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Editorial Open Access

Marine Knottins with Remarkable Biological Functions Cast a Promising Outlook on Clinical Translation

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Editorial

Ocean, which covers approximately 71% of the earth's surface, is an ancient and mysterious habitat with diversiform marine ecosystem. Millions of unique marine organisms survive in this high-salt, hypertonic, tenebrous, and microthermal liquid environment [1,2] and these extreme living environments endowed them the special capacity for producing structurally and biofunctionally unique metabolites. Especially marine-derived knottin peptides, which play an important role in the chemical defense or invasion when creatures are struggling to survive in nature, exhibited amazing bioactivities against diverse human diseases. Thus, discovery of marine knottins opened a new chapter in peptide drug leads research as well as in pharmaceutical industry.

Knottins are intriguing mini-proteins, also known as cystine knot peptides, typically containing 30~50 amino acids and rich in cysteines. The most distinctive characteristic of knottin is that three disulfide bonds interlace into the knot in the core of the structure. The first two disulfide bonds together with the interconnecting peptide backbone form a macrocycle where the third disulfide bridge crossed, generating a "cystine knot motif", which endows knottin extraordinary chemical, thermal, and enzymatic stability. Loops with variable length and composition constrain anti-parallel β-sheet formation and are important for functional maintenance [3,4].

Knottin-like peptides were highly valued owing to the fame of conotoxins, a kind of neurotoxin from venom of marine cone snails. The cone snails are distributed throughout tropical and subtropical waters; generally prey on small fish, worms, or mollusks depending on different species. The slow-moving creatures can successfully catch the prey relying on their secret weapon-venom. The venom can be instantly injected into prey by a harpoon-like radular tooth causing an instantaneous paralysis to the victim. The venom is a kind of complex mixture including a large number of neuroactive cysteine-rich peptides termed conotoxin [5]. A portion of conotoxins are classified as knottin peptides due to knotted arrangement of three disulfide bonds (CysI-CysIV, CysII-CysV, CysIII-CysVI). They mainly target various voltage-gated ion channels corresponding to the ω, κ, μ, and δ pharmacological families [6] The well-known ω-conotoxin MVIIA with high selectivity to the mammalian N-type calcium channel (Cav2.2) was developed into a novel type of analgesic [7], the first marine-derived peptide drug approved by FDA in 2004 for the treatment of chronic pain. Other conotoxins ω-GVIA and ω-CVID completed preclinical studies but encountered termination during phase I/II clinical trials due to robust cytotoxicity. However, still a large number of conotoxins are in the pipeline of drug candidates [8].

Not only marine cone snails, but also sea anemones were found to produce knottin peptides, which are used as weapon to prey on small living bodies, as well as defense against predators. Recently, the first cystine knot peptide PhcrTx1 from sea anemones Phymanthus crucifer were reported as an acid-sensing ion channel (ASIC) inhibitor. ASIC is involved in sensory perception, nociception, neurodegenerative disorders, etc [9]. All these indicate that PhcrTx1 possibly make preys painless to prevent them from struggling to escape. A number of sea anemone peptides with various voltage-gated ion channel activities were identified [10], some of which contain six cysteine residues indicating possible cystine knot formation; however, only the primary structures of them were determined . Sea anemones may prove to be a novel source for marine knottins. Tachystatins A, B, and C were isolated from hemocytes of the marine horseshoe crab. Unlike venom-derived knottins, tachystatins show a broad spectrum of antimicrobial activity against G (-) and G(+) bacteria and fungi. Thus, they are supposed to play an important role in innate immunity of the horseshoe crab [11-13].

Interestingly, marine sponges, the simplest multicellular animals, produce knottins as well. The first identified asteropine A, a bacterial sialidase inhibitor, was isolated from the marine sponge Asteropus simplex [14]. Later, a series of sponge-derived knottins (asteropsins) were reported consecutively from the same sponge genus and validated porifera as a source of unusual knottin-like peptides. Asteropsin A did not show direct effect on ion channels, whereas it enhanced and prolonged neuronal Ca^{2+} influx in Na^{+} channels only when it was administered together with veratridine (a Na^{+} channel activator). Asteropsins were subjected to evaluation of various pharmacological activities, but no significant activity was discovered yet. The N-terminal pyroglutamate modification, incorporation of cis prolines, lack of basic amino acid residues, and the highly negative surface charge significantly distinguish them from other knottin families. Though the biological roles of asteropsins in sponges are still in mystery, their remarkable gastrointestinal enzymatic stabilities (trypsin, chymotrypsin, elastase, and pepsin) and serum proteases resistance were exposed in the spotlight recently [15-17].

Discovery of knottins opened a new efficacious way for oral peptide drug development, which is a persistent challenge in peptide pharmaceutical industry. Certain plant cyclic knottins (cyclotides) such as kalata B1 show low proteolytic susceptibility and long-term stability at higher temperature as well as under extreme pH condition [18]. High stability of kalata B1 was largely attributed to its cyclic backbone structure. Owing to these properties, cyclic knottins may be utilized as scaffolds for peptides drugs for oral delivery. As non-cyclic

knottins, asteropsins showed exceptional enzymatic stability. Asteropsins were impregnable to enzymatic hydrolysis even though enzyme cleavage sites were present in the sequence. Moreover, some cystine knot mini-proteins demonstrated good penetrativity toward rat intestinal mucosa and good stability in rat plasma, which is important for drug absorption and transmission in vivo [19]. Thereby, knottins exhibit great potential as scaffolds for oral delivery of peptide-based drugs.

Knottins also play an important role as a molecular probe for tumor imaging, which is exploited for visualization and quantification of biological process at molecular and cellular levels. Integrins have been involved in angiogenesis, tumor growth, and metastasis, making them attractive markers for prognostic and diagnostic purposes in tumor progression [20]. Hence, a series of cystine knot probes that bind with high affinity to specific integrin receptors were successfully developed for imaging of integrins overexpressed in tumors of mouse models. What's more exciting is that knottin-like probes demonstrate high and specific tumor targeting ability, serum stability, rapid blood clearance, and barely accumulate in non-target organs and tissues [21-23]. Furthermore, a large intergrin-binding knottin EETI 2.5F administered by tail vein injection could pass the blood brain barrier and target intracranial brain tumors [21]. This profile makes knottins as excellent candidates for diagnostic applications, particularly for tumor imaging, with high potential for clinical translation.

Knottins, with protein-like structure and peptide-like size, can be produced by chemical synthesis and genetic recombination. As known to all, tertiary structure of cystine knot peptides were highly conserved, tolerating amino acids substitution, insertion, removal, and even whole loop replacement [4], thus, they can be reformed to fit various purposes. The stability of knottins against certain enzyme can be enhanced by site-directed substitution or knockout of cleavage site amino acid residues without loss of inherent function [19]. Besides, grafting functional peptide fragment onto cystine knot framework will bestow novel bioactivities on knottin [24,25]. It means that a variety of artificial knottins can be synthesized for basic research, which breaks through the limitations of natural products.

So far, most of the reported knottins were from terrestrial organisms, including spiders, snakes, scorpions, plants, bugs, and so on. By contrast, the discovery of marine knottins is only tip of the iceberg and they are in need of further intensive exploration and development. To date, only conotoxins and several knottins from sponges, horseshoe crabs, and sea anemones were discovered from marine organisms. Novel knottins those stem from special function of marine organisms will motivate scientists to create more possibilities for knottins' clinical and industrial applications.

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