Chapter 4
Role of MicroRNAs in Stem Cell Regulation and Tumorigenesis in *Drosophila*

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Abstract MicroRNAs (miRNAs) are small noncoding RNAs that modulate the expression of target mRNA. They are involved in many biological processes such as developmental timing, differentiation, cell death, immune response, stem cell behavior, and cancer. Growing evidence suggests that miRNAs play vital roles in regulating several aspects of stem cell biology in *Drosophila* including cell division, self-renewal, and differentiation. In recent years, miRNAs have emerged as collaborating factors that promote the activity of oncogenes in tumor development. Here, we present a brief overview on the role of miRNAs in the regulation of stem cell behavior and tumorigenesis in *Drosophila*.

Keywords MicroRNA · Stem cells · Tumorigenesis · *Drosophila*

1 Introduction

MicroRNAs (miRNAs) are small ~22-nucleotide (nt)-long noncoding RNAs, which bind to the 3′ untranslated region (UTR) of target mRNAs to regulate gene expression through translational repression and mRNA degradation [1–4]. miRNA biogenesis is a multistep process [5, 6]. miRNAs are initially transcribed in the nucleus as a primary miRNA transcript (pri-mRNA) by RNA polymerase II [7], which are then processed into precursor miRNAs (pre-mRNAs) by a microprocessor protein complex, the nuclear RNase III Drosha, and a double-stranded RNA-binding domain (dsRBD) protein Pasha [8–13]. The pre-miRNAs are then exported to the cytoplasm by the guanosine triphosphate-bound Ran (RanGTP)-dependent transporter protein Exportin 5 [14, 15], where they are further cleaved by RNase III enzyme Dicer [16–18] and its dsRBD partner Loquacious (Loqs) [19] to generate ~22-nt-long miRNA:miRNA* duplex. Finally, the one strand of this duplex (miRNA) is transferred to the RNA-induced silencing complex (RISC), containing

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Argonaute-1 (Ago-1) for targeting gene expression, and releases the other strand (miRNA*) that undergoes degradation [20, 21].

The first miRNA gene, *lin-4,* and its target *lin-14* were discovered in a screening for genes that control developmental timing in *Caenorhabditis elegans* [22, 23]. Since then a large number of miRNAs conserved from worms to mammals have been identified [24–33]. Experimental studies in the past two decades have demonstrated that miRNAs play a regulatory role in various biological processes including development, tissue homeostasis, cell proliferation, tissue growth, cell death, neurogenesis, metabolism, immunity, cell fate determination, stem cell maintenance, aging, and several diseases including cancer [4, 24, 34–45]. Dysregulation of miRNA pathway results in developmental defects, several human diseases, and cancer. In this chapter, we will mainly focus on the role of miRNAs in regulation of stem cell self-renewal, differentiation, and tumorigenesis in *Drosophila*.

2 miRNAs in Stem Cell Regulation

Stem cells play a critical role in tissue development and homeostasis. There are two major classes of stem cells reported, embryonic stem (ES) cells and adult stem cells (including somatic and germ line). Stem cells are undifferentiated cells and have an enormous capacity for self-renewal and differentiation to form specialized cell types. Stem cells follow both asymmetric and symmetric division. Asymmetric division of stem cells results in the formation of two daughter cells, one retaining the stem cell characteristics and other one differentiating into specialized cell types [46, 47]. Stem cell self-renewal divisions are controlled by intrinsic and extrinsic (niche cells) factors [46, 47]. Failure of stem cell function of tissue maintenance results in degenerative diseases; on the other hand, overproliferation of stem cells results in tumor development and cancer [47]. Stem cells offer a great opportunity to study the growth and differentiation of individual cells into tissues and recent studies suggest that they can be used in the treatment of degenerative diseases and cancer [47].

Studies in recent years demonstrated that miRNAs play an important role in self-renewal and differentiation of stem cells in a variety of animal model systems [4, 41, 48–56]. Here, we focus only on the role of miRNAs in stem cell self-renewal and differentiation of germ-line stem cells (GSCs) and somatic stem cells (SSCs) in *Drosophila*.

3 miRNAs in *Drosophila* GSCs: Self-Renewal and Differentiation

GSCs are a self-renewing population of germ cells that generate haploid gametes. In *Drosophila* ovary and testis, GSCs are anchored around the niche cells (hub cells in testis and cap cells in ovary). Several signaling pathways regulate both male and

Table 4.1 MicroRNA pathway and its function in *Drosophila* stem cells and tumorigenesis

MicroRNA pathway	Function	References
Stem cells		
dicer-1	Reduction in germ-line cyst production and delayed GSC division in ovary	[57]
	Maintenance of GSC and SSC population in ovary	[59]
loqs	GSC maintenance in ovary	[48, 53]
bantam	GSC maintenance and repress PGC differentiation	[60, 64]
	Intestinal stem cell proliferation	[91]
Ago-1	GSC fate, oocyte formation, and GSC division in ovary	[61, 62, 66]
miR-7, miR-278	GSC division and differentiation in ovary and testis	[65, 68]
Mei-P26	Restricts growth and proliferation in the ovarian stem cell lineage	[63, 71]
	Regulates germ cell differentiation in ovary by genetically interacting with vasa	[67]
miR-184	GSC development and differentiation	[69]
miR-275, miR-306	Control stem cell differentiation by regulating Bam in testis	[73]
miR-310/13	Regulation of germ and somatic cell differentiation in testis	[74]
miR-124, let-7, miR-8/miR-200	Neuroblast stem cell division and differentiation	[87–90]
Tumorigenesis		
bantam	Promotes growth by limiting expression of Socs36E	[112]
	Regulates cell proliferation, cell death, and tissue growth	[107, 108, 115, 116]
miR-278	Misexpression in the developing eye causes massive overgrowth because of inhibition of apoptosis	[109]
miR-8/200	Growth inhibition by inducing apoptosis and blocking cell proliferation	[110]
miR-7	Enhances Notch pathway-induced eye overgrowth	[113]

female GSC systems. Recent studies demonstrated that the miRNA pathway plays a crucial role in the GSCs in *Drosophila* reproductive organs [48, 57–75] (Table 4.1).

3.1 miRNA and Female GSC

In the adult *Drosophila* ovary, the anterior tip of each germarium contains two to three GSCs, escort stem cells (ESCs), and follicle stem cells (FSCs). Each germarium contains five to seven nondividing somatic cap cells that physically anchor GSCs. Anterior to the cap cells are eight to ten terminal filament (TF) cells and inner germarium sheath (IGS) cells. GSC through asymmetric division produces a self-renewing GSC, and a differentiating cystoblast (CB) cell, which form an interconnected 16-cell cyst by incomplete cytokinesis. These germ cells become an oocyte and the nurse cells. In addition to GSCs, two to three FSCs

reside in the middle of each germarium to proliferate and produce an egg chamber and follicle cells [76].

The role of miRNAs in *Drosophila* stem cells was first demonstrated using ovary GSC systems, where they promote cell division and maintenance of GSCs in their niche [48, 57–62] (Table 4.1). Hatfield et al. [57], using *Drosophila* ovarian GSC systems demonstrated that loss of *dicer*-1, the dsRNaseIII required for miRNA biogenesis, results in marked depletion of developing egg chambers because of the reduction in germ-line cyst production. Further, they found that reduction in cyst production in *dicer*-1 mutant GSCs was not only due to loss of GSCs or a change in their identity but due to a delayed G1-S-phase transition that is dependent on the cyclin-dependent kinase inhibitor Decapo [57]. It has been shown that normal processing of pre-miRNA by Dicer-1 required the dsRBD protein Loqs, which is further demonstrated to be involved in GSC maintenance in *Drosophila* ovary [48]. Further, it has been found that Loqs, Dicer-1, and Ago-1 intrinsically control the self-renewal of GSCs [53, 59]. In addition, Jin and Xie [59] found that Dicer-1 is also required for FSC maintenance in *Drosophila* ovary. Yang et al. [61, 62] found that overexpression of Ago-1 protein leads to GSC overproliferation; however, loss of *Ago-1* results in loss of GSCs, which suggests that *Ago-1* plays an essential and intrinsic role in GSC fate, oocyte formation, and GSC division [66]. Further, they showed that *Ago-1* is not required for *bag of marbles (bam)* silencing and proposed that an Ago-1-dependent miRNA pathway may play a crucial role in repressing GSC/CB [61, 62]. In addition to the role of Dicer-1 in adult GSC maintenance, Shcherbata et al. [60] found that *bantam* miRNA is extrinsically required for GSC maintenance.

Several studies suggest that the miRNA pathway regulates GSC maintenance by repressing *bam* in *Drosophila* [53, 59, 61, 62]. However, the miRNA pathway that controls the balance between self-renewal and differentiation was not clear until Neumuller et al. [63] demonstrated that *mei-p26,* a trim-NHL protein, together with *bam* and by interacting with Ago-1 through the NHL domain inhibits miRNA expression and controls germ cell differentiation [63]. Further, they also demonstrated that *mei-P26* regulates several miRNAs including *bantam*. Further, Liu et al. [67] have demonstrated that vasa promotes germ cell differentiation by genetically interacting with Mei-P26 and activating its translation by binding directly to a (U)-rich motif in its 3′ UTR. Furthermore, Li et al. [71] have shown that Mei-P26 regulates the fates of both GSCs and their differentiating daughters by promoting bone morphogenetic protein (BMP) signaling.

Yu et al. [65] reported that extrinsic signals from the insulin receptor (InR) pathway control Dacapo (Dap) expression through Dicer-1 to regulate GSC division. They found that *dicer-1* can directly regulate Dap levels through the *dap* 3′ UTR in GSCs. Further, in a luciferase assay, they found that *dap* 3′ UTR is targeted by *miR-7, miR-278,* and *miR-309*. Among these miRNAs, they showed that the GSC cell cycle is regulated through *dap* 3′ UTR by *miR-7* and *miR-278*. Furthermore, they showed that *miR-7* and *miR-278* and Dap-based cell cycle regulation in GSCs are controlled by InR signaling [65]. Lovino et al. (69) have demonstrated that *miR-184* controls GSC differentiation by translational repression of

decapentaplegic (DPP) receptor Saxophone (Sax) protein levels. Yang et al. [61, 62] have shown that fragile X mental retardation protein (FMRP) interacts with Ago-1 and bantam and is required for GSC maintenance and repressing differentiation, and also needed for repressing primordial germ cell (PGC) differentiation and functions as an extrinsic factor for GSC maintenance in *Drosophila* ovary [64]). Recently, Wang et al. [70] provided the evidence that artificial miRNAs can effectively downregulate endogenous target genes (in this case, *bam, mad, ote,* and *dpp*) in GSCs and somatic cells in *Drosophila* ovary. More recently, Joly et al. [75] identified *mei-P26* mRNA as a direct and major target of Nos/Pum/CCR4-mediated translational repression for *Drosophila* female GSC self-renewal.

3.2 miRNAs and male GSC

The *Drosophila* testis tip harbors two types of stem cells, GSCs and SSCs. Each testis has six to nine GSCs, which are encysted by two SSCs [77, 78]. Both GSCs and SSCs are physically attached to a group of 12 nondividing somatic hub cells [79–82]. Each GSC divides asymmetrically to form two daughter cells, one retaining GSC identity and the other one called gonialblast (GB) initiating differentiation [83, 84] In a similar way, SSCs self-renew and give rise to daughters that differentiate into somatic cyst cells [85]. The GBs undergo four rounds of mitotic division with incomplete cytokinesis to form 16 interconnected spermatogonia; however, the SSCs will grow without further division and form a thin layer around the spermatogonial cyst [86]. Germ cells form spermatocytes and finally undergo meiosis and differentiate into sperm [82].

In addition to their role in GSC self-renewal and differentiation, miRNAs are also known to play a crucial role in GSC and somatic cell differentiation and GSC-niche aging in *Drosophila* testis [68, 72–74] **(**Table 4.1). Pek and colleagues [68] have shown that Maelstrom (Mael) represses the expression of *miR-7* that targets *bam* through its 3′ UTR. They found that overexpression of *miR-7* in *mael* mutant testes leads to Bam repression, resulting in a differentiation defect. This suggests that Mael ensures proper differentiation of GSC lineage by repressing *miR-7* [68]. Recently, Eun et al. [73] have shown that in the *Drosophila* male GSC lineage, *bam* mRNA, but not Bam, is present in spermatocytes. They found that repression of Bam accumulation is attained by *miR-275* and *miR-306* through the *bam* 3′ UTR. Further, they found that failure to block Bam protein expression in spermatocytes results in spermiogenesis defects and male sterility, which suggests that *miR-275* and *miR-306* downregulate Bam expression to ensure proper spermatid terminal differentiation [73]. Pancratov et al. [74] in a functional screen identified *miR-310/13* cluster (*miR-310* to *miR-313*) as a novel antagonist of the Wingless pathway that directly targets the 3′ UTR of *armadillo* (*arm*) and *pangolin* (*pan*). Interestingly, they found that the *miR-310/13* mutant flies show abnormal germ and somatic cell differentiation in the *Drosophila* testis [74]. In addition to the role of miRNAs in male GSC and somatic cell differentiation, Toledano et al. [72] have

demonstrated that the IGF-II messenger RNA-binding protein (Imp) counteracts with Ago-2 and Dicer-2 to regulate *unpaired* (upd) levels and GSC maintenance. Further, they found that Imp expression decreases in the hub cells of aged males because of the targeting of *Imp* by *let-7,* which suggests that proper expression of Imp is essential to protecting *upd* mRNA from degradation [72].

4 miRNAs in *Drosophila* SSCs

In the past few years, miRNAs have emerged as a major player in stem cell regulation in *Drosophila* GSC systems with only very rare reports have described its function in other characterized *Drosophila* stem cell (neuroblast, intestinal and hematopoietic) systems. There are few reports that demonstrated the role of miRNAs in regulation of *Drosophila* neuroblast stem cells; these include *miR-124* [87, 88], *let-7* [89], and *miR-8/miR-200* [90]. Recently, Huang et al. [91] showed that bantam miRNA, which is highly expressed in *Drosophila* intestinal precursor cells (intestinal stem cells (ISCs), enteroblast (EB) cells) and enteroendocrine (ee) cells and weakly expressed in enterocytes (ECs), is essential for *Drosophila* ISC proliferation in response to the Hippo (hpo) signaling pathway. Tokusumi et al. [92] have shown that the germ-line differentiation factor Bam and *miR-7* antagonize the differentiation-promoting function of Yan to maintain the stem-like hematopoietic progenitor state during hematopoiesis in *Drosophila*.

5 miRNAs in Tumorigenesis in *Drosophila*

Emerging evidence suggests that dysfunction of miRNAs is correlated with various human diseases including cancer. It is known that cancer is the result of genetic alternations in oncogenes and tumor suppressors [93, 94]. Recent studies demonstrated that miRNAs are also involved in tumor formation and function as tumor suppressors or oncogenes by modulating the activity of evolutionarily conserved signaling pathways, which are usually dysregulated in human cancers [94–98]. It is also suggested that miRNAs may promote tumorigenesis by regulating the expression of some very important class of genes involved in tumor cell proliferation and apoptosis [99]. Kumar et al. [100] demonstrated that repressing the miRNA maturation by blocking the miRNA biogenesis components, particularly in cancer cells, can promote cell growth, transformation, and tumorigenesis.

Because more than 68% of the genes involved in human cancer are conserved in *Drosophila* [101, 102], it has become a useful model organism to study cancer research [103–106]. Several key cancer events such as loss of cell polarity, the competition between tumor and normal cells, and metastasis have been demonstrated using *Drosophila* as a model system in the recent years. In the past few years, several miRNA pathways have been identified to regulate the tissue growth, cell proliferation, tumorigenesis, and metastasis in the *Drosophila* tumor model

[107–114]. Several studies demonstrated that bantam miRNA interacts with Hippo, and epidermal growth factor receptor (EGFR) pathways to control tissue growth, cell proliferation, and tumorigenesis [108, 111, 112, 114–116]. Herranz et al. [112] identified growth regulatory miRNA *bantam* and its target, Suppressor of cytokine signaling at 36E (Socs36E), a negative regulator of the Janus kinase/signal transducers and activators of transcription (JAK-STAT) signaling pathway, as cooperating factors in EGFR-driven tumorigenesis and metastasis in a *Drosophila* model for epithelial-to-mensenchymal transformation (EMT). In a misexpression study, it has been found that *Drosophila miR-278/mirvana* in the developing eye causes massive overgrowth, which is partly because of the inhibition of apoptosis [109]. In an overexpression screen, Vallejo et al. [110] identified *Drosophila miR-8* as a potent inhibitor of Notch-induced overgrowth and tumor metastasis. They found that *miR-8* could repress growth by inducing apoptosis and blocking cell proliferation via repressing *serrate* (*Ser*), a notch ligand. In a recent study, Da Ros et al. [113] identified the conserved miRNA *miR-7* that enhances Notch pathway-induced eye overgrowth in *Drosophila*. They found that the *interference hedgehog (ihog)* gene is the functional target of *miR-7* in Notch-mediated tumorigenesis. Further, they found that *miR-7* and Notch pathway cooperatively dampen hedgehog (Hh) signaling through downregulation of its receptors *ihog* and *brother of ihog* (boi). Their study suggests that the genetic cooperation of *miR-7,* Notch, and Hh is probably participating in the development of certain human tumors [113].

6 Conclusion

miRNAs are the key regulatory molecules in several biological processes. miRNAs play crucial roles in the self-renewal and differentiation of stem cells. miRNAs function as oncogenes or tumor suppressors. Abnormal expression of miRNAs results in developmental defects, loss of tissue homeostasis, and tumorigenesis. *Drosophila* provides an ideal model system to study stem cell regulation and tumor formation. Since miRNAs regulate stem cells, tumor-initiating cells, tumor growth, and metastasis, they have an enormous potential to be used as therapeutic targets for human cancers.

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