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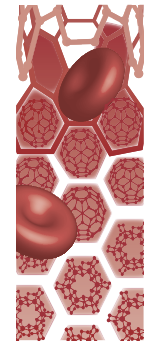

Use of anticancer peptides as an alternative approach for targeted therapy in breast cancer: a review

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Breast cancer is the most common cancer in women worldwide. Traditional therapies are expensive and cause severe side effects. Targeted therapy is a powerful method to circumvent the problems of other therapies. It also allows drugs to localize at predefined targets in a selective manner. Currently, there are several monoclonal antibodies which target breast cancer cell surface markers. However, using antibodies has some limitations. In the last two decades, many investigators have discovered peptides that may be useful to target breast cancer cells. In this article, we provide an overview on anti-breast cancer peptides, their sources and biological activities. We further discuss the pros and cons of using anticancer peptides with further emphasis on how to improve their effectiveness in cancer therapy.

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Breast cancer is one of the most common diseases in women and remains one of their leading causes of death globally [1]. Several factors can effectively reduce or increase a woman's risk for breast cancer including individual differences in age, family history, reproductive history, race or ethnicity. Age and family history are the two most important risk factors for women in relation to breast cancer [2]. Early detection and the use of surgery, radiotherapy and chemotherapy can greatly reduce the risk of metastasis in breast cancer patients; however, these treatments have unfavorable side effects [3,4]. The use of targeted therapy with few side effects has still not been fully explored [5].

Since the invention of hybridoma technology in 1975, investigations for developing monoclonal antibodies (mAbs) as tumor targeting agents have been taking place [6]. The clinical success and approval of some mAbs, such as Herceptin® (an anti-HER2/neu mAb against breast cancer) by the US FDA has further validated their cell surface targeting approach for cancer therapy. Several mAbs that target cell surface receptors are now used in the clinic. Some of them are unconjugated, and they bind to cell surface receptors and inhibit tumor growth by inhibiting their promitogenic function [7]. Some mAbs are conjugated to toxins [8] and many of the antibodies are conjugated to radionuclides [9,10].

Beside the advantages of mAbs in cancer therapy, one major limitation of using them to target tumor cells is that the antibody molecule is relatively large with a high molecular weight. Hence, it has difficulty reaching the interior of a large tumor mass [11–13]. Another major problem is the nonspecific uptake of the antibody molecules into parts of the reticuloendothelial system such as the liver, spleen and bone marrow [14].

Anticancer peptides are small molecules (<50 amino acids) which have been proven to be effective against many cancers. In 1985, cationic peptides isolated from various organisms which had been historically assessed for antimicrobial activities, were studied for the first time as potent anticancer agents [10].

Breast cancer is one of the most studied solid tumors for which new anticancer peptides have been developed. There are several different mechanisms for peptides action against cancer cells (Figure 1) [15]. Some of them can damage cellular membranes leading to the death of the malignant cells by apoptotic or necrotic mechanisms, while others affect the intracellular targets. Some of these peptides act as immune-modulators and may increase T-cell responses or inhibit the regulatory T-cells [16]. Studies have also indicated that one single peptide may employ more than one type of action against cancer cells [17]. Several investigations have demonstrated that factors such

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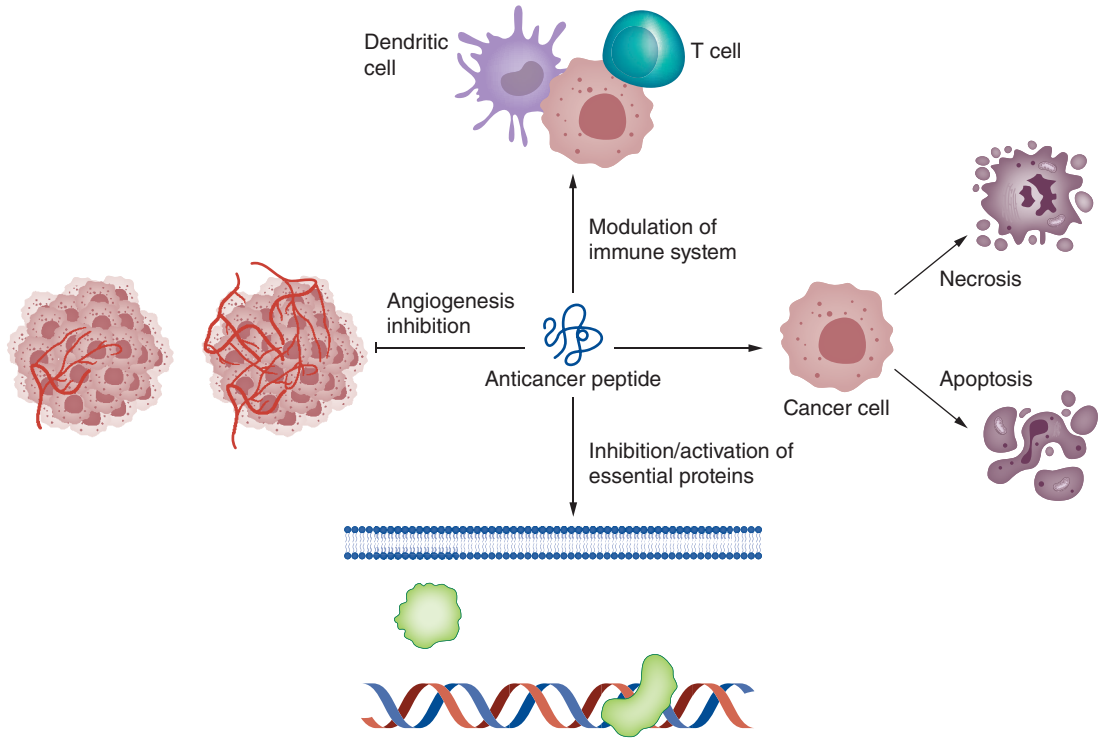

Figure 1. Different mechanisms of action related to anticancer peptides.

Table 1. anti-breast cancer peptides used in clinical trials.

Peptide name	Clinical Phase
Didemnin B	I/II
Aplidine (plitidepsin)	I
Kahalalide F (KF)	I/II
Dolastatin 10	I/II
LTX-315	Ib

as amphipathicity, hydrophobicity, net charge, secondary structure in membrane and oligomerization ability are responsible for these activities in both synthetic and natural anticancer peptides [18,19]. Moreover, it has been reported that since all cell membranes possess a hydrophobic environment, hydrophobicity of peptides plays a critical role during their activities against malignant cells [20].

Compared with antibody drugs, peptides have several advantages, such as small size, ease of synthesis, higher stability, reduced immunogenicity and better organ or tumor penetration [21]. Moreover, with the advent of solid-phase synthesis technology, anticancer peptides have a low cost of production and are easy to modify. However, despite many investigations on discovery or design of anticancer peptides, only a few of them have been studied in clinical trials, particularly for breast cancer treatment (Table 1).

Herein, we categorize the so far studied anti-breast cancer peptides and discuss their effects on breast cancer prevention and treatment. In addition, we discuss the pros and cons of using anticancer peptides with further emphasis on how to improve their effectiveness in cancer therapy. The information collected here may provide some ideas for further research on anti-breast cancer peptides.

Synthetic peptides for treatment of breast cancer

Structure-based designed peptides

The field of structure-based drug design is an area which has greatly progressed in recent years, especially for peptide design. A plethora of genomics, proteomics, and structural information has provided hundreds of new

Table 2. The sequences of the anti-breast cancer structure-based designed peptides.

No.	Name	Sequence	Ref.
(1)	AFPep	*cyclo* EKTOVNOGN	[23]
(2)	PNC-27	PPLSQETFSDLWLLKKWKMRNQFWVKVQRQ	[27]
(3)	–	ATWLPPR	[29]
(4)	HRAP	Ac-PHAHF-NH2	[30]
(5)	–	RASPADREV	[31]
(6)	iPep624	KKKRKVTDSQQPLVWPAWVYCTRYSDRPS	[32]
(7)	iPep682	KKKRKVPLVWPAWVYCTRYSDRPS	[32]
(8)	iPep697	KKKRKVWPAWVYCTRYSDR SNTSESF-NH	[32]
(9)	AUNP-12	SNTSESFKFRVTQLAPKAQIKE-NH2	[33]
(10)	LfcinB	FKCRRWQWRMKKLGAPSITCVRRAF	[36]
(11)	LfcinB (20–25)	RRWQWR	[36]
(12)	LTX-315	KKWWKKDipK	[39]
(13)	Model 6	FLGPTIGKIAKFILKHIVGLGDAALV	[41]
(14)	Model 11	GLFAILKKLVNLVG	[41]

targets and opportunities for discovery of novel drugs. Choice of a target is the primary phase for structure-based drug design. Once a target has been identified, obtaining the accurate structural information is essential. X-ray crystallography, nuclear magnetic resonance and homology modeling are three useful methods for structure determination. Structure-based design begins with identifying a potential ligand binding site on the target molecule. After the identification of structure and target site, computer-aided or experimental methods can be used for developing a good lead based on the structure of the target [21,22].

Several studies have indicated that α-fetoprotein (AFP) interferes with estrogen-dependent responses and affects the growth promotion of estrogen on breast cancer. Accordingly, Bennett *et al.* showed that the active site of AFP, which is an 8-mer sequence consisting of amino acids 472–479 (**EMTPVNPG**), is responsible for its anti-estrotrophic activity [23]. They synthesized this 8-mer peptide, modified it for the purpose of stabilization, and indicated that this new analog, called AFPep **(1)** (Table 2), is stable during long-term storage and, it is able to inhibit the estrogen-stimulated growth of human breast cancer cells both in culture and in implanted xenografts in immune-deficient mice. This peptide interferes with the phosphorylation of the estrogen receptor (ER), particularly at serine 118 in the ER, which is required for full activation after the ER has been liganded with estrogen. AFPep could also prevent the carcinogen-induced breast cancer in a rat model [24–26].

PNC-27 **(2)**, a peptide derived from MDM2-binding domain of P53 (a transcription factor regulating downstream genes involved in cell cycle arrest, DNA repair and apoptosis), has exhibited cytotoxic effects on cancer cells by inducing transmembrane pores, while it had no activity on normal cells. The effects of this peptide were studied on MCF-7 cells, as well as untransformed MCF-10-2A breast epithelial cells [27]. The results indicated the interaction of PNC-27 with specific targets on the membrane of cancer cells, which were not present on untransformed cells. This interaction increases its lifetime allowing for prolonged action on the cell membrane. In normal or untransformed cells, lack of these targets can lead to hydrolysis of the peptide27. In another study, the toxicity of PNC-27 on human breast cancer cell lines including MDA-MB-468 (mutant P53), MCF-7 (overexpressing wild type P53) and MDA-MB-157 (null P53) cells was investigated [28]. The results showed that PNC-27 was able to induce necrosis in these breast cancer cells in a P53-independent manner. In general, this peptide interacts with MDM2 that is highly expressed on many kinds of cancer cells such as MCF-7, but not on normal cells [28].

NRP-1 is a nontyrosine kinase receptor of VEGF165. There is a correlation between the overexpression of NRP-1 with tumor angiogenesis and progression. Starzec *et al.* identified a peptide **(3)** (Table 2) inhibiting the VEGF165 binding to NRP-1. Moreover, the growth of MDA-MB-231 xenografts was inhibited after administration of this peptide to nude mice. Blood vessel density and endothelial cell area of nude mice were also reduced by this peptide, but no changes were observed on tumor proliferation indices [29].

Nakajima *et al.* designed an antagonistic peptide called HRAP **(4)** (Table 2) that binds to HER2 molecule, by their *in silico* design method based on the 3D structure of HER2-antibody complex. HRAP was the first computationally

designed small molecule, which was able to antagonize HER2 signaling by inhibiting its dimerization. This peptide also inhibited the proliferation of HER2 overexpressing human breast cancer cells (KPL-4, BT-474 and SK-BR-3 cell lines) along with a little cellular toxicity. The inhibitory effect of this peptide on cell proliferation was associated with suppression of phosphorylation in PTEN (a tumor suppressor frequently deleted or mutated in various primary cancers) and AKT [30].

Akhoon *et al.* also designed another efficient antibody mimetic oligopeptide **(5)** (Table 2) by computational method which targets HER2. This oligopeptide interacts with pertuzumab (a recombinant humanized mAb) binding sites of HER2. Although they did not study the real effect of this oligopeptide *in vitro* or *in vivo*, the results of *in silico* docking study showed that oligopeptide **(5)** specifically interacts with the dimerization domain of HER2 molecule and possesses high binding affinity toward this marker. In fact, dimerization of HER receptors may happen in some percentage of breast cancers. Among HER receptors, HER2 is a preferred partner for dimerization. Hence, blockade of HER2 dimerization site by oligopeptide **(5)**, can lead to prevention of intracellular signaling by the receptors [31]. Since the overexpression of HER2 is of frequent (20–30%) occurrence in breast cancer, discovering or designing such peptides which target HER2, might be a helpful approach in breast cancer therapy.

In an effort to discover biomarkers suitable for specific targeting of basal-like breast cancer, Beltran and colleagues demonstrated that EN1 was selectively and highly expressed in this kind of tumors and makes the tumor cells resistant to chemotherapy. To block EN1 function, they designed and synthesized the interference peptides (iPeps) comprising the EN1-specific sequences. These synthetic peptides (EN1-iPeps) **(6)**, **(7)** and **(8)** (Table 2) were the mediators of protein-protein interactions essential for EN1 function. They studied EN1-iPeps and iPep controls in SUM149PT breast cancer cells carrying high EN1 expression. These peptides were able to mediate a rapid and strong apoptotic response in EN1-overexpressing tumor cells, with no toxic effects on normal or non EN1-expressing cells. They also showed that low doses of iPeps could further sensitize highly resistant breast cancer cells to chemotherapy agents [32].

In 2014, the companies Aurigene Discovery Technologies Limited and Pierre Fabre started a cooperation to design new cancer therapeutics in immune-oncology, which resulted in the synthesis of AUNP-12 **(9)** (Table 2), a novel immune modulatory peptide (a 29-mer peptide), which targets the PD-1/PD-L1 immune checkpoint to activate T lymphocytes. This peptide strongly inhibited tumor growth and metastasis in preclinical models, and it showed a safe toxicological profile. Inhibition of proliferation in MDA-MB231 tumor cells and 44% reduction of tumor growth in 4T1-bearing mice have been reported for AUNP-12. Moreover, reduction in lung metastasis was occurred in more than 60% of treated mice [33,34].

Bovine lactoferrin (BLF) is a milk protein, which is able to reduce the metastatic properties of both MDA-MB-231 and MCF-7 cell lines [35]. The bovine lactoferricin (LfcinB) **(10)** (Table 2) is a 25 amino acid peptide, which belongs to the N-terminal region of bovine lactoferrin. LfcinB is able to induce apoptosis by direct disruption of the mitochondrial membrane, but is also capable of lyzing the membrane depending on the cancer cell type. LfcinB has exhibited a great cytotoxic effect on human breast cancer cell lines [36]. In 2017, Casanova *et al.* designed different types of peptides including linear, dimeric, tetrameric and cyclic peptides containing sequences derived from LfcinB and tested them against MDA-MB-468 and MDA-MB-231 breast cancer cell lines. The tetrameric peptide **(11)** (Table 2) exhibited a high cytotoxicity on both tested breast cancer lines, while the linear and the dimeric peptides showed a weak and intermediate cytotoxicity, respectively. The cyclic peptide also showed a high toxicity against MDA-MB-468 breast cancer cells [37].

LTX-315 **(12)** (Table 2) is an oncolytic peptide for intratumoral injection, which has been *de novo* designed and was able to stimulate an anticancer immune response in experimental preclinical animal models. LTX-315 induces immunogenic cell death via its membranolytic mode of action, which leads to the release of potent immune stimulants along with tumor antigens. In a variety of different experimental animal models, LTX-315 treatment has led to growth inhibition, complete regression and long-lasting tumor-specific immune responses. In 2019, Camilio *et al.* investigated the effects of LTX-315 in combination with CAELYX® (liposomal doxorubicin) in a preclinical triple negative breast cancer model. This peptide showed a significant additive antitumor effect when combined with CAELYX [38,39]. In addition, in Phase I clinical experiments, LTX-315 led to induction of a partial or complete regression in injected tumors, and a systemic immune response in some patients. Phase Ib study of LTX-315 in combination with pembrolizumab (anti-PD-1) was performed on metastatic breast cancer patients and completed in 2018 (NCT01986426).

Recently, Grisoni *et al.* designed membranolytic anticancer peptides using machine learning techniques. The activity of the twelve peptides was tested on MCF-7 cell line and their selectivity was studied against human

erythrocytes. Six of the peptides showed cytotoxicity on cancer cells with no effect on erythrocytes [40]. In another study, they applied this technique for *de novo* design of anticancer peptides. Fourteen peptides from a total of 1000 *de novo* designs were selected, synthesized and tested on MCF-7 cell line. Six *de novo* designs demonstrated anticancer activity *in vitro*, but two peptides named model 6 **(13)** and model 11 **(14)** (Table 2) showed the highest potency toward MCF-7 cells with IC_{50} values of 2.6 and 2.3 μM, respectively [41].

mRNA-peptide display technology to screen for anti-breast cancer peptides

In vitro peptide selection using mRNA displays leads to directed evolution of new anticancer peptides from constructed libraries [42–44]. In this technique, mRNAs are linked to their encoded peptides via covalent bonds with puromycin molecule. Puromycin molecule which has been covalently attached to the 3′ end of the desired mRNA, is bound to C-terminal end of the encoded peptide in the ribosome. It results in a stable conjugation of a genotype and the corresponding phenotype [45,46]. This technology can be used for creation of a peptide library for finding new ligands such as anticancer peptides.

Recently, an mRNA-peptide display library was constructed by Yang *et al.* and total protein of SK-BR-3 cell line (human breast cancer) was used as a bait to screen for finding specific peptides to treat breast cancer. SA12 **(15)** (**SVPLFNFSVYLA**; patent ZL2013100602261.8, People's Republic of China) was one of the best and highly potent captured peptides, which significantly inhibited the proliferation of SK-BR-3 cells and induced apoptosis. This peptide could interact with tumor-associated proteins MECP2 and CDC20B, changed the bioactivity of these target proteins, and induced apoptosis in tumor cells via the mitochondrial pathway involving BCL-2 family and related caspases. Since mutations or deletions in *P53* and *PTEN* are involved in the development of breast cancer, SA12 can affect breast cancer cells by preventing the inhibition of MECP2 on the transcription of *P53* and *PTEN*, which leads to re-expression of P53 and PTEN, and finally correcting the imbalance between tumor suppressor genes and oncogenes in cancer cells. In fact, activation of P53 and PTEN expression leads to increase in proapoptotic members of BCL-2 family (BAX and BAK) and also, a decrease in antiapoptotic members (BCL-2 and MCL-1). The imbalance of BAX/BCL-2 can cause the release of cytochrome c, which leads to activation of caspase-9 and subsequently the activation of caspase-3. Moreover, this peptide may influence the PI3K/PTEN/AKT signaling cascade, which has a critical role in the initiation and progression of carcinogenesis, to inhibit breast cancer cells. Hence, this novel peptide could potentially be a new candidate or strategy for development of breast cancer targeted therapy [47].

Natural peptides

There are many investigations about potent natural product-derived compounds, which are highly effective on breast cancer cells [48–51]. Herein, we summarize the natural peptides with a potential to act against breast tumor cells.

Marine derived peptides

Bioactive peptides derived from marine sources have a potential for human healthcare and their unique peptides with biological activities make them excellent candidates for drug design and development [52]. Some of the cyclic peptides and their analogues derived from marine sources have demonstrated anticancer activities. Based on the structural variations in the peptides (cyclic oligopeptide, cyclic lipopeptide, cyclic glycopeptide and cyclic depsipeptide), they show different activities and modes of actions. Some of the most reported marine originated peptides which were effective on breast carcinomas include didemnin B **(16)**, aplidine **(17)**, kahalalide F **(18)** and dolastatin 10 **(19)** [53].

Didemnin B **(16)** (Figure 2A), is a potent marine derived compound isolated at first from the Caribbean tunicate *Tridemnum solidum*, but later was obtained from other species of the same genus. Didemnin B, is a 7-amino-acid cyclic polypeptide, which has been shown to inhibit the synthesis of DNA, RNA and proteins [54]. Didemnin B specially affects the eEF1A, which plays a role in protein synthesis. This peptide is the first natural compound obtained directly from a marine source to enter clinical trials. In fact, substantial evidence of activity in preclinical models with dose-dependent and tolerable toxicity profiles led to Phase I clinical trials [55,56]. In 1992, a Phase II clinical trial was investigated for didemnin B in patients with metastatic breast cancer. Due to severe secondary effects, the trials were not successful, and clinical studies were stopped [57].

Aplidine (plitidepsin, dehydrodidemnin B, DDB, aplidin) **(17)** (Figure 2B) isolated from the Mediterranean tunicate *Aplidium albicans*, is another marine anticancer peptide. The antiproliferative activity of this depsipeptide

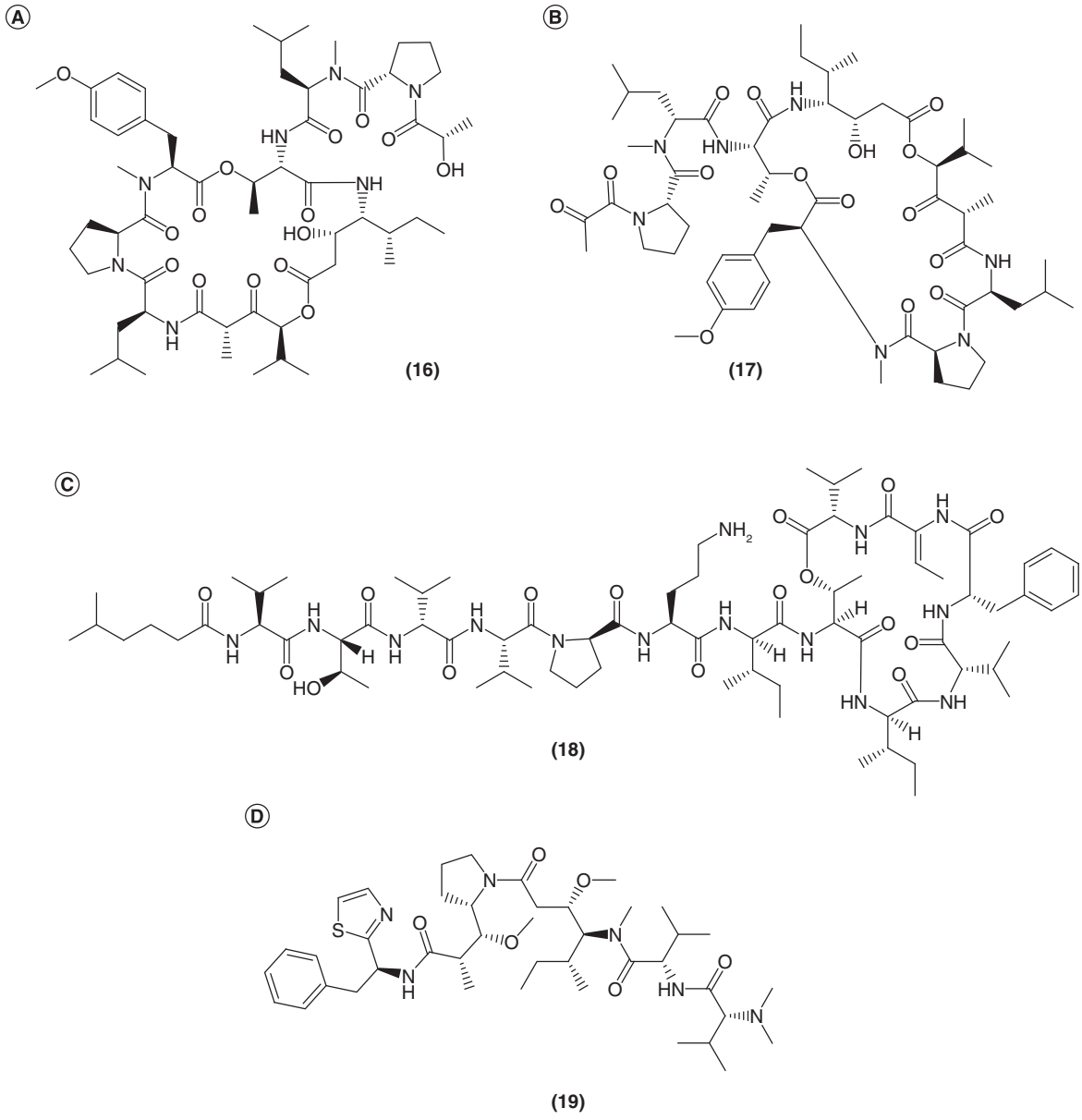

Figure 2. Chemical structures of some marine derived peptides. (A) Didemnin B, **(B)** Aplidine, **(C)** Kahalalide F and **(D)** Dolastatin 10.

against a variety of tumors was evaluated; and freshly explanted breast cancer specimens were sensitive to low concentrations of aplidine [58,59]. This peptide was also well-tolerated with minor toxicity in completed Phase I clinical trials [60,61]. This peptide leads to cell cycle arrest and also inhibition of protein synthesis in cancerous cells. Moreover, aplidine induces early oxidative stress, which causes a rapid and persistent activation of JNK and MAPK phosphorylation, the pathways that regulate cell cycle progression. Activation of these pathways can trigger cytochrome c release and subsequently activate caspase cascades.

One of the marine-derived cyclic depsipeptides is kahalalide F (KF) **(18)** (Figure 2C), a member of the kahalalide family. This peptide was first isolated from *Bryopsis* sp. green alga, as well as *Elysia rufescens* in 1993 and has shown anticancer potency against several cell lines, particularly prostate and breast cancers. This bioactive peptide contains several unusual amino acid residues in its structure. KF has exhibited cytotoxic effects on cancer cells via various mechanisms of action including suppression of HER2 tyrosine kinase activity, inhibiting the expression of the growth factor TGF-α and induction of non-P53-mediated apoptosis; and unlike other anticancer drugs, it did not

induce cell cycle arrest and DNA degradation [62–64]. Sua´rez *et al.* examined the effects of KF on the proliferation of breast cancer cell lines, including SK-BR-3, BT474, MCF-7 and MDA-MB-231. To do so, they estimated the level of DNA synthesis 24 h after treatment with different doses. In all cell lines, a dose-dependent inhibition of DNA synthesis was observed. The cytotoxic effect of KF was triggered very rapidly and did not need continuous presence of this compound on cells. In addition, no change was observed in its cytotoxicity after treatment of BCL-2- or HER2/neu-overexpressing cells. Hence, it is proposed that KF induces oncolytic instead of apoptotic cell death [63]. Kahalalide F is a promising peptide for cancer therapy and is being tested in clinical trials [65] to recommend appropriate doses and treatment times for further Phase II clinical investigations in patients with advanced solid tumors [64]. According to the results, 1000 $\mu g/m^2$ of KF with three hours of treatment per week can be proper; however, prolonged infusion times (i.e., 24-h treatment) are also feasible.

Dolastatin 10 (Aplysia toxin) **(19)** (Figure 2D), a pentapeptide with unusual amino acids, is originated from the marine gastropod mollusk *Dolabella auricularia*. This peptide has antitumor activity against many kinds of cancers and acts by inhibiting microtubule assembly, hydrolyzing the tubulin-dependent GTP, and binding of vinca alkaloids to tubulin through the vinca alkaloid binding domain [66]. Dolastatin 10 or its derivatives have been studied in Phase I and Phase II clinical trials for breast cancer. However, in Phase II study on 21 patients with measurable metastatic breast cancer, no antitumor activities were observed after treatment with dolastatin 10, casting doubt on its efficacy in advanced breast cancers [66–68].

Other marine derived peptides which are effective on breast cancer cells are shown in Table 3.

Food derived peptides

Over the last two decades, food derived peptides have received a considerable attention. Substantial researches have been accomplished to develop anticancer peptides from diverse food sources (milk, egg, fish, rice, soybean, pea, chlorella, spirulina, oyster, mussel *etc*) (Table 4) [76–87].

Some of the aforementioned protein hydrolysates (or peptides) have been reported to possess anticancer activities against breast cancer both *in vitro* and *in vivo* [89]. For example, Lunasin **(28) (SKWQHQQDSCRKQKQGVNLTPCEKHIMEKIQGRGDDDDDDDDD)**, a 43-amino acid peptide that was identified in soybean and other seeds and legumes, is reported as an effective natural peptide against breast tumors in several investigations. Hsieh *et al.* showed that this peptide inhibited cell proliferation in MDA-MB-231 cells and arrested cell cycle at S-phase, decreased tumor generation in DMBA-induced breast cancer in SENCAR (SENsitivity to CARcinogenesis) mice and also induced apoptosis in breast cancer xenografts in nude mice [90–93]. Moreover, Hernández-Ledesma *et al.* demonstrated that lunasin inhibited the histones acetylation, upregulated the *Rb* gene expression, and down-regulated the expression of cell cycle and transduction signaling genes in MDA-MB-231 cells [93].

Other kinds of natural peptides

Mastoparan **(44)** (**INLKALAALAKKIL**) is a peptide isolated from wasp venom, which has 14-residues [105]. Recently, it was shown that nonamidated mastoparan (i.e., mastoparan-COOH) induced apoptosis in melanoma cells [106]. It was also revealed that mastoparan capped with a C-terminal amide (i.e., mastoparan-NH_2), is 8-fold to 11-fold more potent than mastoparan-COOH. Mastoparan exhibited toxicity against human breast cancer cells including MDA-MB-231, SK-BR-3, MDA-MB-468 and T47D, as well as 4T1 murine breast cancer cells. This peptide was also toxic to slow growing and multi drug resistant cancer cells, which can be in association with its direct interactions with the cell membrane. In fact, mastoparan induces mitochondrial-dependent apoptosis in cancer cells and is equally toxic to rapidly dividing and slow growing cells. This effectiveness on slow growing tumors may be an advantage of mastoparan in comparison with conventional chemotherapies, which target rapidly growing tumors. Moreover, this peptide has shown significant selectivity on cancerous cells (selectivity index ~twofold–sixfold) when compared with primary normal cells [107].

RA-V (deoxybouvardin) **(45)** (Figure 3), is another natural peptide derived from the medical plant *Rubia yunnanensis*. In 2013, Fang *et al.* showed that this cyclopeptide could significantly inhibit the growth of human breast cancer cell lines MCF-7 and MDA-MB-231 and also 4T1 mouse mammary carcinoma cells. They also reported the ability of RA-V to trigger the mitochondrial apoptotic pathway, which was indicated by the loss of mitochondrial membrane potential, the release of cytochrome c and the activation of caspase cascade [108].

ICD-85 **(46)** *(venom-derived peptides)* is an active fraction which contains three peptides ranging in size from 10000 to 30000 Da. This fraction is derived from venom of Iranian brown snake (*Gloydius halys*) and yellow scorpion (*Hemiscorpius lepturus*) and has been studied on human breast cancer (MCF-7 and MDA-MB231 cells)

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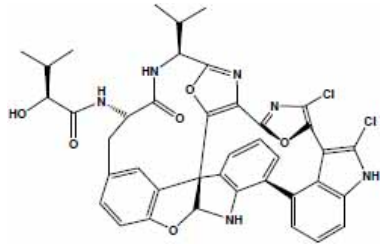
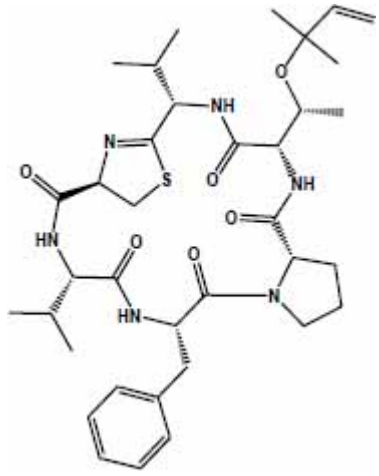
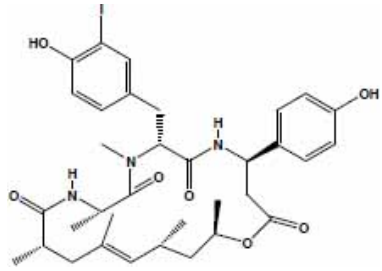
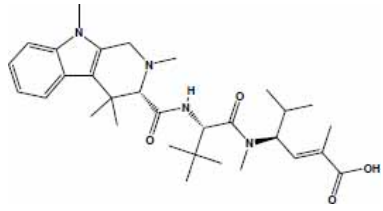
Table 3. Marine-derived anticancer peptides and their activity on breast cancer models.

No.	Source	Peptide name	Sequence/chemical structure	Biological activity	Ref.
(20)	Ascidian (*Diazona angulata*)	Diazonamide A		Showed cytotoxic effects and tubulin polymerization inhibition in MCF-7 cells; at 2–5 nM	[65]
(21)	Ascidian (*Didemnum molle*)	Mollamides B		Showed cytotoxicity against MCF-7 cells at 100 μM	[69]
(22)	Mollusc (*Ruditapes philippinarumk*)	–	AVLVDKNCPD	Induced apoptosis in MDA-MB-231 cells; IC_{50}: 1.58 ± 0.31 μg/ml	[70]
(23)	Brazilian sponge (*Geodia corticostylifera*)	Geodiamolide H		Inhibited the proliferation, migration, and invasion of MCF-7 and Hs578T cells, but not MCF10A (a nontumorigenic epithelial cell line)	[71]
(24)	Sponge (*Cymbastela, Auletta, Siphonochalina*)	Milnamide A		Showed cytotoxic effects, antimitotic activity and tubulin polymerization inhibition on MCF-7 cells	[72]

and normal human dermal fibroblast (HDF) cell lines. The results indicated that ICD-85 had antiproliferative and anti-angiogenic activity on breast cancer cells. ICD-85 decreased the survival of MCF-7 cells in a dose-dependent manner and induced some morphological alterations such as cell shrinkage and rounding in these cells which can

Table 3. Marine-derived anticancer peptides and their activity on breast cancer models (cont.).

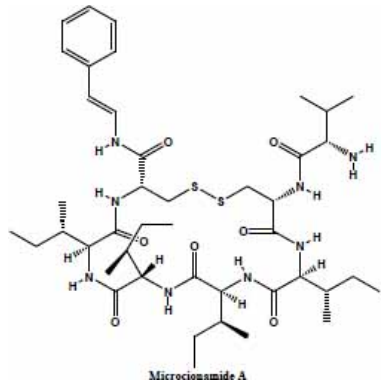
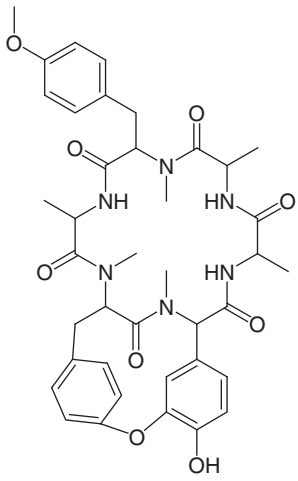
No.	Source	Peptide name	Sequence/chemical structure	Biological activity	Ref.
(25)	Sponge (*Clathria abietina*)	Microcionamides A and B	 Microcionamide A	Exhibited cytotoxic effects on MCF-7 and SKBR-3 cells	[73]
(26)	Shellfish (*Mytilus coruscus)*	–	AFNIHNRNLL	Induced apoptosis in MDA-MB-231 cells	[74]
(27)	Algae (*Spirulina platensis*)	Polypeptide Y2	–	Inhibited cell proliferation in MCF-7 cells	[75]

Table 4. Anticancer activities of various food protein hydrolysates prepared from different food sources.

No.	Sources	Antitumor activities	Ref.
(29)	Corn gluten peptides (dipeptides and tripeptides)	Reduced the tumor incidence in female Sprague–Dawley rats with DMBA-induced mammary tumors	[82]
(30)	Protein hydrolysates from blue whiting, cod, plaice and salmon	Inhibited cell growth in two human breast carcinoma cell lines, MCF-7/6 and MDA-MB-231	[81]
(31)	Rice bran protein hydrolysates	Inhibited cell growth in MCF-7/6 and MDA-MB-231 breast cancer cell lines	[83]
(32)	Gelatin hydrolysates from *Dosidicus gigas* and *Misgurnus anguillicaudatus* muscle	Showed cytotoxic and antiproliferative activities on MCF-7	[94-96]
(33)	Snow crab by-product hydrolysates and blue mussel (*Mytilus edulis*) by-product hydrolysate	Inhibited cell growth of BT549 breast cancer cells	[86,96]
(34)	Tuna dark muscle by-product protein hydrolysates	Demonstrated dose dependent inhibition on MCF-7 cell growth	[73]
(35)	Tuna cooking juice protein hydrolysates	Exhibited antiproliferative activity on MCF-7 cell line and induced cell cycle arrest at S phase	[97]
(36)	*Spirulina platensis* derived peptides	Demonstrated antiproliferative activity on MCF-7 cells	[88]
(37)	An opioid peptide derived from human αS1-casein	Exhibited antiproliferative activity on T47D human breast cancer cells	[98]
(38)	Bovine lactoferricin (LfcinB)	Caused DNA fragmentation and morphological changes consistent with apoptosis in MDA-MB-435 cell line	[99]
(39)	Bovine lactoferrin	Inhibited growth of MCF-7, T47D, MDA-MB-231 and Hs578T breast cancer cells in a concentration-dependent manner	[100]
(40)	Black soybean by-product derived peptide (F2-c)	Showed high cytotoxic potential against MCF-7 cell line	[101]
(41)	Olive seed (peptide LLPSY)	Showed capability to increase the adhesion capacity of MDA-MB-468 cells and decrease their migration capacity and to arrest cell cycle at S phase	[102]
(42)	Walnut residual (peptide CTLEW)	Selectively inhibited MCF-7 cell growth and showed immunomodulatory activity and induced apoptosis and autophagy in these cells	[103]
(43)	Chickpea (*Cicer arietinum* L.)	Increased the level of P53 and showed a high inhibitory activity against MCF-7 and MDA-MB-231 cells	[104]

be considered as markers of apoptosis. Moreover, it was shown that this fraction induced apoptosis in MCF-7 cells through caspase activation [109,110].

BmK AGAP **(47)** (**VKDGYIVDDK NCAYFCGRNA YCDDECEKNG AESGYCQWAG VYGNACWCYK LPDKVPIRVP GRCNG**), an analgesic peptide from the scorpion, *Buthus martensii*, with molecular mass of 7142 Da, has been shown to have antitumor activity. Kampo *et al.* studied the effects of BmK AGAP on cancer cell stemness and epithelial-mesenchymal transition of breast cancer cells both *in vitro* and *in vivo*. This peptide inhibited the growth of breast xenograft tumors, cancer stemness and epithelial-mesenchymal transition in mouse



(45)

Figure 3. Chemical structure of RA-V (deoxybouvardin).

model. It seems that BmK AGAP antitumor activity is originated from down-regulation of PTX3 expression in breast cancer [111].

Antimicrobial peptides & host defense-like peptides

Host defense peptides are a part of the innate immune system, and many of them bind directly to negatively charged membranes [112–115] and lyze them [116,117]. They were initially discovered because of their role in clearance of pathogens [118]. There are many anticancer peptides derived from antimicrobial peptides, which act against tumor cells by selective interfering with them via charge-triggered membrane disruption, so they rarely cause drug resistance. Anticancer peptides also have other modes of action such as interaction with mitochondria and induction of apoptosis, inhibition of tumor angiogenesis and stimulation of host's immune system [119]. In some cases an anticancer peptide may simultaneously adopt multiple anticancer mechanisms [120]. A few of anticancer peptides became inactivated and enzymatically degraded by serum components in *in vivo* studies. To overcome the limitation of these peptides *in vivo* and improve their selectivity against malignant cells, Papo *et al.* designed a short host defense-like lytic peptide **(48)** (**D-K_6L_9**; **LKLLKKLLKKLLKLL**, italic letters are D-amino acid). They inoculated it to immune-deficient mice systemically and this 15-mer D, L-amino acid peptide, showed the inhibition of breast carcinoma growth and prevented spontaneous metastasis. It showed selectivity on cancerous cells both *in vitro* and *in vivo*, which can be partially attributed to its ability to target surface-exposed phosphatidylserine in cancer cells. Given that D-K_6L_9 caused necrosis in cancer cells, a two-step cytolytic effect which leads to necrosis was suggested by the authors. First, D-K_6L_9 binds to distinct sites on the cytoplasmic membrane of the cell and colocalizes with anionic phosphatidylserines. After obtaining a threshold concentration of the peptide, a marked depolarization of the membrane occurs, which leads to cell death [118].

In another study, Hilchie *et al.* reported two cationic antimicrobial peptides of pleurocidin family derived from Atlantic flatfishes, NRC-03 **(49)** (**GRRKRKWLRRIGKGVKIIGGAALDHL**) and NRC-07 **(50)** (**RWGK-WFKKATHVGKHVGKAALTAYL**) as potent peptides for treatment of breast cancer cells. These peptides were toxic to T47-D, MDA-MB-231, MCF-7, SK-BR-3, and MDA-MB-468 human breast cancer cells and also 4T1 cells in a dose-dependent and selective manner. NRC-03 and NRC-07 could sensitize breast cancer cells to chemotherapeutic drugs such as cisplatin (at 10 μM), however, susceptibility of NRC-03 to proteolytic degradation, limits its anticancer potential [121]. D-NRC-03 is a D-amino acid analog of NRC-03 which did not exhibit reduced cytotoxicity in the presence of fetal bovine serum and resisted degradation by human serum proteases, including trypsin. Interestingly, D-NRC-03 demonstrated more cytotoxicity on breast cancer cells, both *in vitro* and *in vivo*. However, D-NRC-03 was less selective than NRC-03 for cancer cells. In addition, MDA-MB-231 cells treated with D-NRC-03, released lactate dehydrogenase which suggested induction of necrosis. Scanning electron microscopy of MDA-MB-231 cells, demonstrated that both NRC-03 and D-NRC-03, killed target cells by causing extensive membrane damage [122].

SVS-1 **(51)** (**KVKVKVKV^{D}P^{L}PTKVKVKVK**), is a designed 18-residue anticancer peptide, which remains unfolded and inactive in aqueous solution. This peptide folds as an amphiphilic β-hairpin structure at the surface of

Table 5. Antimicrobial peptides with anticancer activity against several breast cancer types *in vitro* and *in vivo*.

No.	Peptide	Source	Sequence	Antitumor activities	Ref.
(52)	Pep27anal2	Synthetic	MWKWFHNVLSWWW LLADKRPARDYNRK	Induced cell death in MCF-7 cells (at <10 μM)	[125]
(53)	MAP-04-03	Synthetic	KRLRRVWRRWR	Exhibited inhibitory effects on proliferation (IC_{50} = 61.5 μM) and cell migration (at 5 μM) and also affected the cytoskeleton of MCF-7 cells (at 25 μM)	[126]
(54)	Temporin-1CEa	*Rana chensinensis* (skin secretions)	FVDLKKIANIINSIFGK	Indicated a rapid cytotoxicity in Bcap-37 cells (at 20–40 μM) through membrane-destruction and intracellular mechanisms involving mitochondria	[127]

cancer cells and disrupts their membranes by pore formation [123]. Electrostatic interactions between the peptide and the negatively charged membrane surface of cancer cells induce this kind of folding [119]. SVS-1 showed cytotoxic potency against MCF-7 and MDA-MB-436, and low toxicity on normal cells (HUVEC cell line and erythrocytes). Studies show that SVS-1 folding is electrostatically induced and cell death occurs before the peptide neutralizes the cell's negative membrane charge [123,124]. Other important anti-breast cancer antimicrobial peptides are shown in Table 5.

Delivery systems for anticancer peptides

Despite many advantages of anticancer peptides, their clinical application has several pharmaceutical and biopharmaceutical challenges and limitations. The major route for delivery of such peptides is parenteral administration. However, due to short *in vivo* half-life of proteins and peptides, there is a need for frequent injections [128]. Oral delivery is a noninvasive alternative route for administration of small molecules, but it is not a preferable way for proteins and peptides due to some problems including presence of proteolytic enzymes in gastrointestinal system, their poor stability at lower pH of gastric fluid and also poor permeation across gastrointestinal membrane [129].

Nasal and pulmonary administrations seem to be proper non-invasive routes for using anticancer peptides and proteins, due to their low proteolytic activity relative to oral route, highly vascularized and large absorptive surfaces, however they also face some limitations such as large size and proteolytic instability, which lead to poor absorption of therapeutic proteins across nasal and pulmonary mucosal surfaces. Physiological barriers such as mucociliary clearance may also limit the absorption of these peptides and proteins [129].

To overcome these challenges, encapsulating the therapeutic peptides and proteins in carrier systems such as microparticles, polymeric nanoparticles (NPs), liposomes and solid lipid NPs can deliver the mentioned drugs more efficiently [130,131]. There are several efforts in using NPs for delivery of anti-breast cancer peptides. For instance, the D-amino acid NuBCP-9 peptide, which was shown to specifically induce apoptosis in cancer cells, via a BCL-2-dependent mechanism [132], was encapsulated in polyethylene glycol (PEG)-modified polylactic acid (PLA) diblock copolymer or PEG polypropylene glycol-PEG-modified PLA – tetrablock copolymer by Kumar and his colleagues. They studied the effects of these complexes on growth of BCL-2-expressing MCF-7 cells. The NuBCP-9-encapsulated NPs were highly effective in inhibiting growth of MCF-7 cancerous cells. Moreover, NuBCP-9 NPs were effective in inducing complete regressions of tumors in Ehrlich tumor model in syngeneic mice [133]. In another study, Haggag *et al.* designed RAS protein-regulator of RAN-RCC1 inhibitory peptides for interaction with RAN (a novel therapeutic target in breast cancer). The peptides were encapsulated in polyethylene glycol-poly (lactic-co-glycolic acid) PEG-PLGA polymeric NPs as a delivery system. A PEG-PLGA-NP encapsulating N-terminal peptide exhibited antimetastatic action on MDA-MB-231 breast cancer cells *in vitro* and also reduced tumor volume and inhibited tumor growth in a mouse model of breast cancer [134].

Polymeric NPs offer unique advantages over other carrier systems due to their small size, which makes them a suitable drug carrier for parenteral administration, and also enables them to translocate efficiently across epithelial surfaces as compared with microparticles. Moreover, polymeric NPs showed high stability in biological fluids relative to liposomes and solid lipid NPs. Other important advantages of these NPs include versatility of formulation, protection of encapsulated peptide drugs from enzymatic degradation, sustained release and also tissue biocompatibility [130,131]. It should also be noted that some NPs might be toxic to the cells. For example, carbon nanotubes can be carcinogenic for lung, gastrointestinal tract, CNS and blood. So, awareness of the levels of particles which can cause health problems, is essential for both workers and exposed patients [135].

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Discussion

Current therapeutic strategies for breast cancer treatment have some limitations such as drug-resistance, toxicity to normal tissues and the development of secondary malignancies. Anticancer peptides are potential agents that lack these problems and might be used for treatment of breast tumors and other kinds of cancers [136]. Over the years, peptides have been used in the treatment of bacterial infections (peptide antibiotics), diabetes and cancer and the application of peptides in a variety of other therapeutic areas is under development. Targeting tumor cells by direct use of anticancer peptides with no harmful effect on normal cells (targeted therapy) might be a new alternate strategy to conventional chemotherapy. In this review, we summarized different types of anticancer peptides and discussed about their sources, biological activities on breast cancer cells, modes of action and also delivery systems. Moreover, the pros and cons of using peptides in targeted therapy are further discussed.

Treatment of breast cancer and other kinds of carcinomas by short peptides can be the safest therapeutic strategy, as peptides hydrolyze to their constituent common amino acids in the body and are excreted. Although there have been many efforts to discover potent anti-breast cancer peptides, most studies are stopped in *in vivo* investigations. An important reason for this is often instability and/or enzymatic degradation of peptides *in vivo*. However, to overcome this limitation, as referred in this review, peptides can be modified to D-peptides, the uncommon isoform of amino acids, which cannot be recognized by proteolytic enzymes and as a result, D-peptides have exhibited better *in vivo* anticancer activity [137,138]. Another way to solve this problem might be taking advantage of nanostructured delivery carriers such as nanofibers, metal NPs, MLNPs (multi-layer NPs), nanoliposomes, etc. for delivery of peptide drugs to the site of cancer [139–143]. One of the most important delivery systems are polymeric NPs, the solid colloidal carriers which have been explored for the delivery of protein and peptide therapeutics. Among nanocarriers, polymeric NPs have demonstrated significant advantages over other delivery systems including higher stability in biological fluids compared with liposomes and solid lipid NPs, versatility of formulation and sustained release [131].

The other main problem might be the selectivity of the peptides. Many natural peptides can be very potent anticancer agents, but they may not specifically bind to the expected site on cancerous cells. However, since many of them are not directed to a specific extracellular or intracellular receptor, some mechanisms of resistance can be impaired and these kinds of peptides might act well against multi drug resistant cancer cells.

On the other hand, finding a selective anticancer peptide from natural sources requires the study of a large number of peptides which can be very costly and time consuming. Hence, it seems that in order to obtain a specific and selective peptide for treatment of breast cancer in a relatively short time, computational methods that consider the structures of breast cancer surface markers such as HER2, may pave the way to discover the most specific peptides, which directly target the expected areas on tumor cells. However, the structure of natural anticancer peptides and their effects on breast cancer cells both *in vitro* and *in vivo* might be used as a template for designing new peptides for treatment of cancers. For instance, some of the reported marine originated peptides such as didemnin B, aplidine and kahalalide F that have reached clinical phases are cyclopeptides. This cyclic structure might stabilize the peptide folding in different microenvironments, which can be considered in designing new peptides. Moreover, identification of the specific amino acid sequence in the full sequence of natural peptides, which might be responsible for their anticancer activity and synthesis of shorter fragments that retain full biological activity, will help to reduce their high production cost. These shorter peptides might be more efficient to reach the phospholipid bilayer of the cell membrane leading to increased cytotoxicity.

One of the recent therapeutic strategies for treatment of various cancers such as breast cancer is immunotherapy by using the peptidic checkpoint inhibitors. Inhibition of the anticancer immune response has emerged as an important mechanism of tumor resistance to treatment. So, the development of peptides (instead of mAbs) that block the immune checkpoint receptors such as CTLA4 and PD-1 or its ligand PD-L1, can be a suitable therapeutic strategy. PD-1/PD-L1 signaling pathway might be a proper target for immunotherapy of breast cancer, especially for basal types, as these types of breast carcinoma express greater levels of PD-L1 on their surface. Until now, couples of studies have introduced peptide inhibitors for blockade of PD-1/PD-L1 axis [137,138,144]. However, the only peptide that has been investigated on breast cancer is AUNP12 introduced by Aurigene Discovery Technologies, which has inhibited both primary tumor growth and its metastasis with high efficacy. Based on high PD-L1 expression in basal type breast cancer [145], more attention should be paid to studying the effects of the reported PD-1/PD-L1 blocking peptides on breast cancer models and also designing novel peptide inhibitors for treatment of breast carcinomas.

Although there are many advantages in targeting breast cancer by peptides, a single method alone may not be efficient enough to obtain positive results. Combination therapy might be a proper strategy to achieve synergistic effects in breast cancer treatment. Thus, combining an anticancer peptide with a nonpeptidic cytotoxic drug or with other common treatment modalities such as radiation can lead to a better outcome.

Conclusion

Up to now, despite the need for peptides with anti-breast cancer activity and the numerous studies performed in this area, only a few of them have reached to clinical trials. Discovering or designing more selective and potent peptides is essential to provide more options for the treatment of breast cancer. In order to obtain an optimal anticancer peptide, manipulation of natural peptide sequences, their net charge, secondary structure, oligomerization ability, their amphipathicity and hydrophobicity might be proper approaches. Moreover, *de novo* designs of novel oligopeptides against many identified targets on breast cancer cells, have demonstrated interesting results so far. Despite many advantages in targeting breast cancer by peptides, several studies have shown that a single peptide is not sufficient for cancer treatment and it is better to use the anticancer oligopeptides along with another anticancer strategy.

Future perspective

We believe that peptides will have a major impact on cancer therapy in near future. Based on the advances of rational design of peptides, minimizing or eliminating cytotoxic effects, it would be reasonable to expect that the number of such peptides should tend to increase. Moreover, advances in the large-scale synthesis of peptides have reduced the cost of these agents and make them more accessible to patients. Another strategy for cancer treatment is combination therapy with peptides along with conventional drugs, which has several advantages such as reduction of expenses, minimizing the challenges of drug resistance and preventing recurrence. Hence, using peptides as anticancer agents might have great benefits, both alone and in combination with conventional modalities, mostly due to their effective mechanisms of action on the target cells.

Executive summary

Anticancer peptides & their action mechanisms

- Treatment of breast cancer and other kinds of carcinomas by short peptides can be the safest therapeutic strategy, as peptides hydrolyze to their constituent common amino acids in the body and are excreted.
- There are several different mechanisms for peptides action against cancer cells including disruption of the cellular membranes and then defying the malignant cells by apoptotic or necrotic mechanisms, affecting the intracellular targets, and modulation of the immune system.

Synthetic & natural peptides for treatment of breast cancer

- Structure-based design of oligopeptides has important implications in development of novel cost-effective drugs.
- Marine peptides have unique structures and anticancer activities with various mechanisms of action on cancer cells. Two important marine derived peptides didemnin B and kahalalide F are under Phase I/II clinical trials. Moreover, despite well documented antitumor activities of dolastatin 10 both *in vitro* and *in vivo*, this marine derived peptide has failed in clinical trials in advanced breast cancers.
- Host defense–like peptides rarely cause drug resistance as they act against tumor cells by selective interfering with them via charge-triggered membrane disruption. Their modes of action include the interaction with mitochondria and induction of cell apoptosis, inhibition of tumor angiogenesis and stimulation of host's immune system.

How to overcome the limitations of using anticancer peptides

Using uncommon residues in sequences of the peptides, might decrease their degradation by the proteolytic enzymes in the body, and these kinds of peptides have exhibited better *in vivo* anticancer activities so far.

In order to obtain positive results in breast cancer treatment with peptides, it is better to use them along with other conventional therapeutic approaches.

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