International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2017.677.689

Review Article
Cell Penetrating Peptides: Biomedical/Therapeutic Applications with Emphasis as Promising Futuristic Hope for Treating Cancer

[1]Rekha Khandia, [1]Ashok Munjal, [2]Arvind Kumar, [3]Gaurav Singh, [4]Kumaragurubaran Karthik and [5]Kuldeep Dhama

[1]Department of Biochemistry and Genetics, Barkatullah University, Bhopal-462 026 (M.P.), India
[2]Barkatullah University, Bhopal-462 026 (M.P.), India
[3]School of Biotechnology, Devi Ahilya University, Indore-452 001 (M.P.), India
[4]Central University Laboratory, Tamil Nadu Veterinary and Animal Sciences University, Chennai- 600051, Tamil Nadu, India
[5]Division of Pathology, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly-243 122, Uttar Pradesh, India

Abstract

The intrinsic property of Cell Penetrating Peptides (CPP) is to deliver various molecules including nucleic acids, large plasmids, therapeutic drugs, imaging molecules, liposomes, nano-molecules to various cell and tissues, which indicates about the potential of CPP as therapeutic vehicle molecules. These are easy to prepare, well characterized, versatile and have ability to link with bioactive cargo through covalent and non-covalent bonds. The interaction between cargo and CPP is highly dependent on physiochemical properties of CPP including size, pH and presence of basic residues in the carrier peptide. Primary mechanism of transport of CPP is endocytosis; however, evidences of endocytosis independent mechanisms including carpet model, inverted micelle model, barrel stave pore model and toroidal pore model are also present. Though, the natural CPP are often non- selective and passive, these may be tuned to become specific and targeted by conjugating them with functional groups and chemicals. In fact several natural CPPs such as penetratin, Tat, polyarginies etc have been modified to achieve maximum penetration and desired characters. Linear CPPs may be brought into multi-branched topology to give dendrimeric structures having more cell penetrating capacity, lower toxicity and hemolysis and higher serum stability. Site-specific targeting of CPP might be helpful in achieving several goals including enhancement in protein expression, gene silencing, formation of pleuripotent cells, reduction in inflammation and apoptosis, trans-epithelial transport, neuroprotection, ischemia treatment, treating insulin disorders, delivery of nucleic acids and agricultural pest control. The present review encompasses the detailed information regarding different modes of entry of CPP inside the cells, designing and classes of such peptides, along with theirs versatile therapeutic applications.

Key words: Cell penetrating peptides, carpet model, inverted micelle model, barrel stave pore model, toroidal pore model, therapeutic, cancer, agriculture

Citation: Rekha Khandia, Ashok Munjal, Arvind Kumar, Gaurav Singh, Kumaragurubaran Karthik and Kuldeep Dhama, 2017. Cell penetrating peptides: biomedical/therapeutic applications with emphasis as promising futuristic hope for treating cancer. Int. J. Pharmacol., 13: 677-689.

Corresponding Author: Ashok Munjal, Department of Biochemistry and Genetics, Barkatullah University, Bhopal-462 026 (M.P.), India

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cell Penetrating Peptides (CPP) are typically 3 to 30 amino acids containing peptides having positive charge, which facilitate its interaction with negatively charged glycosaminoglycans (GAGs) and sialic acids. The history of CPP commenced in 1998 with the discovery of Frankel and Pabo[1], who discovered that the trans-activator of transcription (Tat) protein of HIV has ability to translocate across the plasma membrane. In 1991, Drosophila antennapedia homeodomain protein was demonstrated to be introduced inside the cells, which now-a-days referred as penetratin[2]. Since then, numerous CPP have been discovered and still the number is growing. The CPP can be natural, designed, or chimeras. To enhance cell penetrating ability, several strategies including changing of stereochemistry of amino acid from L to D, inclusion of unusual amino acids[3], making them branched or cyclic[4] and usage of β or γ-amino acids[5] are in practice. The present review encompasses the detailed information regarding different modes of entry of CPP inside the cells, designing and classes of such peptides, along with theirs versatile therapeutic applications. It discusses the role of CPP in generation of pluripotent stem cells, anti-inflammatory properties, neuroprotective actions, treating insulin disorders, managing ischemia, delivery of nucleic acids and usages in agricultural pest control.

Design of CPP: The CPP can be divided into different groups based on their origin or distinguish characteristics. Previously, only natural CPP were known such as Tat and penetratin but later several synthetic CPP were also designed possessing the cell penetration properties and sometimes even more efficient than natural ones. Positively charged amino acids are abundantly present in CPP and a stretch of arginine (R) is almost ubiquitous in CPP[6]. Other characters like its secondary structure also play an imperative role during penetration. The CPP can be manipulated to have artificial amino acids for enhanced activity. For example, if lysine (K) residues are replaced with ornithine residue, its susceptibility for degradation is reduced. Alterations in the CPP structure may also aid in utility such as formation of dendrimer or cyclisation is often used to modify side chains[7]. However, while designing and altering, it is important to consider few important points including stability, toxicity, poor delivery, aggregation or poor yield upon synthesis[8].

Formulation of CPP as transport vector: The CPP to cargo peptide conjugation is dependent upon two processes. The one is chemical covalent conjugation, usually achieved by chemical bonds like disulfide bonds, amine bonds or specific linkers[9,10] and the second one is through expression of CPP-cargo as fusion protein in *E. coli* or *Saccharomyces cerevisiae*[11,12]. Physical conjugation is achieved simply by bulk mixing of cargo and CPP; which offers the ratio of cargo and CPP flexibility. The interaction of cargo and CPP is highly dependent on physiochemical properties like size, pH and presence of R residues in the carrier peptide[13].

DIRECT ENTRY OF CPP

There are evidences of endocytosis independent mechanisms of entry of CPP. There are four existing models for direct entry (Fig. 1).

Carpet model: Positively charged CPP are able to cross plasma membrane easily. Hydrophobic residue of CPP faces towards plasma membrane[14]. Accumulation of CPP locally causes disturbance in electric charge of the membrane and micelle formation takes place. The lipid bilayer is disturbed as if it is affected by detergents[15], therefore the mechanism is called detergent-like mechanism[16]. A transient pore is formed, through which the micelle is internalized.

Inverted micelle model: Alain Prochiantz's group presented the inverted micelle model based on NMR studies[2]. In this model, CPP is internalized by the process of receptor mediated endocytosis, that is an energy consuming mechanism[17]. The inverted micelle is a cavity in between two cell membrane bilayers, where CPP is surrounded by hydrophilic environment. This micellar phase is transient and without help of any vesicular body it passes into cytosol and there the peptide is released. Drosophila antennapedia homeodomain proteins, HIV-1 Tat protein and octa-arginine are the CPP having high internalization efficiencies via inverted micelle mechanism[18].

Barrel stave pore model: Barrel stave mechanism the commonest pathway of CPP traffic. Amphipathic α-helices form transmembrane pores. The major steps involved are (a) In α-helical form, peptide bind to the membrane (b) Peptide monomers recognize each other in membrane bound state (c) α-helices further penetrate in hydrophobic core (d) Progressive addition of monomers to the barrel increase the size of barrel. The formed pores are less than 10 nm in size[16]. A peptide lines the pore (Fig. 1).

Toroidal pore model: In toroidal model, peptide molecules are always associated with lipid headgroup even when it is perpendicularly inserted in the membrane. In such a pore, the

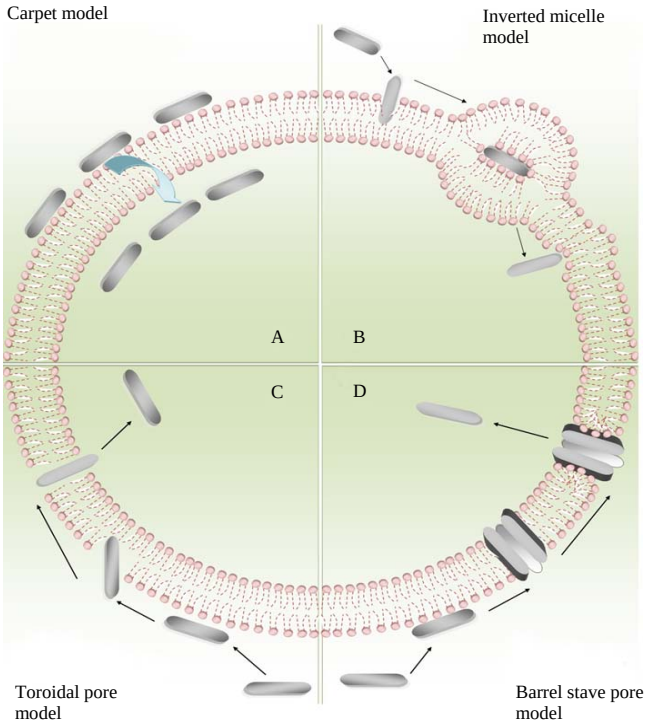

Fig. 1: Possible mechanisms of endocytosis-independent entry of the cell penetrating peptides (CPP)

lipid monolayer bends continuously in a toroidal hole fashion and pore is lined by both the peptide and lipid[19].

CLASSES OF CPP

The CPP have been studied originated from various organisms. The CPP differ in their length, sequence, hydrophobicity and polarity with variable confirmation. These can be broadly classified into cationic peptides, hydrophobic sequences, amphipathic peptides, proline-rich and chimeric or bipartite peptides etc.

Cationic CPP: These are mainly comprised of multiple R and K residues and carry net positive charge at physiological pH with the pKa value ~12. The cationic CPP are generally derived from heparin, RNA and DNA-binding proteins. R possesses the guanidine head group, which stably binds with negatively charged phosphates and sulphates on the surface of cell membranes. K residues also are positively charged but devoid of guanidine head group. A comparison of polymers of R, histidine (H), K, or ornithine revealed that R polymers are most efficacious[20]. The number and the position of R or K residues also determine the CPP transporting efficacy. The R7-R9 peptides are most efficacious in translocation and the efficacy might be reduced by polysulfonated compounds or acidic pH. Tat CPP (RKKRRQRRR) derived from transcriptional activator protein of HIV-1 virus, is a classical cationic CPP and its dimer (RKKRRQRRRRKKRRQRRR) is high in ability to translocate across plant and human cells[21]. The truncated N-terminal Drosophila Antennapedia Transcription Factor (ATF) peptide, a 16 amino acid residue containing peptide is another example of cationic peptide (RQIKIWFQNRRMKWKK) and also referred as penetratin[3]. The seven R residues containing M918, a peptide derived from tumor suppressor protein p14ARF (22 residue long peptide) shares cationic nature as penetratin[22].

Hydrophobic CPP: These are derived from signal peptide sequences. The example of hydrophobic CPP includes the Kaposi Fibroblast Growth Factor (FGF) signal peptide (AAVALLPAVLLALLAP) and integrin β3 signal peptide (VTVLALGALAGVGVG). Less number of Hydrophobic CPP has been reported. The SG3 (RLSGMNEVLSFRWL)[23], contains only two R and one glutamic acid (E) residue and 6 hydrophobic amino acid residues[24]. Hydrophobic CPP contain only non-polar amino acid residues. In comparison to cationic or amphipathic CPP, hydrophobic CPP are less studied.

Amphipathic CPP: Amphipathic CPP contain amphipathic helices with hydrophilic and hydrophobic faces. These CPP insert into the lipid bilayer through hydrophobic interaction. The α-helical structure acquired by the primary amphipathic CPP molecule is responsible for its insertion into plasma

membrane regardless of the strength of ionic interaction. The Model Amphipathic Peptide (MAP) is having α-helical structure with an 18 amino acids (KLALKLALKALKAALKLA). In case of secondary amphipathic CPP with random confirmation, the interaction with plasma membrane is greatly charge dependent and after binding, the CPP attain α-helical structure[25]. Pep-1 is a 21-amino acid long commercially available synthetic CPP, having a tryptophan (W) rich hydrophobic domain, K-rich hydrophilic domain and a spacer domain to separate and maintain the integrity of these two domains. While it prevents degradation of cargo proteins, it is being used for non-covalent transport of proteins.

Proline-rich sequences: Proline (P)-rich peptides are able to attain polyproline I (PPI) and polyproline II (PPII) confirmations. In the presence of aliphatic alcohols, the PPI form is dominating confirmation with right-handed helix with all cis peptide bonds, where in presence of aqueous media or aliphatic acids PPII form is dominating having left-handed extended helix with all trans bonds[26]. By functionalisation of a polyproline helix by O-alkylation of a hydroxyproline monomer, delivery vectors have been prepared. Proline-based leucine (L), K and R mimics also have been prepared. Synthetic proline-derived γ-peptides can be functionalized by acylation, alkylation or guanidylation to get hydrophobic, hydrophilic or amphipathic γ-peptides. Proline-based dendrimers are highly branched structures able to transport drugs, vaccines or DNA. However, at concentrations higher than 60 μM dendrimers are lethal, at lower concentrations these are efficient in carrying DNA even in the presence of serum. Amphipathic pro-rich CPP, appear to be most promising due to reduced toxicity and ease in synthesis[27].

Chimeric or bipartite peptides: Chimeric CPP are the combination of two or more above listed peptides. A chimera containing Ala46-Tyr51 amino acid residue from β-lactamase inhibitory protein (BLIP) and LLIIL residues from cell-penetrating vascular endothelial-cadherin (pVEC) sequence, decreased the number of viable cells in presence of antibiotic. Thus, have greater importance in conquering β-lactamase-mediated ampicillin drug resistance[28]. Lysins are phage derived hydrolases, targeted to digest bacterial peptidoglycan cell wall in order to release the progeny. Because lysins are highly species specific and rarely are subjected to develop bacterial resistance, are now considered as potent alternative to antibiotics. The lysins have a typical structure of N-terminus catalystic domain and C-terminus cell-binding domain. A chimera of lysins could be prepared to improve the host range, activity, solubility and intracellular uptake. Such one chimera staphylolytic chimeolysin (ClyF) is able to lyse all clinical isolates of *S. aureus* including Methicillin-resistant *Staphylococcus aureus* (MRSA)[29].

APPLICATIONS OF CPP

After the discovery of CPP, these fetch the attention of scientific community for targeted drug delivery across the plasma membrane. Many applications of CPP, in field of medicine have been invented and are in phase of clinical trials.

CPP for generation of pluripotent stem cells: Wilmut and his colleagues, demonstrated for the first time that adult somatic cell may be converted to undifferentiated embryonic stem cells using Somatic Cell Nuclear Transfer (SCNT) technique[30]. However, a less complicated technique was discovered later for reprogramming somatic cells to pluripotency by introducing transcription factors Oct4, Sox2, Klf4 and c-Myc through retroviral vectors[31]. However, the technique is impeded by integration of retroviral genomes in the host genomes and resulting risk of mutagenesis and genetic dysfunction. The CPP have provided a safer way to deliver these transcription proteins into human cells. If 9 R residues containing CPP is conjugated to these reprogramming proteins by fusion, it successfully trafficked these factors into fibroblasts from human origin and convert them into pluripotent stem cells[5,32]. In the experiment of Kaitsuka and Tomizawa[33], both the mice and human iPS cells were differentiated to obtain a pancreatic lineage using transcription factors including Pdx1, NeuroD and MafA. A fusion of Tat peptide with nucleus localization signal is used to transduce four transcriptional factors (Oct4, Klf4, Sox2 and cMyc) and resulting embryoids express the most common pleuripotency markers such as SSEA1, Oct4, Sox2, Klf4 and Nanog[34]. Oct4 alone is also able to induce pleuripotency without the requirement of additional transcriptional factors[32]. The transcription factor Oct4 is having a 16 amino acid long peptide which has 68% amino acid homology with the CPP penetration and when tagged with fluorescein isothiocyanate (FITC), revealed its efficient uptake via endocytic pathway. Also, unmodified Oct4 self-penetrated in CVI-5B cells and human BJ foreskin fibroblasts in the experiment of Kim *et al.*[32] revealed the potential of Oct4 CPP as the pluripotency reprogramming factor (Table 1).

CPP against inflammation: There are several mediators of inflammation including TNFα and IL-1β, reactive oxygen species, reactive nitrogen species and NF-κB pathway is upregulated65. A series of evidences suggest that NF-κB inhibition may block inflammation and the associated tissue

Table 1: Cell penetrating peptides and their functions

Property	Name of peptide	Modus operandi	Function owing to	References
Differentiation of induced pluripotent stem cells	11R fused with MafA	Differentiation of iPS cells into insulin-producing cells	Reprogramming of iPS cells	Kaitsuka and Tomizawa[33], Lu *et al.*[35]
	Tat fused with nucleus localization signal and transcription factors; Oct4, Sox2, Klf4 and cMyc fused two columns)	Embryoids seeded in micro-well plated coated with gelatin	Reprogramming of iPS cells (Differentiation into) cardiac cells	Nemes *et al.*[34]
		Embryoids seeded in plated coated With -2- hydroxyethyl methacrylate	Reprogramming of iPS cells (Differentiation into neural cells)	
	RPARPAR peptide or R9 fused with SOX2	SOX2 induce reprogramming of retinal pigmented epithelium cells to functional neurons	Protein cargo transfer mechanism	Hu *et al.*[36]
	16-amino-acid peptide of OCT4	Transfer of MYOD myogenic factor		Kim *et al.*[32]
Anti-inflammatory	TAT	Transfer of Nrf2 in monocytes	Suppression of cytokine TNF	Steel *et al.*[37]
	AIP6	Inhibition of p65 subunit	Downstream NF-κB signaling stopped	Wang *et al.*[38]
	FGF-4- SOCS3 conjugate	SOCS3 delivery	Suppression of cytokine-mediated acute inflammation and liver apoptosis	Hawiger[39], Wang and Jauch[40]
	Tat- Nrf2 conjugate	Activation of Nrf2 and downstream target gene heme-oxygenase-1 (HO-1)	Inhibition of pro-inflammatory cytokine TNF	Steel *et al.*[37]
Anticancer peptide	Melittin, LL-37, Cecropin B, Magainin 2, NRC-03, NRC-07, Hepcidin TH2-3, Dermaseptin B2,	Membrane lysis	Increased anionicity of the cytoplasmic membrane of cancer cells	Schweizer[41], Gaspar *et al.*[42]
	D-K6L9, Polybia-MPI, Temporin-1CEa	Membrane destabilization	Cancer cell membranes contains more fluid than normal cells	Gaspar *et al.*[42]
	Cecropin, melittin	Non-membranolytic activities	Non-receptor mediated mode of action	Winder *et al.*[43]
	Pentastatin-1, chemokinostatin-1, properdistatin	Reduction in tumor vasculature	Receptors expressed on angiogenic endothelial cells	Koskimaki *et al.*[44]
	AMP temporin-1CEa	Breast cancer cell death	Membrane disruption, intracellular calcium release and ROS over production	Wang *et al.*[45]
	FK-16 peptide	Kills colon cancer cells	Autophagic cell death, an additional cell death pathway, while reducing cost production	Ren *et al.*[46]
	Pep2 and Pep3	Kill cells from human leukemia	Inhibition of X-linked inhibitor of apoptosis (IAP) protein (a caspase inhibitor)	Edison *et al.*[47]
	BIM peptide	Selective activation of cell death in a human AML xenograft model.	Target Bcl2 pathway by inhibiting antiapoptotic interactions, directly triggered proapoptotic activity and induced dose-responsive and BH3 sequence-specific cell death of hematologic cancer cells	LaBelle *et al.*[48]
	Magainins	Rapidly and irreversibly lyse hematopoietic tumor and solid tumor target cells	Polymerize and form Cl^- ion permeable pore	Cruciani *et al.*[49]
	Cecropin B (derived from the hemolymph of Hyalophoracecropia)	Selectively kill leukemia and stomach carcinoma cells	Peptide flexibility provides efficient insertion for lytic activity	Wu *et al.*[50]

Table 1: Continue

Property	Name of peptide	Modus operandi	Function owing to	References
	Tachyplesin (from the horseshoe crab)	Bind to hyaluronan or related glycosaminoglycans on the surface of tumor cells	Activation of the classic complement pathway leading to the disruption of the plasma membrane	Chen *et al.*[51]
	CR1166	Interferes in interactions disturbing the events involved with GIPC activity	Decreased proliferation and cytotoxic effects on breast and pancreas cancer cells	Patra *et al.*[52]
	MMIS: buforinIIb fusion (derived from histone H2A)	Cells expressing high amounts of MMPs are killed (approximately 60 cell line respond to the peptide including melanoma, human fibrosarcoma and gliobastoma)	Accumulate primarily in the nuclei and induce mitochondria-dependent apoptosis.	Lee *et al.*[53]
	Human neutrophil peptides HNP-1 to 3	Breast and colon tumors are targeted	Recruitment and activation of dendritic cells	Wang *et al.*[54]
	A9K	Selectivity for leukemia, uterine cervix and kidney cancer cells	Membrane disruption and apoptosis	Xu *et al.*[55]
Neuroprotection	R15	Reduces excitotoxic calcium influx and its toxicity at high concentrations	Prevent neuronal cell stroke	Cardozo *et al.*[56]
	TAT			
	Penetratin	Efficient delivery of iron siderophore (Desferrioxamine-B) in cells	Suppression of redox activity of Fe in HeLa and RBE4 cell lines	Goswami *et al.*[57]
Ischemia	Tat	Delivery of anti-apoptotic BH4-peptide	Reduction in staurosporine-induced apoptosis in primary cardiomyocytes	Boisguerin *et al.*[58]
	(RXR)4			
	Bpep			
	Pip2b			
Nucleic acid delivery	pepR of DENV	Delivery of nucleic acid through endocytic pathway	Translocation of siRNA and ssDNA molecules in mammalian cell	Freire *et al.*[59]
	pepM	Directly pass through membrane		
	S4(13)PV	Heparan sulphate proteoglycan mediated internalization and nucleic acid delivery.	Endocytosis and endocytosis independent direct penetration across cell membranes.	Mano *et al.*[60], Trabulo *et al.*[61]
Agricultural pest control	Lyt-1	Forms a pore and lyse pest cells by reducing ion and voltage gradient	Reduces ion and voltage gradient	Yan and Adams[62]
	Puroindoline PINA and PINB proteins (tryptophane rich)	Entry inside the cell carpet method	Membranolysis of bacterial and fungal cells	Alfred *et al.*[63]
	JH 1.6	Conjugate to various RNA, DNA and proteins	High relative internalization rate and low cellular toxicity	Kim[64]

damage. Anti-inflammatory peptide-6 (AIP6), interacts directly with p65 subunit and inhibit DNA-binding and transcriptional activities of NF-κB and the downstream cascade of generation of inflammatory mediators[38]. In the acute organ injury animal model, pathogen-derived inducers including staphylococcal enterotoxin B (SEB), lipopolysaccharide (LPS), or lectin concanavalin A (ConA) cause inflammation and apoptosis of the liver, which is dependent on the signalling pathways by tumor necrosis factor-alpha (TNF-α), IFN-γ and Fas-Fas ligand interaction. Suppressor of cytokine signalling (SOCS)1 and SOCS3 are rapidly produced to conquer proinflammatory signalling and then degraded. In case of acute inflammation SOCS are insufficient to curb proinflammatory signalling. Khandia *et al*.[65] and Jo *et al*.[66], reasoned the replenishment of exogenous SOCS3 as intracellular protein therapy. Recombinant cell penetrating form of SOCS3 having 12 amino acids long hydrophobic CPP derived from fibroblast growth factor-4[39], effectively suppressed cytokine-mediated acute inflammation and liver apoptosis[40]. Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a regulator molecule in cancer progression and inflammation. Tat peptide conjugated with Nrf2 sequence has been shown to activate Nrf2 and its downstream target gene heme-oxygenase-1 (HO-1) in THP-1 monocytes. Also, the Nrf2 has anti-inflammatory role via inhibition of the TNF[37].

CPP in targeting cancer: The CPP may play an important role in both the screening as well in targeting of tumor cell[67]. Specialized CPP named as tumor penetrating peptides are used to specifically target tumor cells[68]. The EPPT1 peptide linked to cationic polyacrylamide (CPAA) and FITC labelled is capable of targeting transmembrane MUC-1 protein present in colorectal cell lines[69]. Magainins, is a peptide derived from *Xenopus laevis*, which forms amphipathic α-helical structure. It causes pore formation in cancer cells and selectively kills them exempting lymphocytes or fibroblasts. Conjugation of Magainins with tumor homing peptide Bombesin, the anticancer effect is exemplified[70]. Defensins are small (3.5-4 kD), cysteine-rich peptides, produced by body as part of innate defence system. α-defensins (HNP1, HNP2, HNP3) are present in granules of neutrophils and their membrane permeabilization character is attributed to its ability to make pores in lipid bilayers[71] and is effective against squamous cell carcinoma[72] owning to its DNA damaging effects. However, the cytotoxicity of HNPs is not confined to cancers cells only and also harm normal leukocytes or epithelial cell[73]. CopA3 a synthetic peptide derived from defensin like molecules of insects has anticancer potential[74]. Bax is a pro-apoptotic peptide family, having nine α-helices. α5, α6 and α9 helices have ability to bind to cellular membrane and α5 and α6 helices of Bax, involving arginine lysine residues, are able to make toroidal like pore structure. The structure of Bax protein explains how it permeated mitochondrial membrane and cause apoptosis[75]. Designing a poro-peptide encompassing the pore forming region of Bax is able to induce caspase mediated apoptosis in cancer cells. Its peri-tumoral application regressed tumor in a nude mice xenograft model, thus effective ion anticancer therapy[76]. Bacteriorhodopsin C helix derived peptide called as pH (low)-dependent Insertion Peptide (pHLIP), is present in 3 forms. The first one is water soluble form at pH 7 or above; at neutral pH, it resides at the lipid bilayer and at acidic pH below 6, it is inserted into the lipid bilayer as α helix[77]. With the drop of pH folding of the peptide changes and the energy released in this confirmation change, is used to move the cargo present along with peptide across the membrane. pHLIP is a peptide which uses the inherent acidic character of cancer cells to target them[78]. (KLAKLAK)2 synthetic peptide, when fused with CPP or THPs, enter inside the cell and disrupt mitochondria and efficiently caused apoptosis in tumor cells[79,80] (Fig. 2). More such CPP[41-55] have been summarized in Table 1.

CPP in neuroprotective role: R rich CPP have been shown to be neuroprotective, with or without fusion to a neuroprotective cargo peptide and their efficacy increases with increasing length. The charge present on the CPP and number of R residue is important in the neuroprotective role of CPP, which is evident by the fact that charge neutral CPP i.e., polyglutamic acid (E9) and poly-lysine K10 peptide are only weakly protective in E induced neuronal death[81]. Maximum protection is achieved by R15 peptide, which is dependent on the endocytic property of peptide[82]. Meloni *et al*.[83] demonstrated the neuroprotective role of Tat (GRKKRRQRRR), penetratin (RQIKIWFQNRRMKWKK) and oligoarginine-9 (R9: RRRRRRRRR) in *in vitro* neuronal cell stroke models and reduces excitotoxic calcium influx and its toxicity at high concentrations[56]. Iron is an element essential for oxygen transport, electron transfer and redox processes, transported by transferrin protein. Iron overload may result in accumulation of iron in redox active iron, toxic to cell. It causes oxidation of amino acids, proteins and DNA and thereby causes oxidative stress and cell death[84]. Iron overload in central nervous system leads to brain degenerative diseases including Alzheimer's and Parkinson's diseases[85]. With iron if Al, Cu and Zn metals are present, it further increases the reactive oxygen species[86]. Desferrioxamine-B (DFO), is an iron siderophore having significant affinity for Al and Zn too. In clinical trials DFO has shown anti-Alzheimer activity but poor bioavailability reduces its utility for use in neurodegenerative

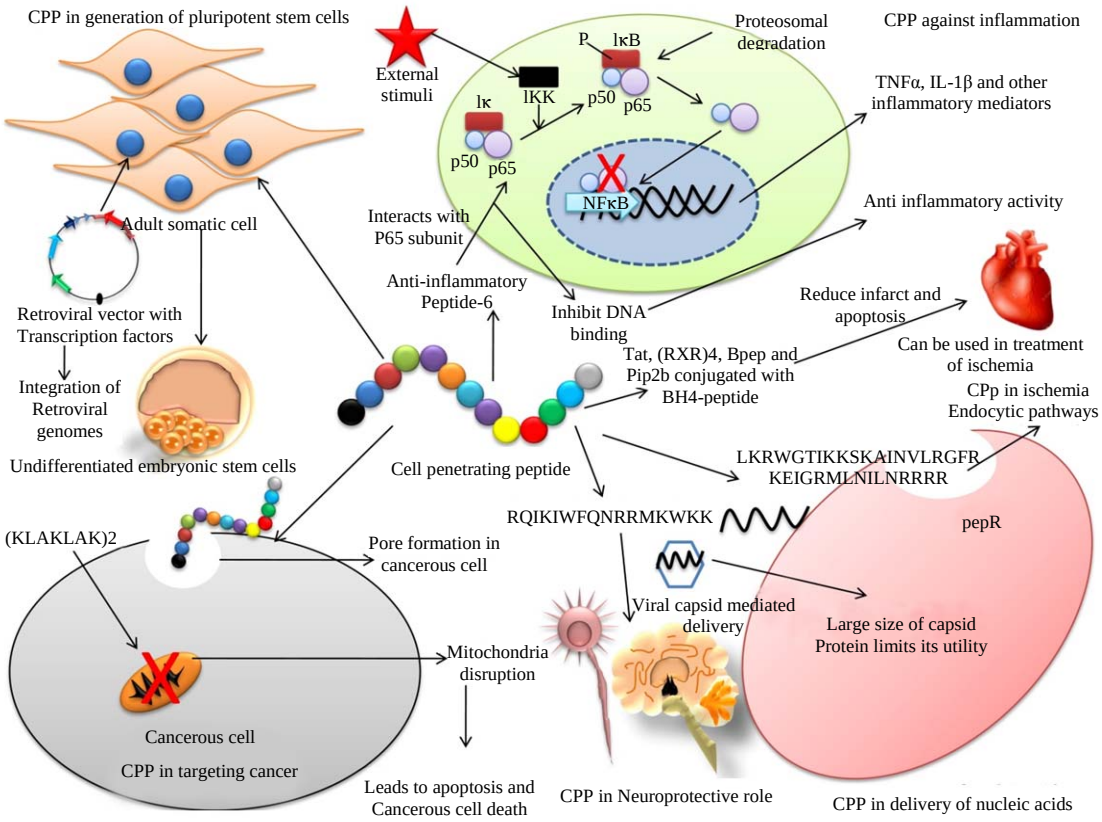

Fig. 2: Beneficial applications of cell penetrating peptides (CPP)

disorders[87]. To improve its bioavailability CPP may be very useful. In the experiment of Goswami *et al.*[57], DFO has been covalently conjugated with TAT and penetratin and the redox activity of Fe was suppressed in HeLa and RBE4 cell lines.

CPP in ischemia: Blockage in the coronary artery of heart leads to ischemia. Even after restoring the revascularization by thrombolysis or angioplasty, it ends up in apoptosis of cardiac cells[88]. The BH4-peptide, derived from the Bcl-xL anti-apoptotic protein, conjugated with four different CPP [Tat, (RXR)4, Bpep and Pip2b] and these conjugates minimized staurosporine-induced apoptosis in primary cardiomyocytes. Intravenous administration of Tat-BH4 and Pip2b-BH4 conjugates, at a single dose of 1 mg kg^{-1}, before reperfusion, was able to reduce infarct size by 50% and apoptosis by 60%. Such targeted delivery of antiapoptotic peptide to myocardium may be of greater therapeutic potential in clinical conditions[58].

CPP in delivery of nucleic acids: Delivery of nucleic acid into the cell is a comparatively difficult task due to negative charge on them, their comparatively higher molecular weight and propensity to degradation. Viral capsid proteins, which have inherent tendency to pack, protect and deliver the nucleic acid into its capsid may be an useful tool to deliver nucleic acid inside the cell[89]; however large size of capsid protein limits its utility. Hence the consideration is to search for capsid domains instead full protein, which can serve as CPP peptides. DENV C protein is large protein of 100 amino acid size and can facilitate large nucleic acid molecules translocation. pepR and pepM are the peptides, derived from DENV C protein, which have tendency to bind with RNA and membrane respectively[59]. pepM is highly hydrophobic, where pepR is highly cationic (LKRWGTIKKSKAINVLRGFRKEIGRMLNILNRRRR)[90] (Fig. 2). pepR translocate through endocytic pathway, where pepM directly pass through lipid membranes (KLFMALVAFLRFLTIPPTAGILKRWGTI)[91]. All the three-dengue virus capsid protein C, pepM and pepR can translocate small interfering RNA (siRNA) and ssDNA molecule in mammalian cell[59]. The S413PV, Penetratin, Pep-1, Polyarginines, Transportan and Tat peptides are few CPP that are able to translocate nucleic acid[61]. The CPP and nucleic acids are often

conjugated non-covalently, due to virtue of negatively charged nucleic acid and positively charged CPP. *In vivo* such conjugation offers greater reproducibility[92].

CPP for agricultural pest control applications: There are some peptides which share similar characters with that of CPP. These are Membrane Acting Microbial Peptides (MAMP) having antimicrobial properties with carrying positive charge. Because some of the peptides are pathogenic to insects, these can be further exploited as biopesticides[93]. Small amphipathic peptide lycotoxin-1 (Lyt-1) from wolf spider (*Lycosa carolinensis*) forms a pore in the membrane and increase cell permeability thereby bringing the lysis of the cell by reducing ion and voltage gradient[62]. Amphipathic α-helical structure occurred in Lyt-1 peptide due to repetition of lysine residue at each 4th or 5th position in the peptide. This amphipathic alpha helical structure is present in other pore forming pore-forming peptides, including magainins, dermaseptins and adenoregulin[94]. Mutant Lyt-1 peptide has been identified through scanning mutant analysis technique, which is specific against armyworms, which causes damage to corn and other crops. The puroindoline proteins (PINA and PINB) of wheat, has a lipid binding ability and due to tryptophan rich domain it exhibits antibacterial and antifungal properties. Based on tryptophan rich domain, constructed synthetic peptide also exhibit the antibacterial and antifungal ability sparing mammalian cells. The PIN based peptides enter inside the cell by carpet method of entering[63]. Bipartite nuclear localization sequence virE2 protein, of *Agrobacterium* is able to mediate ssDNA transfer to nucleus of plant cells. In plant protoplasts, CPP like Tat, *p*VEC and transportan have shown concentration dependent, non-saturable and endocytosis independent internalization[95].

CPP in trans-epithelial transport: Intestinal absorption of insulin has been seen to be enhanced by co administrating CPP like penetratin, however high amounts of penetratin is needed to stimulate intestinal absorption of insulin. In a study of Kamei *et al*.[96], 26 penetratin analogues were evaluated for the absorption-enhancing efficiency based on chain length, hydrophobicity, basicity and amphipathicity. Oligoarginie significantly improved delivery of insulin from the intestine to the systemic circulation without the requirement of interlinking. Conjugation of nona-arginine (R9) to biologically active part of parathyroid hormone [PTH(1-34)], was toxic to the intestinal enterocytes. R rich CPP like R8, penetratin, pVEC and RRL helix significantly increase insulin absorption from intestine, however different CPP exhibited variation in potency while using D or L form[97]. Higher amount of CPP with insulin lead to formation of aggregates in solution and L-penetratin enhanced the insulin absorption even in aggregated form.

CPP in treating insulin disorders: Hyperglycemia is the feature of Diabetes. Patients who are unable to balance critical glucose level in blood Patients with diabetes are unable to effectively manage blood glucose levels, often suffer different complications like heart diseases, an increased risk of blindness and renal failure. The ailment is due to insufficient insulin. When insulin was conjugated to different cationic CPP like Tat, oligoarginine or oligolysine, the transmembrane transport to cultured rat alveolar epithelial cell monolayer was maximum for oligoarginine conjugate with 27 folds increase in comparison to native insulin[98]. Insulin is often taken by oral route; however enzymatic barrier rapidly degrades it and secondly mucosal barrier limits its bioavailability. CPP enhance transport ability as well as bioavailability up to 50% in comparison to subcutaneously administered insulin[99]. Also, low molecular weight protamine (LMWP) acts like CPP peptide, with a cell translocation efficiency equivalent to TAT[100]. Oligoarginine (R8), conjugated insulin transport efficacy was 5-7 times higher and co administration of oligoarginie-hydroxypropyl-β-cyclodextrin (HP-β-CD)- insulin was 8-10 times higher than normal insulin across the Caco-2 cell monolayer[101].

SIGNIFICANCE STATEMENT

- Present review gives a brief account for the guidelines to design Cell penetrating peptides (CPP) as well as describes the mode of CPP trafficking inside the cell
- Different categories of CPP including cationic, hydrophobic, amphipathic, proline-rich sequences and chimeric/bipartite peptides have been described in detail
- Various applications of CPP encompass their use as anticancer peptide, anti-inflammatory, neuroprotective, anti-ischemic, trans-epithelial transporter of insulin, in treating insulin disorders, nucleic acid delivery vehicle and differentiation tool for induced pluripotent stem cells and in agricultural pest control

CONCLUSION AND FUTURE PERSPECTIVE

The numbers of CPP are continuously expanding since day of its discovery. Several CPP have been designed to deliver various cargos not only inside the cells but also across the epithelial and endothelial barriers. The individual amino acid's structure and any other factors are responsible for its intrinsic property to cargo therapeutic molecules and tailor made CPP

might be there with improved characteristics. However, a proper mechanism of its delivery through which epithelial, endothelial and blood brain barrier are crossed and interaction with mucus is carried out, need to be elucidated. More reliable techniques are required to elucidate, those are enabled to tell the precise quantity of CPP delivered to target organ. The safer introduction of CPP into target tissue demands enhanced tissue specificity to reduce possible detrimental effects due to off-target delivery of cargo.

ACKNOWLEDGMENTS

Authors of the manuscript thank and acknowledge their respective Universities/Institutes.

REFERENCES

1. Frankel, A.D. and C.O. Pabo, 1988. Cellular uptake of the tat protein from human immunodeficiency virus. Cell, 55: 1189-1193.
2. Derossi, D., A.H. Joliot, G. Chassaing and A. Prochiantz, 1994. The third helix of the *Antennapedia homeodomain* translocates through biological membranes. J. Biol. Chem., 269: 10444-10450.
3. Kato, T., H. Yamashita, T. Misawa, K. Nishida and M. Kurihara *et al*., 2016. Plasmid DNA delivery by arginine-rich cell-penetrating peptides containing unnatural amino acids. Bioorg. Med. Chem., 24: 2681-2687.
4. Angeles-Boza, A.M., A. Erazo-Oliveras, Y.J. Lee and J.P. Pellois, 2010. Generation of endosomolytic reagents by branching of cell-penetrating peptides: Tools for the delivery of bioactive compounds to live cells in cis or trans. Bioconjug. Chem., 21: 2164-2167.
5. Kristensen, M., D. Birch and H.M. Nielsen, 2016. Applications and challenges for use of cell-penetrating peptides as delivery vectors for peptide and protein cargos. Int. J. Mol. Sci., Vol. 17. 10.3390/ijms17020185
6. Diener, C., G.G.R. Martinez, D.M. Blas, D.A.C. Gonzalez, G. Corzo, S. Castro-Obregon and G. del Rio, 2016. Effective design of multifunctional peptides by combining compatible functions. PLoS C omput. Biol., Vol. 12. 10.1371/journal.pcbi.1004786
7. Oskolkov, N., P. Arukuusk, D.M. Copolovici, S. Lindberg and H. Margus *et al*., 2011. NickFects, phosphorylated derivatives of transportan 10 for cellular delivery of oligonucleotides. Int. J. Peptide Res. Ther., 17: 147-157.
8. Mandal, D., A.N. Shirazi and K. Parang, 2011. Cell-penetrating homochiral cyclic peptides as nuclear-targeting molecular transporters. Angewandte Chemie Int. Edn., 50: 9633-9637.
9. Lee, S.J., S.H. Yoon and K.O. Doh, 2011. Enhancement of gene delivery using novel homodimeric tat peptide formed by disulfide bond. J. Microbiol. Biotechnol., 21: 802-807.
10. Herce, H.D., W. Deng, J. Helma, H. Leonhardt and M.C. Cardoso, 2013. Visualization and targeted disruption of protein interactions in living cells. Nat. Commun., Vol. 4. 10.1038/ncomms3660
11. Caldinelli, L., D. Albani and L. Pollegioni, 2013. One single method to produce native and Tat-fused recombinant human α-synuclein in *Escherichia coli*. BMC Biotechnol., Vol. 13. 10.1186/1472-6750-13-32
12. Kristensen, M., H. Franzyk, M.T. Klausen, A. Iversen and J.S. Bahnsen *et al*., 2015. Penetratin-mediated transepithelial insulin permeation: Importance of cationic residues and pH for complexation and permeation. AAPS J., 17: 1200-1209.
13. Kristensen, M., A.M. de Groot, J. Berthelsen, H. Franzyk, A. Sijts and H.M. Nielsen, 2015. Conjugation of cell-penetrating peptides to parathyroid hormone affects its structure, potency and transepithelial permeation. Bioconjug. Chem., 26: 477-488.
14. Li, Z.L., H.M. Ding and Y.Q. Ma, 2016. Interaction of peptides with cell membranes: Insights from molecular modeling. J. Phys. Condens. Matter., Vol. 28. 10.1088/0953-8984/28/8/083001/meta
15. Hinz, A. and H.J. Galla, 2005. Viral membrane penetration: Lytic activity of a nodaviral fusion peptide. Eur. Biophys. J., 34: 285-293.
16. Mudhakir, D. and H. Harashima, 2009. Learning from the viral journey: How to enter cells and how to overcome intracellular barriers to reach the nucleus. AAPS J., Vol. 11. 10.1208/s12248-009-9080-9
17. Trehin, R. and H.P. Merkle, 2004. Chances and pitfalls of cell penetrating peptides for cellular drug delivery. Eur. J. Pharm. Biopharm., 58: 209-223.
18. Kawamoto S., M. Takasu, T. Miyakawa, R. Morikawa, T. Oda, S. Futaki and H. Nagao, 2011. Inverted micelle formation of cell-penetrating peptide studied by coarse-grained simulation: Importance of attractive force between cell-penetrating peptides and lipid head group. J. Chem. Phys., Vol. 134. 10.1063/1.3555531
19. Yang, L., T.A. Harroun, T.M. Weiss, L. Ding and H.W. Huang, 2001. Barrel-stave model or toroidal model? A case study on melittin pores. Biophys. J., 81: 1475-1485.
20. Wender, P.A., D.J. Mitchell, E.T. Pattabiraman, E.T. Pelkey, L. Steinman and J.B. Rothbard, 2000. The design, synthesis and evaluation of molecules that enable or enhance cellular uptake: Peptoid molecular transporters. Proc. Natl. Acad. Sci. USA., 97: 13003-13008.
21. Vives, E., P. Brodin and B. Lebleu, 1997. A truncated HIV-1 Tat protein basic domain rapidly translocates through the plasma membrane and accumulates in the cell nucleus. J. Biol. Chem., 272: 16010-16017.
22. El-Andaloussi, S., H.J. Johansson, T. Holm and U. Langel, 2007. A novel cell-penetrating peptide, M918, for efficient delivery of proteins and peptide nucleic acids. Mol. Ther., 15: 1820-1826.

23. Zahid, M. and P.D. Robbins, 2015. Cell-type specific penetrating peptides: Therapeutic promises and challenges. Molecules, 20: 13055-13070.
24. Gao, S., M.J. Simon, C.D. Hue, B. Morrison and S. Banta, 2011. An unusual cell penetrating peptide identified using a plasmid display-based functional selection platform. ACS Chem. Biol., 6: 484-491.
25. Zaro, J.L. and W.C. Shen, 2015. Cationic and amphipathic cell-penetrating peptides (CPPs): Their structures and *in vivo* studies in drug delivery. Molecules, 20: 13055-13070.
26. Pujals, S. and E. Giralt, 2008. Proline-rich, amphipathic cell-penetrating peptides. Adv. Drug Deliv. Rev., 60: 473-484.
27. Sanclimens, G., H. Shen, E. Giralt, F. Albericio, M.W. Saltzman and M. Royo, 2005. Synthesis and screening of a small library of proline-based biodendrimers for use as delivery agents. Peptide Sci., 80: 800-814.
28. Alaybeyoglu, B., B.S. Akbulut and E. Ozkirimli, 2015. A novel chimeric peptide with antimicrobial activity. J. Peptide Sci., 21: 294-301.
29. Yang, H., H. Zhang, J. Wang, J. Yu and H. Wei, 2017. A novel chimeric lysin with robust antibacterial activity against planktonic and biofilm methicillin-resistant *Staphylococcus aureus*. Scient. Rep., Vol. 7. 10.1038/srep40182
30. Wilmut, I., A.E. Schnieke, J. McWhir, A.J. Kind and K.H.S. Campbell, 1997. Viable offspring derived from fetal and adult mammalian cells. Nature, 385: 810-813.
31. Takahashi, K. and S. Yamanaka, 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell, 126: 663-676.
32. Kim, D., C.H. Kim, J.I. Moon, Y.G. Chung and M.Y. Chang *et al*., 2009. Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell Stem Cell, 4: 472-476.
33. Kaitsuka, T. and K. Tomizawa, 2015. Cell-penetrating peptide as a means of directing the differentiation of induced-pluripotent stem cells. Int. J. Mol. Sci., 16: 26667-26676.
34. Nemes, C., E. Varga, Z. Polgar, N. Klincumhom, M.K. Pirity and A. Dinnyes, 2014. Generation of mouse induced pluripotent stem cells by protein transduction. Tissue Eng. Part C: Methods, 20: 383-392.
35. Lu, J., L. Lin, H. Dong, X. Meng and F. Fang *et al*., 2017. Protein therapy using MafA fused to a polyarginine transduction domain attenuates glucose levels of streptozotocin-induced diabetic mice. Mol. Med. Rep., 15: 4041-4048.
36. Hu, Q., R. Chen, T. Teesalu, E. Ruoslahti and D.O. Clegg, 2014. Reprogramming human retinal pigmented epithelial cells to neurons using recombinant proteins. Stem Cells Transl. Med., 3: 1526-1534.
37. Steel, R., J. Cowan, E. Payerne, M.A. O'Connell and M. Searcey, 2012. Anti-inflammatory effect of a cell-penetrating peptide targeting the Nrf2/keap1 interaction. ACS Med. Chem. Lett., 3: 407-410.
38. Wang, Y.F., X. Xu, X. Fan, C. Zhang and Q. Wei *et al*., 2011. A cell-penetrating peptide suppresses inflammation by inhibiting NF-κB signaling. Mol. Ther., 19: 1849-1857.
39. Hawiger, J., 1999. Noninvasive intracellular delivery of functional peptides and proteins. Curr. Opin. Chem. Biol., 3: 89-94.
40. Wang, X. and R. Jauch, 2014. OCT4: A penetrant pluripotency inducer. Cell Regen., Vol. 3. 10.1186/2045-9769-3-6.
41. Schweizer, F., 2009. Cationic amphiphilic peptides with cancer-selective toxicity. Eur. J. Pharmacol., 625: 190-194.
42. Gaspar, D., A.S. Veiga and M.AR.B. Castanho, 2013. From antimicrobial to anticancer peptides. A review. Front. Microbiol., Vol. 4. 10.3389/fmicb.2013.00294
43. Winder, D., W.H. Gunzburg, V. Erfle and B. Salmons, 1998. Expression of antimicrobial peptides has an antitumour effect in human cells. Biochem. Biophys. Res. Commun., 242: 608-612.
44. Koskimaki, J.E., E.D. Karagiannis, E.V. Rosca, F. Vesuna and P.T. Winnard *et al*., 2009. Peptides derived from type IV collagen, CXC chemokines and thrombospondin-1 domain-containing proteins inhibit neovascularization and suppress tumor growth in MDA-MB-231 breast cancer xenografts. Neoplasia, 11: 1285-1291.
45. Wang, C., L.L. Tian, S. Li, H.B. Li and Y. Zhou *et al*., 2013. Rapid cytotoxicity of antimicrobial peptide tempoprin-1CEa in breast cancer cells through membrane destruction and intracellular calcium mechanism. PLoS ONE, Vol. 8. 10.1371/journal.pone.0060462
46. Ren, S.X., J. Shen, A.S.L. Cheng, L. Lu and R.L. Chan *et al*., 2013. FK-16 derived from the anticancer peptide LL-37 induces caspase-independent apoptosis and autophagic cell death in colon cancer cells. PLoS ONE, Vol. 8. 10.1371/journal.pone.0063641
47. Edison, N., T.H. Reingewertz, Y. Gottfried, T. Lev and D. Zuri *et al*., 2012. Peptides mimicking the unique ARTS-XIAP binding site promote apoptotic cell death in cultured cancer cells. Clin. Cancer Res., 18: 2569-2578.
48. LaBelle, J.L., S.G. Katz, G.H. Bird, E. Gavathiotis and M.L. Stewart *et al*., 2012. A stapled BIM peptide overcomes apoptotic resistance in hematologic cancers. J. Clin. Invest., 122: 2018-2031.
49. Cruciani, R.A., J.L. Barker, M. Zasloff, H.C. Chen and O. Colamoni, 1991. Antibiotic magainins exert cytolytic activity against transformed cell lines through channel formation. Proc. Natl. Acad. Sci. USA., 88: 3792-3796.
50. Wu, J.M., P.S. Jan, H.C. Yu, H.Y. Haung and H.J. Fang *et al*., 2009. Structure and function of a custom anticancer peptide, CB1a. Peptides, 30: 839-848.
51. Chen, J., X.M. Xu, C.B. Underhill, S. Yang and L. Wang *et al*., 2005. Tachyplesin activates the classic complement pathway to kill tumor cells. Cancer Res., 65: 4614-4622.

52. Patra, C.R., C.N. Rupasinghe, S.K. Dutta, S. Bhattacharya, E. Wang, M.R. Spaller and D. Mukhopadhyay, 2012. Chemically modified peptides targeting the PDZ domain of GIPC as a therapeutic approach for cancer. ACS Chem. Biol., 7: 770-779.
53. Lee, H.S., C.B. Park, J.M. Kim, S.A. Jang and I.Y. Park *et al*., 2008. Mechanism of anticancer activity of buforin IIb, a histone H2A-derived peptide. Cancer Lett., 271: 47-55.
54. Wang, Y.S., D. Li, H.S. Shi, Y.J. Wen and L. Yang *et al*., 2009. Intratumoral expression of mature human neutrophil peptide-1 mediates antitumor immunity in mice. Clin. Cancer Res., 15: 6901-6911.
55. Xu, H., C.X. Chen, J. Hu, P. Zhou, P. Zeng, C.H. Cao and J.R. Lu, 2013. Dual modes of antitumor action of an amphiphilic peptide A_9K. Biomaterials, 34: 2731-2737.
56. Cardozo, A.K., V. Buchillier, M. Mathieu, J. Chen and F. Ortis *et al*., 2007. Cell-permeable peptides induce dose-and length-dependent cytotoxic effects. Biochim. Biophys. Acta-Biomembr., 1768: 2222-2234.
57. Goswami, D., M.T. Machini, D.M. Silvestre, C.S. Nomura and B.P. Esposito, 2014. Cell penetrating peptide (CPP)-conjugated desferrioxamine for enhanced neuroprotection: Synthesis and *in vitro* evaluation. Bioconjug. Chem., 25: 2067-2080.
58. Boisguerin, P., C. Redt-Clouet, A. Franck-Miclo, S. Licheheb, J. Nargeot, S. Barrere-Lemaire and B. Lebleu, 2011. Systemic delivery of BH4 anti-apoptotic peptide using CPPs prevents cardiac ischemia-reperfusion injuries *in vivo*. J. Controlled Release, 156: 146-153.
59. Freire, J.M., A.S. Veiga, T.M. Conceicao, W. Kowalczyk and R. Mohana-Borges *et al*., 2013. Intracellular nucleic acid delivery by the supercharged dengue virus capsid protein. PLoS ONE, Vol. 8. 10.1371/journal.pone.0081450
60. Mano, M., C. Teodosio, A. Paiva, S. Simoes and M.C.P. de Lima, 2005. On the mechanisms of the internalization of $S4_{13}$-PV cell-penetrating peptide. Biochem. J., 390: 603-612.
61. Trabulo, S., A.L. Cardoso, A.M.S. Cardoso, N. Duzgunes, A.S. Jurado and M.C.P. de Lima, 2012. Cell-penetrating peptide-based systems for nucleic acid delivery: A biological and biophysical approach. Methods Enzymol., 509: 277-300.
62. Yan, L. and M.E. Adams, 1998. Lycotoxins, antimicrobial peptides from venom of the wolf spider *Lycosa carolinensis*. J. Biol. Chem., 273: 2059-2066.
63. Alfred, R.L., E.A. Palombo, J.F. Panozzo and M. Bhave, 2013. The antimicrobial domains of wheat puroindolines are cell-penetrating peptides with possible intracellular mechanisms of action. PLoS ONE, Vol. 8. 10.1371/journal.pone.0075488
64. Kim, J.H., 2016. A novel cell penetrating peptide carrier for the delivery of nematocidal proteins drug. http://adsabs.harvard.edu/abs/2016APS..MAR.T1353K.
65. Khandia, R., A.K. Munjal, H.M. Iqbal and K. Dhama, 2016. Heat shock proteins: Therapeutic perspectives in inflammatory disorders. Recent Pat. Inflamm. Allergy Drug Discov., 10: 94-104.
66. Jo, D., D. Liu, S. Yao, R.D. Collins and J. Hawiger, 2005. Intracellular protein therapy with SOCS3 inhibits inflammation and apoptosis. Nat. Med., 11: 892-898.
67. Xiao, Y.F., M.M. Jie, B.S. Li, C.J. Hu, R. Xie, B. Tang and S.M. Yang, 2015. Peptide-based treatment: A promising cancer therapy. J. Immunol. Res., Vol. 2015. 10.1155/2015/761820
68. Khandia, R., S. Sachan, A.K. Munjal, R. Tiwari and K. Dhama, 2016. Tumor Homing Peptides: Promising Futuristic Hope for Cancer Therapy. In: Topics in Anti-Cancer Research, Atta-ur-Rahman, F.R.S. and K. Zaman (Eds.). Bentham Science Publishers, Sharjah, UAE., pp: 43-86.
69. Bloch, M., Y. Kam, E. Yavin, D. Moradov, A. Nissan, I. Ariel and A. Rubinstein, 2012. The relative roles of charge and a recognition peptide in luminal targeting of colorectal cancer by fluorescent polyacrylamide. Eur. J. Pharm. Sci., 47: 904-913.
70. Liu, S., H. Yang, L. Wan, H.W. Cai and S.F. Li *et al*., 2011. Enhancement of cytotoxicity of antimicrobial peptide magainin II in tumor cells by bombesin-targeted delivery. Acta Pharmacol. Sin., 32: 79-88.
71. Kagan, B.L., M.E. Selsted, T. Ganz and R.I. Lehrer, 1990. Antimicrobial defensin peptides form voltage-dependent ion-permeable channels in planar lipid bilayer membranes. Proc. Natl. Acad. Sci. USA., 87: 210-214.
72. McKeown, S.T.W., F.T. Lundy, J. Nelson, D. Lockhart, C.R. Irwin, C.G. Cowan and J.J. Marley, 2006. The cytotoxic effects of human neutrophil peptide-1 (HNP1) and lactoferrin on oral squamous cell carcinoma (OSCC) *in vitro*. Oral Oncol., 42: 685-690.
73. Nishimura, M., Y. Abiko, Y. Kurashige, M. Takeshima and M. Yamazaki *et al*., 2004. Effect of defensin peptides on eukaryotic cells: primary epithelial cells, fibroblasts and squamous cell carcinoma cell lines. J. Dermatol. Sci., 36: 87-95.
74. Kim, I.W., S.J. Kim, Y.N. Kwon, E.Y. Yun, M.Y. Ahn, D.C. Kang and J.S. Hwang, 2012. Effects of the synthetic coprisin analog peptide, CopA3 in pathogenic microorganisms and mammalian cancer cells. J. Microbiol. Biotechnol., 22: 156-158.
75. Qian, S., W. Wang, L. Yang and H.W. Huang, 2008. Structure of transmembrane pore induced by Bax-derived peptide: Evidence for lipidic pores. Proc. Natl. Acad. Sci. USA., 105: 17379-17383.
76. Valero, J.G., L. Sancey, J. Kucharczak, Y. Guillemin and D. Gimenez *et al*., 2011. Bax-derived membrane-active peptides act as potent and direct inducers of apoptosis in cancer cells. J. Cell Sci., 124: 556-564.

77. Andreev, O.A., D.M. Engelman and Y.K. Reshetnyak, 2010. pH-sensitive membrane peptides (pHLIPs) as a novel class of delivery agents. Mol. Membr. Biol., 27: 341-352.
78. Andreev, O.A., D.M. Engelman and Y.K. Reshetnyak, 2009. Targeting acidic diseased tissue: New technology based on use of the pH (Low) Insertion Peptide (pHLIP). Chim. Oggi., 27: 34-37.
79. Mai, J.C., Z. Mi, S.H. Kim, B. Ng and P.D. Robbins, 2001. A proapoptotic peptide for the treatment of solid tumors. Cancer Res., 61: 7709-7712.
80. Boohaker, R.J., M.W. Lee, P. Vishnubhotla, J.M. Perez and A.R. Khaled, 2012. The use of therapeutic peptides to target and to kill cancer cells. Curr. Med. Chem., 19: 3794-3804.
81. Meloni, B.P., L.M. Brookes, V.W. Clark, J.L. Cross and A.B. Edwards *et al*., 2015. Poly-arginine and arginine-rich peptides are neuroprotective in stroke models. J. Cerebral Blood Flow Metab., 35: 993-1004.
82. Meloni, B.P., D. Milanic, A.B. Edwards, R.S. Anderto and R.L.O. Doig *et al*., 2015. Neuroprotective peptides fused to arginine-rich cell penetrating peptides: Neuroprotective mechanism likely mediated by peptide endocytic properties. Pharmacol. Ther., 153: 36-54.
83. Meloni, B.P., A.J. Craig, N. Milech, R.M. Hopkins, P.M. Watt and N.W. Knuckey, 2014. The neuroprotective efficacy of cell-penetrating peptides TAT, penetratin, Arg-9 and Pep-1 in glutamic acid, kainic acid and *in vitro* ischemia injury models using primary cortical neuronal cultures. Cell. Mol. Neurobiol., 34: 173-181.
84. Fleming, R.E. and P. Ponka, 2012. Iron overload in human disease. N. Engl. J. Med., 366: 348-359.
85. Raven, E.P., P.H. Lu, T.A. Tishler, P. Heydari and G. Bartzokis, 2013. Increased iron levels and decreased tissue integrity in hippocampus of Alzheimer's disease detected *in vivo* with magnetic resonance imaging. J. Alzheimer's Dis., 37: 127-136.
86. Liu, G., P. Men, G. Perry and M.A. Smith, 2010. Nanoparticle and iron chelators as a potential novel Alzheimer therapy. Methods Mol. Biol., 610: 123-144.
87. Lynch, S.G., T. Fonseca and S.M. LeVine, 2000. A multiple course trial of desferrioxamine in chronic progressive multiple sclerosis. Cell. Mol. Biol., 46: 865-869.
88. Choi, D., K.C. Hwang, K.Y. Lee and Y.H. Kim, 2009. Ischemic heart diseases: Current treatments and future. J. Controlled Release, 140: 194-202.
89. Wagner, E., 2012. Polymers for siRNA delivery: Inspired by viruses to be targeted, dynamic and precise. Acc. Chem. Res., 45: 1005-1013.
90. Milletti, F., 2012. Cell-penetrating peptides: Classes, origin and current landscape. Drug Discov. Today, 17: 850-860.
91. Freire, J.M., A.S. Veiga, I.R. de Figueiredo, B.G. Torre and N.C. Santos *et al*., 2014. Nucleic acid delivery by cell penetrating peptides derived from dengue virus capsid protein: Design and mechanism of action. FEBS J., 281: 191-215.
92. Heitz, F., M.C. Morris and G. Divita, 2009. Twenty years of cell penetrating peptides: From molecular mechanisms to therapeutics. Br. J. Pharmacol., 157: 195-206.
93. Wimley, W.C. and K. Hristova, 2011. Antimicrobial peptides: Successes, challenges and unanswered questions. J. Membr. Biol., 239: 27-34.
94. Hughes, S.R., P.F. Dowd and E.T. Johnson, 2012. Cell-penetrating recombinant peptides for potential use in agricultural pest control applications. Pharmaceuticals, 5: 1054-1063.
95. Eudes, F. and A. Chugh, 2008. Cell-penetrating peptides: From mammalian to plant cells. Plant Signal. Behav., 3: 549-550.
96. Kamei, N., M. Morishita, Y. Eda, N. Ida, R. Nishio and K. Takayama, 2008. Usefulness of cell-penetrating peptides to improve intestinal insulin absorption. J. Controlled Release, 132: 21-25.
97. Kamei, N., S. Kikuchi, M. Takeda Morishita, Y. Terasawa and A. Yasuda *et al*., 2013. Determination of the optimal cell-penetrating peptide sequence for intestinal insulin delivery based on molecular orbital analysis with self-organizing maps. J. Pharm. Sci., 102: 469-479.
98. Patel, L.N., J. Wang, K.J. Kim, Z. Borok, E.D. Crandall and W.C. Shen, 2009. Conjugation with cationic cell-penetrating peptide increases pulmonary absorption of insulin. Mol. Pharm., 6: 492-503.
99. Liu, E., J. Sheng, J. Ye, Y. Wang and J. Gong *et al*., 2014. CPP mediated insulin delivery: Current status and promising future. Curr. Pharm. Biotechnol., 15: 240-255.
100. He, H., J. Ye, J. Sheng, J. Wang and Y. Huang *et al*., 2013. Overcoming oral insulin delivery barriers: Application of cell penetrating peptide and silica-based nanoporous composites. Front. Chem. Sci. Eng., 7: 9-19.
101. Zhang, L., L. Song, C. Zhang and Y. Ren, 2012. Improving intestinal insulin absorption efficiency through coadministration of cell-penetrating peptide and hydroxypropyl-β-cyclodextrin. Carbohydr. Polym., 87: 1822-1827.