Review

Anti-Mycobacterial Peptides: From Human to Phage

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Key Word

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Abstract

Mycobacterium tuberculosis is the major pathogen of tuberculosis (TB). With the growing problem of *M. tuberculosis* resistant to conventional antibiotics, especially multi-drug resistant tuberculosis (MDR-TB) and extensively-drug resistant tuberculosis (XDR-TB), the need for new TB drugs is now more prominent than ever. Among the promising candidates for anti-TB drugs, anti-mycobacterial peptides have a few advantages, such as low immunogenicity, selective affinity to prokaryotic negatively charged cell envelopes, and diverse modes of action. In this review, we summarize the recent progress in the anti-mycobacterial peptides, highlighting the sources, effectiveness and bactericidal mechanisms of these antimicrobial peptides. Most of the current anti-mycobacterial peptides are derived either from host immune cells, bacterial extraction, or mycobacteriophages. Besides trans-membrane pore formation, which is considered to be the common bactericidal mechanism, many of the anti-mycobacterial peptides have the second non-membrane targets within mycobacteria. Additionally, some antimicrobial peptides play critical roles in innate immunity. However, a few obstacles, such as short half-life *in vivo* and resistance to antimicrobial peptides, need overcoming before clinical applications. Nevertheless, the multiple functions of anti-mycobacterial peptides, especially direct killing of pathogens and immune-modulators in infectious and inflammatory conditions, indicate that they are promising candidates for future drug development.

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Introduction

Recent statistics from the World Health Organization (WHO) estimate that one-third of the world's population is latently infected with *M. tuberculosis*, and from this pool, there are 9 million cases of active tuberculosis, resulting in 2–3 million deaths every year. Multi-drug

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resistant tuberculosis (MDR-TB), extensively-drug resistant tuberculosis (XDR-TB) and co-infection with human immunodeficiency virus (HIV) are the causes of driving the increase in incidence and death. Except the novel anti-TB drug bedaquiline approved in 2012, no novel drugs have been marketed for TB in the past 40 years [1]. To counteract the emerging MDR-TB threats, identification and development of novel fast-acting and affordable compounds for TB treatment is now more prominent than ever.

Antimicrobial peptides are gene-encoded peptides produced by genera from all domains, showing direct activity against a wide range of microorganisms and playing major roles in host defense [2]. Some features of antimicrobial peptides, such as low immunogenicity, selective affinity to prokaryotic negatively charged cell envelopes, and diverse modes of action make anti-mycobacterial peptides promising anti-TB therapeutics [3]. In this review, we summarize the anti-mycobacterial peptides derived from different sources, including host immune cells, bacterial extraction, mycobacteriophages, as well as synthetic peptides (Table 1).

Human immune cells-derived anti-mycobacterial peptides

During mycobacterial infections, antimicrobial peptides, such as cathelicidins, defensins, and granulysins, are produced in innate immune cells. These peptides are also important signaling molecules in regulation of both innate and adaptive immunity, exhibiting immunomodulatory functions in host defense.

LL-37

LL-37 peptide is derived from Human Cationic Antimicrobial Protein 18 (hCAP18), which consists of a highly conserved N-terminal signal domain and a structurally variable cationic antimicrobial domain at the C-terminal [4]. hCAP18 is synthesized in activated neutrophils and epithelial cells, then released into extracellular milieu [5]. After that, hCAP18 is cleaved by proteinase-3 to form biologically active LL-37 peptide, which is the C-terminal of hCAP18 and then taken up by neutrophils [6]. The expression of hCAP18 was induced by 1, 25-dihydroxyvitamin D3 (1,25D3), which also induced the co-localization of mycobacterial phagosomes with autophagosomes in human macrophages through a cathelicidin-dependent manner [7].

The exogenous addition of LL-37 or endogenous over-expression of LL-37 in macrophages significantly reduces the intracellular survival of *M. tuberculosis*. After incubation of macrophages infected by *M. tuberculosis* H37Rv and *M. bovis* BCG with LL-37, more than 60% of both strains could be killed [8]. At the same time, no cytotoxic effects on macrophages were observed. The above results indicated that LL-37 exhibited anti-mycobacterial activity in vitro as well as inside macrophages. LL-37 also plays an important role in the innate immune response against *M. tuberculosis*. It functioned as an immune-regulatory agent through the activation of multiple receptors, such as a G protein-coupled receptor N-formylpeptide receptor-like-1 (FPRL-1) [9]. FPRL-1 can induce the secretion of chemokines including IL-8 and MCP-1 to chemically attract human peripheral blood neutrophils, monocytes, and T cells to the injury site [10]. The mechanism of LL-37 against *M. tuberculosis* in vitro is pore formation or membrane barrier disruption. While in vivo, LL-37 involves in both Toll-like receptor (TLR) activation of monocytes to induce the killing of *M. tuberculosis* and vitamin-D mediated human antimicrobial responses against *M. tuberculosis*. Furthermore, LL-37 could potentially reverse the ability of lipopolysaccharide (LPS) to stimulate the production of pro-inflammatory cytokines, such as TNF- α [11].

Two derivative peptides of LL-37 with higher anti-mycobacterial activity have been synthesized [12, 13]. One derivative peptide is a truncated variant of LL-37 named as LLKKK-18, in which the polar uncharged residues glutamine (Q22), asparagine (N30) and negatively charged aspartic acid (D26) are substituted by positively charged lysine [8]. LLKKK-18 was found to be more active against *M. tuberculosis* compared with LL-37, and no

cytotoxic effects on macrophages. LLKKK18 also showed increased killing of *M. tuberculosis* in combination with the biogenic-silver nanoparticles [14]. The other derivative peptide is the D-enantiomer of LL-37 named as D5, in which three alanine residues are substituted with more hydrophobic leucine residues, and valine at position 16 is substituted with lysine for avoiding the high level of self-association [12]. The minimum inhibitory concentration (MIC) values of D5 against *M. tuberculosis* were 35.2 µg/ml and 49 µg/ml against the MDR-TB strain [12]. D5 also showed a significantly better hemolytic activity and therapeutic index value than the parent LL-37 peptide. The higher activity of D5 could be due to that D-enantiomer has better action of membrane-disrupting [15, 16] and bacterial biofilm disassembly [17].

In addition to its direct anti-mycobacterial activity, LL-37 also possesses antifungal and anti-viral activity [18]. Moreover, it is capable of inducing wound healing [19], angiogenesis and modulating apoptosis [20]. The cathelicidin peptide was also found able to suppress tumor growth [21]. All these studies suggested that LL-37 peptide would have enormous potential to be a therapeutic agent not only for the treatment of mycobacterial infections.

Human defensins

The defensins belong to the family of small antimicrobial peptides having six highly conserved cysteine residues, resulting in three disulfide linkages. According to the structural differences of the mammalian defensins, they can be divided into three main subfamilies: α -defensins, β -defensins and the recently described θ -defensins. The defensins are key components of the innate immune response in phagocytic cells against intracellular pathogens, such as *M. tuberculosis*, by an oxygen-independent mechanism [22].

Human neutrophil peptide 1 (HNP-1) is one of the α -defensins present in the azurophilic granules of polymorphonuclear neutrophils. *In vitro* studies showed that HNP-1 was active against *M. tuberculosis* H37Rv in a concentration-dependent manner. The MIC value of HNP-1 against *M. tuberculosis* H37Rv was 2.5 µg/ml, which was higher than that of isoniazid (0.3 µg/ml) and rifampicin (0.2 µg/ml). However, HNP-1 had been shown to have synergistic activity in combination with the first-line anti-TB drugs. The combination of HNP-1 with either isoniazid or rifampicin could reduce the MICs of isoniazid and rifampicin against *M. tuberculosis* H37Rv to 0.0375 and 0.025 µg/ml, respectively [23]. In a mouse model, combination with low dosage of isoniazid and rifampicin, HNP-1 resulted in significantly better clearance of *M. tuberculosis* H37Rv than the same dose of drugs alone [24]. Transmission electron microscopy suggested that HNP-1 killed *M. tuberculosis* H37Ra mainly by binding to the plasma membrane, followed by permeabilization. DNA was suggested to be the intracellular secondary target for HNP-1 [25], which resulted in general inhibition in the macromolecular biosynthesis, with maximum inhibition for lipid biosynthesis [26]. HNP-1 also plays an important role in the pulmonary innate host defense system during mycobacterium infection, such that HNP-1 can mediate macrophages to secrete TNF- α , which stimulates neutrophil mycobactericidal activity, and recruits monocytes to the infection site [27, 28].

There are four different human β -defensin (HBD1–4) that are expressed in both leukocytes and epithelial cells [29]. All β -defensins are expressed inducibly, except HBD-1, which is expressed constitutively. HBD-2 is an antimicrobial peptide that plays an important role in innate immunity. It is well known that HBD-2 is associated with intracellular *M. tuberculosis* lysis [30]. Furthermore, *in vitro* studies indicated that HBD-2 could suppress *M. tuberculosis* growth. In combination with first-line anti-TB drugs isoniazid and rifampicin, HBD-2 could reduce *M. tuberculosis* load significantly. A study by Kisich et al. [31] showed that *M. tuberculosis* could be killed in human monocyte derived macrophages transfected with HBD-2 gene, implicating that HBD-2 plays a role in immunity against *M. tuberculosis*. In a mouse model, it was found that β -defensins induced by L-isoleucine showed improved therapeutic efficiency to TB [32]. It was also found that HBD-2 expression was significant higher in latent infection than in progressive infection, suggesting that HBD-2 could be used as a biomarker for re-activation [33].

It has been proposed that human defensins act on microbial membranes and form membrane pores, which are the mechanism for their antimicrobial activity. Besides this, defensins are broad-spectrum antimicrobial agents capable of anti-viral, anti-fungal, and having immune- modulatory and chemokines activity. However, before using defensins therapeutically, we must pay attention to some concerns. First, defensins showed a non-specific cytotoxicity to normal and malignant mammalian cells [34]. Second, the genome of *M. tuberculosis* contains a gene *mprF*, which was found associated with resistance to several antimicrobial peptides including defensins [35].

Granulysin

Granulysin is a member of the saposin-like protein family, and has highly homologous to NK-lysin. Granulysin contains four helical bundle motifs, with the helices enriched with positively charged arginine and lysine residues. Granulysin is a cationic small glycoprotein, synthesized as a secretory 15 kDa precursor. Then the precursor is enzymatically processed into a granular 9kDa protein, which is cytolytic against extracellular and intracellular *M. tuberculosis* [36]. Granulysin localizes in human CTL and NK cell granules and is the only antimicrobial peptide identified in CTL cells, in which both 15kDa (15K) precursor and 9kDa (9K) granulysin could be detected [37].

The 15K granulysin could enter into human macrophages and kill *M. tuberculosis* in the cytoplasm of macrophages. Recombinant 15K granulysin contributed directly to immune defense against TB, resulted in decreased number of bacilli in the lungs of the mice infected with *M. tuberculosis* by aerosol challenge [38]. Granulysin as candidate immune marker for TB was also investigated during childhood and adolescence. Compared with controls, the results indicated that granulysin remained high in individuals newly diagnosed with active TB or latent TB infection [39].

Two granulysin-derived peptides granF2 [40] and G13 [41] were found having a particularly high activity against clinical isolates of both drug-susceptible *M. tuberculosis* and MDR-TB. More importantly, the bactericidal activity against MDR-TB was better than that against drug susceptible strains. GranF2 showed a significant synergistic effect with ethambutol against susceptible clinical isolates. Although both of them have an anti-TB effect through permeating the cell wall, GranF2 and ethambutol act at different sites of the *M. tuberculosis* cell wall. In combination with streptolysin O, granF2 could efficiently reduce the growth of MDR-TB, which can survive and persist within macrophages [42]. It was believed that streptolysin gained access to and killed the intracellular bacteria after destructing the host cell walls by GranF2.

It has been reported that acting on mycobacterial membranes, disrupting synthetic liposomes and inducing apoptosis of mammalian cells are the antimicrobial mechanisms for granulysins. Granulysins also have potent broad-spectrum antimicrobial activity against bacteria, fungi, and tumor cell. Granulysins containing either helix2 or helix3 can lyse bacteria, while only granulysins containing helix3 can lyse tumor. Both intra- and intermolecular disulfide bonds are necessary for the peptides to lyse tumor targets, whereas lysis of bacteria is unaffected by reduction of the disulfide bonds.

From human non-immune cells

Different types of human non-immune cells, such as liver hepatocytes, keratinocytes and eosinophils, can also produce antimicrobial peptides including ubiquitin-derived peptides, hepcidin and RNase-derived peptides. These peptides exert antimycobacterial effects through direct killing activities, but without immuno-modulatory activities.

Lysosomal ubiquitin-derived peptides

Ubiquitin (Ub) is a post translational modification protein and usually acts as a signal for proteasome degradation and endocytosis. However, some studies showed that ubiquitin-

derived peptides located in the lysosome contribute to anti-mycobacterial activity [43].

When macrophages are activated by mycobacterial infection, mycobacteria are delivered to the lysosome and to be killed through both oxidative and non-oxidative mechanisms. Oxidative mechanisms include the action of reactive oxygen and nitrogen intermediates on bacteria. Non-oxidative mechanisms are linked to the ubiquitin-derived peptides. Although ubiquitin exists in the lysosome, which contains some other proteases with direct anti-mycobacterium activity, the intact ubiquitin itself has no bactericidal activity to mycobacteria *in vitro*. Autophagy plays a key role for the mycobactericidal activity of ubiquitin-peptides, because it can promote the fusion of *M. tuberculosis*-containing phagosome with lysosome, and then enhance the delivery of ubiquitin to lysosome [44]. Ubiquitin also has been used as a vaccine component. Fusing to mycobacterium antigens, such as ESAT-6 [45], Ag85A [46] and MPT64 [47], could significantly increase the efficacy of the vaccines against TB.

Ub2 is a ubiquitin-derived peptide, which was identified in lysosome by Kieffer et al. [48], exerted significant antibacterial activity against *M. Tuberculosis* with a MIC of 5 μ M [44]. It has been shown that the secondary structure β -sheet of Ub2 was vital to its membrane targeting ability and mycobactericidal activity [49]. Point mutations showed that the crucial residues of Ub2 for bactericidal activity were charged residues positioned at the C-terminal end of the peptide. Some mutants such as Ub2H4A, Ub2R8A, Ub2R10A, Ub2G11A, and Ub2G12A would significantly reduce the net charge of the peptide and decreased their mycobactericidal activity [49], though the point-mutated peptides retained the same membrane targeting capacity. The mycobactericidal mechanism of Ub2 was associated with its outer membrane permeability [50]. Subcellular localization studies showed that Ub2 not only inserted into the bacterial membrane but also reached the cytoplasm. Loss of membrane integrity in lysosome environments exposed the bacterium to adverse conditions, including additional bactericidal molecules. Incorporation of Ub2 into the bacterial membrane was explained using a micellar aggregate channel model, in which a peptide at high local concentrations induced the cytoplasmic membrane to form micellar-like structures that effectively generated channels in the membrane [50]. Ub2 exhibited the greatest bactericidal activity at pH 5.5 and pH 6, which was in line with the pH values of mature mycobacteria-containing phagosomes.

In addition to Ub2, Cathepsin cleavage of ubiquitin proteins generates other peptides, such as Ub17.1 and Ub21, which have α -helical secondary structures with lower net charges than Ub2. However, both of them could not disrupt the mycobacterial membrane and had little mycobactericidal activity. Moreover, a transcriptional regulator *msmeg_0166* located in *M. smegmatis* genome, was found able to lead to the resistance of mycobacteria to Ub2 [51].

Hepcidin

Hepcidin is a key regulator of iron recycling by inhibiting iron absorption in response to iron overload and inflammatory stimuli [52]. The precursor is synthesized in macrophages and liver hepatocytes possessing 84 residues, but then cleaved to yield the mature 25 residues hepcidin and 35 residues proregion. Both of them have antibacterial activity against mycobacteria.

M. tuberculosis and its bacterial constituent, such as lipoarabinomannan and culture filtrate proteins, could strongly induce hepcidin mRNA expression in human alveolar epithelial cells and dendritic cells [53]. The production of hepcidin by cells present in the lungs supports the importance of hepcidin in innate host defense against *M. tuberculosis* infection. Iron is essential for the growth and the survival of mycobacteria. During infection, both host macrophage and the pathogen compete for iron. And then hepcidin will be secreted by macrophage as a key role in restricting the growth of mycobacteria though inhibiting release of the recycled iron by macrophages.

In vitro, hepcidin could inhibit *M. tuberculosis* growth and caused structural damage to the mycobacteria [54]. After incubation with hepcidin, the colony form units (CFUs) of *M. tuberculosis* were reduced by 50% compared with that of the control. Transmission electron microscopy (TEM) revealed that hepcidin could lead to structural damages to the mycobacteria. A qRT-PCR study [55] found that Hepcidin mRNA could be induced by

mycobacteria and IFN- γ in macrophages, but not pro-inflammatory cytokines or iron overload. Hepcidin expression was also found regulated by TLR2 and TLR4 receptors in macrophages via the MyD88-dependent signaling pathway [56]. These findings provided clues to understand the roles of Hepcidin in host defense strategies against mycobacterial infections.

Human host defense ribonucleases (RNase)

RNase 3 and RNase 7 are the representative members of the vertebrate RNase superfamily secreted by innate cells, such as stimulated keratinocytes and eosinophils, which are potent host defense effector cells activated by mycobacterial infection. Both RNase 3 and RNase 7 are small cationic proteins with a well characterized cytotoxic action contributing to the host defense against mycobacterial infections.

RNase 3 is secreted by eosinophil secondary granules and also called the eosinophil cationic protein (ECP). RNase 7 is abundantly secreted by keratinocytes and mainly involved in the skin defense against infective microorganisms, like *M. leprae*. RNase 3 and RNase 7 inhibited mycobacterial growth in micromolar range and the MIC values against *M. tuberculosis* were 20 μ M. The first 45 residues of the N-terminal of RNase (RN3(1-45) or RN7(1-45)), encompassing three α -helices, retain most anti-*M. vaccae* activity of the full protein. The RN7(1-45) peptide showed similar MIC value and cell viability to the parental RNase 7 [57]. While, the peptide RN3 (1-45) has better antimicrobial activity than the parental protein. The MIC value of RN3 (1-45) was below 10 μ M. The better activity may be owing to a higher pI (pI = 12.61 versus 10.94) and more positive net charges (+8 versus +7) in RN3(1-45) than in RN7(1-45). Ultrastructural analysis showed that the envelope integrity of *M. vaccae* cells was completely disrupted after interaction with both RNases and their derived peptides.

HCL2

HCL2 is part of a seven-bundle helix of the human cytochrome c oxidase subunit3 (COX3) proteins, with a helical structure similar to an early secreted antigenic target protein ESAT-6. HCL2 can interact with culture filtrate protein 10 (CFP10) through hydrophobic interactions and disrupt ESAT-6:CFP10 complex. ESAT-6 and CFP10 proteins are secreted into the extracellular matrix during broth culture of *M. tuberculosis* and play critical roles in mycobacterial pathogenesis [58-60]. HCL2 could disrupt the heterodimeric interaction between ESAT-6 and CFP10 *in vivo*, and significantly inhibited the growth of both extracellular and intracellular *M. tuberculosis*. HCL2 could strongly hinder the survival of *M. tuberculosis* inside human macrophagial THP-1 with no cytotoxicity. In the presence of HCL2, either endogenous or exogenous, cell wall and cell morphology of *M. tuberculosis* showed distinct degeneration by electron microscopy.

Anti-mycobacterial Peptides Produced by Bacteria

Unlike most antibiotics, which are secondary metabolites, bacteriocins are ribosomally synthesized antimicrobial peptides produced by Gram-positive or Gram-negative bacteria. Bacteriocins can kill other closely-related or non-related microbiotas, but will not harm the bacteria themselves because of their specific immunity. Bacteriocins are unique in their large diversity of structures and functions, wide resources, and heat stability.

Lacticin 3147

Lantibiotics are antimicrobial peptides that undergo extensive posttranslational modifications, characterized by the unusual lanthionine and/or methyllanthionine residues, dehydrated serines and/or threonines [61]. Among them, lacticin 3147 produced by *Lactococcus lactis* had inhibitory activity against *M. tuberculosis* H37Ra, *Mycobacterium avium* and *Mycobacterium kansasii*, with MIC₉₀ values of 7.5 μ g/ml, 60 μ g/ml and 15 μ g/

ml, respectively [62]. Lacticin 3147 is a two-peptide (LtnA1 and LtnA2) lantibiotic with LtnA1 and LtnA2 at 1:1 ratio and effective at nanomolar concentrations [63, 64]. Three D-alanines present in lacticin 3147 are vital for its antimicrobial activity. The conversion of L-alanine to D-alanine in lacticin 3147 was performed by dha reductase [65, 66]. The ability to create D-residues in lacticin 3147 may provide a novel model of designing D-amino acid-containing peptides for novel antimicrobial activities. Lacticin 3147 possesses more than one mechanism of action, inhibiting peptidoglycan synthesis as well as forming pores in the membrane of target cells [67, 68]. Both activities were facilitated by binding to the peptidoglycan precursor, lipid II [69]. It is notable that the structure of mycobacterial lipid II differs from all other structures in bacteria investigated to date [70], which is due to an alternative synthesis pathway as well as modifications on both the N-acetylmuramic acid and peptide side chain components.

E50-52

E50-52 was obtained from isolates of *Enterococcus faecalis*, and belongs to class IIa bacteriocins. E50-52 inhibited mycobacterial growth within macrophages at a non-cytotoxic concentration of 0.1 mg/L, but inhibition stronger than rifampicin could be achieved at a high concentration of 1 µg/ml [71]. In macrophages, E50-liposome complex was able to inhibit mycobacterial growth without damaging the membrane of macrophages. In a mouse model, E50-liposome was able to significantly increase the life span of the infected animals, which were injected once every 24 h for 5 consecutive days. E50 may also serve as a good supplement to antibiotics in the treatment of MDR-TB [71]. Nevertheless, the efficacy against MDR-TB in mouse model of chronic TB needs testing.

Lassomycin

Lassomycin was found by screening extracts from uncultured species against *M. tuberculosis* [72]. Lassomycin is a cyclic peptide, synthesized and secreted by the environmental microbe *Lentzea kentuckyensis*. Its structure is generated by cleavage of a ribosomal product, cyclization, and C-terminal esterification. Except *M. tuberculosis*, lassomycin exhibits little activity against other bacteria or mammalian cells. This specificity is due to its unique target, ClpC1P1P2, which is an essential ATP-dependent protease for viability of mycobacteria [73], but not in other bacteria. Lassomycin can bind to an acidic N-terminal pocket of ClpC1P1P2 and activate its ATPase activity, which leads to protein degradation within mycobacteria. It is unusual for an antibacterial peptide to activate rather than inhibit its target enzyme. The intriguing mechanism of action is relatively rare, showing lassomycin is a very unusual bactericidal agent. Lassomycin had a MIC of 0.8–3 mg/ml and MBC of 1–4 mg/ml against *M. tuberculosis* including MDR-TB and XDR-TB isolates [72]. Lassomycin would be a promising anti-TB drug, though its cytotoxic and anti-mycobacterial activity in animal models need to be tested.

Mycobacteriophage-derived anti-mycobacterial peptides

Bacteriophages are the viruses of their host bacteria, and viewed as potential antibacterial therapeutics [74]. Ren Lai et al. [75] tested more than 200 presumed antibacterial peptides on the basis of genomic information of mycobacteriophages. One of them, PK34 showed *M. tuberculosis*-killing activity with a MIC of 50 µg/ml *in vitro*, and exerted strong anti-mycobacterial activity *in vivo*. PK34 could bind to trehalose-6, 6-dimycolate (TDM), which is an important target for anti-mycobacterial agents. In a mouse model, PK34 showed significant inhibition on inflammatory cytokines secretions by inactivating MAPK and PKB signals, while maintained certain pro-inflammatory cytokine production. In addition to its direct *M. tuberculosis*-killing ability, PK34 might be a useful adjunct in immuno-regulation. PK34 exerted potential protective effects against *M. tuberculosis* infection by both direct *M. tuberculosis* killing and inflammation inhibition. A few peptides derived from other phages

Table 1. The sequences and properties of anti-mycobacterial peptides.* Represent MIC₉₀; Rare amino acids are shown in bold

Name	Sequence	MIC	cytotoxicity	Test	Ref.
LL-37	NH ₂ - LLGDFFRKSKEKIGKEFKRIVQRIKDFL RNLVPRTES-OH	2.1 µg/ml	No	mice	[6, 8, 12, 18, 103-108]
LLKKK-18	KEFKRIVKRIKKFLRKL	25 µg/ml *	No	In vitro	[8, 109]
D5	Ac- KWKSFLKTFKSLKKTCLHTLLKLISS- amide	35.2 µg/ml	No	Ex vivo	[12]
G13	QRSVSNAAATRVCRTRGRSRW	-	Yes	Ex vivo	[42, 110]
GranF2	VCRTGRSRWRDVCNFMRRYQSR	-	Yes	Ex vivo	[42, 110]
proregion	SVFPQQTGQLAELQPQDRAGARASW MPMFQRRRRR	-	No	Ex vivo	
HNP-1	DCYCRIPACIAGERRYGTCTIYQGRWL FCC	2.5 µg/ml	Yes	mice	[111]
HCL2	ESTYQGHHTPPVQKGLRYGIILFITSEV FFFAGFF	-	No	In vitro	[112]
MIAP	GIGKFLKSKGKFGKA	300 µg/ml	No	Ex vivo	[113, 114]
Ub2	STLHLVLRLRG	5 µM	No	Ex vivo	[44, 49, 115]
RNase 3(1-45)	RPPQFTRAQWFAIQHISLMPPRCTIA MRAINNYRWCKNQNTFLR	10 µM	Yes	In vitro	[57]
RNase 7(1-45)	KPKGMTSSQWFKIQHMQPSPQACNS AMKNINKHTKRCKDLNTFLH	20 µM	Yes	In vitro	[57, 116]
Hepcidin	DTHFPICIFCCGCHRSKCGMCKCT	-	No	Ex vivo	[55, 117, 118]
D-LFcin17-30	FKCRRWQWRMKKLG	14.4 µg/ml *	No	mice	[119]
Lassomycin	GLRRLFADQLVGRRI-CO ₂ CH ₃	0.8 µg/ml	-	In vitro	[72]
Nisin A	IDhb AIDL Dha PGAK Abu GALMGAN MK Abu A Abu ANASIHVD ha L	60 µg/ml *	-	In vitro	[120]
lactacin 3147	AAD hb N Dhb FALADYWGNGAWAA bu L Abu HEAMAWAK	7.5 µg/ml *	-	In vitro	[62, 65, 121, 122]
E50-52	TTKNYNGVGCNSVNWQCQGNVWASC NLATGCAAWLCKLA	0.1 µg/ml *	No	In vitro	[71]
pK34	PRVIETKVHGREVTGLARNVSEENV DLAKRWIK	50 µg/ml	No	Mice	[75]
1-C134mer	H-Ntridec-NLys-Nspe-Nspe-NLys-NH 2	6.3 µM	Yes	In vitro	[123]
Ci-MAM-A24	WRSLGRTLRLSHALKPLARRSGW- NH ₂	-	No	Ex vivo	[124]

could also kill their hosts directly. Bacteriophage-derived peptidase CHAP_K, containing only the cysteine, histidine-dependent amidohydrolases/peptidases (CHAP) domain of endolysin LysK, possessed lytic activity against live clinical staphylococcal isolates and could eliminate staphylococcal biofilms [76, 77]. PlyG₁₀₆₋₁₆₅ (residues 106 to 165) was found able to specifically recognize *B. anthracis* spores, through binding to the exosporium of the spores [78]. These data demonstrated that bacteriophage-derived peptides represented attractive approaches for the future design of antibacterial agents [79].

Future Directions and Concluding Remarks

As shown in Table 1 and Fig. 1, anti-mycobacterial peptides reported so far displayed considerable diversity in their primary sequences, lengths, biological activities, sources and antibacterial mechanisms.

Fig. 1. The targets of anti-mycobacterial peptides

However, it is expected that there are still some obstacles to overcome before developing any of them into a therapeutic drug. A major challenge is that these peptides have a short half-life *in vivo* and may lose activity in physiological conditions. Secondly, these peptides have a larger size than traditional antibiotics, and how to deliver them and use them to clear intracellular mycobacteria infections would need special attentions. However, several recent studies have shown promising results. Liposomal vectors could be used to enhance these peptides intracellular delivery, reduce immunogenicity, and confer some protection from neutralizing antibodies [80]. Thirdly, the attributes of charge, hydrophobicity, salt-resistance, biocompatibility, as well as the resistance to antimicrobial peptides (AMPs) [81, 82], may hinder AMPs into clinical applications. For maximum efficacy *in vivo* and *in vitro*, these attributes would need optimizing for an antimicrobial peptide. Some strategies have been used to generate peptides with improved antimicrobial activity, such as site-saturation mutagenesis [83], D-amino acids isomerization [84], synthetic modifications [85-87], *De novo*-design AMPs [87-89] and high-throughput screening [90, 91]. These methods provide excellent ways for searching novel and efficient anti-mycobacterial peptides.

Another direction might be using anti-mycobacterial peptides as adjunct chemotherapy with conventional drugs against TB [92]. Combination of conventional anti-mycobacterial drugs with anti-mycobacterial peptides has been found able to prevent drug-resistance and enhance bactericidal action [93-95]. In combination with gold [96] or silver [97] nanoparticles, anti-mycobacterial peptides were enhanced to kill mycobacteria without harming the host cells. Other combinations have also been tried to develop more selective and/or more potent agents against antibiotics-resistant pathogens. One example was conjugating AMPs to antibiotics like vancomycin using click chemistry [98-100]. Another example was fusing the binding domain of FyuA, which is an outer membrane transporter protein of *Yersinia pestis*, to the N-terminus of T4 lysozyme similar to pesticin [101, 102]. Further understanding the mechanisms of action, toxicity and immunogenicity of the combinations will help to develop anti-TB drugs based on anti-mycobacterial peptides.

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