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Secretory and circulating bacterial small RNAs: a mini-review of the literature

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Abstract

Background: Over the past decade, small non-coding RNAs (sRNAs) have been characterized as important post-transcriptional regulators in bacteria and other microorganisms. Secretable sRNAs from both pathogenic and non-pathogenic bacteria have been identified, revealing novel insight into interspecies communications. Recent advances in the understanding of the secretory sRNAs, including extracellular vesicle-transported sRNAs and circulating sRNAs, have raised great interest.

Methods: We performed a literature search of the database PubMed, surveying the present stage of knowledge in the field of secretory and circulating bacterial sRNAs.

Conclusion: Extracellular bacterial sRNAs play an active role in host-microbe interactions. The findings concerning the secretory and circulating bacterial sRNAs may kindle an eager interest in biomarker discovery for infectious bacterial diseases.

Keywords: Small non-coding RNA, Bacterial RNA, Secretory RNA, Outer membrane vesicle, Circulating biomarker

Background

Small non-coding RNAs (sRNAs) are a class of post-transcriptional regulators in bacteria and eukaryotes. Bacterial sRNAs usually refer to non-coding RNAs approximately 50–400 nt in length that are transcribed from intergenic regions of the bacterial genome [1]. The first characterized bacterial regulatory sRNA was MicF RNA from *Escherichia coli*, which can down-regulate the major outer membrane protein OmpF [2]. Since then, the abundance of bacterial sRNAs and their significance in physiological responses have been much better appreciated, due to the application of a combination of cloning-based techniques and computational methods [3, 4]. Integrated data concerning bacterial-specific sRNAs have contributed greatly to unveiling the regulatory networks of major bacterial pathogens [1, 5]. However, a main question that remains to be addressed is how study results should be translated into clinical benefits.

Interestingly, recent advances in the characterization of sRNA-containing microvesicles have provided important insights to this field of research. Extracellular sRNAs in membrane-enclosed vesicles represent a novel class of active players in host-microbe communications and potential circulating biomarkers for infectious diseases. In this review, we survey the current stage of knowledge concerning secretory sRNAs in pathogenic bacteria, their detection in the circulation, and discuss their potential clinical applications.

Bacterial sRNAs in extracellular vesicles

Secretory products of microorganisms play active roles in microbe-microbe and host-microbe communications. Extracellular vesicles (EVs) are major vehicles for secretory products in both bacteria and eukaryotes [6]. In Gram-negative bacteria, EVs usually go by the name "outer membrane vesicles (OMVs)", which are generally produced by Gram-negative bacteria as part of their normal growth [7]. OMVs package a variety of bacterial products, including proteins, lipopolysaccharides (LPS), DNA fragments, and RNAs [7, 8]. OMVs were found to deliver virulence factors [9–11] and bacterial antigens within the human host [12–14]. The roles of OMVs in immune modulation have been studied intensively [8]; however, the biological significance of bacterial RNAs in OMVs or those of other secreted factors remain largely undetermined.

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In 2015, Ghosal et al. characterized the extracellular component of *Escherichia coli*, a model for Gram-negative bacteria [15]. The study demonstrated that the OMVs secreted by *Escherichia coli* substrain MG1655 contain abundant bacteria-derived, small non-coding RNAs. In the same year, Sjöström et al. reported that purified OMVs of *Vibrio cholerae* comprise sRNAs transcribed from intergenic regions [16]. To date, secretory sRNAs from a range of Gram-negative bacteria, including *Pseudomonas aeruginosa*, uropathogenic *Escherichia coli* strain 536, and *Porphyromonas gingivalis*, have been characterized in vitro [17–20]. In addition, Resch et al. reported for the first time the identification of non-coding RNAs enriched in EVs (reported as membrane-derived vesicles, MVs) from Gram-positive bacteria, group A *Streptococcus* [21].

To date, secretory bacterial sRNAs remain much less understood compared to their well-documented intracellular counterparts. Their sorting mechanisms, cellular targets, and involvement in biological regulation are largely unknown. Recently, Koeppen et al. demonstrated that sRNA52320 from *Pseudomonas aeruginosa* OMVs can be transferred into airway epithelial cells, and may attenuate the LPS-induced immune response by targeting interleukin (IL)-8 mRNA [17]. This is the first description of inter-kingdom regulation by sRNAs via bacterial OMVs. The studies presented above have preliminarily revealed the biological and pathological significance of secretory bacterial sRNAs.

Characterization of secretory microRNA-sized sRNAs

Interestingly, recent studies have identified a distinct set of secretory sRNAs, microRNA (miRNA)-sized sRNAs (msRNAs), which are comparable in size (~ 22 nt) to eukaryotic miRNAs. First systemically characterized by Lee et al. in *Streptococcus mutans*, msRNAs were found to be expressed by diverse bacterial species [22–25]. Recently, Choi et al. characterized secreted msRNAs in membrane vesicles from Gram-positive bacteria *Streptococcus sanguinis*, and in OMVs from three Gram-negative periodontal pathogens, including *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Treponema denticola* [19]. They also found that OMVs may deliver specific msRNAs to recipient T cells and suppress the production of IL-5, IL-13, and IL-15. This phenomenon is similar to the exosome-mediated transfer of miRNAs in eukaryotes but is less commonly observed. In addition, Gu et al. identified the msRNA Sal-1 in *Salmonella*, a model intracellular bacterial pathogen [26]. Sal-1 shares a number of biological features with eukaryotic miRNAs and can be released into the cytoplasm of host epithelial cells. Sal-1 can target iNOS in a miRNA-like manner and is likely to facilitate the intracellular survival of *Salmonella* [27]. In conclusion, secretory msRNAs are a class of active players in host-microbe interactions that deserves more attention in future studies.

Identification of bacterial sRNAs in human circulation

Circulating RNAs, which have been intensively studied in recent years, consist of a wide range of RNA species, including miRNAs and other non-coding RNAs [28]. Over the past decade, circulating miRNAs have become a class of promising minimal-invasive biomarkers for cancers and other diseases [29, 30]. It is remarkable that cell-free exogenous RNAs, including miRNAs encoded by DNA viruses [31, 32], sRNAs from parasites [33, 34], and plant- and food-derived RNAs [35–37], were also readily detected in the human circulation. However,

Table 1 A summary of the reviewed studies on secretory bacterial sRNAs

Origin	Secretion	Remark	Reference
Escherichia coli substrain MG1655	OMVs	The first detailed study on the secretory sRNA of *Escherichia coli*	[15]
Vibrio cholerae	OMVs	Transcribed from intergenic regions	[16]
Pseudomonas aeruginosa	OMVs	sRNA52320 targets host's immune response gene	[17]
Uropathogenic *Escherichia coli* strain 536	OMVs	sRNAs can be transferred into epithelial cells	[18]
Group A *Streptococcus*	MVs	From Gram-positive bacteria, miRNA-sized	[21]
Streptococcus sanguinis	MVs	From Gram-positive bacteria, miRNA-sized	[25]
Aggregatibacter actinomycetemcomitans, *Porphyromonas gingivalis*, and *Treponema denticola*	OMVs	From periodontal pathogens, miRNA-sized	[19]
Salmonella	Undetermined	Sal-1 targets iNOS in a miRNA-like manner	[26, 27]
Phylum *Firmicutes*	Circulation	Gut microbiome	[38]
Genera *Escherichia* and *Acinetobacter*	Circulation	Gut microbiome	[39]
Phylum *Proteobacteria*	Circulation	Gut microbiome	[36]
Mycobacterium tuberculosis	Culture supernatant and circulation	ASdes is detectable in the plasma from active tuberculosis patients	[41]

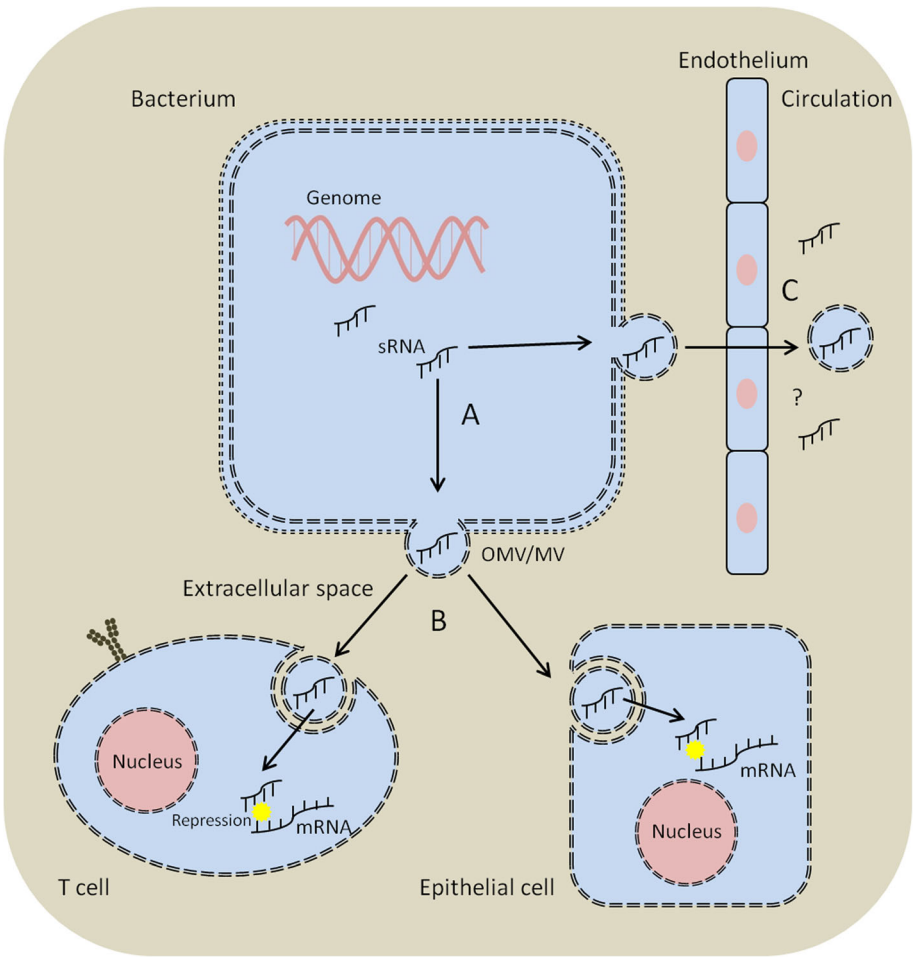

Fig. 1 The biological activities of secretory bacterial sRNAs. A. Bacterial sRNAs can be sorted into the OMVs in Gram-negative bacteria or MVs in Gram-positive bacteria; B. sRNAs carried by OMVs/MVs can be released into the extracellular space, taken up by recipient cells, and repress host mRNAs; C. Secretory bacterial sRNAs are detectable in the circulation of infected host; however, the mechanisms remain largely unknown

expression profiling of bacterial sRNAs in circulation, especially for pathogen-encoded sRNAs in patients with infectious diseases, has not been systemically investigated.

In 2012, Wang et al. studied host-microbiome interaction by analyzing plasma RNAs originating from exogenous species in detail using a next-generation sequencing technique [38]. The results showed that a significant amount of the reads were mapped to diverse microbial species, including phylum *Firmicutes*, a major bacteria phylum present in the human gut microbiome. Semenov et al. also stably detected sRNAs matching bacterial non-coding RNAs attributed to the genera *Escherichia* and *Acinetobacter* as well as other microorganisms in plasma from healthy donors [39]. Subsequently, Beatty et al. conducted a detailed study analyzing the expression of circulating exogenous sRNAs from 6 participants, which showed that the majority of the bacterial reads were from phylum *Proteobacteria*, indicating that their origin was the gut [36]. Another recent study assessing cell-free RNAs in the circulation of pregnant women has also drawn a similar conclusion [40]. The studies presented above suggest that sRNAs that originate from the gut microbiome are likely to be a main constituent of the circulating "bacterial footprints" under physiologic conditions.

Expression profiling of disease-associated bacterial sRNAs in vivo has yet to be systematically studied. However, several recent studies have helped to gain further insight into this field of research. Fu et al. conducted a series of experiments to identify the sRNAs secreted by *Mycobacterium tuberculosis* (MTB) [41]. Four sRNAs previously characterized by Arnvig et al., including ASdes, ASpks, AS1726, and AS1890, were readily detected in the supernatant of cultured MTB using quantitative polymerase chain reaction (qPCR) assays [42]. Interestingly, the sRNA ASdes was also detected in the plasma of patient with active tuberculosis; the detection rate was 55.56% (15/27). This inspiring discovery

suggests that cell-free bacteria-specific sRNAs can be released into the circulation, possibly from infected tissues. Notably, tuberculosis is known for a lack of early-stage diagnostic biomarkers. To our knowledge, a plethora of MTB-encoded sRNAs have been identified previously [42–46]; therefore, further investigations regarding the secretion of bacterial sRNAs may provide novel insight into the discovery of sRNA-based biomarker for tuberculosis and other bacterial infectious diseases. However, more questions concerning the secretory mechanisms and the tissues of origin of circulating bacterial sRNAs remain to be answered.

Conclusions

Recent extensive studies have unveiled novel aspects regarding the identification (Table 1) and the biological activities (Fig. 1) of secretory bacterial sRNAs, which have drawn increasing attention. First, the massive datasets obtained using deep-sequencing techniques and bioinformatics have shown that regulatory sRNAs can be transferred to host cells via membrane-enclosed vesicles from both Gram-negative and Gram-positive bacteria, representing a class of cross-species virulence factors of bacterial pathogenicity. Second, bacterial miRNA-sized sRNAs analogous to eukaryotic miRNAs were found to be secreted as active players in host-microbe interactions. Finally, accumulating evidence suggests that blood circulation is the interface between the host and microbiome under physiological and pathological conditions; therefore, bacterial sRNAs released into the circulation may be active players in, and even diagnostic biomarkers for, related diseases. Much like circulating miRNAs as biomarkers for cancer, the detection of circulating bacterial sRNAs should undergo rigorous investigation; however, the findings may kindle an eager interest in biomarker discovery for infectious bacterial diseases that are difficult to diagnose in the early stages.

Abbreviations

EV: extracellular vesicle; IL: interleukin; LPS: lipopolysaccharides; miRNA: microRNA; msRNA: microRNA-sized small non-coding RNA; MTB: *Mycobacterium tuberculosis*; MV: membrane-derived vesicle; OMV: outer membrane vesicle; qPCR: quantitative polymerase chain reaction; sRNA: small non-coding RNA

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Authors' contributions

Y.W and J. F collected the data. J. F wrote the manuscript. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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