Biological Properties, Current Applications and Potential Therapeutic Applications of Brevinin Peptide Superfamily

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Abstract
The Brevinin peptides are antimicrobial agents obtained from frog skin secretions. Brevinin-2R has attracted many attentions due to its very low hemolytic activity, cationic property, and high affinity to cancer cells. Moreover, it has shown little toxicity against normal mammalian cells, while having killed several tumor cell lines by activation of lysosome-mitochondrial death pathway. In this review, we introduced the Brevinin superfamily with a focus on its therapeutic applications. Next, some unique properties of Brevinins were briefly discussed, including their ability to stimulate insulin secretion, dendritic cell maturation, and wound healing. In this context, we also provide information about the decoration of nanoparticles, such as cerium nano-oxide, by Brevinins. Finally, we addressed their potential for anti-tumor and drug design applications.

Keywords Antimicrobial peptide · Brevinin · Bioactive peptide · Cancer · Frog skin secretions

Introduction
Bioactive peptides are biomolecules with different effects on cellular behavior in a way that could promote the biological functions of the body, thereby might ultimately improve human health (Perez Espitia et al. 2012). Numerous properties have been introduced for bioactive peptides, such as, antimicrobial (Li et al. 2012), anti-oxidant (Power et al. 2013), anti-thrombotic (Khiari et al. 2014), anti-hypertensive (Pokora et al. 2014), and immunomodulatory (Haney and Hancock 2013) activities.

Attention to bioactive peptides has dramatically increased in recent years to design and develop effective drugs with minimal side-effects for the treatment of different diseases, like cancer, diabetes, inflammatory and infectious disorders (Seo et al. 2012). Commercial development of peptide drugs has raised from 14.1 million dollars in 2011 to 25.4 million dollars in 2018, and led researchers to carry out extensive studies in this field. More than 500 peptide drugs are under study in different clinical trial phases. About 60 drugs, based on peptides, have confirmed up to now, and according to ongoing studies, this number will have strong potential to in the near future (Fosgerau and Hoffmann 2015).

One of the main sources of bioactive peptides, including antimicrobial peptides (AMPs), is the skin of amphibian that acts as a physical and chemical barrier against pathogens. In fact, most of AMPs are cationic and some are anionic (Lai et al. 2007). Generaly, the AMP peptides contain less than 50 amino acids in length and many studies have demonstrated the contribution of these peptides to immune defense (Daum et al. 2012; Sadredinamin et al. 2016). Brevinines are natural AMPs and have been found in various species of frogs. According to database of anuran and defense peptides (DADP), over 350 types of Brevinines are identified with two main families: Brevinine 1 and Brevinine 2 (Novkovic et al. 2012).

This review was intended to introduce the Brevinin superfamily and its therapeutic properties. Next, we briefly dealt with some unique properties of Brevinins, such as their activity to provoke insulin secretion, dendritic cell maturation, and wound healing. Ultimately, some recommendations were suggested with regard to their therapeutic potentials.
Structure of Brevinin Superfamily

Morikawa et al. isolated these unique AMPs from the skin of the frog, *Rana brevipoda* parsa for the first time in 1992, and introduced them as Brevinin-1 and -2. Brevinine-1 has 24 amino acid with FLPVLA GIAAKVPALFCKITKCC sequences, and Brevinine-2 is composed of 33 amino acid with GLDSSLKGFAATAGKGVLQSSLSTASCKLAKTC sequences (Morikawa et al. 1992). The Brevinin super-family presents common characteristics, however several studies have revealed that the amino acid sequences of Brevinin-1 and -2 are poorly conserved across species with at least four invariant residues (Conlon et al. 2005, 2004). Members of this superfamily contain “Rana box”, which consists of disulfide-bridged cyclic heptapeptide (Cys18-(Xaa)4-Lys-Cys24), and shows cationic and amphipathic properties.

Different kinds of frogs from the genus “Rana” were investigated to isolate Brevinin peptide, such as, *Rana okinavana*, *Rana septentrionalis*, and *Rana boylii*, which have led to the identification of B1R, B1RP, Brevinin-1BYa, and B2RP-ERa, respectively (Hossain et al. 2011). These cationic peptides lack a well-defined secondary structure in water, even though having the potential to form amphipathic α-helices in biological solution (Kwon et al. 1998; Powers and Hancock 2003). The presence of Rana box was thought to be responsible for the antibacterial activity of the Brevinin family in preliminary experiments; nevertheless, this idea has been refuted in later studies (Conlon et al. 2005). Nowadays, it has been postulated that the interaction of α-helical structures with anionic lipid bilayers of bacterial cell membranes is mainly related to antimicrobial properties (Conlon et al. 2014). To confirm this idea, a study by Won et al. showed that the N-terminal domain of Brevinin-1 (residues 1–13) is the critical region for antimicrobial activity (Won et al. 2004). Since Brevinin-2 was separated from the skin of the rain frog *R. brevipoda* parsa (reclassified as *Pelophylax porosus*), divergent members of this family are widely discovered. To date, Brevinin-2 peptides have not been identified in any North American frogs of the Ranidae family (Conlon et al. 2009b). The Brevinin-2 related peptides (B2RPs) are 21-amino-acid peptide purified by ultrafiltration and ion exchange chromatography from the skin of *Rana* frog species (*R. septentrionalis*) which can be found in the north shores of the Caspian Sea and Russia (Li et al. 2013). Although B2RPs exhibit sequence similarity to Brevinin-2 peptides, the C-terminal heptapeptide domain (Cys-Lys-Xaa4-Cys) is not present as compared with Eurasian species. Besides, some studies have demonstrated that this cyclic domain in Brevinin-2 is not necessary for its antimicrobial activity (Bevier et al. 2004).

Synthetic Analogues of Brevinin Peptides

All living cell sorounded by one or two membranes composed of lipids and proteins. Cell and bacteria membranes provide protection against extracellular envoirnment and play a key role in the exchange phenomena between the cytoplasm and the outer space. The AMPs serve as the nonspecific innate immunity system by interacting with lipids of the bacterial and fungal cell membranes. Despite the great potential of AMPs as natural antibiotics, their application as pharmaceuticals are limited due to their lack of selectivity on exposed serum membranes. There have been some studies to reduce the hemolytic activity of B2RP, for example, some analogues of B2RP were developed by the substitution of Leu 18 to Lys cause decreases in both antimicrobial and hemolytic activity. In contrast, substitution of Asp 4 to Lys cause sensible increases in antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* without significant changes in hemolytic activity against human erythrocytes (Conlon et al. 2009a; Deslouches and Di 2017). More recently, Vineethkumar et al. (2017) reported the C-terminal amidation of Brevinin1 HYba1 and HYba2 improved their antimicrobial activity against *Aeromonas sobria* (a fish pathogen). Exploiting this post-translational modification, the positive charge of the peptides increased and intensified the electrostatic interaction of peptides with the anionic membrane of bacteria. Besides, they revealed that the 3D-structure of these peptides was stabilized by internal disulfide bridge (Tv et al. 2017). Also, another study reported the synthesis of 17 analogs of B1CTcu5 peptide by C- and N-terminal amino acid substitution or deletion and concluded that both regions are crucial for peptide function. It was demonstrated that the N-terminal region attributed to antibacterial property, while the C-terminal contributes to conformational stability (Parvin et al. 2015). Hossain et al. prepared an analogue of Brevinin-1BYa (isolated from *R. boylii*) through replacing disulphide bridge with a stable dicarba bond. Dicarba-Brevinin-1BY increased antimicrobial activity against both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria in comparison with unmodified Brevinin-1BYa (Fig. 1). Growth inhibitory potential against methicillin-resistant *S. aureus* (MRSA) and multidrug resistant *Acinetobacter baumannii* (MDRAB) was observed, as well (Hossain et al. 2011).

Antimicrobial Effect of Brevinin Peptide

Antimicrobial peptides protect the host against pathogens (Conlon et al. 2009a; Holroyd 2003), and modulate harmful effects of the inflammatory responses (Ali et al. 2002). These peptides have different action levels in vertebrates and
mostly exist in the skin, mucous surfaces and granules of the immune cells (Rao 1995). The AMPs do not only belong to vertebrates, but also are produced in all kinds of animals (Hancock 1997; Jenssen et al. 2006). Some AMPs are stored in skin granular glands of amphibian. They frequently have the α-helical structure and are released on the skin surface when the sympatic nerve is damaged or in the presence of other environmental stimulations (Chan et al. 2006). Antimicrobial effects arise from the interplay between cationic peptides and the anionic membrane of pathogens (Pálffy et al. 2009). The number and distribution of positive charges can be a selective advantage for the microbial membrane.

There are three main mechanisms for the interaction between the plasma membrane and Brevinin family (Chan et al. 2006; Sitaram and Nagaraj 1999):

1. Barrel stave model: In this model, antimicrobial activity of peptide is based on the pore formation in the membrane. The polar residues constitute the inner part of the channel, while the hydrophobic ones are in contact with the membrane phospholipids in the outer part (Savelyeva et al. 2014).
2. Carpet like model: Integration and uniformity of the membrane are disrupted in this model (Chan et al. 2006; Shai 1999).
3. Toroidal model: A mechanical stress and replacement of monomers on both sides of the membrane result in the membrane instability and thereby loss of its integrity.

Almost all Brevinin superfamily possess high antimicrobial activity against Gram-positive and Gram-negative bacteria, as well as fungal pathogens. The application of Brevinins as antimicrobial agents is limited because of their strong hemolytic property, however some studies have attempted to moderate this negative effect via structure modification. A good example is the replacement of the C-terminal box to central position in Brevinin-1E, which, in turn, decreased hemolytic activity and maintained antibacterial property (Kumari and Nagaraj 2001).

Among this family of peptides, Brevinin-2R is an exception; it shows low hemolytic activity and has a broad-spectrum antimicrobial property (Biever et al. 2004). The multidrug-resistant bacteria (MDRB) infections have spread in recent years owing to extensive application of antibiotics in the food industry, medicine, and agriculture. It is of utmost importance to develop new antibacterial agents for the treatment of life threatening health problems caused by MDRB, including MRSA, Gram-positive MDRB resistant to not only the beta-lactam antibiotics, but also erythromycin, ciprofloxacin, and gentamicin (Borde and Kern 2012), and extended-spectrum β-lactamase (ESBL) E. coli, Gram-negative MDRB with restricted antibiotic options for its treatment (Nuotio et al. 2013). Some Brevinin family members, such as dicarba derivative of bevinin-1BYa (Hossain et al. 2011), Brevinin-2TS (Conlon et al. 2006), and Brevinin-2CE (Zhang et al. 2014), have been investigated for the treatment of MRSA, as the most difficult infection to treat. In conjunction with that, Brevinin-2CE and B2RP have been found to have antimicrobial potential against multidrug-resistant A. baumannii (Al-Ghaferi et al. 2010; Zhang et al. 2014). In addition to antibacterial action of the Brevinins family, some scholars have addressed their antiviral properties. Yasin et al. confirmed the antiviral activity of Brevinin-1 (isolated from the skin of Asian frog P. porosus) against herpes simplex virus type 1 and 2. They exhibited that the Brevinin-1 anti-viral properties were maintained even after reduction and carboxamidomethylation, while cysteine modification diminished hemolytic and cytotoxic effects (Yasin et al. 2000). Brevinin-1BYa from R. boylii and B2RP-ERa from Hylarana erythraea were documented to inhibit HSV-1 infection of MDBK cells. Brevinin-1BYa prevented HSV-1 infection via inactivation of free virions or disturbance intracellular virus replication. However, it caused high cytokotoxicity in the MDBK cells (Conlon et al. 2014). Some derivatives of Brevinin-1 revealed remarkable antiviral activities against HSV by direct impacts on the viruses lipid membrane, suggesting new therapeutic potentials of Brevinins for
treatment of resistance in viral diseases, like Ebola, SARS or HIV/AIDS (Yasin et al. 2000).

In a study, Wang et al. examined anti-HIV-1 activities of about 30 peptides. They developed a library by rearrangement of Aurein residues as a template peptide. The sequence pattern of their B5 peptide was similar to Brevinin-2DYd, but this peptide appeared ineffective for HIV-1 inhibition (Wang et al. 2010). It seems that more studies are needed to elaborate on the efficiency of the Brevinin family against viruses.

The Role of Brevinin Peptides in the Innate Immunity System

The innate immune system is activated by the release of pro-inflammatory cytokines, namely interferon-γ (IFN-γ), tumor necrosis factor-alpha (TNF-α), interleukin (IL)-8, and so forth from mononuclear cells (Semple and Freedman 2010). Popovic et al. explored the role of Brevinin-2GU (isolated from *Hylarana guentheri*) (Conlon et al. 2008) and B2RP-ERa in producing pro-inflammatory and anti-inflammatory cytokines from peripheral blood mononuclear cells (PBMCs). They stimulated these cells by concanavalin A (ConA), a plant mitogen that triggered cytokines production under specified in vitro conditions. Both Brevinins declined the release of TNF-α from ConA-stimulated PBMCs at 20 µg/mL, while none exerted a significant effect on the IFN-γ release in these cells (Table 1). Only B2RP-ERa provoked anti-inflammatory cytokines, including TGF-β, IL-4, and IL-10 (Popovic et al. 2012). It seems that there is no correlation between antimicrobial efficiency and anti-inflammatory properties; for instance, IDR-1, an antimicrobial peptide derived from human cathelicidin, unraveled antimicrobial activity against both Gram-positive and Gram-negative pathogens, while reducing pro-inflammatory cytokine responses (Scott et al. 2007). Recently, one study reported the stimulation of pro-inflammatory IL-1β and -8 cytokines at different concentrations of Brevinin-2R in human lung epithelial adenocarcinoma cell line (A549). As well documented in the pertained literature, Brevinin-2R is speculated to possess low hemolytic activity however the viability of A549 cells decreased non-considerably (20%) following the treatment with 5–20 µg/mL of B2R peptide (Asoodeh et al. 2013). Similarly, it was showed that Brevinin-2R increased the expression of IL-1β and -6 genes in human liver carcinoma cells (HepG2) in a dose-dependent manner (Homayouni-Tabrizi et al. 2015). Further research should be carried out to elucidate immunogenicity of Brevinins.

### Anti-cancer Effects of Brevinin

Since Brevinin-2R does not form pores in the cell plasma membrane (like many toxins) and exhibits low hemolytic activity against human erythrocytes, it has begun to attract interest from scholars for further investigations as a novel anti-cancer peptide. A preliminary study revealed anti-cancer properties of B2R in a semi-selective manner. It was proposed that B2R could afford to kill cancer cells (Jurkat, BJAB, MCF-7, L929, A549) via lysosome-mitochondrial cross talk during cell death in the caspase independent pathway. Nevertheless, cells, which either over-expressed Bcl2 or suppressed BNIP3 expression, were observed to resist anti-cancer effects of B2R. Furthermore, in this study the cytotoxicity influence of individual B2R (10 µg/mL) were also compared to its combination with doxorubicin and cisplatin (50 µg/mL) in Jurkat and MCF-7 cells over short time periods (4 h). The results revealed that B2R was more toxic than doxorubicin and cisplatin in these cells. Brevinin-2R triggered cell death by reduction of the mitochondrial membrane potential as well as cellular ATP levels, and yet elevation of reactive oxygen species (ROS) generation (Ghavami et al. 2008). Interestingly, B2R showed low cytotoxicity against non-cancerous cells, such as PBMCs and human CD3 + T cells (Ghavami et al. 2008). This phenomenon

| Peptide          | Action | Cell source        | References                            |
|------------------|--------|--------------------|---------------------------------------|
| Pro-inflammatory |        |                    |                                       |
| TNF-α            | B2RP-ERa | Inhibit           | ConA-stimulated PBMC                  | Popovic et al. (2012) |
| INF-γ            | B2RP-ERa | Inhibit           | Unstimulated PBMC                     | Popovic et al. (2012) |
| IL-1β            | Brevinin-2R | Stimulate       | A549/HepG2                           | Homayouni-Tabrizi et al. (2015) |
| IL-6             | Brevinin-2R | Stimulate       | HepG2                                | Asoodeh et al. (2013) |
| IL-8             | Brevinin-2R | Stimulate       | A549                                 | Homayouni-Tabrizi et al. (2016) |
| Anti-inflammatory|        |                    |                                       |
| TGF-β            | B2RP-ERa | Stimulate         | Both ConA-stimulated/unstimulated PBMC | Popovic et al. (2012) |
| IL-4             | B2RP-ERa | Stimulate         | Both ConA-stimulated/unstimulated PBMC | Popovic et al. (2012) |
| IL-10            | B2RP-ERa | Stimulate         | ConA-stimulated PBMC                  | Popovic et al. (2012) |
might arise from variations of the outer membrane surface of cancer cells unlike normal cells. Some studies have demonstrated that the negatively charged phospholipid phosphatidylyserines (PS) increase in the outer leaflet of the human tumor cell membranes (Ran et al. 2002; Utsugi et al. 1991). Others have substantiated these findings and suggested the PS presence to detect metastasis (Riedl et al. 2011). It is speculated that B2R low cytotoxicity against non-cancerous cells, however direct studies to investigate this mechanism are needed for confirmation. Similar studies have proposed some analogues of Brevinin-1, namely Brevinin-1CEa, to carry anti-cancer impacts against the growth of MCF-7 (breast cancer) and HeLa (cervical cancer) cells (Yu et al. 2009). Additionally, anti-cancer properties of Brevinin-1EMa derivatives were investigated against seven tumor cell lines, such as A498 (kidney), A549 (lung), HCT116 (colon), MKN45 (stomach), PC-3 (prostate), SK-MEL-2 (skin), and SK-OV-3 (ovary). (Kang et al. 2012). There was a report concerning the anti-cancer function of modified Brevinin, like dicarba-Brevinin-1BYa. Elevated cytotoxicity against human erythrocytes, MDA-MB-231 (breast carcinoma), and HepG2 (hepatoma) cancer cells occurred following treatment with dicarba-Brevinin (Hossain et al. 2011).

**Effect of Brevinins on Insulin Release**

The prevalence of diabetes (largely type 2) is on the rise due to the worldwide increase in obesity, insulin resistance, and dyslipidemia. The main strategy for treatment regards hypoglycemia control using a different kind of drugs, namely thiazolidinedione, metformin, and sulphonylureas. These medicines have limited efficacy, limited tolerability, and several side effects (Moller 2001). Consequently, it sounds essential to research and develop new natural types of therapeutic agents (Leidig-Bruckner et al. 2014). Surprisingly, beyond the antimicrobial activities of the Brevinin superfamily, a host of these peptides has shown to stimulate insulin secretion in vitro. In this way, therapeutic potentials of Brevinins for the treatment of diabetes mellitus (type 2) patients have been explored in few studies. The BRIN-BD11 is a rat insulin-secreting β-cell line and nominated as an approved model for insulin-release studies (McClanaghan et al. 1996). Most experiments have utilized BRIN-BD11 cells to indicate the insulin-release stimulation as a result of Brevinin peptides, such as Brevinin-1 Pa (Marenah et al. 2005), Brevinin-1E, -2Ec (Marenah et al. 2006), Brevinin-1CBB (Mechkarska et al. 2011), Brevinin-2GUb (Conlon et al. 2008), and Brevinin-2-related (B2RP) peptide (Abdel-Wahab et al. 2010). In another study, gaegurin-6, a peptide from the Brevinin-1 family, was presented to trigger insulin release from rat RINm5F insulinoma-derived cells (Kim et al. 2010). In a recent study, it was revealed that two members of the Brevinin-1 family, Brevinin-1ITa and -1ITb, could carry stimulative effects on insulin release from BRIN-BD11 clonal β-cells in a concentration-dependent manner. Although, Brevinin-1ITa failed to exhibit antimicrobial activity against Gram-negative bacterium *E. coli* (Conlon et al. 2017). The mechanism of insulin release by Brevinins could be due to the protective nature of these peptides along with their antimicrobial activities. Brevinins stimulate insulin secretion and reduce blood glucose in their predators as a defense mechanism since hypoglycemia might be fatal to some species, namely snakes and birds (Marenah et al. 2004). The exact molecular mechanism underlying this action is not fully elucidated however it appears that an increase in intracellular Ca²⁺ concentrations may induce insulin secretion (Kim et al. 2010). Since B2RP lacks the C-terminal cyclic heptapeptide domain that enhances antimicrobial activity and reduces cytotoxicity, it has received much more attention than others. In this regard, some have intended to modify this peptide for insulin secretion. Abdel-wahab et al. modified B2RP by Asp4 → Lys substitution and accordingly insulin-releasing potency enhanced owing to elevation of cationicity. Nonetheless, other analogues, which were produced by amino acid substitution and contained more hydrophobic residues, developed reduced insulin-releasing abilities (Abdel-Wahab et al. 2010). Despite the evidence supporting the potential of Brevinins as novel insulinotropic peptides, it is worth mentioning that they are rapidly cleared from the circulation. This event is perceived as a negative factor for this kind of treatment. Therefore, additional studies are required to assess and validate Brevinins as potential therapeutic agents for the treatment of diabetes mellitus (Table 2).

**Brevinin Effect on Wound Healing**

Wound healing is a complex process containing cellular and molecular stimulation, which may cause inflammation, tissue formation, and ultimately tissue regeneration. People in Vietnam and south America apply amphibian skins for wound healing and inflammation treatment for years (Shores et al. 2007). Modern science has just recently investigated the wound healing properties of frog skin secretions and extractions (Liu et al. 2014; Piccolo et al. 2008). One of the these studies reported that the extract obtained from *Rana ridibunda* frog skin secretions (especially those products under 10 kDa) could accelerate the wound healing process (Mashreghi et al. 2013). Likewise, the efficacy of dermal (FS) and epidermal (RFS) sides of this frog skin was examined as wound dressing (Rezazade Bazaz et al. 2015). The main limitation of these studies regards the lack of sufficient characterizations of special compounds to determine the factor(s) involved in such properties. Furthermore, no study...
is available to compare the efficacy of Brevinin peptides and to focus on the approved standard of wound healing medicines. This process have been attributed to the collagen and lipid content of the frog skin (Kumar et al. 2002; Raghavan et al. 2010). What is more, the antimicrobial property of the skin secretion probably reduces wound infection and thus inducing the proliferation and migration of endothelial cells to the damaged site. Popovic et al. (2012) examined the therapeutic potential of the Brevinin-2GU, isolated from Hylarana guentheri (Al-Ghaferi et al. 2010) as a wound healing agent for common skin rashes. They found out that these peptides exhibited the bactericidal effect against Propionibacterium acnes as a major pathogenesis in acne vulgaris development (Popovic et al. 2012). Therefore, the influences of Brevinins on the wound healing process can be explained by their antimicrobial activities, to date.

Brevinin peptides have the advantages of low or no hemolytic activity even at high concentrations (no cytotoxicity against normal cells), and strong antimicrobial activity that provide a basis for their implication in wound healing. However, the short half-life of Brevinins in the circulation could significantly restrict their systemic administration and topical application.

Brevinin Functionalized Nanostructure

Ligand conjugation to nano-particles is a widely used technique that allows to extend application of functionalized nano-structures in diverse fields, such as gene or drug delivery systems (Askarian et al. 2017, 2015), biomedical imaging process, drug design, and vaccine development strategies (Sapsford et al. 2013). Casciaro et al. (2017) covalently conjugated an Esculentin-1a derivative, Esc (1–21) NH2, to gold nanoparticles (AuNPs) using poly(ethylene glycol) linkers. The Esculentin-1a is a frog skin antimicrobial peptide and isolated from Pelophylax lessonae/ridibundus. The antimicrobial activity of peptide-coated AuNPs increased about 15-fold against Pseudomonas aeruginosa bacterium more than free peptides (Casciaro et al. 2017). In accordance to extant studies, it seems that peptide-functionalized nano-particles can considerably enhance the antibacterial activity of biomolecules (Veerapandian and Yun 2011). Recently, cerium oxide nanoparticle (CNP) was conjugated to B2RP. The resultant complex (CNP-B2R) showed a higher cytotoxicity against cancer cell line (A549) than normal (HFLF-pI5) cell line (Homayouni-Tabrizi et al. 2016). Furthermore, this study benefited from the semi-selective anti-cancer activity of B2RP and the anti-oxidant property of cerium oxide nanoparticles, but further studies are necessary on CNP-B2R nano-structure stability and the validation of results by in vivo experiments. In general, the numbers of studies in which amphibian peptides undergo modification or functionalization with nano-particles are scarce. Given the potential of nano-structures for target modification of peptides, designing novel Brevinin-derived peptides through variant nanoparticle or different modifications holds promise for improving their current use or even extending their application in other fields (Fig. 2).

Table 2 Origins and primary structures of Brevinin peptides with stimulative impacts on insulin release

| Peptides                  | Species                  | Primary structures                                                                 | Rate of action                                                   | References                  |
|---------------------------|--------------------------|-----------------------------------------------------------------------------------|-----------------------------------------------------------------|-----------------------------|
| Brevinin-1Pa              | Lithobates pipiens       | FLPIIAGVAAKVPKIF-CAISKKC                                                          |                                                                | Marenah et al. (2005)       |
| Brevinin-1E, Brevinin-2Ec | Pelophylax saharicus     | FLPLLGLAANFLPKIF-CITRKCGILDLKKNFAKTAG-KGVLQSLBNTASCKLSQGC                      |                                                                | Marenah et al. (2006)       |
| Brevinin-1CBb             | Lithobates catesbeianus  | FLPLLGLAANFLPKIF-CITRKCGILDLKKNFAKTAG-KGVLQSLBNTASCKLSQGC                      | 119% of basal rate at a concentration of 30 nM                  | Mechkarska et al. (2011)    |
| Brevinin-2GUb             | Hylarana. guentheri      | GVIIDTLGAAKT-VAEELLRKHCK-LTNSC                                                    | 139% of basal rate at a concentration of 0.1 µM                 | Conlon et al. (2008)        |
| Brevinin-2-related peptide(B2RP) | Lithobates septentrionalis | GIWDTIKSMGKVFAG-KILQNL                                                           | 148% of basal rate at a concentration of 1 µM                  | Abdel-Wahab et al. (2010)  |
| Gaegurin-6                | Glandirana emeljanovi    | FLPLLGLAANFLPKIF-CITRKCGILDLKKNFAKTAG-KGVLQSLBNTASCKLSQGC                      |                                                                | Kim et al. (2010)           |
| Brevinin-1 ITa Brevinin-1 ITb | Rana italica            | IVFPLLGMVPKLVCLIT-KKC VFLGAIQALTSLLG-KLNH                                        | The basal rate in the presence of 5.6 mm glucose at a concentration of 10^{-10} M of Brevinin-ITa | Conlon et al. (2017) |
Conclusions

The search for novel bioactive peptides of therapeutic promise is a never-ending story as human health permanently faces the complications caused by ongoing diseases as well as emerging disorders. Antimicrobial peptides (AMPs) are biomolecules found in skins of almost all organisms and act as a natural defense mechanism against pathogens. Some of these peptides have other unique characteristics besides antimicrobial properties, which make them intriguing effector molecules to employ in different applications. The Brevinin superfamily consists of AMPs derived from frog skins and captivates many attentions owing to their protection against cancer cells, low hemolytic activity, insulin release stimulation, and wound healing properties. Brevinins also can counteract the infectious effects of bacteria, viruses, and pathogenic fungi. Furthermore, Brevinin 2R has presented a strong potential to become a cancer-specific peptide with low affinity toward non-malignant cells as well as nonhemolytic activity. Brevinin-1 and -2 derivatives (with or without peptide modifications) have been proven to stimulate insulin release, though the exact molecular mechanisms remain unknown. Wound healing properties of Brevinins are more likely to arise from their antimicrobial activity in spite of few studies. Another interesting aspect of AMPs generally and Brevinins exclusively is the possibility of chemical modifications by conjunction to nano-particle. Some studies have demonstrated that design of smart targeting delivery of these modified peptides affords both anti-cancer and antimicrobial activities. Despite the numerous investigations on different members of the Brevinin superfamily, the molecular mechanisms underlying their actions against pathogens and cancer cells are still in need for further research prior to the clinical applications. Of note, the precise and detailed mechanisms of their cellular uptake are unknown.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval The article does not contain any studies with human participants or with animals performed by any of the authors.

Informed Consent The article is a review manuscript and does not contain any photograph. However, the figures and tables are also prepared by the authors themselves.

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