Cell-Penetrating Peptides Applications

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Since their identification over twenty-five years ago, the plethora of cell-penetrating peptides (CPP) and their applications has skyrocketed. These 5 to 30 amino acid in length peptides have the unique property of breaching the cell membrane barrier while carrying cargoes larger than themselves into cells in an intact, functional form. CPPs can be conjugated to fluorophores, activatable probes, radioisotopes or contrast agents for imaging tissues, such as tumors. There is no singular mechanism for translocation of CPPs into a cell, and therefore, many CPPs are taken up by a multitude of cell types, creating the challenge of tumor-specific translocation and hindering clinical effectiveness. Varying strategies have been developed to combat this issue and enhance their diagnostic potential by derivatizing CPPs for better targeting by constructing specific cell-activated forms. These methods are currently being used to image integrin-expressing tumors, breast cancer cells, human histiocytic lymphoma and protease-secreting fibrosarcoma cells, to name a few. Additionally, identifying safe, effective therapeutics for malignant tumors has long been an active area of research. CPPs can circumvent many of the complications found in treating cancer with conventional therapeutics by targeted delivery of drugs into tumors, thereby decreasing off-target side effects, a feat not achievable by currently employed conventional chemotherapeutics. Myriad types of chemotherapeutics such as tyrosine kinase inhibitors, antitumor antibodies and nanoparticles can be functionally attached to these peptides, leading to the possibility of delivering established and novel cancer therapeutics directly to tumor tissue.

Keywords: cell-penetrating peptides ; protein transduction domains ; tumor imaging ; targeted therapies

1. Introduction

As so often happens in science, the discovery of cell-penetrating peptides (CPP) was a serendipitous one. Two independent groups of researchers working on the human immunodeficiency virus (HIV) viral coat Trans-activator of Transcription (Tat) protein observed the protein's ability to cross cell membrane barriers without any transfection reagents [1][2]. Similarly, the homeobox Antennapedia (Antp) transcription factor of *Drosophila melanogaster* was demonstrated to enter nerve cells in a receptor-independent manner, where it could then regulate neural morphogenesis [3]. Further mapping studies of the domains within Tat and Antp responsible for the observed transduction led to the identification of the first two CPPS: the 11 amino acid cationic, arginine- and lysine-rich domain of Tat protein (YGRKKRRQRRR) [4] and the 16 amino acid sequence from the third helix of the Antennapedia domain (RQIKIWFQNRRMKWKK) termed Antp, also known as penetratin [5]. The next big development in the field of CPPs came with the demonstration of Tat peptide's ability to cross cell membrane barriers while carrying cargo many times its size in a functional form [6].

Since this initial description, the plethora of CPPs has expanded exponentially. Although the first two CPPs identified were non-cell specific, researchers have utilized phage-display methodologies to identify multiple tissue-specific peptides. Phage display was a technique developed by Smith in 1985 [7], and for which he subsequently received the Nobel prize for chemistry in 2018 [8]. The technique of phage display was initially utilized to identify NRG and RGD motifs targeting tumor cells, and the utility of these peptides in delivering chemotherapeutic agents specifically to tumor vasculature was demonstrated [9]. Phage display has also been used to identify peptides targeting vascular endothelium [10], synovial tissue [11], dendritic cells [12], pancreatic islet cells [13] and cardiac myocytes [14]. Additionally, this list continues to grow every year.

2. Clinical Trials Using CPPs as Cancer Therapeutics

The authors currently know of ten clinical trials involving CPPs to treat cancer. This number is expected to increase as additional advancements are made in the arena of targeted cancer therapeutics. Of the ten clinical trials discussed in the table below (Table 1), six have completed at least Phase 1a and are continuing onto Phase 1b and Phase 2; the other four are in the process of recruiting and completing Phase 1. Aileron therapeutics appear to be a leader in innovation with their ALRN-6924 peptide that is being successfully employed in half of the clinical studies discussed below. ALRN-6924 is a

CPP that disrupts interaction between p53 tumor suppressor protein and its inhibitors MDMX and MDM2 [15][16][17][18][19]. This peptide has been tested alone for safety and efficacy as well as combined to many anticancer drugs such as cytarabine, paclitaxel and topotecan [15][16][17][18][19]. Phase 1 clinical trials have shown that ALRN-6924 alone and in combination is safe and increases the therapeutic index of the covalently attached drugs [15][16][17][18][19]. ALRN-6924 is currently being employed in two clinical trials that are in Phase 2 and have shown promising results in cancer treatment including pediatric cases [18][19]. Another peptide currently in clinical trial is BT1718, designed to target and inhibit the function of MT1-MMP by recognizing and attaching itself to the MT1-MMP protein [20]. Once it is attached it is internalized into cancer cells [20]. P28 is another CPP being evaluated currently in two cancer clinical trials. It is derived from azurin and targets solid tumors that resist standard methods of treatment [21][22]. Both of these trials have completed Phase 1 and look promising at treating solid tumors resistant to conventional chemotherapeutics. PEP-010 is another peptide about to begin enrollment into a Phase I trial to assess its safety profile [23].

Table 1. Summary of various clinical trials utilizing CPPs in anticancer therapies.

Sponsor	ClinicalTrials.govIdentifier	Study Stage	CPP Employed	Cancer Targeted	Drug Employed with CPP	Study Size
Aileron Therapeutics [15]	NCT02264613	Phase 1—Completed Phase 2a—Completed	ALRN-6924	Solid tumor, lymphoma, and peripheral T-cell lymphoma	ALRN-6924—alone and in combination withpalbociclib	149
Aileron Therapeutics [16]	NCT02909972	Phase 1—Completed	ALRN-6924	Acute myeloid leukemia, and advanced myelodysplastic syndrome	ALRN-6924—alone and in combination with cytarabine	55
Aileron Therapeutics [17]	NCT03725436	Phase 1	ALRN-6924	Advanced, metastatic or unresectable solid tumors	ALRN-6924—in combination with paclitaxel	45
Aileron Therapeutics [18]	NCT03654716	Phase 1	ALRN-6924	Pediatric leukemia, pediatric brain tumor, pediatric solid tumor, pediatric lymphoma	ALRN-6924—alone or in combination with cytarabine for patients with leukemia	69
Aileron Therapeutics [19]	NCT04022876	Phase 1a—Completed Phase 1b Phase 2	ALRN-6924	Small cell lung cancer	Phase 1b—ALRN-6924 with topotecan Phase 2—topotecan alone and in combination with ALRN-6924	120
Cancer Research UK [20]	NCT03486730	Phase 1 Phase 2	BT1718	Advanced solid tumors, non-small cell lung cancer, non-small cell lung sarcoma, and esophageal cancer	BT1718—alone	130
CDG Therapeutics and Dr. Tapas K. Das Gupta [21]	NCT00914914	Phase 1—Completed	P28	Refractory solid tumors	P28—alone	15
Pediatric Brain Tumor Consortium/National Cancer Institute (NCI) [22]	NCT01975116	Phase 1—Completed	P28	Recurrent or progressive central nervous system tumors	P28—alone	18
Institut Curie [23]	NCT04733027	Phase 1	PEP-010	Metastatic solid tumor cancer	PEP-010—alone PEP-010—in combination with paclitaxel	56

Sponsor	ClinicalTrials.govIdentifier	Study Stage	CPP Employed	Cancer Targeted	Drug Employed with CPP	Study Size
Amal Therapeutics [24]	NCT04046445	Phase 1a — Completed Phase 1b	ATP128	Stage IV colorectal cancer	ATP128—alone and in combination with BI 754091	32

3. Summary

Since their identification nearly twenty-five years ago, the number and applications of CPPs, both in the arena of tumor diagnostics and therapeutics, continue to grow at a brisk pace. Combining them as novel vectors for targeted delivery of both established and emerging therapeutics has the potential to reduce drug doses, decrease tumor resistance and reduce off-target adverse effects that so often limit dosage of chemotherapeutics, as well as adversely affect patient quality of life. While the future of CPPs in medicine is promising, there are still many issues and challenges that need to be addressed to make their future in medicine feasible. One such challenge will be endosomal entrapment of the peptides; this could potentially be overcome by conjugating drugs or peptides to a peptide that causes endosomal lysis. Another issue to overcome is the human body's natural immune response and the body's generation of antibodies that target antitumor drugs. This could be overcome by delivering immunosuppressants with the peptide–drug combination, or finding ways to locally administer the conjugate. Finding a way to deliver peptides successfully to specific organelles within specific types of cells is another challenge. While CPPs have shown promise at delivering cargo to specific cell types, there needs to be more work on targeting specific organelles. One possible solution to this is to add organelle localization sequences to peptides as well as adding endosome-lytic peptides. Finally, proteases are also a concern as they could break down the peptide. This could be overcome by using the D-enantiomers of the amino acid forming the peptide or protecting the CPPs in liposomal or nanoparticle formulations. All of this goes to show that CPPs have immense potential in both cancer diagnostic and therapeutic applications; however, further research is needed for them to become truly efficacious in the field of oncology.

References

1. Frankel, A.D.; Pabo, C.O. Cellular uptake of the tat protein from human immunodeficiency virus. Cell 1988, 55, 1189–1193.
2. Green, M.; Loewenstein, P.M. Autonomous functional domains of chemically synthesized human immunodeficiency virus tat trans-activator protein. Cell 1988, 55, 1179–1188.
3. Joliot, A.; Pernelle, C.; Deagostini-Bazin, H.; Prochiantz, A. Antennapedia homeobox peptide regulates neural morphogenesis. Proc. Natl. Acad. Sci. USA 1991, 88, 1864–1868.
4. Green, M.; Ishino, M.; Loewenstein, P.M. Mutational analysis of HIV-1 Tat minimal domain peptides: Identification of trans-dominant mutants that suppress HIV-LTR-driven gene expression. Cell 1989, 58, 215–223.
5. DeRossi, D.; Joliot, A.H.; Chassaing, G.; Prochiantz, A. The third helix of the Antennapedia homeodomain translocates through biological membranes. J. Biol. Chem. 1994, 269, 10444–10450.
6. Schwarze, S.R.; Ho, A.; Vocero-Akbani, A.; Dowdy, S.F. In vivo protein transduction: Delivery of a biologically active protein into the mouse. Science 1999, 285, 1569–1572.
7. Smith, G.P. Filamentous fusion phage: Novel expression vectors that display cloned antigens on the virion surface. Science 1985, 228, 1315–1317.
8. Smith, G.P. Phage Display: Simple Evolution in a Petri Dish (Nobel Lecture). Angew. Chem. Int. Ed. Engl. 2019, 58, 14428–14437.
9. Arap, W.; Pasqualini, R.; Ruoslahti, E. Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model. Science 1998, 279, 377–380.
10. Nicklin, S.; White, S.J.; Watkins, S.J.; Hawkins, R.E.; Baker, A.H. Selective Targeting of Gene Transfer to Vascular Endothelial Cells by Use of Peptides Isolated by Phage Display. Circulation 2000, 102, 231–237.
11. Mi, Z.; Lu, X.; Mai, J.C.; Ng, B.G.; Wang, G.; Lechman, E.R.; Watkins, S.C.; Rabinowich, H.; Robbins, P.D. Identification of a synovial fibroblast-specific protein transduction domain for delivery of apoptotic agents to hyperplastic synovium. Mol. Ther. 2003, 8, 295–305.
12. Chamarthy, S.P.; Jia, L.; Kovacs, J.R.; Anderson, K.R.; Shen, H.; Firestine, S.M.; Meng, W.S. Gene delivery to dendritic cells facilitated by a tumor necrosis factor alpha-competing peptide. Mol. Immunol. 2004, 41, 741–749.

13. Rehman, K.K.; Bertera, S.; Bottino, R.; Balamurugan, A.N.; Mai, J.C.; Mi, Z.; Trucco, M.; Robbins, P.D. Protection of islets by in situ peptide-mediated transduction of the Ikappa B kinase inhibitor Nemo-binding domain peptide. J. Biol. Chem. 2003, 278, 9862–9868.
14. Zahid, M.; Phillips, B.E.; Albers, S.M.; Giannoukakis, N.; Watkins, S.C.; Robbins, P.D. Identification of a Cardiac Specific Protein Transduction Domain by In Vivo Biopanning Using a M13 Phage Peptide Display Library in Mice. PLoS ONE 2010, 5, e12252.
15. Aileron Therapeutics. ALRN-6924 in Patients with Advanced Solid Tumors or Lymphomas; National Library of Medicine: Bethesda, MD, USA, 2014; NCT02264613 (ClinicalTrials.gov Identifier).
16. Aileron Therapeutics. Safety Study of ALRN-6924 in Patients with Acute Myeloid Leukemia or Advanced Myelodysplastic Syndrome; National Library of Medicine: Bethesda, MD, USA, 2016; NCT02909972 (ClinicalTrials.gov Identifier).
17. Aileron Therapeutics. ALRN-6924 and Paclitaxel in Treating Patients with Advanced, Metastatic, or Unresectable Solid Tumors; National Library of Medicine: Bethesda, MD, USA, 2018; NCT03725436 (ClinicalTrials.gov Identifier).
18. Aileron Therapeutics. Phase 1 Study of the Dual MDM2/MDMX Inhibitor ALRN-6924 in Pediatric Cancer; National Library of Medicine: Bethesda, MD, USA, 2018; NCT03654716 (ClinicalTrials.gov Identifier).
19. Aileron Therapeutics. A Study of ALRN-6924 for the Prevention of Topotecan-induced Myelosuppression During Treatment for Small Cell Lung Cancer; National Library of Medicine: Bethesda, MD, USA, 2019; NCT04022876 (ClinicalTrials.gov Identifier).
20. Cancer Research, UK. BT1718 in Patients with Advanced Solid Tumours; National Library of Medicine: Bethesda, MD, USA, 2018; NCT03486730 (ClinicalTrials.gov Identifier).
21. Das Gupta, T.K. Safety Study of a Cell Penetrating Peptide (p28) to Treat Solid Tumors That Resist Standard Methods of Treatmen; National Library of Medicine: Bethesda, MD, USA, 2009; NCT00914914 (ClinicalTrials.gov Identifier).
22. Pediatric Brain Tumor Consortium. p28 in Treating Younger Patients with Recurrent or Progressive Central Nervous System Tumors; National Library of Medicine: Bethesda, MD, USA, 2013; NCT01975116 (ClinicalTrials.gov Identifier).
23. Institut Curie. First-in-human Phase I to Evaluate PEP-010 as Single Agent and in Combination with Paclitaxel (CleverPeptide); National Library of Medicine: Bethesda, MD, USA, 2021; NCT04733027 (ClinicalTrials.gov Identifier).
24. Amal Therapeutics. Phase 1b Study to Evaluate ATP128, With or Without BI 754091, in Patients with Stage IV Colorectal Cancer (KISIMA-01); National Library of Medicine: Bethesda, MD, USA, 2019; NCT04046445 (ClinicalTrials.gov Identifier).