peptide nanomaterials for tumor therapy and correlative treatment strategies (Scheme 1). An overview on this burgeoning field is helpful to comprehend the state of the art of self-assembling peptides and explores new avenues for putting forward the peptide-based materials for applications in tumor therapy.

2 | ADVANTAGES OF SELF-ASSEMBLING PEPTIDES

2.1 | High biocompatibility

The biocompatibility of nanomaterials is crucial to further apply for clinic therapy. Peptides derive from some parts of natural protein, thus peptides are composed of different amino acids that are indispensable for human body. Obviously, peptides possess excellent biocompatibility and are preferred to choose for preparing nanomaterials. Nanomaterials based on self-assembling peptides cannot arouse intractable problems of toxicity and degradation-resistance compared with other inorganic nanomaterials. For example, rapid degradation of zeolitic imidazolate framework-8 nanoparticles causes high toxicity and silica nanorattles are difficult in metabolism. Multifunctional nanomaterials based on self-assembling peptides enhance biocompatibility compared with free hydrophobic drugs, which effectively facilitate tumor therapy. Self-assembling peptide nanomaterials exhibit high biocompatibility, which is the most significant part for biomedicine.

2.2 | Tumor microenvironment response

Tumor microenvironment exhibits several barriers so that nanomaterials cannot be transported into the tumor, which limits the bioapplication of nanomaterials for tumor therapy. Enhanced permeability and retention effect alone cannot overcome this difficulty. Meanwhile, the properties of the tumor microenvironment are often various owing to the type, location, and progression stage of tumor microenvironment. However, nanomaterials are still applied on the “one size fits all” strategy, rather than different nanomaterials to be used based on the physiological properties of different tumors. To achieve favorable therapeutic effect, nanomaterials need to be intelligently designed for the various properties of tumor microenvironment.

Each microvasculature possesses a specific pore-size distribution, for example, breast or pancreatic tumor may be 50-60 nm, brain tumor ~7 nm, thus it is critical to design nanomaterials with corresponding size for effective tumor therapy. It has been reported that the size of the nanomaterials determines the penetration depth in the tumor tissue.
and smaller nanoparticles can penetrate deeper in tumor tissue. However, very small particles (<11 nm) are easily and quickly cleared, which is not beneficial for tumor therapy. Therefore, it is crucial to design corresponding and suitable size nanomaterials for specific tumors according to a balance between maximizing tumor tissue penetration and minimizing renal clearance. It is worth mentioning that peptides can self-assemble into nanomaterials with different sizes by changing ionic strength, co-solvents, pH, and temperature, which is favorable for permeation into tumor microenvironment.

In addition, non-spherical such as rod-like and disc-shaped nanomaterials more rapidly penetrate and accumulate in tumor site than various size spheres, and are appropriate for tumors with smaller vessel-pore-sizes due to the shortest dimension of the particles. Importantly, the morphology of nanomaterials are determined by the interplays between multiple non-covalent interactions, including $\pi-\pi$ stacking, hydrogen bonding, electrostatic, and hydrophobic interactions codetermine. Therefore, peptides are ideal choice that can self-assemble into nanomaterials with appropriate morphology for specific tumors by modulating noncovalent interactions.

The cationic or anionic characteristic of nanomaterials can also affect the permeability at the tumor site, cationic nanomaterials more easily penetrate into tumors than anionic or neutral ones. Tiny metal elements that are essential in living organisms, such as Zn$^{2+}$, Mn$^{2+}$, and Fe$^{3+}$ can be introduced to the self-assembling peptide nanomaterials, which can tune the surface charge of nanomaterials. These are beneficial for improved tumor permeation and enhanced diagnostic and therapeutic performance.

Meanwhile, presenting partial acidic property and secreting more glutathione (GSH) in tumor microenvironment, which has been already known to the general public. Self-assembling peptides nanomaterials can degrade in partial acidic environment and disassemble via reacting with GSH due to the protonation and the breaking of disulfide bond, exhibiting great responsiveness on tumor microenvironment. Hence, it highlights the potential of self-assembling peptides as smart nanoplatforms for antitumor therapy due to the ultrasensitive responsiveness to pH and GSH.

In summary, peptides can subtly self-assemble into suitable nanomaterials by changing synthesis condition

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**Scheme 1** Schematic illustration about advantages and antitumor applications of self-assembling peptides
and noncovalent interactions to response actively on tumor microenvironment. It is demonstrated that self-assembling peptides possess high biocompatibility, good flexibility, and easy operability compared with other nanomaterials, which is worth researching for tumor therapy.

2.3 | Multivalency

The multivalency is a significant characteristic of self-assembling nanostructures, which can produce multivalent interactions that obviously improve binding affinity of weakly specific interactions. This makes self-assembly process of high effectiveness, low cost, and simple operation. Peptides fabricate self-assembling nanoparticles by a bottom-up self-assembling process, thus self-assembling peptides possess the important feature of multivalency. The multivalent interactions play an important role on bioactive functionalization and can activate immune system owing to multivalent antigens recognized by B cells. Moreover, multivalency plays a significant role in biological systems, which performs more obvious avidity and specificity than monovalency. Multivalency can reorganize some receptors on cell surfaces, which is able to enhance the associativity of receptor. Thus, the self-assembling peptides with multivalency can be used for activating immunogenicity to promote tumor immunotherapy. Meanwhile, it is the big thrust in the development of the self-assembling peptides with multivalency as vaccines or vaccine adjuvants against tumor cells. For example, multivalent R-helical glycopoly peptides exhibited more obvious inhibition effect of the cholera toxin B pentamer than monovalent galactose owing to multiple saccharide ligands combining with CT B5. The multivalency of self-assembling peptides acts as unique advantage, exhibiting great application value in immunotherapy and other fields.

2.4 | Diverse structure

Peptides can form various nanostructures via self-assembly in different environmental conditions in aqueous solution. For example, nanofibers are rapidly formed from short peptides dissolved in solution with low pH and osmotic pressure. Thus, it is conducive to understand the structures of self-assembling peptides and self-assembly mechanism for the rational design of objective structures. The structures of self-assembling peptides involve secondary structure including α-helix, β-sheet, poly-L-proline type II (PPII) helices, and tertiary structure including micelles, vesicles, fibers, tubes, tapes, and ribbons. It has been reported that some structures of self-assembling peptides are stable, which is favorable to apply for biological application, for example, coiled-coil is a more stable structure than other α-helices. Various structures of self-assembling peptides have different bioapplications. Studies mention that the structure of nanomaterials can affect cells uptake and recognition, and even affect the immune response. For example, nanofibers can facilitate different kinds of mammalian primary cells’ attachment, growth, and differentiation, and fibrillized peptides can enhance antibody responses and produce specific antibodies without supplying any immune adjuvants compared with β-sheet fibrils and hydrogels formed by peptides.

3 | SELF-ASSEMBLING PEPTIDES FOR BIOLOGICAL APPLICATION

Self-assembling peptides play a significant role on the application of tumor therapy, which tactfully overcome different shortcomings existing in various treatment methods.

3.1 | Self-assembling peptides for delivery carriers

Self-assembling peptides can be used as delivery carriers for cells and drugs owing to high biocompatibility and chemical versatility. Delivery cells, called cell transplantation, have been emerging in the field of medicine in recent years. More and more diseases’ treatments use cell transplantation for therapy. However, current methods exist with many drawbacks, for example, the low survival rate of transplanted cells and lack of proper delivery system with oxygenation and nutrient. The cell injection therapy is injecting cells into the lesion, which is considered as a useful method and provides suitable environment for growing. Nanofibers self-assembled from peptides possess unique properties, such as supporting attachment and growth of cells and modifying growth factors and cellular signals on the surface. Especially, self-assembly nanomaterials support living space for cells and bioactive signals due to controlled architecture and dimensions. Thus, self-assembling peptides are considered as suitable carriers to transport cells, even employed to construct
tissue-specific model in vitro and reconstruct the tumor microenvironments in 3D cell cultures for tumor therapy.\textsuperscript{32b} Webber et al. reported peptide-based hydrogelation can make cells homogeneously distribute in the hydrogels with maintaining cell viability for cell transplantation and targeting biological sites.\textsuperscript{37} Self-assembling peptides with skeletal structure that can support living space for cells, which exhibit more potential for cell delivery in the area of medicine.

Drug delivery is one positive and effective strategy for chemotherapy, which not only reduces toxicity of free drugs but also enhances target ability of free drugs by active or passive transport. Some studies choose inorganic nanomaterials as drug carriers, such as, metal–organic framework materials or core-shell structured materials.\textsuperscript{38} However, these nanomaterials have a number of shortcomings, such as toxicity and poor drug loading.\textsuperscript{13a} Therefore, it is a meaningful strategy to find both materials and improve drug loading. Peptides, composed of amino acids, are high biocompatibility. Most importantly, peptides can encapsulate hydrophobic drugs during self-assembling process due to their amphiphilicity, achieving higher drug loading rate. Self-assembling peptides can form various nanostructures, such as micelles, vesicles, and rods, which promote cell uptake.\textsuperscript{2b,20c} Self-assembling peptides as drug carriers not only possess high loading rate but also response on tumor microenvironment to achieve effective release. For example, cell-penetrating peptide-based nanoparticles can be used to load hydrophobic drugs DOX (PNP-D). Basing on programmability of peptides, monoclonal antibodies (mAb) that could target human fibroblast activation protein-α were modified onto the surface of self-assembling peptides (PNP-D-mAb). The whole preparation process is showed in Figure 1A (PNP-D-IgG as control group). As shown as Figure 1B, when the stimulus-responsive PNP-D-mAb to tumor microevironment, the release rate of DOX from PNP-D-IgG or PNP-D-mAb reached up to 80% for 48 h, while 30% of DOX was released for 12 h, demonstrating that DOX could be released effectively and controlled to release. The NIR tumor imaging and growth curves of tumors have showed that PNP-D-mAb possessed good targeting ability and remarkable therapeutic effect (Figure 1C,D). In addition, peptides can also self-assemble into fibrils or hydrogels, which is suitable for sustained drug release.\textsuperscript{4b} Hence, self-assembling peptides as drug carriers both possess high loading rate and effectively release drugs. Additionally, the new strategy of recognition-reaction-aggregation is adopted and combined with addressable self-assembling peptides for enhancing the sensitivity of chemodrugs and perturbing the permeability of cell membranes.\textsuperscript{1d} Importantly, self-assembling peptides as drug carriers improve targeting and reduce toxicity compared with free drugs, which is beneficial for further research and clinic application. Abraxane is the first medicine that employed protein nanoparticles to load anticancer drug, which was approved by the Food and Drug Administration in 2005.\textsuperscript{4g} These efforts have been well recognized, more and more drugs based on self-assembling peptides will be brought to clinics in the future.

3.2 | Self-assembling peptides for PDT, PTT, and SDT

PDT and PTT belong to phototherapies, which are well known for its non-invasive feature for tumor therapy. Achieving phototherapies need two necessary parts: photosensitizers and specific light. When photosensitizers are
transported to tumor site, they are activated under light irradiation to produce reactive oxygen species (ROS) during PDT or thermal energy through effective photothermal conversion during PTT for destroying tumor cells. PDT and PTT are widely applied for tumor therapy through taking advantages of their non-invasive feature. SDT acts as an emerging and noninvasive therapy method, which displays deep penetration compared with laser therapy. The mechanism of SDT is similar to that of PDT, that sonosensitisers are activated by ultrasound to produce ROS for arousing tumor cell death.

To further improve the efficiency of phototherapies and further facilitate clinical application, design and preparation of new nanomaterials is a significant strategy. It is well known that photosensitizers have many disadvantages, such as poor water solubility, no special selectivity, and low bioavailability, which limit their further bioapplications. The new strategy in which peptides and photosensitizers assemble into nanomaterials in a controllable manner, not only overcome the disadvantages of photosensitizers but also take full advantages of peptides. Recently, our group has designed and developed smart peptide-based supramolecular metallo-nanodrugs for PDT.21a Short peptides were selected for designing self-assembled materials due to its versatility and flexibility. Fluorenylmethoxycarbonyl-l-histidine (Fmoc-H) and N-benzyloxycarbonyl-L-histidine-l-phenylalanine (Z-HF) were selected to co-assembly with Zn2+ through coordination interactions. Moreover, self-assembling nanomaterials consisted of photosensitizer chlorin e6 (Ce6) was successfully prepared (Figure 2A). The results demonstrated that metallo-nanodrugs possess many distinctive advantages for bioapplication, such as robust stability, high loading capacities, and prolonging the lifetime of blood circulation. During PDT, owing to responding on pH and GSH in tumor microenvironment, molecular Ce6 were released explosively. Then molecular Ce6 was activated with light irradiation and reacted with molecular oxygen to produce burst release of ROS that effectively destroy tumor cells. Experimental result demonstrated that antitumor efficacy was visibly improved. This research offered new insights into
the design of smart photosensitizing nanomedicines with multifunctions and flexibility for enhancing tumor targeting therapy and improving therapeutic effects.

Meanwhile, our group also reported the emerging application of self-assembling peptides for PTT. Photothermal nanoagents formed through assembly of biliverdin (BV), short peptide (Z-Histidine-Obzl, ZHO), and Mn\(^{2+}\). BV was chosen due to its advantages, such as clear sources and metabolic pathways without biosafety concerns, intense near-infrared absorption for photothermal conversion. However, the hydrophobicity and non-stability of BV monomers limited its bioapplication. Hence, ZHO and BV assembled BV nanoagents not only improved the heat conversion efficiency due to the enhanced NIR absorption, but also improved the bioavailability (Figure 2b).

Liu et al. prepared composite peptide amphiphile-ICG nanomicelles (PAIN) that ICG were encapsulated into the contracture of self-assembling peptides C18GR7RGDS for achieving the multimodality therapy of SDT-PDT-PTT.\(^40\) The preparation process and experimental mechanism are shown in Figure 2c that PAIN and \(\alpha_\beta_3\)-overexpressed tumor-targeting are applied for tumor therapy under NIR laser or ultrasound irradiation. When PAIN were exposed to ultrasound and NIR laser irradiation, ROS was produced tenderly and the temperature also increased. Interestingly, PAIN exhibited better stability of photothermal and sonothermal, and produced more ROS than free ICG with the same concentration. The results implied that the ICG encapsulated by self-assembling peptides overcome the limitation of hydrophobicity of ICG. It could be further applied for tumor therapy based on the response to tumor microenvironment, achieving SDT-PDT-PTT under NIR laser or ultrasound irradiation. It has demonstrated the effective, multimodal, non-invasive therapy strategy is based on self-assembling peptides.

Peptides and photosensitizers or sonosensitizers self-assemble into nanomaterials for PDT, SDT, and PTT, which not only overcome the limitation of hydrophobicity and non-targeting shortcomings of photosensitizers and sonosensitizers but also enhance material stability. The biocompatibility, biodegradability, and programmability advantages of peptides facilitate the achievement of maximum therapeutic effect.

3.3 Self-assembling peptides for tumor immunotherapy

Immunotherapy exhibits enormous potential to effectively eliminate tumors by activating immune system.\(^41\) The nanovaccines play an increasing significant role on immunotherapy, which has attracted increasing attention.\(^11d,42\) There have been different kinds of nanovaccines, including polymer, biopolymer, liposome, nanovesicles, hydrogel, and inorganic nanoparticles.\(^4b,43\) These nanovaccines are formed through loading antigens with nanomaterials, which has disadvantages of low loading rate and poor therapeutic efficiency. In addition, ordinary peptides cannot elicit immune responses or tissue inflammation, while artificial amino acid sequences and highly oligomerized structures can cause theoretical immunogenicity.\(^44\)

To enhance immunotherapy, the peptides are self-assembled into nanomaterials as nanovaccines, which are also called peptide-based nanovaccines. For example, peptide epitopes are used to self-assemble into nanofibers, which cause immunogenicity via eliciting high antibody without any adjuvant.\(^1a\) In addition, peptides without nonimmunogenicity need to be programed from programmable primary structure or antigen peptides.\(^31,45\)

Considering that peptides contain such unique advantages, which is promising for the immunotherapy applications. The peptide-based nanovaccines can response to immune system owing to peptide specificity.\(^11b\) More importantly, peptide-based nanovaccines effectively reduce antigen degradation, and are more easily recognized by dendritic cells (DCs). It shows enormous potential for peptide-based nanovaccines during immunotherapy.\(^46\)

Moreover, it has been reported that nano-scaled micelles self-assembled from peptide amphiphiles (PAs) can effectively respond to immune system due to activating Toll-like receptors (TLRs) on DCs.\(^3a\) Therefore, to avoid PAs activating immune response, PAs with a synthetic lipid tail need to be designed. Antigen delivery system was developed to protect antigens from degrading and improve uptake by DCs. In the experimental design, a dialkyl tail with two palmitic (C\(_{16}\)) as hydrophobic, lipid-like tail, and antigen ovalbumin (OVA) as hydrophilic, biofunctional peptides self-assemble into DiC\(_{16}\)-OVA cylindrical micelles (Figure 3A). The DiC\(_{16}\)-OVA cylindrical micelles exhibited uniform rod morphology, as verified by TEM and AFM imaging (Figure 3B). To further prove experimental purpose that DiC\(_{16}\)-OVA cylindrical micelles can arouse an immune response in vivo, OVA peptides were not completely coated by Freud’s adjuvant (IFA), which was chosen as one of control groups. Finally, the result from percentage of CD8+ cells demonstrated that DiC\(_{16}\)-OVA cylindrical micelles could induce and prolong immune response (Figure 3C,D). It proved that antigenic peptides obtained the effective protection for immunotherapy. Inspired by this research, various antigen peptides are chosen to prepare self-assembling nanovaccines, which both activate immune system and protect antigen peptides from degrading. The development of self-assembling antigen
peptides as nanovaccines for immunotherapy will become an important direction.

3.4 | Self-assembling peptides for synergistic therapy

Although immunotherapy has the ability to arouse long-term resistance against tumor by systemic immunity, it is difficult to eradicate primary tumors. Hence forecasts, immunotherapy need to combine with other complementary therapies against malignant tumors.

3.4.1 | The synergistic treatment of immunotherapy with PTT

PTT is capable of providing robust thermal energy to effectively destroy tumor cells in short time. Mass necrosis of tumor cells attracts more macrophages and elicits some degree of immune responses. To enhance synergistic treatment of immunotherapy with PTT, Sun’s group reported nanovaccines consisted of model antigen OVA and ICG, which employed antigen-directed strategy based on the combination treatment of immunotherapy and PTT (Figure 4A). This nanovaccine OVA–ICG showed high loading rate of antigens. The antigen-directed synergistic treatment of immunotherapy with PTT achieves remarkable treatment effect. In PTT, different concentrations of OVA–ICG solution exhibited obvious temperature increasing compared with pure water under laser irradiation at 808 nm. In immunotherapy, DC cells were incubated with OVA, ICG, and OVA–ICG nanovaccine for 24 and 48 h, which secreted significant TNF-α and IL-6 after 24 h and further increased for 48 h. And expression levels of MHC-II, CD80, and CD83 were significantly higher than OVA–ICG group than pure OVA, ICG groups (Figure 4B). The result demonstrated that OVA–ICG nanovaccines exhibited enhanced immunotherapy combined with PTT for tumor therapy. These nanovaccines employed a new strategy in which antigen peptides are selected for self-assembly to build multifunctional nanovaccines, which has high antigen loading rate and overcomes hydrophobicity problem of ICG. Moreover, these nanovaccines also employed synergistic treatment that photothermal therapy for tumor ablation and immunotherapy for antigen overexpression activating immune system.

3.4.2 | The synergistic treatment of immunotherapy and dynamic therapy

It has been reported that PDT was able to elicit antitumor immune responses due to immunogenic cell death (ICD) of tumor cells. Meanwhile, damage-associated molecular patterns (DAMPs) that can stimulate antitumor immune
FIGURE 4 Self-assembling peptides for therapeutic methods of combining PTT with immunotherapy. (A) Schematic illustration of the preparation process of OVA–ICG nanovaccine and the mechanism of photothermal-immunotherapy for tumor therapy. (B) The amount of TNF-α and IL-6 and the levels of MHC-II, CD80, and CD83 secreted and expressed by DC cells incubated with OVA, ICG, and OVA-ICG for 24 and 48 h. Reproduced with permission. Copyright 2018, Wiley-VCH

responses as immunogenic signals, are released during ICD process. Hence, it is new thought to develop the burst release of ROS in PDT, which effectively activates antitumor immune responses. The plasma membrane (PM) as targeted chimeric peptide has been introduced by a research. The fast release of DAMP under the action of specific PM damage enhanced photodynamic immunotherapy. The treatment mechanism of research is shown in Figure 5A in which enzyme-driven tumor cell PM-targeted chimeric peptide destroyed specific PM,
then DAMPs were released, finally antitumor immune system was activated, including the presentation of tumor antigens, the maturation of DC, and the activation of cytotoxic T cell. As shown in Figure 5B, the level of HMGB1, ATP release, IL-6, TNF-α, and matured DCs (CD11c⁺, CD80⁺, CD86⁺) obviously increased, which showed better immune response. To enhance immune activation, checkpoint blockade inhibitor (anti PD-1) was introduced, which obviously improved therapeutic effect. The efficacy of PDT was improved through targeted chimeric peptides damaging PM, and then released DAMP elicited antitumor immune responses, achieving clever combination for PDT cooperating with immunotherapy for antitumor applications. Therefore, functional peptides are used for self-assembling nanoparticles target to destination, which promote effective combination of various therapy methods from the mechanism. It is potential for functional peptides to self-assemble for tumor therapy.

Ultrasound possesses remarkable superiority, such as non-invasiveness and depth tissue penetration compared with NIR laser, hence SDT is considered as a promising and effective therapeutic modality. SDT is capable of arousing immune response that the mechanism is similar to PDT. SDT has been used to combine with other treatments, including PDT, PTT, chemotherapy, and immunotherapy. Recently, synergistic therapy of SDT with immunotherapy for tumor therapy has been reported. Nano-sonosensitizers (HMME/R837@Lip) were developed through using liposomes (Lip) to co-encapsulate sonosensitizers (HMME) and immune adjuvant (R837) for the synergistic therapy of SDT and immunotherapy. The result demonstrated that the combination of SDT with anti-PD-L1 was able
to arouse antitumor immune response, which effectively suppressed primary tumor and prevented tumor metastasis. The synergistic therapy of SDT and immunotherapy has advantages of deep penetration of ultrasound and long-term immunological memory of immunotherapy. If self-assembling peptides with functions are introduced in experimental design and got the utmost out of their advantages, achieving promising synergistic therapy for withstanding malignant tumors in the future.

In summary, antigen peptides and other functional peptides directly prepare nanovaccines for immunotherapy or indirectly elicit immune response, resulting the synergistic therapy of immunotherapy and dynamic therapy. Antigen peptides self-assembling nanomaterials possess excellent stability and high drug loading compared with other nanomaterials. Functional peptides self-assembling nanomaterials skillfully combine dynamics therapy with immunotherapy for maximizing the therapeutic effect.

3.4.3 The other synergistic treatment methods

Chemotherapy is still one of the main methods of clinical treatment, but it is limited by drug resistance, poor targetin.48 In immunotherapy, immune checkpoint blockade (ICB) therapy is applied for immune escape. However, ICB therapy is limited due to poor immunogenicity, and even immunosuppressive tumor microenvironment (ITM). The multifunctional nanomedicine was reported by Fenget al, which overcame not only immune escape by chemotherapy drugs but also the hydrophobicity of photosensitizers. In this experiment, ICG as template could self-assemble with paclitaxel (PTX) to prepare two-in-one nanoparticles (ISPN). PDT effect was generated from ICG of ISPN under laser irradiation, which could elicit antitumor immune response. Meanwhile, the regulatory T lymphocytes (Tregs) were suppressed by PTX loading in ISPN, which was capable to available combat ITM. Finally, immune checkpoint blockade (αPD-L1) was also introduced against recurrence prevention and metastasis inhibition. The whole experiment employed the synergistic treatment of three therapy methods, including PDT, immunotherapy, and immune checkpoint blockade therapy, which exhibited favorable effect of suppressing tumor growth and lung metastasis. Most importantly, the strategy of ICG as template to self-assemble with small molecular drugs was able to extend to other small drugs, especially antigen peptides. Overall, this study employed ICGtemplated, selfassembling nanoparticles for chemotherapy-PDT-immunotherapy, exhibiting a potential strategy for tumor therapy. This further promotes the design and development of peptide-templated self-assembling nanomaterials for tumor therapy in clinic application.

Inspired by this, NIR dyes (IR820), chemodrugs (DTX), and pre designed peptides (CF27) could self-assemble into nanomaterials for synergistic therapy of PTT, chemotherapy, and immunotherapy. IR820 loaded DTX with high drug encapsulation by supramolecular assembly. Then as-obtained nanoparticles made self-cross-linking by CF27 (Figure 6A). The stimuli-responsive property of DTX-IR820-CF27 was favorable to release DTX for chemotherapy (Figure 6B). As Figure 6C showed that tumor-targeting property of DTX-IR820-CF27enhanced for targeting drug delivery and release. In addition, in order to enhance the immune response against tumor cells, the immune checkpoints PD-1/ PD-L1 were introduced by designing CF27 as PD-1/ PD-L1. The experiment achieved a satisfying purpose that drug-dye-peptide nanomaterials were prepared by assembly and responded for PTT/chemotherapy/immunotherapy (Figure 6D).14a Self-assembling nanomaterials based on functional peptides, photosensitizer, and chemodrugs possess many advantages, such as high drug encapsulation, targeting to the tumor site, and controlled drug release. During synergistic therapy, peptides play a crucial role owing to their programmability and biodegradability.

During synergistic therapy, experimental design principle is adopting the strategy of promoting strengths and avoiding weaknesses of different treatment strategies for achieving maximized synergistic therapy. The significant advantages of self-assembling peptides are very attractive, especially, tumor microenvironment response. Self-assembling peptides play an important role on combining with other treatment methods for maximizing therapeutic efficiency.

4 SUMMARY AND OUTLOOK

We mainly expounded unique advantages and biomedical application of self-assembling peptides for tumor therapy. Peptides display biocompatibility, nonimmunogenicity, tissue permeability, rapid metabolism from body, and most importantly programmability, which have huge potential for biological researches and clinical applications. In particular, self-assembling peptides with high drug loading achieve effective drug release due to responsiveness to tumor microenvironment, which shows remarkable therapeutic effect and overcomes toxicity and nontargeting of free drugs. Despite many advantages, some issues still exist, which are worth to ameliorate for self-assembling peptides against malignant tumors in the future.
Finding more functional peptides and researching the functions of peptides are crucial to further apply for tumor therapy; (b) Designing functional peptides according to the programmability of peptides to achieve a specific treatment of tumors; (c) With an in-depth understanding of the tumor microenvironment, developing specific nanomaterials based on self-assembling peptides that play a significant role at tumor site; (d) Finding the peptides directly related to the tumor microenvironment, and adjusting the tumor microenvironment to achieve the maximum efficiency of tumor inhibition; (e) Constructing appropriate nanomaterials based on self-assembling peptides by controlled methods for specific types of tumor tumors to provide new insights into tumor therapy. (f) More importantly, modifying peptide chains with other materials for protecting peptides from degrading by enzymes in physiological conditions and avoiding immune response of peptides in vivo. In conclusion, more and more functional peptides have been applied for tumor theranostics. We believe that self-assembling peptides, especially functional peptides, will have great application value in biology and medicine in the near future.

Conflicts of Interest
The authors declare no conflict of interest.

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