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RESEARCH HIGHLIGHT

Towards the understanding of "Herbal RNA Code" for traditional medicine

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Recent emerging studies have unveiled the presence of millions of herbal small RNAs (sRNAs) in decoction of traditional medicines, suggesting their roles in cross-kingdom communication. Investigating the origins of herbal sRNAs, their delivery mechanisms, and modes of action in alleviating diseases could pave the way for innovative pharmaceutical engineering and novel clinical treatments.

Traditional herbal medicines, utilized for millennia in Asia, have demonstrated potency in treating and preventing various diseases, notably seen in artemisinin's Nobel-winning revolution in malaria treatment.[1] Beyond ongoing efforts to discover essential chemical compounds in these remedies, recent exploration has turned towards other complex components such as herbal sRNAs. The potential role of herbal sRNAs, mostly derived from fragmentation of longer RNAs (e.g., tRNAs and rRNAs),[2] has become a burgeoning area of interest.

In a recent effort, Cao et al. began to establish a comprehensive herbal sRNA expression database referred to as "Bencao sRNA Atlas", by analyzing water decoctions of 245 types of traditional Chinese medicines from the Chinese Pharmacopeia. They extracted and sequenced sRNAs, proposing a sequence-based nomenclature for each sRNA in the Bencao sRNA Atlas, which contains $>2 \times 10^7$ sRNA sequences detected in the decoctions.[3] Using the Rfam database for sRNA mapping, the authors annotated ~5% of the sequences, with a majority of them (>99%) derived from ancient structured RNAs such as tRNAs, rRNAs and snRNAs, and a small portion (<1%) from miRNAs and lncRNAs. Notably, 278 unique sRNA sequences were ubiquitously found across 208 species, 274 of which were mapped to known plant tRNAs and rRNAs (called tRNA-derived small RNAs (tsRNAs) and rRNA-derived small RNAs (rsRNAs),[4] respectively), suggesting sequence conservation of tRNAs and rRNAs across plant species. This is also consistent with the view that the fragmentation of structured RNAs (e.g., tRNA and rRNA) into sRNAs is an ancient way of sRNA biogenesis ubiquitously existing in all domains of life[4,5]; the resulting sRNAs could exert functions in different biological systems.[4,5]

Since >95% of the sRNAs remain unannotated, elucidating the Bencao sRNA Atlas in the future is a massive undertaking that requires a reliable reference database for the source herbs and more advanced tools to specifically annotate tsRNAs and rsRNAs.[6] Moreover, the Bencao sRNA Atlas is built upon a traditional sRNA sequencing method; tsRNAs and rsRNAs are known to carry various RNA modifications from their precursors, and some of these modifications interfere with adapter ligation and reverse transcription during cDNA library construction for RNA sequencing.[4] Recent methodological advancements could address these issues and enable generation of sRNA reads with less bias, particularly empowering the discovery of highly modified sRNAs such as tsRNAs and rsRNAs.[7] The application of these advanced methods would lead to a more comprehensive sRNA landscape for the Bencao sRNA Atlas. Given the regulatory functions of RNA modifications on RNA stability, structure and binding potential,[8,9] the sRNA modifications should be considered as an integral part of the "Herbal RNA Code", awaiting more comprehensive study with emerging technologies.[4]

Although herb-derived sRNAs are found in host circulation and cells, how herbal sRNAs enter the host system after oral administration remains largely unresolved. Several possibilities have been suggested, including delivery via encapsulation of sRNAs into extracellular vesicles, membrane RNA transporters, forming specific RNA structures, and/or forming complexes with RNA binding proteins.[5,10] Each pathway is supported by some evidence, but none can fully explain the delivery of these sRNAs. Jiang's group identified plant-derived exosome-like nanoparticles in herbal decoctions after boiling, which consist of lipids, sRNAs, small-molecule metabolites, and proteins.[11] They further showed that co-assembly of synthesized sRNAs with a lipid layer (which the team dubbed bencaosomes) can increase the stability of sRNAs and enable more effective in vivo delivery of the sRNAs. Interestingly, heating can facilitate the co-assembly process, suggesting that it might be harnessed as a powerful method for in vivo RNA delivery, which could be used in disease treatment in the future.

Drawing on the knowledge gained from the Bencao sRNA Atlas and the use of bencaosomes, Jiang's group further examined the functional and therapeutic effects of selected herbal sRNAs. In a mouse model of angiotensin II-induced hypertension, they demonstrated that oral administration of the bencaosome composed of sphingosine and a herbal rsRNA named XKC-sRNA-h3, can prevent angiotensin II-induced hypertensive cardiac damage and reduce kidney injury.[12] The anti-hypertensive effect potentially involves the rsRNA's complementarity to the 3′-UTR of mRNA encoding ACE, a key enzyme for generating angiotensin II, the active vasoconstrictor. Interestingly, a previous study reported that a honeysuckle-encoded rsRNA (originally reported as mir2911) can inhibit H1N1 viral replication through inhibiting H1N1-encoded PB2 and NS1 protein expression in a sequence-dependent manner. Administration of this rsRNA or honeysuckle decoction was shown

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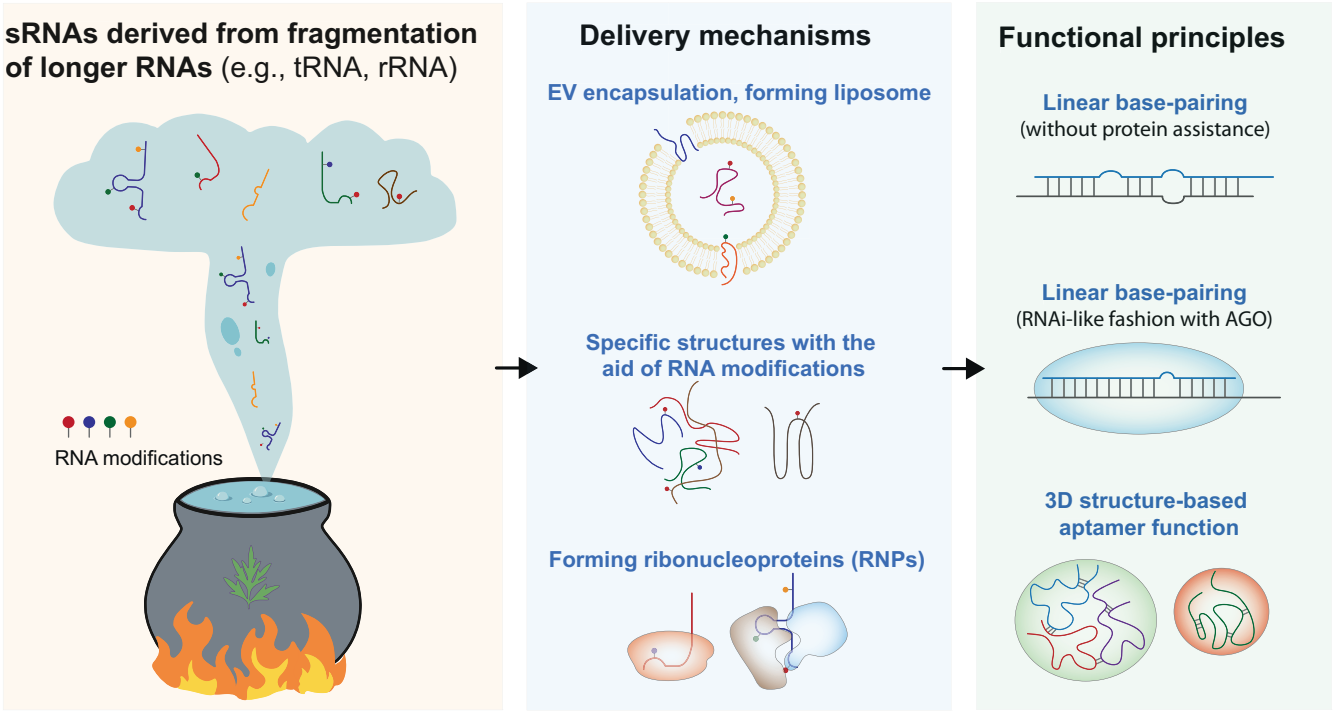

Fig. 1 Illustration depicting the generation of herbal sRNAs through the fragmentation of longer RNAs (e.g., tRNAs, rRNAs) in herbal decoctions, the possible pathways for their entry into the host system, and their potential mechanisms for regulating host cell functions. AGO Argonaute, EV extracellular vesicle.

to reduce mouse lethality by H5N1 infection.[13] These cases highlight the functional and therapeutic potential of rsRNAs, the most highly expressed sRNA category in the Bencao sRNA Atlas.

Notably, rsRNAs are also found to be the most highly expressed sRNAs in many mammalian tissues and cells.[4] Although they have not been extensively studied compared to other types of sRNAs such as miRNAs, their functional potential should not be overlooked. Notably, the modes of action of rsRNAs (and other sRNAs) may extend beyond the reported conventional RNAi-like mechanisms based on linear base pairing, but might adopt secondary or tertiary structures to bind a range of proteins or metabolites, or assemble higher-order structures with other RNAs and proteins to form large ribonucleoprotein complexes, functioning in an aptamer-like manner.

In fact, considering the vast sRNAs present in herbal decoctions (either from a single herb or combined herbs), it is most likely that the observed therapeutic effects of herbal medicine result from the combined action of multiple sRNAs. Indeed, in another study, thousands of types of herbal sRNAs were detected in the blood cells of COVID patients after consuming Toujie Quwen granules. The treatment led to a decrease in the number of differentially expressed genes, and gene target prediction analysis based on base pairing suggests that the observed effects are derived from combined actions of numerous sRNAs.[14] An important step towards understanding the complexity of the "Herbal RNA Code" would include the consideration beyond the effect of individual sRNAs but encompass the intricate information represented by the entire repertoire of sRNAs and RNA modifications, similar to other complex biological systems.[15]

To fully decode the herbal RNA code, future research should not only focus on the complete repertoire of sRNAs and their RNA modifications using advanced technologies, but also gain a better understanding of sRNA delivery into the host system, and explore a broader spectrum of functional principles beyond linear base pairing and reductionist approaches (Fig. 1). These would require understanding of the inherent complexity of biological systems from a systems biology perspective.

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ADDITIONAL INFORMATION

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