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Advance in Research of microRNA in *Caenorhabditis elegans*

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ABSTRACT

microRNA (miRNA) is a family of small, non-coding RNA first discovered as an important regulator of development in *Caenorhabditis elegans* (*C. elegans*). Numerous miRNAs have been found in *C. elegans*, and some of them are well conserved in many organisms. Though, the biologic function of miRNAs in *C. elegans* was largely unknown, more and more studies support the idea that miRNA is an important molecular for *C. elegans*. In this review, we revisit the research progress of miRNAs in *C. elegans* related with development, aging, cancer, and neurodegenerative diseases and compared the function of miRNAs between *C. elegans* and human. J. Cell. Biochem. 114: 994–1000, 2013.

KEY WORDS: microRNAs; *Caenorhabditis elegans*; DEVELOPMENT; LIFESPAN; HUMAN DISEASE

MicroRNA (miRNA) is a family of 21–25 nucleotides small RNA which regulates expression of characterized targets at the post-transcriptional level. The biogenesis of miRNA was well delineated by some reviews [Ambros, 2004; Bartel, 2004; Carthew and Sontheimer, 2009]. In detail, miRNA is initially processed from introns of protein-coding genes or RNA polymerase II (RNAPII) specific transcripts of independent genes. The nascent miRNA transcripts (pri-miRNA) are first cleaved to be precursors (pre-miRNA) by a protein complex Drosha and the double-stranded RNA-binding protein Pasha in the nucleus [Carthew and Sontheimer, 2009; Kim et al., 2009]. Thereafter, the pre-miRNAs are exported to the cytoplasm by exportin-5, and transformed to 21–25 nucleotides mature miRNAs by nuclease Dicer. Finally, mature miRNAs incorporated into miRNA-induced silencing complex (miRISC), and imperfectly bind with complementary sequences in the 3′-untranslated regions (UTRs) of target mRNAs and negatively regulate gene expression through translational inhibition (Fig. 1) [Cullen, 2004; Kim, 2005].

The existence of miRNA in *C. elegans* was first identified by Lee et al. [1993], and the other 154 miRNAs were found in *C. elegans* subsequently by scientists [Grad et al., 2003; Lim et al., 2003]. Noteworthy, recent studies suggested that miRNAs in *C. elegans* have the similar properties with mammalian. According to the miRBase (release 10.1) database, approximately 62% or 55–62% miRNAs of *C. elegans* relate to Drosophila and human [Ibanez-Ventoso et al., 2008], more importantly 34 miRNAs in *C. elegans* are conserved through other species [Lim et al., 2003]. In *C. elegans* the expression levels of miRNAs are varied in different developmental periods [Karp et al., 2011]. The wide conservatism and timing expression suggests miRNAs may play an important role in evolution and development. The present review will highlight advantages of *C. elegans* as a model organism for miRNAs studies and recent findings of miRNAs in *C. elegans*.

THE ADVANTAGES OF *C. elegans* AS A MODEL ORGANISM

The discovery of miRNAs in *C. elegans* has its inevitability. *C. elegans*, as a subtype of nematodes, is the first animal with known whole genome sequence. Its full genome encodes about 20,000 genes, of which at least 40% has homolog genes in the human genome [Sternberg, 2001]. It has two sexes, the hermaphrodite and the male. Based on the sexual character, *C. elegans* display

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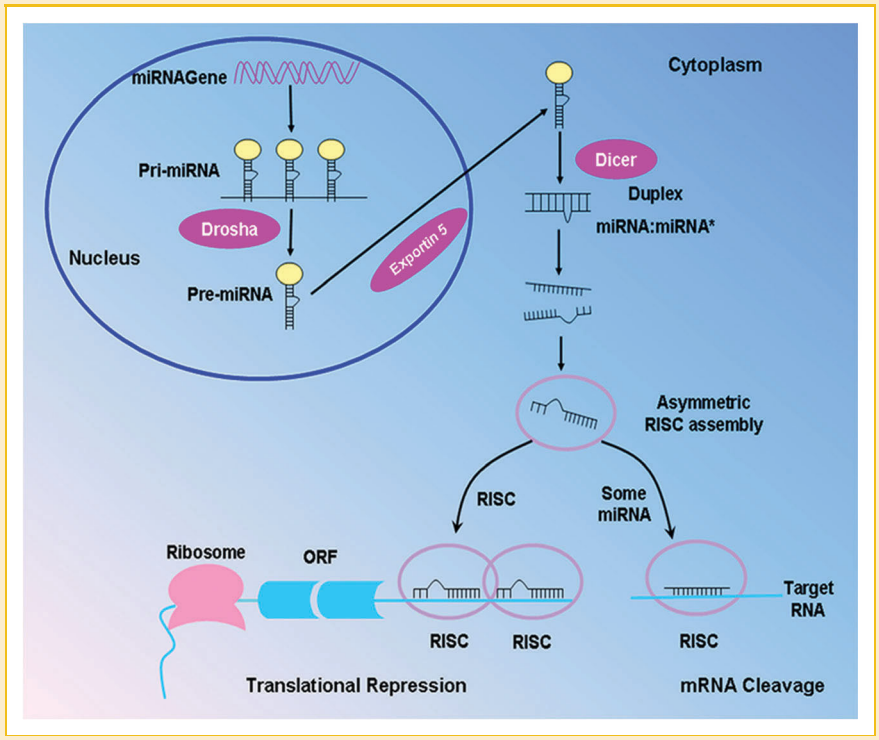

Fig. 1. Model of the biogenesis and post-transcriptional suppression of miRNAs. The pri-miRNA transcripts are first processed into pre-miRNA by Drosha inside the nucleus. Pre-miRNAs are transported into the cytoplasm by Exportin 5 and then become miRNA:miRNA* duplexes by Dicer. Next, one strand of the miRNA:miRNA* duplex is assembled into the miRNA-induced silencing complex (miRISC). Finally, miRNA acts on its target by translational repression or mRNA cleavage.

unparalleled advantages in genetic studies. Hermaphrodite *C. elegans* are common used to conduct genetic mating and analysis due to their breed true by self-fertilization and keep the traits without mating [Strange, 2006]. *C. elegans* also used to study developmental mechanism because of their clear developmental cycle (embryogenesis and four larval stages: L1–L4), which was punctuated by molts [Luo, 2004]. In a favorable environment, larval development is rapid and continuous [Sulston and Horvitz, 1977]. However, in unfavorable environment *C. elegans* will enter a developmental arrested, long-lived, and highly stress-resistant stage called dauer diapause [Cassada and Russell, 1975]. Furthermore, recent studies have found that some miRNA and proteins in dauer larvae were different from non-dauer animals in various developmental stages [Jones et al., 2001; Wang and Kim, 2003]. These features of *C. elegans* provided an excellent animal model to study mechanisms of cell differentiation, development, and aging. It is worth mentioning that there are many landmark discoveries using the small worm. In 1974, Brenner used Ethyl Methanesulphonate (EMS) chemical mutagenesis to induce wild-type *C. elegans* and established ~300 mutants with characterized behavior and morphology [Brenner, 1974]. It laid the foundation for *C. elegans* in the animal behavior and development genetic study. In 1977, Sulston used the differential interfering contrast (DIC) technology to study different phenotypes of cell characteristics in *C. elegans* and painted a unique cell fates lineage diagram [Sulston and Horvitz, 1977; Sulston et al., 1983], which provided a platform for scientists to study the genetic development regulatory mechanism on the single cell in *C. elegans*. Based on above work, Horvitz cloned dozen genes that regulated the programmed cell death in *C. elegans* and the following studies found these genes were similar with mammals [Ellis and Horvitz, 1986; Horvitz, 1999]. In 2002, Sydney Brenner shared the Nobel Prize in Physiology and Medicine with John Sulston and H. Robert Horvitz for their discovery of genes in *C. elegans* that regulate organ development and programmed cell death. These achievements display the irreplaceable position of *C. elegans* in the field of biomedical research.

FUNCTION OF miRNAs In *C. elegans* DEVELOPMENT

Animal development is a complex, strictly regulated process. Recent studies discovered miRNAs important roles in development. It was report that 12 miRNAs expression increases dramatically and eight miRNAs expression decreases dramatically in the lifespan from L1–L4 [Karp et al., 2011]. Among these miRNAs, the lin-4 and let-7 family are well studied. The lin-4 was found to control the L1 to L2 development of *C. elegans* by targeting on lin-14 and lin-28 mRNAs and then the two mRNAs regulate hbl-1 expression directly [Ambros, 1989; Wightman et al., 1993]. The let-7 was reported to control cell fate of hypodermal in the time of late-larval development [Reinhart et al., 2000]. Over-expression of let-7 can prevent the development of *C. elegans* from the L4 to adult by directly inhibiting the expression of lin-41, hbl-1, daf-12, and pha-4

mRNAs. These mRNAs were further found to regulate the development of *C. elegans* by depressing the transcription factor lin-29 [Slack et al., 2000; Lin et al., 2003; Grosshans et al., 2005]. Furthermore, miR-48, miR-84, and miR-241 were found to participate in the L2 to L3 development of *C. elegans* by targeting on hbl-1 mRNA. In addition, miR-48 and miR-84 also acted on cessation of the larval molting cycle at the adult stage, but their target genes are unknown (Fig. 2) [Abbott et al., 2005].

FUNCTION OF miRNAs IN *C. elegans* LIFESPAN

At the achievement of the larval development, aging is following and along with miRANs change. de Lencastre et al. [2010] reported that comparing with Day 0 of adulthood wild-type *C. elegans*, 7 miRNAs were significantly up-regulated, and 23 miRNAs greatly down-regulated on day 10, suggesting miRNAs may involved in aging pathways to regulate the lifespan of *C. elegans*.

Insulin/IGF-1 signaling (IIS) is the first pathway identified in regulating *C. elegans* lifespan [Kenyon et al., 1993]. In this pathway, DAF-2 (insulin receptor-like protein) is the key component to control the lifespan of *C. elegans* by regulating the expression of both transcription factor abnormal dauer formation-16 (DAF-16) and heat shock factor-1 (HSF-1) [Lin et al., 1997; Hsu et al., 2003]. The loss of daf-2 function in *C. elegans* is considered to increase lifespan, while gain of daf-16 function is required for the longevity which can be antagonized by daf-2 in wild-type *C. elegans* [Lin et al., 2001]. In 2005, Boehm and Slack first reported that over-expression of lin-4 can induce a longevity in *C. elegans*, whereas loss of lin-4 function has an opposite phenomenon. Thereafter, they found the lin-4 induced longevity by targeting on the lin-14 which act on the daf-16 and hsf-1 of IIS pathway [Boehm and Slack, 2005; Boehm and Slack, 2006]. Lin-4 is the first and a classical example of miRNA to regulate lifespan. These phenomena suggest miRNAs could regulate *C. elegans* lifespan by affecting different stage. However, whether miRNAs affect the lifespan of *C. elegans* by direct acting on Insulin/IGF-1 signaling pathway is unknown.

Dietary Restriction (DR) is another molecular mechanism in *C. elegans* lifespan regulation [Lakowski and Hekimi, 1998]. Studies indicated that the extension of lifespan in the eat-2 (a nicotinic acetylcholine receptor subunit that acts in the pharyngeal muscle) mutant *C. elegans* by DR was dependent on a transcription factor named by pharynx development defect-4 (PHA-4) [Panowski et al., 2007] rather than DAF-16 [Houthoofd et al., 2003]. Interest, based on the miRNAs databases (TargetScan), pha-4 is a potential target of let-7. However, there is no report that let-7 can affect lifespan through down-regulating pha-4 in aging *C. elegans* until now. Therefore, whether miRNAs involved in DR pathway is still unclear up-to-now.

It is well known that oxidative stress can affect lifespan [Kenyon, 2005]. However, whether aging-associated miRNAs could influence *C. elegans* lifespan through affecting the response of *C. elegans* to

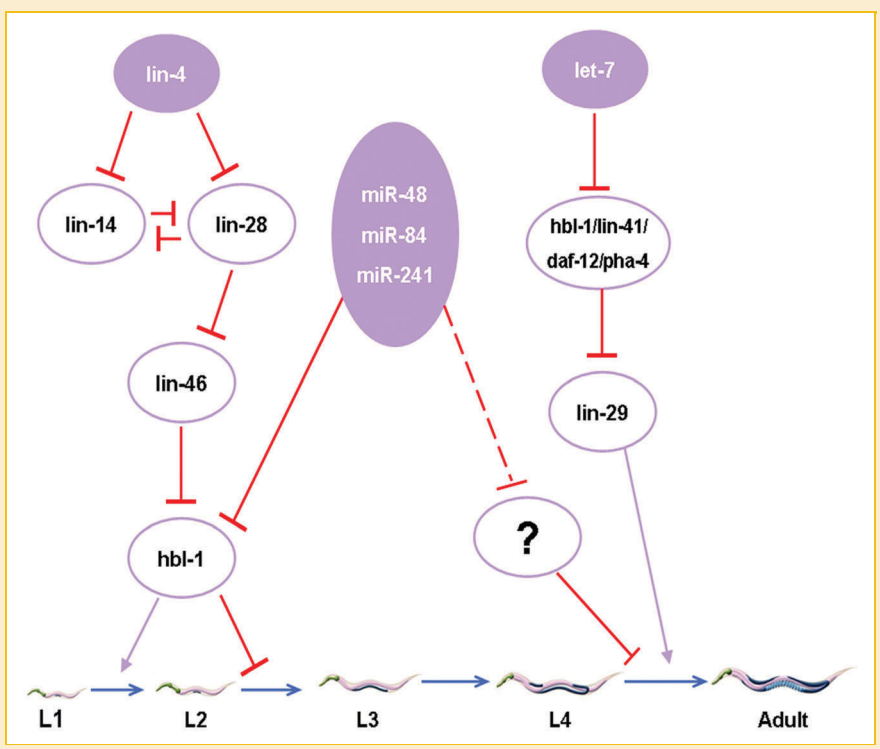

Fig. 2. The function of the miRNAs in *C. elegans* development. The lin-4 miRNA targets lin-14 and lin-28, which interact in early larval development. Lin-46 acts downstream of lin-28 and regulates the expression of hbl-1. The let-7 miRNA targets the lin-41, hbl-1, daf-12, and pha-4 mRNAs, which lead to the expression of the transcription factor lin-29. miR-48, miR-84, and miR-241 which are let-7-like miRNAs regulate the hbl-1 mRNA at the early development time. The dotted line represents a possible role of mir-48, mir-84, and mir-241 during development.

stress is unknown. Previous studies found that deletion of miR-239 enhanced resistance to both heat stress and oxidative stress finally resulted in longevity, while deletion of miR-71 was converse. In contrast, deletions of miR-238 and miR-246 induce increased sensitivity to oxidation and heat stress, respectively [de Lencastre et al., 2010]. However, the exact mechanism is still unknown. The transcriptional factor skn-1 plays a key role in the oxidative stress pathway, and is also necessary for intestinal development in *C. elegans* [An and Blackwell, 2003; Park et al., 2009]. Though skn-1 is a target for all miR-71, miR-238, and miR-239, whether these miRNAs act on skn-1 and regulate the *C. elegans* response to stress and lifespan need to be further studied (Fig. 3).

miRNA AND DISEASE: *C. elegans* TO HUMAN

Since human share the common laws of development and differentiation with *C. elegans*, studies about miRNAs in *C. elegans* would provide invaluable hints to understand the role of miRNAs on physiological and pathophysiological process in human.

In human, inappropriate cell proliferation and differentiation are reminiscent of cancer, and recent studies indicated that many miRNAs were involved in development of various human malignancies [Lu et al., 2005]. Importantly, some miRNAs, which are associated with organism development, cell proliferation, and differentiation in *C. elegans*, are found to be potential predictors for tumors in human. For example, let-7 is found associated with cancer. Vitro experiments have revealed that let-7 is reduced frequently in lung cancer and over-expression of let-7 can inhibit the growth of lung cancer cells in human [Takamizawa et al., 2004]. Whereas, though miR-34 up-regulated in both *C. elegans* and mammalian cells in post-radiation, studies found that the up-regulated miR-34 level was p53-independent in *C. elegans* [Kato et al., 2009], but p53-dependent in human [Hermeking, 2009]. The difference suggests that the role of miRNAs in *C. elegans* could provide valuable information for human diseases but not completely used for human diseases. Besides let-7 and miR-34, other miRNAs involved in *C. elegans* development are also associated with cancer (Table I).

C. elegans is also an excellent animal used for studying neurodegenerative disease, such as Alzheimer's disease (AD) and Parkinson's disease (PD). In AD patient, dysfunction of the amyloid precursor protein (APP) is one of the major risk factor [Selkoe, 2007]. In *C. elegans*, apl-1 is an APP-related gene, which is conserved in evolution [Daigle and Li, 1993]. Though, no study reported the directly regulation action of miRNAs on apl-1, apl-1 was found have significant genetic interactions with let-7 family [Niwa et al., 2008]. These results indicate that apl-1 expression is temporally regulated by miRNAs. It may provide new insights into the time dependent progression of AD. In PD-associated *C. elegans* model, miR-64 and miR-65 are co-expressed in α-synuclein transgenic strain and cat-1

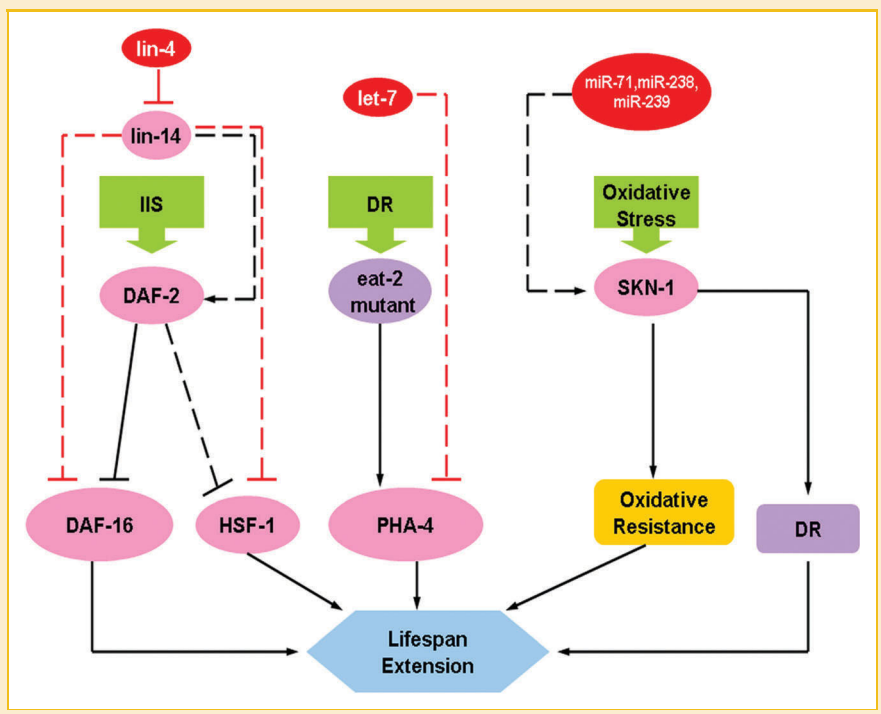

Fig. 3. Pathways that influence lifespan extension in response to miRNAs regulation. In IIS pathway, DAF-16 is antagonized by the DAF-2. The low level of lin-14 expression enhances the longevity promoters DAF-16 and HSF-1 expression. In DR pathway, defection of eat-2 enhances expression of transcription factor PHA-4 which is required to mediate DR induced longevity specifically and it is also the validated target of the let-7. SKN-1 is a transcription factor required for responding to oxidative stress in intestine, and also required for DR-induced lifespan extension [Bishop and Guarente, 2007]. The miR-71, miR-238, and miR-239 are involved in stress resistance suggesting possibility of these miRNAs function through stress resistance to mediate lifespan in *C. elegans*. Dotted lines represent possible roles in these pathways.

TABLE I. Some miRNAs Function in *C. elegans* Development Associate With Cancer

miRNA	Target(s)	Function	Homology	Property	Malignancy	Reference
let-7, miR-48, miR-84, miR-241	lin4, hbl-1, daf-12, pha-4, let-60	Stem cell difference, cessation of molting	Hs-let-7, Hs-miR-98, Hs-miR-196	TS	CLL, lymphoma, gastric, lung, prostate, breast, ovarian, colon, leiomyoma, melanoma	[Reinhart et al., 2000; Slack et al., 2000; Abrahante et al., 2003; Lin et al., 2003; Abbott et al., 2005; Alvarez-Garcia and Miska, 2005; Grosshans et al., 2005; Li et al., 2005; Calin et al., 2008; Roush and Slack, 2008; Peter, 2009; Garofalo and Croce, 2010; O'Day and Lal, 2010; Osada and Takahashi, 2010]
miR-34a, miR-34b, miR-34c	SIRT1	Cell death	Hs-miR-34	TS	CLL, lymphoma, pancreatic, colon, lung, neuroblastoma, glioblastoma	[Alvarez-Garcia and Miska, 2005; Calin et al., 2008; Cole et al., 2008; Garofalo and Croce, 2010; Li et al., 2009; Wiggins et al., 2010; Yamakuchi et al., 2008; Yamakuchi and Lowenstein, 2009]
lin-4, miR-273	lin-14, lin-28, die-1	Stem cell difference, left-right asymmetry	Hs-miR-125b	TS	breast, ovarian, lung, prostate, urothelium, colorectum	[Lee et al., 1993; Wightman et al., 1993; Negrini et al., 1995; Rasio et al., 1995; Chang et al., 2003; Alvarez-Garcia and Miska, 2005; Iorio et al., 2005; Ozen et al., 2008; Veerla et al., 2009; Nishida et al., 2011]
lys-6	cog-1	Left-right asymmetry				[Johnston and Hobert, 2003; Alvarez-Garcia and Miska, 2005]

miRNAs that are down-regulated in malignancies are named as tumor-suppressor (TS), but their function in malignancy is not all experimentally validated.

strain. Additionally members of let-7 family are co-expressed in the α-synuclein transgenic strain and pdr-1 strain [Asikainen et al., 2010]. These results suggest that different miRNAs may express on different PD models of *C. elegans* and lead to different PD pathogenesis.

FUTURE DIRECTIONS

Up-to-today, there are some of miRNAs that could regulate organism development, cell proliferation, and differentiation as well as lifespan in *C. elegans*, but the function of miRNAs in *C. elegans* is largely unknown. Therefore, there will be an extensive future in miRNAs research in *C. elegans*. Since the homology of miRNAs in *C. elegans* and mammals, it will be a huge challenge to study the correlation of miRNAs between *C. elegans* and mammals even human. We believe *C. elegans* will help us further understand miRNAs function and may discover new areas of small RNA world.

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