Peptides and Drug Delivery

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Abstract

Peptides have been used as drugs to treat various health conditions, and they are also being developed as diagnostic agents. Due to their receptor selectivity, peptides have recently been utilized for drug delivery to target drug molecules to specific types of cells (*i.e.* cancer cells, immune cells) to lower the side effects of the drugs. In this case, the drug is conjugated to the carrier peptide for directing the drug to the target cells (*e.g.* cancer cells) with higher expression of a specific receptor that recognizes the carrier peptide. As a result, the drug is directed to the target diseased cells without affecting the normal cells. Peptides are also being developed for improving drug delivery through the intestinal mucosa barrier (IMB) and the blood-brain barrier (BBB). These peptides were derived from intercellular junction proteins such as occludins, claudins, and cadherins and improve drug delivery through the IMB and BBB via the paracellular pathways. It is hypothesized that the peptides modulate protein-protein interactions in the intercellular junctions of the IMB and BBB to increase the porosity of paracellular pathways of the barriers. These modulator peptides have been shown to enhance brain delivery of small molecules and medium-sized peptides as well as a large protein such as 65 kDa albumin. In the future, this method has the potential to improve oral and brain delivery of therapeutic and diagnostic peptides and proteins.

Keywords

Targeted drug delivery • Peptide-drug conjugate • Peptide-particle conjugate • Blood-brain barrier • Intestinal mucosa barrier • Modulator of intercellular junctions • Brain delivery

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Abbreviations

AJ	adherens junction
ANG	angiopep
APaseP	aminopeptidase P
APP	amyloid precursor protein
BBB	blood brain barrier
BBMEC	bovine brain microvessel endothelial cells
CAD	*cis*–asconitic anhydride–DOX
C–CPE	*clostridium perfringens enterotoxin*
CNS	central nervous systems
CPP	cell penetrating peptide
CPT	camptothecin
DOX	doxorubicin
DSDS	drug self-delivery system
DSPE-PEG	1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-methoxy-(polyethylene glycol)
EC-1	extracellular domain-1
ECM	extracellular matrix
EGF	epidermal growth factor
EGRF	epidermal growth factor receptor
EGTA	ethyleneglycol-bis-(β-aminoethyl ether)-N, N′-tetraacetic acid
ERK	extracellular signal-regulated kinases
FACS	fluorescence-activated cell sorting
FDA	Food and Drug Administration
FITC	fluorescein isothiocyanate
GABA	γ-aminobutyric acid
Gd-DTPA	gadopentetic acid
GIP	glucose-dependent insulinotropic peptide
GLP-1	glucagon-like peptide-1
GMB	glioblastoma multiforme
GnRH	gonadotrophin-releasing hormone
HEK	human embryonic kidney cells
HIV-1	human immunodeficiency virus 1
HPMA	(2-hydroxypropyl) methacrylamide
ICAM-1	intercellular adhesion molecule-1
IMB	intestinal mucosa barrier
JAM	junction adhesion molecules
LFA-1	lymphocyte function-associated antigen-1
LHRH	luteinizing hormone-releasing hormones
LRP-1	lipoprotein-related protein 1
MDCK	Madin-Darby Canine Kidney Epithelial Cells
MDR1	multi drug resistance-1
MEK	mitogen-activated protein kinase
MMAF	monomethyl auristatin F
MMPs	matrix metalloproteinases
MRI	magnetic resonance imaging
NB	nanobubbles
NLS	nuclear localization signal
NMR	nuclear magnetic resonance
NRP-1	neuropilin-1
OCT	octreotide
ODN	octadecanol
OVCAR-3	ovarian cancer cell-3
PAMAM-PAsp-PEG	poly(amidoamine)-b-poly(aspartic acid)-b-poly(ethylene glycol)
pAntp	antennapedia homeodomain protein of Drosophila
PEG	polyethylene glycol
PET	positron emission tomography
pHLIP	pH low insertion peptide
PI3K	phosphoinositide 3-kinase
PTX	paclitaxel
RES	reticuloendothelial system
RGD	Arg-Gly-Asp
SA	stearylamine
SCCHN	squamous cell carcinoma of head and neck
SI	secretase inhibitor
siRNA	small interfering RNA
STTR	somatostatin receptors
TAMRA	tetramethylrhodamine
TAT	*trans*-activating transcriptional activator
TEER	*trans*-epithelial electrical resistance
TfR	transferrin receptor
TJ	tight junction
TPP	triphenylphosphonium
ZO	*zonula occludin*
Zot	*zonula occludens toxin*

8.1 Introduction

Peptides have been successfully developed as therapeutic and diagnostic agents because of their selectivity to bind the respective target receptors (Kaspar and Reichert 2013; Uhlig et al. 2014; Fosgerau and Hoffmann 2015). Currently, there are more than 60 peptide drugs approved by the US Food and Drug Administration (FDA). Thus, development of peptide drugs has increased significantly in the past decades and, as of today, approximately 140 peptides are in clinical trials as potential drugs. In addition, more than 500 therapeutic peptides are in preclinical development (Fosgerau and Hoffmann 2015). Available peptide drugs which include oxytocin, calcitonin, octreotide, and exenatide are being used to treat various conditions. Some bioactive peptides have been derived from endogenous substances; however, some peptides were derived from truncation of the active region(s) of the parent proteins. For example, opioid peptides such as enkephalins, endorphins, and dynorphins that are found in the brain have been used as drugs. Oxytocin, an endogenous hormone released by the posterior pituitary, is a cyclic peptide synthesized in the paraventricular nucleus of the hypothalamus (Bell et al. 2014). Oxytocin and its analogs work as neurotransmitters in the brain to facilitate breastfeeding, induce labor, and treat postpartum hemorrhage. Calcitonin peptide is a hormone produced by the thyroid gland to control calcium and potassium levels in the blood. A synthetic salmon-calcitonin peptide has been used to treat osteoporosis; this peptide was first developed as a nasal spray (Tella and Gallagher 2014). Tumors can be treated by octreotide, which inhibits the release of growth hormones (Broder et al. 2015). Type-2 diabetes is treated successfully with exenatide (Table 8.1), which is a derivative of a glucagon-like peptide-1 agonist (GLP-1; Table 8.1) (Knop et al. 2017). Exenatide was developed to increase the *in vivo* half-life because GLP-1 was ineffective in clinical trials for diabetes treatment due to its short half-life. Exenatide binds to GLP-1 receptor and regulates glucose metabolism and insulin secretion. Both GLP-1 and glucose-dependent insulinotropic peptide (GIP) hormones are produced upon ingestion of food to stimulate insulin secretion; however, only GLP-1 causes insulin secretion in a diabetic state.

Bioactive peptides can also be derived from the active region(s) of large functional proteins. One example is "Arg-Gly-Asp" (RGD), which is derived from the sequence of extracellular matrix (ECM) proteins such as fibronectin, fibrinogen, vitronectin, von Willebrand factor, laminin, and collagen (Ruoslahti and Pierschbacher 1987). RGD sequence on the ECM is recognized by various integrin receptors on the cell surface for cell adhesion to ECM. The ECM-integrin binding is essential in various disease processes such as thrombosis, angiogenesis and tumor metastasis (Ruoslahti 1994). In thrombosis, the process of vascular blood clotting prevents the normal blood flow from the heart, which involves platelet aggregation. Platelet aggregation results from interactions of fibrinogen and platelets, which are mediated by recognition of RGD sequences on the α- and γ-subunits of fibrinogen by gpIIb/IIIa integrin receptors on platelet surfaces (Dunehoo et al. 2006). Therefore, RGD peptides (*e.g.* integrilin or eptifibatide) and peptidomimetics (*e.g.* aggrastat or tirofiban) have been used as antithrombic agents in the clinic. Integrilin and aggrastat are selective and potent ligands for gpIIb/IIIa receptors, blocking platelet aggregation during thrombosis. Angiogenesis in solid tumors can be inhibited by RGD peptides (Table 8.1), which are designed to bind to cell-surface $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrins that are overexpressed during tumor angiogenesis (Mas-Moruno et al. 2010). Other cell adhesion peptides, such as those derived from intercellular adhesion molecule-1 (ICAM-1) and lymphocyte function-associated antigen-1 (LFA-1) receptors, have been shown to inhibit T-cell adhesion by adhering to epithelial and endothelial cells during inflammation (Yusuf-Makagiansar et al. 2002).

In addition to their use as drugs, bioactive peptides have been used as targeting moieties for the delivery of drug payloads (*e.g.* anticancer and anti-inflammatory) to specific types of cells in tissues, and ultimately, to reduce their adverse side effects. Some of these peptides are internalized by their respective receptors into cells via a

Table 8.1 Peptide names and sequences

Peptide	Sequence
Exenatide	HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS-NH_2
GLP-1	HDEFERHAEGTFTSDVSSYLEGQAAKEFIAWLVKGR - NH_2
ALOS4	Cyclo1,9(CSSAGSLFC)
RGD-1	Cyclo(RGDyK)
Transportan	GWTLNSAGYLLGKINLKALAALAKKIL
Penetratin	RQIKIWFQNRRMKWKK
PAF-26	RKKWFW
Octreotide	Cyclo2,7(fCFwKTCT)
GnRH-1	EHWSYkLRPG-NH_2
GnRH-2	EHWSHkWYPG-NH_2
GnRH-3	EHWSHDWKPG-NH_2
pHLIP	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
MMP-hexapeptide	PVGLIG
ANG	TFFYGGSRGKRNNFKTEEY
ANG-SI	TFFYGGSRGKRNNFK–EVN-sta-VAEF
ANG-PEG-S	TFFYGGSRGKRNNFK–PEG–EVN-sta-VAEF
ANG-TAT	TFFYGGSRGKRNNFK–TEEYGRKKRRQRRRPPQQ
Biotin-ANG	TFFYGGSRGKRNNF([Biotin)TEEY
Biotin-ANG-TAT	TFFYGGSRGKRNNFK(Biotin)TEEYGRKKRRQRRRPPQQ
T10	HAIYPRH
ERK	MPKKKPTPIQLNP
T10-ERK	HAIYPRH–GGCG–MPKKKPTPIQLNP
PEGA	Cyclo1,10(CPGEPEGAGC)
A54	AGKGTPSLETTP
G3-C12	ANTPCGPYTHDCPVKR
KLA	$D(KLAKLAK)_2$
RGD-2	Cyclo(RGDfC)
Pep-1	Cyclo1,9(CGEMGWVRC)
EGRF	YHWYGYTPQNVI
CPP	CKRRMKWKK
YSA	YSAYPDSVPMMS
RGD-3	CRGDK
OCC2	GVNPQAQMSSGYYYSPLLAMC(Acm)SQAYGSTYLNQYIYHYC(Acm)TVDPQE; Acm = Acetamido methyl
$OP_{90–135}$	DRGYGTSLLGGSVGYPYGGSGFGSYGSGYGYGYGYGYGYGGYTDPR-NH_2
$OP_{90–103}$	DRGYGTSLLGGSVG
Lip-$OP_{90–103}$	Lipid-C12-DRGYGTSLLGGSVG
C1C2	SSVSQSTGQIQSKVFDSLLNLNSTLQATR-NH_2
HAV6	Ac-SHAVSS-NH_2
ADTC5	Cyclo1,7Ac-(CDTPPVC)NH_2
cIBR7	Cyclo1,8(CPRGGSVC)
cLABL	Cyclo1,12(PenITDGEATDSGC)
C-CPE	SSYSGNYPYSILFQKF
AT-1002	FCIGRL
PN-78	FDFWITP
PN-159	KLALKLALKALKLAALKLA-NH_2

receptor-mediated endocytosis process. Others have been conjugated to drug-loaded nanoparticles for specific delivery to corresponding cell targets of the peptide. Receptor selective peptides were also investigated as diagnostic agents by conjugating them to dyes, radioisotopes, or magnetic resonance imaging (MRI) contrast agents.

The ultimate goal of targeted drug delivery is to direct a drug to diseased cells or organs (*e.g.* cancer cells), while avoiding normal cells. This results in a drug construct with lower side effects than the free form of its parent drug. This chapter describes the roles of peptides in drug delivery, including the use of peptides as: (a) peptide-drug and peptide-particle conjugates for targeting molecules to a specific type of cells and (b) modulators of biological barriers for improving the oral and brain delivery of drugs and diagnostic agents.

8.2 Peptide-Drug Conjugates for Targeted Drug Delivery

Drug targeting methods are normally explored to reduce side effects by directing toxic drugs to cells involved in disease states, leaving normal cells minimally affected. Conjugation of the drug to its peptide carrier (targeting agent) can be done directly or through a chemical linker**.** Thus, as a peptide carrier selectively binds to a specific receptor on the surface of targeted cells (*i.e.* cancer cells), it carries along the drug or diagnostic molecule with it. As an example, cancer cells have a certain upregulated receptor(s) (*e.g.* HER-2, EGFR) compared to normal cells. These upregulated receptors become distinguishing and exploitable features to selectively direct populations of conjugated drug molecules to accumulate in cancer cells over normal cells. Binding of the conjugate to the target receptor is followed by cellular uptake of the ligand-receptor complex into the early endosomes via receptor-mediated endocytosis (Majumdar and Siahaan 2012). From the endosomes, the conjugate reaches the lysosomes where the drug is released as a result of lowered pH and/or enzyme degradation in the lysosomes. The rate of release of the drug can be controlled by designing the appropriate linker between the drug and the peptide (Hamann et al. 2002; Majumdar and Siahaan 2012; Buyuktimkin et al. 2016). This method has been successfully applied in antibody-drug conjugates such as Adcetris® and Kadcycla® to treat cancer patients (Leal et al. 2014; Buyuktimkin et al. 2016). Peptides are smaller than proteins, and can be rapidly synthesized using solid-phase methods. Unlike proteins, most peptides have only primary and secondary structures. Thus, most peptides do not suffer as much from physical instability as do proteins, which often leads to the formation of aggregates that generate immunogenicity. Due to their small size, peptide-drug conjugates are potentially less immunogenic than protein-drug conjugates. Although their formulation remains challenging, peptide conjugate formulation is usually less complicated than that of protein-drug conjugates.

In designing a peptide-drug conjugate, a functional group (*i.e.* amine, carboxylic acid, alcohol, or thiol) within the structure of the drug can be used to link the drug directly to a targeting peptide's N- or C-termini, or a side-chain functional group of the Lys, Asp, Glu, Ser, Thr, or Cys residue(s) on the peptide. This direct drug-peptide linkage may be in the form of an amide, ester, or thioether bond. In many cases, the drug may be conjugated to the peptide using a molecular linker (*e.g.* PEG, maleimido, etc.). Other than bridging drug and targeting components of the construct, the vital role of the linker is to provide a distance between the peptide and the drug, which is often crucial to the overall activity and potency of the construct. In one respect, ample distance between the drug and targeting-peptide components prevents interference (or steric hindrance) in binding to their respective receptors. This is because both drug and peptide have a molecular surface recognized by their respective receptors for biological activity. Normally, the conjugation is done at the functional group away from the bioactive region of the peptide or the drug. In addition, the linker can be designed to control the release of the drug from the conjugate upon reaching the cell targets within the tissue. Premature release of the drug in the systemic or lymphatic circulation before reaching the respective target cells can be harmful, and ultimately, defeats the purpose of drug-targeting.

8.2.1 Peptide-Drug Conjugates for Cancer Therapy and Diagnostics

Chemotherapy remains the treatment of choice for cancer patients (Song et al. 2015). Most chemotherapeutic agents are cytotoxic and kill not only cancer cells but also normal cells in the body. Some drugs have poor solubility, are highly toxic, or cannot cross the cancer cell membranes into the intracellular space. Most cancer cells eventually generate resistance to anticancer drugs after multiple treatments. One of the drug-resistance mechanisms is due to the overexpression of efflux pumps *(i.e.* Pgp, MRP, MDR1) that expel the anticancer drug from the cancer cell membranes. Therefore, there is a need to develop an alternative method to deliver drugs into cancer cells and overcome drug resistance by avoiding the efflux pumps. One way to increase drug penetration across cell membranes is by utilizing receptor-mediated endocytosis mechanisms. Thus, drugs have been conjugated to peptides, proteins, nanocarriers, carbon nanotubes, and dendrimers. As an example, paclitaxel (PTX), which is widely used to treat breast, ovarian, testicular, and cervical cancers, is known to have poor water solubility (Majumdar et al. 2016). Thus, conjugation to a targeting peptide via a polyethylene glycol (PEG) linker increases solubility and improves selectivity to target cancer cells.

8.2.1.1 Cell Adhesion Peptides

Cell adhesion peptides have been used to target drugs and radioisotopes for cancer treatments and diagnostics. RGD peptides are cell adhesion molecules that have been extensively explored as carriers in peptide-drug conjugates. Certain cyclic RGD peptides bind selectively to $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrin receptors, which are upregulated during angiogenesis in tumors. Thus, these selective cyclic RGD peptides have been used to deliver radioisotopes such as ^{18}F, ^{99m}Tc, ^{125}I, or ^{64}Cu as cancer diagnostic agents. For example, ^{18}F-containing galactose was incorporated to RGD-1 peptide (Table 8.1) via an amide bond to the D-Lys side chain (Haubner et al. 2005). The ^{18}F-labeled conjugate binds selectively to upregulated $\alpha_V\beta_3$ integrin receptors on tumor vasculature as observed by positron emission tomography (PET). Thus, the conjugate can be used as an imaging tool to detect angiogenesis and tumor metastasis *in vivo* (Haubner et al. 2005). The levels of $\alpha_V\beta_3$ receptors on human tumor cells have been detected with ^{18}F-labeled conjugates and observed using PET. It was found that the detected levels of $\alpha_V\beta_3$ receptors were similar to those detected using immunohistochemistry (Beer et al. 2006). Accumulation of ^{18}F-labeled RGD-1 peptide in human tumors showed intra- and inter-variability of conjugate accumulation due to different levels of $\alpha_V\beta_3$ across different individuals (Haubner et al. 2005). The various levels of $\alpha_V\beta_3$ found in humans can be used to predict populations of cancer patients most likely to respond to treatments with RGD-anticancer drug conjugates. RGD peptides have been used to selectively deliver the anticancer drugs doxorubicin (DOX) (Arap et al. 1998) and PTX (Chen et al. 2005) to cancer cells *in vitro* and *in vivo*. RGD-1-DOX conjugate suppressed the growth of breast cancer xenographs in mice better than DOX alone, suggesting that cyclic RGD peptide improves the targeting of DOX to breast cancer cells *in vivo* (Arap et al. 1998).

ALOS4 peptide (Table 8.1) is a non-RGD peptide that also binds to $\alpha_V\beta_3$ integrin. The peptide was linked to camptothecin (CPT) and fluorescein isothiocyanate (FITC) via a GABA linker to give ALOS4-CPT and ALOS4-FITC, respectively (Redko et al. 2016). FACS analysis showed a strong binding of ALSO4-FITC to WM-266-4, a malignant melanoma cell line. *In vivo* studies confirmed that tumor-bearing WM-266-4 cells in mice intravenously administered with ALOS4-FITC showed accumulation of ALOS4-FITC specifically in tumors rather than organs as observed after 24 hours. Next, ALOS4-CPT enhanced drug cytotoxicity to tumor cells better than CPT and other anticancer drugs. CPT activity has been known to be deactivated by the hydrolytic opening of a vital lactone ring in its structure. In contrast to its free form, the stability of this lactone ring is increased in the ALOS4-CPT conjugate (Redko et al. 2016). At 10 μM,

CPT alone kills high percentages of both malignant WM-266-4 and non-malignant HEK-293 tumor cells (human embryonic kidney cells) while ALOS4-CPT kills 70% of malignant WM-266-4 cells compared to 30% of the non-malignant HEK-293 cells. The activity of the conjugate is dose-dependent (Redko et al. 2016). These results affirm that ALOS4 selectively targets and delivers conjugates to malignant tumor cells.

8.2.1.2 Cell-Penetrating Peptides

Generally, most peptides cannot readily cross the cell membranes due to their physicochemical properties; however, cell-penetrating peptides (CPPs with 6–30 amino acids) are capable of crossing membranes and entering the cytoplasm of the cells. A detailed mechanism of the cellular uptake of CPPs is not well understood, but it may take place either by direct translocation or by endocytosis. The early CPPs identified were *trans*-activating transcriptional activator (TAT) peptides, derived from human immunodeficiency virus 1 (HIV-1), and antennapedia homeodomain protein of drosophila (pAntp). These long sequences have been reduced to 6–7 amino acid peptides, which maintain similar cell penetrating behavior. CPPs have been used for cellular delivery of small drug molecules (*i.e.* doxorubicin, methotrexate and taxol), proteins, nucleic acids, and contrasting agents. Apart from the natural CPPs, synthetic and semi-synthetic CPPs, including the chimeric 27-amino acid transportan, penetratin, and PAF-26, were designed to facilitate drug delivery (Table 8.1).

8.2.1.3 Peptide Hormone for Drug Delivery

Peptide hormones such as octreotide (OCT), gonadotrophin-releasing hormone (GnRH), and epidermal growth factor (EGF) peptides (Table 8.1) have been investigated for delivering drugs to cancer cells. GnRH receptors are overexpressed in malignant tumor of ovarian, breast, prostate cancers as part of the paracrine/autocrine regulatory system of malignant tumors (Bajusz et al. 1989; Limonta et al. 2003; Muranyi et al. 2016). OCT and other somatostatin peptides bind to somatostatin receptors (STTRs) especially STTR2, which are upregulated in breast, cervical, colon, lung, ovarian cancers cells (Hejna et al. 2002). OCT peptide has a long half-life in systemic circulation with good tissue penetration due to its uptake by the STTR2 receptor. OCT has also been used for targeting radiotherapies (Muranyi et al. 2016). PTX-OCT conjugate was designed to improve the biological properties of PTX and overcome the issue of cancer resistance. Ovarian cancer is treated with PTX, but normally through multiple sessions/doses, often causing the emergence of drug resistance (Chen et al. 2016). Localization of OCT peptide after delivery has been monitored using FITC-labeled OCT peptide (FITC-OCT), injected into nude mice bearing a xenografted tumor. Localization of FITC-OCT on the xenografted tumor confirmed abnormally high levels of STTR2 receptor expression in tumors. PTX-OCT also suppressed tumor growth in mice xenografts better than in those treated with free PTX, OCT, and mixtures of PTX + OCT (Chen et al. 2016). This result demonstrates the selectivity of PTX-OCT to tumor cells on the basis of high expression of STTR2. In addition, the conjugate downregulates the expression of multi drug resistance-1 (MDR1) protein.

GnRH or LHRH peptides effectively deliver anticancer drugs such as DOX and CPT to cancer cells (Nagy et al. 1996; Dharap 2003). FITC-labeled GnRH analogues have been used to compare targeting efficiencies of GnRH-1, GnRH-2, and GnRH-3 peptides (Table 8.1) in human breast, colon, pancreas, and prostate cancer cells to that in the non-tumor cell line such as Madin-Darby canine kidney epithelial (MDCK) cells. This study also revealed that human pharynx tumor cells similarly overexpress GnRH receptors on cell surfaces such as human breast, colon and prostate cancer cell lines. In contrast, pancreatic tumor cells (BxPC-3) do not present GnRH-1 receptors on their membranes (Muranyi et al. 2016). As expected, GnRH peptides are internalized by tumor cells via active transport mechanisms. Although different cancer cell lines vary in their uptake properties for the three different GnRH peptides, uptake by all tumor cells was

significantly higher than in the control MDCK cell-line, thus indicating the role of GnRH receptor upregulation in tumor cells.

8.2.1.4 pH Low Insertion Peptide (pHLIP)

A pH low insertion peptide (pHLIP; Table 8.1) was developed as a pH-dependent cell-penetrating peptide for drug delivery (Burns et al. 2017). pHLIP is a water-soluble membrane peptide that interacts weakly with cell membranes at neutral pH; however, when the cell surface is slightly acidic, the pHLIP peptide is inserted into the cell membranes as a stable transmembrane α-helix. Its primary sequence is characterized by acidic residues (*i.e.* Asp or Glu) that can be protonated at the low extracellular pH observed in tumors. In testing the concept, six pHLIP derivatives were conjugated to monomethyl auristatin F (MMAF) to make pHLIP-MMAF conjugates and the MMAF is attached to the pHLIP C-terminus via a S-S bond that can be cleaved in the cytoplasm (Burns et al. 2017). The efficacy of six pHLIP-MMAF conjugates was evaluated *in vitro* against cultured cancer cells to find the lead conjugate. *In vivo*, the lead conjugate showed significant therapeutic efficacy in mouse models without overt toxicities. pHLIP-MMAF was localized in cancer cells and inhibited the proliferation of cancer cells in a pH-selective and concentration-dependent manner.

8.2.1.5 MMP Peptides

Tumor cells have a high expression of MMPs (MMP-2 and MMP-9) that are important in tumor proliferation and metastasis (Paez Pereda et al. 2000). One MMP-hexapeptide, PVGLIG, has a high binding affinity to matrix metalloprotease-2 (MMP-2) enzyme. Conjugated to PTX at the C-terminus of the MMP peptide via an ester bond, PTX-MMP was found to deliver PTX in a tumor-specific manner (Huang et al. 2016). Incubation of PTX-MMP with MMP2, as well as with cancer cells (*i.e.* HT-1080 and U87MG), releases PTX from the conjugate. PTX release was higher in HT-1080 and U87MG cells compared to negative control cells (*i.e.* Hep-2 and Hep G2), suggesting the involvement of MMPs in both cancer cells (Huang et al. 2016). PTX-MMP shows significantly higher cytotoxicity in HT-1080 and U87MG cells compared to PTX alone, with no difference in toxicity between PTX-MMP and PTX on Hep-2 and Hep G2 cells, which have low expression of MMPs. Mice implanted with HT-1080 or U87MG cells have a higher survival rate when treated with PTX-MMP compared to those treated with PBS, PTX, and the MMP hexapeptide (Huang et al. 2016). These results support a role for the peptide and MMP-2 in the activity of the PTX-MMP conjugate against tumor cells.

8.2.1.6 A Combination of Peptides

A combination of two peptides has been used to target drugs to certain cells. Angiopep (ANG) peptide has been used alone or in combination with other peptides (*e.g.,* TAT peptide) to deliver drugs to neuronal cells. ANG peptide (Table 8.1) was derived from the ligand of a low-density lipoprotein-related protein 1 (LRP1) receptor that is involved in the uptake and processing of amyloid precursor protein (APP) in the intracellular compartment inside endosomal vesicles (Kim et al. 2016). LRP-1 has been shown to mediate transport of various ligands across the BBB (Li et al. 2016). To prove the concept, ANG peptide alone was conjugated to β-secretase inhibitor (SI) (*i.e.* ANG-SI and ANG-PEG-SI; Table 8.1) for endosomal delivery of neuronal cells to inhibit the formation of amyloid-beta (Aβ) (Barve et al. 2016; Kim et al. 2016). Neuroblastoma cells internalize the ANG-SI conjugate better than SI peptide alone, suggesting that the uptake is through receptor-mediated endocytosis. Conjugation of ANG to an SI peptide alters the recognition of the ANG peptide by LRP1 receptors because the uptake of the ANG-SI conjugate is unaffected in the decrease of LRP1 receptors. This suggests the involvement of another receptor in the uptake of the conjugate (Kim et al. 2016).

A combination of ANG and TAT peptides was used to deliver PTX as a conjugate (ANG-TAT-PTX; Table 8.1) across the BBB, and this conjugate was developed to treat glioblastoma brain tumor (Li et al. 2016). ANG-TAT-PTX is expected to bind and be internalized by the LRP-1

receptor across the BBB. In previous studies, a conjugate of PTX with angiopep-2 and -3 (ANG1005) has been shown to cross the BBB and was investigated in clinical trials (Regina et al. 2008; Thomas et al. 2009). The cellular uptake of ANG-TAT by U87 glioblastoma cells was higher than that of ANG alone (Li et al. 2016). It is interesting to find that, although ANG-TAT and TAT-ANG were both internalized by U87 glioblastoma cells, only ANG-TAT crossed the BBB (Li et al. 2016). The brain delivery studies were done using Biotin-ANG-TAT (Table 8.1), which was detected in brain tumor tissue. Biotin-ANG-TAT has significantly higher deposition (1.8 times) than Biotin-ANG; in this case, the TAT peptide improved brain tumor uptake. ANG-TAT-PTX-treated mice with implanted U87 glioblastoma cells in the brain have better survival rate than diseased mice treated with ANG-PTX or PTX alone (Li et al. 2016). Therefore, TAT peptide is important in improving the conjugate brain delivery.

A combination of T10 and extracellular signal-regulated kinases (ERK) peptides (Table 8.1) was also used to deliver DOX molecule to breast cancer cells to overcome drug resistance (Sheng et al. 2016). T10 peptide binds to and can be internalized by transferrin receptor (Tfr), which is overexpressed in tumor cells. ERK peptide can prevent activation of ERK by inhibiting phosphorylation and its binding to mitogen-activated protein kinase (MEK). T10 peptide was conjugated to ERK peptide via a spacer (GGCG), and the thiol group on the Cys residue was linked to DOX to give T10-ERK-DOX conjugate. The DOX cellular uptake in MCF7/ADR cancer cells was increased when attached to the conjugate. The conjugate reversed the drug resistance by downregulating Pgp expression and inhibiting ERK phosphorylation. Although T10-DOX delivered DOX to MCF7/ADR cancer cells and suppressed MCF7/ADR xenograft in nude mice, T10-ERK-DOX had better efficacy than T10-DOX in suppressing growth of MCF7/ADR tumor xenografts in nude mice (Sheng et al. 2015). This indicates that a combination of two peptides with different mechanisms improves the outcome of tumor suppression activity.

The concept of dual peptide targeting was also applied to PEGA peptide (Table 8.1) that binds a membrane-bound proline-specific aminopeptidase P (APaseP). APaseP is expressed approximately 100-fold higher in vasculature and malignant lesions in breast cancer than in normal tissues (Cordova et al. 2016). Thus, PEGA-TAT-TAMRA conjugate was used to evaluate cellular delivery and localization of the peptide in breast cancer cells (Cordova et al. 2016). The conjugate was internalized by cancer cells *in vitro* and *in vivo* in tumor xenografts. Although conjugation of PEGA to TAT resulted in reduced selectivity for PEGA to APaseP, the overall results of uptake and localization of the dual peptide conjugate showed selective delivery to breast cancer tissue (Cordova et al. 2016). Thus, this dual peptide has the potential to delivery cytotoxic drugs to tumor cells *in vivo*.

8.3 Peptide-Particle Conjugates for Drug Delivery

Nanoparticles are another emerging technology to improve drug delivery to a specific type of cells. One potential advantage of nanoparticles is that they can be used to store the drug and deliver it in a controlled-release fashion. The drug release can be coupled to the different redox conditions between the extra- and intra-cellular environments of the cell because of elevated concentrations of reductive substances in tumor cells, which differentiate them from normal cells (McEligot et al. 2005; Jones 2010). Certain types of nanoparticles are generated due to self-assembly and micelle formation of the components in water because of their low critical micelle concentration. The micelles normally have high drug encapsulation efficiency. One example is PEGylated chitosan-based glycolipid, which can form a redox-responsive nanocarrier system called A54-PEG-CSO-ss-SA. The nanoparticles were studded with A54 peptide (Table 8.1) conjugated to a PEG moiety. The nanoparticles were

loaded with DOX and directed to human hepatoma cells by A54 peptide (Liu et al. 2016). The PEG moiety also serves to increase the *in vivo* half-life of nanoparticles by avoiding uptake by the reticuloendothelial (RES) system. *In vitro* and *in vivo* studies of nanoparticles show that DOX can be released via reduction of the disulfide bond depending on the amount of reductive substances in the tumor cells (Liu et al. 2016).

Recently, some efforts have been shifted from targeting drugs to specific types of cells to targeting them to subcellular organelles (*e.g.* the nucleus or mitochondria). In this case, the drug delivery systems are decorated with ligands that are specific for subcellular compartments, including the nuclear localization signal (NLS) and the lipophilic triphenylphosphonium (TPP) cation. Both NLS and TPP can penetrate the nucleus because of their high affinity for nuclear pore complexes and can anchor to mitochondria via electrostatic interactions (Smith et al. 2003; Kang et al. 2010). Previously, drugs conjugated with NLS or TPP failed to reach the nucleus or mitochondria because the design of these conjugates was not favorable for entering cancer cells from the extracellular space (Jensen et al. 2003; Callahan and Kopecek 2006). To overcome this problem, a (N-(2-hydroxypropyl) methacrylamide (HPMA) polymeric delivery system was conjugated to G3-C12 peptide (Table 8.1), a galectin-3-targeting ligand. The ligand was used for cellular uptake by cancer cells as well as subcellular mitochondria inside the cells (Sun et al. 2017). An antibiotic KLA peptide (Table 8.1) was also conjugated to HMPA to give a G3-C12-HPMA-KLA delivery system. The *in vitro* studies showed increased receptor-mediated internalization into PC-3 cells with overexpressing galectin-3. Moreover, the specific binding between galectin-3 and the G3-C12 peptide directed HPMA-KLA conjugates to the mitochondria with enhanced cytotoxicity. An *in vivo* study revealed that the G3-C12 peptide significantly enhanced the tumor accumulation of the polymer conjugate, exhibiting the best therapeutic efficacy and an improved survival rate in animals (Sun et al. 2017).

Carboplatin has been used to treat ovarian cancer; however, the uptake of carboplatin by ovarian cancer cells becomes poor because of drug resistance upon multiple treatments of cancer cells. Thus, the poly(amidoamine)-*b*-poly(aspartic acid)-*b*-poly(ethylene glycol) (PAMAM-PAsp-PEG) system was designed to improve carboplatin delivery to ovarian cancer cells (OVCAR-3). The nanoparticles utilize RGD-2 peptide (Table 8.1) to direct them to OVCAR-3 cells that have overexpression of cell surface $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrin receptors. Carboplatin molecules were attached to the polymer via a coordination complex with two carboxylic acid on the poly-aspartic acid chains tethered to the polymer. The release of carboplatin was pH-dependent, and 88% of carboplatin was released from the polymer over 50 h at pH 5.5, while only 18% of carboplatin was released over 50 h at pH 7.4. To track the cellular uptake and localization of the polymer in OVCAR-3 cells, Cy5-dye was also connected to the particles via PEG linker (Wang et al. 2016). The results showed that the particles containing RGD-2 peptide were efficiently internalized by the cells compared to particles without RGD-2 peptide. The targeted particles have significantly higher toxicity to the cells than carboplatin alone. It was proposed that carboplatin was occurring in the lysosome due to pH change and protonation of the carboxylic acid of the Asp residues (Wang et al. 2016).

A new drug self-delivery system (DSDS) was designed as nanocarrier for delivering PTX; in this case, PTX was conjugated to octadecanol via a disulfide bond to produce PTX-ODN. The PTX-ODN can self-assemble to form nanoparticles. Then, Pep-1 (Table 8.1) recognized by overexpressed interleukin-13 receptor α2 (IL-13Rα2) was used to direct the particles to glioblastoma multiforme (GBM) and for crossing the BBB and blood-brain-tumor barrier (Jiang et al. 2017). In this case, the Pep-1-PEG-DSPE conjugate is used to incorporate Pep-1 on the DSDS. The PTX-loaded nanoparticles were engulfed by IL-13Rα2 receptor-mediated endocytosis into glioblastoma cells and disintegrated in the endosomes to

release the PTX-ODN component (Jiang et al. 2017). The disulfide bond of PTX-ODN was reduced in the endosomes by glutathione to release PTX (Jiang et al. 2017). To follow the uptake and movement of the nanoparticles inside U87MG cells, the particles were labeled with coumarin-6 fluorophore. It was confirmed that the nanoparticles were internalized by U87MG cells in a receptor-mediated manner. *In vivo*, the nanoparticles can be detected in the U87MG glioma brain tumor grafted in nude mice (Jiang et al. 2017). Brain tumor mice treated with Pep-1-PTX-nanoparticles showed a higher survival population than those treated with vehicle, taxol, and PTX-octadecanol conjugate (Jiang et al. 2017).

Nanosize particles (PEG-EGFR-PTX) were constructed using branched PEG conjugated to PTX and an epidermal growth factor receptor (EGRF) peptide (Table 8.1). The nanoparticles were designed to improve PTX delivery to cancer cells overexpressing EGFR (Majumdar et al. 2016). The roles of PEG were to increase drug-water solubility and half-life of the particles. The abilities of PEG-EGFR-PTX, PTX-PEG, and PTX to inhibit cell growth were evaluated in squamous cell carcinoma of the head and neck (SCCHN) cell line. The results showed that the IC_{50}s of PEG-EGFR-PTX, PTX-PEG, and PTX were 21.74, 8.05, and 1.47 nM, respectively (Majumdar et al. 2016). The lower activity of PEG-EGFR-PTX compared to PTX alone may be due to the less efficient uptake of the PEG-EGFR-PTX particles rather than to passive diffusion of PTX. Unfortunately, the toxicities of PEG-EGFR-PTX particles and PTX were not compared between EGFR overexpressing cancer cells and normal cells to prove particle targeting by EGFR peptide. Therefore, it is difficult to evaluate the usefulness of the particles in treating tumors *in vivo*.

A conjugate of peptide in nanobubbles (NBs) was designed to deliver small interfering RNA (siRNA) molecules, which have high specificity for the oncogenic mRNA in cancer cells. siRNA molecules are known to have unfavorable physicochemical properties (*e.g.,* size and anionic charges) for partitioning and crossing the cellular membranes to enter the intracellular space and exert their activity. To overcome this problem, Myc siRNA was conjugated to CPP (Table 8.1) to give CPP-Myc siRNA, which is encapsulated in ultrasound sensitive NBs. Ephrin peptide (YSA peptide, Table 8.1) was attached to the surface of NBs using 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-methoxy-(polyethylene glycol) (DSPE-PEG) to give CPP-siRNA/YSA-NB (Xie et al. 2015). YSA peptide selectively binds to overexpressed EphA 2 protein on the cell surface (Xie et al. 2015). After cellular uptake, the CPP-Myc siRNA was released from NBs upon exposure to ultrasound that induced apoptosis of MCF-7 breast cancer cells *in vitro*. CPP-Myc siRNA/YSA-NB administered in conjunction with ultrasound significantly suppressed tumor growth of MCF-7 xenografts in mice compared to without ultrasound (Xie et al. 2015). The CPP-Myc siRNA/YSA-NB + ultrasound was significantly more effective than controls CPP-NC-siRNA/YSA-NB + ultrasound or CPP-siRNA alone, suggesting that YSA and ultrasound improved the efficacy of the CPP-Myc siRNA.

To reduce premature drug release, prodrug nanomedicine was developed to increase stability and solubility and to reduce injection of inactive carriers (Song et al. 2015). Clinical trials of prodrug carriers such as albumin-PTX have shown promise. When formulating prodrug nanomedicine, PEGylation of the drug is often used as carrier because PEGylation is neither toxic nor immunogenic, and can improve the half-life of the drug in circulation (Song et al. 2015). A cleavable linker between the drug and the carrier is also an essential component. The *cis*-asconitic anhydride-DOX (CAD) was conjugated to a PEG group via an amide bond to make a PEG-CAD prodrug. Then, the PEG-CAD prodrug was conjugated to RGD-3 peptide (Table 8.1), which selectively binds to neuropilin-1 (NRP-1) receptors. NRP-1 receptors have been shown to be overexpressed in tumor vessels as well as in many human cancer cell lines. The conjugation utilized a thiol-ene reaction to give an acid-labile prodrug called PEG-CAD-RGD-3. Due to the amphiphilic nature of PEG-CAD-RGD-3, it

assembled into nanoparticles in water. During *in vitro* and *in vivo* administration, the release of DOX was triggered in acidic pH, but was restricted in neutral pH environment. Compared to DOX alone, the presence of RGD-3 peptide improved nanomedicine endocytosis and cytotoxicity into tumor cells. In Balb/c mice, PEG-CAD-RGD-3 nanomedicine has shown prolonged accumulation of DOX in tumors (Song et al. 2015).

8.4 Peptide Modulation of Biological Barriers to Improve Drug Delivery

Biological barriers such as the intestinal mucosa barrier (IMB) and blood-brain barrier (BBB) are present to protect the body from infections entering into the systemic circulation and the central nervous systems (CNS), respectively (Deli 2009). IMB is composed of a single layer of epithelial cells at the luminal side of the gastrointestinal tract followed by the lamina propria and the *muscularis mucosae*. The BBB is comprised of the luminal and abluminal membranes of the brain capillary endothelium as the major route for molecules (drugs and diagnostic agents) to enter the brain (Pardridge 2012). The IMB and BBB function as selective filters to allow needed substances and nutrients to enter the systemic circulation or brain, respectively, while preventing unwanted substances such as toxins from crossing the barriers. The gastrointestinal tract, skin, kidney, and lung barriers are made up of epithelial cells while the BBB microvessels are composed of endothelial cells. The delivery of molecules through the IMB and BBB is normally via the transcellular and paracellular pathways. Passive diffusion of drugs through the transcellular pathway depends on physicochemical properties of the drugs, and the passive diffusion of these drugs is normally regulated by Lipinski's rules of five. In general, peptide and protein drugs cannot cross the transcellular pathways due to their size, hydrophilicity, and hydrogen bonding potential. However, some hydrophilic small and large molecules (*e.g.* peptides and proteins) can cross the biological barriers via the transcellular pathway using receptor-mediated transporters.

Alternatively, drug molecules could cross the IMB and BBB via the paracellular pathway, where molecules pass through the intercellular space between the cells (O'Donnell and Maddrell 1983; Laksitorini et al. 2014). The paracellular space or intercellular junctions are connected by cell-cell adhesion proteins, forming a contiguous membrane connection (Anderson and Van Itallie 2009; Van Itallie and Anderson 2014). Therefore, there is a size limit for molecules to cross the paracellular pathway; normally, only ions and molecules with hydrodynamic radius <11 Å can cross this pathway (Lutz and Siahaan, 1997b). This limitation is imposed by the tight junctions that are mediated by cell-cell adhesion proteins such as occudins, claudins, and junction adhesion molecules (JAMs), which act as a fence to prevent free diffusion of molecules. Below the tight junctions there are adherens junctions (AJ), which are mediated by nectin and calcium-binding cadherins (Zheng et al. 2006). Beneath the adherens junctions lie the desmosomes, which are composed of desmoglein and desmocollin proteins; these proteins are also part of the cadherin family of cell-cell adhesion molecules with calcium-dependent binding properties (Garrod and Chidgey 2008).

Modulation of the intercellular junctions of the IMB and BBB have shown promise in enhancing paracellular permeation of molecules. A hypertonic mannitol solution is used clinically to deliver anticancancer drugs to treat terminally ill brain tumor patients (Neuwelt et al. 1979, 1984, 1987). This method is called osmotic delivery because the hypertonic solution shrinks the BBB vascular endothelial cells and modulates the intercellular junctions to increase their porosity. Various chemicals as specific and nonspecific junction modulators (*i.e.* sodium caprate, sodium decanoate, oleic acid, ethyleneglycol-bis-(β-aminoethyl ether)-N, N′-tetraacetic acid (EGTA)) have successfully improved penetration of molecules through *in vitro* models of biological barrier (Lutz and Siahaan 1997a, b). Due to

uncontrolled paracellular opening, many toxic and unwanted side effects were observed with some of these methods. Thus, much research is focused on designing synthetic peptides that selectively modulate the protein-protein interactions in the intercellular junctions to improve paracellular permeation of delivered molecules.

8.4.1 Peptide Modulation of Tight Junction Proteins

One way to improve delivery of drug molecules via paracellular pathways of IMB and BBB is by modulating the interactions of cell-cell adhesion proteins in the intercellular junctions to increase the porosity of the paracellular pathways (Laksitorini et al. 2014; Bocsik et al. 2016). Several peptides derived from occludins have been synthesized and evaluated for this purpose. Occludins are 60 kDa membrane proteins that are involved in maintaining tight junction integrity. They are composed of four transmembrane domains, three cytoplasmic domains, and two extracellular loops of approximately similar size. One of these extracellular loops contains more Tyr and Gly residues (Gonzalez-Mariscal et al. 2003). The OCC2 peptide (Table 8.1) derived from extracellular loop 2 has been shown to modulate the tight junctions of A6 cell monolayers; the peptide lowers the transepithelial electrical resistance (TEER) values of the monolayers (Wong and Gumbiner 1997). OCC2 also enhances the penetration of paracellular markers such as inulin, dextran 3000, and dextran 40,000 across the A6 cell monolayers, indicating that the peptide increases paracellular porosity. $OP_{90–135}$ peptide (Table 8.1) derived from the first loop of occludin can lower the TEER values of Caco-2 cell monolayers, a model for IMB (Tavelin et al. 2003). The peptide also enhanced the transport of a paracellular marker, ^{14}C-mannitol, across the Caco-2 cell. A smaller $OP_{90–103}$ peptide (Table 8.1) has better modulatory activity than the parent $OP_{90–135}$ in Caco-2 cell monolayers. In addition, Lip-$OP_{90–103}$ peptide (Table 8.1) that is an N-terminus lipid-alkylated peptide has about 11 times higher modulatory activity than the parent $OP_{90–103}$.

Besides the occludins, claudins (Cldn-1, -2, -3, and -4) are also responsible for forming tight junctions; claudins have a transmembrane structure similar to that of occludins (Gonzalez-Mariscal et al. 2003; Anderson and Van Itallie 2009; Van Itallie and Anderson 2014). They have a short cytoplasmic N-terminus, two extracellular loops, and a C-terminal cytoplasmic domain. Both occludins and claudins interact via their C-terminus to zonula occludin-1 (ZO-1), ZO-2, and ZO-3 to stabilize the cytoskeleton membranes of the tight junctions (Schneeberger and Lynch 2004). Knocking down the expression of Cldn-1, Cldn-4, occludin, and ZO-1 increases the paracellular permeation of molecules and ions across the cell monolayers (Van Itallie and Anderson 2014). This result confirms their importance in maintaining the tight junctions. A 29-amino acid C1C2 peptide derived from the extracellular loop-1 of claudin-1 can enhance the permeation of small and large paracellular markers (*i.e.* Lucifer Yellow and FITC-Dextran 10 KDa) across the cell monolayers (Zwanziger et al. 2012). *In vivo*, the peptide increases the brain delivery of tetrodoxin and enkephalin peptides, suggesting that the peptide modulates the tight junctions of the BBB. The proposed mechanism of action of C1C2 peptide is via binding to claudins followed by induction of claudin endocytosis into the cytoplasmic domain (Staat et al. 2015). Therefore, this internalization lowers the population of claudin in the tight junction to make the tight junctions looser.

8.4.2 Peptide Modulators of Adherens Junction Proteins

Peptides derived from the extracellular domain-1 (EC1) domain of E-cadherin (*i.e.* HAV and ADT peptides) have been shown to modulate the intercellular junctions of MDCK and Caco-2 cell monolayers (Makagiansar et al. 2001; Sinaga et al. 2002). HAV and ADT peptides have been shown to inhibit E-cadherin-mediated cell-cell adhesion of single cells of bovine brain microves-

sel endothelial cells (BBMEC) as well as the intercellular junctions of BBMEC monolayers (Lutz and Siahaan 1997a, b; Pal et al. 1997). HAV6 and/or ADTC5 peptides (Table 8.1) increase the *in vivo* brain delivery of molecules in mice and/or rats; the delivered molecules include paracellular markers (*i.e.* ^{14}C-mannitol, 25 kDa IRdye800cw-Polyethylene glycols or PEG), anticancer drugs (*i.e.* ^{3}H daunomycin, Glu-CPT), efflux pump substrates (*i.e.* rhodamine 800 (R800), ^{3}H daunomycin), magnetic resonance imaging (MRI) enhancing agents (*i.e.* gadopentetic acid or Gd-DTPA), peptides (*i.e.* IRdye800cw-cLABL and cIBR7; Table 8.1) and proteins (*i.e.* 65 kDa galbumin) (Kiptoo et al. 2011; On et al. 2014; Laksitorini et al. 2015; Alaofi et al. 2016; Ulapane et al. 2017). The brain depositions of radioactive molecules such as ^{14}C-mannitol and ^{3}H-daunomycin were detected and quantified in the brain homogenates using a radioactive counter, while the quantity of brain deposition of R800, IRdye800cw-PEG, and IRdye800cw-cLABL was determined in the intact isolated brain using near IR fluorescence imaging. MRI was used in living animals to detect the brain distribution of Gd-DTPA and galbumin. Finally, the amounts of brain-delivered non-labeled Glu-CPT and cIBR7 peptide in rats were detected using LC-MS/MS. The duration of modulation of the *in vivo* BBB for small molecules was less than 1 h for HAV6 peptide and between 2 and 5 h for ADTC5 peptide (On et al. 2014; Laksitorini et al. 2015). However, the duration of BBB modulation by HAV6 and ADTC5 was too short for delivering a large molecule such as 65 kDa galbumin, less than 10 min for HAV6 and from 10–40 min for ADTC5 peptide (Ulapane et al. 2017). The results suggest that the peptides create small pores in the intercellular junctions with a long-time duration compared to a short duration for large pores in the BBB intercellular junctions. The results also indicate that the BBB modulation is reversible.

The mechanism of action of HAV and ADT peptides is potentially due to their binding to E-cadherin to inhibit cadherin-cadherin interactions in the intercellular junctions of the BBB. Using nuclear magnetic resonance spectroscopy (NMR) and molecular docking studies, HAV and ADT peptides were shown to bind at different sites on the EC1 domain of E-cadherin (Alaofi et al. 2017). It is proposed that the HAV6 peptide binds to the EC1 domain and inhibits the binding of the EC1 domain from one E-cadherin to the EC2 domain of another cadherin on the same membranes, which is the *cis*-cadherin interaction. In contrast, ADTC5 peptide binds to the EC1 domain to prevent the *trans*-EC1 domain from swapping between two E-cadherins from opposite cell membranes or *trans*-cadherin interactions.

8.4.3 Other Peptide Modulators Tight Junctions

Bocsik et al. have shown that C-CPE, AT-1002, PN-78, and PN-159 peptides (Table 8.1) could modulate the intercellular junctions of the IMB and BBB in cell culture models (Bocsik et al. 2016). These peptides were not derived from the sequence of proteins from the intercellular junctions of the IMB and/or the BBB. C-CPE and AT-1002 peptides were respectively derived from *clostridium perfringens enterotoxin* (C-CPE) and zonula occludens *toxin* (Zot). Both peptides modulate the penetration of molecules through the paracellular pathways of IMB and BBB *in vitro* and/or *in vitro* (Sonoda et al. 1999; Bocsik et al. 2016). PN-78 and PN-159 peptides were discovered using phage display and they increased the paracellular permeation of molecules through the lung epithelial cell monolayer (Herman et al. 2007). C-CPE, AT-1002, PN-78, and PN-159 modulate the intercellular junctions of Caco-2 cell and brain endothelial monolayers as models of IMB and BBB, respectively. This junction modulation was reflected in the lowering of *trans*-epithelial/endothelial electrical resistance (TEER) upon peptide treatment. The paracellular transport of fluorescein across the Caco-2 cell monolayers was enhanced by C-CPE, AT-1002, and PN-159 but not PN-78 peptides. However, all four peptides enhanced the penetration of albumin across the Caco-2 cell monolayers. The paracellular permeation of both

fluorescein and albumin across the BBB cell monolayers was increased by AT-1002, PN-78, and PN-159 but not C-CPE. Thus, these peptides can be used to deliver drug molecules across the IMB and the BBB in *in vivo* studies.

8.5 Conclusion

Peptides have been successfully developed as drugs. Now, peptides have been extensively investigated to deliver drugs to specific cells to lower their unwanted side effects. Similarly, conjugation of peptides to labeled molecules or atoms was shown to be useful for potential diagnostic agents to locate diseased cells within the body using various detection methods such as MRI and PET. Finally, delivery across the intestinal mucosa and the blood-brain barrier can also be enhanced by modulation of the protein-protein interactions in the intercellular junctions of these barriers using peptides. Modulation of the BBB using cadherin peptides can enhance the brain delivery of small-to-large molecules to the brains of living animals. Thus, modulation of the intercellular junctions can be exploited to clinically deliver drug and diagnostic molecules through the IMB and BBB in the future.

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