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REVIEW ARTICLE

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Tumor-homing peptide and its utility for advanced cancer medicine

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Abstract

Cell-penetrating peptides, such as antibodies, have gained great attention as tools for the development of specific delivery systems for payloads, which might be applied as non-invasive carriers in vivo. Among these, tumor-homing peptides recently have been studied for use in tumor medicine. Tumor-homing peptides are oligopeptides, usually consisting of 30 or fewer amino acids that are efficiently and specifically incorporated into tumor cells, suggesting their potential use in establishing novel non-invasive tumor imaging systems for diagnostic and therapeutic applications. Here, we briefly introduce the biological characteristics of our tumor-homing peptides, focusing especially on those developed using a random peptide library constructed using mRNA display technology. The advantage of the tumor-homing peptides is their biological safety, given that these molecules do not show significant cytotoxicity against non-neoplastic cells; lack serious antigenicity, which alternatively might evoke unfavorable immune responses and inflammation in vivo; and are rapidly incorporated into target cells/tissues, with rates exceeding those seen for antibodies. Given their small size, tumor-homing peptides also are easy to modify and redesign. Based on these merits, tumor-homing peptides are expected to find wide application in various aspects of tumor medicine, including imaging diagnostics (eg, with dye-conjugated probes for direct visualization of invasive/metastatic tumor lesions in vivo) and therapeutics (eg, using peptide-drug conjugates [PDCs] for tumor targeting). Although further evidence will be required to demonstrate their practical utility, tumor-homing peptides are expected to show great potential as a next-generation bio-tool contributing to precision medicine for cancer patients.

KEYWORDS
cancer, diagnostics, drug-delivery system (DDS), peptide, therapeutics

1 | INTRODUCTION

Recently, the term "precision medicine" has been used frequently in the field of oncology to describe a mode of advanced medicine. The term usually indicates specific tumor therapeutics that use antitumor agents such as molecular-targeting chemical agents that function in a gene-specific manner, or agents that enable the delivery of an antitumor payload to specific

target cells/tissues using a drug-delivery system (DDS) technology. Some recently developed small molecule inhibitors, especially kinase inhibitors that inactivate kinase-involved signaling pathways, are representatives of the former group (antitumor agents); antibody medications or antibody-drug conjugates (ADCs), including rituximab, trastuzumab, and cetuximab, are representatives of the latter class.[1,2] These ADCs exert a therapeutic effect by precisely regulating the dynamics of the drug in vivo, enabling efficient accumulation of the payload at target cells through binding of the conjugated antibody to specific cell surface antigens.

Tumor cell-permeable oligopeptides are an example of biocarriers for antitumor targeting agents. Namely, such oligopeptides, which can consist of several to scores of amino acids, can serve as useful bio-tools to regulate the dynamics of antitumor payloads in vivo, and also can be linked to anticancer moieties to create DDS-based antitumor drug conjugates. In this context, we here present a brief introduction to our tumor-homing peptide system and its expected use in advanced cancer medicine.

2 | PHYSIOLOGICAL INCORPORATION OF CELL-PENETRATING PEPTIDES IS SUMMARIZED IN SEVERAL MAJOR PATHWAYS

Since the Tat (YGRKKRPQRRR) peptide was first identified,[3,4] partial amino acid sequences encoded by certain viral and cellular proteins have also been reported to exhibit similar cell-permeable functions, having the ability to penetrate various lineages of cells via the surface plasma membrane; collectively, such peptides are called "cell-penetrating peptides" (CPPs). In addition to Tat, several representative CPPs, including penetratin (RQIKIWFQNRRMKWKK) and poly-arginine (eg, R_9; RRRRRRRRR), are shown as "*Cell-penetrative*" in the upper panel in Table 1.[5-7] CPPs generally exhibit permeability not only against a single lineage of cells but also against cells from diverse origins such as epithelial, neuronal, and mesenchymal cells.[5,6] Accordingly, these conventional CPP sequences have been used to generate CPP-molecular fusions, leading to the development of cell-permeable peptide/protein, peptide/nucleic acid, and peptide/

TABLE 1 Representative cell-penetrating peptides and tumor-homing peptides that have been reported recently. Tat, penetratin, transportan, SV40, prion, and poly-arginine are representative CPPs. Molecules listed from PL3 to iRGD have been reported as peptides with tumor-homing properties. All peptides are shown as the encoded amino acid sequences. For representative CPPs, sequence origins are shown. The reported tumor-homing peptides are indicated with their respective target molecules as well as the cell/tissues against which the peptides are generally permeable

Cell-penetrative	Sequence	Origin	Target cell/tissue
Tat	YGRKKRPQRRR	HIV-1 Tat	Non-neoplastic/neoplastic cells
Penetratin	RQIKIWFQNRRMKWKK	pAntp (43-58)	Non-neoplastic/neoplastic cells
Transportan	GWTLNSAGYLLGKINKALAALAKKIL	artificial CPP	Non-neoplastic/neoplastic cells
SV40	PKKKRKV	SV40 NLS	Non-neoplastic/neoplastic cells
Prion	MANLGYWLLALFVTMWTDVGLCKKRPKP	N-term(1-28) of prion protein	Non-neoplastic/neoplastic cells
Poly-Arg	$R_{4\text{-}16}$	artificial; sequential arginines	Non-neoplastic/neoplastic cells
Tumor-homing	**Sequence**	**Target molecule**	**Target cell/tissue**
PL3	AGRGRLVR	Tenascin-C	Glioblastoma, prostate cancer
Angiopep2	TFFYGGSRGKRNNFKTEEY	LRP1	Glioma/glioblastoma
EETI 2.5F	GCPRPRGDNPPLTCKQDSDCLAGCVCGPNGFCG	α/β Integrin	Various lineage tumors
DWVAP	WVAP (D-amino acid)	GRP78	Glioblastoma
Lin TT1	AKRGARSTA	p32	Murine breast cancer
PL1	PPRRGLIKLKTS	fibronectin/tenascin-C	Prostate cancer
LN-1	CTGTPARQC	unidentified	Prostate cancer
LyP-1	CGNKRTRGC	NRP receptor	Glioma
CREKA	CREKA	fibrin-fibronectin complex	Tumor vessel/stroma
UNO	CSPGAKVRC	M2 macrophage	Peritoneal carcinomas
iRGD	CRGDK/RGPD/EC	$\alpha_v\beta_3$ integrin→NRP-1	Various tumors

small chemical compound complexes, as well as imaging probes for diagnostics.[4,5]

The mode of intracellular penetration was revealed to be in a physiological manner mediated by endocytosis which is energy (ATP)-dependent, micropinocytosis/pinocytosis, or attachment to proteoglycan/lipid, and the main mode was dependent on individual CPP sequence, molecular size, electric charge, or complexed manners via multiple pathways even by the same CPP[6,8,9] (Figure 1). For instance, TAT has been shown to be incorporated in the different pathways depending on its peptide form. However, this process is complicated and remains controversial. Specifically, evidence has suggest that Tat alone is internalized by a micropinocytosis-mediated pathway,[10] while the protein alone or a Tat-protein fusion is internalized by receptor-mediated endocytosis, including the clathrin/caveolae-dependent pathway.[11,12] Therefore, uptake of these conventional CPPs into cells appears to be complex, in contrast with designed oligopeptides corresponding to subsequences of native (full-length) proteins that serve as ligands for specific receptors.

3 | TUMOR-HOMING PEPTIDES

3.1 | Tumor-homing peptides identified in the recent studies

Several peptides with properties of tumor cell penetration were recently reported as "tumor-homing peptides," as shown in the lower panel "*Tumor-homing*" in Table 1. All of these tumor-homing peptides were oligopeptides, most of which were isolated by biopanning phage display libraries, ranging in size from 5 to approximately 30 amino acids in length. These peptides were not surface anchored, but were incorporated intracellularly through affinity binding to specific cell surface molecules such as receptors and receptor-associated proteins that were identified based on characteristic overexpression on target tumor or tumor microenvironment cells.[13-16] Accordingly, unlike the mode of intracellular uptake of the conventional CPPs that showed comprehensive permeation to diverse lineages of cells, these were eventually incorporated into specific target cells by endocytosis via specific receptors. Most of these tumor-homing peptides are able to penetrate cells without affecting cell function as well as conventional CPPs such as TAT, but some exert both penetration and antitumor effects against target tumor cells. For example, iRGD (CRGDKGPDC), the stereotypical tumor-homing peptide, has been reported to penetrate tumor cells of diverse origins, as well as endothelial cells, while also inhibiting cancer metastasis in vivo.[16,17] Naturally, the detailed mode of each tumor-homing peptide incorporation is molecularly different from each other (its molecular target and cellular target are different), but their uptake was mediated by endocytosis by binding to the cell surface receptor. Giving one example, iRGD first binds to the $\alpha_v\beta_3$ integrin, following entrapment by neuropilin-1 (NRP-1) after its cleavage, and subsequently incorporated by endocytosis.[16] PL3 bound both to Tenascin-C and NRP-1 and was incorporated via endocytosis;[13] intracellular uptake of Angiopep-2 was mediated by endocytosis by binding to the low-density lipoprotein receptor related protein-1 (LRP-1).[14] As further details on the mode of action for each tumor-homing peptide should be omitted, choice of the suitable tumor-homing peptide should be considered carefully following the biological characteristics of target tumor cell of the interest.

3.2 | Tumor-homing peptide isolated from mRNA display-based random peptide library

Various methods have been used to isolate novel tumor-homing peptides. The first, most-basic, approach is trimming the amino acid sequences from proteins that serve as specific ligands for the interactive receptors, followed by screening the resulting subsequences

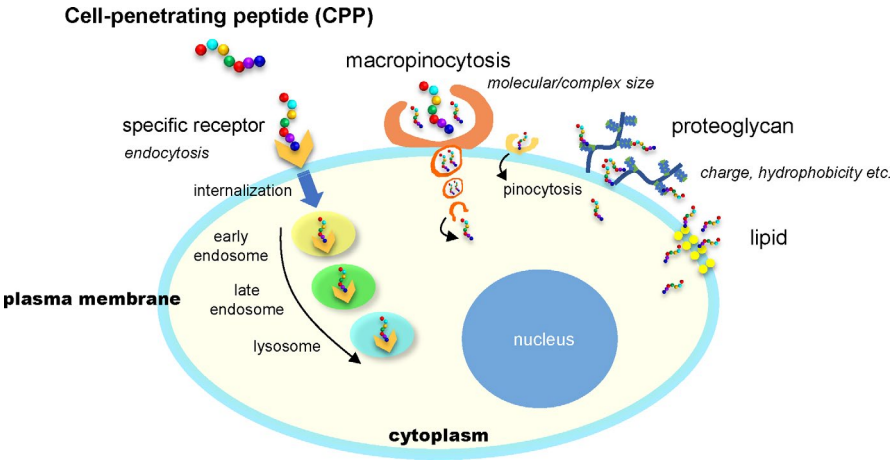

FIGURE 1 Physiological incorporation of cell-penetrating peptides (CPPs). There are several pathways for incorporation of CPPs by physiological cellular activity. Internalization of CPPs across a plasma membrane by binding to specific receptor is by an endocytic pathway, which is an energy (ATP)-dependent process. Macropinocytosis mediates uptake of CPPs depending on molecular size, CPPs also are internalized by attachment to surface proteoglycan complexes or to lipids on the cell surface. All of these mechanisms are based on metabolic activities used by cells for their survival and proliferation

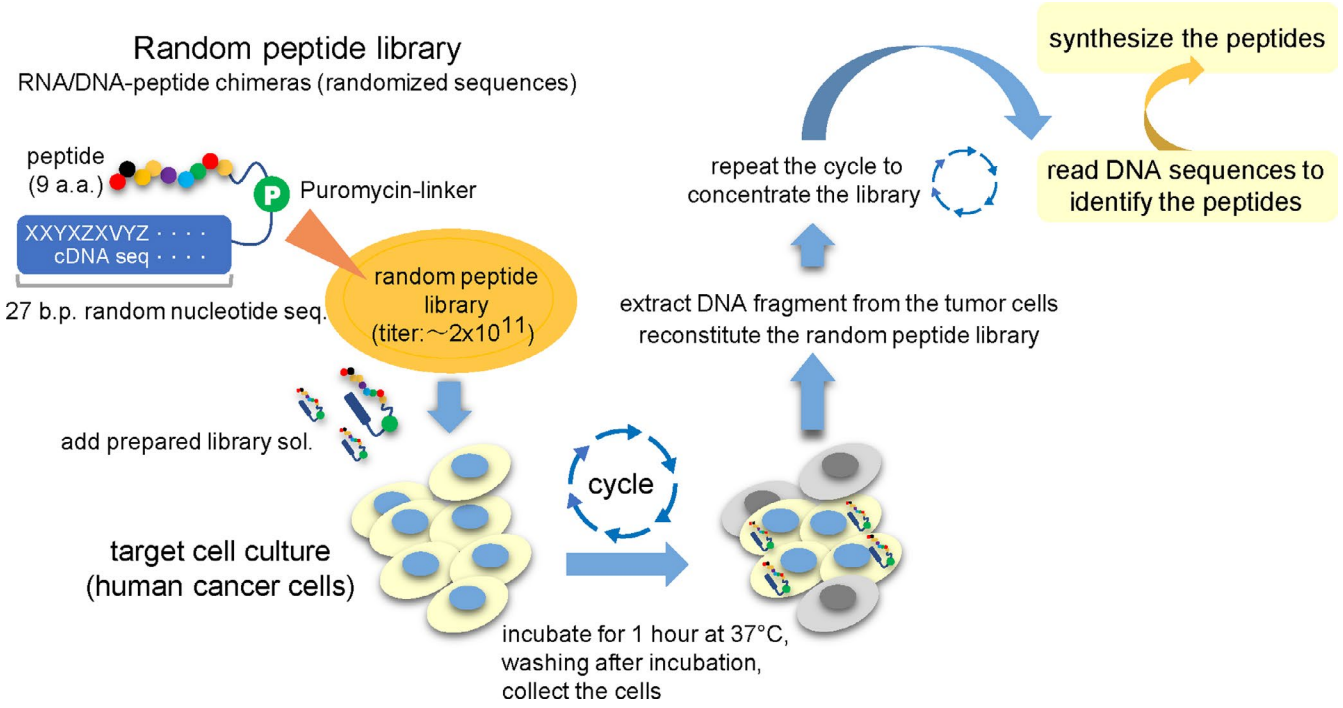

FIGURE 2 A schematic of the technique for isolating tumor-homing peptides from a random peptide library constructed by mRNA is displayed. Briefly, DNA templates that encoded random nucleotide sequences (27 bp) were fused to a T7 promoter fragment bearing a ribosomal binding site and were transcribed to mRNA, ligated to a puromycin (Puro)-linker, and then translated to oligopeptides (9 amino acids in length) with amino acid sequences corresponding to those encoded by the templates. Sequentially, cDNA was synthesized using reverse transcriptase at the mRNA part in mRNA/peptide chimeric molecules, which eventually formed mRNA/cDNA hybrids in the chimeras. This pool was used as a source of the random peptide library. The chimeric molecules were incubated with target cancer cells, and internalized cDNAs then were extracted from the cells. The recovered cDNAs were used as templates for the next library pools, and the cycle was repeated several times, depending on the library titer. Finally, the DNA from the pool was sequenced and the corresponding amino acid sequences were used to identify the encoded oligopeptides that were enriched by multiple rounds of screening[21,22]

for recognition of the appropriate binding site on the cognate receptor.[18,19] A second approach is to screen a random peptide library constructed using established techniques such as phage display, ribosomal display, and mRNA display.[20] To obtain peptides with selective permeability for specific tumors, we chose mRNA display to prepare a random peptide library as a source for isolating the tumor-homing peptides (Figure 2). We considered mRNA display to be more suitable for this purpose because this technique has several advantages compared with phage display and ribosomal display in the context of recovering high-performing peptides.[21,22] First, mRNA display uses mRNA/peptide chimeras of small molecular size (< 100 nm), in contrast with the larger molecular components required for phage display (approximately 1 μm) and ribosomal display (<400 nm); this size difference directly affects the efficiency of isolating the encoded peptide (Figure 2). Second, it is easier to establish high-titer libraries with mRNA display compared with the other 2 techniques. Third, mRNA display permits flexible changes to be made in the size of the peptide (number of amino acids) when preparing the library. Other aspects of the mRNA display (eg, choice of a suitable cell-free translation system, PCR amplification) also improved the convenience of construction of the random peptide library. The method for isolating tumor-homing peptides using our system is summarized in Figure 2. The library (approximate titer, 5×10^{10} to 1×10^{11} transcripts) prepared by this technique was added to the culture medium containing target tumor cells and the mixture was incubated for 1 h. Unincorporated chimeric complexes that remained in the medium were then removed by washing several times. Tumor cells were collected and library-derived cDNA fragments were recovered from the cells; the resulting reconstructed library was then used for another round of screening. Following several cycles of this process, the peptides that we sought were enriched efficiently.[21,22] Consequently, the peptide with maximal performance among several candidates was chosen after an in vitro cell penetration assay and an in vivo peptide delivery assay in a tumor-bearing mouse model.

3.3 | Unique biological characteristic of tumor-homing peptide, differed from antibody

To understand the unique biological characteristics of tumor-homing peptides, an initial comparison was made between tumor-homing peptides and antibodies. One marked difference is the time required for binding/incorporation into target tumor cells. The homing peptides are quickly bound and incorporated into target cells via a process that requires less than 120 min; in contrast, the process

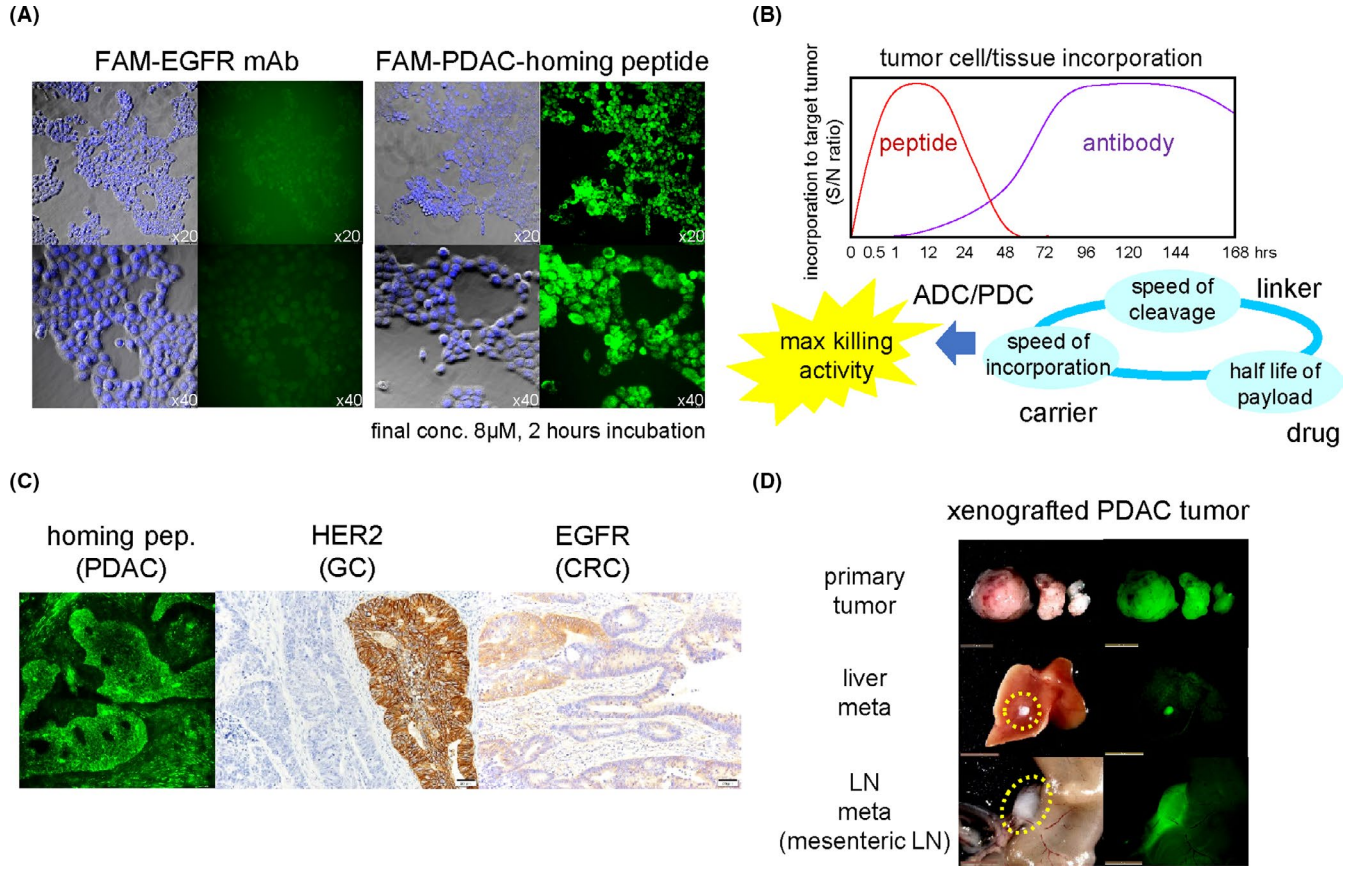

FIGURE 3 Biological characteristics of the tumor-homing peptide. A, Rapid penetration/internalization of 5/6FAM-labeled PDAC-homing peptide in comparison with Alexa488-labeled anti-human EGFRmAb at the same molecular ratio (8 μmol/L), as imaged after 2 h of incubation with BxPC3 cells. B, General trend of the in vivo dynamics when comparing between tumor-homing peptide and antibody (upper panel; modified from E. Kondo, *Drug Delivery System* 35(3), 2020). The 3 shared components are necessary for generating both ADCs and PDCs. Schematic showing the rate-determining stages of ADC/PDC that are determined by each component. Together, these properties affect the total efficiency of the ADC/PDC (lower panel). C, Distribution of the intravenously administrated PDAC-homing peptide inside human PDAC tumor tissue grown in human PDAC cell line-derived tumor xenograft (CDX)-mice. The peptide showed broad incorporation, as depicted by fluorescent signals covering entire cancer nests (left). Conversely, expression of HER2 in gastric cancer tissue of the patient and EGFR in colorectal cancer patient tissue were partially observed inside tumor tissues (middle and right). ×20 magnification. D, Mode of uptake of the PDAC-homing peptide in vivo. Incorporation of the fluorescently labeled peptide was observed in liver metastasis and lymph node (LN) metastasis as well as in primary PDAC tumors

of binding and incorporation of antibodies is much slower. For example, the signal indicating binding and internalization of fluorescently labeled anti-human epidermal growth factor receptor (EGFR) antibody by BxPC3, a human pancreatic ductal adenocarcinoma cell (PDAC) line, was not visible after 120 min, whereas our PDAC-homing peptide was sufficiently incorporated by these cells in the same time frame (Figure 3A). The maximum signal/noise (S/N) ratio was observed from approximately 2 h to 12 h with peptide administration, and from approximately 4 d to 6 d with antibody (Figure 3B). This result suggested that (all other variables being equal) a peptide-drug conjugate (PDC) incorporating this tumor-homing peptide would exert an antitumor effect more quickly than would an ADC (Figure 3B). In this context, we expect that tumor-homing peptides will be more effective therapeutically when combined with drugs that have short half-lives. A second property distinguishing our homing peptide from antibodies is that the homing peptide was broadly incorporated into targeted tumor cells, covering almost completely whole tumor nests in vivo (in a mouse tumor model). Figure 3C shows one such result using our PDAC-homing peptide (5/6-Carboxyfluorescein hydrate (5/6-FAM)-labeled); the peptide was efficiently incorporated into target cells inside PDAC tumor tissue, covering every tumor nest. This result indicated that PDCs incorporating this peptide might exert potent antitumor effects without missing the target cells. Conversely, biologicals such as anti-HER2 and anti-EGFR antibodies, which are utilized as carrier tools in current ADCs, are limited to targeting specific antigen-positive tumor cells, meaning that negative cells internal to tumor tissues will be spared, excepting bystander effects from the payload. A third important aspect of our tumor-homing peptides is that these molecules generally are able to target both primary tumors and metastatic foci, given that the peptide is adsorbed equally to both lesions. Figure 3D shows an example of a PDAC-homing peptide that was efficiently and selectively incorporated into target pancreatic cancer lesions, including both primary and metastatic sites.

4 | PROSPECTED USE OF TUMOR-HOMING PEPTIDE FOR ADVANCED CANCER MEDICINE

Given that tumor-homing peptides are available to target tumors with specific lineages in vivo, these molecules are expected to have use for advanced tumor medicine. One application of tumor-homing peptides would be their use for in vivo tumor imaging, for instance using a peptide probe chemically labeled with dyes such as fluorescein, near infrared dye (NIR dye) such as indocyanine green (ICG), and 5-aminolevulinic acid (5-ALA). Such an application may lead to the development of novel photodynamic diagnosis (PDD) for cancer patients, especially for PDD used during surgical operations to determine the range of cancer invasion or to find micro-metastatic foci by visualization with the probes. As a practical example, using fluorescein-labeled PDAC-homing peptide, we demonstrated that PDAC dissemination was clearly delineated within the abdominal cavity of a tumor-bearing mouse (Figure 4A). Rapid incorporation (within 60 min) of the peptides into the target tumor lesions is a unique physiological characteristic of tumor-homing peptides, making these molecules attractive for application in PDD, which may lead to an indispensable role in precision medicine. In the context of using tumor-homing peptides for live tumor imaging in vivo, the peptide probes are expected to be suitable for diagnostics that depend on direct-viewing systems (viewed through the operator's eye),[22,23] but not for radiolabeled systemic imaging systems such as Positron emission tomography (PET) or single photon emission computed tomography (SPECT). Notably, the magnitude of peptide incorporation into target tumor lesions in vivo is not still sufficient for use as a PET tracer. Specifically, PET tracers such as ^{18}F-fluorodeoxyglucose (FDG) require more than scores to 100-fold accumulation in the tumor tissues in vivo to permit detection of small tumor lesions, whereas the peptides, even when incorporated into target tissue, show approximately 5-fold to 20-fold accumulation (data not shown).

The homing peptides also are expected to be useful for the development of PDCs. As shown in Figure 4B, a PDC comprises a homing peptide (carrier), a protease-cleavable linker (linker), and an anticancer agent (payload), an overall architecture similar to that of ADCs. The design of the peptide can be diverse, depending on this carrier's chemical characteristics. For example, the peptide might take a linear or circular form for stability in vivo, and can vary in peptide length (molecular size, specificity, and affinity), amino acid modification (glycosylation, methylation, acetylation, succinylation), and the use of D-amino acids to obtain maximum performance as a targeting tool.[24-27] Of course, the structure must be determined or improved without impairing its target specificity or efficiency of intracellular incorporation. In addition to the design of the carrier peptide, optimization is necessary for the cleavable linker and the anticancer agent, and the

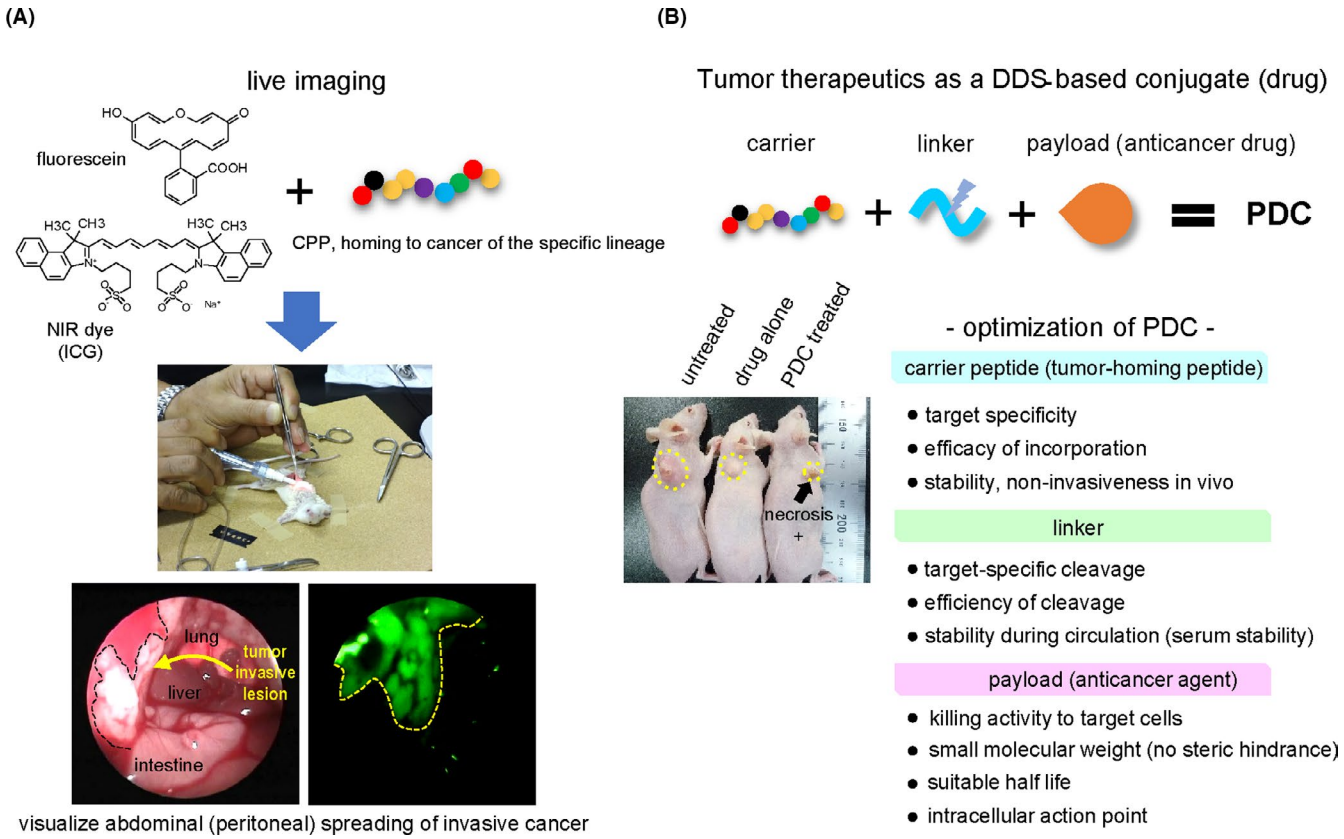

FIGURE 4 Practical use of the tumor-homing peptide. A, Tumor imaging probe is possibly made by labeling with various kinds of dye. Using an endoscope, intra-abdominal spreading of cancer lesions was directly visualized as a fluorescent signal after intravenous administration of the fluorescently labeled tumor-homing peptide. B, Construction and requirements in each component of PDC. Total balance of each part is critical to the development of effective tumor-targeting PDCs

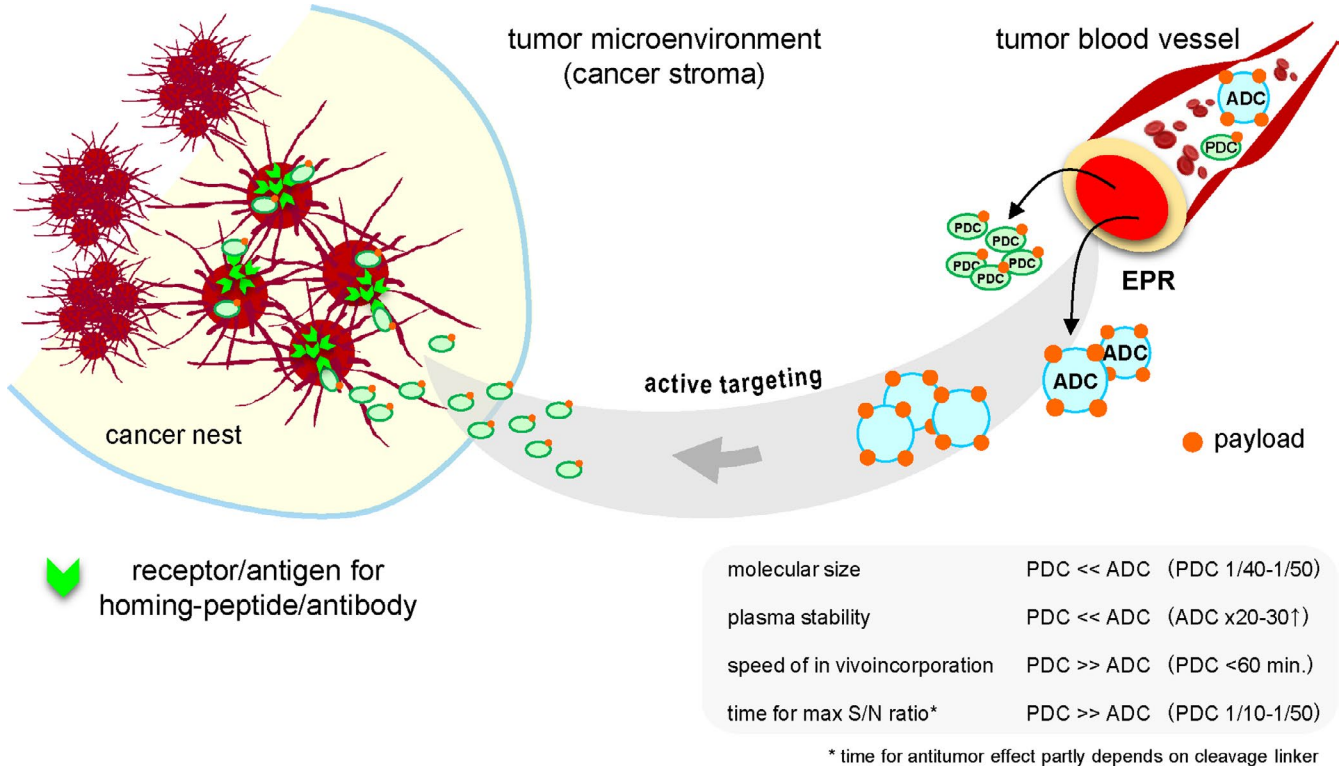

FIGURE 5 How might PDCs be used in tumor therapeutics? There are several different aspects that distinguish the biological/chemical characteristics of PDCs from those of ADCs. To make the best use of PDC for patient therapy, it will be necessary to consider the application, taking into consideration both the advantages and weak points

most suitable form of the PDC as a total complex needs to provide high performance against the target tumor (Figure 4B). All of these aspects must harmonize to permit the PDC to function in a tumor-specific manner. Concretely, the linker plays a critical role in efficient payload release, and its cleavage should be triggered predominantly or even only in a tumor-dependent manner by a specific protease active in the target cells. The choice of the antitumor agent as a payload for PDC is also important, namely the aspects such as the intracellular point of action, half-life, and in vivo dynamics have to be considered. Furthermore, the final form of the PDC should itself have low toxicity and possess minimal off-target effects, properties that are essential for the PDC's practical utility in tumor therapeutics. When we consider CPPs from the viewpoint of advanced cancer therapeutics, there are still diverse utilities applicable, not being restricted to use as a drug carrier, but as bio-tools for various aspects in cancer medicine. For example, the ZEBRA-CPP could deliver multiepitope peptides as a cargo in its fused form to dendritic cells, which resulted in activation of tumor immunity through promoting cytotoxic T-cells. This peptide does not have a tumor-homing property, but it also contributes to cancer medicine through CPP technology.[28]

5 | CONCLUSION

Oligopeptides have great advantages in medical application. Generally, they are physiologically non-invasive and easily absorbed and well metabolized in vivo. They also show lower antigenicity compared with other foreign proteins in vivo because of their rapid degradation by endogenous proteases, quick absorption at the small intestine as a single amino acid, and lower production of specific neutralizing antibody, except for tumor antigenic oligopeptides. These dynamics were also observed in our experiments, which resulted in the persistent efficacy of our tumor-homing peptides by their repeated administration in vivo (unpublished data). Indeed, functional peptides are physiologically generated in vivo. Many such molecules, notably including endocrine hormonal peptides (polypeptide hormones) such as insulin, glucagon, and ghrelin, are widely recognized to be essential for regulation of homeostasis. Therefore, oligopeptides are considered to be highly useful in various fields in cancer medicine. One of the major potential applications of such tools would be the development of peptides that affect cellular function by targeting molecules located on the tumor cell surface or intracellularly (eg, receptors, oncogenic/tumor suppressor proteins).[29,30] In this brief review, we introduced our tumor-homing peptides as a tumor-targeting tool. These peptides are CPPs that were isolated from an mRNA display-based random peptide library. Based on the biochemical advantages for medical application mentioned above, we expect that these tumor-homing peptides represent a class that will become available for development and use as novel agents in tumor diagnosis, therapeutics, or even theranostics. We propose that a peptide probe can be used for tumor imaging/diagnostics, a PDC can be used as a novel type of therapeutic agent as well as ADC.

In considering the application of PDCs to cancer therapy, we must carefully consider the use of these peptides from the point of view of their unique biological and chemical characteristics and in vivo dynamics (Figure 5). Tumor-homing peptides still need to overcome several important issues for the purpose of practical application, especially with regard to in vivo stability and detailed molecular mechanisms for uptake into tumor cells/tissues. Nonetheless, we expect that tumor-homing peptides will be important technological tools for use in advanced cancer medicine.

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DISCLOSURE

The authors have no conflict of interest.

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REFERENCES

1. Wu P, Nielsen TE, Clausen MH. FDA-approved small-molecule kinase inhibitors. *Trends Pharmacol Sci*. 2015;36:422-439.
2. Khongorzul P, Ling CJ, Khan FU, et al. Antibody-drug conjugates: a comprehensive review. *Mol Cancer Res*. 2020;18:3-19.
3. Schwarze SR, Ho A, Vocero-Akbani A, et al. In vivo protein transduction: delivery of a biologically active protein into the mouse. *Science*. 1999;285:1569-1572.
4. Waida JS, Dowdy SF. Transmembrane delivery of protein and peptide drugs by TAT-mediated transduction in the treatment of cancer. *Adv Drug Deliv Rev*. 2005;57:579-596.
5. Fonseca S, Pereira MP, Kelley SO. Recent advances in the use of cell-penetrating peptides for medical and biological applications. *Adv Drug Deliv Rev*. 2009;61:953-964.
6. Guidotti G, Brambilla L, Rossi D. Cell-penetrating peptides: from basic research to clinics. *Trends Pharmacol Sci*. 2017;38:406-424.
7. Futaki S, Nakase I, Tadokoro A, et al. Arginine-rich peptides and their internalization mechanisms. *Biochem Soc Trans*. 2007;35:784-787.
8. Alves ID, Jiao C-Y, Aubry S, et al. Cell biology meets biophysics to unveil the different mechanisms of penetratin internalization in cells. *Biochem Biophys Acta*. 2010;1798:2231-2239.
9. Wadia JS, Stan RV, Dowdy SF. Transducible TAT-HA fusogenic peptide enhances escape of TAT-fusion proteins after lipid raft micropinocytosis. *Nat Med*. 2004;10:310-315.
10. Kaplan IM, Wadia JS, Dowdy SF. Cationic TAT peptide transduction domain enters cells by micropinocytosis. *J Control Release*. 2005;102:247-253.
11. Ben-Dov N, Korenstein R. The uptake of HIV Tat peptide proceeds via two pathways which differ from micropinocytosis. *Biochim Biophys Acta*. 2015;1848:869-877.
12. Richard JP, Melikov K, Brooks H, et al. Cellular uptake of unconjugated TAT peptide involves clathrin-dependent endocytosis and heparin sulfate receptors. *J Biol Chem*. 2005;280:15300-15306.
13. Lingasamy P, Tobi A, Kurm K, et al. Tumor-penetrating peptide for systemic targeting of Tenascin-C. *Sci Rep*. 2020;5089.
14. Demeule M, Régina A, Ché C, et al. Identification and design of peptides as a new drug delivery system for the brain. *J Pharmacol Exp Ther*. 2008;324:1064-1072.
15. Laakkonen P, Akerman ME, Biliran H, et al. Antitumor activity of a homing peptide that targets tumor lymphatics and tumor cells. *Proc Natl Acad Sci USA*. 2004;101:9381-9386.
16. Sugahara KN, Teesalu T, Karmali PP, et al. Tissue-penetrating delivery of compounds and nanoparticles into tumors. *Cancer Cell*. 2009;16:510-520.
17. Sugahara KN, Braun GB, de Mendoza TH, et al. Tumor-penetrating iRGD peptide inhibits metastasis. *Mol. Cancer Ther*. 2015;14:120-128.
18. Davenport AP, Scully CCG, de Graaf C, et al. Advances in therapeutic peptides targeting G protein-coupled receptors. *Nat Rev Drug Discov*. 2020;19:389-413.
19. Ruan H, Chai Z, Shen Q, et al. A novel peptide ligand RAP12 of LRP1 for glioma targeted drug delivery. *J Control Release*. 2018;279:306-315.
20. Pitt A, Nims Z. Peptide display technologies. *Methods Mol Biol*. 2001;2019:258-298.
21. Kamide K, Nakakubu H, Uno S, et al. Isolation of novel cell-penetrating peptides from a random peptide library using in vitro virus and their modifications. *Int J Mol Med*. 2010;25:41-51.
22. Kondo E, Saito K, Tashiro Y, et al. Tumor lineage-homing cell-penetrating peptides as anticancer molecular delivery systems. *Nat Commun*. 2012;3:951.
23. Higa M, Katagiri C, Shimizu-Okabe C, et al. Identification of a novel cell-penetrating peptide targeting human glioblastoma cell lines as a cancer-homing transporter. *Biochem Biophys Res Commun*. 2015;457:206-212.
24. Grunwald J, Rejtar T, Sawant R, et al. TAT peptide and its conjugates: proteolytic stability. *Bioconjug Chem*. 2009;20:1531-1537.
25. Liu M, Li C, Pazgier M, et al. D-paptide inhibitors of the p53-MDM2 interaction for targeted molecular therapy of malignant neoplasms. *Proc Natl Acad Sci USA*. 2010;107:14321-14326.
26. Verdine GL, Hilinski G. Stapled peptides for intracellular drug targets. *Methods Enzymol*. 2012;503:3-33.
27. Park SE, Sajid MI, Parang K, et al. Cyclic cell-penetrating peptides as efficient intracellular drug delivery tools. *Mol Pharm*. 2019;16:3727-3743.
28. Belnoue E, Di Berardino-Besson W, Gaertner H, et al. Enhancing antitumor immune responses by optimized combinations of cell-penetrating peptide-based vaccines and adjuvants. *Mol Ther*. 2016;24:1675-1685.
29. Vinogradov AA, Yin Y, Suga H. Macrocyclic peptides as drug candidates: recent progress and remaining challenges. *J Am Chem Soc*. 2019;141:4167-4181.
30. Saito K, Iioka H, Kojima C, et al. Peptide-based tumor inhibitor encoding mitochondrial p14(ARF) is highly efficacious to diverse tumors. *Cancer Sci*. 2016;107:1290-1301.

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