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Review

Biomimetic Peptides for the Treatment of Cancer

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Abstract. *Cancer remains one of the leading causes of death worldwide, indicating that current cancer therapies are ineffective. Therefore, new treatments with high specificity and low toxicity are needed. Cancerous cells can be distinguished from normal cells based on expression of key proteins, namely surface proteins, scaffold proteins and signaling molecules. Moreover, cancer cells communicate with the tumor micro-environment consisting of a heterogenous population of cells, extracellular matrix components and soluble factors such as cytokines/chemokines and growth factors. Most therapeutic interventions have been designed to specifically target these proteins of interest. Biomimetic peptides (BPs) are artificially designed peptides that imitate the action of parent proteins or peptides. BPs can be classified into at least three types based on their target molecule: BPs that target (i) cell-surface molecules, (ii) intracellular molecules, and (iii) cancer cell–tumor microenvironment interactions. In this review, we analyze/discuss the current strategies for targeting tumors using BPs.*

Cancer is one of the leading causes of death worldwide and this is due to high morbidity, recurrence rate and mortality (1). In general, tumorigenesis is a highly complex multi-step process. Tumor cells exhibit unique characteristics, such as the capacity to continually undergo unregulated proliferation, evade apoptosis, promote abnormal angiogenesis and invade other organs after migrating through blood and lymphatic vessels (2, 3). Although surgery, chemotherapy and radiotherapy are widely accepted as the main therapeutic approaches for treating cancer, these treatments often have severe adverse effects that significantly reduce the quality of life for the patient. Hence, new, more effective therapeutic interventions that have a high specificity for cancer cells and low toxicity to normal, healthy tissue are urgently needed.

The term 'biomimetics', was first coined by the American neurophysiologist Otto Schmitt, and refers to "a creative form of technology that uses or imitates nature to improve human lives" (4, 5). This concept has since been applied to several scientific fields including chemistry, nanotechnology, mechanics, robotics and medicine. In the medical field, numerous studies have been published, utilizing biomimetic applications such as artificial organ and dental implant surface modifications to improve the biocompatibility of these materials (6-8).

Peptides are critical molecules that regulate many biological events. For example, hormonal peptides serve as modulators of homeostasis within the body (9-11). Bioactive peptides derived from digested dietary proteins also play an important role in the cardiovascular (12, 13), gastrointestinal (12, 14) and nervous systems (12, 15). Moreover, antimicrobial peptides (AMPs) are important components of the innate immune system (12, 16). Artificially synthesized peptides can be easily and rapidly mass produced within the laboratory. Therefore, numerous studies have attempted to develop artificially designed biomimetic peptides (BPs) that mimic the actions of the parent proteins or the peptides from which they originate and may be novel therapeutic candidates for a range of conditions (17-25). BPs can be classified into at least three types, based on their derivation and molecular targets: BPs targeting (i) cell-surface molecules, (ii) intracellular molecules, and (iii) interactions between cells and the tissue microenvironment (*e.g.* extracellular matrix components, cytokines, other tissue-resident cells).

Cancer cells can generally be distinguished from normal cells based on the activity of specific proteins, and most current therapeutic agents specifically target these proteins

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or signaling molecules (26). This review will highlight the current strategies for targeting tumors using BPs, focusing on the relationship between the protein of origin and the molecular target of the peptide (Table I).

Targeting Molecules at the Cell Surface

A major component of the plasma membrane of a cell is the phospholipid bilayer which contains several proteins (including receptors) that are either integrated into the lipid bilayer or bound to the membrane indirectly through protein–protein interactions. Certain key receptors are more highly expressed on cancer cells than normal cells and induce cancer cell proliferation upon ligand binding (27). As such, many BPs that bind to target receptors are hormone analogs, such as somatostatin analog peptides (28-32) and gonadotropin-releasing hormone (GnRH) analog peptides (33, 34). These analogs share sequence homology and contain the almost same number of amino acids as the parent peptides. Thus, hormonal analogs were classically investigated for clinical application of BPs in the field of oncology. These have been approved for clinical use and provide great benefit for patients with cancer, more specifically with prostate cancer (28-34). Fragments of the parent protein have also reportedly been used (35-37). Synthesized as fragment peptides, human epidermal growth factor receptor (HER)2- and vascular endothelial growth factor (VEGF)-mimicking peptides used in combination with metronomic paclitaxel for treatment significantly reduced tumor burden and prolonged survival rates in both transgenic and inoculated tumor models of human breast cancer (35, 36). The FOXY5, wingless-type MMTV integration site family (WNT)-5A-derived hexapeptide, impaired the migration and invasion of murine breast cancer cells without affecting their apoptosis or proliferation. The peptide also inhibited metastasis in a transplantable tumor model of murine breast cancer (37).

The integrin family of proteins are usually expressed at very low levels (or are even undetectable) in most normal cells and their expression is often highly up-regulated in most tumors (38). Integrins interact with the extracellular matrix (ECM) glycoprotein and immunoglobulin superfamily molecules. A wide variety of integrins support the proliferation, survival and migration of cells within tumors. Specific integrins preferentially bind to distinct ECM proteins containing an adhesive arginine-glycine-aspartic acid (RGD) sequence. Therefore, RGD peptides and RGD mimetic peptides have been utilized as carriers to deliver biological cargo such as DNA (39), small interfering (si) RNA (40), saccharides (41), fatty acids (42), inhibitors (43) and drugs (44) to tumors. RGD peptides have been shown to suppress proliferation and induce apoptosis of cancer cells directly *in vitro* (45). Such peptide was also found to reduce the density of functional vessels within tumor, causing retardation of tumor growth and inhibiting metastasis *in vivo* (46).

Targeting Intracellular Molecules

A key step in tumorigenesis is the evasion of growth signaling and loss of tumor suppressors. Many oncogenes have been identified (47-49), the aberrant activation of these proteins can affect signaling cascades within cancer cells at several different stages (50). Thus, a wide variety of intracellular molecules have been investigated as potentially promising therapeutic targets for cancer treatment. To access the intracellular target molecules, anticancer drugs against these molecules must be able to penetrate tumor tissue and enter cancer cells. All AMPs have the capacity to interact strongly with cell membranes, some are able to achieve this interaction without membrane permeabilization (51). Certain AMP-derived BPs are also able to penetrate cell membranes. For example, the synthetic peptide LTX-315, derived from bovine lactoferricin, was shown to be internalized by cells and accumulate close to the mitochondria (52). The peptide was found to kill the cells by permeabilizing the mitochondrial membrane (53). The oncolytic property of LTX-315 has been shown in several cancer lines (54), while displaying low cytotoxicity against human red blood cells (55). In an *in vivo* murine model of transplantable melanoma, LTX-315 induced complete regression of tumors (55). Recently, the peptide was also found to induce immunogenic cell death and the induction was comparable with well-known immunogenic cell death-inducible agents, anthracyclines (56). LTX-315 is currently being investigated in clinical phase I/IIa studies (57).

AMPs and some of their analogous BPs have the ability to undergo cellular internalization. However, other peptides need to be modified to improve their permeability through the plasma membrane. One strategy to enhance their uptake into cells is to use cell-penetrating peptides (CPPs). CPPs can carry many different therapeutic agents into cells, including small molecules, plasmid DNA, siRNA, proteins, viruses, imaging agents, and other various nanoparticles (58). Several studies have reported the use of CPP-conjugated BPs (59-63). One example of this is the bcl-2-associated x protein (BAX) segment peptide, R8-BAX [106-134] which was designed from the α5 helices of BAX (residue Asn106 to Arg 134) and conjugated to the cell-penetrating arginine octapeptide (R8) (59). R8, a well-known CPP, is concentrated at the cell surface by interacting with membrane-associated proteoglycans which then induce macropinocytosis, stimulating active and efficient cellular uptake (64, 65). The BAX protein plays a key role in the mitochondrial apoptosis pathway (66), and its α5 helices forms the mitochondrial membrane insertion and pore formation domain of the protein (67). R8-BAX induced caspase-dependent apoptotic cell death *in vitro* and in a murine mammary carcinoma xenograft model significantly reduced tumor volume by partially mimicking the functions of BAX. In addition, BIM stabilized

Table I. *Representative biomimetic peptides with anticancer properties*.

Name	Sequences	Protein of origin	Anticancer mechanism	References
Targeting cell-surface molecule				
Octreotide	fCFwKTCT-ol	Somatostatin	Mimics the actions of somatostatin	28-32
Leuprorelin	*pE*HWSyLLRP-NHC_2H_5	GnRH	Mimics the actions of GnRH	33, 34
Cyclized HER2-D-peptide mimetic	CH_3CONH-VCSAGFTYRGEPNP-MSEFTDTNYTVLAPCHL-$CONH_2$	HER2	Blocks receptor–ligand interactions	35, 36
Cyclized VEGF-L-peptide mimetic	CH_3CONH-FSMECIMRIKPHQG-QHIGCQMTI-$CONH_2$	VEGF	Blocks receptor–ligand interactions	35, 36
FOXY5	*formyl*MDGCEL	WNT5A	Mimics the actions of WNT5A	37
Targeting intracellular molecule				
LTX-315	KKWWKKW*Dip*K-NH_2	Bovine lactoferricin	Induces apoptosis through mitochondrial membrane permeabilization and immunogenic cell death	52-54
R8-BAX[106-134]	RRRRRRRRGNWGRVVALFY-FASKLVLKALCTKVPELIR	BAX	Partially mimics the functions of BAX and induces apoptosis	59
BIM $SAHB_A$	IWIAQELR*IGD*FNAYYARR	BIM	Partially mimics the functions of BIM and induces apoptosis	70, 72
Targeting the tumor microenvironment				
PSAP peptide	DWLP(K)	Prosaposin	Stimulates p53 and the protein TSP1 in bone marrow-derived cells	75-77

pE: Pyroglutamic acid; *Dip*: diphenylalanine; GnRH: gonadotropin-releasing hormone; HER2: human epidermal growth factor receptor-2; VEGF: vascular endothelial growth factor; WNT5A: wingless-type MMTV integration site family-5A; BAX: bcl-2-associated x protein; SAHB: stabilized α-helix of BCL2 domains; PSAP: Prosaposin; *hydrocarbon staple site. Lower case letters in sequences indicate D amino acids.

α-helix of bcl-2 domains (SAHB)A is a synthetic segment BP derived from the BH3 domain of BIM (68). BIM $SAHB_A$ is a stapled hydrocarbon α-helix that combines two distinct conformational stabilization strategies previously found to individually induce α-helical structures, namely, α,α-di-substitution and macrocyclic bridge formation (69-71). Using fluorescein isothiocyanate-conjugation, stapled peptides were found to be able to enter the cell depending on certain factors, such as charge, hydrophobicity and α-helical structure (70). BIM $SAHB_A$ has also been shown to have membrane-penetrating capability (72). Treatment of hematological cancer cells with BIM $SAHB_A$ directly triggered pro-apoptotic activity and induced BH3 sequence-specific cell death *in vitro* and significantly reduced tumorigenesis in a human xenograft model of acute myeloid leukemia (72).

Targeting the Tumor Microenvironment

Carcinomas are not only composed of tumor cells but are in fact complex tissues, to which many other cells are recruited that can be 'corrupted' by transformed cells within the tumor (73). As evidence that cancer cells are able to communicate with a wide range of cell types, ECM components and soluble factors (*e.g.* cytokines/chemokines and growth factors) has accumulated, the concept of the tumor microenvironment (TME) has gradually been accepted. As a result, recent studies have not only focused on the tumors themselves but also on the TME and are currently targeting tumor–TME interactions using BPs. Prosaposin (PSAP) is a glycoprotein proteolytically cleaved in late endosomes/lysosomes that functions as a regulator of lysosomal enzyme and a secreted factor that exerts neuroprotective and glioprotective effects (74). PSAP stimulates the expression and production of the anti-tumorigenic protein thrombospondin-1 (TSP1) in cluster of differentiation (CD)$11b^+$/granulocyte-differentiation antigen-1^+/$Ly6C^{high}$ monocytes and was found to inhibit tumor metastasis in lung and breast tumor models (75, 76). On the basis of these finding, quadropeptide DWLP and cyclic pentapeptide DWLPK derived from PSAP (named PSAP peptide) were developed to inhibit metastatic ovarian cancer (77). In fact, PSAP peptide was found to induce tumor regression in a patient-derived tumor xenograft model of metastatic ovarian cancer (77). PSAP peptide has been shown to stimulate the expression and production of TSP1 in bone-marrow cells. Three distinct mechanisms of inhibiting ovarian cancer progression by PSAP peptide-induced up-regulation of *TSP1* have been proposed: TSP1 released from bone-marrow cells, (i) directly kill ovarian cancer cells, (ii) acts as an antiangiogenic effector, or (iii) blocks CD47-signal

regulatory protein α interaction by competitively binding to CD47, thus disrupting the "do not eat me" signal within CD47-expressing cells (77).

Conclusion

In this review, we mainly discussed the anticancer BPs which imitate naturally-occurring molecules. Due to the large body of evidence on the mechanisms governing tumorigenesis and the potential application of novel peptides, many anticancer BPs have been developed. Some parent amino acid sequences have been modified to enhance the efficacy of the BPs, while other peptides have been modified to enhance their cell permeability. Due to their efficacy and low toxicity, some peptides have already have been approved as cancer therapies and are currently being used in clinical practice. The safety and efficacy of several other BPs are also currently being tested in clinical trials. While further work is needed to develop this technology for use in other clinical settings, BPs represent a promising therapeutic strategy to treat cancer.

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