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Neurotoxins and pore forming toxins in sea anemones: Potential candidates for new drug development

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Summary. There are two kinds of toxins in sea anemones: neurotoxins and pore forming toxins. As a representative of the sodium channel toxin, the neurotoxin ATX II in neurotoxin mainly affects the process of action potential and the release of transmitter to affect the inactivation of the sodium channel. As the representatives of potassium channel toxins, BgK and ShK mainly affect the potassium channel current. EqTx and Sticholysins are representative of pore forming toxins, which can form specific ion channels in cell membranes and change the concentration of internal and external ions, eventually causing hemolytic effects. Based on the above mechanism, toxins such as ATX II can also cause toxic effects in tissues and organs such as heart, lung and muscle. As an applied aspect it was shown that sea anemone toxins often have strong toxic effects on tumor cells, induce cancer cells to enter the pathway of apoptosis, and can also bind to monoclonal antibodies or directly inhibit relevant channels for the treatment of autoimmune diseases.

Key words: Sodium channel toxins, Potassium channel toxins, Cytolysin, Organs, Diseases

Introduction

Most of the members of the cnidarian of sea anemones have special stinging organelles, which are used to capture prey. In the process of catching prey, stinging animals will release spiny organelles, cnidarians that penetrate the prey's skin, and release toxins, which result in the loss of resistance (Bosmans and Tytgat, 2007). As a representative of stinging animals, sea anemones are closely related to human life. Some sea anemone toxins can cause strong inflammatory responses (Anderluh and Macek, 2002; Honma and Shiomi, 2006). As one of the most toxic marine animals on earth, sea anemone toxins have similar effects than other animal toxins such as scorpion toxin and tetrodotoxin (Catterall and Beress, 1978; Molgo et al., 1986). However, there are more kinds of sea anemone toxins and more affected organs deserving attention.

There are several toxins in sea anemones, among which neurotoxins and cytolysins are the most thoroughly studied. Among them, neurotoxins are mainly sodium channel toxins represented by ATX II and potassium channel toxins represented by BgK and ShK. The toxins of these two channels can substantially affect the action potential of nerve endings by affecting the state of the channel (Bergman et al., 1976; Castaneda et al., 1995; Cotton et al., 1997). Sodium channel toxins such as ATX II can also cause the increase of the second messenger cGMP and cAMP to affect the downstream pathway (Ahnert et al., 1979). Toxins such as ATX II and BgK can also directly affect the release of acetylcholine from the presynaptic membrane, and also affect the action potential of cardiomyocytes (Metezeau et al., 1979; Boutjdir and El-Sherif, 1991; Aneiros et al., 1993; Salinas et al., 1997). Most of cytolysin toxins in cytolysin have been fully characterized, the amino acid sequences of basic toxins in these article have been sequenced (Kem and Dunn, 1988; Macek and Lebez, 1988). The structure of some toxins such as cytolysin EqTx has also been clarified (Belmonte et al., 1994; Athanasiadis et al., 2001), and the pore forming mechanism has also been studied thoroughly. However they are not included in this review due to page number restrictions. Cytolysin often causes cell lysis or calcium influx and protein leakage in the membranes by forming holes in the cell membrane (Rios et al., 1998; Frangez et al., 2000; Meunier et al., 2000). Toxins such as EqTx can also cause hemolysis and platelet aggregation (Teng et al., 1988; Bunc et al., 1994). In recent years, the function of sea anemone toxins has also been comprehensively studied. In addition to affecting the heart, lung and other organs in different ways, neurotoxins such as ShK and its analogues may become effective drugs for the treatment of autoimmune diseases by affecting the potassium channel (Pennington et al.,

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1995; Kem et al., 1999; Wulff et al., 2003). Moreover, the sea anemone toxin can also effectively kill tumor cells, induce cancer cells to enter the stage of apoptosis (Ramezanpour et al., 2014b; Abdzadeh et al., 2020), affect the cell cycle (Ramezanpour et al., 2014a), and have synergistic effects with various anticancer drugs (Soletti et al., 2008), which also provides the direction for cancer treatment in the future. In this paper, we reviewed the mechanism of tumor and the potential function of several toxins, and paid less attention to the self-expression of malignant diseases. The structures of related toxins have been reviewed and introduced in detail (Honma and Shiomi, 2006).

Mechanism of neurotoxins affecting sodium and potassium channels

Sodium channel toxin can slow down channel inactivation and affect the release of transmitters

Origin of the toxin

At present, the relatively comprehensive toxins studied mainly include ATX II in ATX from anemone *Anemonia sulcata* and Anthopleurin A and Anthopleurin B in *Anthopleurin xanthogrammica* (ApA and ApB for short). This review discusses the main functions of the three toxins. ATX II is a polypeptide isolated from sea anemone *Anemonia sulcata*. It has been isolated from sea anemone through a series of chemical methods (Beress et al., 1975a). Sequencing (Beress and Beress, 1971; Beress et al., 1975a,b; Wunderer et al., 1976) and toxin lethal dose detection (Beress et al., 1975a) were carried out by the Edman method. There is no reported homology between ATX and other neurotoxin residues (Wunderer et al., 1976), indicating that it may be composed of 47 amino acids (Wunderer et al., 1976). ApA and ApB are high-efficiency cardiotonic polypeptides isolated by chromatography from anemone Antholeura xanthogrammica (Norton et al., 1976; Norton, 1981), and their amino acid sequences were determined (Tanaka et al., 1977; Reimer et al., 1985). ApA is a single chain composed of 49 amino acid residues (Tanaka et al., 1977), and ApB is also a single chain composed of 49 amino acid residues (Tanaka et al., 1977), which is different from ApA in 7 amino acid positions, these differences are also important reasons for the different effects of the two toxins (Reimer et al., 1985). Other studies have shown that Leu-18 and Arg-14 rings and nearby residues in ApB residues have an important contribution to the high affinity of toxin (Dias-Kadambi et al., 1996; Seibert et al., 2004), while Lys-48 mainly plays a key role in toxin recognition (Khera and Blumenthal, 1996). The difference of affinity between ApA and ApB toxins is not only related to the types of residues, but also related to their dissociation constants (Benzinger et al., 1997). There are also relevant studies on the role of many charged residues and residues interacting with sodium channels (Kelso et al., 1996; Khera and Blumenthal, 1996).

Effect on neurotransmitters

Early studies have shown that ATX II can promote the release of neurotransmitters like acetylcholine (Metezeau et al., 1979). The half maximum effect of the toxin on synaptosomes was reported to be $K_{0.5}$=0. 02pM (Abita et al., 1977). On this basis, the effect of ATX II on transmitter release was further studied to distinguish the effect of toxin on presynaptic membrane and postsynaptic membrane. In the experiment, it was found that the amplitude of EPP and EPC increased significantly after a few minutes of toxin application. Further observation found that the increase of EPP amplitude was due to the effect of the toxin on the presynaptic membrane, which was not related to the postsynaptic membrane (Erxleben and Rathmayer, 1984). Relevant experiments also show that ATX II can increase the quantum content of the repeated endplate potential and phase endplate potential by 3-4 times (Molgo and Mallart, 1985) as is shown in Fig. 1, and can also increase the frequency of MEPP, but the effect of toxin is significantly weakened when the toxin is exposed to a sodium deficient environment (Molgo et al., 1986). In this process, TTX can significantly inhibit the action of ATX II (Abita et al., 1977; Molgo et al., 1986). A cytolytic fraction from the sea anemone *Bunodosoma caissarum* called Bc2 was found to promote the release of glutamate from synaptosomes in a dose-dependent manner, which is independent of extracellular calcium ions, but Bc2 has no effect on sodium channels (Migues et al., 1999).

Effect on action potential

Since ATX II was found and successfully isolated, it has been found that the toxin can affect the sodium current and potassium current, and it also indicates that ATX II acts on the relevant receptor sites of sodium conductance (Bergman et al., 1976). Subsequent studies showed that ATX II could rapidly reduce the inactivation rate of sodium conductance. Through kinetic studies, it was found that the toxin played a role by changing the energy distribution between the open configuration and the closed configuration of the channel (Bergman et al., 1976). In addition, the main effect of ATX II on sodium channels is that it can prolong the opening time of activated sodium channels (Ahnert et al., 1979; Habermann and Beress, 1979; Hartung and Rathmayer, 1985; Nagy, 1987), and slow down the inactivation of sodium current (Erxleben and Rathmayer, 1984; Nagy, 1988; Boutjdir and El-Sherif, 1991). Beside that different sodium channel subtypes can also have different responses to ATX II (Oliveira et al., 2004; Snape et al., 2010; Klinger et al., 2012). Kinetic studies showed that ATX II modified some sodium channels in an all or no manner (Neumcke et al., 1980). The effect of ATX II on sodium channel is intuitively reflected in the

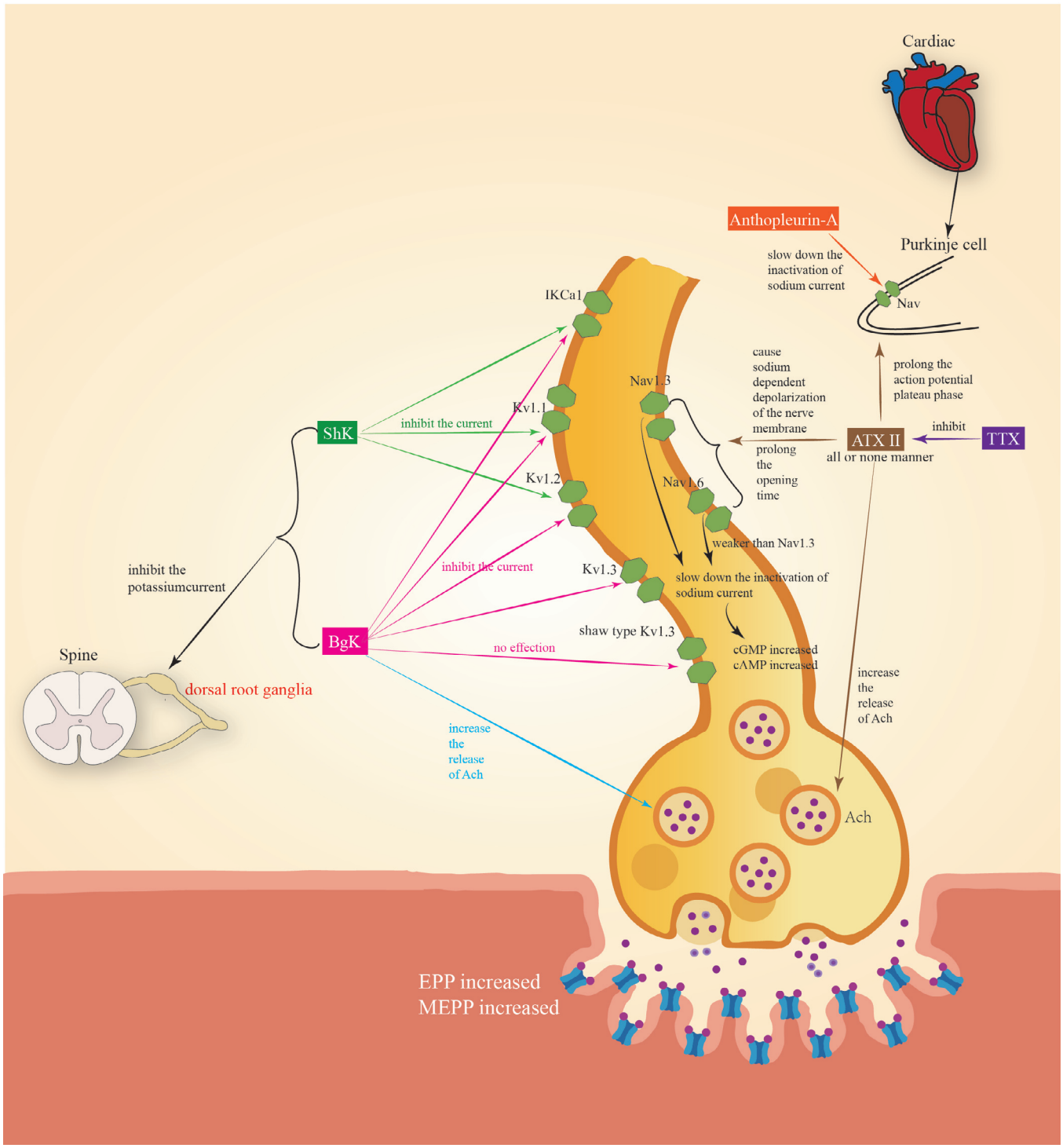

Fig. 1. Effect of anemone neurotoxin on excitable cells. Toxin ATX II has an effect on axon terminals and presynaptic membrane. It can selectively affect sodium channel subtypes, cause sodium ion dependent depolarization in nerve terminals, prolong the opening time of sodium channel, and increase the content of intracellular second signals such as cGMP and cAMP by slowing down sodium ion current. ATX II can also increase the content of acetylcholine released from the protrusion anterior membrane, resulting in the increase of EPP and MEPP in the postsynaptic membrane. In cardiomyocytes, ATX II can also prolong the action potential plateau of Purkinje cells. Anthopleurin-a, a toxin from another sea anemone, can also affect the action potential of cardiomyocytes, which can slow down the inactivation of sodium current. In addition, Anthopleurin-A can also affect the action potential of axons. The toxins ShK and BgK that affect potassium channels can specifically recognize potassium channel subtypes and inhibit the corresponding potassium currents. The potassium subtypes inhibited by them are different, but both can inhibit IKCa 1 channel currents and potassium currents in dorsal root ganglia. Similar to ATX II, BgK can also increase the content of acetylcholine released from presynaptic membrane.

change of action potential and the change of the second messenger such as cGMP content (Ahnert et al., 1979) as is shown in Fig. 1. First, the use of ATX II can cause sodium dependent depolarization of the nerve terminal membrane (Harris and Tesseraux, 1984; Boutjdir and El-Sherif, 1991), followed by an extension of the duration of action potentials (Schmidtmayer et al., 1982; Chang et al., 1983; Isenberg and Ravens, 1984), which process is reversible (Schmidtmayer et al., 1982). It can also significantly prolong the action potential plateau phase in cardiac muscle cell, which has been well studied in Purkinje cells (Boutjdir and El-Sherif, 1991). Compared with action potential, stretch induced receptor potential and resting membrane potential were not significantly affected by ATX II (Rathmayer, 1979; Isenberg and Ravens, 1984). Secondly, because ATX II will keep the activated sodium channel open, some studies have shown that in mouse brain slices, after using ATX II, the content of cGMP increased about 35 times and the content of cAMP doubled, which degree is greater than that of other known toxins (Ahnert et al., 1979). Experiments show that if ATX II was injected into the ventricle of mice, it would also produce convulsions and excessive excitement (Habermann and Beress, 1979). Studies in myelinated nerve fibers of frogs also show that ATX II can reversibly prolong the duration of nerve action potential by partially inactivating the biphasic activity of sodium channels (Ohizumi and Shibata, 1982).

Compared with the effect of ATX II in both heart and muscle, Anthopleurin-A (ApA) from *Anthopleura xanthogrammica* mainly plays a role in the heart and is an effective cardiotonic polypeptide (Norton et al., 1976; Norton, 1981). In a certain concentration range, ApA can modify the sodium channel in myocardium and slow down the inactivation of Na current (Wasserstrom et al., 1993), but this modification is selective (Hanck and Sheets, 1995). The toxin can also prolong the duration of action potential and refractory period of cardiac Purkinje cells (Shimizu et al., 1979). However, APA can also play a role in nerve axons. Experiments on the axons of crayfish show that APA can also affect the resting potential, action potential and membrane current of axons (Pa et al., 1979).

Potassium channel toxins can specifically inhibit potassium channels and key residues

There are many potassium channel toxins isolated from sea anemone, which are basically included in the Table 1, but the toxins which are well studied are mainly ShK from sea anemone *Stichodactyla helianthus* and BgK from sea anemone *Bunodosoma granulifera*. BgK is a kind of toxin that affects potassium channel isolated from sea anemone *Bunodosoma granulifera* (Aneiros et al., 1993). Sequencing and homology comparison show that it is a new potassium channel toxin (Aneiros et al., 1993). BgK can promote the release of acetylcholine at the neuromuscular junction of birds, experiments in rats found that the toxin can also inhibit the potassium current in dorsal root ganglia (Aneiros et al., 1993). In a follow-up study, in addition to modifying the sequence of the toxin, it was also found that BgK can distinguish different subtypes of potassium channels (Cotton et al., 1997). In Xenopus oocytes, BgK was found to inhibit Kv1. 1-1. 3 current but does not affect Shaw type Kv3. 1 current to support the above view (Cotton et al., 1997). BgK also has an effect on cardiomyocytes, which will be reflected in the subsequent discussion (Salinas et al., 1997).

After BgK was isolated and studied, some researchers isolated the toxin ShK from the sea anemone *Stichodactyla helianthus* to study whether it is similar to BgK (Castaneda et al., 1995). After ShK was separated and sequenced, all its amino acid residues and positions were obtained, which proved that it was a new potassium channel toxin (Castaneda et al., 1995). After the 27th amino acid Phe was changed to Ala, the potency decreased significantly, and the potency of using other substitutes also decreased, indicating its importance (Pennington et al., 1996a). It was found that ShK could also inhibit the potassium current of rat dorsal root neurons, and after reaching a certain concentration, ShK could promote the convulsive response of chick biventer cervicis preparations, but had no effect on acetylcholine and carbachol (Castaneda et al., 1995). In the follow-up study, it was found that ShK mainly acts on Kv1. 3 and Kv1. 2 channels (Pennington et al., 1995, 1996a,b) as is shown in Fig. 1. It was found that ShK can inhibit Kv1. 3 channal in Jurkat T lymphocytes at a very low concentration (Pennington et al., 1996b). The effect of this channel is concentration dependent, and Arg11 in this toxin plays a major role in this process (Pennington et al., 1996b), which provides a new direction for the development of immune drugs (Pennington et al., 1995; Kem et al., 1999). In addition, ShK is a potassium channel blocker independent of channel opening (Pennington et al., 1995). The study of the interaction between toxin and potassium channel shows that Lys9 has a great effect on the blocking effect, while the monosubstituted of ShK analogues at other non cysteine positions have a great effect on Kv1. 2 and Kv1. 3, which blocking effect of these channels is basically similar (Pennington et al., 1996b). Subsequent studies also showed that Lys22 and tyr23, two conserved residues of ShK, are very key to the interaction with potassium ion channels (Kem et al., 1999).

The study also found that both ShK and BgK can block the Ca^{2+}-activated potassium channel IKCa1, but for ShK, blocking Kv1. 3 channel is more effective than blocking IKCa1 channel (Rauer et al., 1999). In this process, ShK has eight essential residues, namely Arg11, His19, Ser20, Met21, Lys22, Tyr23, Arg24 and Phe27, while BgK has only five essential residues, namely His19, Ser20, Lys22, Tyr23 and Arg24 (Rauer et al., 1999).

Table 1. Chemical composition and toxic effect of sea anemone toxin.

Name	Chemical essence	Species	Function	Author
ATX II	It contains 44 amino acids with a molecular weight of 4197. It is composed of 47 amino acid residues, which are interconnected by three disulfide bonds	*Anemonia sulcata*	Change the inactivation rate of sodium conductance, prolong the opening time of sodium channel, act on presynaptic membrane and cause the release of transmitters. It has different effects on different sodium channel subtypes, has positive inotropic effect in muscle and heart, can also prolong the isometric contraction of muscle fibers, increase the resting tension of muscle and induce early depolarization in heart	Beress et al., 1975a; Alsen et al., 1976; Bergman et al., 1976; Ravens, 1976; Wunderer et al., 1976; Ahnert et al., 1979; Alsen et al., 1981; Schmidtmayer et al., 1982; Erxleben and Rathmayer, 1984; Khan et al., 1986; Hoey et al., 1994; Snape et al., 2010
ATX III	The polypeptide contains 27 amino acids and three disulfide bonds. Rich in aromatic amino acid residues, Eight amino acids are not contained: Met, Leu, Ile, Phe, Thr, His, Asp and Ala	*Anemonia sulcata*	Slow down the inactivation of sodium channels	Beress et al., 1975a; Beress et al., 1977; Martinez et al., 1977; Warashina et al., 1988
Anthopleurin-A	A soda ash polypeptide with a molecular weight of about 5500, consisting of a single chain composed of 49 amino acid residues	*Anthopleura xanthogrammica*	Slowing down the inactivation of sodium channels has little effect on the activation and inactivation of channels. Changing the self discharge rate and causing positive inotropic effect in cardiomyocytes can also enhance the contractility of some muscles.	Norton et al., 1976; Tanaka et al., 1977; Shibata et al., 1978; Pa et al., 1979; Scriabine et al., 1979; Shimizu et al., 1979
Anthopleurin-B	A single chain polypeptide composed of 49 amino acid residues. The amino acid sequence is different from anthopleurin-a in seven positions: residue 3 (Pro for Ser), 12 (Arg for Ser), 13 (Pro for Val), 21 (Ile for Thr), 24 (Phe for Leu), 42 (Asn for Thr) and 49 (Lys for Gln).	*Anthopleura xanthogrammica*	At a certain concentration, it will lead to its relaxation in the isolated ileum of guinea pigs. It can also cause the contraction of the isolated vas deferens and increase the content of Na released by the vas deferens in vitro	Norton, 1981; Norton et al., 1981; Ohizumi and Shibata, 1981; Ohizumi and Shibata, 1982; Reimer et al., 1985
Anthopleurin-C	Polypeptide	*Anthopleura elegantissima*	The duration of action potential was increased and the voltage dependence of sodium channel inactivation was reduced	Norton, 1981; Salceda et al., 2006
Bg II; Bg III	Asparagine at position 16 in Bg II is replaced by aspartic acid in BgIII	*Bunodosoma granulifera*	The effect of voltage-gated sodium channel is similar to that of ATX II, but the effect is different. It will prolong the action potential and change the resting potential. The effect of BG II is stronger than that of BG III, which reduces the selectivity of sodium channel.	Goudet et al., 2001; Bosmans et al., 2002; Salceda et al., 2002
AFT-I AFT-II	AFT-I and AFT-II are composed of 47 and 48 amino acid residues with three disulfide bonds.	*AntleopltJUV jwroviridis*		Sunahara et al., 1987
Ae I	Polypeptide, rich in Gly, lacking Glu and Ala, containing 54 amino acid residues	*Actinia equina*	It has lethal activity to crabs	Lin et al., 1996
RpI; RpII RpIII; RpIV	A polypeptide consisting of 48 or 49 amino acids; The sequence of RpII has been determined.	*Radianthus paumotensis*	It acts on the sodium channel and slows down the inactivation process of the channel	Schweitz et al., 1985
PaTX	The polypeptide is composed of 31 amino acid residues and cross-linked with four disulfide bridges.	*Parasicyonis actinostoloides*	It acts on sodium channel and slows down the inactivation process of channel. Processing for a long time will cause the channel to close abnormally.	Warashina and Fujita, 1983; Nishida et al., 1985
GRX	Polypeptide, no cysteine residues in some sequences	*Bunodosoma granulifera*	It has serious neurotoxic effects in mice	Santana et al., 1998
Bc-III	Polypeptide. Has great homology with ATX II	*Bunodosoma caissarum*	Induce sustained activation of sodium channel subtypes 1. 3 and 1. 6.	Oliveira et al., 2004
HTX-I	Polypeptide	*Homostichanthus duerdemi*	It can delay the inactivation of TTX sensitive sodium current, and the effect is reversible in a short time	Kryshtal et al., 1982
RTX-VI	Polypeptide composed of two sulfur free peptide chains. Arg-13 is not included	*Heteractis crispa*	A neurotoxin	Kalina et al., 2020
ShK	Polypeptide	*Stichodactyla helianthus*	It can inhibit the potassium current in rat dorsal root ganglion and block Kv1. 3 channel in jurkat T lymphocytes at low concentration, which is mainly through the combination of Kv1. 2 channels works. It can also block IKCa1 channel.	Castaneda et al., 1995; Pennington et al., 1995; Pennington et al., 1996a; Rauer et al., 1999

Table 1. Continued.

BgK	Polypeptide, C-terminal tetrapeptide (34-37) is Cys Glu Leu Cys	*Bunodosoma granulifera*	Can inhibit Kv1 expressed in Xenopus oocytes 1、Kv1. 2 and Kv1 3 current without affecting Shaw Kv3 1 current. It can promote the release of acetylcholine at the neuromuscular junction of birds. In a certain concentration range, it can enhance the myocardial contractility of rats, and has a positive inotropic effect on cardiac multicellular preparation.	Aneiros et al., 1993; Cotton et al., 1997; Salinas et al., 1997; Rauer et al., 1999
HmK	Composed of 35 amino acid residues include 6 cysteine residues. The molecular weight is 4055	*Heteracitis magnifica*	Reduce the Current of Kv1. 2 channel	Gendeh et al., 1997
AsKs AsKC	Polypeptide	*Anemonia sulcata*	Inhibit K1 channel activity. AsKC has inhibitory effect on trypsin, but AsKS has no inhibitory effect on it.	Schweitz et al., 1995
Ate1a	A 17 residue peptide with two disulfide bonds and one amidated C-terminal. The single isotope mass is 1887. 93 da	*Actinia tenebrosa*	It is an effective shaker type Kv channel inhibitor with nano molar titers to Kv1. 1, Kv1. 2 and Kv1. 6.	Madio et al., 2018
EqTx I	Polypeptide containing 158 amino acids	*Actinia equina*		Macek and Lebez, 1988
EqTx II	Polypeptide, contains about 29-33% of the α Spiral structure, 53-58% β folding chain and β Corner and 10-16% random structure		It can cause hemolysis, platelet aggregation and dissolution, form holes in the cell membrane and produce calcium influx, but it will be inhibited by sphingomyelin. It has cardiotoxic effect in the heart and can cause arrhythmia and other phenomena, accompanied by respiratory arrest. It can also affect the permeability of pulmonary vessels and increase resting tension. It also has toxic effects on some tumor cells.	Sket et al., 1974; Giraldi et al., 1976; Macek and Lebez, 1981; Lafranconi et al., 1984; Macek and Lebez, 1988; Teng et al., 1988; Zorec et al., 1990; Belmonte et al., 1994; Bunc et al., 1994; Horvat-Znidarsic and Suput, 1996; Anderluh et al., 1999; Athanasiadis et al., 2001
EqTx III	Polypeptide containing 159 amino acids		The toxins can cause arrhythmias, usually accompanied by respiratory arrest, and lead to platelet aggregation and degranulation. Increase the tension of smooth muscle	Bunc et al., 1994; Macek and Lebez, 1988; Suput et al., 2001; Anderluh et al., 1999
EqTx IV	Polypeptide		Sphingomyelin can inhibit it	Anderluh et al., 1999
EqTx V	The sequence of mature toxin is preceded by a 19 amino acid signal peptide and a 16 amino acid hydrophilic pre peptide, and its end is a pair of basic residues		Sphingomyelin can inhibit it	Pungercar et al., 1997; Anderluh et al., 1999
Sticholysin I	There are more Gly, Lys, Tyr and many non-polar amino acids in the amino acid sequence, but there is no Cys. The molecular weight is 19401da	*Stichodactyla helianthus*	Sphingomyelin promotes the interaction between toxin and monolayer membrane and increases permeability. The size of pores formed in the film is basically constant.	Devlin, 1974; Bernheimer and Avigad, 1976; Kem and Dunn, 1988; Tejuca et al., 2001; Valcarcel et al., 2001
Sticholysin II	There are more Gly, Lys, Tyr and many non-polar amino acids in the amino acid sequence, but there is no Cys. The molecular weight is 19290da		The size of pores formed in the film is basically constant. It has hemolytic effect.	Devlin, 1974; Bernheimer and Avigad, 1976; Kem and Dunn, 1988; Lanio et al., 2001; Tejuca et al., 2001; Valcarcel et al., 2001
Sticholysin III	Polypeptide with 153 amino acids lacking cysteine		The hemolytic activity of the toxin depends entirely on the extracellular pH value	Devlin, 1974; Bernheimer and Avigad, 1976; Blumenthal and Kem, 1983; Kem and Dunn, 1988; Doyle and Kem, 1989; Lanio et al., 2001
HMgs	Polypeptide, without Cys	*Heteracitis magnifica*	It has cytolytic activity and can inhibit the binding of brain synaptosomes to GABA and choline.	Khoo et al., 1993, 1995; Wang et al., 2000, 2008
PsTX-60A	The molecular weight is about 60 kDa. N-terminal sequence is clarified	*Phyllodiscus semoni*	It can cause hemolysis of sheep red blood cells	Nagai et al., 2002b
PsTX-60B	The molecular weight is about 60 kDa, The N-terminal sequence is clarified			
PsTX-20A	Polypeptide			Nagai et al., 2002a
PsTX-T	Polypeptide, sequenced		Damage the kidney and destroy the glomerulus and stroma	Mizuno et al., 2007
Gigantoxin I Gigantoxin II Gigantoxin III	Polypeptide, sequenced	*Stichodactyla gigantea*	Effect on A431 cell line	Shiomi et al., 2003

Toxic effects of sea anemone toxin at cell level and organ level

Pore forming toxins exhibit cytotoxicity by forming pores

In addition to the above neurotoxins, another large part of the toxin isolated from sea anemone is cytolysin. Compared with neurotoxins acting on ion channels to show toxicity, cytolysin usually shows toxicity by forming holes on the surface of cell membrane. This chapter mainly discusses several well studied cytolysins found in sea anemones. So far, the sea anemone cytolysins that have been found and studied have been mostly listed in the table below. The cytolysins discussed in this chapter mainly include the following: Equinatoxin and its subtype Equinatoxin II from sea anemone *Actinia equina*, Sticholysins and its subtype from sea anemone *Stoichactis helianthus*, and other toxins.

The most thoroughly studied toxin is Equinatoxin (short for EqTx) isolated from the sea anemone *Actinia equina*. Early studies have proved that EqTx is a protein with properties similar to those toxins existing in snakes through some methods like enzyme digestion and heat inactivation (Ferlan and Lebez, 1974). At present, EqTx II has been reviewed and discussed (Frangez et al., 2017). Further separation of EqTx in follow-up study found that the number of amino acids of EqTx I-III had

Table 1. Continued.

Gigantoxin-4			It has strong hemolytic activity. It can cause hypotension and bradycardia in the heart, as well as liver and lung injury.	Hu et al., 2011
tenebrosin-A	Polypeptide, sequenced, containing 186 residues, Mr 20000 Da, lack of Cys	*Actinia tenebrosa*	It can produce positive inotropic effect in guinea pig atrium. It has hemolytic activity.	Thomson et al., 1987; Norton et al., 1990
tenebrosin-B	Polypeptide, sequenced, lacking Cys		It can produce positive inotropic effect in guinea pig atrium. It has hemolytic activity.	Norton et al., 1990
tenebrosin-C	Polypeptide, sequenced, lacking Cys		It has hemolytic activity and will be inhibited by sphingomyelin. It can produce positive inotropic effect in guinea pig atrium.	Galettis and Norton, 1990; Norton et al., 1990; Simpson et al., 1990
bandaporin	Polypeptide containing 49 amino acids	*Anthopleura asiatica*	It has hemolytic activity and inhibited by sphingomyelin.	Kohno et al., 2009
Avt120	Polypeptide containing 8 Cys	*Actineria villosa*	High ATP degradation activity.	Uechi et al., 2011
Avt I	Polypeptide, Mr19 kDa, isoelectric point of 9. 2, contains 226 amino acids.	*Actineria villosa*	It has hemolytic activity and inhibited by sphingomyelin.	Uechi et al., 2005a,b
Bcg-3	Polypeptide	*Bunodosoma cangicum*	Induce hemolysis in a dose-dependent manner. Prevent the combination of TTX and sodium channels.	Lagos et al., 2001
RTX-A	Polypeptide	*Heteroactis crispa*	Has obvious cytotoxic effect on several human cancer cell lines	Fedorov et al., 2010
RTX-S II	Polypeptide containing 177 amino acids	*Radianthus macrodactylus*	Cytotoxicity and hemolytic activity	Klyshko et al., 2004
Or-A	The polypeptide has a mass of 15353. 7 DA and a molecular weight of 18 kDa	*Oulactis orientalis*	It has low cytotoxicity and hemolytic activity	Ilina et al., 2005
Or-G	The polypeptide has a mass of 16035. 9da and a molecular weight of 18kDa			
Condylactis toxin	Polypeptide	*Ccmdylactis gigantea*	hemolytic activity, human platelets are relatively resistant, inhibited by sphingomyelin.	Bernheimer et al., 1982
parasitoxin	The total number of amino acid residues is 160 and the molecular weight is 17200	*Parasicyonis actinastoloides*	Hemolytic activity	Shiomi et al., 1985
epiactins A epiactins B epiactins C	Polypeptides, sequenced, lack Pro and Met	*Epiactis prolifera*		Bernheimer and Avigad, 1982
keratin	A polypeptide with a molecular weight of about 18000	*Stolchactis kenti*	Hemolytic activity, inhibited by sphingomyelin.	Bernheimer and Lai, 1985
variolysin	Polypeptide, protein with molecular weight of 19500 and isoelectric pH value of 9. 8, lacking methionine and cysteine	*Psaufartinia vans*	Hemolytic activity, inhibited by sphingomyelin.	Bernheimer et al., 1984
Bc2	Polypeptide	*Bunodosoma caissarum*	Promote the release of glutamate from synaptosomes in a dose-dependent manner	Migues et al., 1999

little difference, and only the content of methionine had a significant difference (Macek and Lebez, 1988). The most thoroughly studied toxin EqTx II has been sequenced and the secondary structure has been estimated (Belmonte et al., 1994). The study shows that the toxin contains about 29-33% of the α Spiral structure, 53-58% β folding chain and β Corner and there is also 10-16% random structure (Belmonte et al., 1994). Relevant studies also show that EqTx II was measured its crystal structure of soluble form at 1. 9 A˚. EqTx II is a 12 chain based β three dimensional folded form with hydrophobic core and a pair of α Spirals, each associated with the surface of the sheet (Athanasiadis et al., 2001). In order to study the cytolytic activity and cytotoxicity of the toxin, EqTx II has been cloned and expressed in *E. coli* to further study its function (Anderluh et al., 1996). In addition, the study on the amino acid residues of EqTx II found that Arg and Tyr residues play a key role in the hemolytic activity of the toxin (Turk et al., 1989), while Trp residues play a key role in the natural conformation of the toxin (Turk et al., 1992). At the same time, it was also found that the hemolytic activity of some mutant toxins such as EqtIIK77C, EqtIIS54C and EqtIIH67C changed significantly, indicating that the region of S54C, H67C and K77C play an important role in the hemolytic activity of natural toxins (Anderluh et al., 2000). In addition, the hemolytic activity of EqtIIK77C increased significantly, which was found to be related to the positive and negative charge at position 77 (Anderluh et al., 2000). The type of cysteine side chain of this mutant also determined the pore forming activity of the toxin (Anderluh et al., 2000).

In the study of pore forming toxins, relevant reviews have discussed the main mechanism of pore forming in detail (Kristan et al., 2009). When studying the cytolytic activity of the toxin EqTx, it was found that the hemolytic activity of the toxin was significantly enhanced in the presence of calcium ion, but the hemolytic activity of the toxin was significantly inhibited in the presence of lithium ion (Macek and Lebez, 1981). Further experiments using EqTx II isolated from EqTx also showed that EqTx II caused calcium influx through the formation of cation channels in the planar lipid bilayer membrane, so as to play a cytotoxic role and cause cell morphological changes, such as swelling of neuroblastoma cells (Frangez et al., 2000; Meunier et al., 2000) as is shown in Fig. 2, which is closely related to the increase of calcium activity in cell solution (Zorec et al., 1990). In the study of artificial lipid membrane and human red blood cells, through the change of conductivity after the use of toxin, it was found that the cytolysis caused by toxin was the result of the channel, which the toxins entered the cells. Toxin activity was also closely related to the pH value of the solution (Belmonte et al., 1993). EqTx II can also affect the fluidity of the membrane, specifically reducing the high fluidity area and increasing the low fluidity area (Sentjurc et al., 1996). More studies have revealed that there is lipid in the channel of toxin formation (Anderluh et al., 2003), and the interaction between toxin and cell membrane is also related to the components of the cell membrane (Schon et al., 2008). Between the liquid disordered phase and the liquid ordered lipid phase rich in SM, EqTx II preferentially combines with the former, where SM plays a synergistic role in the combination of EqTx II and membrane (Schon et al., 2008). When EqTx II was applied to V-79-379A cell line, it was also found that the toxin could significantly cause cell lysis and had significant cytotoxic effect on cells (Batista et al., 1986), which further confirmed the pore forming effect of the toxin (Batista et al., 1987). Subsequently, the interaction between toxin and the process of cell exocytosis was well studied (Suput, 1994).

Another major function of cytolysin was to cause hemolysis in tissues. The toxin EqTx can cause rabbit platelet aggregation in a dose-dependent manner and is an effective aggregation inducer. However, the whole aggregation process does not involve or change the formation of thromboxane B2 and is not inhibited by heparin, which also shows that the aggregation caused by the toxin is different from the classical platelet aggregation pathway (Teng et al., 1988). The remaining unaggregated platelets are enlarged and degranulated, the number of neutrophils in the blood is reduced, and basophils are almost absent. However, the specific mechanism is not clear (Bunc et al., 1994) as is shown in Fig. 2. Using the toxin EqTx II, it was found that the lethality of the toxin was related to the effects of the toxin on organs and blood (Bunc et al., 2000).

Compared with EqTx, studies related to the toxin Sticholysins from the sea anemone *Stoichactis helianthus* and its subtype Sticholysins I-III (StI, StII and StIII for short) mainly focus on the role of toxins in the cell membrane and the structure of pore formation. Sticholysin is a hemolytic toxin in the sea anemone *Stoichactis helianthus*, where there are four subtypes, sticholysin I-IV (Kem and Dunn, 1988). The first purified part of the toxin was obtained by gel electrophoresis and its hemolytic activity was measured in red blood cells of sheep (Devlin, 1974). Sequencing showed that the amino acid sequences of toxin I-III were similar, and the molecular size was between 17400 and 18200, while toxin IV was larger, about 19600. The isoelectric points of the four toxins were greater than or equal to 9 (Kem and Dunn, 1988). However, in subsequent studies, it was found that the sequences of StII and StIII were similar, the newly found peptide segments in StII showed that StII and StIII were actually the same molecule (Lanio et al., 2001). However, this paper still discusses the two separately in the follow-up discussion. After the toxin was separated, the molecular weights of the two toxins were obtained by electrophoresis, chromatography and sequencing analysis. StI was 19401 Da and StII was 19290 Da (Lanio et al., 2001). In addition, the amino acid positions of STI and StII were also obtained. The data showed that both toxins were rich in Gly, Lys, Tyr and non-polar

amino acids such as Ala, Leu and Val (Lanio et al., 2001). StIII was sequenced and its amino acid sequence was obtained (Blumenthal and Kem, 1983). The data showed that StIII lacked cysteine (Blumenthal and Kem, 1983). The primary structure was determined by the Edman degradation method and the secondary structure was predicted (Blumenthal and Kem, 1983).

Before the separation of STI and StII, experiments were carried out with the hemolytic toxin sticholysin. It was found that sphingomyelin can inhibit the sticholysin (Linder et al., 1977). It was also found that sphingomyelin inhibited the ability of sticholysin to lyse rabbit red blood cells, while other types of phospholipids had no such inhibitory effect, and the inhibitory effect changed with different animal species (Bernheimer and Avigad, 1976). After adding toxin to the lipid bilayer membrane, it can be found that the conductivity of the lipid bilayer membrane increases significantly, suggesting the emergence of channel (Michaels, 1979) as is shown in Fig. 2. In subsequent experiments with liposomes, it was found that the toxin only damaged the surface of the membrane (Shin et al., 1979). In planar lipid bilayers, the sensitivity of the membrane to toxins is affected by sphingomyelin, which can increase the sensitivity in the presence of sphingomyelin, and the toxin can also cause the increase of lipid dependent

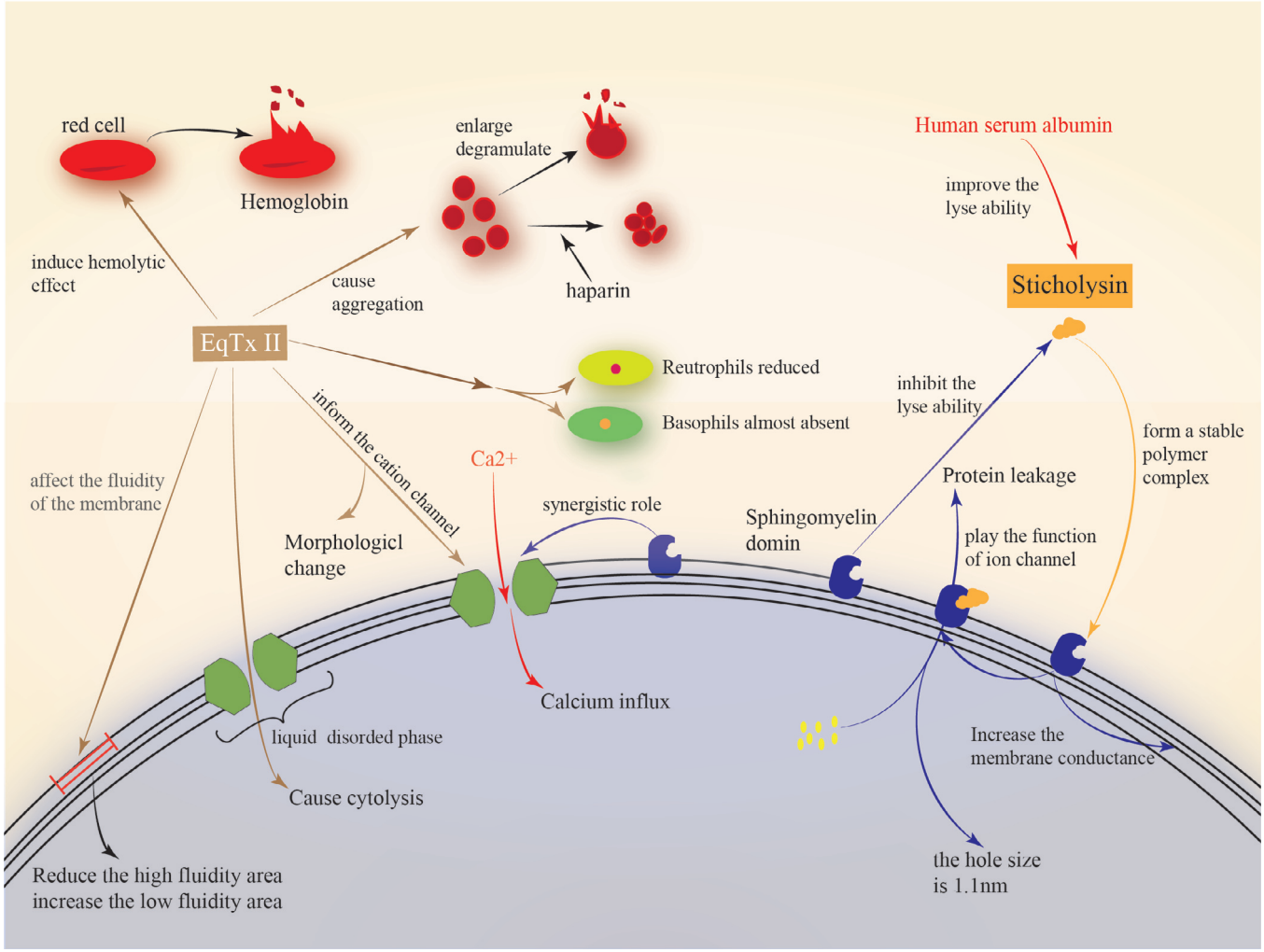

Fig. 2. Effect of anemone cytolysin on cells. The pore forming mechanism and effect of toxin EqTx II and toxin Sticholysin are different. The former will form pores in the liquid disordered phase of the cell membrane, and sphingomyelin will play a synergistic role, while the latter takes sphingomyelin on the cell membrane as the receptor. After sphingomyelin increases the sensitivity of the cell membrane, it inserts into the cell membrane through the domain of sphingomyelin to play the role of ion channel. After the toxin forms holes in the cell membrane, EqTx II will lead to calcium influx, and the toxin will also enter the cell through the holes, resulting in cell lysis. However, sphingomyelin can inhibit the lytic activity of toxin, but its hemolytic ability can be improved in the presence of HSA. In addition to pore forming on the cell membrane, EqTx II can also change the fluidity of the cell membrane, increase the low fluidity area and reduce the high fluidity area. Its hemolytic activity can lead to the lysis of red blood cells and the release of intracellular hemoglobin. EqTx II can also lead to platelet aggregation and degranulation, which does not involve the formation of thromboxane B2 and is therefore not inhibited by heparin. Its hemolytic activity is promoted in the presence of calcium ion and inhibited in the presence of lithium ion.

membrane conductance (Varanda and Finkelstein, 1980). The single channel activity of toxin producing holes can be observed at low concentrations (Varanda and Finkelstein, 1980). The relevant mechanism shows that the initial binding of the toxin is nonspecific and reversible, and then it is inserted into the membrane through the sphingomyelin domain in the membrane to form a stable polymer complex and play the function of ion channel (Doyle et al., 1989). After the separation of STI and StII, it was also found that sphingomyelin can promote the interaction between toxins and membrane (Tejuca et al., 1996). Whether StI or StII, the hole size in the artificial plasma membrane is basically the same as that in the natural cell membrane, which is about 1. 1nm and compared with the combination of lecithin (PC) and sphingomyelin (SM), the toxin is easier to combine with phosphatidylic acid (PA) and sphingomyelin (Tejuca et al., 2001; Valcarcel et al., 2001). Using StII for experiment alone, it was also found that the toxin produced membrane leakage through an all or no mechanism, and the degree of leakage depended on the protein concentration in the vesicle components (Rios et al., 1998). Methods such as monoclonal antibody and Western blot techniques were used to prove that sphingomyelin is indeed the receptor molecule of StII in cell membrane (Garcia et al., 2012). When human serum albumin (HSA) is present in the environment, the ability of StII to dissolve red blood cells is improved, but the pore forming rate in liposomes and red blood cells is reduced (Celedon et al., 2013). Nevertheless, StII still maintains its hemolytic activity in complex environment (Celedon et al., 2013). In the process of StII binding to the cell membrane, it was found that the distribution ratio of the toxin between membrane and water phase was relatively constant, and the toxin also had certain hemolytic activity (Doyle and Kem, 1989). The interaction between StI, StII and membrane (Alvarez et al., 2009) and the structure and function of StI and StII and StIII are reviewed (Rivera-de-Torre et al., 2020), and will not be discussed in detail here.

Toxic effects of two types of toxins on organs

Effects on heart

Many toxins in sea anemones have significant effects on the hearts of mammals and arthropods. The toxins with significant effects mainly include the following: ATX II, ApA, EqTx, Gigatoxin-4 and Tenebrosins-A, Tenebrosins-B and Tenebrosins-C. Using ATX II in the hearts of guinea pigs, rats and cats, it was found that ATX II had dose-dependent positive inotropic effects in different cardiac preparations (Alsen et al., 1976, 1982; Hoey et al., 1994) as is shown in Fig. 3, and this toxic effect of ATX II depended on the concentration of extracellular calcium ion (Alsen et al., 1976). It can cause myocardial contracture and arrhythmia in guinea pig atrium (Alsen et al., 1976, 1982), while it can lead to abnormal cardiac activity time in cat heart (Alsen et al., 1976). Using patch clamp method, it was found that the activation state of cardiac sodium channel was significantly prolonged (Schreibmayer et al., 1987; El-Sherif et al., 1992) and at the same time prolonged the action potential of ventricular cells in rats and guinea pigs under the action of toxin (Hoey et al., 1994), but the detailed mechanism is not clear.

ApA, a toxin from sea anemone *Anthopleura xanthogrammica*, can also produce a strong positive myogenic effect on isolated myocardium of guinea pigs and rabbits and anesthetized dogs, restore myocardial contractility, and the effect is basically not weakened in the presence of many calcium influx antagonists (such as nifedipine and verapamil) (Shibata et al., 1978). It was also found that ApA can prolong the duration of action potential (Kodama et al., 1981). ApA also has a positive inotropic effect on isolated cardiac papillary muscles of cats (Scriabine et al., 1979). After injecting the toxin into dogs, ApA can also enhance myocardial contractility, and when the dose is too high, the experimental animals will die of ventricular fibrillation (Scriabine et al., 1979). Similar experiments also show that the positive inotropic effect produced by ApA may have a therapeutic effect on heart failure related diseases (Gross et al., 1985).

After injection of Equinatoxin, a toxin from sea anemone Actinia equina, into rats, significant bradycardia was observed, accompanied by apnea (Sket et al., 1974). Toxins can produce dysrhythmia, ventricular contractions, paroxysmal ventricular tachycardia, and finally can cause irregular ventricular beats and ventricular fibrillation (Sket et al., 1974) as is shown in Fig. 3. EqTx also inhibits both right atrial pulsation and left atrial drive (Ho et al., 1987). EqTx II isolated from EqTx has cardiotoxic effects on rabbit heart, resulting in myocardial contracture (Bunc et al., 1999). It also has positive inotropic effect in the hearts of guinea pigs and rats (Norton et al., 1992). But different from ATX II, EqTx II can also cause a dose-dependent decrease in coronary flow (CF), which also depends on the extracellular calcium concentration (Alsen et al., 1976; DrevenSek et al., 2000). EqTx III of the same species can also increase the contractility of myocardium (Suput et al., 2001).

As a protein with cardiac stimulation and hemolytic activity, the toxins Tenebrosins-A, Tenebrosins-B and Tenebrosins-C from sea anemone *Actinia tenebrosa* show a strong positive inotropic effect on the isolated but spontaneously pulsating guinea pig atrium, and phospholipase A2 blocker can inhibit the cardiac activity of Tenebrosin-c (Galettis and Norton, 1990; Norton et al., 1990). In addition, the toxin gigantoxin-4 from sea anemone *Stichodactyla gigantea* can also cause hypotension, bradycardia and myocardial congestion (Hu et al., 2011).

Effects on lung

The toxins that can damage the lung mainly include Equinatoxin and Gigatoxin-4, and EqTx and EqTx II

purified from EqTx also have different emphasis on lung damage. After using EqTx, it will significantly affect the pulmonary vascular permeability, damage the venous septum, resulting in pulmonary edema, and increase the dry wet weight ratio of the lung in a dose-dependent manner (Lafranconi et al., 1984) as is shown in Fig. 3. However, if EqTx II is used, the most significant effect is to reduce the cell survival rate of lung fibroblasts and

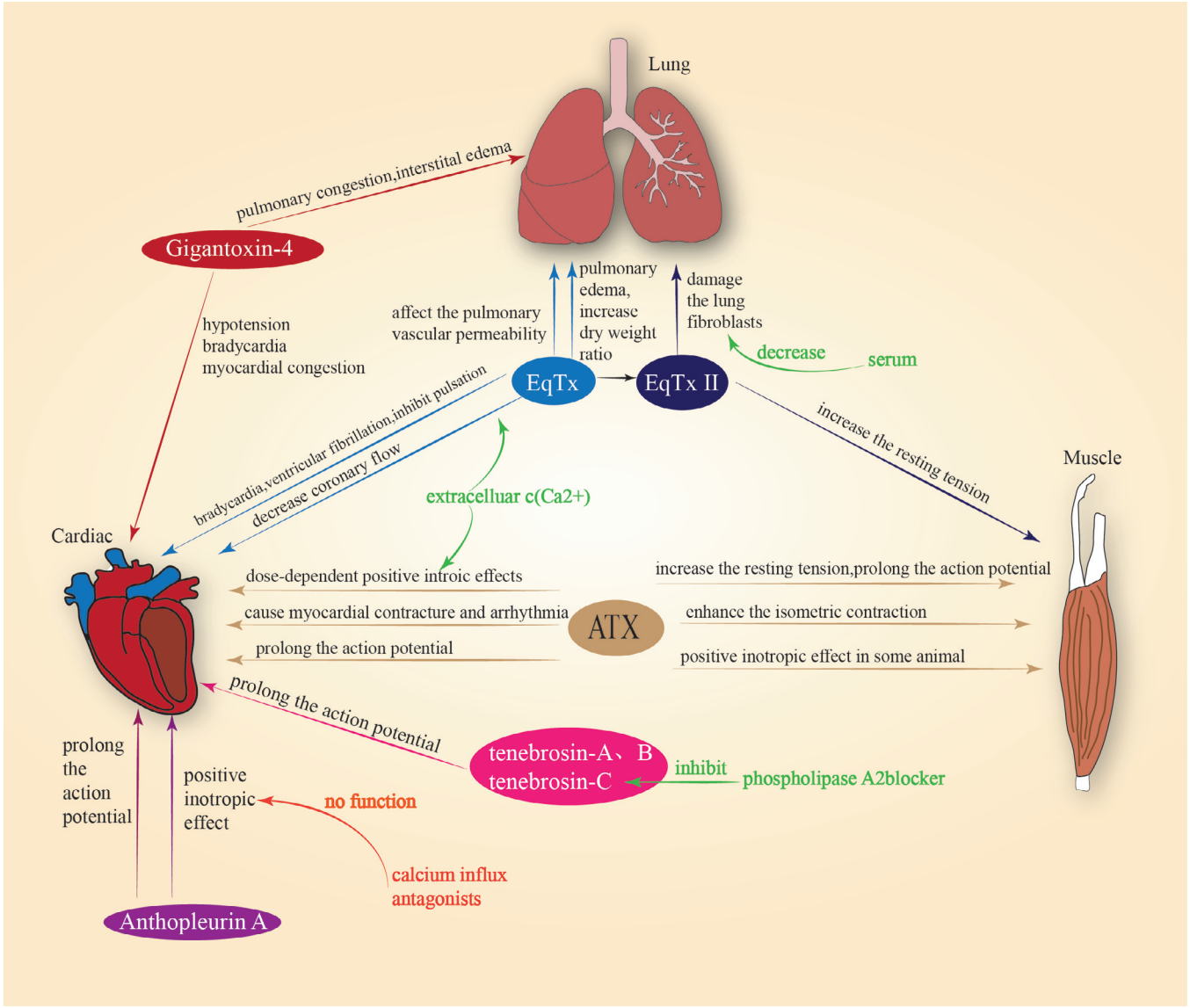

Fig. 3. Toxic effects of sea anemone toxin on important organs of the body. The main organs affected by sea anemone toxin are lung, cardiac and muscle. Among the toxins discussed, EqTx is the most widely affected. In the cardiac, it can cause bradycardia, ventricular fibrillation and inhibit pulsation. Under the promotion of external calcium ions, it can also reduce coronary flow. In the lung, it will mainly affect the pulmonary vascular permeability, form pulmonary edema and increase the lung dry wet ratio in a dose-dependent manner. EqTx II isolated from EqTx can also reduce the survival rate of lung fibroblasts, which is inhibited by serum. In muscle, EqTx II can also increase the resting tension of muscle. Compared with EqTx, ATX II mainly plays a role in the cardiac and then muscles. In the cardiac, ATX II can produce positive inotropic effect, which is also regulated by the concentration of exogenous calcium ions. ATX II can also cause myocardial contracture and arrhythmia and prolong action potential. In muscle, ATX II mainly increases resting tension, enhances isometric contraction of muscle, prolongs action potential, and produces positive inotropic effect in some animals. As a kind of gigatoxin-4, which can produce significant toxic effects on many organs in the body, the lung is the most seriously damaged organ. It can cause symptoms such as lung congestion and interstitial edema. In the cardiac, gigatoxin-4 can cause hypotension, bradycardia and myocardial congestion. In addition to the above three toxins, some toxins such as Anthopleurin-A and tenebrosin also have effects on the cardiac. As a cardiotonic polypeptide, Anthopleurin-A cannot only prolong the action potential, but also produce positive inotropic effect in the cardiac without being affected by calcium influx inhibitors. Tenebrosin-ABC can show strong positive inotropic effect in the cardiac, but phospholipase A2 can inhibit the cardiac activity of tenebrosin-C.

in this process, serum can neutralize the toxicity of toxin (Batista et al., 1990). As a toxin that can cause damage to a variety of organs, the lung is the most seriously damaged organ caused by this venom (Monroy-Estrada et al., 2013). After administration of gigatoxin-4, it will cause pulmonary congestion, interstitial edema and other symptoms in a short time (Hu et al., 2011).

Effects on muscle

During the research, the effect of toxin on muscle is usually analyzed in combination with action potential. The above discussion has shown that ATX II can modify sodium channels and can also prolong action potential. Experiments in isolated guinea pig papillary muscle showed that when the concentration of external calcium increased, the time of toxin ATX II prolonging action potential decreased, but the interaction mechanism between them is not clear (Ravens, 1983). By using the muscles of frogs, guinea pigs and rats, it is found that when the venom was applied to rat sarcolemma, it can increase the resting tension (Alsen et al., 1981; Ravens and Schollhorn, 1983) and can also increase the convulsive response of the muscles (Ravens and Schollhorn, 1983; Erxleben and Rathmayer, 1984), as is shown in Fig. 3. However, some articles also show that it can inhibit the convulsive response of the muscles (Ravens and Wiese, 1985). When the venom was applied to frog muscle, it can also prolong the action potential, but it will not affect the resting potential, and these effects are reversible (Erxleben and Rathmayer, 1984). In addition, ATX II can also enhance the isometric contraction of muscle fibers in a time-dependent manner (Khan et al., 1986). Positive inotropic effects can also be produced in guinea pig papillary muscles (Ravens, 1976), but not in rat papillary muscles, although action potential can be prolonged both in guinea pig papillary muscles and rat papillary muscles (Hoey et al., 1994). Experiments in mice also found that the toxin could bind to the tendon of the mouse diaphragm without being innervated by the diaphragm (Horvat-Znidarsic and Suput, 1996). In addition, EqTx II, a toxin from sea anemone *Actinia equin*a, can also increase resting tension in muscle fibers (Horvat-Znidarsic and Suput, 1996). EqTx III of the same species can also increase the tension of smooth muscle (Suput et al., 2001).

Pathology and pharmacology of anemone toxin

Toxin kills cancer cells through apoptosis

Since the isolation and purification of sea anemone toxin, up to now, the research on the use of sea anemone toxin in human cancer therapy has been carried out. So far now many human cancers such as lung cancer, breast cancer, skin cancer and glioblastoma have been studied, and at the same time Ehrlich ascites tumors have been studied in mice.

Current studies have shown that the crude extracts of three kinds of sea anemone *Bunodeopsis globulifera*, *Stichodactyla haddoni* and *Heteroactis magnifica* and the components separated by gel running of these sea anemone have obvious cytotoxic effects on human lung cancer A549 cell line (Monroy-Estrada et al., 2013; Ramezanpour et al., 2014a; Abdzadeh et al., 2020). Different components of sea anemone *Bunodeopsis globulifera* have different killing effects on human lung cancer cells. They can damage cell membrane and mitochondria, but the specific mechanism is not clarified (Monroy-Estrada et al., 2013). *Heteroactis magnifica* inhibits the growth of the cell and induces apoptosis of cancer cells by mediating apoptosis and intercepting cancer cells in G0/G1 phase without entering DNA replication phase, which means it can increase the proportion of cells in G0/G1 phase and decrease the proportion of cells in S phase (Ramezanpour et al., 2014a). And its lethality to normal cells is much less than cancer cell (Ramezanpour et al., 2014a). Using sea anemone *Stichodactyla haddoni* for study could found that under the treatment of different sea anemone crude extracts, the expression of Bcl2 gene which inhibiting apoptosis was down regulated in A549 cells, on the contrary, the expression of BaK gene which promoting apoptosis was significantly increased, and even exceeded the effect caused by anticancer drugs (Abdzadeh et al., 2020). In addition, the sea anemone crude extracts could also cause the up regulation of Bax gene expression, which was similar to that caused by anticancer drug doxorubicin, thus mediated mitochondrial pathway of apoptosis (Abdzadeh et al., 2020).

The study of sea anemone *Heteroactis magnafica* shows that the toxin in sea anemone can significantly reduce the survival rate of human breast cancer T47D cell line. It was found in the experiment that the subG1 phase of cancer cells increased and at the same time the G1 phase shortened, and the release of cytochrome C increased by using relevant technologies (Ramezanpour et al., 2014b). It was also found that the toxin can also cut caspase-8 and caspase-9 to activate caspase-3, which indicates that the toxin can induce apoptosis by mediating the endogenous mitochondrial pathway and the exogenous pathway triggered by death receptor (Ramezanpour et al., 2014b).

For human blastoma cell lines, toxins Bc2 and EqTx II can play a great cytotoxic role, and can also fight cancer drugs like arabinoside, doxorubicin, and vincristine, which has different promoting effects (Soletti et al., 2008).

Heteroactis magnifica (toxins are magnificalysins I and II), Heteroactis crispa (RTX-A), Heteroactis malu, *Cryptodendrum adhaesivum* and *Entacmaea quadricolor* these five kinds of sea anemones are also used in human lung cancer, skin cancer and breast cancer these three major cancers, the results show that lung cancer A549 cell line is the most sensitive and the most easily killed,

and the growth of breast cancer cells is most likely to be inhibited by two kinds of sea anemones such as *H. malu* and *E. quadricolor*. The growth of skin cancer cells is most easily inhibited by four kinds of sea anemone toxins other than toxin RTX-A from *H. crispa* (Ramezanpour et al., 2012).

The study of sea anemone *Stoichactis kenti*, EqTx II of *Actinia equina* and RTX-A of *Heteroactis crispa* in mice found that the Ehrlich ascites tumor in mice could be effectively controlled when using a very low dose of protein extracted from sea anemone Stoichactis kenti (Norton and Kashiwagi, 1972). EqTx II has significant cytotoxic effect on L1210 leukemia cell line and ascitic Ehrlich carcinoma cell line in vitro, and can also significantly prolong the survival time of mice suffering from ascitic Ehrlich carcinoma, but it has no effect on L1210 leukemia cell line in mice (Giraldi et al., 1976). RTX-A can inhibit the transformation of JB6P$^+$ CL41 into malignant tumor in mice by inhibiting the activities of tumor factors AP-1 and NF-kB (Fedorov et al., 2010). The experiment with EqTx also found that the toxin can cause the extensive lysis of tumor cells in vitro and prolong the survival time of diseased mice (Giraldi et al., 1976).

Toxins treat autoimmune diseases by specifically inhibiting the activity of potassium channels

In addition to inducing different cancer cells to enter the apoptosis pathway, sea anemone toxin can also reduce autoimmune diseases. In many autoimmune diseases, patients' activated T_{EM} cells usually have Kv1.3^{hig}hIKCa1^{low} phenotype. Because ShK is a potassium channel blocker, the study of applying ShK to autoimmune diseases found that ShK can significantly inhibit the proliferation of activated TEM cells without damaging naive T cells (Wulff et al., 2003). Because MBP leads to the proliferation of CD4$^+$ T_{EM} and the occurrence of autoimmune encephalomyelitis (short for AT-EAE), it is necessary to inhibit the growth of CD4$^+$ T_{EM} cells to prevent AT-EAE. After ShK and ShK-Dap22 (Kv1.3 blocker) were used in the experiment, the results showed that ShK alone could significantly reduce the severity of the disease, while using ShK-Dap22 alone had no such effect. If used together, it would have a greater effect (Beeton et al., 2001). Due to CD4$^+$ T_{EM} cells is sensitive to the changes of Kv1.3 Channel but not sensitive to the change of IKCa1 channel, so the treatment effect of using TRAM-34 alone is not ideal, but the combination of ShK, ShK-Dap22 and TRAM-34 can significantly improve symptoms (Beeton et al., 2001). Using ShK analogue: ShK (L5)-amide (SL5 for short), it was found that SL5 could almost completely block Kv1.3 Channel and inhibits the function of T_{EM} cell by inhibiting the production of IL2 and IFN by GAD65 specific CD4$^+$ T_{EM} clone, without affecting antigen-specific IgG and IgM response (Beeton et al., 2006). It didn't affect the naive T cells, but can inhibit the proliferation of T_{EM} cells and the delayed type hypersensitivity (DTH) caused by T_{EM} cells (Beeton et al., 2005).

Hemolytic toxin (short for HT) isolated from sea anemone *Stoichactis helianthus* is mainly used to form conjugates with immature T lymphocytes (IOR-16) and monoclonal antibodies (MAbs), target recipient cells such as human leukemia cells, and produce cytotoxicity after adding DTT to restore hemolytic activity (Avila et al., 1988). HT can also directly combine with MAbs of monoclonal antibodies such as carcinoembryonic antigen through artificial disulfide bridge to form hybrid molecules, and restore hemolytic activity after the reduction of disulfide bridge, so as to have cytotoxic effect on carcinoembryonic antigen and inhibit its growth (Avila et al., 1989).

Discussion

Neurotoxins affect tissues and organs via changing action potential

The mechanisms of action of different toxins from different sea anemones are different, and the main effects are also different. Neurotoxins mainly include ATX II, BgK and ShK. Although they are all neurotoxins, the ion channels they affect and effects are different. We also discussed the effects of some neurotoxins on different tissues and organs.

ATX II, the most important neurotoxin, is the most important sodium channel toxin. It can distinguish different sodium channel subtypes and significantly prolong the action potential of related subtypes, which is embodied in slowing down the inactivation of channels, increasing conductivity and prolonging the opening time. Then it will cause the increase of intracellular secondary messengers such as cGMP and cAMP, so as to regulate the downstream pathway. In addition to affecting the nerve endings, ATX II also directly leads to an increase of acetylcholine released from the presynaptic membrane of the neuromuscular junction, which leads to an increase of EPP and MEPP of the postsynaptic membrane. In addition to inducing effects in muscle, ATX II also has a similar effect in cardiomyocytes. It can significantly prolong the plateau period of action potential of Purkinje cells. Compared with ATX II, toxins ShK and BgK from other sea anemone species are specific potassium channels blockers that block the neuromuscular junctions. Both have selectivity for potassium channel subtypes, but both can inhibit the opening of the IKCa 1 channel and the potassium current in the dorsal root ganglion. Like ATX II, BgK can also promote the release of acetylcholine from the presynaptic membrane. The neurotoxin of sea anemone mainly affects sodium and potassium channels, and can affect the downstream pathways through the intracellular secondary messenger. In addition to the electrochemical impact on the body, it also has a strong

signal pathway impact.

Effects of pore forming toxins on different tissues and organs

Pore forming toxins mainly include EqTx and Sticholysin. Here the pore forming mechanisms of toxins and their effects on different tissues and organs are discussed. The mechanism of pore forming toxins is quite different from that of neurotoxins. Both EqTx and Sticholysin show the formation of pores in the cell membrane, so as to exert the effect of ion channels. The difference is that EqTx is more likely to form holes directly in the liquid disordered phase, and the sphingomyelin on the membrane will play a synergistic role, while Sticholysin is formed by inserting the sphingomyelin domain into the membrane. The channels formed by EqTx can cause calcium influx, or directly cause toxins to enter cells and cause cell lysis, while the channels formed by Sticholysin can mediate the leakage of proteins in the membrane. Because sphingomyelin can inhibit the cleavage ability of toxins, once the channels are formed, the probability of cell lysis will decrease. In addition, EqTx can also lead to red blood cell fragmentation, hemolysis, platelet aggregation and platelet fragmentation and degranulation. These processes can be promoted by calcium ions. Since it will not lead to the formation of thromboxane B2, platelet aggregation will not be affected by heparin. The two toxins can be connected with appropriate antibodies to inhibit their activity. When the antibodies encounter the corresponding antigens, such as cancer cells or other abnormal cells in the body and anchor, the toxins recover their activity and break down the cells or lose their corresponding functions by forming holes in the cells. The new coagulation pathway shown by EqTx can also develop corresponding coagulation drugs for related treatments.

The above toxins have shown that they can affect the heart, lungs and other organs of experimental animals. The positive inotropic effect and prolonging action potential are the main effects on the heart, but their toxicity will also cause phenomena such as arrhythmia and rhythm disorder. Neurotoxin also has a significant effect on muscle cells, which can significantly increase resting tension and isometric contraction.

Conclusion and perspectives

Based on the action of sodium channel toxins and potassium channel toxins, sodium channel toxins can be considered to regulate the downstream signal pathways through the effect on action potential, which has a prospect for the treatment of many diseases related to nerves, muscles and cardiovascular diseases. Since the action sites of many anesthetics are also related to sodium channels, ATX II can also be included in one of the raw materials of anesthetics. In addition, since tetrodotoxin TTX is a blocker of ATX II, we can also explore whether ATX II can block the role of TTX and provide a new treatment for patients with tetrodotoxin poisoning. Because potassium channels are linked to many autoimmune diseases, the blocking of potassium channels by sea anemone toxin obviously provides a new method for the treatment of autoimmune diseases and a new idea for drug research and development. In addition, because neurotoxins and pore forming toxins have different toxic effects on various organs, these two toxins also have potential therapeutic effects on some diseases. The sea anemone toxin has a possible therapeutic effect on symptoms such as insufficient myocardial contractility, but follow-up research is needed to remove the effect of its own toxicity on cardiomyocytes. The neurotoxin may also have a therapeutic effect on symptoms such as muscle tension and muscle atrophy. Therefore, neurotoxins such as ATX II can interact with sodium channels, so they can be used as one of the sources of new anesthetics, so as to develop new anesthetics for clinical medicine. As many autoimmune diseases are related to potassium channels, potassium channel toxins such as ShK and BgK can also be used as one of the drug sources for the treatment of immune diseases. This new kind of drugs can be used for the treatment of potassium channel related autoimmune diseases. Neurotoxins also have significant effects on muscle cells, so they may have therapeutic effects on muscle tension and muscle atrophy. EqTx in pore forming toxins can be studied as a new coagulant because of its different coagulation pathways.

In the existing studies, it has been shown that sea anemone toxin has prospects in the treatment of immune system diseases and cancer treatment. Through the specific combination of toxin and antibody, it can treat autoimmune diseases. On this basis, it can also be considered to modify sea anemone toxin to make it more lethal and more affinity to target cells. In the treatment of cancer, it is found that the toxin not only has cytotoxic effects on cancer cells, up regulates the expression of various apoptosis related genes and causes apoptosis, but also plays a synergistic role with various anticancer drugs to accelerate the death of cancer cells. Therefore, in the follow-up related research, we can conduct more in-depth research on the synergy between toxins and drugs, and also use sea anemone toxins to study more cancer types to observe whether they are also specific for different cancer cell types.

At present, most studies on sea anemone toxins focus on several toxins, and a large number of toxins have not been studied or studied in depth. Many toxins were found to cause damage to kidneys and other organs in a small number of studies, but they are not included in the scope of this discussion due to too few studies and no research mechanism. Obviously, the toxic effects of sea anemone toxin are far more than those discussed in this paper, and the related diseases are also more than those listed in this paper. For example, there is not much research on cardiovascular diseases, but it can be seen from the mechanism of toxin action that it is necessary

to study related diseases. In addition, because there are too few types of toxins and a large number of toxin studies are not in-depth, the research of sea anemone toxins is limited and are not consistent with the treatment of human diseases. Therefore, in the follow-up research, in addition to continuing to explore the pathogenic mechanism and pharmacological research of the studied toxins, focus should be on those toxins that have not been studied in depth, in addition to the basic purification, separation and sequencing. There should further be an emphasis the mechanism of toxin action and pharmacological applications.

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