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Peptide-Driven Nanotheranostics: Simultaneous Targeting, Imaging, and Therapy of Malignancies

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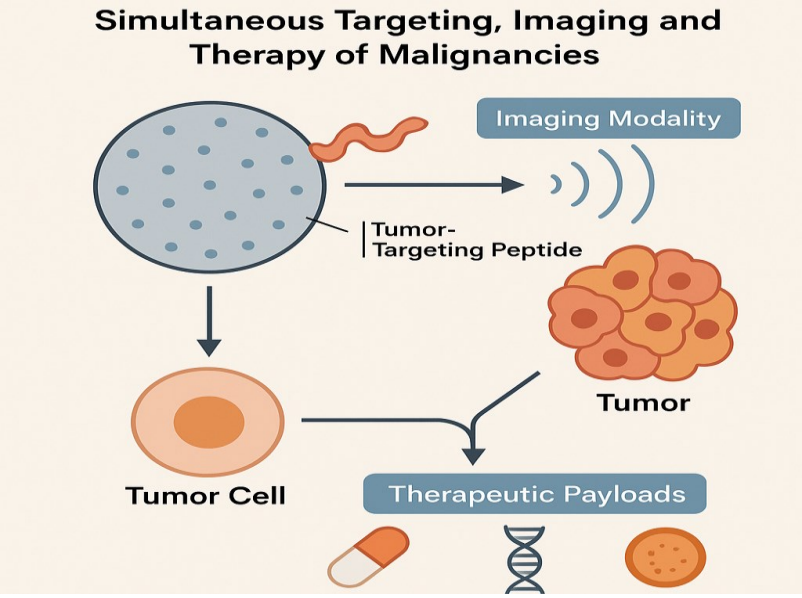
ABSTRACT

Peptide-guided nanotheranostics is a revolutionary strategy in oncology that combines targeted therapy, imaging, and diagnostics on a single platform to overcome the drawbacks of traditional cancer therapies. Even with the advancement of surgery, chemotherapy, and radiotherapy, the issues of low drug solubility, systemic toxicity, and multidrug resistance remain. Peptide-functionalized nanoparticles (peptide-f-NPs) are a potential solution that takes advantage of the biocompatibility, biodegradability, and tumor-targeting specificity of peptides to improve drug delivery and imaging accuracy. These nanocarriers (liposomes, polymeric nanoparticles, dendrimers, and inorganic NPs) are designed with tumor-penetrating peptides (e.g., RGD, NGR) or receptor-targeting peptides (e.g., DOTATATE, bombesin analogs) to impart active targeting, bypassing the heterogeneity of tumor microenvironments. Sophisticated imaging modalities like fluorescence, MRI, PET/SPECT, and photoacoustic imaging are combined with therapeutic payloads (chemotherapeutics, siRNA, photothermal agents, and radionuclides) for real-time monitoring and precision therapy. But despite challenges of peptide instability, RES-mediated quick clearance, immune response, and production complexity, innovation in peptide engineering (cyclization, PEGylation) and nanocarrier design for multifunctionality seeks to advance stability, diminish toxicity, and increase clinical translatability. This review captures the potential of peptide-based nanotheranostics to revolutionize cancer therapy while meeting present limitations and future prospects for clinical translation.

INTRODUCTION

Cancer continues to be one of the world's toughest health challenges, with approximately 2.5 million existing cases in India and around 800,000 new cases and 550,000 deaths occurring each year (1,2). In the United States, cancer causes almost a quarter of all deaths, with 1.5 million new cases and 569,490 deaths predicted for 2010 (3). Even though there have been major developments in traditional therapies—like surgery, chemotherapy, and radiation therapy—their methods have major drawbacks like poor solubility of drugs, non-tumor specificity, and multidrug resistance (MDR) via efflux pumps like P-glycoprotein (P-gp) (4). Recent advancements in nanotechnology have transformed cancer therapy by transcending the disadvantages of traditional therapy. Nanoparticles (NPs) improve drug delivery by enhancing solubility, targeted biodistribution, and minimizing systemic toxicity (5, 6). Of these, peptide-functionalized nanoparticles (peptide-f-NPs) have been proposed as a valuable strategy because of their biocompatibility, biodegradability, and capability to enable targeted drug delivery (7). Peptides, which are short chains of amino acids, perform important functions in biological systems and are engineered to specifically interact with cancer cells (8). Peptides are perfectly suited for therapeutic use due to their tunable properties like low toxicity and excellent penetration efficiency (9). Supramolecular chemistry has also advanced peptide therapeutics by facilitating the formation of nanostructures by way of ionic interactions, hydrogen bonding,

and π–π stacking (10,11,). Such peptide-f-NPs have shown promise for drug delivery, biosensing, gene therapy, and even COVID-19 vaccine (12, 13). Further, also FDA-approved are therapeutic peptides some of which demonstrate the potential of cancer cells with minimal cytotoxicity on regular tissues. Peptide-NP conjugates represent the opportunities explored by scientists aiming to generate innovative therapeutics designed for the focused targeting of the tumor, selective release of medications, as well as simultaneous visualization (14, 15).



Peptides as Targeting Moieties

Peptides have also become extremely useful targeting ligands in nanotheranostics because of their high specificity, biocompatibility, and facile modification. They can be generally categorized into three broad categories based on their mechanism of action:

1. cell-penetrating peptides (CPPs),
2. tumor-homing peptides
3. Receptor-specific peptides.

1. Cell-Penetrating Peptides (CPPs) in Nanotheranostics

Cell-penetrating peptides (CPPs) are amphipathic, hydrophobic, or cationic, short peptides that are capable of penetrating biological membranes, rendering them precious for intracellular delivery of nanotheranostic agents. Established CPPs, including TAT (GRKKRRQRRRPQ) from HIV-1 transactivator of transcription protein and penetratin (RQIKIWFQNRRMKWKK) from Drosophila Antennapedia homeodomain, allow cellular uptake via energy-dependent (endocytosis) or energy-independent (direct translocation) processes (16). In contrast to traditional targeting ligands that take advantage of receptor overexpression, CPPs utilize electrostatic attraction with negatively charged cell membranes and cause reversible membrane perturbations to facilitate cargo internalization without the need for specific cellular markers (17). This feature makes them ideal for delivering a broad variety of payloads, such as chemotherapeutic drugs, nucleic acids (siRNA, miRNA), and imaging agents (e.g., fluorescent dyes, radionuclides). While efficient, a key weakness of CPPs is that they are not tumor-specific, and this allows for unwanted uptake into normal cells and systemic toxicity. To address this issue, scientists have introduced double-targeting approaches in which CPPs are combined with tumor-targeting peptides (e.g., RGD, NGR) or they are covered with pH-sensitive or protease-degradable linkers that only become active in the TME (18). For example, a TAT-RGD hybrid peptide has been employed to increase nanoparticle accumulation in αvβ3 integrin-expressing tumors with decreased off-target effects (19). Likewise, charge-reversal CPPs that only become cationic under acidic TME conditions (pH ~6.5) have been designed to enhance selectivity (20). Advances in recent times in high-throughput screening and computational modeling have further rationalized CPP sequences towards greater stability, lower immunogenicity, and better endosomal escape (21). Further, CPP-tagged nanocarriers, like liposomes, polymeric nanoparticles, and quantum dots, have shown preclinical efficacy in targeting tumors by combined imaging and drug delivery (22). Issues still lie in large-scale manufacturing, achieving in vivo stability, and immune evasion, for which more work will be required prior to mass clinical application.

2. Tumor-Homing Peptides for Targeted Nanotheranostics

Tumor-homing peptides are a type of targeting ligand that specifically accumulate in tumor tissues via specific binding to angiogenic markers or tumor-associated ECM components, allowing for site-specific delivery of nanotheranostic agents. One of the best-studied examples is the RGD (Arg-Gly-Asp) peptide, which has high affinity for αvβ3 and αvβ5 integrins that are strongly overexpressed on the surface of tumor vasculature as well as on numerous cancer cell surfaces (23). This differential binding property has been used comprehensively to amplify the tumor deposition of diverse nanocarriers such as liposomes and polymeric nanoparticles for both diagnostic imaging and drug delivery.Another clinically useful tumor-targeting peptide is the NGR (Asn-Gly-Arg) motif, which targets CD13/aminopeptidase N - a cell surface peptidase that is highly expressed on tumor neovasculature (24). The NGR sequence has been shown to be of particular value for delivering therapeutic payloads to solid tumors with minimal systemic exposure. Of interest, NGR-modified nanoconstructs have been observed to exhibit increased permeability and retention effects within tumor tissue owing to their ability to target tumor endothelial markers. The LyP-1 (CGNKRTRGC) peptide provides a unique targeting mechanism by interacting with p32/gC1qR, a mitochondrial protein that gets translocated to the cell surface in most tumor types and tumor-associated lymphatic vessels (25). This feature allows LyP-1-conjugated nanoparticles to target not only primary tumors but also metastatic lesions, making it especially useful for holistic cancer management. These tumor-targeting peptides enable active targeting of nanotheranostics by receptor-mediated endocytosis, substantially enhancing tumor-to-background contrast ratios in molecular imaging and increasing therapeutic index over passive targeting strategies (26). Due to their compact size (<3 kDa), synthetic ease, and high binding selectivity, they are suitable targeting moieties for designing multifunctional nanoplatforms that can enable concurrent tumor detection and treatment.

3. Receptor-Specific Peptides for Precision Nanotheranostics:

Receptor-specific peptides are a highly efficacious category of targeting ligands designed to specifically bind overexpressed cancer cell-bound receptors with high selectivity, allowing for highly selective tumor targeting of nanotheranostic probes. One such excellent example is somatostatin analogs like octreotide and DOTATATE, which have nanomolar binding affinity for the somatostatin receptor subtype 2 (SSTR2) which is highly expressed in neuroendocrine tumors (27). This particular interaction has been translated into both diagnostic imaging (Ga-68 labeled DOTATATE PET) and targeted radiotherapy (peptide receptor radionuclide therapy, PRRT) clinically, showing the double diagnostic-therapeutic capability of receptor-targeting peptides. Another clinically relevant example is the family of bombesin analogs, which act against gastrin-releasing peptide receptors (GRPR) overexpressed in prostate cancer (in >85% of cases) and some subtypes of breast cancer (28). These peptides have been of specific interest when linked to nanoparticles for multimodal imaging and targeted delivery of drugs, with recent studies showing their capability to enhance sensitivity of tumor detection while minimizing off-target effects in preclinical models.

The therapeutic success of these targeting approaches is evidenced by Lutathera® (177Lu-DOTATATE), an FDA-approved peptide-receptor radionuclide therapy consisting of the SSTR2-targeting peptide DOTATATE linked with the beta-emitting radionuclide Lutetium-177 (29). This game-changing therapy has provided dramatic enhancements in progression-free survival for midgut neuroendocrine tumors, proving the receptor-specific peptide strategy correct.

These receptor-targeting peptides have several benefits in nanotheranostic applications:

- High binding specificity (nanomolar affinity for target receptors)
- Early tumor penetration with molecular size-related high permeability
- Easy conjugation chemistry to allow nanoparticle functionalization
- Demonstrated clinical translation capability, exemplified by the multiple FDA-approved peptide-based radiopharmaceuticals

Ongoing research extends this method to engage other cancer-restricted receptors, such as CCK2R in medullary thyroid carcinoma and PSMA in prostate carcinoma, and numerous peptide-nanoparticle conjugates are under clinical trials (30).

Mechanisms of action:

The therapeutic effectiveness of peptide-guided nanotheranostics mainly functions via two basic mechanisms: receptor-mediated binding and differential targeting strategies (passive vs. active). These mechanisms act synergistically to increase tumor specificity with reduced systemic toxicity.

1. **Receptor-Mediated Binding Mechanisms**

Peptide ligands facilitate specific tumor targeting by high-affinity interactions with cell surface receptors that are overexpressed. Two major receptor families are specifically targeted:

- **Integrins (**e.g., αvβ3, αvβ5): RGD-containing peptides target these receptors, which are overexpressed in tumor vasculature and most cancer cell types. The interaction induces receptor-mediated endocytosis, enabling intracellular delivery of nanotheranostic agents (31). Integrin activation also regulates tumor cell survival and angiogenesis pathways, potentially improving therapeutic responses.
- **G Protein-Coupled Receptors (GPCRs):** Biotargeted by peptides such as somatostatin analogs (SSTR2) and bombesin analogs (GRPR), these receptors are commonly overexpressed in most malignancies. GPCR binding triggers internalization mechanisms that effectively deliver peptide-nanoconjugates into cells while at the same time triggering downstream signaling cascades that can be targeted for therapy (32). The large copy number of GPCRs on cancer cells (usually >10,000 receptors/cell) facilitates strong tumor accumulation.

2. **Targeting Strategy Paradigms**

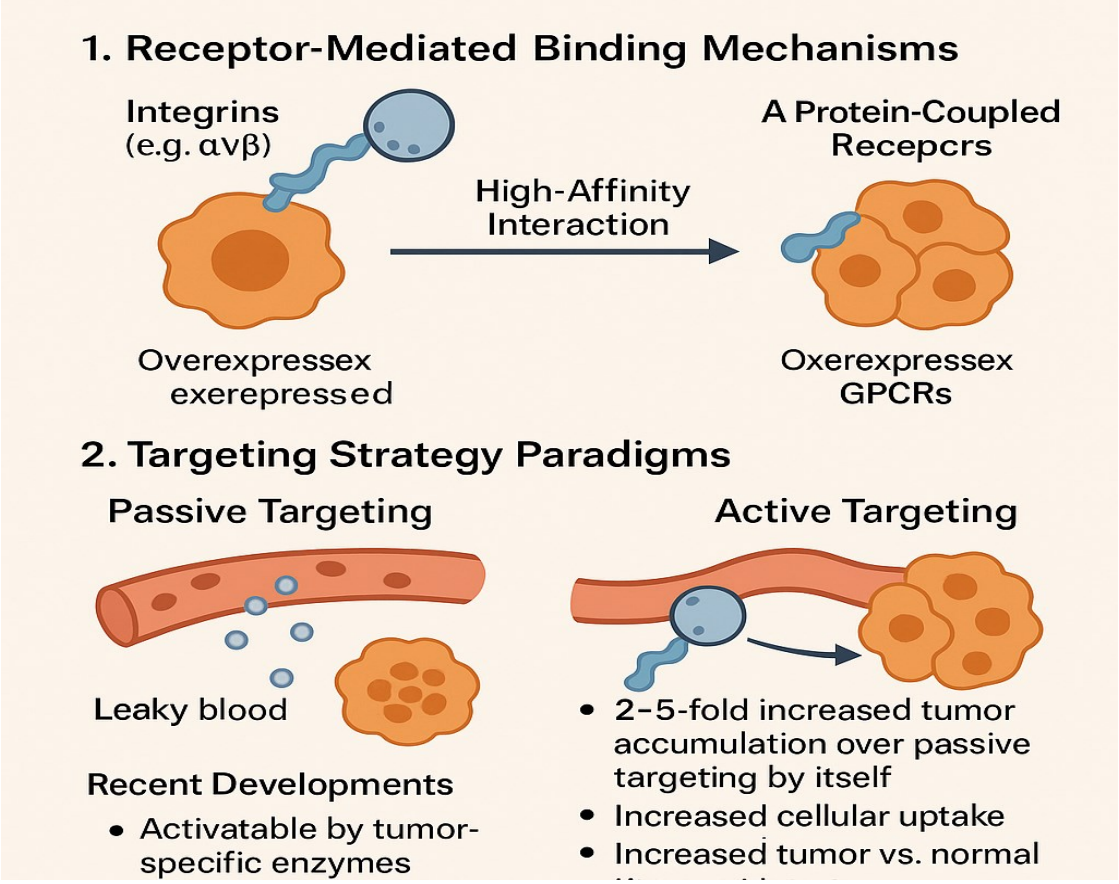
- **Passive Targeting:** Depends on the Enhanced Permeability and Retention (EPR) phenomenon, wherein nanocarriers (generally 50-200 nm) extravasate across leaky tumor vasculature and converge as a consequence of impaired lymphatic drainage (33). Although capable for certain cancers, EPR exhibits marked variability between patients as well as different tumor types.
- **Active Targeting:** Marries passive accumulation and specific molecular interaction by surface-conjugated target peptides (34). This two-step approach generally evidences:
    - 2-5-fold increased tumor accumulation over passive targeting by itself
    - Increased cellular uptake by receptor-mediated endocytosis
    - Increased ratios of tumor vs. normal tissue in imaging processes
    - Decreased therapeutic doses for efficacy

The selection between these approaches is determined by tumor biology, with active targeting especially beneficial for:

- Low-EPR tumors (e.g., pancreatic cancer)
- Metastatic lesions with intact vasculature
- Tumors with well-defined receptor overexpression profiles
- Applications with precise subcellular localization

Recent developments use "smart" targeting strategies in which peptides are:

- Activatable by tumor-specific enzymes (e.g., MMP-cleavable linkers)
- pH-sensitive for tumor microenvironment activation
- Dual-targeting to treat tumor heterogeneity(35)



Peptide Engineering: Modifications for Stability, Affinity and Multivalency

Peptide engineering is key to the improvement of peptide-based targeting systems for nanotheranostics by improving their stability, binding affinity, and multivalency. Native peptides, although very specific, are usually plagued by proteolytic enzyme-mediated rapid degradation and unfavorable in vivo pharmacokinetics. To address these drawbacks, various chemical and structural modifications are utilized.

Improvement of stability is essential to preserve peptide integrity in the biological environment. Typical approaches include D-amino acid incorporation, N-methylation, cyclization, and PEGylation. Cyclization—head-to-tail or side-chain—bends peptide structure, enhancing resistance to enzymatic hydrolysis and longevity of receptor binding. For instance, cyclic RGD peptides (cRGD) have displayed significantly enhanced stability and tumor-targeting activity than their linear versions (36). Likewise, PEGylation (polyethylene glycol chain attachment) not only stabilizes peptides against degradation but also extends the time of circulation by diminishing renal clearance and immune detection (37).

In order to enhance binding affinity, engineering attempts aim at optimizing the peptide sequence for more tight and specific interaction with the target receptor. High-affinity ligands can be designed by methods such as phage display, computational modeling, or rational design. Engineered peptides show improved receptor occupancy and retention in tumor tissues, which is of paramount importance for successful imaging and therapeutic use (38).

Multivalency is another very effective strategy, where several units of peptide are displayed on a single nanoparticle or scaffold so that simultaneous binding to several receptors or several sites on the same receptor can occur. This not only enhances avidity through cooperative binding but also enhances selectivity and signal amplification. For example, multivalent display of RGD peptides on nanoparticles has been found to greatly increase targeting efficiency and cellular uptake through integrin clustering and internalization (39).

Peptide-Functionalized Nanocarriers

Peptide-functionalized nanocarriers have transformed cancer theranostics with the integration of site-specific tumor targeting along with improved drug delivery. Engineered systems utilize the exclusive characteristics of various nanoplatforms to traverse biological obstacles and enhance treatment efficacy

1. **Liposomes**

Liposomes are phospholipid bilayer spherical vesicles that can entrap both hydrophilic and hydrophobic agents. Due to their biocompatibility and their capacity to fuse with cellular membranes, liposomes are excellent drug delivery vehicles. Targeting with modified peptides at the surface (e.g., RGD, NGR, or TAT) facilitates site-specific targeting to tumor tissues. Enhanced targeting to αvβ3 integrin-bearing tumor vasculature and intracellular uptake have been reported using RGD-modified liposomes, for instance (Torchilin, 2011). Furthermore, PEGylation of peptide-functionalized liposomes extends there in vivo circulation time and stability even further(40).

2. **Polymeric Nanoparticles**

Polymeric nanoparticles, particularly those derived from biocompatible polymers such as PLGA, PLA, or chitosan, provide controlled drug release, adjustable degradation rates, and simple surface functionalization. Peptide conjugation to polymeric nanoparticles enhances their tumor tissue accumulation by active targeting processes. For example, PLGA nanoparticles with T7 peptide surface modification have been found to cross the BBB and preferentially accumulate in glioma tissues through transferrin receptor-mediated endocytosis (41). Moreover, dual-targeting approaches based on two peptides (e.g., RGD and NGR) improve specificity in heterogeneous tumor microenvironments.

3. **Dendrimers**

Dendrimers are a special type of nanocarrier with highly branched, monodisperse architecture. Polyamidoamine (PAMAM) dendrimers, for example, offer multiple surface functional groups for multivalent peptide conjugation (42). Demonstrated that RGD-conjugated generation-5 PAMAM dendrimers had dramatically increased binding avidity to

integrin-rich tumors as a result of the cluster effect. Fourth-generation dendrimers have shown specific potential for penetrating biological barriers, and recent research has looked into their application for brain tumor targeting when coupled with blood-brain barrier-penetrating peptides.

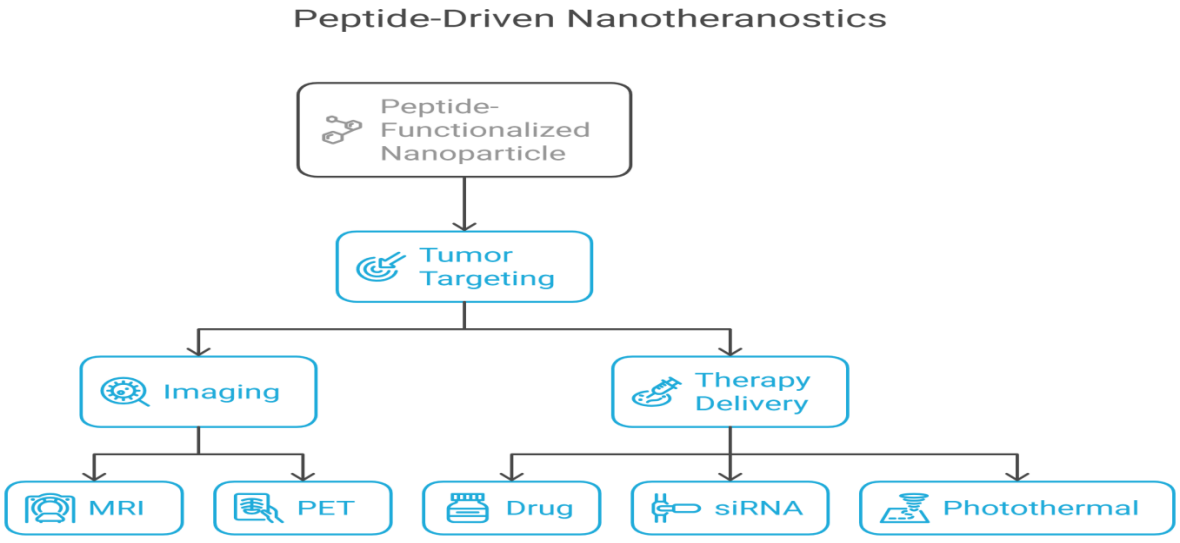
4. **Polymeric micelles**

Polymeric micelles based on amphiphilic block copolymers such as PEG-PLA have unique advantages for tumor penetration because of their nanoscale size (10-100 nm). Core-shell micelles can be modified with cell-penetrating peptides (e.g., TAT) or receptor-targeting peptides (e.g., GE11 for EGFR) to increase tumor accumulation. Kedar et al. (2010) showed that TAT-conjugated micelles delivered paclitaxel 8-fold more intracellularly in drug-resistant tumors than non-targeted systems. Recent studies aim at "smart" micelles that respond to tumor microenvironment stimuli by changing their morphology for improved drug release.

5. **Inorganic Nanoparticles**

Inorganic nanoparticles, including gold nanoparticles (AuNPs), silica nanoparticles (SiNPs), magnetic nanoparticles, and quantum dots, have found widespread applications owing to their specific physical properties—optical, thermal, or magnetic—that provide diagnostic and therapeutic capabilities.

- **Gold nanoparticles** with RGD or NGR peptides functionalized allow integrin-targeted photothermal therapy and CT imaging because of their high absorbance in the NIR range (43).
- **Mesoporous silica nanoparticles (MSNs)** provide high surface area and pore size tunability for high drug loading. Tumor-homing peptide functionalization provides tumor selectivity and controlled release.
- **Superparamagnetic iron oxide nanoparticles (SPIONs)** are utilized extensively as MRI contrast agents. Targeting peptides (e.g., chlorotoxin or cRGD) decoration provides improved tumor imaging with reduced off-target distribution.



Imaging Modalities Integrated with Therapy:

Combining imaging and therapy within an integrated nanotheranostic platform allows for accurate diagnosis, treatment planning, monitoring, and assessment of response to therapy in cancers. Peptides, when used as a vector for attaching imaging probes and nanocarriers, allow for target-specific imaging of tumors while concurrently delivering therapeutic agents

1. **Fluorescence Imaging**

Fluorescence imaging is a real-time, non-invasive optical imaging method commonly employed for intraoperative tumor detection and therapy guidance. Fluorescent dyes or quantum dots (QDs) are conjugated with peptides for specific tumor targeting. A notable example is integrin αvβ3-binding Cy5.5-labeled RGD peptides (Cy5.5-RGD). The latter have been applied to image overexpressing tumor vasculature and stroma during surgery or treatment. Quantum dots, like CdSe/ZnS nanoparticles, provide high brightness, photostability, and emission tunability, making them ideal for long-term imaging. When tumor-homing peptides like iRGD or NGR are conjugated to QDs, they can illuminate tumor margins and metastatic nodules, aiding in diagnosis and treatment planning(44).

2. **Magnetic Resonance Imaging (MRI):**

MRI offers high-resolution anatomical images with great soft-tissue contrast. Superparamagnetic iron oxide nanoparticles (SPIONs) are most commonly used as contrast agents that reduce T2 relaxation times, resulting in dark regions on MRI corresponding to nanoparticle accumulation.Peptide functionalization of SPIONs with cyclic RGD (cRGD) increases their targeting efficacy to integrin αvβ3-expressing tumors(45). have demonstrated that cRGD-SPIONs dramatically increased T2-weighted contrast in tumor tissues, allowing for accurate demarcation of tumor margins and evaluation of therapeutic response. SPIONs may also be loaded with drugs or conjugated with photothermal agents, combining therapeutic functions with imaging functions.

3. **PET and SPECT Imaging:**

Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) are extremely sensitive nuclear imaging methods employed to visualize molecular and physiological processes in tumors. Isotopically radiolabeled peptides like ^{68}Ga, ^{99}mTc, or ^{18}F are frequently utilized for targeted cancer biomarker imaging.

One commonly used agent is ^{68}Ga-DOTATATE, an analog of somatostatin binding to SSTR2 in neuroendocrine tumors. It provides better sensitivity and specificity for the imaging of primary and metastatic lesions, and permits theranostic matching with therapeutic isotopes such as ^{177}Lu for peptide receptor radionuclide therapy (PRRT). Likewise, ^{99}mTc-labeled RGD peptides have been employed to visualize angiogenic activity in tumors by binding to integrin receptors(46).

4. **Photoacoustic Imaging:**

Photoacoustic imaging (PAI) is an interdisciplinary method involving optical excitation and ultrasound detection to produce high-contrast, deep-tissue imaging. PAI is particularly well suited to tumor imaging owing to its sensitivity to vascular networks, hypoxia, and molecular targets.

Gold nanorods (AuNRs), whose NIR absorption and photothermal behavior make them effective photoacoustic contrast agents, can be conjugated with MMP-2-cleavable peptides. These activated AuNRs in the tumor environment where there is overexpression of matrix metalloproteinases have been shown by Jokerst et al. to result in tumor-targeted signal amplification, permitting imaging and therapy (e.g., photothermal ablation) with increased accuracy(47).

Therapeutic Payloads in Peptide-Based Nanotheranostics

Peptide-functionalized nanotheranostatic platforms not only facilitate accurate tumor targeting and imaging but also function as effective vehicles for the delivery of varied therapeutic payloads. These include chemotherapeutics, gene-silencing vectors (siRNA/miRNA), photothermal/photodynamic agents, and radionuclides, each playing a distinct role in treating cancer. The incorporation of these vectors in peptide-decorated nanocarriers improves therapeutic efficacy while reducing off-target toxicity via receptor-specific targeting.

1) Chemotherapeutics

Conventional Traditional chemotherapeutic agents usually experience non-specific distribution, causing systemic toxicity and decreased therapeutic index. Peptide-functionalized nanoparticles have the ability to deliver these agents in a targeted manner, enhancing accumulation at the target site and minimizing collateral tissue damage. As an example of this, the use of liposomes loaded with doxorubicin and RGD peptides conjugated to them has been demonstrated to target selectively αvβ3 integrin-expressing tumors. This leads to increased cellular uptake, increased rates of apoptosis in tumor cells, and better therapeutic response than with non-targeted formulations (48). In addition, paclitaxel and cisplatin were also entrapped in polymeric nanoparticles or micelles bearing tumor-homing peptides such as LyP-1 or NGR, where the circulation half-life was increased and tumor penetration improved (49).

2) siRNA/miRNA Delivery:

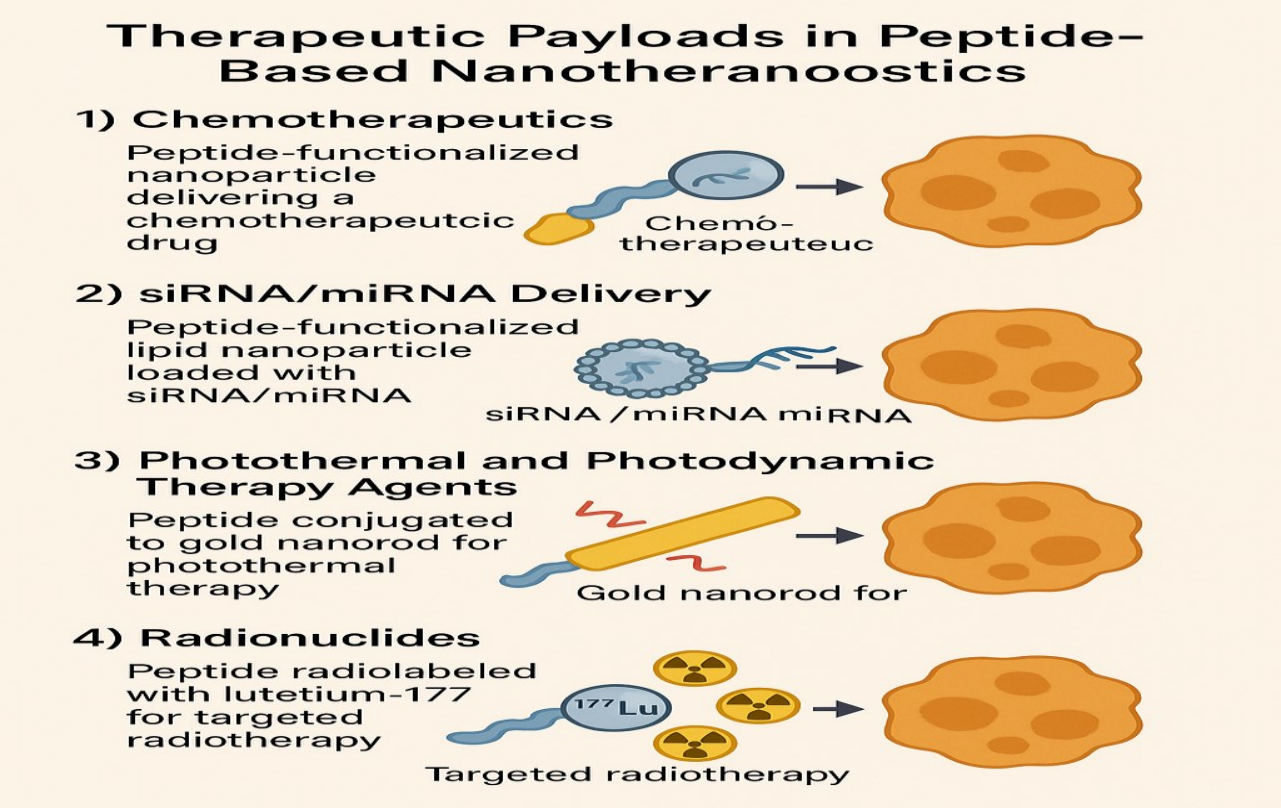
Gene-silencing treatments involving small interfering RNA (siRNA) or microRNA (miRNA) may have potential for cancer treatment through suppression of oncogenes or reconstitution of tumor suppressor activity. These nucleic acids are, however, susceptible to degradation and need protective, targeted delivery systems. Peptide-functionalized nanocarriers, including cationic lipid nanoparticles or dendrimers, offer a stable delivery platform for siRNA/miRNA. For instance, iRGD-modified lipid nanoparticles carrying siRNA against VEGF profoundly inhibited tumor angiogenesis and growth in vivo, showing the advantage of receptor-targeted gene silencing (50). NGR peptide-coated chitosan nanoparticles have been applied to deliver miRNA to the vasculature of tumors in order to promote the stability and cell uptake of the cargo (51).

3) Photothermal and Photodynamic Therapy Agents:

Photothermal therapy (PTT) and photodynamic therapy (PDT) take advantage of light-sensitive agents to cause local tumor ablation. PTT acts through photothermal agents such as gold nanorods or carbon nanotubes that produce heat upon illumination with NIR light, whereas PDT utilizes photosensitizers that generate cytotoxic reactive oxygen species (ROS) upon activation with light. When conjugated with peptides, these agents can be targeted specifically to tumors, thus reducing damage to the surrounding healthy tissue. For instance, RGD-conjugated gold nanorods target integrin-expressing tumor cells selectively and cause extensive tumor ablation under NIR laser irradiation (52). Likewise, chlorin e6 (Ce6)-loaded micelles that are tumor-homing peptide-modified with iRGD or LyP-1 have shown improved tumor localization and efficient PDT in various cancer models (53).

4) Radionuclides:

Radionuclide therapy, especially with peptide-receptor radionuclide therapy (PRRT), is a method of administering radioactive isotopes to tumors through receptor-targeted peptides. The payloads release beta or alpha particles that destroy cancer cells with minimal effect on surrounding normal tissue. example is ^{177}Lu-DOTATATE, somatostatin analog lutetium-177 radiolabeling, aimed at treating neuroendocrine cancers expressing SSTR2. Such a drug has already been granted clinical approval and offers imaging (gamma emission) and therapy (beta emission) in one compound (54, 55). In a similar manner, ^{131}I-RGD or ^{90}Y-labeled bombesin analogs are being explored for the treatment of prostate and breast cancers, delivering targeted radiotherapy depending on integrin and GRP receptor expression respectively.



Challenges in Peptide-Based Nanotheranostics

1. **Peptide Stability: Proteolytic Degradation and Short Half-Life**

Peptides encounter major stability problems in vivo because they are quickly degraded by proteolytic enzymes in tissues and blood. Their generally short half-life (hours to minutes) restricts therapeutic activity and necessitates repeated administration. In response to this, researchers have developed a number of approaches to stabilization. Cyclization of peptides (e.g., cyclic RGD) with disulfide bonds or head-to-tail linkages produces substantial enhancement of resistance to enzymatic digestion without loss of target binding affinity. Introduction of N-methylation or non-biocyclic D-amino acid replacements reduces peptide susceptibility to protease recognition. PEGylation by addition of polyethylene glycol chains induces steric hinderance against degradative agents along with elongating circulation duration. These technologies have yet to conquer the remaining top challenge for systemic peptide drug applications: keeping achieved stability gains along with requisite retention of in vitro biological potency (56).

2. **Tumor Heterogeneity: Variable Expression of Receptors**

The dynamic and heterogeneous character of tumors is a major challenge for peptide-targeted therapies. Receptor expression may vary greatly between the different areas of the same tumor, between metastatic and primary lesions, and over time with the development of the tumor. For example, although RGD peptides are excellent at targeting αvβ3 integrins in most tumors, there may be areas or metastases that do not express this target. Such heterogeneity may result in imperfect tumor targeting and treatment resistance. Present strategies to overcome this involve the creation of multi-targeting peptides that recognize multiple tumor markers at once, the generation of microenvironment-responsive peptides that respond only in tumor tissue, and the implementation of combination therapies targeting multiple pathways. Comprehensive treatment of tumor heterogeneity is still an ongoing area of research (57,58).

3. **Toxicity and Clearance of Nanoparticles:**

Uptake of RES and Immune Response Nanoparticle delivery systems are subject to a number of biological barriers that restrict their therapeutic potential. The reticuloendothelial system (RES), and especially liver and spleen macrophages, rapidly clears numerous nanoparticles from the bloodstream before they have a chance to target tumors. Nanoparticles can also initiate immune responses, such as complement activation and anti-drug antibodies. PEGylation has been extensively employed to design "stealth" nanoparticles that avoid RES clearance, but repeated dosing can cause anti-PEG immunity. Other approaches involve biomimetic coatings with cell membranes or "self" peptides such as CD47 mimetics that block phagocytosis. A balance between extended circulation and efficient tumor penetration, and reduced immunogenicity, is still a major challenge in nanoparticle design (59,60).

4. **Cost and Manufacturing Complexity**

The translation of peptide-nanotheranostics from lab to clinic faces significant manufacturing and economic hurdles. Peptide synthesis, especially for complex modified peptides, is expensive and difficult to scale while maintaining purity and consistency. Nanoparticle production must overcome batch-to-batch variability in critical parameters like size, peptide density, and drug loading. Regulatory requirements for sterility, stability, and quality control add further complexity and cost. These challenges contribute to the high failure rate of nanotherapeutics in clinical translation, with only about 12% of candidates reaching Phase III trials. Emerging solutions include continuous manufacturing approaches like microfluidics, artificial intelligence-assisted design, and platform technologies that can be adapted for multiple therapeutic payloads (61,62)

CONCLUSION

Peptide-guided nanotheranostics has tremendous promise to revolutionize cancer diagnosis and treatment by providing an integrated, targeted, and imaging-guided therapeutic approach. With the incorporation of tumor-targeting peptides into multifunctional nanocarriers, this platform overcomes numerous drawbacks of traditional treatments such as non-targeted drug delivery, systemic toxicity, and poor tumor visualization. Utilization of peptides like RGD, NGR, and DOTATATE facilitates targeted tumor imaging, while conjugation with imaging agents like MRI, PET/SPECT, and photoacoustic imaging provides real-time imaging of therapeutic response. Moreover, therapeutic payloads from chemotherapeutics to siRNA and photothermal agents are delivered more efficiently, improving the efficacy of treatment. However, major challenges including peptide instability, tumor heterogeneity, nanoparticle clearance, and translational complexity need to be resolved. Ongoing developments in peptide engineering, surface modification of nanocarriers, and scalable synthesis processes are crucial for minimizing safety, efficacy, and reproducibility concerns. Overall, peptide-functionalized nanotheranostics is an exciting new area of precision oncology with the ability to facilitate more personalized, potent, and minimally invasive cancer therapies.

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