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MicroRNAs and the Genetic Network in Aging

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Abstract

MicroRNAs (miRNAs) comprise a class of small RNAs important for the posttranscriptional regulation of numerous biological processes. Their combinatorial mode of function, in which an individual miRNA can target many genes and multiple miRNAs share targets, makes them especially suited for regulating processes and pathways at the "network" level. In particular, miRNAs have recently been implicated in aging, which is a complex process known to involve multiple pathways. Findings from genome-wide miRNA expression profiling studies highlight three themes in miRNA function during aging: many miRNAs are differentially expressed, many such miRNAs target known aging-associated pathways, and there are global trends in miRNA expression change over time. In addition, several miRNAs have emerged as potentially coordinating multiple pathways during aging. Elucidating the underlying network structure of genes and miRNAs involved in aging processes promises to advance our understanding of not only aging and associated pathogenesis but also how miRNAs can connect disparate pathways.

Introduction

MicroRNAs (miRNAs) are endogenous small RNAs that regulate gene expression largely at the posttranscriptional level and have gained increasing attention in the last decade. Since their discovery in the nematode *Caenorhabditis elegans*, miRNAs have been identified in a variety of animals and plants, and their reported functions span many pathways and processes including timing of developmental decisions, cell differentiation, oncogenesis, and organismal aging.[1,2] The human genome codes for over a thousand miRNAs (miRBase release 19),[3–6] and computational predictions suggest that miRNAs may regulate nearly one-third of all human genes,[7] making miRNAs one of the largest "families" of regulatory molecules. Understanding how miRNAs fit into and interact with the overall genetic landscape is a major goal in miRNA research.

The role of miRNAs in aging has only recently begun to be investigated. Aging was long considered a result of random, accumulating molecular deterioration, but research in the last three decades since the isolation of the first genetic lifespan mutants in *C. elegans*[8–10] has shown a large genetic component influencing organismal aging rates. We now know that aging is shaped by a network consisting of multiple partially overlapping genetic pathways, many of which center on insulin signaling (see Refs. 11 and 12 for review). The involvement of miRNAs in aging therefore is particularly interesting in light of two key features of miRNA-mediated regulation: a single miRNA can target many genes, and multiple miRNAs can target the same mRNA.[13] This mode of function suggests that miRNAs act as "network-level" regulators modulating complex phenotypes by integrating multiple inputs and outputs. Thus, in the context of aging research, miRNAs may help us understand how diverse genetic pathways are connected and regulated.

This review will present evidence for several conserved roles of miRNAs in determining organismal aging rates. We will highlight some themes learned from genome-wide miRNA expression studies during aging. Other reviews have discussed specific miRNAs and how they have been associated with particular aging-related biological processes.[14,15] Here, we will instead focus on how miRNAs connect these processes as potential upstream regulatory factors.

Genetic pathways in aging and *lin-4*

The genes and pathways that are involved in aging rate and lifespan determination have been studied extensively. Basic research conducted in model organisms, particularly *C. elegans*, has identified many single-gene mutants demonstrating lifespan phenotypes, and genome-wide expression studies have revealed distinct expression profiles associated with aging.[16–18] We know from these studies that specific biological processes are affected with advancing age and that a network of multiple, yet specific, genetic pathways are involved (for reviews, see Refs. 11 and 12).

The best-characterized aging-associated pathway is insulin/insulin-like growth factor 1 (IGF-1) signaling (IIS). The core components of this pathway and their effects on lifespan are highly conserved: from nematodes to mice, reduced insulin signaling results in extended lifespan.[19] For example, in *C. elegans*, a mutation in the *daf-2* insulin receptor ortholog more than doubled the 2-week lifespan of wild-type worms.[10] Inactivation of the IGF-1 receptor gene as well as the insulin receptor substrate gene IRS1, which acts downstream of the IGF-1 receptor, in female mice similarly extended lifespan significantly.[20,21] Furthermore, longevity in humans has been associated with variations in IIS pathway genes, particularly FOXO3A transcription factor.[22–26]

IIS plays a central role in orchestrating many cellular processes involved in aging. (We refer readers to Ref. 19 for a review on IIS.) At the core of IIS is a phosphorylation cascade, triggered by the binding of insulin or an insulin-like molecule (e.g., IGF-1) to the insulin receptor, culminating in the phosphorylation of forkhead (FOXO) transcription factor that prevents it from localizing to the nucleus. Multiple internal and external signals that influence aging rate converge on the IIS pathway, often on FOXO.[27] Some examples of lifespan-determining signals that require DAF-16/FOXO activity include reproductive status[28] and environmental stress via *sir-2.1* and Jun kinase signaling pathway.[29,30] FOXO in turn regulates the transcription of many downstream effector genes that affect various cellular processes including metabolism, apoptosis, and stress resistance.[31–33] It is important to note that IIS-independent pathways that affect lifespan have been reported; these include DNA damage checkpoint signaling, developmental and behavioral timing pathway, and mitochondrial genes.[34–36] We do not know whether or how these pathways are connected to influence aging.

One of the earliest reports of miRNAs associated with lifespan determination also invoked insulin signaling. Our laboratory reported that a mutation in *lin-4*, a heterochronic miRNA studied extensively in the regulation of developmental timing in *C. elegans*,[37] shortened adult lifespan.[2] This phenotype was dependent on the expression of a *lin-4* target, *lin-14* transcription factor. In turn, the effect of LIN-14 on lifespan was mediated by the insulin signaling pathway, requiring the functions of DAF-2 and DAF-16 FOXO homolog, and by the heat-shock transcription factor HSF-1.[2] HSF-1 acts downstream of IIS and couples signaling with stress response; *hsf-1* mutants demonstrated shortened lifespan.[38,39] This study not only demonstrated that a miRNA can influence lifespan but does so through pathways with known importance in aging. Further, these results suggest that *lin-4* links upstream IIS with downstream heat stress response, possibly to better coordinate the outputs of both pathways.

Genome-wide studies of miRNAs in aging

In an effort to identify other miRNAs affected by aging, genome-wide miRNA expression has been profiled extensively in *C. elegans* (whole animal).[40–42] Profiling has also been conducted in a variety of tissues and cell types in additional systems. These include the fruit fly *Drosophila melanogaster* brain[43]; mouse brain,[44–46] liver,[47,48] and cardiac tissue[49]; and human skeletal muscle[50] and blood cells.[51–53] These studies found that many miRNAs are differentially expressed between old and young subjects. We henceforth refer to these differentially expressed miRNAs as "aging-associated" miRNAs, summarized in Table 1.

One caveat of studies in specific tissue/cell types is the extent to which they represent aging of the whole organism. It is known that tissues age at different rates.[54] While it can be difficult to extrapolate findings from specific tissues to aging of the whole organism, there are, nonetheless, valuable lessons to gather about both tissue-specific and organismal aging.

Several themes arise from these genome-wide studies. First is that multiple studies have identified common, and often evolutionarily conserved, aging-associated miRNAs. These miRNAs may represent particularly important regulators during aging. Second, aging-associated miRNAs often target pathways and processes that influence aging (Fig. 1). Indeed, some miRNAs target multiple aging-related pathways suggesting that miRNAs may have a role in coordinating the outputs of those pathways. Finally, there are clear global trends in miRNA expression change during aging. This suggests that a common process, such as miRNA transcription or biogenesis, may be altered with aging, changing large numbers of miRNA levels simultaneously.

Identification of common aging-associated miRNAs

Common aging-associated miRNAs have been found between published reports. Three separate studies of miRNA expression change with age have

Table 1. Summary of aging-associated miRNAs and their associated species and functions

miRNAs	Species	Aging-associated pathways	References
miR-34	*C. elegans*, *D. melanogaster*, *Mus musculus*, *Rattus norvegicus*	DNA damage response, senescence, cell death	40–43, 45, 60–63
let-7	*C. elegans*, *D. melanogaster*, *M. musculus*, *Homo sapiens*	DNA damage response, mitochondrial respiration, germline maintenance	40–42, 45, 48, 50, 53, 69, 70, 75
miR-71	*C. elegans*	DNA damage response, IIS, germline signaling	40–42, 67
lin-4	*C. elegans*	IIS, stress response	2
miR-239	*C. elegans*	IIS	40–42
miR-35	*C. elegans*	?	40–42
miR-38	*C. elegans*	?	40–42
miR-43	*C. elegans*	?	40–42
miR-70	*C. elegans*	?	40–42
miR-30d	*M. musculus*	?	44, 47
miR-468	*M. musculus*	?	44, 47
miR-669b	*M. musculus*	?	44, 47
miR-709	*M. musculus*	?	44, 47
miR-246	*C. elegans*	?	41

been performed in *C. elegans*.[40–42] While not all reported aging-associated miRNAs overlap, all three studies saw changes in the expression levels of *let-7*, miR-34, miR-35, miR-38, miR-43, miR-70, miR-71, and miR-239. It is plausible that these miRNAs function cooperatively to regulate processes during aging. However, it is important to point out that though alterations were consistent, the directions of change, surprisingly, were not. Notably, some of these miRNAs are conserved in human (*let-7*, miR-34, and miR-43)[55] and thus may be important for human aging and lifespan determination.

In mouse, Li *et al*. and Maes *et al*. found commonly upregulated miRNAs in two different aging tissues—the liver and the brain.[44,48] They suggested that these miRNAs (miR-30d, miR-34a, miR-468, miR-669b, and miR-709) may contribute to the general aging process, whereas miRNAs found only in one tissue may be implicated in tissue-specific aging.[44] However, of these core aging miRNAs, only one (miR-669b) was found to be upregulated in old *versus* young brains of the long-living Ames dwarf mice.[46] None were found in a study of aging heart tissue[49] or a different study on the aging brain.[45]

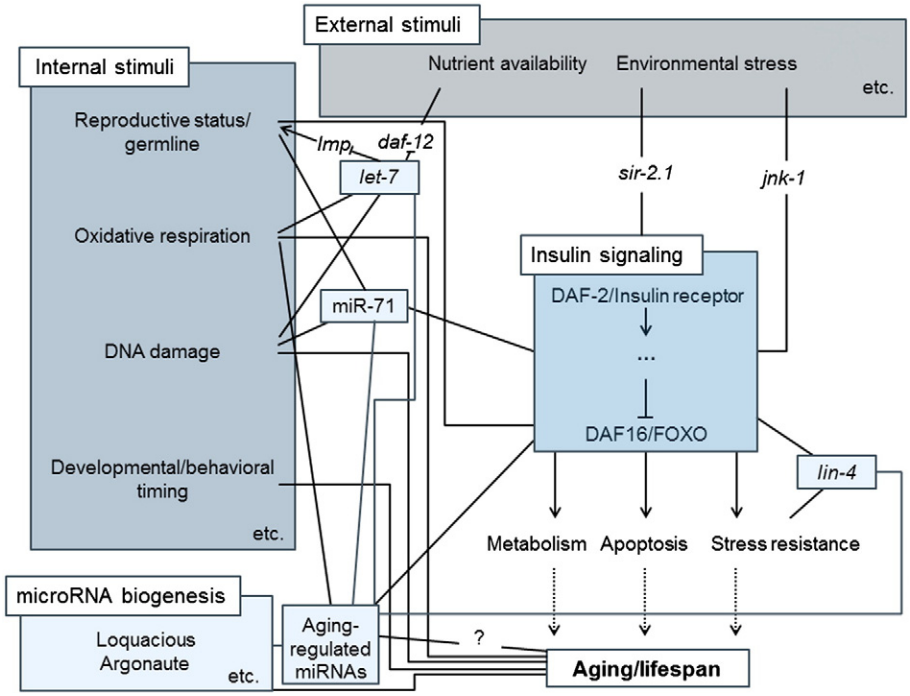

Fig. 1. miRNAs are part of a complex genetic network implicated in aging. Individual and groups of miRNAs are associated with aging and aging-related pathways that mediate signals from the external and internal environments and center on the insulin signaling pathway. In particular, *let-7*, miR-71, and *lin-4* are connected to multiple pathways.

The different experimental methods and statistical thresholds applied in each study may partly explain why they did not identify overlapping sets of aging-associated miRNAs.

One highly conserved miRNA was identified in multiple aging studies across various organisms and tissues. miR-34 belongs to 1 of only 19 miRNA families conserved at a sequence level from nematode to mammals.[56] Moreover, the functions of miR-34 in DNA damage response, senescence, and cell death are highly conserved,[57–59] and as DNA damage signaling is a known pathway affecting lifespan,[34] this suggests that conserved regulation by miR-34 may also exist for aging-associated processes. Its reported expression changes and effects on lifespan, however, have not been consistent. Increased expression with age was reported in *D. melanogaster* brain, *C. elegans* whole animal, rat liver, and several mouse tissues,[40–42,60,61] while decreased expression was observed in the mouse brain.[45,62] In *C. elegans*, *mir-34* mutants demonstrated extended lifespan in one study[63] (but not in another),[41] but in *D. melanogaster*, overexpression of miR-34 extended lifespan and protected flies from neurodegenerative disease.[43] Nonetheless, studies point to the importance of miR-34 in lifespan determination across phyla, and further research may unveil conserved regulation mediated by miR-34 that is crucial to normal aging.

Aging-associated pathways are targeted by aging-associated miRNAs

Many aging-associated miRNAs seem to interact with genes and pathways that are relevant to aging. It is worth noting here that the functions of most miRNAs are not known due to the fact that the vast majority of these were identified through cloning and computational prediction alone.[56,64–66] The functions of some age-associated miRNAs have been inferred from classic genetic characterizations of individual miRNA mutants while others have been predicted solely from mRNA target predictions coupled with bioinformatics analyses of those targets. Experimentally validating miRNA functions and their putative associations with specific pathways remains to be a challenge.

Many *C. elegans* strains with mutations for aging-associated miRNAs have been characterized in aging,[41,67] made possible by a large collection of miRNA loss-of-function mutants.[68] When characterized during development, most of these strains did not demonstrate gross abnormalities[68]; nevertheless, mutations for some aging-associated miRNAs have resulted in lifespan phenotypes[41,67]: *mir-71* and *mir-246* mutants demonstrated shortened lifespan, and *mir-239* mutants showed extended lifespan. Overexpressing these miRNAs resulted in the respective opposite phenotypes, suggesting specific involvement in aging. Further, the lifespan phenotypes of *mir-71* and *mir-239* mutants were dependent on IIS[41] and FOXO.[67] miR-71 interacts with at least two other aging-associated pathways: its activity was necessary for the long lifespan of both the DNA damage response gene *cdc-25.1* knockdown animals and of germline-ablated animals.[41,67] These findings suggest that miR-71 may act as a link between IIS, the DNA damage checkpoint pathway, and germline signaling. Particularly, the connection with the DNA damage checkpoint pathway is interesting because it functions independently of IIS in lifespan determination.[34]

The miRNA *let-7* may also link multiple aging-associated pathways. *let-7* is differentially expressed in many aging studies.[40–42,45,48,50,53,69,70] Nuclear hormone receptor *daf-12* is a known *let-7* target,[71] and mutations in *daf-12* affect longevity.[72] Further, DAF-12 activity is necessary for the germline signal that determines lifespan.[28] *let-7* has also been associated with DNA damage checkpoint genes and mitochondrial respiration genes[42,50]; though direct genetic interactions have not been validated, *let-7* may directly or indirectly coordinate these pathways. Furthermore, *let-7* and *daf-12* have been implicated in feedback signaling that is important for the specification of developmental fate in response to environmental cues in *C. elegans*.[73,74] While there is currently no evidence for this interaction during aging, if it exists, it may act to reinforce a specific lifespan phenotype. Unfortunately, most *C. elegans* *let-7* alleles cause developmental defects making aging studies difficult to interpret.

A recent study described a role for *let-7* in the age-related decline of the *Drosophila* testis stem cell niche. Toledano *et al*. found that, in old *Drosophila* testis, *let-7* targets *Imp* (*I*GF-II *m*essenger RNA-binding *p*rotein), which has a role in germline stem cell maintenance by stabilizing the self-renewal factor, *upd*, from being degraded by a short interfering RNA.[75] Other miRNAs that function to link multiple pathways may also have dual functions as genetic switches during aging.

Most effects of individual miRNAs on target expression levels are thought to be subtle.[76] Thus, in addition to examining individual miRNAs and their putative targets, it is also informative to investigate the processes that aging-associated miRNAs target as a collective. In a study in the aging mouse brain, Li *et al*. found that 27 out of 70 upregulated miRNAs target mitochondrial genes.[44] They showed by mass spectrometry that these genes are indeed affected during aging and correlated with the expression changes of the miRNAs that putatively target them. Ibañez-Ventoso *et al*. found that 31 out of 50 age-associated miRNAs target gerontogenes, which they identified from the literature; furthermore, many aging-associated miRNAs were predicted to target genes in the IIS pathway.[40] Our laboratory

has shown that nearly one-sixth of genes predicted to be targeted by one or more age-associated miRNAs are necessary for normal lifespan in *C. elegans*.[42]

Systems approaches such as pathway and Gene Ontology term analyses have also identified aging-associated processes as putative targets of aging-associated miRNAs. These include cell cycle control, cell growth, cell proliferation, mitochondrial respiration, and IIS.[42,45,48,50,52,70] Particularly, our laboratory has shown that each of the core genes in the IIS pathway may potentially be targeted by multiple aging-associated miRNAs in the mouse brain,[45] again suggesting the centrality of IIS in aging.

Global changes in miRNA expression during aging

Global trends in miRNA expression with increased age have been noted in multiple studies, with many specifically reporting decreases in miRNA abundance.[40,41,43,44,47,48,51,53,70] There were no tissue-specific differences in trends. This observation suggests that, in addition to miRNA-specific regulation, there may be more general mechanisms changing the levels of many or most miRNAs over time, such as age-related alterations in miRNA transcription and steps in miRNA biogenesis.

A few miRNA biogenesis genes have been investigated in the context of aging. Liu *et al*. characterized the lifespan phenotype of hypomorphic *loquacious* (*loqs*) mutants in *D. melanogaster*.[43] Loqs is a homolog of the human TAR RNA-binding protein and associates with RNase-III enzyme Dicer to facilitate efficient pre-miRNA processing.[77–79] The *loqs* hypomorph resulted in a significant decrease in *D. melanogaster* lifespan,[43] suggesting that miRNAs are required for normal lifespan. How *loqs* expression is affected during natural aging has not been investigated. If its expression is indeed diminished with age, then this may account for the decreased expression of miRNAs during aging in the fly brain.[43]

Two studies investigated the requirement for Argonaute proteins in aging. Argonautes are key components of the effector complex for miRNA-mediated gene regulation. Our laboratory studied the effects of adult-specific RNAi-mediated knockdown of *alg-1* (*A*rgonaute-*l*ike *g*ene *1*, the miRNA-specific Argonaute in *C. elegans*), which resulted in a significant reduction in lifespan compared to control, providing evidence that miRNAs are required for normal adult lifespan.[42]

Zhang *et al*. examined expression changes of Argonaute (Ago) 1 and 2 in mouse cardiac ventricular tissue with increased age.[49] The authors found that *Ago1* and *Ago2* mRNA expression increased through mid-adulthood, at which point it peaked, and then gradually decreased with advancing age. As Argonautes can stabilize mature miRNAs and thereby contribute to increased miRNA abundance,[80] their diminished expression in late adulthood may result in a global reduction in miRNA expression, thereby affecting functions of aging-associated pathways and influencing lifespan.

We know little about how other miRNA biogenesis pathway components are affected during aging. Although many studies have identified specific miRNAs important for aging, perturbations in miRNA biogenesis may too impact aging-associated pathogenesis and decline. Indeed, miRNA biogenesis is frequently deregulated in cancer.[81–83] Further research is needed to elucidate how aging impacts the upstream regulators of miRNA expression.

Conclusions and future perspectives

Aging is a complex process involving multiple genetic pathways. An outstanding goal of the field is to understand how these pathways interact and result in the final output of aging. The IIS pathway certainly has a central role in coordinating different external and internal signals.[19] However, there are also pathways that affect aging independently of IIS. The implication of miRNAs in regulating processes involved in aging sheds light on a possible mechanism linking these pathways.

Current research suggests that a large number of miRNAs are differentially expressed during aging. Genetic interactions and computational target predictions suggest that many miRNA may influence aging and lifespan by targeting aging-associated pathways. Furthermore, some miRNAs may regulate multiple pathways, suggesting a role in coordinating the functions of disparate pathways during aging. We have only begun to investigate the depth and breadth of miRNA-mediated regulation during aging. There are still many questions to be addressed such as how miRNAs themselves are regulated during aging and how miRNAs are connected to each other.

Investigating the network structure of pathways in aging will allow us to understand how perturbations in a specific part of the network can affect the overall output (i.e., aging), and it promises to advance our knowledge of aging-related pathogenesis. Some miRNAs have been implicated in genetic feedback loops in the context of development[84] and many more have been predicted.[85] Further research is needed to identify the exact wiring of the aging network to better understand the systems-level role of miRNAs.

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IIS, insulin/insulin-like growth factor 1 signaling; miRNA, microRNA; IGF-1, insulin-like growth factor 1.

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