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Review

Functionalized Peptide-Based Nanoparticles for Targeted Cancer Nanotherapeutics: A State-of-the-Art Review

Ritika Sharma, Shikha Jyoti Borah, Bhawna, Sanjeev Kumar, Akanksha Gupta,* Poonam Singh, Vijay Kumar Goel,* Ravinder Kumar, and Vinod Kumar*

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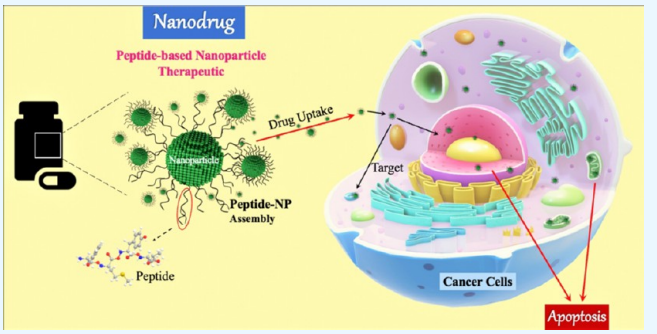
ABSTRACT: Cancer mortality is increasing at an alarming rate across the globe. Albeit, many therapeutics are available commercially, they are not effective and have no cure up to today. Moreover, the knowledge gap in cancer therapy persists, representing a potential blind spot for the innovation of effective anticancer therapeutics. This review presents an update on current advancements in nanopeptide therapeutics. Herein, a detailed exploration of peptide-functionalized nanoparticles for the development of nanotherapeutics was carried out. Different approaches that include self-assembly nanostructures, solid phase peptide synthesis, ligand exchange, chemical reduction, and conjugation methods for assembling peptides for functionalizing nanodrugs are also highlighted. An outlook on biomedical applications is also reviewed. Additionally, a comprehensive discussion on targeted cancer cell therapy and mechanism of action are provided. The present review reflects the functional novelty of nanodrugs to improve stability, accessibility, bioavailability, and specificity toward cancerous cells. Finally, it summarizes the current challenges and future perspectives on the formulation of these nanodrugs.



1. INTRODUCTION

Cancer is a global health issue with limited combinatorial treatment possibilities such as chemotherapy and radiation complemented by adverse effects.[1] However, there is a lack of understanding and clinical translation in cancer pathophysiology. Despite technological advancements and breakthrough achievements in cancer research, there is no cure for cancer. Furthermore, there are certain limitations associated with conventional treatment strategies.[2] Accumulated literature throughout the past decade has provided cardinal insights into the regulatory mechanisms underlying cancer biology and malignancy.[3] Peptides embody a short stretch of amino acids linked by an amide bond (−CONH−) and are involved in various biological functions.[1,4] These biomolecules play a pivotal role in the human body through various biological processes and form an intriguing biological material due to multiple properties such as biocompatibility, bioactivity, and biodegradability.[5,6] The structure−function relationship of peptides have offered several platforms for futuristic breakthroughs in the realm of biomedical applications for various disorders, which has piqued researchers' interest.[5−7] Additionally, therapeutics based on peptides have been of immense interest to researchers due to several features such as low toxicity, better penetration, tunability, and many more.[4] However, there are certain demerits associated with peptide drugs, such as being less stable and prone to degradation.[1,8,9] The concept of supramolecular chemistry and enhanced peptide-based therapeutics through the formation of interactions such as ionic,[10] hydrogen bonding (H-bonding),[11] and even $\pi-\pi$ interactions,[12] results in a systematic hierarchical nanostructure. Despite the lack of understanding of the peptide-nanoparticle (NP) interaction and the parameters that determine its effectiveness, intense research has persisted in achieving ground-breaking results in anticancer medications.

Cancer, being a worldwide incurable terminal disease, creates a global demand for the creation of new materials that show features such as biocompatibility, minimum toxicity, bioavailability, etc. The universe of NPs provides a wide range of materials, among which peptide-functionalized NPs (peptide-f-NPs) has been one of the ideal biomolecules that enhance the efficiency of drug delivery,[13,14] biosensors,[15] COVID-19 vaccines,[16] biotemplated NP catalysts,[17] active ingredients of personal care items (toothpaste and hair

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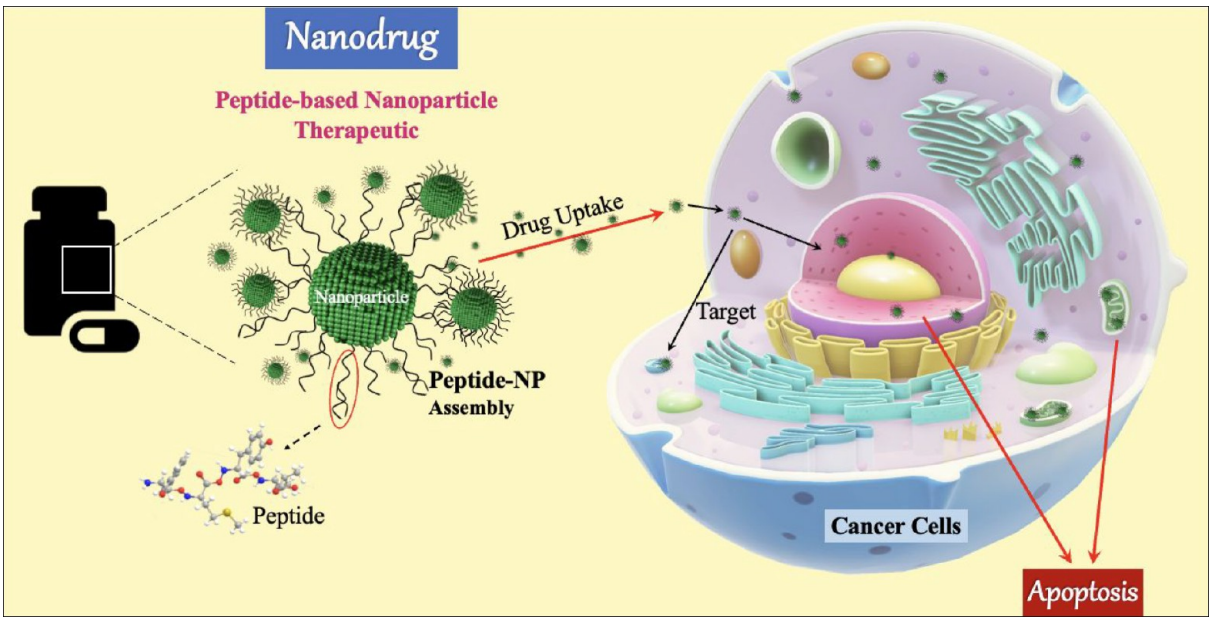

Figure 1. Schematic representation of the peptide-functionalized nanodrug mediating targeted anticancer activity.

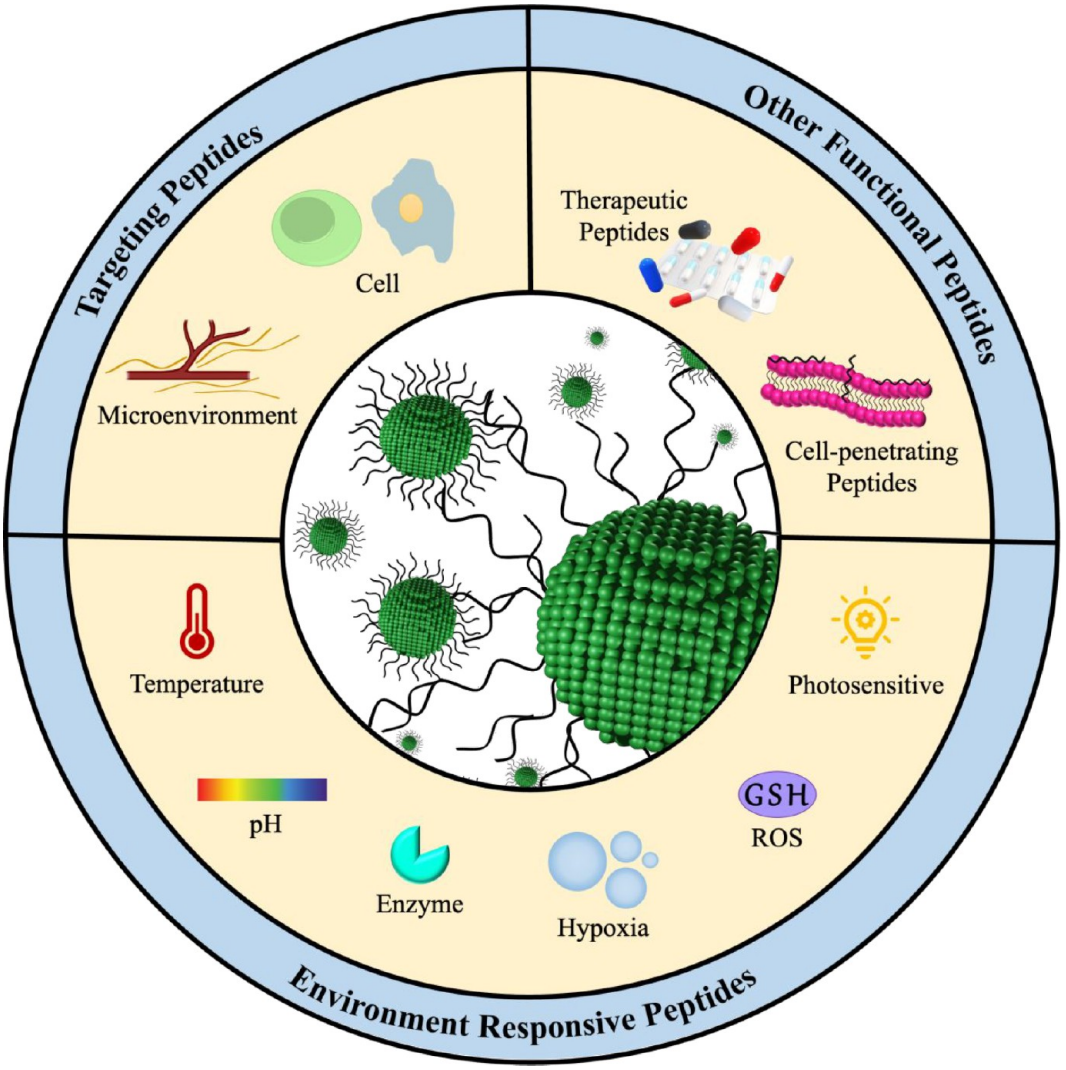

Figure 2. Schematic illustration of peptide-functionalized nanotherapeutics classified into three categories: (1) environment responsive peptides, (2) targeting peptides, and (3) other functional peptides.[23]

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coloring),[18] gene therapy,[19] and many other biomedical applications.[20] Consequently, the diverse implementation has urged researchers to explore the *in situ* construction of *in vivo* self-assembly that can be used to develop peptide-f-NPs and determine the possible areas of improvisation.

Therapeutics' based on the combination of peptides and NPs have emerged as a replacement for cancer therapy as they demonstrate the potential for targeted cancer treatment and tracking (Figure 1). Various NPs have been conjugated or functionalized with a peptide moiety via several approaches. The mechanism of targeted cancer treatment is as follows with reduced side effects on healthy cells.[2,21] The peptide-NP assembled therapeutics were injected. Upon injection, the nanopeptides migrate to the tumor cells via penetration or endocytosis. It causes cellular or DNA damage leading to the onset of apoptotic pathways, for example, mitochondria-targeted apoptosis.

From the view of drug discovery and delivery mechanisms, very limited therapeutics are clinically approved.[3] Most of those were not specific to cancerous cells. Moreover, only limited literature explores the functionalization of peptide nanotherapeutics for potential application in targeted cancer therapy.[22] A significant emphasis was provided on the functionalization of NPs. They are summarized with an up-to-date view of anticancer therapy.[23,24] Furthermore, the functionalization of self-assembled nanostructure peptides was also discussed.[25] This work reflects a viewpoint on the functional novelty of nanodrugs with improved stability, accessibility, and bioavailability with cancerous cell specificity.[26] Moreover, the mechanism of action of these nanotherapeutics was also comprehensively discussed. The current challenges and future trends in the formulation of these nanodrugs, along with the future trends are also explored.[27]

2. PEPTIDE ASSEMBLY APPROACHES FOR NANOPARTICLE FUNCTIONALIZATION

Before understanding the application of peptide-f-NPs, an exquisite focus must be brought to their synthesis. Most often, the ideal approach toward the synthesis of NPs is through hydrothermal or solvothermal methods.[28,29] The functionalization of prepared NPs to obtain appropriate efficiency as therapeutic peptide-f-NP drugs is through different strategies: (1) self-assembled nanostructures, (2) solid phase peptide synthesis (SPPS), (3) ligand exchange, (4) chemical reduction, and (5) chemical conjugation for peptide assembly.

2.1. Self-Assembly. For an efficient drug delivery system, a smart binding peptide is required, which can recognize and trigger targeted drug accumulation and release drugs upon appropriate stimuli. Various functional peptides have been determined and are further distinguished based on their functionalities (Figure 2).[23] For instance, targeting peptides can bind to different tissues and lesions (bones, muscles, tumors, etc.), as a consequence of which they can effectively bind to receptors. Examples of such targeting peptides are muscle-targeting peptides (Ala-Ser-Ser-Leu-Asn-Ile-Ala; ASSLNIA) for heart and skeletal muscle diseases,[30] kidney-targeting peptides,[31] and liver targeting peptides.[32] Another class of functional peptides is the responsive peptides, which are produced as a result of changes in pH, light, enzyme, and temperature showing enhanced impact and scope in several applications such as biosensors, tissue engineering, and drug delivery. Zhang et al. synthesized pH and temperature-dependent peptide series, which exhibited reversible transitions between α-helix and β-sheets.[33] In addition to targeting and responsive peptides, therapeutic peptides are the major type of functional peptides, which have presented a promising field for the invention of the drug delivery approach. Many therapeutic peptides have been rigorously studied through clinical trials, besides which many of such peptides have also been Food and Drug Administration (FDA) approved.[34] Having an extensive range of applications in diseases such as cancer, allergies, and metabolic and immunological diseases, peptide-f-NP therapeutics have gained international importance in the global market.[35]

2.2. Solid Phase Peptide Synthesis (SPPS). SPPS is an effectively explored approach, developed by Merrifield.[36] The iterative cycles of deprotection, activation, and coupling are performed using different protecting agents bound to the resin molecule (Figure 3).[24] A frequently used protecting agent is

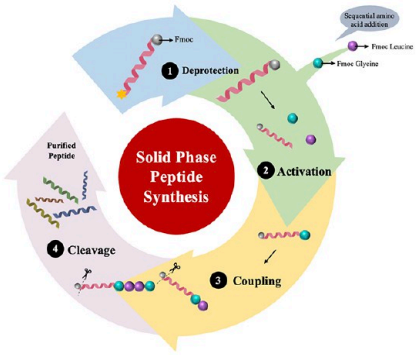

Figure 3. Schematic representation of repeated cycles of Fmoc-based SPPS.[24]

the 9-fluorenylmethoxycarbonyl (Fmoc) group and the *tert*-butyloxycarbonyl (Boc) group, to avoid an undesired reaction. Typically, the Fmoc bound side chain attaches to the resin. Next, the Fmoc deprotection is achieved. Then during activation, amino acids are added sequentially. Subsequently, derivatives of Fmoc-amino acid are hitched using suitable coupling reagents. This coupling step is followed by subsequent deprotection, cleavage, and finally peptide purification.[24,36]

2.3. Ligand Exchange. This simple method proceeds through the displacement of an original present ligand on the surface of the NP by one or a mixture of specifically chosen peptide ligands and has often been seen as a convenient method.[37,38] Ligand exchange occurs under mild conditions of ligands exhibiting multifold functionality and excellent colloidal stability. This displacement has often been successfully observed in peptides having cysteine (Cys; C). For instance, Hu et al. observed that the thiol group can quickly form a stable covalent bond with the gold (Au) atoms on the surface of Au NPs. However, these Au−S covalently bonded interactions may further undergo decomposition by thiols like glutathione, as a result of which modification of Cys using selenium (Se) to produce Au−Se bond formation has been explored by many researchers.[39,40] Alternatively, in mild conditions, phosphorylated amino acids can form stable coordinate bonds when in combination with metallic cations such as Fe^{3+} and Zn^{2+}. The phosphorylated amino acids, like phosphorylated serine (Ser-P), induce an easy exchange of metallic NPs through metal-phosphate coordinate bonds.[41,42] An excellent example of this has been reported by Liu et al.

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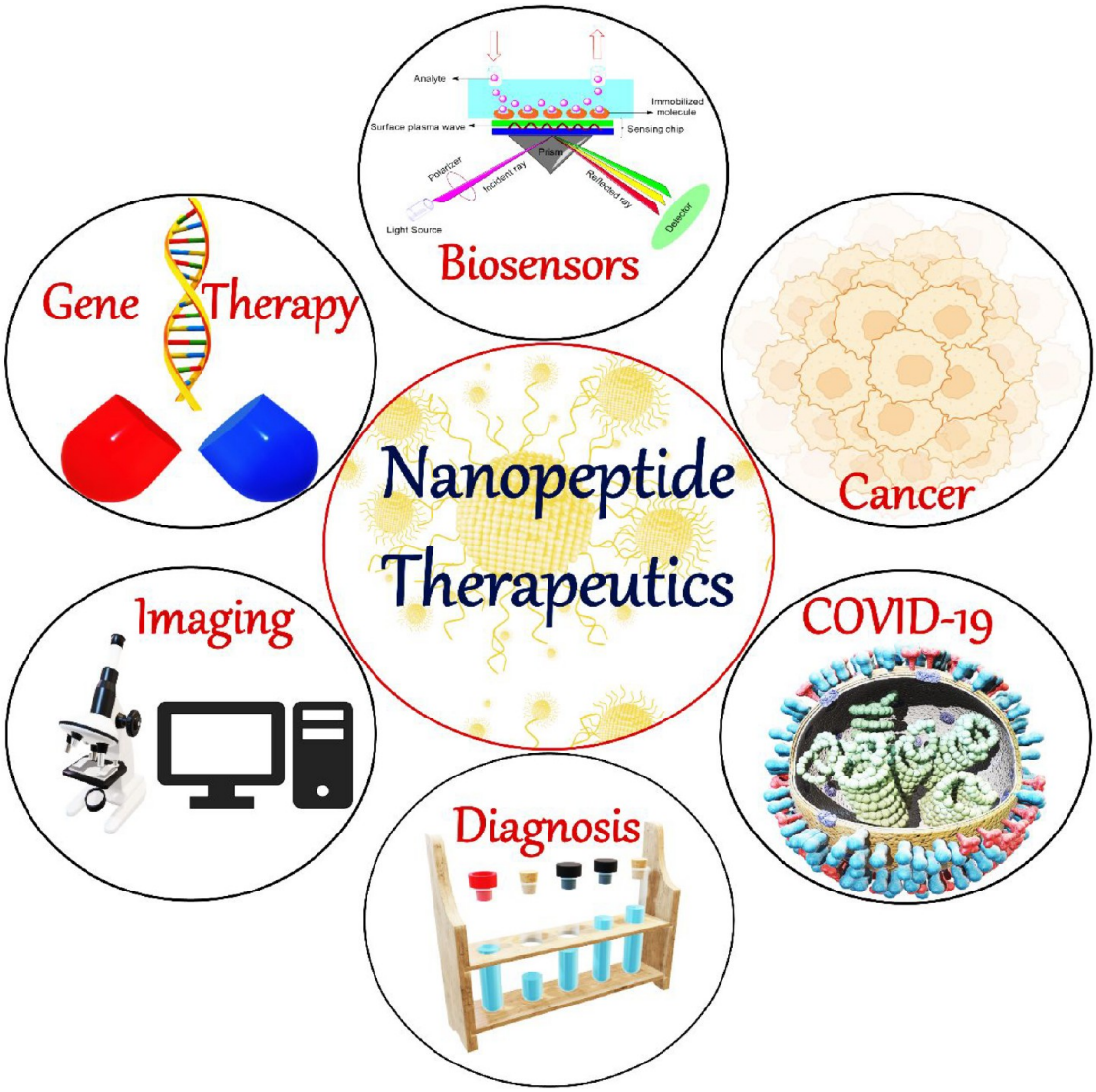

Figure 4. Schematic illustration of nanopeptide therapeutics in biomedical applications.

showing a simple tryptone-induced ligand exchange for achieving functionalization of various NPs such as zinc oxide (ZnO) NPs, silver (Ag) NPs, and $NaGdF_4$ nanodots.[42]

2.4. Chemical Reduction. Chemical reduction is a simple and direct method of synthesizing peptide-f-NPs, which follows three general steps. This method begins with premixing a metal precursor and an appropriate peptide in a solution, followed by the optional second step of adding a reducing agent in a lower quantity. Finally, the synthesized peptide-f-NPs are purified. The peptide incorporated in this method determines the reduction of metal ions as well as the stability of the synthesized NPs.[43] However, the peptide often acts as a stabilizing agent, while several chemical compounds, such as $NaBH_4$ or ascorbic acid, act as the reducing agent. Residual amino acids, like tyrosine (Tyr; Y), tryptophan (Trp; W), proline (Pro; P), and many others, can reduce metal ions to their respective metals via the mechanism of electron transfer.[43,44] This has been seen in recent works by Si and colleagues, who showed a successful reduction of $HAuCl_4$ or $AgNO_3$ via a Trp residue to obtain tripeptide-functionalized Au NPs or Ag NPs.[45] These NPs can be further modified by improvisations based on reaction conditions such as rate of reaction, peptide sequence, metal to peptide ratio, and pH.

2.5. Chemical Conjugation. In the chemical conjugation method, there is a two-step approach to produce desirable peptide-f-NPs. The first step is to cap the NPs with suitable stabilizers such as polyethylene glycol (PEG) derivatives, which proceed via ligand exchange or interactions such as H-bonding, or silica (Si) shells, which proceed via the water-in-oil microemulsion method.[29,46,47] Capping is followed by the conjugation of peptides on the surface of the NPs which can be modulated by different operational parameters. Bartczak and Kanaras successfully conjugated the Lys-Pro-Gln-Pro-Arg-Pro-Leu-Ser (KPQPRPLS) peptide (positively charged) to Au NPs using the N-(3-dimethyl aminopropyl)-N-ethyl carbodiimide hydrochloride (EDC)/*N*-hydroxysuccinimide (NHS) method. Here, the Au NPs were stabilized by carboxy-terminated oligoethylene glycol.[48] Fu et al. developed monocyclic peptides (MCP)-functionalized Mn-doped iron oxide (MnIO NPs) by using a bifunctional PEG-based ligand (3,4-dihydroxy benzyl-amine-PEG-NH_2) to transfer the MnIO NPs into aqueous solution.[49] A recent work conducted by Li et al. exhibited the fabrication of hydrophobic upconversion NPs (UCNPs) using

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a SiO_2 shell from the organic to an aqueous phase and additionally conjugated peptide ligands having an excellent affinity toward tumors. The synthesized peptide-f-NP conjugate was effectively used for diagnosing and bioimaging of colorectal cancer (CRC). Therefore, it holds great significance and has a dual role in cancer therapy.[28]

3. BIOMEDICAL APPLICATIONS OF NANOPEPTIDES

Recent studies have confirmed that peptide-f-NPs have effective potential in antitumor nanomedicines,[50] drug delivery in the central nervous system (CNS)-tuberculosis (TB) treatment,[51] drug delivery,[13,14] nanobiosensors,[15] nanovaccines,[16] gene therapy,[19] cancer, and many other diseases (Figure 4). Regardless, the application diversity has urged researchers to explore other rationales for designing peptide-f-NPs for possible areas of improvisation. A few of them are briefly discussed below. However, the literature already holds a significant number of publications that demonstrated the role of nanopeptides in most applications. Albeit, the literature is scarce on cancer therapy, and in the following section, therefore, an emphasis on the implementation of nanopeptide drugs for focused cancer therapy is elaborated.

3.1. Biosensors and Diagnosis. Multifunctional nanomaterials (MNMs) are the essential foundation for biosensors which are sophisticated tools capable of sensitive and accurate detection. These novel (simple, durable, and sensitive) MNMs used for biosensors have been upgraded, robust, and downsized as nanobiosensors. Recent studies have shown that low molecular weight peptides coupled with nanoscale graphene derivatives are suitable building blocks for analytical formulations with diagnostic capabilities.[52] Peptide-functionalized graphene derivatives have been reported to have multiple biosensing applications such as cancer cell detection,[53] ATP determination,[54] monitoring protease activity,[55] etc. When a targeting peptide is conjugated to the cargo surface for gene delivery, transfection and cell specificity frequently increases. The correct formulations can effectively target *in vivo* gene expression limited to the target tissue and even have beneficial medicinal effects.[56] Li et al. synthesized MoS_2 nanosheets which they functionalized with the Met-Pro-Pro-Pro (MPPP) peptide with the help of polydopamine (PDA) and PEG. The MoS_2-PDA-PEG-MPPP was achieved via facile chemical synthesis. The MoS_2-PDA-PEG-MPPP fluorescent biosensor has been effectively demonstrated for caspase-3 detection with a detection limit of 0.33 ng/mL.[57]

3.2. Imaging. Imaging probes based on nanotechnology are used to quantitatively identify living tissues and cells. Compared with conventional contrast agents, the inclusion of nanoscale materials in the procedure improves the contrast of the interface.[58] In a recent study, Lin et al. developed dual-modal optical imaging rare earth NP (RENP) probes with peptide functionalization for targeted tongue squamous cell carcinoma (TSCC) imaging and therapy.[59] More research has also been explored for applying peptide-f-NPs in targeted drug delivery. For instance, nearly two decades ago, De la Fuente and Berry developed a method to target the cell nucleus by linking the HIV-derived CPP Tat (Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg; GRKKRRQRRR) to Au-NPs. They reported Tat peptides functionalized with Au@tiopronin NPs to demonstrate the capacity to enter cells and target their nuclei.[60] Li et al. used graphene oxide (GO) for the fabrication of a peptide that is specific for gastric receptors, i.e., AF750-6Ahx-Sta-BBN. The biofunctionalization was achieved using H-bonding and $\pi-\pi$ interactions. The affinity for ligand–receptor binding, internalization, and cellular uptake were assessed for HSC-3 cells, which are human oral squamous carcinoma cells. It demonstrated an effectiveness for fluorescent imaging utilizing near-infrared dyes. Additionally, it appears as the potential to offer an NP-based delivery strategy for therapeutic agents.[61]

3.3. COVID-19 Therapy. The most recent global emergency posed by COVID-19 has created a magnificent requirement for advances through the use of nanobased technology and science. This outbreak has put a strain on healthcare and raised important issues regarding their management using traditional strategies and diagnostic devices. In this context, the applications of peptide-f-NPs present a new chance for creating prevention, diagnosis, and treatment solutions against the COVID virus. In a detailed review, Campos et al. have discussed the challenges and drawbacks that need to be addressed when using nanotechnology to manage the COVID-19 virus through the development of nanobased materials, such as disinfectants, personal protective equipment, diagnostic tools, nanocarriers systems, treatment, and vaccine development.[62] Thus, it is clear that peptide-f-NPs have a broad range of applications that have been continuously examined and analyzed over the last three decades. However, further research is necessary to fully comprehend and utilize this potential.

3.4. Gene Therapy. A recent investigation by Tarvirdipour et al. demonstrates the smart assembly of NPs that are functionalized with peptide molecules. They comprehensively presented the potential for the implementation of peptide-f-NPs for their application in gene therapy and tracking. The demonstration of nanobased delivery of nucleic acids thereby enhances the probe sensitivity for targeted therapy.[19] One study demonstrated PEG along with octaarginine (R8) peptide. The Arg-rich peptide molecule is used as a cell-penetrating peptide that can be a potential cargo for delivery to different cells. Their investigation showed pH-responsive behavior of the synthesized PEG-plasmid DNA (pDNA)-R8 peptide as well as synergistic effects.[63] Shtykalova et al. investigated magnetic NPs that are biofunctionalized with the hexaarginine (R6) peptide, which is bound electrostatically with the $\alpha v\beta 3$ integrin targeted peptide. The functionalization was achieved and was conjugated with pDNA. It reduces transfection time and improves delivery of pDNA encoding thymine kinase gene for the herpes simplex virus type I (HSV-1). Therefore, it shows a potential nonviral-based approach for the treatment of uterine leiomyoma.[64]

4. CANCER THERAPY

Nanoscale intervention over the past decades has provided insightful information for peptide-f-NP based on therapeutic approaches for cancer treatment and diagnosis. Several NPs, such as Au, GO, quantum dots (QDs), and others, have been integrated with peptide drugs to enhance the stability of peptides. Furthermore, peptides combine immense potential and large protein selectivity, possessing specific pros of small molecules, like stability, oral bioavailability, and availability. Therefore, various peptide-f-NPs have been developed, such as self-assembly, supramolecules, cell-penetrating peptides, peptide-based nanopores, peptide-based hormones, stimuli-responsive peptides, and layered double hydroxide (LDH)-based peptides.[1,4,8,13,24,65−68] Recent advances in the peptide-f-NP nanodrug would increase their potential applications in

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Table 1. Overview of Peptide-f-NP Drugs Used As Anticancer Therapeutics

peptide	f-NP or loaded drug	synthesis method	application in cancer therapy	ref
PS NPs				
CpG; E7; WT1; SV	PS NPs	covalent conjugation	elicit CD8+ T cell specificity with minimal cross-reactivity in gynecological cancers	69
FMYL	PS NPs	GLIDE docking	anticancer agent	21
		facile SPPS	inhibit the DHFR pathway in cancerous cells	
PEG-based Materials				
TAT	PEG−PCL	ring-opening polymerization	encapsulation of siRaf-1 and camptothecin in the formed peptide-NP	70
			exhibit higher uptake and beneficial effect in glioma cells	
MP9-aPDL1	PEG	SPPS	enhanced CRC immunotherapy	71
Au NPs				
CRGDK; P12; Nrp-1	Au NPs	EDC/NHS	improved therapeutic P12 peptide delivery to cancer cells	72
			highly effective breast cancer treatment (upregulating p53 expression)	
LA-WKRAKLAK	Au NPs	facile mixing	induces intrinsic apoptotic pathway in cancer cells	73
Other Materials				
SMAC	DOX	EDC/NHS	treatment of drug-resistant cancers	74
FRRG			highly specific to cancer cell	
D-penetratin	DOX	SPPS	exhibits increased uptake in brain perfusion studies	75
SynB1			enhanced DOX (20-fold) transported into the brain parenchyma	
			reduced DOX concentration in heart tissue	
aPDL1 and cathepsin-B cleavable peptide	DOX	intermolecular interactions	targeted cancer immunotherapy	76
			induced ICD	
RGDK receptor	DOX	EDC/NHS method	efficient delivery of cytostatic drugs	77
			tumor inhibition upon single dose administration	
Fe(III)-DA	DOX	SPPS	efficacy of CDT and PTT	78
mussel-derived peptide			photothermal ablation	
			multimodal synergistic cancer therapy	
P435 HER2/neu-derived	Maleimide-PEG2000-DSPE	covalent binding with a thiol group	nanoliposomal vaccine delivery systems against HER2-positive breast cancer cells	79
streptavidin-coated peptide (TLR9 and CpG ON)	PLGA	double emulsion solvent evaporation	potential as a therapeutic cancer vaccine	80,81
			effective against melanoma or prostate cancer cells	
F-KLAK	poly-CB[7]	facile mixing	∼97% encapsulation without affecting the peptide activity	82
			enhanced stability and anticancer potency of the peptides *in vivo*	
FFYSV	PpIX	facile SPPS	tumor PA imaging	83
			chemo-photothermal combination therapy	
PTX; CPK	PLGA	emulsion solvent evaporation	high affinity for MCT1 receptor	84
			potential antineoplastic nanodrug	
			specific for CRC cancer	
			inhibit angiogenesis	
MiRGD	Graphene QDs	hydrothermal method	multifunctional for targeted drug delivery and tracking	85
	DOX or Curcumin	chemical synthesis		

anticancer activity. Table 1 summarizes the peptide-f-NP drugs along with their application in tumor targeting.

To address this, peptide-f-NPs have been of interest in association with cancer detection, imaging, and therapy due to the aforementioned characteristics. As tabulated above, the recent trends associated with cancer therapy have been classified based on the carrier materials: (1) polystyrene (PS) NPs, (2) PEG-based, (3) Au NPs, and (4) other nanomaterials.

4.1. Polystyrene NPs. Singh et al. have fabricated human dihydrofolate reductase (hDHFR) for the first time. In their study, they performed molecular docking studies for the hDHFR along with its substrate (PDB ID 4M6K) and docked the peptides via the GLIDE docking program. Their results demonstrated a tetrapeptide conjugated with carboxylated PS NPs, enhanced delivery at the tumor site, and enhanced growth inhibitory effects. They designed a tetrapeptide (Phe-Met-Tyr-Leu; FMYL) that can efficiently bind at the substrate binding site of hDHFR, instead of folic acid or methotrexate. Therefore, conjugation of FMYL to PS NPs improves the delivery of the optimized peptide and growth-inhibiting effects. Therefore, this rationally designed peptide-f-NP drug possesses the ability to overcome the limitations of a conventional anticancer agent, i.e., methotrexate.[21] Xiang et al. have formulated a peptide vaccine with the integration of PS NPs. The peptides used are the E7 protein of the human papillomavirus, 5′-cytosine-phosphate-guanine-3′ (CpG), surviving (SV), and Wilms Tumour antigen 1 (WT1). These peptides were covalently conjugated with PS NPs and were tested for anticancer activity. Their results demonstrated that

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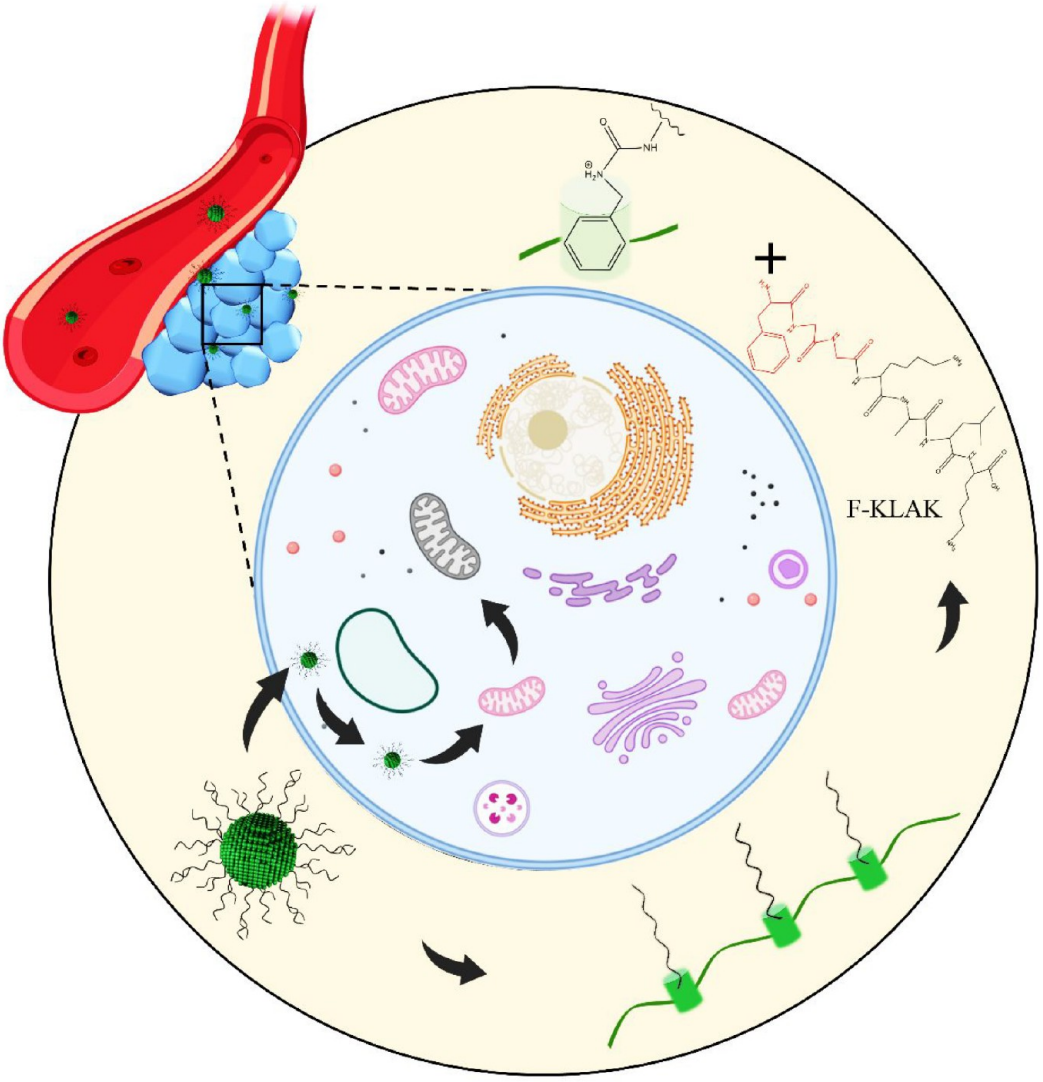

Figure 5. Schematic illustration of the development of supramolecular peptide therapeutic (SPT) for enhanced stability and anticancer ability of the peptides *in vivo*.[82]

the formed nanovaccine elicits a CD8+ T cell response without any inflammation. Therefore, these are effective against gynecological tumor cells.[86]

4.2. PEG-Based Materials. Wang et al. have demonstrated a cucurbituril- and phenylalanine (Phe; F)-functionalized peptide-f-NP drug via the facile mixing method. In the synthesis, curcubit[7]uril (CB[7]) was copolymerized with PEG. Further, the N-terminal F-containing peptide (Phe-Lys-Leu-Ala-Lys; F-KLAK) and poly-CB[7] were mixed in equimolar ratios and were stirred under ambient temperature and pressure. The poly-CB[7]/F-KLAK (PCB/FK) composite was synthesized, and the degree of polymerization was measured through proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. The PCB/FC complexed nanodrug demonstrated ∼97% encapsulation without affecting peptide activity, and the designed nanodrug was studied *in vivo* and their potential role in anticancer efficacy was observed (Figure 5).[82] A recent investigation demonstrated enhanced CRC immunotherapy using PEG conjugation with mastoparan (MP1) and anti-programmed cell death 1-ligand 1 (aPDL1) via SPPS. The prodrug was conjugated using a matrix metalloproteinase-2 (MMP-2) linker. The PEG-MPL1-aPdL1 conjugate exhibited oncolytic and pharmacokinetic properties along with the immune checkpoint block (ICB). The novel peptide-f-NP are accumulated in tumor cells demonstrating target specificity and enhancing infiltration of lymphocytes to induce oncolytic immunotherapy.[71]

4.3. Au NPs. One of the studies by Akrami et al. demonstrated the synthesis of pro-apoptotic peptides, i.e., α-lipoic acid (LA) and peptide conjugate (Leu-Ala-Trp-Lys-Arg-Ala-Lys-Leu-Ala-Lys; LA-WKRAKLAK) which was functionalized with Au NPs via facile mixing. The formed LA-peptide-Au NP conjugate could induce intrinsic apoptosis in anticancer cells. Furthermore, they demonstrated that the performance of the formed conjugate depends on the morphology features, like size and shape, of the NPs. For example, nanospheres showed cytotoxicity and morphological changes over nanorods. Similarly, nanorods demonstrate higher hemolytic activity over nanospheres.[73]

Kumar et al. have functionalized Au NPs along with Cys-Arg-Asp-Gly-Lys (CRDGK) and p12/PMI, that is, targeted and therapeutic peptides, respectively. The Au@P12-CRDGK was synthesized via the EDC/NHS method (Figure 6A,B).[72] On the basis of the available literature, p12 is known to

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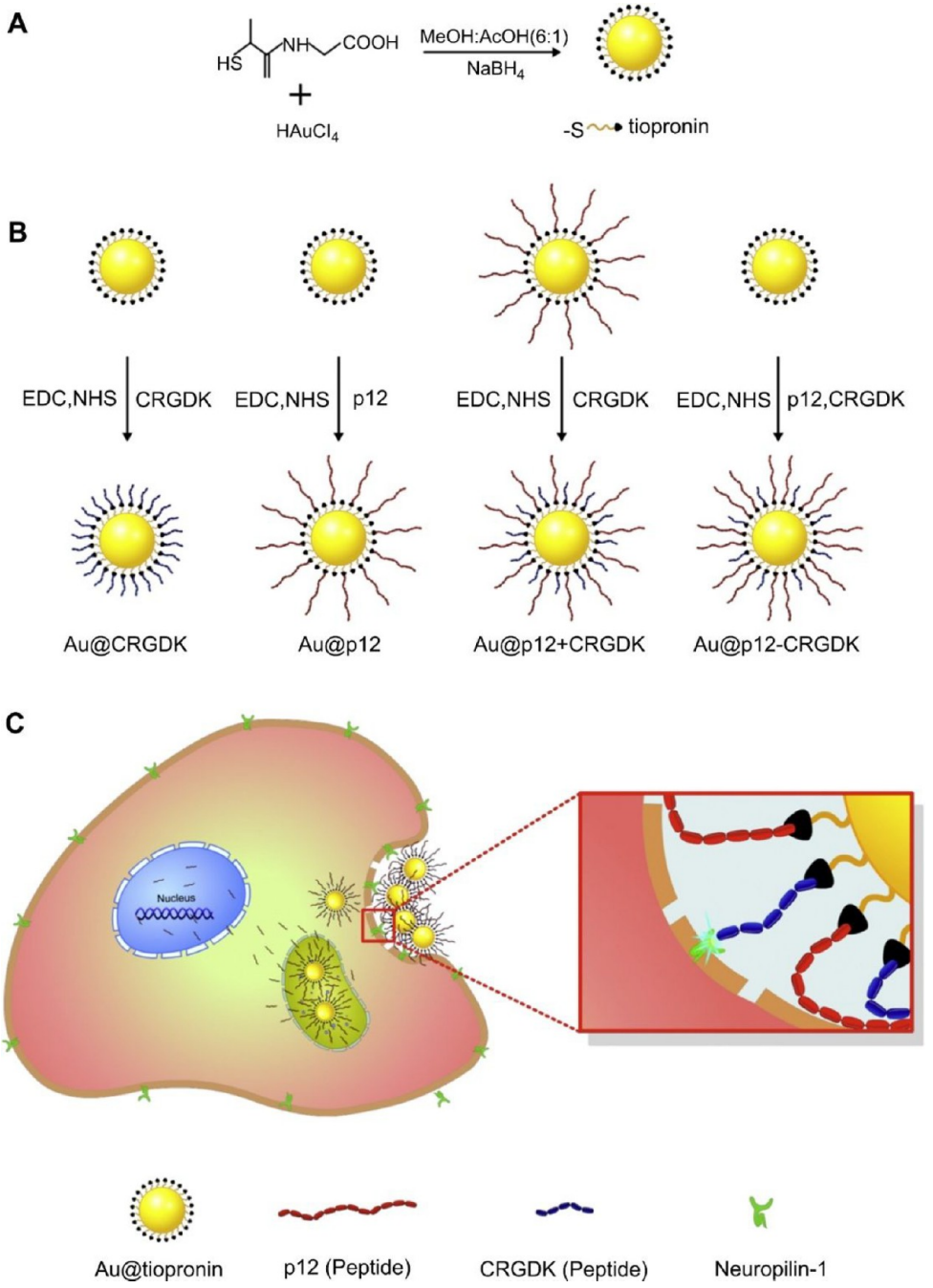

Figure 6. Schematic representation for Au@tiopronin synthesis and peptide functionalization for cancer treatment. (A) Synthesis of Au@tiopronin. (B) Au NPs functionalized with two types of peptides: p12 and CRGDK. (C) Receptor−ligand interaction enhances intracellular entry and increases response to the intracellular release of the therapeutic peptide into the cells.[72] Copyright 2011 Elsevier Ltd.

regulate p53 (a tumor suppressor protein) and is thereby responsible for cell cycle inhibition, DNA damage, and apoptosis. Furthermore, neuropilin (Nrp-1) binds with the targeted peptide that interacts with the protein tyrosine kinase receptor and thereby results in angiogenesis in breast cancer.[87−92] The conjugation of Au NPs enhances the uptake of the therapeutic peptide, and the selective binding of the targeted peptide with the Nrp-1 receptor causes a targeted effect on the cancerous cells without harming healthy neighboring cells (Figure 6C).[72]

4.4. Other Nanomaterials. Shim et al. have fabricated a second mitochondria-derived activator of caspase/Phe-Arg-Arg-Gly/doxorubicin (SMAC/FRRG/DOX) and cathepsin-B (SMAC/Phe-Gly-Phe-Arg (FGFR)/DOX) specific conjugates via the EDC/NHS method and further sieved through reverse-phase high-pressure liquid chromatography (RP-HPLC). The chemical structures for chemical conjugation, demonstrating hydrophobic interaction, and $\pi-\pi$ assembly between the peptide and DOX NPs were developed (Figure 7a).[74] SMAC-FRRG/FRGR-DOX was fabricated, and the drug−drug (D-D) NPs accumulated in cancerous cells via enhanced permeability and retention (EPR) effect, i.e., the accumulation of peptide-f-NP drugs in the tumor cells thereby enhancing permeability (Figure 7b). The NP composite got cleaved, and free DOX causes pro-apoptosis in cancerous cells mediated by cathepsin-B (Figure 7c). Briefly, the formed conjugate specifically cleaves

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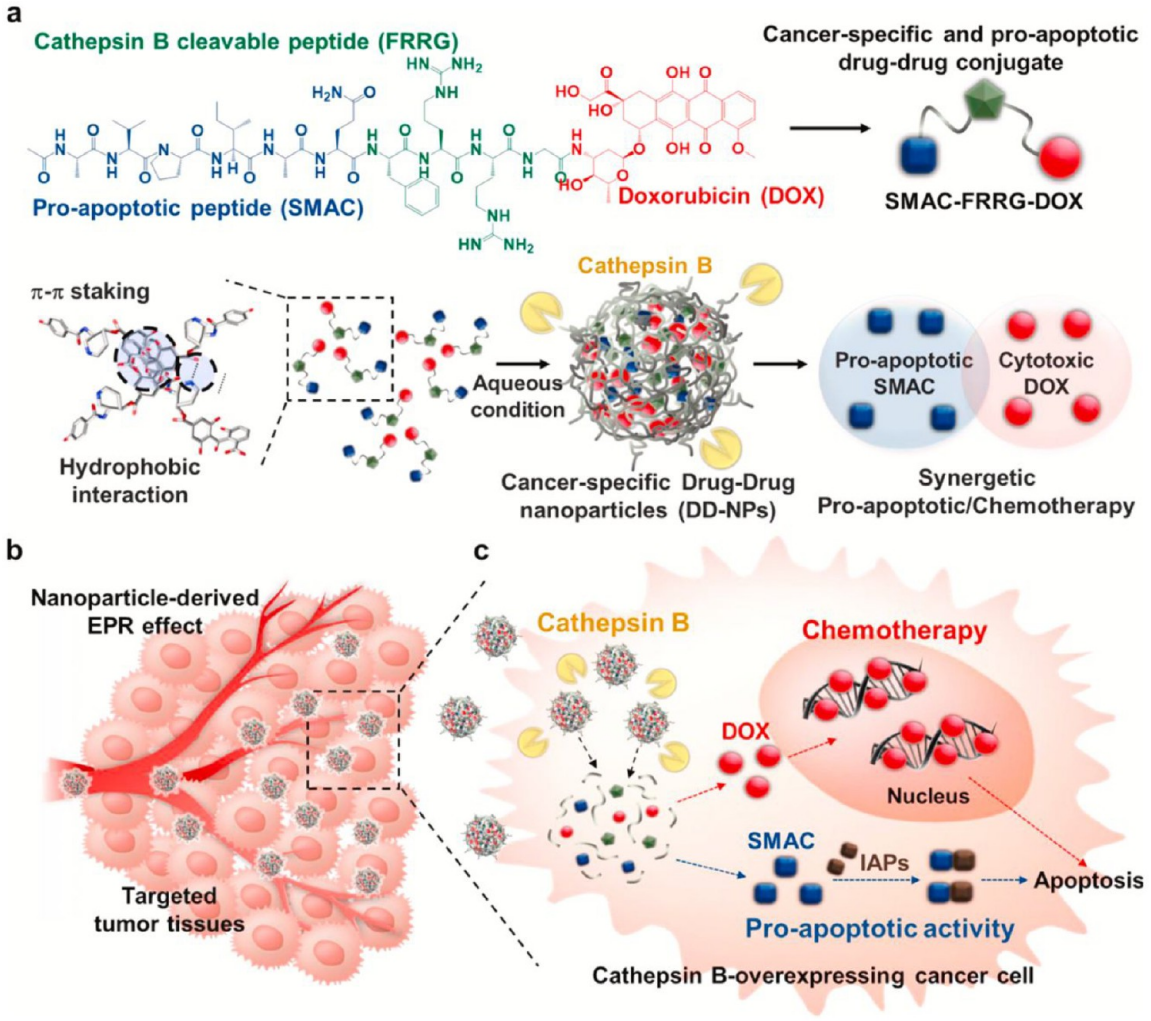

Figure 7. Synergetic pro-apoptotic/chemotherapy by cancer-specific and pro-apoptotic DD-NPs. (a) Chemical structure of cancer-specific and pro-apoptotic D-D conjugate that can form stable NPs. (b) The DD-NPs highly accumulate in the targeted tumor tissues via the EPR effect. (c) The conjugated NPs are specifically cleaved, resulting in drug-resistant cancer cells. Copyright 2020 Elsevier Ltd.[74]

pro-apoptotic SMAC and cytotoxic DOX in cancer cells that are overexpressing cathepsin B, and then pro-apoptotic/chemotherapy is influenced via the synergic effects of the chemotherapy and the inhibitor of apoptosis proteins (IAPs) inhibition. Contrastingly, due to the reduced cytostatic cathepsin-B expression in normal cells, the novel strategy depicted reduced toxicity in normal cells. Therefore, the novel nanotherapy is effective for pro-apoptotic and chemotherapy, with the potential to tame drug resistivity in cancerous cells.[74] Another investigation demonstrated an efficient utilization of D-penetratin or SynB1 in conjugation with DOX via SPPS. The formed conjugate showed a 20-fold enhancement in the uptake to the blood-brain barrier.[75]

Another study explored cathepsin-B cleavable peptide and aPDL1 by conjugating them to DOX through intermolecular interactions. The novel prodrug entered the tumor cells via a receptor (programmed cell death-ligand 1; PDL-1) mediated endocytosis. After entry, DOX was released, which induced immunogenic cell death (ICD). Synchronously, pathways responsible for immune suppression were disrupted, resulting in the infiltration of T lymphocytes. The prodrug particles accumulated in the cancerous cells and resulted in innumerate T cell recruitment.[76]

A recent investigation demonstrated the fabrication of DOX that was stacked with mesoporous Si NPs. Furthermore, the formed nanocarrier was conjugated with an aptamer (AS1411) as well as the Arg-Gly-Asp-Lys (RGDK)-octa-arginine (RGDK receptor) peptide. The formed conjugate was elucidated for efficient delivery of cytostatic drugs (Figure 8). The results elucidated breast cancer tumor inhibition upon administration of a single dose via *in vivo* analysis.[77]

A research group explored DOX-loaded NPs for synthesizing mussel-derived peptides via SPPS. The complex has the ability for self-assembly to form the Fe(III)-dopamine (DA) complex. The complex demonstrates chemodynamic therapy (CDT) and photothermal therapy (PTT). As the pH changes, the DOX is released in the cell (in an acidic environment), inducing apoptosis and increasing ROS through Fenton reactions. Therefore, it has potential for cancer therapy with diminished side effects (Figure 9).[78]

Ren et al. have fabricated porphyrin (PpIX) and Phe-Phe-Tyr-Ser-Val (FFYSV) peptides via SPPS, possessing the ability to self-assemble supramolecular nanodrugs (Figure 10a).[83] The PpIX-FFYSV nanodrug gets accumulated in cancerous tissue. Upon irradiation, PpIX converted light to heat for imaging tumors (Figure 10b). Furthermore, peptides acted as

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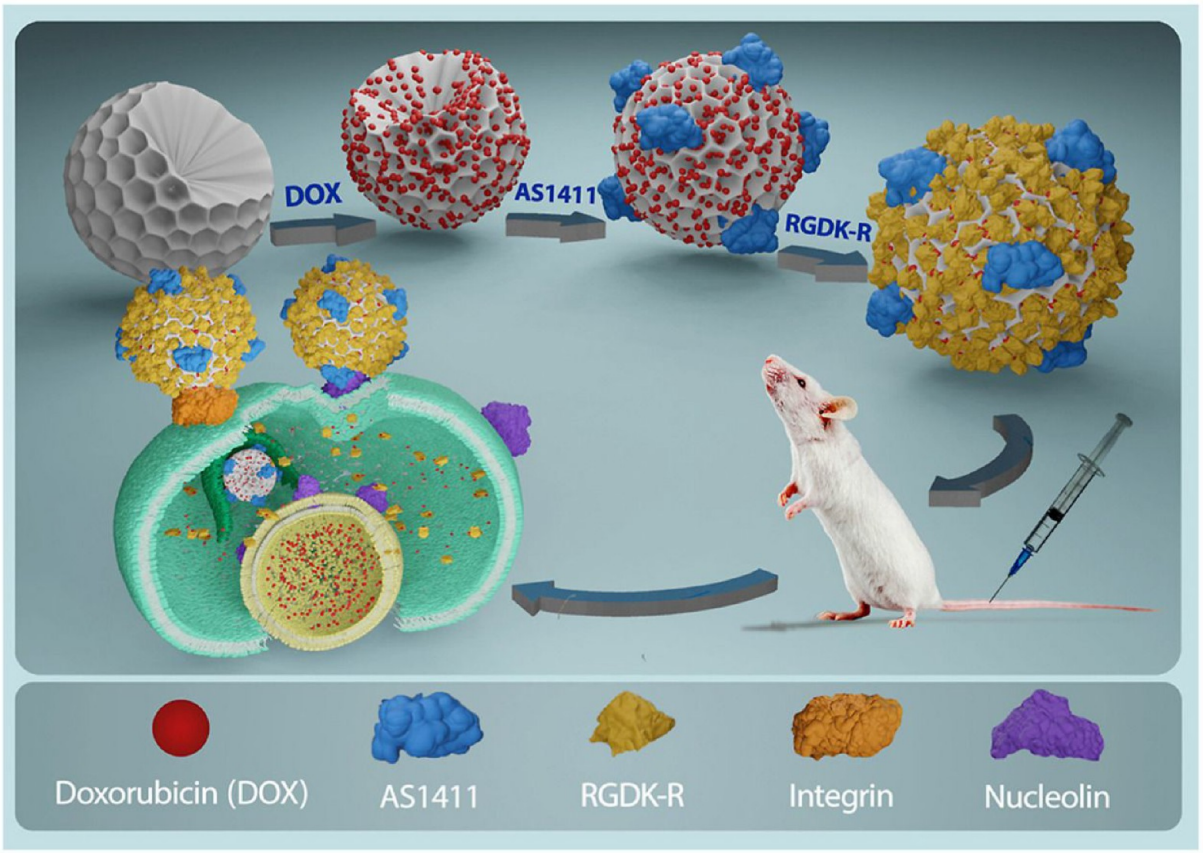

Figure 8. Structure of RGDK receptor-Apt-DOX-MSN.COOH (MDAP) and its function.[77] Copyright 2022 Elsevier B.V.

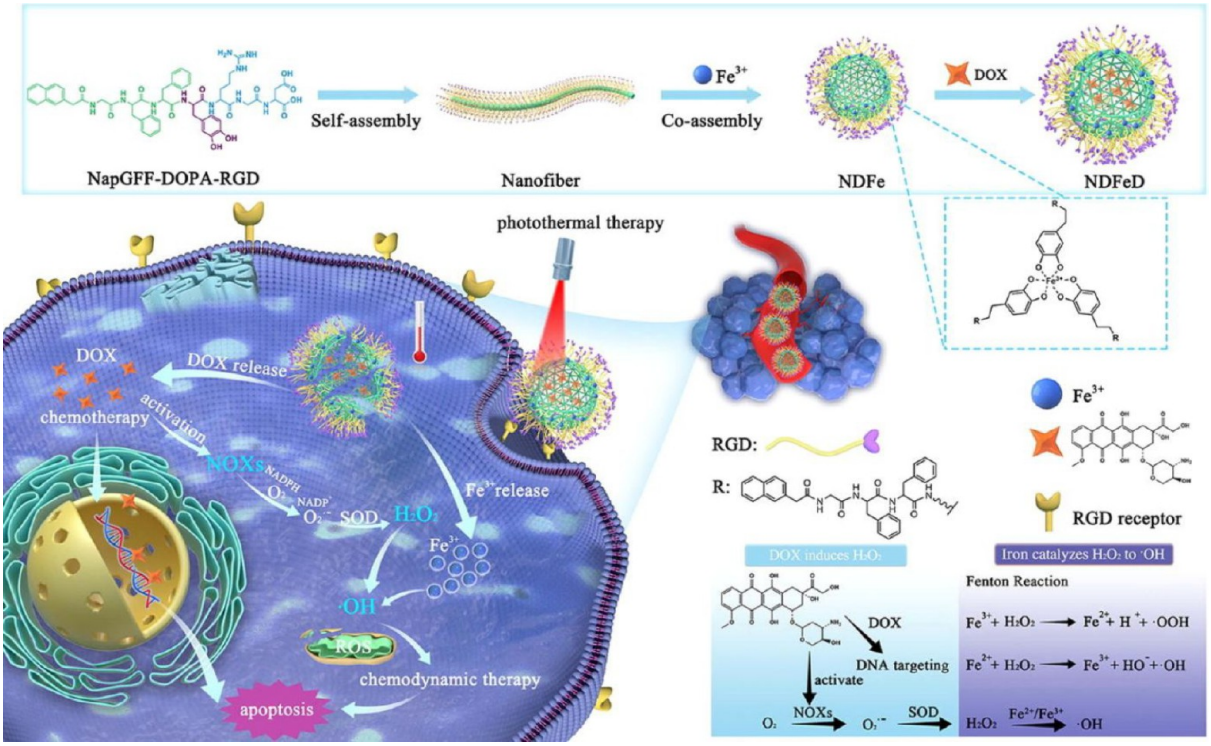

Figure 9. Schematic illustration of the self-assembly process of the mussel-derived peptide (NapGFF-DOPA-RGD), Fe^{3+}, and DOX for the generation of multifunctional NMs with potential for synergistic PTT-CDT-chemotherapy.[78] Copyright 2022 Elsevier B.V.

histone deacetylase inhibitors, which led to apoptosis and increased acetylation of histone. Hence, the designed supra-

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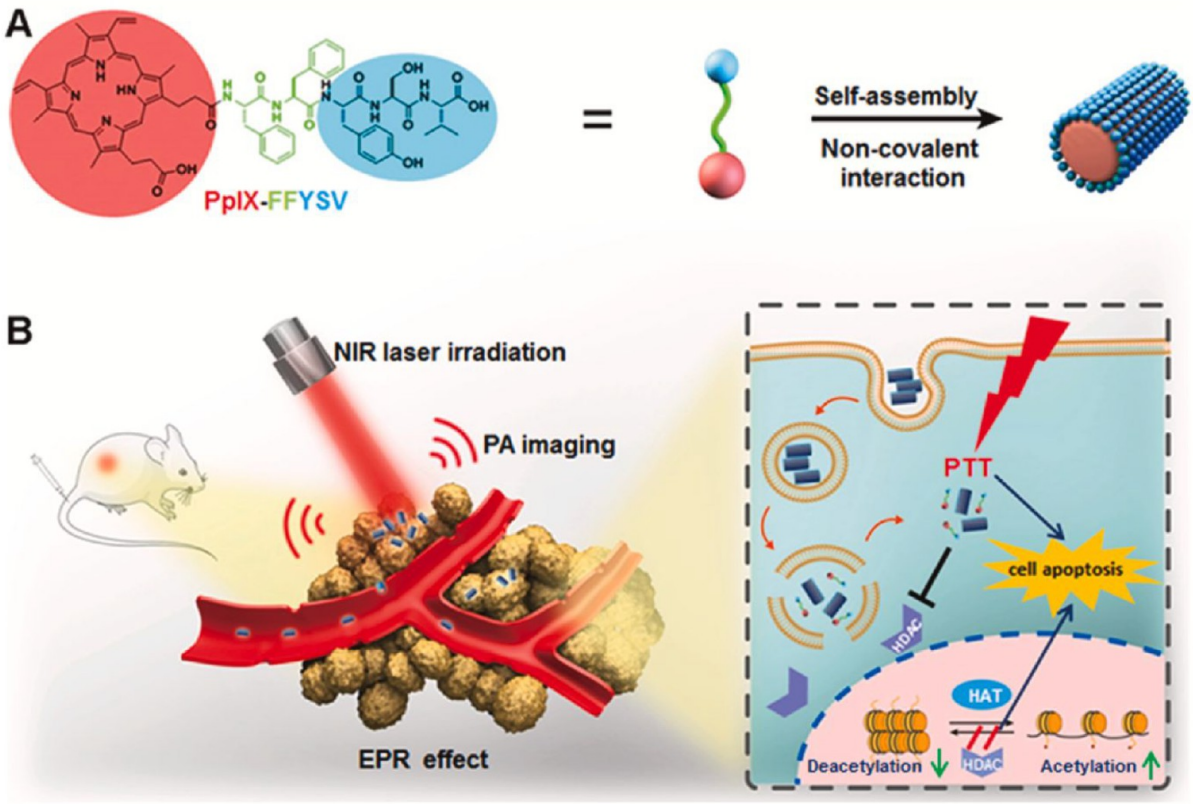

Figure 10. (a) Molecular structure of PpIX-FFYSV and illustration of the monomeric self-assembly into PpIX NAs. (b) Schematic illustration of the self-assembled PpIX NAs for *in vivo* tumor photoacoustic imaging and chemo-photothermal combination therapy.[83] Copyright 2021 Elsevier B.V.

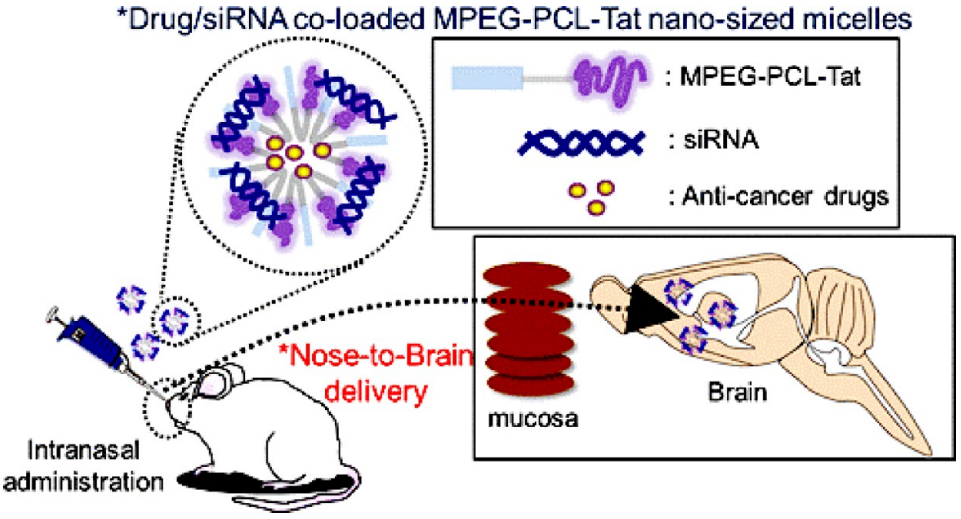

Figure 11. Schematic illustration of intranasal drug/siRNA co-delivery to the brain with cell-penetrating peptide-modified nanomicelles. Reproduced from ref 70. Copyright 2014 American Chemical Society.

molecular nanodrugs with self-assembly ability could be a potential platform for chemo-photothermal nanotherapy and photoacoustic (PA) imaging of cancerous cells.[83]

Kanazawa et al. have fabricated a nanomicelle-based intranasal drug using an altered poly(ethylene glycol)-*co*-poly(ε-caprolactone)-Tat block copolymer (MPEG-PCL-TAT) via a ring-opening polymerization approach. These nanomicelles were demonstrated to be stable with a particle size range between 60 and 200 nm and were found to exhibit potential application as an intranasal therapeutic for delivering the drug to the brain and CNS (Figure 11).[70]

Farzad et al. have fabricated the P435 HER2/neu-derived (IRGRILHDGAYSLTLQGLGIHGGGC) peptide that was linked to maleimide-PEG2000-DSPE (1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine) via an adjuvant: 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) and monophosphoryl lipase A and covalent linkage between the conjugate and thiol group. The formed conjugate of the nanoliposomal

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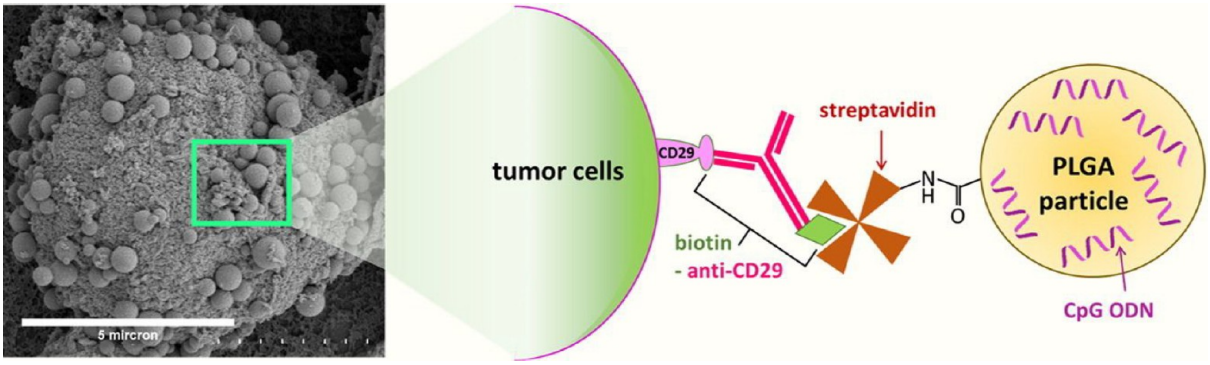

Figure 12. Schematic representation of cell-surface engineered particle with potential as a therapeutic cancer vaccine.[81] Copyright 2017 Elsevier B.V.

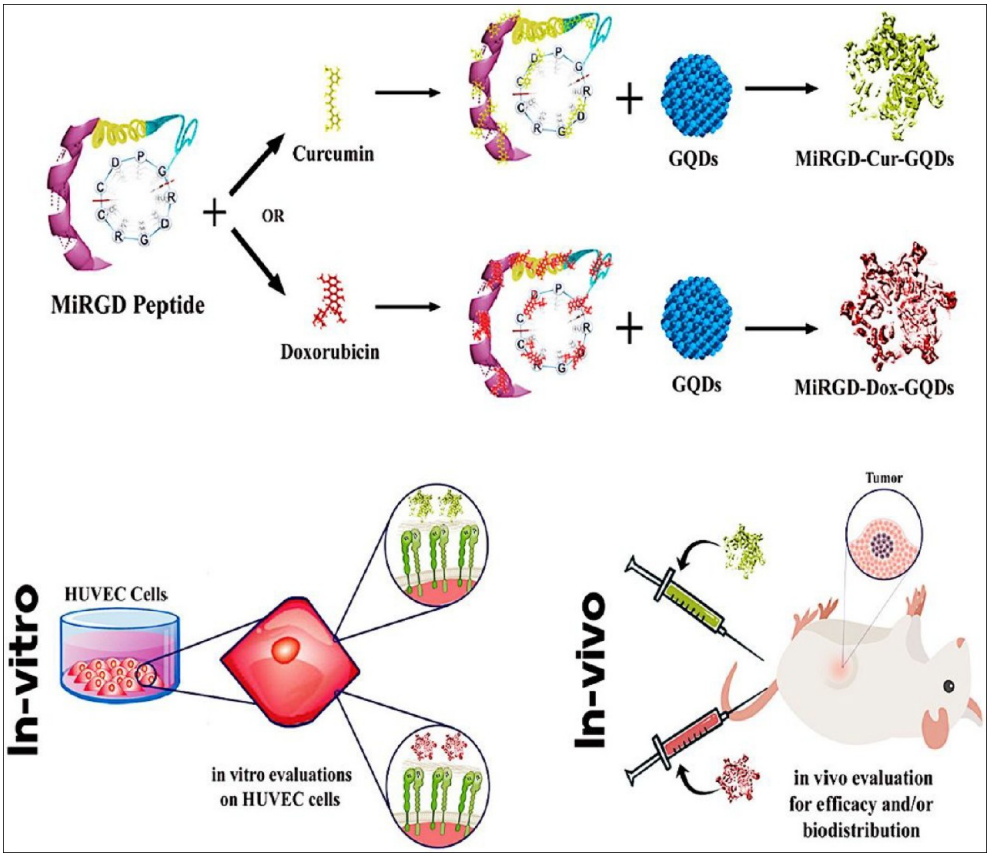

Figure 13. Schematic illustration of peptide-functionalized with graphene QDs and drug molecules, i.e., DOX and Curcumin along with their dual-application in targeted cancer therapy and tracking.[85] Copyright 2022 Elsevier Inc.

vaccine demonstrated potential anticancer efficacy and would enhance the survival time in the TUBO mice model.[79] A recent investigation conducted by Ahmed et al. has synthesized a Toll-like receptor 9 (TLR9) and CpG oligonucleotide (CpG ON) that were lyophilized and linked to streptavidin. These streptavidin-bound peptides were then conjugated with poly lactic-*co*-glycolic acid (PLGA) (Figure 12). Furthermore, the *in vitro* and *in vivo* studies were conducted on murine melanoma cell lines (B16.F10) and C57BL/6 and BALB/c mice, respectively. Their results demonstrated efficient application as a cancer vaccine in prostate cancer cell models.[81,93]

Yakati et al. demonstrated PLGA NP conjugation with tumor targeting peptides: CPKSNNGVC (CPK) and paclitaxel (PTX). Herein, the encapsulation took place using the emulsion solvent evaporation method, and the association between CPK peptide and PLGA NP was achieved via maleimide−thiol chemistry. The formed prodrug exhibited a high affinity for the monocarboxylate transporter 1 (MCT1) receptor, which mediated endocytosis selectively in CRC cells due to overexpression of MCT1 receptors. To further confirm the therapeutic potential of the nanopeptide drug, an assessment of angiogenesis was carried out in chick embryos. They observed a remarkable reduction in angiogenesis, i.e., new blood vessel formation. Therefore, it exhibits immense potential as antineoplastic nanodrugs specific to CRC.[84] A recent study conducted by Ghafary et al., adopted a hydrothermal and chemical synthesis route for the fabrication of MiRGD peptide (a chimeric peptide with Cys-Arg-Gly-Asp-

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Lys-Gly-Pro-Asp-Cys; CRGDKGPDC motif) and functionalized it with graphene QDs. These are then conjugated with cytostatic drugs such as DOX, cisplatin, curcumin, etc. as carrier moieties (Figure 13). However, for their study, they selected DOX and curcumin as hydrophobic and hydrophilic cytostatic drugs, respectively. On the basis of the qualitative and quantitative analysis, the formed peptide-f-NP drug exhibited enhanced and targeted drug delivery as well as tracking of tumor cells via *in vivo* studies conducted in mice.[85]

5. CHALLENGES AND OUTLOOK

The expanding literature demonstrates intricate nanoassemblies and biomolecule (nanobio) interactions, heretofore, the interceding for therapeutic and clinical applications are still in the exploratory stage.[94] For the development of peptide-f-NP drug conjugates with efficient potential for clinical manifestation, several important criteria need to be taken into consideration such as composition, target affinity, dynamic properties, stability, distribution, permeability, retention, cytotoxicity, and biocompatibility.[24] These criteria are arduous for evaluating nanobio interactions. Evaluation of the NPs for basic biological processes, including absorption, distribution, metabolism, excretion, and toxicity (ADMET) play crucial insights into the development of novel nanopeptide drugs.[94] Henceforth, effective strategies are needed for the synthesis of peptide molecules with therapeutic ability.

The peptides are sensitive to elimination from undesired *in vivo* interactions due to their short *in vivo* half-life and limited stability. A method to enhance its stability has been through the formation of self-assembled peptide-f-NPs. Ionic, H-bonds, and $\pi-\pi$ interactions are the major interactions in these nanostructures, with $\pi-\pi$ interactions playing a key role in the production of peptide-f-NPs. Unfortunately, progress is slow in this sector, resulting in a significant lack of understanding of these structures and their interconnections.[26]

Another important obstacle is the circulation time of peptides which is a major drawback for peptide synthesis. This is caused by the proteolytic cleavage of these therapeutic peptides. Therefore, studies based on enzymatic stability, profiling of peptide degradation, and pharmacokinetic and *in vivo* studies are crucial for further innovation and clinical intervention. To improve the circulation time of peptides for therapeutic intervention, certain approaches such as modification of peptides can be considered effective. For example, N- and C-terminal modifications like acetylation and amidation, respectively, could be adopted. Another aspect could be peptide cyclization which would reduce proteolytic degradation and thereby increase circulation time.[24]

Another facet is the accurate dosage of the formulated peptide-f-NP drugs. Due to the nanobio interactions in peptide-f-NP drugs, the nature of the drug molecule is heterogeneous. Thereby, the evaluation of precise dosage is crucial to elucidate various characteristics of a proposed peptide-f-NP drug. In addition to this, several parameters such as surface area and molar concentration also influence the accurate assessment of such drugs. Unfortunately, the obstacles for quantification are progressively increasing as the surface area has gone from single particle to complex nanoassemblies.[94−97]

Furthermore, the intervention of NPs for peptide-based drugs would also enhance the stability and biocompatibility and would be an excellent source for delivering mechanisms.[1] Henceforth, the amalgamation of NPs into peptide-derived therapeutics for anticancer activity would benefit clinical translation in the field of cancer treatment.

6. CONCLUSION

With advances in the understanding of cancer biology and clinical intervention, several strategies for peptide-based nanotherapeutics have tremendously increased and emerged as a potential role in oncology research. However, only a handful of nanopeptide drugs have made it to clinical trials. Our current research lacks a complete understanding of peptide-NP interactions and the various parameters (peptide length, amino acid sequence, NP size, surface chemistry, etc.) involved in the functionalization of peptide-NPs and therefore needs many detailed studies and vigorous analysis. This issue is thought to be due to the certainty that each NP-peptide interaction is exclusive, necessitating detailed individual evaluations for each of these superstructures. Furthermore, examination of only one of these interactions does not allow for generalization to other types of peptide-NP superstructures. Additionally, studies have to be extended toward an analysis of the relationship between the primary amino acid structures of the peptide and the binding NPs, which is an essentially difficult task that remains a huge challenge to current peptide therapeutics. Therefore, this continuously expanding universe of peptide-f-NPs needs more detailed studies and vigorous analysis. The lack of reliable and advanced spectroscopic and computational techniques also hinders our ability to fundamentally understand these complex nanostructures. Consequently, only a limited number of peptide-f-NP therapeutics have been FDA approved for cancer therapy. However, due to the urgent need for a medication to combat the steadily rising number of cancer mortality worldwide, the integration of nanostructured platforms for peptide-based drugs is poised to grab researchers' attention for the development of new anticancer therapeutics. Thus, the new innovative combinatorial intervention of NPs and peptide drugs would overcome the demerits of conventional cancer therapy strategies and, therefore, fast-track the clinical translation of peptide-f-NP nanotherapy for anticancer treatment.

AUTHOR INFORMATION

Corresponding Authors

Akanksha Gupta − *Department of Chemistry, Sri Venkateswara College, University of Delhi, Delhi 110007, India*; orcid.org/0000-0003-1398-2085; Email: akankshashachem05@gmail.com

Vijay Kumar Goel − *School of Physical Science, Jawaharlal Nehru University, Delhi 110067, India*; Email: vijaykgoel@jnu.ac.in

Vinod Kumar − *Special Centre for Nano Sciences, Jawaharlal Nehru University, Delhi 110067, India*; orcid.org/0000-0002-8362-8009; Email: kumarv@mail.jnu.ac.in

Authors

Ritika Sharma − *Department of Biochemistry, University of Delhi, Delhi 110021, India*

Shikha Jyoti Borah − *Special Centre for Nano Sciences, Jawaharlal Nehru University, Delhi 110067, India*

Bhawna − *Department of Chemistry, University of Delhi, Delhi 110007, India*

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Sanjeev Kumar − *Department of Chemistry and Department of Chemistry, Kirori Mal College, University of Delhi, Delhi 110007, India*
Poonam Singh − *Department of Applied Chemistry, Delhi Technological University, Delhi 110042, India*
Ravinder Kumar − *Department of Chemistry, Gurukula Kangri (Deemed to be University), Haridwar 249404 Uttarakhand, India*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c03974

Notes

The authors declare no competing financial interest.

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