Molecular Therapy
Nucleic Acids
Commentary

Plant/food-derived small non-coding RNAs: Critical determinants to protect heart against ischemia/reperfusion injury

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Traditional Chinese medicine (TCM), born in the third millennium BC, is linked to the emperors Huang Di and Shen Nong. Huang Di is said to have established its general principles and contributed to the development of acupuncture, while Shen Nong established the first knowledge of dietetics and herbalism. Plants have been used in medicine, providing many drugs, from the earliest times to the present, and Chinese herbal products have been studied for many medical problems, including stroke, heart disease, mental disorders, and respiratory diseases. They furnish natural compounds (lipids, micro-elements, vitamins, saponins, alkaloids, and flavonoids) that may modulate gene expression (e.g., microRNAs) in treated organisms, but until a few years ago, it was unthinkable that they could directly supply RNA molecules. It is known that microRNAs (miRNAs) can survive outside the cell, allowing intercell communication and supporting their higher stability compared with mRNAs. Although still debated, it was shown that small RNA molecules may be absorbed by the epithelium of the digestive tract,[1] and preclinical data (not yet clinical studies) support the potential use of the oral route for the local delivery of formulated nucleic-acid-based drugs.[2]

Kua Hu and colleagues[3] discovered the presence of a small RNA, derived from the processing of $tRNA^{Gln(UUG)}$ of ginseng, that, modulating the expression of a specific long non-coding RNA (MIAT), induces the expression of the vascular endothelial growth factor (VEGF), an important angiogenic factor. VEGF is critical for initiating early cellular responses to hypoxia caused by ischemic events.

Their work supports the now-accepted importance of non-coding RNAs in gene regulation and highlights new compounds (small RNAs derived from tRNAs) derived from ginseng that function in a miRNA-like manner in mammalian systems. This work opens a new view in TCM and more in general in diet importance: the possibility of having active compounds in herbals holding great potential as RNAi-based therapeutics.

The study leaves open some important questions related to the specific ability of ischemia/reperfusion (H/R)-injured cells to assimilate the nude small RNA, avoiding its toxicity in non-injured cells, and the possibility of alternative functions of the small RNA in association with the pleiotropic activity of miRNAs. For example, the authors showed the impact on mitochondrial network of the tRNA-derived small RNA without discussing its ability to modulate important genes regulating mitochondrial shape like optic atrophy protein 1 (OPA1), mitofusin 1 (MFN1), MFN2, dynamin 1-like (DNM1L), or fission, mitochondrial 1 (FIS1). An important therapeutic development highlighted from the authors is based on the ability of $tRNA^{Gln(UUG)}$-derived small RNA to modulate cytoskeletal disorganization. Cytoskeletal disorganization is typical of not only cardiac diseases but also neurodegenerative diseases or cancer. Therefore, we might think of applying this small RNA also to evaluate the treatment of diseases other than H/R injury.

Ischemia occurs when the necessary blood supply (and consequently oxygen) in organs or tissues is lost. It can depend on various causes such as compression of blood vessels, atherosclerosis, blood clot formation (thrombosis), or embolism and results in myocardial infarction (MI) when it affects the heart for a prolonged time. This is a serious problem considering that each year about 1 million people in the USA die from heart attack. The most common cause of myocardial ischemia is atherosclerosis, which is caused by the deposition of cholesterol on the arterial walls. Therefore, many drugs deputed to control atherosclerosis base their activity on reducing circulating cholesterol. The limited or absent ability of adult cardiac tissue to regenerate results in loss of function once it suffers damage with the substitution of cardiac muscle with fibrotic tissue. In lower vertebrates, where cardiac regeneration is present, formation of new vasculature following injury is vital for regeneration. Therefore, a functioning dynamic vasculature is critical for a successful regenerative response. To this purpose, it is essential that fine-tuning of gene expression that can be regulated also by non-coding RNAs (ncRNAs). Less than 2% of the human transcriptome encodes protein-coding RNAs, while the majority are ncRNAs, including ribosomal RNAs, tRNAs, miRNAs, long ncRNAs (lncRNAs), circular RNAs (circRNAs), and other small RNAs. ncRNAs play a crucial role in both the regulation of cardiac development and the treatment of myocardial ischemia.[4] miRNAs (sncRNAs 21–24 nt long) act as signaling molecules strongly involved in cardiovascular disorders and have been successfully used to treat diseases associated with cardiovascular syndromes (Table 1). miRNAs can originate either from the canonical pathway, based on the transcription of specific genes and maturation of the synthesized transcripts by specific RNAses, or through alternative pathways that process tRNAs, small

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Table 1. Small RNAs (antisense and short interfering RNAs) used in the treatment of cardiovascular-related syndromes

Product (commercial name; developer/manufacturer)	Length	Modifications	Route of administration	Indication	Target organ	Target gene and mechanism	Effects	Approval
Antisense oligonucleotides (ASOs)								
Mipomersen (Kynamro; Ionis Pharmaceuticals, Kastle Therapeutics)	20-mer	PS, 2′-MOE, GapmeR	subcutaneous	familiar hypercholesterolaemia (FH)	liver	apolipoprotein B (ApoB) mRNA	reduce severe hypercholesterolaemia	2013 (FDA); 2019 (withdrawn)
Inotersen (Tesgedi; Ionis Pharmaceuticals, Akcea Therapeutics)	20-mer	PS, 2′-MOE, GapmeR	subcutaneous	hereditary transthyretin amyloidosis	liver	transthyretin (TTR) mRNA	transthyretin is the target of this ASO as in the case of patisiran	2018 (EMA), 2018 (FDA)
Volanesorsen (Waylivra; Ionis Pharmaceuticals, Akcea Therapeutics)	20-mer	PS, 2′-MOE, GapmeR	subcutaneous	familiar chylomicronaemia syndrome (FCS)	liver	apolipoprotein C3 (ApoC3I) mRNA	reduces triglyceride levels	2019 (EMA)
Small interfering RNAs (siRNAs)								
Patisiran (Onpattro; Anylam Pharmaceuticals)	21-nt ds	2′-*O*-Me	intravenous	hereditary transthyretin amyloidosis	liver	transthyretin mRNA	modulate the accumulation of amyloid deposits in peripheral nerves, the heart, and other organs, allowing reductions in left-ventricular wall thickness, improved longitudinal strain, and reduced pro-BNP levels.	2018 (EMA), 2019 (FDA)
Inclisiran (Leqvio; Novartis Pharmaceuticals)	22-nt ds	PS, 2′-*O*-Me, 2′-F, GalNAc conjugated	subcutaneous	primary hypercholesterolaemia or mixed dyslipidaemia	liver	proprotein convertase subtilisin/kexi)*n* type 9 (PCSK9) mRNA	Upregulation of hepatocyte low-density lipoprotein (LDL) receptors and consequent reduction of plasma LDL-cholesterol levels	2020 (EMA), 2021 (FDA)

GalNAc, N-acetylgalactosamine; PS, phosphorothioate modification; 2′-O-methoxy-ethyl Bases, 2′-MOE; 2′-Fuoro, 2′-F.

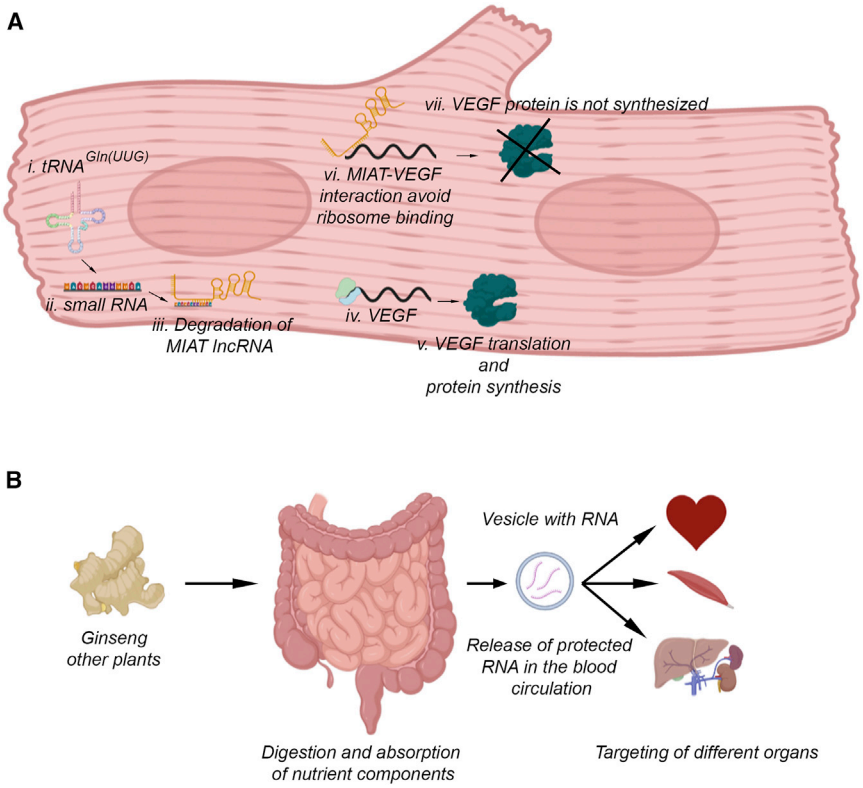

Figure 1. Activity of small RNAs

(A) Image representing a cardiomyocyte with two nuclei and the exemplification of the activity of the small RNA derived from the $tRNA^{Gln(UUG)}$ as proposed by Hu [3]. The maturation of $tRNA^{Gln(UUG)}$ (i) allows the production of a small RNA (ii) that is able to interact with the lncRNA MIAT inducing its degradation (iii). The absence of the lncRNA MIAT allows ribosome binding to the mRNA coding for VEGF (iv) and therefore the synthesis of the corresponding protein (v). The translation of the mRNA for VEGF is blocked in the presence of the lncRNA MIAT. MIAT is able to bind with VEGF mRNA, avoiding the binding of ribosomes to the same mRNA (vi) and consequently the production of the protein (vii). (B) Small RNAs from ginseng or other plants may pass through the digestive tract and can be adsorbed by the digestive epithelium.

nucleolar RNAs, or introns of coding genes.[5] They are able to bind to both DNA and RNA, inducing translational repression or mRNA degradation.[6] miRNA biology is further complicated by the possibility that lncRNAs or circRNAs interact with miRNAs, avoiding regulation of their targets, or lncRNAs may modulate mRNA translation directly interacting with the mRNA. Furthermore, miRNAs may be encapsulated in cytoplasmic bodies with proteins that protect them or in vesicles permitting not only intracellular gene regulation but also extracellular programs. These aspects provide opportunities to assess their presence in plant cells, which are often eaten raw, limiting nucleic acid degradation by cooking even if they are stable also in the cooked food,[7] and to study stochastic interactions in the complex cellular environment. Interactions between exogenous plant sncRNAs and host mRNAs could allow alterations in the genetic regulation of host cells, where these orally acquired plant sncRNAs find their complementary targets and modulate transcriptional or post-transcriptional processes. Recent findings place two classes of sncRNAs, miRNAs and tRNA-derived small fragments, in such a scenario, acting as key pieces in *trans*-individuals/species cross-talk.

Hu and colleagues[3] have demonstrated the importance of this *trans*-specific regulation, evidencing its ability to interfere with the interaction between the mRNA for VEGF and the lncRNA MIAT allowing the production of VEGF (Figure 1A). The maintenance of VEGF expression during the ischemic event is therefore particularly important since it regulates angiogenesis, which is essential for the salvage of ischemic myocardium at the early stage of MI. If we think of herbals as foods enriched with natural RNA "drugs," we have to face the problem of their assimilation through the digestive tract, where the stomach pH remains a barrier. Interestingly, the 2′-O-methyl groups on the ribose of the last nucleotide, a modification only present in plant miRNAs, confer that they are more stable with respect to mammalian miRNAs, and therefore their deterioration rate is kept to a minimum, allowing their distribution to other districts in the body (Figure 1B). Blood is an excellent vehicle to transport to other tissues miRNAs that are absorbed from the intestinal epithelium. Therefore, to test the possibility of inter-kingdom communication via miRNAs, plant miRNAs, for example, may be searched in the blood of individuals who have ingested them. Keeping in mind the possible artifacts and errors in the evaluation of the presence of miRNA plant-derived in the blood of treated organisms, here we discussed miRNA transport from plants to animals as a mode of cross-kindom gene regulation. According to WHO, more than 70% of the population of developing countries rely upon traditional medicinal sources for the treatment of their ailments due to paucity of modern medical facilities, and they are probably the principal beneficiary of this cross-kingdom gene regulation. There are also industrial realities that are developing this concept, (https://www.mirnagreen.com/) but since investigations in this area have been initiated very recently and limited information are available, the experimental validation with animal models or human cell lines is also necessary to examine the protective or ameliorative effects of food-borne miRNAs.

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DECLARATION OF INTERESTS

The author declares no competing interests.

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