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New evidence of cross-kingdom regulation by plant microRNA: *Lycium barbarum* L.-derived miR162a for osteoporosis treatment

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In a recent work published in *Engineering*, Gu *et al.* have proposed that miR162a derived from *Lycium barbarum L.* can alleviate osteoporosis [1]. This study complements the theory of *Lycium barbarum L.* reinforcing kidney essence and strengthening the bones and further substantiates the significant role of microRNAs (miRNAs) derived from traditional Chinese medicine (TCM) in disease treatment.

Early investigations have revealed the important role of non-coding RNAs, including miRNA and siRNA, in maintaining cellular homeostasis, the regulation of physiological processes, and the intricate path toward disease progression [2–4]. RNAs are commonly acknowledged to predominantly reside within the confines of the cellular milieu, mainly to safeguard their integrity from the presence of numerous nucleases found in the extracellular domain. In our study published in *Cell Research* in 2008, we revealed a striking phenomenon: the stability of miRNAs within the serum and plasma of both humans and animals [5]. The discovery not only shed light on a previously unknown realm of extracellular RNA research but also paved the way for subsequent breakthroughs in the field. Furthermore, the subsequent identification and documentation of stable, cell-free miRNAs in various bodily fluids, such as saliva, urine, and breast milk, provided evidence for the widespread presence and functional significance of these extracellular RNA molecules (Table 1).

TCM has a rich history spanning several millennia in its application for disease prevention and treatment. However, the complex composition of TCM has hindered a clear understanding of their specific mechanisms of action and active compounds. In 2015, our team found that certain miRNAs in honeysuckle remained stable in the decoction even after boiling for 30 minutes (verified by sequencing and qPCR) [6]. MiR2911, the miRNA with the highest content in the honeysuckle decoction, was subsequently proven to have broad-spectrum antiviral properties [6,7]. These investigations demonstrate the phenomenon of

cross-regulation by plant-derived miRNA and suggest that miRNA may serve as a vital active component in the therapeutic effects of TCM (Figure 1). It also provided a referential preparation process for the prior treatment of TCM. Similarly, the latest research conducted by Gu and his team suggests that miR162a might be the medicinal composition of *Lycium barbarum L.*

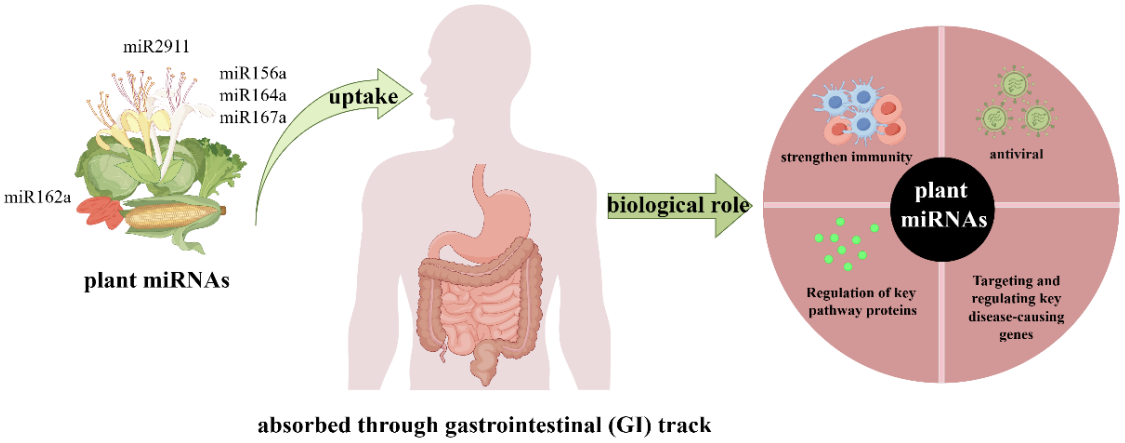

Figure 1. The uptake process of plant miRNAs and their biological functions. Dietary-derived miRNAs are absorbed through the gastrointestinal tract and then transported to target cells via the circulatory system to carry out their biological functions.

For thousands of years, *Lycium barbarum L.* has been recognized as an effective treatment for osteoporosis, yet the substance contributing to its activity remains unknown. To search for potential active substances of *Lycium barbarum* L., Gu *et al.* utilized high-throughput sequencing and observed elevated levels of miR162a in the blood of mice fed with *Lycium barbarum L.*. These *Lycium barbarum L.*-derived miR162a promoted osteogenic differentiation in bone marrow mesenchymal stem cells, zebrafish, and a mouse model of osteoporosis. Fluorescein-labeled tracing experiments demonstrated that miR162a was mainly absorbed through systemic RNA interference defective transmembrane family member 1 (SIDT1) in the stomach. Bioinformatics predictions and luciferase reporter assays identified that miR162a targeted the nuclear receptor corepressor (NCoR) (Figure 2). Finally, Gu *et al.* introduced a transgenic technology to deliver miR162a by using *N. benthamiana leaves* and confirmed that it can vigorously protect against osteoporosis in mice. The therapeutic effect of miR162a on osteoporosis is comparable to other miRNA therapies currently reported [8,9]. Gu's work further validates our previous findings: miRNAs derived from TCM can exist stably in the blood of both humans and mice and have therapeutic effects. More importantly, this research reaffirms the cross-regulatory properties of plant-derived miRNAs and provides additional evidence for miRNAs as key active components in TCM. It also offers a potential strategy for developing drugs based on miRNAs from TCM.

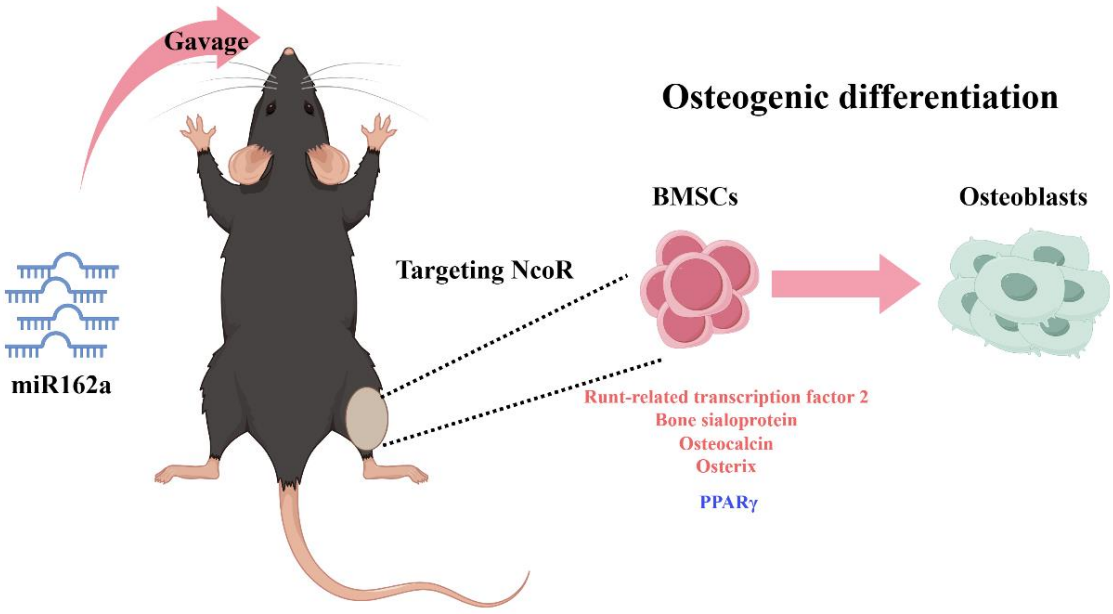

Figure 2. The mechanism of miR162a in treating Osteoporosis. miR162a induces osteogenic differentiation of BMSCs by directly targeting NcoR. After treatment with miR162a, the expression of NcoR and PPARγ in mice decreased (indicated in blue) while classical osteogenic markers: runt-related transcription factor 2 (RUNX2), bone sialoprotein (BSP), osteocalcin (OCN), and osterix were significantly upregulated (indicated in red). BMSCs: bone marrow mesenchymal stem cells; NcoR: nuclear receptor corepressor; PPARγ: Peroxisome Proliferator-Activated Receptor Gamma.

Currently, the delivery of miRNA drugs primarily relies on multifunctional nanoparticles, liposomes, and viral vectors, which can enhance the in vivo delivery efficiency of miRNA [10,11]. These exogenous carriers or molecules may give rise to potential issues such as cell toxicity and immunogenicity. The stable, efficient, and safe delivery of miRNA drugs *in vivo* holds great potential for enhancing their clinical application. Our previous research reported the production of small interfering RNA in lettuce, which can inhibit the expression of the HBsAg gene and reduce liver damage in a mouse model [12]. Meanwhile, we have developed a controllable siRNA self-assembly and delivery system that can control gene expression in a target-driven model [13]. Gu and his colleagues introduced a novel miRNA delivery system by developing miR162a-overexpressed transgenic *N. benthamiana* leaves which were selected for its easy planting, short life cycle, and large-scale production [1]. These studies have important implications for the selection of novel miRNA drug delivery vectors and the development of transgenic miRNA-based plant medicines.

Table 1. Plant-derived miRNA studies of cross-kingdom regulation.

miRNA	Source	Target cell or animal	Target gene	Function	Reference
miR168a	Rice	Humans/mice	LDLRAP1	Decreases LDL removal from	[14]
Mol-miR168a	Moringa oleifera	Human hepatoma cell line G2 (HepG2) cells	SIRT1	Absorbed	[15,16]
miR166a, miR156a, miR157a, miR172a	Tomato	Human breast milk	/	Absorbed	[17,18]
miR156a	Cabbage, spinach, and lettuce	Human aortic endothelial cells	JAM-A	Suppresses the progression of atherosclerosis	[19]
miR2911	Honeysuckle	Mice	/	Targets various influenza A subtypes	[6]
miR375	Murine milk	Mice	/	Rapid degradation of milk miRNAs in the intestinal fluid	[20]
miR156a, miR164a, miR167a	Corn	Mice	/	Absorbed	[21]
miR172, miR166, miR167, miR168	Soy/fruit substance	Macaques	/	Absorbed	[22,23]
zma-miR164a-5p	Maize	Pigs	OTX1, PLAGL2, CSPG4	Absorbed	[24,25]

In summary, the research conducted by Gu *et al.* further validates the cross-regulatory nature of plant-derived miRNAs. It also suggests that miRNAs are very likely to be one of the effective components of TCM, enriching the scientific connotation of Traditional Chinese Medicine. The development of genetically engineered plants for the synthesis of artificial

miRNA has also provided a novel, safe, effective, and economically feasible delivery system for miRNA drugs, and simplifies the administration method, making oral administration possible. However, there are still challenges for this study to transition into clinical application. While confirming miR162a absorption into the blood, questions remain about whether genetically modified plants or *Lycium barbarum L.* can deliver adequate amounts of miR162a for absorption and therapeutic effect in humans. Investigations into the pharmacokinetics and biodistribution of miR162a in vivo are also necessary. Additionally, further basic research on miR162a is required to broaden its scope for clinical application.

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Conflicts of interests

The authors declare no conflicts of interest.

Authors' contribution

Conception and design: Y.W.; Writing and revision of the manuscript: S.Z.

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