REVIEW ARTICLE

Recent Progress of Marine Polypeptides as Anticancer Agents

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Abstract: *Background*: Marine environment constitutes an almost infinite resource for novel anticancer drug discovery. The biodiversity of marine organisms provides a rich source for the discovery and development of novel anticancer peptides in the treatment of human cancer. Marine peptides represent a new opportunity to obtain lead compounds in biomedical field, particularly for cancer therapy.

Objective: Providing an insight of the recent progress of patented marine peptides and presenting information about the structures and mechanistic mode of anticancer activities of these marine peptides.

Methods: We reviewed recent progress on the patented anticancer peptides from marine organisms according to their targets on different signal pathways. This work focuses on relevant recent patents (2010-2018) that entail the anticancer activity with associated mechanism and related molecular diversity of marine peptides. The related cellular signaling pathways for novel peptides that induce apoptosis and affect tubulin-microtubule equilibrium, angiogenesis and kinase activity that are related to the anticancer and related pharmacological properties are also discussed.

Results: The recent patents (2010-2018) of marine peptides with anticancer activity were reviewed, and the anticancer activity of marine peptides with associated mechanism and related molecular diversity of marine peptides were also discussed.

Conclusion: Marine peptides possess chemical diversity and display potent anticancer activity via targeting different signal pathways. Some of the marine peptides are promising to be developed as novel anticancer agents.

Keywords: Anticancer, apoptosis, angiogenesis, marine organisms, peptides/polypeptides, tubulin-microtubule equilibrium.

1. INTRODUCTION

The ocean has received much attention as a new useful source of bioactive compounds, and several kinds of drugs including marine peptides have been discovered and derived from marine natural products currently, with some drugs used clinically to treat cancer [1]. Recently, the success of discovery and development of these peptides from marine has provided a new prospect for pharmaceutical industry [2]. Anticancer peptides derived from marine organisms kill cancer cells through a variety of mechanisms including promoting apoptosis, inhibiting tubulin-microtubule equilibrium,

angiogenesis and kinase activity, etc. [3]. These findings have helped us with a better understanding of novel chemical structures with potent and selective anticancer efficacy as well as novel mechanisms for the pharmacological activity of these anticancer peptides from marine sources.

This work will focus on recent patents (2010-2018) that entail the antitumor polypeptides from marine sources in cancer therapy. A number of antitumor polypeptides with different mechanisms have been isolated and extracted from different marine sources as listed in Table **1**. Peptides that induce apoptosis, inhibit angiogensis and impact tubulin-microtubule equilibrium will be discussed. The problem and prospect in developing marine peptides as novel anticancer agents is also presented.

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Table 1.   Summary of Anticancer Peptides Derived from Marine and Their Mode of Actions.

Name of Peptides	Sources	Class/Types	Mode of Action and Patent Number
Jaspamide (Jasplakinolide)	Marine sponge *Jaspis johnstoni*	Cyclic depsipeptide	Activation of caspase-3 and decrease of Bcl-2 protein expression
Chondramides	*Chondromyces* sp.	Cyclodepsipeptides	Induction of G-actin polymerization (US20150291658) [4]
Sepia ink oligopeptides	*Sepia* ink	Oligopeptides	Activation of p53 and caspase-3, upregulation of Bax, and downregulation of Bcl-2, (CN101983968) [5]
Somocystinamide A (ScA)	*Lyngbya majuscula/Schizothrix* sp. assemblage of marine cyanobacteria	Lipopeptide	Activation of caspase-8 (US9045401) [6]
C-phycocyanin (*C*-PC)	Cyanobacteria *Agmenellum quadruplicatum, Mastigocladus laminosus,* S*pirulina platensis*	Tetrapyrrole-protein complex	Caspases-dependent apoptosis
Aplidine (dehydrodidemnin B, DDB, Aplidin)	Tunicate, *Aplidium albicans*	Cyclic depsipeptide	Activation of JNK and p38 MAPK phosphorylation (US7576188) [7]
Iturin A	Marine *Bacillus* sp.	Lipopeptide	Inhibition of Akt pathway (DE60324948) [8]
P11A, P11B and valinomycin	Marine actinomycete *Streptomyces* sp. P11-23B	Cyclodepsipeptides	Apoptosis, but mechanism unclear (CN106167517) [9]
Sansalvamide A	Marine fungus	Cyclic depsipeptide	Apoptosis, up-regulation of p21, down-regulation of cyclins D1, E, and A, and cdk4 (US7709443) [10]
Didemnin B	Tunicate, *Trididemnum solidum*	Cyclic depsipeptide	Apoptosis but mechanism unclear (US9644005) [11]
SBP	*Brevibacillus* sp. S-1	Cyclic peptide	Apoptosis but mechanism unclear (CN1035556) [12]
PBN11-8	*Marine Bacillus* sp. N11-8	Linear polypeptide	Targeting focal adhesion kinase pathways (CN104610432) [13]
Dolastatin 10	Marine mollusk, *Dolabella auricularia*	Linear peptide	Microtubule assembly inhibition (US6034065) [14]
Diazonamide	Marine ascidian, *Diazona angulata*	Macrocyclic peptide	Tubulin polymerization inhibition (US7960420) [15]
Hemiasterlin	Marine sponges, *Auletta* sp. and *Siphonochalina* sp.	Tripeptide	Tubulin polymerization inhibition (US7192972) [16]
Mere15	Coelomic fluid, *Meretrix meretrix*	Protein	Tubulin polymerization inhibition (CN102125686) [17]
Mycothiazole	*Petrosaspongia mycofijiensis* marine sponge	Mixed polyketide/peptide derived compound	Suppression of HIF1 (WO2010014240) [18]
CS5931	*Ciona savignyi*	Linear polypeptide	Block of VEGF production and release of cytochrome c and activation of the caspases 9 and caspases 3 (CN102172394) [19]
Phakellistatins	Marine sponges	Cyclic heptapeptides	Unknown mechanism (US5646246) [20]

2. ANTICANCER PEPTIDES THAT INDUCE APOPTOSIS

As most of the anticancer agents, there are large amount of anticancer peptides derived from marine with pro-apoptotic activity to cancer cells. Apoptosis plays an important role in cellular development, normal physiology and homeostasis. The unbalance by decreased pro-apoptotic signals or increased anti-apoptotic signals could cause various diseases including inflammation and cancer etc. resulting in the failure of treatment due to drug resistance [21]. In general, there are two main apoptotic pathways, the extrinsic (death receptor-mediated) pathway and the intrinsic (mitochondrial) pathway with substantial crosstalk. There are numerous molecules that are capable of triggering or inhibiting apoptosis by pro-apoptosis or anti-apoptosis. As a consequence, developing anticancer peptides for these molecules represents an important strategy in the development of novel anticancer therapy.

The equilibrium between the pro-apoptotic gene Bax and the pro-survival gene Bcl-2 plays a pivotal role for cell survival. Thus, it represents a promising strategy for discovery and development of anticancer drugs via induction of Bax and/or inhibition of Bcl-2. The identification of caspase activators represents a different approach to discover novel anticancer drugs because caspases are involved in both the internal and external apoptotic pathways. Some anticancer peptides derived from marine are able to promote the release of cytochrome c (Cyt C) from the mitochondria through the activation of the Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) pathways [22]. Apoptosis is an intricate process involved in multiple signaling pathways and molecules. One of the main obstacles in cancer therapy is the inability to induce apoptosis. Therefore, to identify and develop the agents that can target multiple apoptosis-regulating genes is a good strategy for discovering and developing novel and effective anticancer drugs.

2.1. Anticancer Peptides that Activate the Intrinsic (Mitochondrial) Pathway in Apoptosis

The intrinsic (mitochondrial) pathway is initiated when apoptogenic molecules are released from mitochondrial into the cellular cytosol as a result of permeabilization of mitochondrial outer membrane. The release of Cyt C and other pro-apoptotic factors from mitochondria into cytosol to form a multimeric complex known as apoptosome and initiates the activation of caspases cascades. The intrinsic pathway plays a key role in normal cellular homeostasis and is very important for the pathogenesis of many diseases [23, 24]. There are at least 18 anti-apoptotic and pro-apoptotic proteins in the Bcl-2 family are involved in the apoptotic process in the intrinsic pathway. Bax, as a pro-apoptotic member in the Bcl-2 family, plays a crucial role in maintaining the equilibrium of anti-apoptotic and pro-apoptotic signals because of its ability to regulate mitochondrial function [25, 26].

Jaspamide (Jasplakinolide; NSC-613009) is a cyclic depsipeptide containing a 15-carbon macrocyclic ring with 3 amino acid residues (Fig. **1A**), which was separated from marine sponge *Jaspis johnstoni* [27]. It has been demonstrated that Jasplakinolide induces apoptosis by formation of nucleosomal DNA ladder and enhancement of the activity of caspase-3 in Jurkat T cells [28]. Study also showed that the transformed cells are more impressionable to the apoptosis induced by Jasplakinolide compared to the non-transformed cells [28]. Apoptosis induced by Jaspamide is related to the activation of caspase-3, decreased expression of Bcl-2 protein, and increase of Bax protein. Jaspamide seems to induce cell death in a caspase-independent pathway, evidenced by the apoptotic cells changes in the cytoplasmic and membrane, as well as caspase-dependent cell death characterized by PARP proteolysis [29]. A study indicates that jaspamide also possesses the ability of other functions affecting the Cav1.2, Cav 3.2, and HCN2 but the impact of Kv11.1 (hERG) channel is minimally affected [30]. A narrow margin of safety (effective dose *versus* toxic dose) for Jaspamide was observed in mouse tumor models. Jaspamide (10 μM) inhibited Kv1.5 activity by 98.5%. These results suggested that the observed toxicity in rats and dogs results from the toxic effect of jaspamide on myocardial cells [30]. A recent study also found that jaspamide can affect tubulin polymerization (US20150291658) [4].

Chondramides A to D are cyclodepsipeptides, which were originally isolated from terrestrial strains of *Chondromyces crocatus*. Structurally, these compounds are similar to jasplakinolide and jaspamide. Both jasplakinolide and chondramides can lead to G-actin polymerization *in vitro* in non-polymerizing (low salt) conditions (US20150291658) [4]. There are more than 30 new chondramide derivatives separated from *Chondromyces* sp. MSr9030. These cyclic depsipeptides target actin filaments with a binding pattern similar to the mushroom toxin phalloidin. Brominated analogues of chondramide derivatives have been prepared by improving fermentation conditions, and these brominated analogues have better anticancer efficacy than other chondramides [31].

A new linear anti-prostate cancer peptide with amino acid sequence LKYYDDESTQHGAKRMVPYKIFLNRAATRG (CN103897050) was isolated from *Sepia*. *In vitro* studies have demonstrated that the peptide can significantly inhibit the proliferation of human prostate PC-3 cancer cells [32]. Another oligopeptide with the N-terminus of the amino acid sequence Gln-Pro-Lys was also isolated from the *Sepia* species (CN101983968) and it also had a significant effect of cytotoxicity on the human prostate cancer DU-145 cells [5]. A protein isolated from *Sepia* ink was hydrolyzed by pepsin and a novel antitumor peptide, termed SHP with the sequence of Leu-Lys-Glu-Glu-Asn-Arg-Arg-Arg-Arg-Asp was isolated from the digested mixture. SHP dose-dependently inhibited the proliferation of PC-3 cells. Study showed that the early-stage apoptotic cells of PC-3 were increased from 9% to 29% following exposure to 10-15 mg/mL SHP for 24 h by Annexin V/PI double-staining analysis. The apoptosis induced by SHP was accompanied by the down-regulation of apoptosis regulator Bcl-2, up-regulation of apoptosis regulator Bax and activation of caspase-3 and p53 [33]. A cyclomimetic peptide based on SHP, called SIO, was designed by introducing a disulfide bond to stabilize the native peptide. SIO showed increased stability and more potent anticancer activity than SHP [34].

Other peptides derived from marine organisms, such as C-phycocyanin (C-PC) and Somocystinamide A (ScA, US9045401), display potent caspases-dependent pro-

apoptotic activity against a variety of cancer cells. ScA, a lipopeptide, was isolated from *Lyngbya majuscula/ Schizothrix* sp., an assemblage of marine cyanobacteria (Fig. **1B**) [35]. Compositions of ScA and its analog lipopeptides including liposomes and nanoparticles of ScA are useful in treating or ameliorating melanoma (US9045401) [6]. ScA induces apoptosis in various cancer cells through both the intrinsic and the extrinsic pathways [36]. C-PC, a tetrapyrrole-protein complex seperated from the cyanobacteria *Agmenellum quadruplicatum, Mastigocladus laminosus* and *Spirulina platensis,* induces pro-apoptotic genes activation, and anti-apoptotic genes down-regulation, leading to apoptosis in human cervix carcinoma HeLa cells [37, 38]. Treatment of C-PC in HeLa cells activated caspases 2, 3, 4, 6, 8, 9, and 10, indicating that C-PC-induced apoptosis is caspase-dependent. C-PC treatment of HeLa cells also promotes the release of Cyt C from the mitochondria into the cytosol [38]. C-PC/CMC-CD59sp nanoparticles can inhibit the growth of HeLa cells *in vitro* and tumors *in vivo*. Furthermore, the nanoparticles induce cellular apoptosis through down-regulating Bcl-2 and cyclin D1 proteins [39].

2.2. Anticancer Peptides that Target the p38 MAPK or JNK Pathway

MAPKs and JNKs play a key role in cellular signaling that regulates cell responses to cellular stress [40, 41]. Non-scheduled proliferation is a characteristic of cancer, and the p38 MAPK and JNK pathways have profound impact on the development of a variety of cancers by regulating the progression of cell cycle at different sites through both transcription-dependent and transcription-independent mechanisms. The role of pro-apoptosis or anti-apoptosis of JNKs is dependent on the stimuli, as well as cellular signals. Activation of the JNK and p38 MAPK pathways is able to induce the release of Cyt C and subsequently activate caspase cascades [40].

Aplidine (dehydrodidemnin B, DDB, Plitidepsin)(US7576188) [7], isolated from the Mediterranean tunicate *Aplidium albicans,* is a cyclic depsipeptide and called as plitidepsin in clinical studies (Fig. **1C**). A variety of cancer cells including breast cancer, non-small-cell lung cancer, and melanoma are sensitive to the treatment of aplidine at low concentrations [42, 43]. Aplidine and its analogues have potent anticancer effects on lymphomas and leukemias, especially with the combination therapies. The modes of mechanistic action of aplidine is involved multiple actions including protein synthesis inhibition and cell cycle arrest. Aplidine induces oxidative stress at early stage and results in a rapid and persistent phosphorylation of p38 MAPK and JNK and activation of the related kinases much earlier than the induction of apoptosis, and the full process of activation only needs less than 5-10 min of drug treatment in HeLa cells. The activation of p38 MAPK and JNK leads to the release of Cyt C and activation of caspase-9, caspase-3 and PARP cleavage in the downstream, indicating the role in mediation of the mitochondrial apoptotic pathway. Protein kinase C delta (PKC-δ) also plays a crucial role in aplidin-induced cell apoptosis and mediation of the cytotoxicity [44]. Aplidin activates the serine/threonine kinases, non-receptor protein-tyrosine kinase Src, p38 MAPK and JNK leading to apoptosis in human breast cancer MDA-MB-231 cells. The

two mechanisms of JNK activation by aplidin lead to the rapid activation of Rac1 small GTPase and the down regulation of MKP-1 phosphatase. Aplidine is well tolerated with only minor toxicity in the completed phase I clinical trials [45-48]. A recent investigation demonstrated that plitidepsin exerts its antitumor activity by targeting eEF1A2 [49]. Nanoparticles with poly (trimethylene carbonate)-bock-poly(L-glutamic acid) derived polymersomes and plitidepsin were designed to target epidermal growth factor receptor (EGFR) and results have showed that the new designed composite exhibited significant antitumor activity against cancer cells overexpressing EGFR both *in vitro* and *in vivo* [50].

2.3 Anticancer peptides that Target the PI3K/Akt Pathway

Akt kinase as an important composition of the PI3K/Akt signaling pathway is often overexpressed in human cancers, especially in breast cancer. It would be a useful approach to develop a therapeutic regimen with aberrant Akt activity in the treatment of breast cancer. Iturin A (DE60324948) [8]. A marine *Bacillus* microbe-derived lipopeptide was based on this approach and evaluated the anticancer effects on human breast cancer *in vitro* and *in vivo* through the disruption of the Akt pathway [51]. Iturin A significantly inhibits the growth of human breast cancer MCF-7 and MDA-MB-231 cells [51]. Studies also indicated that Iturin A inhibited the phosphorylation (Ser473 and Thr308) of Akt induced by EGF and its downstream targets of GSK3β and FoxO3ab. Iturin A inactivates MAPK and Akt kinases resulting in the translocation of FoxO3a from cytoplasm to nucleus. Gene silencing of Akt reduced the sensitivity of cancer cells to the treatment of Iturin A in human breast cancer MDA-MB-231 and MCF-7 cells. However, overexpression of Akt in cancer cells leads to high susceptibility to Iturin A-induced apoptosis. Iturin A inhibited tumor growth with reduced expressions of Ki-67, P-MAPK, P-Akt, CD-31, P-FoxO3a and P-GSK3β in a xenograft model of breast cancer [39]. Iturin F_1, iturin F_2, iturin A_8 and iturin A_9 were separated from *Bacillus* sp. KCB14S006 derived from a saltern and also showed moderate cytotoxicity against HeLa and src(ts)-NRK cells [52].

2.4. Anticancer Peptides Induce Apoptosis with Unknown Mechanism

Some peptides derived from marine organisms are capable to induce cell death with apoptotic characteristics such as DNA fragmentation, cell membrane swelling and nucleus shrinkage. However, the precise mechanism of cytotoxic activity and apoptotic induction is unclear. Three cyclodepsipeptides of P11A, P11B and valinomycin, were isolated from the cultured marine actinomycete *Streptomyces* sp. P11-23B (CN106167517) [9]. Both P11A and P11B can inhibit the proliferation of several glioma cell lines, with the IC_{50} values of 0.1 to 1.4 μM [53]. Streptodepsipeptide P11A induced apoptosis and inhibited the cell cycle at the G0/G1 phase in glioma cells [53]. Further study showed that streptodepsipeptide P11A downregulated the expressions of important tumor metabolic enzymes such as HK2, GLS, PKM2, PFKFB3, and FASN [53]. Sansalvamide A (US7709443) [10], a cyclic depsipeptide produced by a ma-

rine fungus, has potent antitumor activity [54]. Sansalvamide A causes cell cycle arrest in G0/G1 phase by flow cytometry analysis and is able to up-regulate p21, down-regulate of cyclins D1, E, and A, and cdk4 by Western blot analyses in human pancreatic cancer AsPC-1 and CD18 cells [55]. Sansalvamide A-amide with unique structure affects cancer-related pathways associated with heat shock protein 90 (HSP90) [56].

Didemnins, first identified and reported in 1981 [57], belong to the depsipeptide family with antiviral, antitumor, and immunosuppressive activities. These compounds are mainly isolated from Caribbean tunicate *Trididemnum solidum*, but were also found from other species of the same genus.

Didemnin B (US9644005), a branched *N*-methylated cyclic peptolide, was isolated from the *Trididemnum* genus of marine tunicates (Fig. **1D**) [11]. Didemnins B displays its anticancer activity and induction of apoptotic effect in cancer cells. However, its mechanism is unknown.

Our laboratory has isolated a new cytotoxic peptide, named as SBP (CN103555622) from the fermentation broth of the marine bacterium *Brevibacillus* sp. S-1, by ion-exchange chromatography and reverse-phase HPLC chromatography. The molecular weight of SBP was 1570 Da as determined by MALDI-TOF mass spectrometry. The structure of SBP was a cyclic peptide determined by the methods of MALDI-TOF/TOF mass spectrometry and *de novo* sequencing. MTT test indicated that SBP displayed cytotoxicity against human colon carcinoma RKO, human lung cancer A549, human hepatocellular carcinoma BEL-7402, human breast carcinoma MCF-7 and human glioma U251 cells [49, 50]. It is very interesting that SBP exhibits low cytotoxicity

against human normal lung fibroblast HFL1 cells [58, 12]. A novel linear polypeptide, PBN11-8, was also isolated in our laboratory recently from a *Bacillus* sp. N11-8 of Antarctic Ocean water (CN103667097) [59]. PBN11-8 has relatively high cytotoxic activity against a verity of cancer cells such as human hepatoma carcinoma BEL-7402, human ovarian carcinoma NIH:OVCAR-3, human renal clear cell carcinoma 786-0, and human large cell lung cancer NCI-H460 cells (CN104610432) [13]. PBN11-8 was also found to inhibit the growth of human hepatocellular carcinoma cells by targeting focal adhesion kinase pathways.

3. ANTICANCER PEPTIDES THAT AFFECT THE EQUILIBRIUM OF TUBULIN-MICROTUBULE

Microtubules belong to the intracellular organelles which are formed with the protein tubulin. They have many basic cellular functions such as chromosome segregation, maintenance of cell shape, motility, transport, and distribution of organelles [60]. Drugs affecting the balance of tubulin-microtubule has the potential to be developed as anticancer agents [60]. Since the successful application of taxanes in cancer therapy and the common use of vinca alkaloids, vincristine and vinblastine in clinical oncology, tubulin binding molecules are considered as an important class of anticancer agents. These kinds of compounds suppress cellular mitosis via binding to the mitotic spindle of tubulin, blocking tubulin polymerization into microtubules (MTs). Therefore, there is a great motivation for discovery and development of novel antimitotic agents interacting with tubulin at the sites different from those of vinca alkaloids and taxanes from natural resource [61].
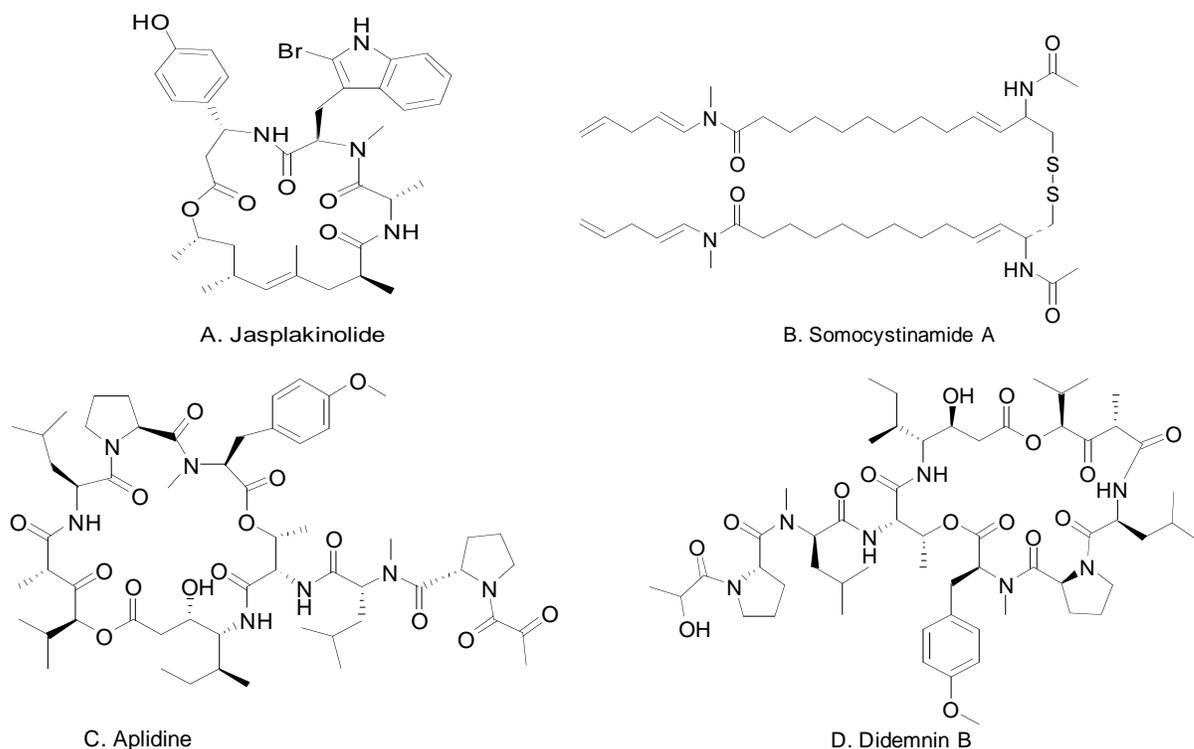

Fig. (1). Chemical structures of the peptides derived from marine with pro-apoptotic activity: Jasplakinolide (**A**), Somocystinamide A (**B**), Aplidine (**C**) and Didemnin B (**D**).

Dolastatin 10(US6034065) [14], a linear pentapeptide containing several unique amino acid subunits (Fig. **2A**), is found from the marine mollusk *Dolabella auricularia*. The compound is considered as the most potent member of a large class of related peptides with anticancer activity (US6323315) [62, 63]. Both Dolastatin 10 and a tripeptide segment of Dolastatin 10 are capable of inhibiting tubulin polymerization and GTP hydrolysis. Recently, a novel patent of "the synthesis and elucidation of nineteen heterocyclic or halophenyl amide derivatives of dolastatin 10" was disclosed (WO1996018408) [64]. A dolastatin derivative, auristain E, was linked to a CD30 monoclonal antibody to develop as a novel antibody drug conjugate (ADC), called Adcetris. Adcetris has been approved for the treatment of Hodgkin's lymphoma in 65 countries [63, 65-67].

Some other marine peptides, such as diazonamides (US7960420) [15] and hemiasterlins (US7192972) [16], showed potent inhibitory effect on tubulin polymerization in several cancer cell lines. Diazonamide A (Fig. **2B**) is a complex of cytotoxic peptide isolated from the marine ascidian, *Diazona angulata* [68]. Diazonamide A and its analog bind weakly to the unpolymerized tubulin but strongly to the ends of microtubule [69]. A US patent (7960420) for the description of the composition of Diazonamide A analogs and their esters, salts, and conjugates, has been published recently [62]. Studies have indicated that some of the compositions are effective in the treatment of proliferative diseases such as cancer (US7538129) [70]. The synthetic diazonamide analogue, DZ-2384 showed strong anticancer activity at effective plasma concentrations against various of cancers with high safety margin without neurotoxicity in rats [71]. Compared to other vinca-binding compounds, DZ-2384 binds to the vinca domain of tubulin in a distinct way, impairing structural and functional microtubule dynamics. Studies by X-ray crystallography and electron microscopy have demonstrated that DZ-2384 could cause straightening of curved protofilaments which favors polymerization of tubulin. The growth rate of microtubules was inhibited by DZ-2384. However, study has reported that DZ-2384 increased the rescue frequency and preserves the microtubule network in nonmitotic cells and primary neurons [71]. BF65 and BF78 derived from Hemiasterlin are highly effective in killing cancer cell at the concentrations of nanomolar range through the inhibition of tubulin polymerization, similar to the effect of vinca alkaloids [72]. These compounds exhibit synergistic anticancer activity with the inhibitors of colchicine site microtubule both *in vitro* and *in vivo* [72].

We isolated a new linear polypeptide called Mere15 from *Meretrix meretrix* (CN102125686) [17]. Mere15 significantly inhibited the growth of human lung adenocarcinoma A549 xenografts bearing in nude mice. Mere15 is able to induce the release of Cyt C and cleavage of procaspase-9, procaspase-3, and poly ADP-ribose polymerase (PARP) in a dose-dependent manner. Mere15 also leads to the decline of Bcl-2 protein and the increase of the Bax level in a concentration-dependent manner in cancer cells [73]. Mere15 inhibits the proliferation of K562 cells with the IC_{50} of 38.2 μg/mL. Treatment of cancer cells with Mere15 leads to concentration-dependent apoptosis, with overproduction of reactive oxygen species and loss of mitochondrial membrane potential in cells. Studies from our laboratory showed that
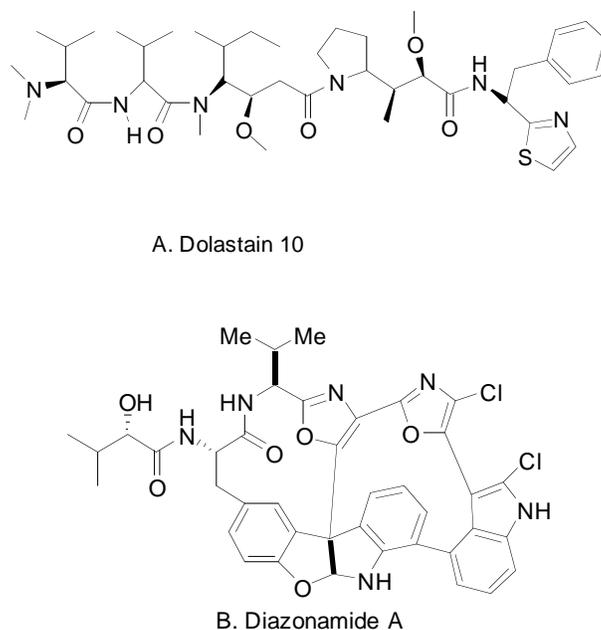

A. Dolastain 10

B. Diazonamide A

Fig. (2). Chemical structures of the peptides derived from marine to affect the equilibrium of tubulin-microtubule: Dolastatin 10 (**A**) and Diazonamide A (**B**).

Mere15 also causes the microtubule cytoskeleton disassembly in human chronic myelogenous leukemia K562 cell lines and inhibits tubulin polymerization in a cell-free system [74]. Further investigations demonstrated that Mere15 is also efficient in the inhibition of the metastasis of cancer cells [75].

4. ANTICANCER PEPTIDES THAT INHIBIT ANGIOGENESIS

Angiogenesis plays a vital role in the carcinogenesis, cell growth, proliferation, invasion and metastasis of cancer. The growth and metastasis of cancer depend on the extension of vasculatures into cancer tissues via angiogenesis. Several growth factors, including vascular endothelial growth factor (VEGF) and receptor (VEGFR) Flk-1 (VEGFR-2, KDR), play a vital role in angiogenesis of cancer and carcinogenesis [76, 77]. Disrupting angiogenesis pathways and downstream of VEGF-VEGFR-2 intracellular signaling affect tumor growth. The angiogenesis pathways include VEGF-induced phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2), the promoter of C-X-C chemokine receptor type 4 (CXCR4), hypoxia inducible factor 1alpha (HIF1α) and serine/threonine protein kinase family protein kinase B (Akt) [78, 79]. The subunit of HIF1 transcription factor HIF1α not only controls various cellular functions under normoxia condition, but also adaptive responses to hypoxia. HIF1α induces the aggregation of VEGF which is very important for endovascular differentiation and cell survival [80, 81].

Mycothiazole (WO2010014240) [18], a central thiazole compound with mixed polyketide / peptide, was obtained from *Petrosaspongia mycofijiensis* marine sponge and the compound suppresses hypoxic HIF1 signaling correlated with the inhibition of the expression of VEGF in cancer cells. Further studies showed that mycothiazole selectively inhibits mitochondrial respiration chain complex I (NADH-ubiquinone oxidoreductase) [82-84].

A novel linear polypeptide CS5931 with strong anti-tumor activity was isolated from *Ciona savignyi* (CS5931) in our laboratory. The effects of CS5931 on antitumor are through significant induction of apoptosis and inhibition of the proliferation in cancer cells (CN102172394) [19]. Mechanistic study revealed that CS5931 could activate caspase-9 and caspase-3 and mediate the release of Cyt C in colon cancer HCT-8 cells [85]. We have increased the production of CS5931 by improved fermentation conditions with response surface methodology [86]. Furthermore, the recombinant CS5931 could significantly block the growth of tumor in nude mice bearing colon cancer HCT-116 xenografts *in vivo* [85]. We also found that CS5931 can inhibit angiogenesis in cancer cells *in vitro* and tumor xenografts *in vivo*. CS5931 can inhibit the formation of capillary-like structures, proliferation and migration in human umbilical vein endothelial cells (HUVECs) in a dose-dependent manner. Moreover, CS5931 inhibited spontaneous angiogenesis in the vessels of zebra fish [87]. Further mechanistic studies revealed that CS5931 can also block the production of VEGF and downregulate the expression of matrix metalloproteinases (MMP-2 and MMP-9) at both levels of mRNA and protein in the HUVECs [87]

5. ANTICANCER PEPTIDES WITH AN UNKNOWN MECHANISM

Although great efforts have been made to develop anti-cancer drugs from marine sources in the last decades, this field of research is still a virgin land. So far, only very fewer peptides have been identified and useful in the clinics compared to those found from other sources. The exact mechanisms of many peptides with anticancer activity are still unknown, which may be due to its complex mode of action. Several peptides with cytotoxic activity against various cancer cells have been separated from ascidians including botryllamides A-D, eusynstyelamide, patellamides Styelin D, cyanobactins, Lissoclinamides and Mollamides [88, 89]. Eusynstyelamide, a dimer peptide found from ascidian *Eusynstyela misakiensis*, was identified as a potent cell cycle inhibitor [90]. Eusynstyelamide B, a novel topoisomerase II poison, is able to induce cell growth inhibition, cell cycle arrest and DNA damage in breast and prostate cancer cells [91].

Sponge is another rich resource from marine, and many peptides have been isolated from sponges including microcionamides A and B, koshikamide B, haligramides milnamide A, halicylindramides, orbiculamide A, phakellistatins, geodiamolides A-G, corticiamide A, taumycins A, koshikamide A1 and A2, as well as theopapuamide etc. Studies have shown that efrapeptin G displays strong cytotoxicity against various cancer cells but the exact targets for its anticancer activity have not yet been elucidated. Phakellistatins, isolated from two Indo-Pacific sponges, *Stylotella aurantium* and *Phakellia costata,* are a group of proline rich cyclic heptapeptides (US5646246) [20, 92, 93]. Phakellistatin 1 exhibits strong inhibition of melanoma and P388 murine leukemia cells [94]. Depsipeptides, a halicylindramides Farnesoid X receptor antagonist, was isolated from the *Petrosia* sp. marine Sponge in Korea [95]. Scopularide A and B [96] andkulokekahilide-2 [97, 98] were found from fungus *Scopulariopsis brevicaulis*, *Cephalaspidean mollusk*, *Phili-*

nopsis speciosa, respectively, and they display anticancer activity but their mechanisms are still unclear.

Apratoxins including Apratoxin H and apratoxin A sulfoxide were isolated from the Red Sea cyanobacterium *Moorea producens* [99]. Report has described the method for total synthesis of apratoxin A and its analogues as well as their biological activity such as anticancer effect [100]. A new effective apratoxin A/E hybrid was designed from systematic chemical mutagenesis and it exhibited improved *in vivo* antitumor activity in a tumor xenograft model of colorectal cancer [101].

Recently, the new cyclic depsipeptides, Kohamamides A, B, and C, belonging to the kulolide superfamily, were isolated from a marine cyanobacterium *Okeania* sp. The structures of Kohamamides were identified by spectroscopic analysis and degradation reaction. Kohamamide B has moderate cytotoxic effect against leukemia HL60 cells [102]. Although many natural products in the kulolide superfamily have been isolated from cyanobacteria around the world, kohamamides A-C are the first members to be identified and isolated from the East Asian marine environment [102]. Furthermore, unlike other members in the kulolide super family, kohamamides A-C have a Leu residue adjacent to the Pro residue not another lipophilic amino acid [102].

6. DISCUSSION

The anticancer peptides derived from marine source discussed along with their mechanistic actions are summarized in here. However, it should be kept in mind that many peptides derived from marine exhibit antitumor activity through multiple targets. For example, Dolastatin 10 not only affects microtubule assembly, but also induces apoptosis accompanying by decreasing the expressions of Bcl-2 and p53 and enhancing in lymphoma cells. The mechanisms of action of aplidine are involved in several signal pathways associated with protein synthesis inhibition and cell cycle arrest, as well as anti-angiogenic activity. Mere15 can induce apoptosis via release of Cyt C, cleavage of procaspase-9, procaspase-3 and poly ADP-ribose polymerase (PARP). The polypeptide is also capable of inducing the disassembly of the microtubule cytoskeleton in human immortalised myelogenous leukemia K562 cells and inhibit the polymerization of tubulin in a cell-free system. Our recent study also showed that CS5931 is able to inhibit angiogenesis via blocking the production of VEGF and downregulation of the expression of matrix metalloproteinases (MMP-2 and MMP-9). It has been well established that the apoptosis is considered a main process in drugs induced cell death. Like other anticancer agents, most marine anticancer peptides induce cell death via apoptotic pathway. Although some of the marine peptides are able to target other pathways, like kinases, angiogenesis and tubulines etc, in most cases these peptides inhibit the growth of cancer cells by apoptosis. Anticancer effects of peptides from venom have been intensively studied, and some of them also inhibit cancer cell growth by targeting multiple signal pathways [103, 104].

CONCLUSION

Marine peptides are able to target different signal pathways of cancer development. The diversity of marine pep-

tides provides a rich source for developing novel anticancer agents. Some of the marine peptides are promising to be developed as novel anticancer agents.

CURRENT & FUTURE DEVELOPMENTS

Studies on the marine peptides for anticancer and other pharmaceutical purposes are still in the stage of infancy. More novel peptides with anticancer activity could be found from the marine organisms with the advancement of modern deep-sea technology. These marine peptides appear to be very useful and promising resource for discovery and development of novel drugs for the treatment of cancer and other diseases clinically. Therefore, it is highly relevant to deepen the study of anticancer mechanisms of marine peptides to develop new candidate compounds. Indeed, further study of the diversity of marine peptides in differences of structure and modes of action will provide a rich resource for the design of very specific and potent new pharmaceuticals.

The anticancer peptides reviewed here were found from different kinds of marine organisms and showed diverse mechanisms for their anticancer activities. Due to the peculiar life of these organisms in the sea, some of these compounds could be found only from a single source. For example, Jaspamide (Jasplakinolide) could be only obtained from marine sponge *Jaspis* species [10-12], and the only source for didemnin B is marine tunicate. Although the underlying mechanism of these anticancer peptides derived from marine is unclear, it is conceivable that the special marine environment offers the diversity of marine natural products to provide unique anticancer peptides with promising clinical value over the anticancer peptides found from other sources.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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