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CELL-PENETRATING PEPTIDES (CPP); AN EFFECTIVE TOOL IN THE MODERN TREATMENT

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ABSTRACT
The present picture of novel therapeutic improvements relies mostly on gene-targeted technologies, facilitating to fight rare genomic diseases, from infections to cancer and inherited diseases. Although, reaching the action-site for this novel therapy needs to reach to nucleic acids, or other macromolecules into cells, which may face complex tasks to pharmaceutical companies. To overcome this technological shortfall, wide ranges of vectors have been developed in the past years and have confirmed to be doing well in delivering differenttherapeutics. Cell-penetrating peptides (CPP) have been one of the distinct technologies widely detail studied and have been ever more used to transfer small RNA/DNA, plasmids, antibodies, and nanoparticles into cells. Although CCP already showed huge potential that these peptide-based techniques are involve in few advances which have been put to pharmacological or clinical application. This review will portray the origin, classification, mechanism, development, and practical application of CPP to deliver therapeutic agents into cells.

KEY WORDS: Efficient vector, Future medicine

INTRODUCTION
Background: Cell-penetrating peptides (CPPs) are a class of diverse peptides, classically with 5–30 amino acids,that unlike most peptides can penetrate into the cellular membrane and are currently pursued for their assembly of biomedical applications [Drug Delivery , Anticancer, Antiviral, Antimicrobial]. Assigned to different terminologies such as ‘protein transduction domains’,‘membrane translocating peptides’ and ‘Trojan Horse Peptides’ the terminology CPP introduced by Pooga et al.[1] And is the most accepted terminology for the set of peptides capable of interacting with, getting into, and eventually transposing membrane structures.[2] CPP are usually short, typically 5–30 amino acids residues long, and can be isolated from naturally present proteins, modified or designed de novo.[3,4] As cellular, endosome, and other organelle membranes are tough barriers in the quest for delivering drugs to impending intracellular therapeutic targets, CPP are now considered as frontrunners for the development of such reliable DDS.[3,4] They can subordinate to molecules such as nucleic acids, proteins, drugs, or imaging agents by covalent or non-covalent binding, and deliver the cargo to the cytoplasm or nuclei of cells.[3,4,5,6] CPP research had its origins in the study of protein transduction domains, which established the ability to shuttle transcription factors inside and between cells, together with the observation by Frankel and Pabo[7] that the HIV-1 transcription-transactivation protein (TAT) could go into cells and translocate into the nuclei.[7] Later, in 1991, another peptide sequence demonstrated the ability to affect cells, Drosophila Antennopediahomeodomains, which in 1994, yielded the first so called CPP–Penetrating[8, 9].The following years reproduced a boom in CPP research and numerous new sequences were designed and created such as MPG–the firstCPP capable of deliver non-covalent nucleic acid[10,11]; and Pep-1 for which deliverprotein and peptide.[12,13] Afterwards, Lebleu and coworkers[14] identified Tat peptide, having the minimum, nine residues long, sequence from TAT protein required for cellular uptake, and the first evidences-of-concept for the in vivo application of CPP were knowledgeable for the delivery of Peptides and proteins in 1999.[6,15] Another important revolution was the demonstration by the groups of Wender and Futaki that octa-arginine (R8) was sufficient for prompting cellular and in vivo peptide uptake.[16,17] Ever since the discovery of Tat, researchers have been progressively increasing the pool of known CPP, with entries derivative from natural, chimeric and later synthetic sources. Together with CPP, Homing peptides (HP) are molecules that deliver their cargo to the cell surface with

cell specificity that are deficient in CPP by targeting specific markers on cell surface but with no membrane internalization. A class of CPP derivatives, CPHP (Cell-penetrating HP) that conjugate both translocation and cell specificity features of CPP and HP, respectively, was successfully considered, combining specific delivery with cell internalization ability.[18] To maximize the design of new CPP, current advances in bioinformatics gave labor to tools that allow the identification of protein regions with membrane translocation areas (i.e., CPP[19-20] to maximize the design of new effective CPP. However, if one goes deep into CPP cataloguing, one can find different categories under which they can be classified, by their physical chemical properties, by its origin or by its linkage to their cargo. In addition, due to its chemical composition and acquired secondary structure, they also can be classified into different modes of uptake. Chimeric and synthetic CPP can be attained either by modification of functional groups. Replacement lysine by argininethatenhances the affinity for the cell surface, while using histidines helps the endosomal escape by creating a "proton sponge effect".

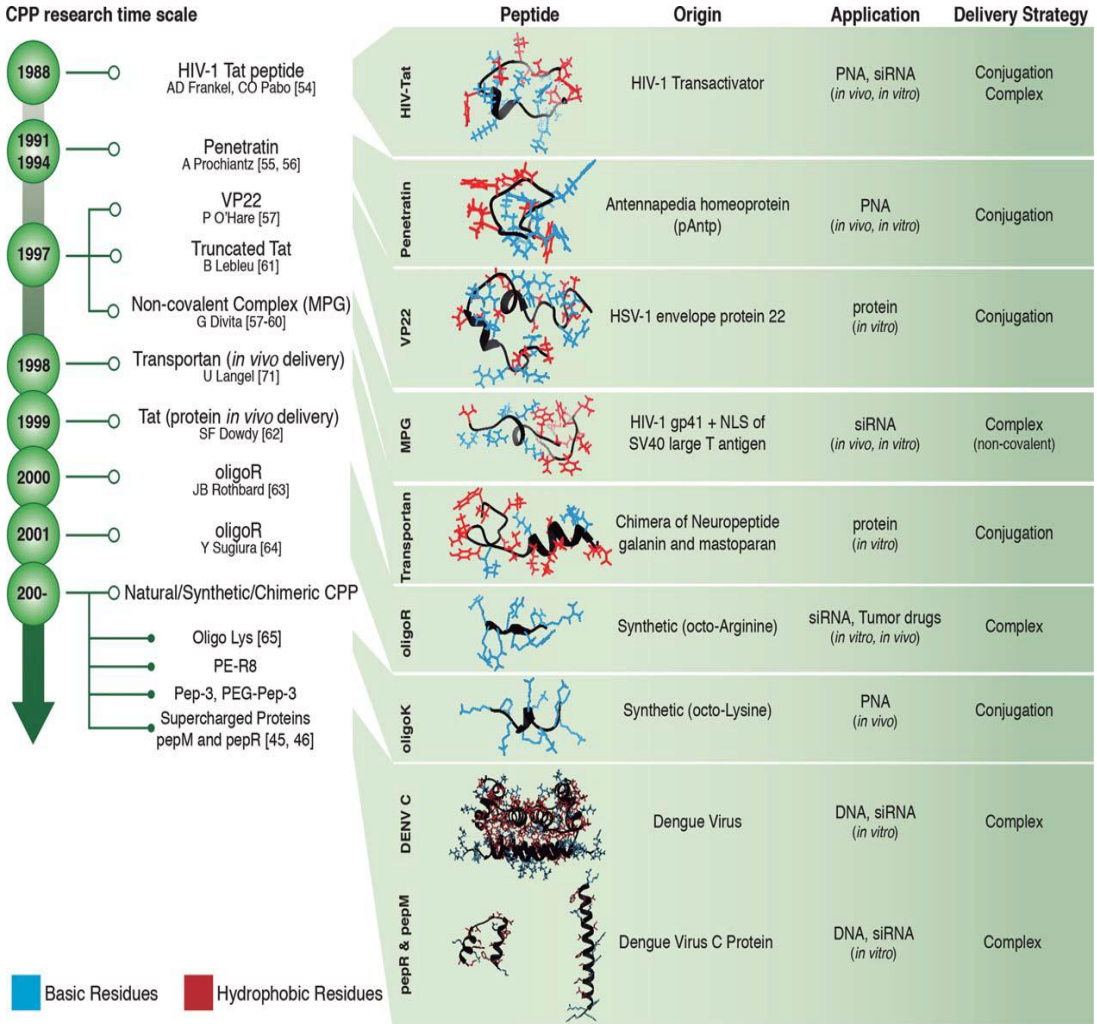
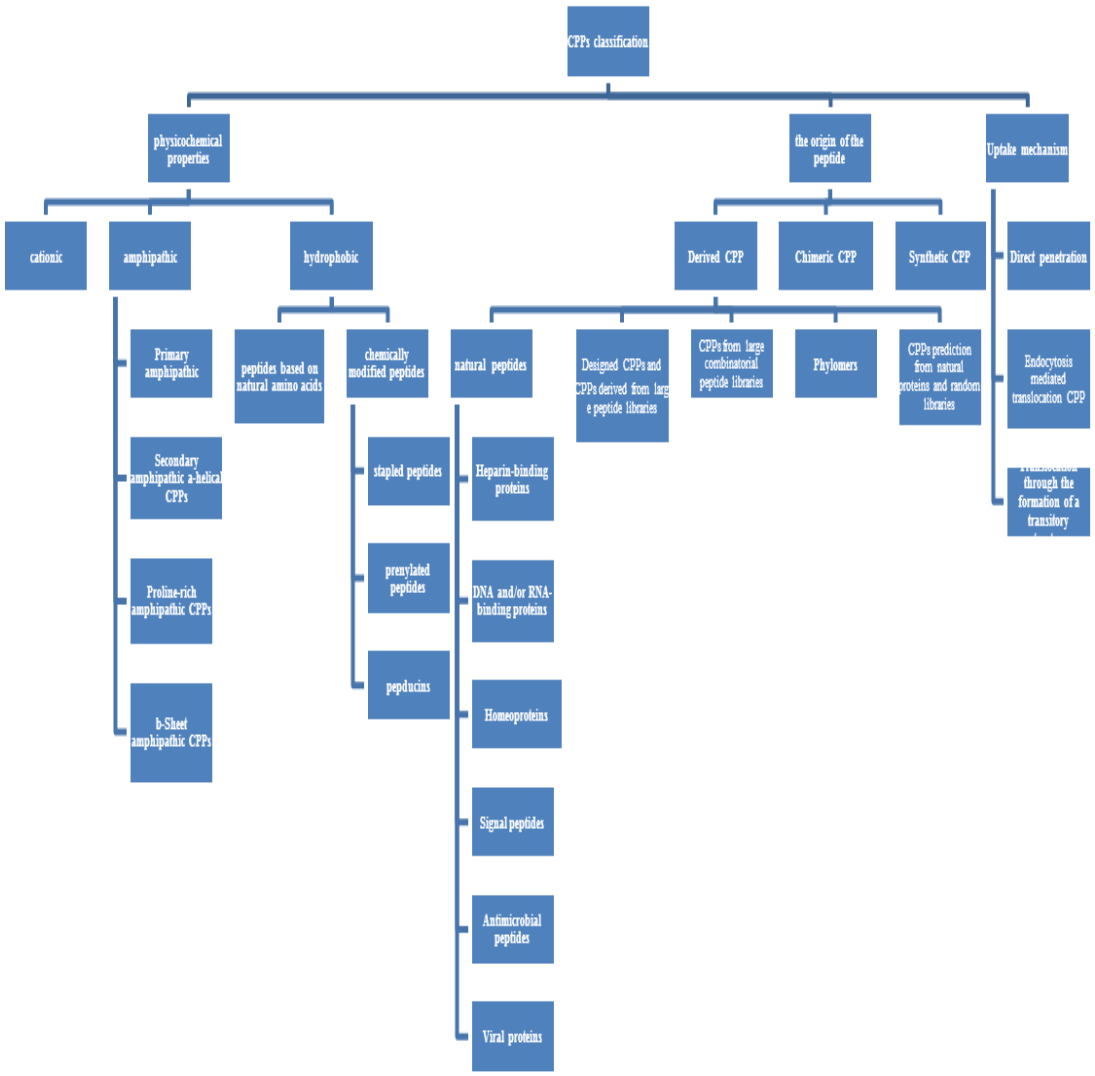


Figure 1: The Timeline of The CPP research, Left panel: primary stage discoveries that contributed to the enhancement of CPP field as well as some existing outgoing research. Right panel: examples of the greatest representative CPP discovered and developed for nucleic acid delivery are made known. Peptide name with the tridimensional structure (black–ribbon; blue–basic residues; red–hydrophobic residues) and arrangement, origin (protein/synthetic), delivery application, and strategy is providing. A: All PDB files of the CPP structures represented were obtained from CPPite[21]:HIV-Tat, VP22, MPG, OligoR: OligoK; from RCSB PDB: Penetratin–2NZZ, Transportan.1SMZ, DENV C–1R6R; or predicted: pepR and pepM[22,23]:predicted by I-TASSER

Classification of CPPs

CPPs classification based on

A. physicochemical properties
B. the origin of the peptide
C. Uptake mechanism



I. Physicochemical properties classification

Cationic CPPs

Most CPPs are cationic because of their own positive charge.[67] The first CPP identified was cationic and was derived from the HIV-1 protein Tat (RKKRRQRRR).[28] Studies on arginine-based peptides (from R3 to R12) have presented that the minimal sequence for cellular uptake is octa-arginine (R8), and that increasing the number of arginines increases the level of uptake.[25] Studies propose that at least eight positive charges are needed for effective uptake of several other cationic CPPs.[29] A special case of cationic CPPs are nuclear localization sequences (NLSs). NLSs are tiny peptides based on lysine-, arginine- or Proline-rich motifs that able to be transported to the nucleus through the nuclear pore complex, which is a multimeric complex containing 50–100 dissimilarproteins. NLSs can be classified into monopartite and bipartite signals, which consist, respectively, of one or two groups of four or more basic amino acids.[31] Due to the number of charges in most NLSs is well lowerthan eight, most NLSs are not good CPPs[32], but they can be covalently bonded to a hydrophobic peptide sequence to get an amphipathic CPP with a good uptake profile.

1. Amphipathic CPPs

Amphipathic CPPs are sequences that have non-polar and hydrophobic amino acids.

a. Primary amphipathic

Numerous primary amphipathic CPPs are chimeric peptides obtained by covalently bonding a hydrophobic domain for effective targeting to cell membranes to a NLS. For example, MPG (GLAFLGFLGAAGSTMGAWSQPKKKRKV) depend on the SV40 NLS (PKKRKV). The hydrophobic domain of MPG was created from the combination sequence of the HIV glycoprotein 41 (GALFLGFLGAAGSTMGA) which has high attractionto membranes.[33] Other primary amphipathic CPPs are entirely derived from natural proteins, such as pVEC, ARF(1–22), and BPrPr(1–28).Thusproposing that the properties of the signal peptide are also important for uptake.[33]

b. Secondary amphipathic a-helical CPPs

A common structural motif in several peptides and proteins that attached to membranes is the amphipathic □-helix, in which hydrophilic and hydrophobic amino acids are clustered in separate sides of the helix.[34] Secondary amphipathic □-helical CPPs have aextremely hydrophobic patch on one face , while on the other side can be cationic, anionic, or polar. Even though almost of amphipathic CPPs are cationic, improvements suggest that the membrane translocation is a concern of amphiphilicity and not of positive charges.[35] Furthermore, there are several other examples of anionic amphipathic CPPs: GALA (WEAALAEALAEALAEHLAEALAEALEALAA)[37], designed depend on another cationic CPP, KALA; p28, a peptide derived from Azurin[24] P28 is a helical peptide with aexpanse of hydrophobic amino acids grouped on one side of the net charge of amphipathic CPPs. It is unclear whether a least length is required for the uptake of amphipathic CPPs. Although studies on MAP[36] originally recommended that a minimum of four helix turns is critical, shorter amphipathic CPPs are known today. The grade of hydrophobicity of different amino acids should be studied further to understand the uptake of amphipathic CPPs.[38]

c. b-Sheet amphipathic CPPs

An amphipathic □-sheet peptide is createddepending on one hydrophobic and one hydrophilic stretch of amino acids exposed to the solvent. Cellular uptake studies on VT5 (DPKGDPKGVTVTVTVTVTGKGDPKPD) propose that its ability to form □-sheets is critical for uptake, given that analogs where a little residues where mutated using D-amino acids had no susceptibility to adopt a □-sheet conformation and had very poor uptake.[39]

d. Proline-rich amphipathic CPPs

Proline has several unique features among the 20 natural amino acids: it is very rigid due to of its pyrrolidine ring; its tertiary nitrogen refuse accept H-bonds like the secondary nitrogen of the other amino acids; if it is high rich in a peptide structure, in pure water it creates a well-defined secondary structure, polyproline II (PPII). PPII is a left-handed extended helix of 3.0 residues per turn, as opposite to the 3.6 residues of a standard right-handed alpha helix. Several Pro-rich CPPs have been informed. These include bactenecin- 7 (Bac7)[40], synthetically derived peptides (PPR)n and (PRR)n (where n = 3, 4, 5, and 6)[41], arginine-rich peptides origin from the PPII helix of the avian pancreatic polypeptide[42], andsyntheticallyderivativepolyproline-helix based peptides[43,44] with different R-groups attached to the pyrrolidine ring.

2. Hydrophobic CPPs

Hydrophobic peptides that whichever: contain only non-polar residues; have a small net charge (less than 20% of the sequence); or have a hydrophobic styles or chemical group that is critical for uptake regardless of the rest of the sequence.[67] Compared with cationic and amphipathic peptides, only a small number of hydrophobic CPPs have been discovered, however this maybe a mere reflection of an historical bias towards cationic and amphipathic CPPs, it were discovered previous. Indeed, it looks that random library-based methods have shown quite a few new hydrophobic CPPs.[67]

Hydrophobic CPPs are further divided into

a. peptides based on natural amino acids

This subclass contains anionic and cationic BIP penta-peptides[45–47]. Examples include PMLKE, VPALR, VSALK, IPALK, IPMLK. Those peptides does not affectsignificantly on the cellular uptake[46], opposing to what is observed for most amphipathic and cationic CPPs. The insufficient of sensitivity to sequence scrambling has also been reported for other hydrophobic peptides, like PFVYLI, which is the least cell-penetrating sequence of the longer peptide C105Y (CSIPPEVKFNKPFVYLI).[48] Recent research proposes that some hydrophobic CPPs able to translocate directly through membranes. Marks et al.[49] defined a series of 18 peptides that spontaneously translocate through synthetic lipid membranes by screening a library of 24,000 randomly selected sequences.

b. Chemically modified peptides.

1. Stapled peptides

Gained by ring-closing olefin metathesis.[50] The stapling converses cell penetration, and increases peptide helicity by rigidifying the peptide structure. Numerous publications[51,52] have shown that the stapling increases helicity to varying grades depending on the peptide structure and on the position of the staple.

2. Prenylated peptides

The adding of either a farnesyl (C15) or geranylgeranyl (C20) isoprenoid moiety, well-known as prenylation, has been reported to provide peptides inherent cell-penetrating ability over and done with an ATP independent, non-endocytic pathway.[53]

3. Pepducins

Pepducins are a subclass of N-terminally lipidated peptides that able to cross the cell membrane and attach to the cytosolic region of a multiplicity of transmembrane proteins (GPCRs, MMPs, among others).[54] The N-terminal lipidation contains either palmitoyl or other fatty acids.

Origin of the peptide classification.

A. Derived CPPs

1- CPPs derived from natural proteins or peptides.

a) Heparin-binding proteins

Heparin is a type of the glycosaminoglycan (GAG) family of carbohydrates, which contains also the closely related heparan sulfate. For several cationic CPPs, attached with GAGs is an essential step before uptake and for these reason proteins with domains that attached

withGAGs can be a source of CPPs. For example, a class of cationic CPPs named Vectocell1 was discovered from human heparin attached toproteins.[55]

b) DNA and/or RNA-binding proteins: Due to the DNA and RNA is rich with negatively charged phosphates; many DNA and RNA binding with proteins contain highly cationic motifs. Possibly because they have the same feature is also important for uptake through binding with glycosaminoglycans, numerous CPPs are derived from DNA and/or RNA binding proteins. CPPs origins from RNA binding proteins include Tat, Rev, and FHC coat.[56]

b) Homeoproteins

A unique class of DNA binding proteins arehomeoproteins, which produce a large family of transcription factors including a conserved DNA-binding motif called homeodomain.[57]

c) Signal peptides

Signal peptides[58] are small peptides of 5–30 amino acids at the N-terminus of secreted proteins.And their own function is to target the nascent protein for excretion or to specific organelles for further processing. Signal peptides are analyzed once they reach the targeted location. Even though the targeting function of signal peptides and the cell-penetrating ability of CPPs are notbased on the same mechanisms, almost of signal peptides have a hydrophobic central region that maybe confer affinity for cell membranes. With a great hydrophobic character, certain signal peptides able to cross the cell membrane using a mechanism that is endocytosisand receptor-independent.[59]

d) Antimicrobial peptides

Antimicrobial peptides (AMPs) are a sub-group of peptides, several of which were discovered from toxin venoms, with the capability to kill a diverse spectrum of microorganisms. Higher organisms secrete AMPs on epithelial surfaces or in endothelial and phagocytic cells asprotection mechanisms against infections. Most AMPs are amphipathic peptides have a net positive charge and numerous hydrophobic residues.[60]

e) Viral proteins

Many viral proteins have advanced mechanisms to gain cell entry, and numerous CPPs were derived from motifs found in viral proteins. Examples: Inv3 (TKRRITPKDVIDVRSVTTEINT) was recognized from a Mycobacterium tuberculosis membrane protein known as Mycobacterium cell entry protein (Mce1A 130–151).[61]

2- Designed CPPs and CPPs derived from large peptide libraries

While the majority of considered peptides have a characteristic amphipathic structure, peptides discovered from highdata screening on large peptide libraries are much more diverse, with several having a larger number of hydrophobic, rather than cationic amino acids, and no pure preference for amphipathic structures.

3- CPPs from large combinatorial peptide libraries: Randomized peptides libraries able to obtained through DNA encoded peptide libraries and thus enable the creation of billions of peptides. Numerous methods are available to bind peptides to their encoding DNA, such as phage display, plasmid display, micro-organism surface display and ribosome display.[62]

4- Phylomers: Phylomers are example of natural peptides encoded by natural genes of diverse bacterial genome.[63] New CPPs created from this tactic have also been reported, some cationic, others amphipathic with a totally negative charge, and others mainly hydrophobic.

5- CPPs prediction from natural proteins and random libraries

Only a little number of prediction models for CPPs have been suggested, including that by Hansen et al.[64] established based on Sandberg z descriptors for amino acids[65], and that by Sanders et al. depend on biochemical properties of peptides (such as amino acid composition, peptide length and net charge).[66]

Uptake mechanism classification

1) Direct penetration

This pathway includes interactions between positively charged CPPs, the phosphate groups on two sides of the lipid bilayer of cellular membrane, the creation of cavities in order to facilitate conversion and the direct penetration of CPPs into the cytoplasm.[68,69]

2) Endocytosis mediated translocation (energy-dependent pathway)

Through the process of endocytosis, cells captive materials from the outside of the membrane and absorb them.[68,69]

3) Translocation through the formation of a transitory structure

This mechanism is depending on the formation of the inverted micelles that are also named as aggregates of colloidal surfactants. The structure of the inverted micelles permits the peptide to be stable in a hydrophilic environment. In this exemplary, a penetrating dimer bind with the negatively-charged phospholipids that lead to the creation of an inverted micelle inside of the lipid bilayer.[68,69]

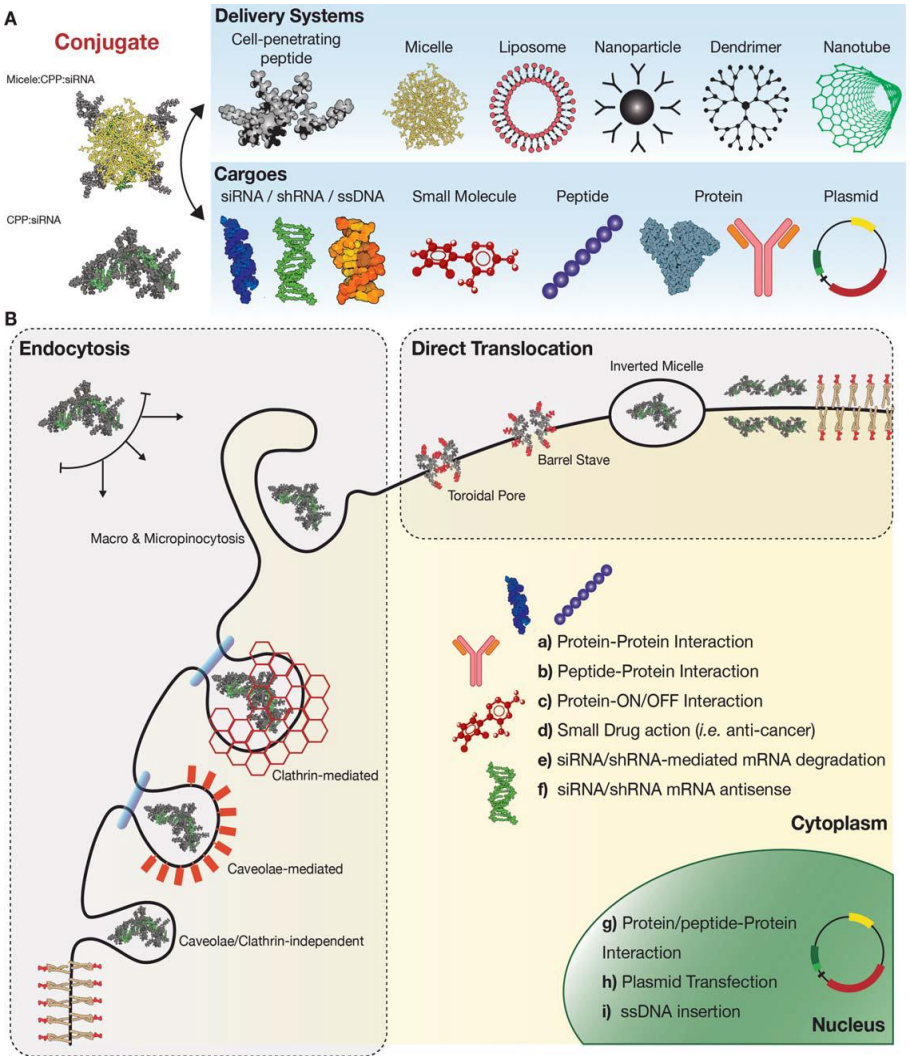


Figure 2: drug delivery technics and cellular entry mechanisms suggested. a: variability of drug delivery systems able to conjugate with diverse payloads to be reach intothe cells. the conjugation able to be covalent or non-covalent binding, combination between two or more drug delivery systems is also possible (i.e., micelles/liposomes/nanoparticles conjugated with cpp to enhance cell specificity and entry). b: delivery methods that the conjugate may take to reach to the cell cytoplasm. entry pathways range from endocytic mechanisms (clathrin-/caveolae-dependent or independent and macropinocytosis/micropinocytosis) to nonendocytic methods by different proposed lipid membrane direct translocation models: toroidal pore, barrel stave pore, reversed micelle, or carpet models. once enter the cell permitting to the payload delivered it may achieve different functions and trigger a varied range of cellular processes and reach several cellular compartments.

Importance of CPPs

CPPs can act as vectors for siRNA nucleotides, small molecules, proteins, and for other peptides, both in vitro and in vivo. Importantly, not only can a CPP be used to carry a functional peptide inside the cell, but it can also incorporate a functional motif. Until recently, transport of hydrophilic macromolecules into cells was not possible without interruption of the plasma membrane. This problem was resolved with the discovery of peptides. Cell-penetrating peptides (CPPs) provide a promising solution to the problems commonly related with drug delivery of conventional cancer chemotherapeutics as well as oligonucleotide based treatments.

CPPs as a Novel Drug Delivery System

CPPs as Nano-carriers

The impenetrability of biological membranes is a maindifficulty in drug delivery, the presence of phospholipid membrane is essential for cells survival and their function; it is a maincomplication for intracellular cargo delivery. Until recently, transport of hydrophilic macromolecules into cells was impossible without interruption of the plasma membrane. This problem was solved with the discovery of peptides. CPPs are biologically potent tools for non-persistent cellular internalization of cargo molecules. Nevertheless, the non-specificity properties of these peptides make a restriction for targeting drug delivery; so that, a peptidic-nanocarrier sensitive to matrix metalloproteinase (MMP) has been synthesized, called Activatable cell-penetrating

peptide (ACPP). Furthermore to the cell-penetrating peptide Dendrimer (DCPP), other similarities of CPPs have been synthesized.

Conjugation of nanoparticles with the cell-penetrating peptides

With the beginning of nanotechnology and its wide spectrum applications in the medical field, huge advances have been prepared in the treatment of various diseases including different types of cancers, AIDS and hepatitis.

Nanoparticles arematerials with a size in the range of 1-1000 nanometers and contain a group of compounds such as metals; semiconductor quantum dots (QDs), oxides, polymers, vesicles (e.g., micelles/liposomes), carbon-based materials (e.g., nanotubes, fullerenes and nanodiamonds) and protein- and nucleic acid-based particles.

The following we will introduces conjugated nanoparticles with CPPs and their analogues such as activatable cell penetrating peptide (ACPP) and cell-penetrating peptide dendrimer (DCPP).[70,71]

Conjugation of quantum dots with cell-penetrating peptides

QDs are fluorescent colloidal semiconductor nanocrystals. These inorganic nanoparticles, because of their remarkable properties countingwide excitation, depend on fluorescent emission on the QD composition and main size and narrow size distribution, are used broadly in drug delivery, labeling and imaging fields[72, 73]. QDs have been used for real intracellular delivery of fluorescent proteins, counting yellow fluorescent protein (YFP) and the multichromophore b-phyco-erythrin complex (b-PE).[74]

Conjugation of superparamagnetic iron oxide nanoparticles with CPPs

Superparamagnetic iron oxide nanoparticles (SPIONs), because of their valuable magnetic properties and low toxicity, have been comprehensively studied in drug delivery, gene deliveryand contrast-enhancing agents in MRI.[75-76] Furthermost available SPIONs cannot penetrate into cells; sothat, in order to advance uptake of these nanoparticles by cells, Wang et al proposed that conjugation of TAT peptide to SPIONs could increase the translocation of these nanoparticles.[77]

In some study conducted, conjugation of Ɣ-amino-proline-found cell-penetrating peptide, in a real synthetic CPP, with SPIONs indicated higher translocation of theses nanoparticles in a much HeLa and COS-1 cells in comparison to the analogue TAT–SPION. In addition, this new CPP was used to make efficient bimodal imaging.[78]

Conjugation of gold nanoparticles with CPPs

Beside metal nanoparticles, gold nanoparticles (GNPs) ordered great interest in drug delivery systems. Due to the special properties that possessed, example as induced minimum toxicity, high solubility, easy synthesis, bioconjugation, strong absorption, efficient clearance from the body and scattering, they have made an encouragement of many medical researchers[79-80].In previous relationship, GNPs have a positive charge; furthermore, they can accompany cationic CPPs in translocating into the cells by an maximum energy-independent. The α-helix peptides of 17-amino acids conjugated to gold nanoparticles played an importantrole as carriers for delivery of the anti-cancer drug doxorubicin (DOX); it has indicated that this system has much efficiency in cell-selective drug delivery than free DOX, which is associated to the cell-selective of an internal s activity of the chosen peptides.[81]

Conjugation of polymeric nanoparticles with CPPs

Chitosan (CS) is a natural cationic co-polymer that due to excellent biocompatibility, biodegradability and low cytotoxicity, has been extensively researched as a suitable carrier for drug delivery.[82,83] Therefore, application of this polymer in gene delivery has been reduced by its low gene transfection efficiency.

CPP and penetration conjugated CS as a promising non-viral vector for gene delivery.Results shown that linoleic acid and penetrating dual functional chitosan (CS-Lin-Pen), a modified CS, has been applied successfully for transfection of plasmid DNA (pDNA).[84]

The blood–brain barrier (BBB) is a great obstacle in brain drug delivery. The BBB consists of many endothelial tight junctions; Additionally, it stop the diffusion of therapeutic molecules into the central nervous system (CNS).[85,86] CPPs linked to nanoparticles have been regulate as an appealing carrier for improving brain-targeted delivery.[87,88]

It has been indicated that penetration of functional poly-(ethylene glycol) – poly (lactic acid) (PEG–PLA) nanoparticles successfully were used for brain drug delivery.The Results were revealed that PEG–PLA are coupled to the CPP reduces systemic clearance of nanoparticles. However, penetration of conjugated on the surface of nanoparticles could enhanced the cellular uptake.[89]

Conjugation of lipid nanoparticles with CPPs

Effective and appropriate internalization of few interfering RNA (siRNA) in the gene therapy depends on plasma half-life and biodistribution of siRNA, because of naked RNA is easily degraded by RNase in the living organisms. Mostly non-invasive technique in the transition of siRNA. SiRNA is trapped within nanoparticles for protection from enzymatic degradation in the cell.[90,91]

Additionally, protamine peptide coupled with cholesterol nanoparticles have been designed as an effective vector for improvement and delivery half-life and efficiency of siRNA delivery. The Interaction of CPP with the endosomal membrane in which the positive zeta potential of CPP-lipid nanoparticles may facilitate the transfer of siRNA into the cytoplasm.[92]

Bioconjugates of CPPs

The negative charge ofplasma membrane and oligonucleotides is positively-charged vector which is necessary for efficient delivery of many biomaterials. The cationic nature of CPPs may facilitates the transduction and promotes stability of nucleic sequences.[93,94]

The High molecular weights, hydrophilicity and enzymatic degradation of peptides and proteins decrease the parameters of intestinal absorption in the oral delivery of peptide and protein drugs.[95,96]

The co-administration of insulin as a peptide drug and D-R8 (D-form arginine octamer, a typical CPP) enhances intestinal absorption of the peptide drugs because of intermolecular binding between the D-R8 and the insulin.[97]

Efficient delivery of antibodies in the intracellular membrane is confined because of the hydrophobic nature and high size of these biomolecules.Hence, the antibody molecule is degraded within the lysosome; however, to prevent the lysosomal breakage, it needs to be freely into the cytoplasm by denaturing the endosome.[98]

There are two dominant approaches for efficacy entrance into the antibody fragments to target to precise compartments. The first delivery of DNA encoding for an antibody fragment within the cell andThe second is the delivery of the antibody molecule into the cytoplasm by suitable vectors.[99,100]

In addition, CPPs have been employed as a vector for introducing the biomolecules into cells.[101,102] The modulatedentry complex (a CPP derived from an N-terminal region of the X-protein of the hepatitis B virus), using an antibody and siRNA, in order to expand the uptake of antibodies and increase the capacity for killing B-raf-dependent melanoma cells,[103]

Activatable cell-penetrating peptides

CPPs, despite the have many significance, and have some limited number in vivo application due to its non-specificity. A novel strategy of CPPs, called activatable cell- penetrating peptides (ACCPs), new CPPs with high permeability, are comprised of a polycationic cell-penetrating peptide attached to the polyanionic peptide through a cleavable linker and sensitive to metalloproteinase (MMP).[104,105] As a result of high level of expression of MMP in tumor cells, ACPPs can be used for site-specific targeting and delivery of anticancer drugs. Despite MMPs are disease biomarkers which can be used to improvement of many diagnosis or application in imaging -guiding surgery with radiolabeled MMP binding ligands, such as antibodies or small molecules.[106,107]

Paclitaxel (PTX) is used in cancer chemotherapy as one of the most appropriate anti-proliferative agents that can be prevents some cell proliferation by stabilization of microtubules and tubulin polymerization, leading to cell apoptosis. Xia et al investigated ACCPs functionalized nanoparticles (NPs) with increased permeability for site-specific targeting of PTX in the tumor tissue. The findings demonstrated that PTX loaded by ACCPs-NP may have large accumulation in the tumor.[108]

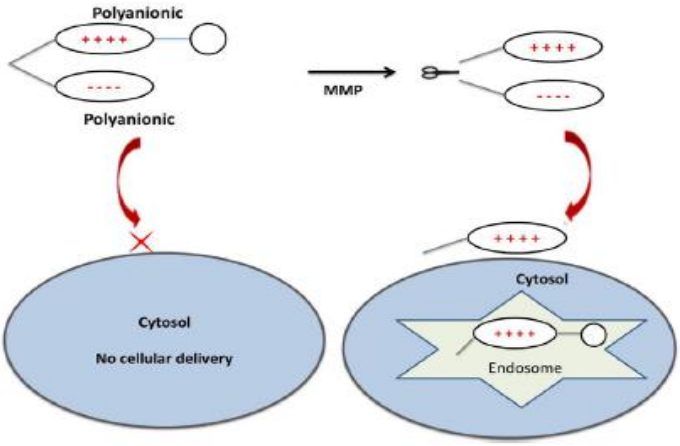


Figure 3: Demonstration of Activatable cell-penetrating peptides

Cell-penetrating peptide dendrimer (DCPP)

Despite that, the therapeutic peptides have a typically linear structure, but branched peptides are also found in nature. Large branched structure, the so-called dendrimer. Dendrimers, in comparison to many linear peptides, which have important such as resistance to rapid biodegradation, many loading capacities and large-scale production capability.[109,110]

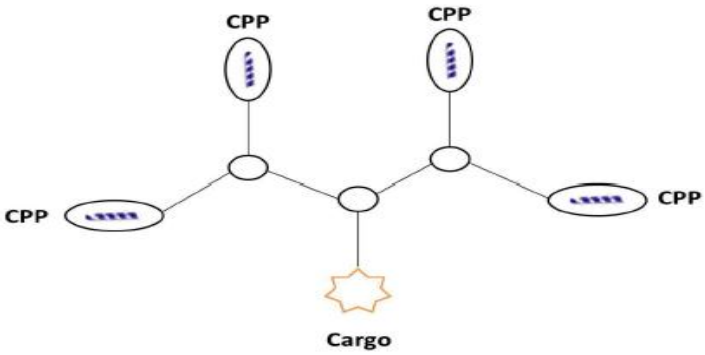


Figure 4: Cell-penetrating peptide dendrimer (DCPP)

Demonstrates the dendritic structure of DCCP. In Research conducted, polyester-based dendritic guanidine with a focal point alkyne conjugated to Fe3O4 nanoparticles has been shown significance used for enhancement of the cellular uptake of these particles. Study demonstrated that, the functionalization of Fe3O4 nanoparticles with branched guanidine led to the increased of cell uptake and the enhancement of the ability to detect the cells by MRI.[111]

Zhao et al employed different generations (G) of lysine dendrimers (G1–G3) as the carriers for delivery of anticancer drug 5-fluorouracil.[112] Flow cytometric analysis of the new peptide sequences indicated that, its have the capacity for effective translocation into cells. In addition, investigations revealed that these new CPPs have more advantages such as; stable drug release, low toxicity to normal cells, and moderate inhibition of tumor cells.

Drug Loading

Huge number of CPPs have been described so far, the can be grouped into two different classes, the first requiring chemical linkage for the drug in cellular internalization and the second involving formation of stable, non-covalent complexes with drugs. CPPs constitute very promising things for thenon-invasive cellular delivery of cargo and had been successfully applied for in vitro and in vivo delivery of therapeutic molecules differed from small molecules such as; Nucleic acids, proteins and peptides toliposomes, and nanoparticles.[113]

Clinical applications of CPPs

Cancer application

During the previous decades, the potential of peptides for drug delivery into cells has been known by the discovery of many CPPs.[114] Much number of CPP-conjugated therapies (CTTs) indicates strong promise for clinical efficacy[115]and had been employed to enhance extracellular and intracellular internalization of different small molecules and biomolecules including plasmid DNA, siRNA, oligonucleotide and peptide nucleic acid (PNA).[116] The deficient of cell specificity remains the major problems for the clinical development of CPPs.[117]

There are more strategies to selectively target cancer cells with CPPs conjugated with targeting ligands:

a. Cell targeting peptides
b. Activatable cell-penetrating peptides
c. Transducible agents.

a. Cell-Targeting Peptides

The targeted delivery by cell-targeting peptides (CTPs) together the ability to recognize cancer cells is particularly attractive for cancer therapy.[115,118] Proper Screening of phage-display libraries has resulted in the discovery of a number of homing peptides that selectively detects molecular markers on tumor blood vessels.[115,118] One such homing peptide is cyclic peptide PEGA that has shown to accumulate in breast tumor tissue in mice. PEGA peptide conjugated to the cell-penetrating peptide pVEC was taken up by another breast cancer cells in vitro. Furthermore, the capacity of the PEGA-pVEC was conserved in vivo, in which the conjugated mainly accumulates in blood vessels in breast tumor tissue or breast cancer vasculature, and later it was taken up by tumor cells.[119,120] However, the conjugation of the anticancer drug chlorambucil to pVEC-PEGA was shown to increase the drug efficacy over four times.[120] The linear five amino acid long peptide CREKA, which was identified in breast tumors in MMTV-PyMT transgenic mice, This new peptide.[115,118] CREKA-pVEC, is more efficient to synthesize and it is more convenient in translocating cargo molecules into cancer cells as compared to last the published PEGA-pVEC peptides. A recent study demonstrated that CREKA-pVEC is a suitable vehicle for targeted intracellular delivery of a DNA alkylating agent, chlorambucil, as the chlorambucil-peptide conjugate was important more effective at killing cancer cells in vitro than the anticancer drug alone.[115,118,120]

In different study, the target ligand folic acid (FA) and the cell penetrating peptide octa arginine (R8) were joined with the gene vector (PEI (600)-CyD, PC) to form nano-vectors for highly efficient gene delivery to tumor cells. The resultant ternary nano-complexes of FA-PC/R8-PC/pDNA produced excellent gene transfection abilities in folate receptor-positive tumor cells in vitro and in vivo.[115,121]

b. Activatable CPPs

The best solution to the problem of non-specificity of CPPs is activatable CPPs (ACPPs).[120] Are novel in vivo targeting agents and also a new class of ensuring molecular imaging they comprise of a polycationic cell-penetrating peptide (CPP) connected via a cleavable linker to a neutralizing polyanion.[122,123] This structure reduces the overall charge to nearly zero and inhibits electrostatic interactions and thereby inhibits uptake into cells.[115]

Many studies have demonstrated a relationship between increased matrix Metalloproteases (MMP) expression and poor clinical outcome in a number of cancers including breast (MMP-11), colon (MMP-1), gastric (MMP-2 and MMP-9), non-small cell lung cancer (MMP-13), esophageal (MMP-7), and small-cell lung cancer (MMP-3, MMP-11, and MMP-14).The synthesis of a conjugate of ACPP with the antitumor drug doxorubicin (DOX) sensitive to matrix metalloproteinase-2 and -9 (MMP2/9) has been used for tumor-targeting therapy purposes. The ACCP-DOX conjugate could be triggered by MMP-2/9, which enabled the activated CPP-DOX to enter cells. The ACCP was designed including three units: A cell-penetrating domain (polyarginine,R9), a cleavable enzyme-specific substrate domain of MMP-2/9 (PLGLAG), and an attenuating peptide domain (DGGDGGDGGDG).[124,125]

c. Transducible Agents of CPPs

Hence a number of lethal tumors are treated by local administration of chemotherapeutics, many tumors are widespread throughout the body, thus necessitating the systemic delivery of anticancer agents. Early in vivo experiments demonstrated that TAT proteins are delivered to a large number of organs after intraperitoneal (IP) injection, suggesting that systemic delivery to a primary tumor or to many metastases should be feasible with transducible agents.[126] In order to develop a potential therapeutic protein drug highly specific for solid tumors, a fusion protein selectively stabilized in hypoxic tumor cells was constructed.[127]

Application of CPPs in Hepatitis B

a. CPP-PNA Conjugates on DHBV Replication

The Advancement in novel therapeutic agents are now capable to inhibit viral replication is a big challenge for the treatment of chronic hepatitis B in future, for the reason that the encapsidation signal" is an important part in the initiation of HBV reverse transcription.[128,129] scrutinizing the ability of an antisense PNA targeting this "to inhibit viral replication in the DHBV infection model.[130]

It can efficiently block viral reverse transcription in a sequence-specific manner. But the main disadvantages in the use of PNA as antiviral agents is their poor transport across the cell membrane, the bioavailability of PNA alone or coupled to a CPP (CPP-PNA conjugate) in vivo is first evaluated in the duck model. Thus fluorescein-labeled PNA targeting DHBV", which was conjugated to D-oligoarginine (FITC-PNA-CPP), was directed into ducklings and the fluorescence was observed in the cells. We have spotted the presence of hepatocytes-associated fluorescence, showing that this FITC-PNA-CPP was able to reach the duck liver.[131]

These results show that conjugation of antisense PNA to CPPs permits flourishing intracellular delivery of PNA which was able to block hepadna-viral replication. Prominently, the antiviral activity of various CPPs may impede with antisense specificity of CPP-PNA conjugates. Thus, results highlight that the choice of CPPs used as carrier for intracellular delivery of PNAs can possibly play a critical role in the antiviral specificity of their cargos.[131]

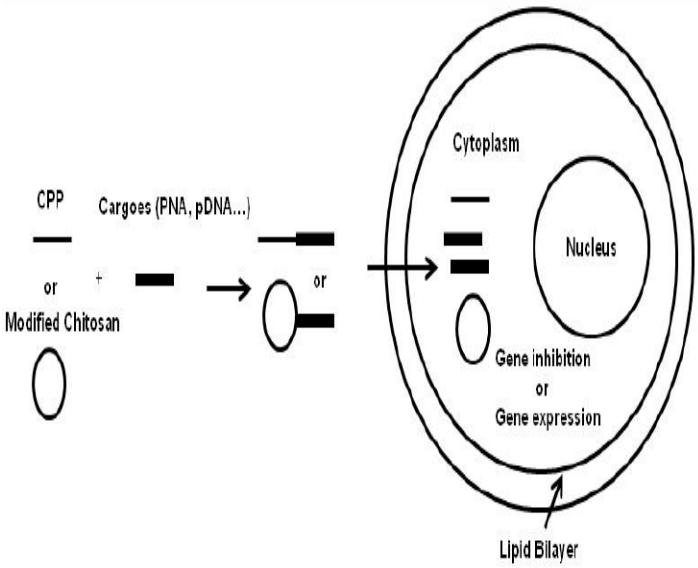


Figure 5: Non-viral gene delivery systems based on CPPs or modified chitosan (CS).

f) **Modified CPPs (CatLip) as Novel Anti-HBV Agents**

CPPs have high biological activity, although their antibacterial activity potentially has been more intensively studied than antiviral activity[132]. The antiviral activities of CPPs have been associated to the interference with both viral adsorption and entry process of different enveloped viruses such as Herpes Simplex Virus (HSV), Vesicular stomatitis Virus (VSV) and Human Immunodeficiency Virus (HIV).[132,133] Data highlights that CatLip, resulting from chemical conjugation of CPPs to a lipid domain (such as fatty acids), have better cellular uptake andendosomal escape[134,135].New discovery of anti-DHBV activity exhibited by oligoarginine, which optimize us to evaluate different CatLip for their ability to block viral replication in vitro, in primary duck hepatocytes cells (PDHs) and in firmly DHBV-transfected chicken hepatoma cells (LMH-D2).[136]

Results recommend that Decanoyl-(DArg)8 is also capable to inhibit, in a dose-dependent method, human HBV morphogenesis. Significantly, when we compare it in contrast to current antiviral drugs that block HBV polymerase leading to resistant variant surfacing, Decanoyl-(DArg)8, by targeting an additional step of viral life cycle, may signify a novel class of therapeutics for chronic hepatitis B treatment. In addition due to the increase immunogenicity of core antigen, the secretion of high quantity of naked nucleocapsids generated via Decanoyl-(DArg)8 treatment may be valuable for immune responses stimulation in HBV patients. Figure 6.

Different CPPs and their therapeutic potential against hepatitis B.

CPPs	Cargo	Cells/Animal Models	DHBV/HBV Infection	Bilogical Activities
$(DArg)_8$	PNA	PDHs, Peking Ducklings	DHBV	Inhibition of DHBV replication
Decanoyl-$(DArg)_8$	-	PDHs, LMH-D2, HepG2.2.15	DHBV/HBV	Drastic Inhibition of late stages of DHBV replication Inhibition of HBV release

Antimicrobial Properties of CPPs

CPPs share a huge number of features with AMP starting with their chemical structure, which elucidates their bond with cell membranes and why some AMPs can be used as CPP, and vice-versa; CPP have AMP usages.

Anti-inflammatory Properties of CPPs

Inflammation plays a role in a variety of diseases, from infections to cutaneous, alergological, and rheumatologic diseases, and is interceded by mostly intracellular pathways of cells of the innate system. Inactivating these pathways has been the purpose of many therapies such as the suppression of TNF α and IL 6, proinflammatory cytokines, with the KAFAK peptide, delivered by nanoparticles or the inhibition of NF-κB by the AIP6 peptide, which has both anti-inflammatory and CPP features.

Concluding remarks

Probably, the chemotherapy will be the first field to get benefit from the utilization of CPPs. Subsequently the targeted delivery systems, activatable CPP can be prepared and also we can develop new therapeutic targets for treatment. CPPs are extremely prospective candidates for therapeutic delivery of siRNA; in theory one single effective peptide sequence could be used to transfer hundreds of different siRNAs, facilitating therapeutic targets as well as an option of personalized cancer treatments. The low toxicity of peptide based delivery system will be a key advantage of this treatment preference, when it is compared to many other means of drug delivery such as mainly in free drugs or in the liposome formulation. CPPs have also a capacity to play a role in combined delivery systems based on other types of nanoparticles or on liposomal dosage forms. Nanoparticles modified with CPPs could potentially have improved cellular uptake, allow crossing of the blood-brain barrier. For these problems, CPP-based technology goes on to draw the attention of organizations around the world so that one day their full potential might be recognized.

REFERENCES

1. Pooga, M., Soomets, U., Hällbrink, M., Valkna, A., Saar, K., et al. Cell penetrating PNA constructs regulate galanin receptor levels and modify pain transmission in vivo. Nat Biotechnol, 1998; 16: 857–861.
2. Jones, A. T., and Sayers, E. J. Cell entry of cell penetrating peptides: tales of tails wagging dogs. J. Control. Release, 2012; 161: 582–591.
3. Milletti, F. Cell-penetrating peptides: classes, origin, and current landscape. Drug Discov. Today, 2012; 17: 850–860.
4. Heitz, F., Morris, M. C., and Divita, G. Twenty years of cell-penetrating peptides: from molecular mechanisms to therapeutics. Brit. J. Pharmacol, 2009; 157: 195–206.
5. Bechara, C., and Sagan, S. Cell-penetrating peptides: 20 years later, where do we stand? FEBS Lett, 2013; 587: 1693–1702.
6. Lehto, T., Kurrikoff, K., and Langel, Ü. Cell-penetrating peptides for the delivery of nucleic acids. Expert Opin. Drug Deliv, 2012; 9: 823–836.

7. Frankel, A. D., and Pabo, C. O. Cellular uptake of the tat protein from human immunodeficiency virus. Cell, 1988; 55: 1189–1193.
8. Joliot, A., Pernelle, C., Deagostini-Bazin, H., and Prochiantz, A. Antennapediahomeobox peptide regulates neural morphogenesis. Proc. Natl. Acad. Sci. USA, 1991; 88: 1864–1868.
9. Derossi, D., Joliot, A. H., Chassaing, G., and Prochiantz, A. The third helix of the Antennapediahomeodomaintranslocates through biological membranes. J. Biol. Chem, 1994; 269: 10444–10450.
10. Morris, M. C., Vidal, P., Chaloin, L., Heitz, F., and Divita, G. A new peptide vector for efficient delivery of oligonucleotides into mammalian cells. Nucleic Acids Re., 1997; 25: 2730–2736.
11. Morris, M. C., Chaloin, L., Méry, J., Heitz, F., and Divita, G. A novel potent strategy for gene delivery using a single peptide vector as a carrier. Nucleic Acids Res., 1999; 27: 3510–3517.
12. Morris, M. C., Depollier, J., Mery, J., Heitz, F., and Divita, G. A peptide carrier for the delivery of biologically active proteins into mammalian cells. Nat Biotechnol, 2001; 19: 1173–1176.
13. Morris, M. C., Deshayes, S., Heitz, F., and Divita, G. Cell-penetrating peptides: from molecular mechanisms to therapeutics. Biol. Cell, 2008; 100: 201–217.
14. Vivès, E., Brodin, P., and Lebleu, B. A truncated HIV-1 Tat protein basic domain rapidly translocates through the plasma membrane and accumulates in the cell nucleus. J. Biol. Chem., 1997; 272: 16010–16017.
15. Schwarze, S. R. In vivo protein transduction: delivery of a biologically active protein into the mouse. Science, 1999; 285: 1569–1572.
16. Wender, P. A., Mitchell, D. J., Pattabiraman, K., Pelkey, E. T., Steinman, L., et al. The design, synthesis, and evaluation of molecules that enable or enhance cellular uptake: peptoid molecular transporters. Proc. Natl. Acad. Sci. USA, 2000; 97: 13003–13008.
17. Futaki, S., Suzuki, T., Ohashi, W., Yagami, T., Tanaka, S., et al. Arginine-rich peptides an abundant source of membrane-permeable peptides having potential as carriers for intracellular protein delivery. J. Biol. Chem., 2001; 276, 5836–5840.
18. Svensen, N., Walton, J. G. A., and Bradley, M. Peptides for cell-selective drug delivery. Trends Pharmacol. Sci., 2012; 33: 186–192.
19. Sanders, W. S., Johnston, C. I., Bridges, S. M., Burgess, S. C., and Willeford, K. O. Prediction of cell penetrating peptides by support vector machines. PLoSComput. Biol., 2011; 7: e1002101.
20. Holton, T. A., Pollastri, G., Shields, D. C., and Mooney, C. CPPpred: prediction of cell penetrating peptides.Bioinformatics, 2013; 29: 3094–3096.
21. Gautam, A., Singh, H., Tyagi, A., Chaudhary, K., Kumar, R., et al. CPPsite: a curated database of cell penetrating peptides. Database, 2012; 2012: bas015.
22. Freire, J. M., Veiga, A. S., Conceição, T. M., Kowalczyk, W., Mohana-Borges, R., et al. Intracellular nucleic acid delivery by the supercharged dengue virus capsid protein. PLoS ONE, 2013; 8: e81450.
23. Freire, J. M., Veiga, A. S., Rego de Figueiredo, I., de la Torre, B. G., Santos, N. C. et al. Nucleic acid delivery by cell penetrating peptides derived from dengue virus capsid protein: design and mechanism of action. FEBS J., 2014; 281: 191–215.
24. Taylor, B.N. et al. Noncationic peptides obtained from azurin preferentially enter cancer cells. Cancer Res., 2009; 69, 537–546.
25. Tu¨nnemann, G. et al. Live-cell analysis of cell penetration ability and toxicity of oligo-arginines. J. Pept. Sci., 2008; 14, 469–476.
26. Verdurmen, W.P.R. et al. Cationic cell-penetrating peptides induce ceramide formation via acid sphingomyelinase: implications for uptake. J. Control. Release, 2010; 147: 171–179.
27. Sasaki, Y. et al. Cell-penetrating peptide-conjugated XIAP-inhibitory cyclic hexapeptides enter into Jurkat cells and inhibit cell proliferation. FEBS J. 2008; 275: 6011–6021.
28. Green, M. et al. 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.
29. Futaki, S. et al. Arginine-rich peptides. J. Biol. Chem., 2001; 276: 5836–5840.
30. Prochiantz, A. Getting hydrophilic compounds into cells: lessons from homeopeptides. Curr. Opin. Neurobiol, 1996; 6: 629–634
31. Ragin, A.D. et al. Cellular import mediated by nuclear localization signal peptide sequences. Chem. Biol., 2002; 9: 943–948.
32. Mueller, J. et al. Comparison of cellular uptake using 22 CPPs in 4 different cell lines. Bioconjugate Chem., 2008; 19, 2363–2374.
33. Oglecka, K. et al. Relevance of the N-terminal NLS-like sequence of the prion protein for membrane perturbation effects. Biochim. Biophys. Acta: Biomembr, 2008; 1778: 206–213.
34. Auger, I. Computational techniques to predict amphipathic helical segments. In The Amphipathic Helix (Epand, R., ed.), 1993; 8–19, CRC Press
35. Oehlke, J. et al. Cellular uptake of an [alpha]-helical amphipathic model peptide with the potential to deliver polar compounds into the cell interior nonendocytically. Biochim. Biophys. Acta: Biomembr, 1998; 1414: 127–139.
36. Scheller, A. et al. Structural requirements for cellular uptake of a-helical amphipathic peptides. J. Pept. Sci., 1999; 5: 185–194.
37. Li, W. et al. GALA: a designed synthetic pH-responsive amphipathic peptide with applications in drug and gene delivery. Adv. Drug Deliv. Rev., 2004; 56: 967–985.

38. Eisenberg, D. et al. The helical hydrophobic moment: a measure of the amphiphilicity of a helix. Nature, 1982; 299; 371–374.
39. Oehlke, J. et al. Extensive cellular uptake into endothelial cells of an amphipathic [beta]-sheet forming peptide. FEBS Lett., 1997; 415: 196–199.
40. Sadler, K. et al. Translocating proline-rich peptides from the antimicrobial peptide bactenecin 7. Biochemistry, 2002; 41: 14150–14157.
41. Daniels, D.S. and Schepartz, A. intrinsically cell-permeable miniature proteins based on a minimal cationic PPII motif. J. Am. Chem. Soc., 2007; 129: 14578–14579.
42. Smith, B.A. et al. minimally cationic cell-permeable miniature proteins via ahelical arginine display. J. Am. Chem. Soc., 2008; 130: 2948–2949.
43. Fillon, Y.A. et al. Cell penetrating agents based on a polyproline helix scaffold. J. Am. Chem. Soc., 2005; 127: 11798–11803.
44. Geisler, I. and Chmielewski, J. Cationic amphiphilicpolyproline helices: side-chain variations and cell-specific internalization. Chem. Biol. Drug Des, 2009; 73: 39–45.
45. Gomez, J.A. et al. Bax-inhibiting peptides derived from Ku70 and cellpenetratingpentapeptides. Biochem. Soc. Trans, 2007; 35: 797–801.
46. Gomez, J.A. et al. Cell-penetrating penta-peptides (CPP5s): measurement of cell entry and protein-transduction activity. Pharmaceuticals, 2010; 3: 3594–3613.
47. Yoshida, T. et al. Bax-inhibiting peptide derived from mouse and rat Ku70. Biochem. Biophys. Res. Commun, 2004; 321: 961–966.
48. Rhee, M. and Davis, P. Mechanism of uptake of C105Y, a novel cellpenetrating peptide. J. Biol. Chem., 2006; 281: 1233–1240.
49. Marks, J.R. et al. Spontaneous membrane-translocating peptides by orthogonal high-throughput screening. J. Am. Chem. Soc., 2011; 133: 8995–9004.
50. Schafmeister, C.E. et al. An all-hydrocarbon cross-linking system for enhancing the helicity and metabolic stability of peptides. J. Am. Chem. Soc., 2000; 122: 5891–5892.
51. Bernal, F. et al. Reactivation of the p53 tumor suppressor pathway by a stapled p53 peptide. J. Am. Chem. Soc., 2007; 129: 2456–2457.
52. Walensky, L.D. et al. Activation of apoptosis in vivo by a hydrocarbon-stapled BH3 helix. Science, 2004; 305: 1466–1470.
53. Ochocki, J.D. et al. Evaluation of a cell penetrating prenylated peptide lacking an intrinsic fluorophore via in situ click reaction. Bioorg. Med. Chem. Lett., 2011; 21: 4998–5001.
54. Covic, L. et al. Activation and inhibition of G protein-coupled receptors by cell-penetrating membrane-tethered peptides. Proc. Natl. Acad. Sci U. S. A., 2002; 99: 643–648
55. De Coupade, C. et al. Novel human-derived cell-penetrating peptides for specific subcellular delivery of therapeutic biomolecules. Biochem. J., 2005; 390: 407–418
56. Ziegler, A. and Seelig, J. High affinity of the cell-penetrating peptide HIV-1 Tat-PTD for DNA. Biochemistry, 2007; 46: 8138–8145.
57. Gehring, W.J. et al. Homeodomain proteins. Annu. Rev. Biochem, 1994; 63: 487–526.
58. Gouridis, G. et al. Signal peptides are allosteric activators of the protein translocase. Nature, 2009; 462: 363–367.
59. Chaloin, L. et al. Conformations of primary amphipathic carrier peptides in membrane mimicking environments. Biochemistry, 1997; 36: 11179–11187.
60. Splith, K. and Neundorf, I. Antimicrobial peptides with cell-penetrating peptide properties and vice versa. Eur. Biophys. J., 2011; 40: 387–397.
61. Lu, S. et al. A cell-penetrating peptide derived from mammalian cell uptake protein of Mycobacterium tuberculosis. Anal. Biochem, 2006; 353: 7–14
62. Sidhu, S.S. and Weiss, G.A. DNA-encoded peptide libraries and drug discovery. In Anticancer Drug Development (Kerr, D.J., ed.), 2002; 237–248. Academic Press
63. Watt, P.M. Screening for peptide drugs from the natural repertoire of biodiverse protein folds. Nat. Biotechnol, 2006; 24: 177–183.
64. Hansen, M. et al. Predicting cell-penetrating peptides. Adv. Drug Deliv. Rev., 2008; 60: 572–579.
65. Sandberg, M. et al. New chemical descriptors relevant for the design of biologically active peptides. A multivariate characterization of 87 amino acids. J. Med. Chem., 1998; 41: 2481–2491.
66. Sanders, W.S. et al. Prediction of cell penetrating peptides by support vector machines. PLoSComput. Biol., 2011; 7. E1002101
67. Deshayes S, Heitz A, Morris MC, Charnet P, Divita G, Heitz F. Insight into the mechanism of internalization of the cell-penetrating carrier peptide Pep-1 through conformationalanalysis. Biochemistry, 2004; 43: 1449–57. doi: 10.1021/bi035682s
68. Deshayes S, Morris M, Divita G, Heitz F. Cell-penetrating peptides: Tools for intracellular delivery of therapeutics. Cell Mol Life Sci., 2005; 62: 1839–49. doi: 10.1007/s00018-005-5109-0
69. Snyder E, Dowdy S. Recent advances in the use of protein transduction domains for the delivery of peptides, proteins and nucleic acids in vivo. Expert Opin Drug Deliv, 2005; 2: 43-51. doi: 10.1517/17425247.2.1.43
70. Danhier F, Ansorena E, Silva J, Coco R, Le Breton A, Preat V. PLGA-based nanoparticles: an overview of biomedical applications. J Control Release, 2012; 161: 505-22. doi: 10.1016/j. jconrel.2012.01.043
71. Nitta S, Numata K. Biopolymer-based nanoparticles for drug/gene delivery and tissue engineering. Int J Mol Sci., 2013; 14: 1629-54. doi: 10.3390/ijms14011629

72. Akinfieva O, Nabiev I, Sukhanova A. New directions in quantum dot-based cytometry detection of cancer serum markers and tumor cells. Crit Rev Oncol/Hematol., 2013; 86: 1-14. doi: 10.1016/j. critrevonc.2012.09.004
73. Hild W, Breunig M, Goepferich A. Quantum dots - Nano-sized probes for the exploration of cellular and intracellular targeting. Europ J Pharm Liu R, Huang Y, Aronstam R. Intracellular Delivery of Nanoparticles and DNAs by IR9 Cell-penetrating Peptides. PLoS One, 2013; 8: 64205-17. doi: 10.1371/journal.pone.0064205
74. Wang C, Qiao L, Zhang Q, Yan H, Liu K. Enhanced cell uptake of superparamagnetic iron oxide nanoparticles through direct chemisorption of FITC-Tat-PEG600-b-poly(glycerol monoacrylate). Int J Pharm, 2012; 430: 372-80. doi: 10.1016/j. ijpharm.2012.04.035
75. Song H, Yang J, Lo S, Wang Y, Fan W, Tang X, et al. Gene transfer using self-assembled ternary complexes of cationic magnetic nanoparticles, plasmid DNA and cell-penetrating Tat peptide. Biomaterials, 2010; 31: 769-78. doi: 10.1016/j. biomaterials.2009.09.085
76. Wang C, Qiao L, Zhang Q, Yan H, Liu K. Enhanced cell uptake of superparamagnetic iron oxide nanoparticles through direct chemisorption of FITC-Tat-PEG600-b-poly(glycerol monoacrylate). Int J Pharm, 2012; 430: 372-80. doi: 10.1016/j. ijpharm.2012.04.035
77. Silvia Cavalli S, Carbajo D, Acosta M, Lope-Piedrafita S, Candiota A. Efficient c-amino-proline-derived cell penetrating peptide– superparamagnetic iron oxide nanoparticle conjugates via aniline-catalyzed oxime chemistry as bimodal imaging nanoagentsw. ChemCommun, 2012; 48: 5322-24. doi: 10.1039/C2CC17937G
78. Egusa S, Ebrahem Q, Mahfouz R, Saunthararajah Y. Ligand exchange on gold nanoparticles for drug delivery and enhanced therapeutic index evaluated in acute myeloid leukemia models. ExpBiol Med., 2014; 30: 1553-70. doi: 10.1177/1535370214536648
79. You J, Zhou J, Zhou M, Liu Y, Robertson J, Liang D. Pharmacokinetics, clearance, and biosafety of polyethylene glycol-coated hollow gold nanospheres. Part FibreToxicol, 2014; 11: 26-33.
80. Park H, Tsutsumi H, Mihara H. Cell penetration and cell-selective drug delivery using a-helix peptides conjugated with gold nanoparticles. Biomaterials, 2013; 34: 4872-79. doi: 10.1016/j. biomaterials.2013.03.049
81. Wang C, Ravi S, Garapati U, Das M, Howell M, Mallela J. Multifunctional Chitosan Magnetic-Graphene (CMG) Nanoparticles: a Theranostic Platform for Tumor-targeted Co-delivery of Drugs, Genes and MRI Contrast Agents. J Mater Chem B Mater Biol Med., 2013; 1: 4396-405. doi: 10.1039/C3TB20452A
82. Battogtokh G, Ko Y. Self-assembled Chitosan-Ceramide Nanoparticle for Enhanced Oral Delivery of Paclitaxel. Pharm Res., 2014; 14: 14-8. doi: 10.1007/s11095-014-1395-2
83. Layek B, Singh J. Cell penetrating peptide conjugated polymeric micelles as a high performance versatile nonviral gene carrier. Biomacromolecules., 2013; 14: 4071-81. doi: 10.1021/bm401204n
84. Li G, Simon M, Cancel L, Shi Z, Ji X, Tarbell J. Permeability of endothelial and astrocyte cocultures: in vitro blood-brain barrier models for drug delivery studies. Ann Biomed Eng., 2010; 38: 2499- 511. doi: 10.1007/s10439-010-0023-5
85. Banks W. Delivery of peptides to the brain: emphasis on therapeutic development. Biopolymers, 2008; 90: 589-94. doi: 10.1002/bip.20980
86. Popov M, Abu Hammad I, Bachar T, Grinberg S, Linder C, Stepensky D. Delivery of analgesic peptides to the brain by nano-sized bolaamphiphilic vesicles made of monolayer membranes. Eur J Pharm Biopharm., 2013; 85: 381-89. doi: 10.1016/j.ejpb.2013.06.005
87. Demeule M, Beaudet N, Regina A, Besserer-Offroy E, Murza A, Tetreault P. Conjugation of a brain-penetrant peptide with neurotensin provides antinociceptive properties. J Clin Invest, 2014; 124: 1199-213.
88. Xia H, Gao X, Gu G, Liu Z, Hu Q, Tu Y. Penetratin-functionalized PEG-PLA nanoparticles for brain drug delivery. Int J Pharm, 2012; 436: 840-50. doi: 10.1016/j.ijpharm.2012.07.029
89. Joo M, Yhee J, Kim S, Kim K. The potential and advances in RNAi therapy: Chemical and structural modifications of siRNA molecules and use of biocompatible nanocarriers. J Control Release, 2014; 24: 333-37. doi: 10.1016/j.jconrel.2014.05.030
90. Fu G, Pan J, Lin N, Hu H, Tang W, Xu J, et al. siRNA Against KIR3DL1 as a Potential Gene Therapeutic Agent in Controlling HIV-1 Infection. Viral Immunol, 2014; 16: 16-22. doi: 10.1089/ vim.2013.0126
91. Tang Z, Zhou Y, Sun H, Li D, Zhou S. Biodegradable magnetic calcium phosphate nanoformulation for cancer therapy. Eur J Pharm Biopharm, 2014. doi: 10.1016/j.ejpb.2014.01.003
92. Lee S, Castagner B, Leroux J. Is there a future for cell-penetrating peptides in oligonucleotide delivery ?Eur J Pharm Biopharm, 2013; 85: 5-11. doi: 10.1016/j.ejpb.2013.03.021
93. Rytkonen J, Arukuusk P, Xu W, Kurrikoff K, Langel U, Lehto V. Porous silicon-cell penetrating peptide hybrid nanocarrier for intracellular delivery of oligonucleotides. Mol Pharm, 2014; 11: 382-90. doi: 10.1021/mp4002624
94. Astriab-Fisher A, Sergueev D, Fisher M, Shaw B. Conjugates of antisense oligonucleotides with the TAT and antennapedia cell-penetrating peptides: effects on cellular uptake, binding to target sequences, and biologic actions. Pharm Res., 2002; 19: 744-54. doi: 10.1023/A:1016136328329

95. Lee S, Castagner B, Leroux JC. Is there a future for cell-penetrating peptides in oligonucleotide delivery ?Eur J Pharm Biopharm, 2013; 85: 5-11. doi: 10.1016/j.ejpb.2013.03.021
96. Noriyasu K, Mariko M, Kozo T. Importance of intermolecular interaction on the improvement of intestinal therapeutic peptide/ protein absorption using cell-penetrating peptides. J Control Release, 2009; 136: 179-86. doi: 10.1016/j.jconrel.2009.02.015
97. Heng B, Cao T. Making cell-permeable antibodies (Transbody) through fusion of protein transduction domains (PTD) with single chain variable fragment (scFv) antibodies: Potential advantages over antibodies expressed within the intracellular environment (Intrabody). Med Hypotheses, 2005; 64: 1105-8. doi: 10.1016/j. mehy.2005.01.011
98. Chen L, Liu J, Yu X, He M, Pei X, Tang Z. The biocompatibility of quantum dot probes used for the targeted imaging of hepatocellular carcinoma metastasis. Biomaterials, 2008; 29: 4170-76. doi: 10.1016/j.biomaterials.2008.07.025
99. Nida D, Rahman M, Carlson K, Richards-Kortum R, Follen M. Fluorescent nanocrystals for use in early cervical cancer detection. GynecolOncol, 2005; 99: 89-94. doi: 10.1016/j.ygyno.2005.07.050
100. Jeyarajan S, Xavier J, Rao N, Gopal V. Plasmid DNA delivery into MDA-MB-453 cells mediated by recombinant Her-NLS fusion protein. Int J Nanomedicine, 2010; 5: 725-33.
101. Kobayashi N, Niwa M, Hao Y, Yoshida T. Nucleolar localization signals of LIM kinase 2 function as a cell-penetrating peptide. Protein PeptLett, 2010; 17: 1480-88.
102. Malhotra M, Tomaro-Duchesneau C, Saha S, Kahouli I, Prakash S. Development and characterization of chitosan-PEG-TAT nanoparticles for the intracellular delivery of siRNA. International journal of Nanomedicine, 2013; 8: 2041.
103. Silhol M, Tyagi M, Giacca M, Lebleu B, Vivès E. Different mechanisms for cellular internalization of the HIV-1 Tat-derived cell penetrating peptide and recombinant proteins fused to Tat. Europ J Biochem, 2002; 269: 494-501. doi: 10.1046/j.0014-2956.2001.02671.x
104. Caminade A, Laurent R, Majoral J. Characterization of Dendrimers. Adv Drug Delivery Rev, 2005; 57: 2130-46. doi: 10.1016/j.addr.2005.09.011
105. Faust A, Waschkau B, Waldeck J. synthesis and evaluation of a novel hydroxamate based fluorescent photoprobe for imaging of matrix metalloproteinases. Bioconjug Chem, 2009; 20: 904-12. doi: 10.1021/bc8004478
106. Schafers M, Schober O, Hermann S. Matrix-metalloproteinases as imaging targets for inflammatory activity in atherosclerotic plaques. J Nucl Med., 2010; 51: 663-66. doi: 10.2967/jnumed.109.065698
107. Xia H, Gu G, Hu Q, Liu Z, Jiang M. Activatable cell penetrating peptide-conjugated nanoparticles with enhanced permeability for site-specific targeting delivery of anticancer drug. Bioconjugate chemistry, 2013; 24: 419-30. doi: 10.1021/bc300520t
108. Medina S, El-Sayed M. Dendrimers as Carriers for Delivery of Chemotherapeutic Agents. Chem Rev., 2009; 109: 3141-57. doi: 10.1021/cr900174j
109. Gillies E, Frechet J. Dendrimers and Dendritic Polymers in Drug Delivery. Drug Discov Today, 2005; 10: 35-43. doi: 10.1016/S1359-6446(04)03276-3
110. Martin A, Bernas L, Rutt B, Foster P. Enhanced Cell Uptake of Superparamagnetic Iron Oxide Nanoparticles Functionalized with Dendritic Guanidines. Bioconjugate chemistry, 2008; 19: 2375–84. doi: 10.1021/bc800209u
111. Zhao J, Zhou R, Fu X, Ren W. Cell-Penetrable Lysine Dendrimers for Anti-Cancer Drug Delivery: Synthesis and Preliminary Biological Evaluation. Arch Pharm Chem Life Sci., 2014; 347: 1-9. doi: 10.1002/ardp.201300415
112. Heitz, F.; Morris, M.C.; Divita, G. Twenty years of cell penetrating peptides: From molecularmechanisms to therapeutics. Br. J. Pharmacol, 2009; 157: 195–206.
113. Yamagata, M.; Kawano, T.; Shiba, K.; Mori, T.; Katayama, Y.; Niidome, T. Structural advantage of dendritic poly(L-lysine) for gene delivery into cells. Bioorg. Med. Chem, 2007; 15: 526–532.
114. Bolhassani, A. Potential efficacy of cell-penetrating peptides for nucleic acid and drug delivery incancer. Biochim. Biophys. Acta, 2011; 1816: 232–246.
115. Munyendo, W.L.L.; Lv, H.; Benza-Ingoula, H.; Baraza, L.D.; Zhou, J. Cell penetrating peptides inthe delivery of biopharmaceuticals. Biomolecules, 2012; 2: 187–202.
116. Vivès, E.; Schmidt, J.; Pèlegrin, A. Cell-penetrating and cell-targeting peptides in drug delivery.Biochim. Biophys. Acta, 2008; 1786: 126–138.
117. Mäe, M.; Myrberg, H.; EL-Andaloussi, S.; Langel, Ü. Design of a tumor homing cell-penetrating peptide for drug delivery. Int. J. Pept. Res. Ther., 2008; 15: 11–15.
118. Myrberg, H.; Zhang, L.; Mäe, M.; Langel, Ü. Design of a tumor-homing cell-penetrating peptide.Bioconjugate Chem, 2008; 19: 70–75.
119. Kersemans, V.; Cornelissen, B. Targeting the tumour: Cell Penetrating peptides for molecularimaging and radiotherapy. Pharmaceuticals, 2010; 3: 600–620.
120. Jiang, Q.Y.; Lai, L.H.; Shen, J.; Wang, Q.Q.; Xu, F.J.; Tang, G.P. Gene delivery to tumor cells by cationic polymeric nanovectors coupled to folic acid and the cell-penetrating peptide octaarginine. Biomaterials, 2011; 32: 7253–7262.
121. Olson, E.; Aguilera, T.; Jiang, T.; Ellies, L.G.; Nguyen, Q.T.; Wong, E.H.; Gross, L.A.; Tsien, R.Y. In vivo characterization of activatable cell penetrating peptides for targeting protease activity in cancer. Integr. Biol., 2009; 1: 382–393.

122. vanDuijnhoven, S.M.J.; Robillard, M.S.; Nicolay, K.; Grüll, H. Tumor targeting of MMP-2/9 activatable cell-penetrating imaging probes is caused by tumor-independent activation. J. Nucl. Med., 2011; 52: 279–286.
123. Zhang, X.X.; Eden, H.S.; Chen, X. Peptides in cancer nanomedicine: Drug carriers, targetingligands and protease substrates. J. Control. Release, 2012; 159: 2–13.
124. Shi, N.Q.; Gao, W.; Xiang, B.; Qi, X.R. Enhancing cellular uptake of activable cell-penetrating peptide-doxorubicin conjugate by enzymatic cleavage. Int. J. Nanomed, 2012; 7: 1613–1621.
125. Snyder, E.L.; Dowdy, S.F. Cell penetrating peptides in drug delivery. Pharm. Res., 2004; 21: 389–393.
126. Harada, H.; Hiraoka, M.; Kizaka-Kondoh, S. Antitumor effect of TAT-oxygen-dependentdegradation-caspase-3 fusion protein specifically stabilized and activated in hypoxic tumor cells. Cancer Res., 2002; 1: 2013–2018.
127. Nassal, M. Hepatitis B viruses: Reverse transcription a different way. Virus Res., 2008; 134: 235–249. [CrossRef] [PubMed]
128. Wang, G.H.; Seeger, C. Novel mechanism for reverse transcription in hepatitis B viruses. J. Virol, 1993; 67: 6507–6512. [PubMed]
129. Robaczewska, M.; Narayan, R.; Seigneres, B.; Schorr, O.; Thermet, A.; Podhajska, A.J.; Trepo, C.; Zoulim, F.; Nielsen, P.E.; Cova, L. Inhibition of hepadnaviral replication by polyethylenimine-based intravenous delivery of antisense phosphodiesteroligodeoxynucleotides to the liver. Gene Ther, 2001; 8: 874–881. [CrossRef] [PubMed]
130. Ndeboko, B.; Buronfosse, T.; Lemamy, G.J.; Narayan, R.; Trepo, C.; Nielsen, P.E.; Cova, L. Role of cell-penetrating peptides in intracellular delivery of peptide nucleic acids targeting hepadnavirus replication, 2015. (submitted).
131. Hancock, R.E.; Diamond, G. The role of cationic antimicrobial peptides in innate host defences. Trends Microbiol. 2000; 8: 402–410. [CrossRef]
132. Jensen, H.L. Herpes simplex virus type 1 morphogenesis and virus-cell interactions: Significance of cytoskeleton and methodological aspects. APMIS Suppl, 2006: 114; 7–55. [CrossRef] [PubMed]
133. Koppelhus, U.; Shiraishi, T.; Zachar, V.; Pankratova, S.; Nielsen, P.E. Improved cellular activity of antisense peptide nucleic acids by conjugation to a cationic peptide-lipid (CatLip) domain. Bioconjug. Chem, 2008; 19: 1526–1534. [CrossRef] [PubMed]
134. Shiraishi, T.; Nielsen, P.E. Improved cellular uptake of antisense peptide nucleic acids by conjugation to a cell-penetrating peptide and a lipid domain. Methods Mol. Biol., 2011; 751: 209–221. [PubMed]
135. Abdul, F.; Ndeboko, B.; Buronfosse, T.; Zoulim, F.; Kann, M.; Nielsen, P.E.; Cova, L. Potent inhibition of late stages of hepadnavirus replication by a modified cell penetrating peptide. PLoS ONE, 2012; 7: e48721. [CrossRef] [PubMed].