Review Article

Lin28 and *let-7* in cell metabolism and cancer

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Abstract: Malignant cells exhibit major metabolic alterations. The regulatory gene networks that regulate metabolism and the impact of these alterations on overall cellular fitness deserve further exploration. The *let*-7 microRNAs and their antagonists, the Lin28 RNA-binding proteins, are well-known for controlling the timing of embryonic development. This pathway has recently been shown to regulate glucose metabolism in adult mice and to reprogram metabolism during tissue injury and repair. In addition, many lines of evidence have established that *Lin28* is an oncogene that drives tumorigenesis in part by suppressing *let*-7. The metabolic underpinnings of this oncogenic program are just beginning to be uncovered. Here, we will review the current understanding of how Lin28 exerts regenerative and oncogenic effects through metabolic mechanisms.

Keywords: *Lin28*, *let*-7; cancer; regeneration; metabolism

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Introduction

Metabolism governs cellular homeostasis, growth, and survival. Alterations in cell metabolism are a common feature of cancer and have been increasingly viewed as one of the hallmarks of malignant transformation (1). In order to support their high proliferative rates, cancer cells adapt to their environment in part by reprogramming the metabolism of all major classes of macromolecules: proteins, carbohydrates, nucleic acids, and lipids (1). This link between metabolism and cancer is not a novel observation. Sixty years ago, Otto Warburg first noted that under normoxic conditions, normal cells metabolize glucose by using mitochondrial oxidative phosphorylation (OxPhos) instead of glycolysis to maximize the production of adenosine triphosphate (ATP), while some cancer cells rely more on aerobic glycolysis, a phenomenon later known as the Warburg effect (2). Since then, interest in this topic has increased and major areas of knowledge have been gained, but fundamentally important questions remain unresolved. The upstream signals that trigger metabolic alterations in cancer cells and what impact these changes have on overall tumor development and progression are still under intense investigation.

Many of the signaling pathways altered in oncogenesis, such as the PI3K-Akt-mTOR pathway (3), can reprogram cell metabolism in a way that promotes malignant growth (1,4). Recently, *Lin28a* and its homolog *Lin28b* (collectively referred to as *Lin28*) and the *let*-7 microRNA family, have been found to play a direct role in regulating glucose metabolism in adult tissues (5-8). In addition, mouse genetic studies have shown that the reactivation of *Lin28* can drive tumor initiation and progression through *let*-7 dependent and independent mechanisms (9-13). Although all the connections have not been made, it is possible that reprogramming cell metabolism could be a major mechanism by which Lin28 exerts its oncogenic effects. Determining how Lin28 regulates metabolic reprogramming may provide an additional way to understand the interplay between oncogenic signaling pathways and cellular metabolism. We will first summarize roles for Lin28 and *let*-7 in regulating self-renewal and differentiation in stem cells and cancer. Then, we will discuss their roles in regulating metabolism.

The *Lin28/let-7* axis temporally regulates self-renewal and differentiation

Lin28 and *let*-7 were first identified through mutagenesis screens as heterochronic genes that govern developmental timing in *C. elegans* (14-17). *Lin28* homologs are RNA-binding proteins that consist of zinc fingers (consisting of cysteine and histidine residues in the order CCHC) zinc fingers and cold shock RNA binding domains (16). Subsequent genetic loss and gain-of-function studies in worms revealed that Lin28 promotes self-renewal and delays differentiation of the hypodermal and vulval progenitor cells (16). As expected from these functions, *Lin28* expression is high during embryogenesis and early larval stage of development, and gradually declines to an undetectable level in adult tissues (18). In contrast, *let*-7 expression increases as *Lin28* expression wanes from late larval stage and remains high thereafter (14,19). Loss-of-function *let*-7 mutations promote the division of seam cells and prevent them from cell cycle exit (20), phenocopying *Lin28* gain-of-function mutants (19). Later, it was shown that *let*-7 promotes differentiation and inhibits self-renewal during the transition from larva to adulthood by repressing *Lin28* expression through its 3'UTR (16,18).

The expression and regulation of *Lin28* and *let*-7 are highly conserved throughout evolution (18). In mice, *Lin28* is expressed at high levels throughout the embryo at early developmental stages (~E6.5) (21). Then, its expression declines through development and remains present in only some adult tissues (21). Whether Lin28 also functions to promote stemness in mammals became more intriguing when overexpressing *Lin28a*, along with *Sox2*, *Oct4*, and *Nanog*, proved to be sufficient to reprogram human somatic fibroblasts into inducible Pluripotent Stem Cells (iPSCs) (22). Nevertheless, the mechanism by which Lin28 exerted this effect remained a mystery until a flurry of studies showed that in both mouse embryonic stem cells (ESCs) and *C. elegans* epithelial stem cells, Lin28 inhibits the post-transcriptional maturation of *let*-7. Lin28 binds to the primary immature form of *let*-7 and sequesters it from being processed by Drosha/DGCR8 (the small RNA generating machinery in the nucleus) (23,24). In the cytoplasm, Lin28 blocks the loading of *let*-7 into Dicer by binding the premature form of *let*-7 and recruiting Tutase4/7, which polyuridylates *let*-7's tail, marking it for degradation by a exonuclease called Dis3l2 (24-35). Thus, when antagonized by Lin28, *let*-7 is rendered inactive.

Studies in mouse ESCs also demonstrated that *let*-7 antagonizes self-renewal and promotes differentiation. Melton *et al*. showed that *Drosha/DGCR8* knockout ESCs, which are unable to produce most mature miRNAs, fail to silence the stem cell self-renewal program when placed under differentiation-inducing conditions. Introduction of mature *let*-7 into these *Drosha/DGCR8* knockout ESCs is capable of rescuing differentiation and even inhibits ESC self-renewal in stem cell culture conditions (36). These phenotypes were in part due to the *let*-7 mediated suppression of pluripotency factors such as *Lin28*, *Sal4*, and *N-Myc* (36). More recently, Worringer *et al*. in Yamanaka's group showed that *let*-7 acts as a barrier to counteract iPSC reprogramming by promoting the expression of differentiation genes (37). Thus, together *Lin28* and *let*-7 form a highly conserved and highly regulated axis that temporally regulates the self-renewal and differentiation of stem cells.

Lin28a and *Lin28b* are oncogenes

In adult mammalian tissues, *let*-7 is one of the most abundant miRNAs (38). Although the exact roles of *let*-7 in adult tissues have not been fully characterized, *let*-7 is known to have tumor suppressor functions. There is copious evidence that *let*-7 expression is downregulated in a large number of cancers (39-43) and that *let*-7 overexpression inhibits growth and transformation of cancer cell lines and tumor xenografts (44-52). These anti-cancer effects are partly due to the suppression of *let*-7 target genes that are critical for cell cycle progression and proliferation, such as *K-Ras*, *Cyclin D1*, *c-Myc*, *Cdc34*, *Hmga2*, *E2f2*, and *Lin28* (45-49,51,53). Most of these findings were discovered in cell lines, and thus our knowledge of *let*-7 functions would benefit from more definitive investigation in animal models.

In contrast to *let*-7, *Lin28* expression is upregulated in multiple tumor types such as neuroblastoma, hepatocellular carcinoma (HCC), Wilms' tumor, and melanoma (13). Several studies have demonstrated that activation of *Lin28* is able to promote tumor development in various mouse tissues in part by suppressing *let*-7 (9-12). Furthermore, we recently showed that genetic deletion of *Lin28a* and *Lin28b* abrogated *c-MYC*-driven hepatocarcinogenesis and improved overall survival in mice (12). Similar results were achieved using *in vivo* siRNA to knockdown *Lin28b*, which resulted in greater levels of cell death in tumor tissues (12). In another study, He *et al*. isolated pre-malignant liver progenitor cells from Diethylnitrosamine mutagenized mice and

showed that these cells have high expression of *Lin28a* and *Lin28b*, suggesting that Lin28 plays a role in malignant transformation within the chronically injured liver (54). These studies have functionally established the role of *Lin28* in tumor initiation and progression and suggest that Lin28 could be a relevant target for either cancer prevention or therapy.

Although many major effects of Lin28 are mediated through *let*-7, Lin28 can also directly bind to and influence the translation of many mRNAs enriched with GGAGA (with G = guanosine and A = adenosine) sequences in their loop structures (55). Many of these mRNAs are oncogenic or growth-promoting genes, such as *Igf2*, *Igf2-mRNA* binding proteins, *Hmga1*, or those encoding ribosomal proteins, cell-cycle regulators, and metabolic enzymes (6, 12,55-59). Whether or not and to what extent these mRNA targets of Lin28 contribute to its oncogenic effects are important areas for future investigation.

The *Lin28/let-7* axis regulates metabolism in mammalian ESCs

Studies in mammalian ESCs provided initial insights into the role of the *Lin28/let*-7 axis in metabolism. Genome-wide studies in human ESCs revealed that Lin28 binds to many mitochondrial enzyme mRNAs and interacts with RNA helicase A to enhance the translation of these mRNAs (56). In mouse ESCs, Wang *et al*. recently showed that threonine (Thr) oxidation into glycine (Gly) and acetyl-CoA catalyzed by threonine dehydrogenase is critical for cell growth (60). A follow-up study by Shyh-Chang *et al*. revealed that the catabolism of Thr also fuels the synthesis of S-adenosyl-methionine (SAM), which is important for methylation reactions and critical for pluripotency (61). Decreased SAM ultimately led to slowed growth and increased differentiation (61). Metabolic profiling also demonstrated that inducing *Lin28* and *let*-7 had dramatic effects on the Thr-Gly-SAM pathway in mouse ESCs (61). Specifically, overexpressing *Lin28* in mouse ESCs led to increased amount of many Thr-Gly-SAM metabolites, while overexpressing *let*-7 led to reduced amount of these metabolites (61). Together, these studies provided the first evidence that the *Lin28/let*-7 axis regulates metabolic networks in ESCs.

Lin28 regulates body size, metabolism, and tissue regeneration in adult mice

Lin28 and *let*-7 also modulate the expression of pathways that directly regulate metabolism in adult mammalian tissues. For gain of function studies, we previously engineered a tetracycline-inducible *Lin28a* transgenic mouse model [*Lin28a* transgenic (Tg)]. Due to leakiness of the transgene, *Lin28a* expression levels are modestly increased in the muscle, skin and connective tissues in the absence of doxycycline induction. We reported that *Lin28a* Tg mice, compared to control mice without the transgene, exhibited increased body size and delayed puberty onset (62). These phenotypes functionally validated a number of genome-wide association studies that identified connections between human height, puberty timing, and the *LIN28B* locus (63,64).

Most interestingly, *Lin28a* Tg mice exhibit enhanced glucose uptake in peripheral tissues (62). Enhanced glucose uptake in *Lin28a* Tg mice also led to higher levels of the glycolytic metabolite lactate (62). Similarly, whole body inducible human *LIN28B* overexpressing mice also exhibit superior glucose tolerance, indicating conserved functions between the *Lin28* paralogs from two species (6). While gain-of-function *Lin28a* results in increased body size, loss-of-function *Lin28a* [*Lin28a* knockout (KO)] caused dwarfism from E13.5 to adulthood (65). Conditional deletion of *Lin28a* in skeletal muscles led to insulin resistance and impaired glucose uptake, indicating that *Lin28* is physiologically required for normal glucose homeostasis (6,65). In contrast to the phenotypes seen in *Lin28a* Tg mice, inducible *let*-7 Tg mice not only have reduced body size and growth retardation, but also have hyperglycemia and glucose intolerance (6). Simultaneous *Lin28a* and *let*-7 whole body overexpression cancels out the glucose phenotype of each factor. Thus, there are likely to be mutually antagonistic effects including the possibility that Lin28a increases glucose uptake by suppressing *let*-7 (6) (*Figure 1A*).

Mechanistically, the overgrowth of *Lin28a* Tg mice could partly be due to the reduction of *let*-7 expression levels in organs where endogenous *Lin28a* is not normally present (62) and global increases in *let*-7-target protein production, such as *Hmga1*, *Igf2*, and *Oct4*—all of which are known to regulate body size (6,66-68). To further illustrate that the enhanced glucose uptake seen in *Lin28a* Tg mice was due to cell autonomous mechanisms, *Lin28a* was overexpressed in C2C12 myoblasts. Compared to control myoblasts, *Lin28a* overexpressing myoblasts take up glucose much faster (*Figure 1A*). This was the result of Lin28a suppressing *let*-7, which in turn suppresses the Insulin-PI3K-mTOR pathway at multiple nodes (namely, Igf1r, Insulin receptor,

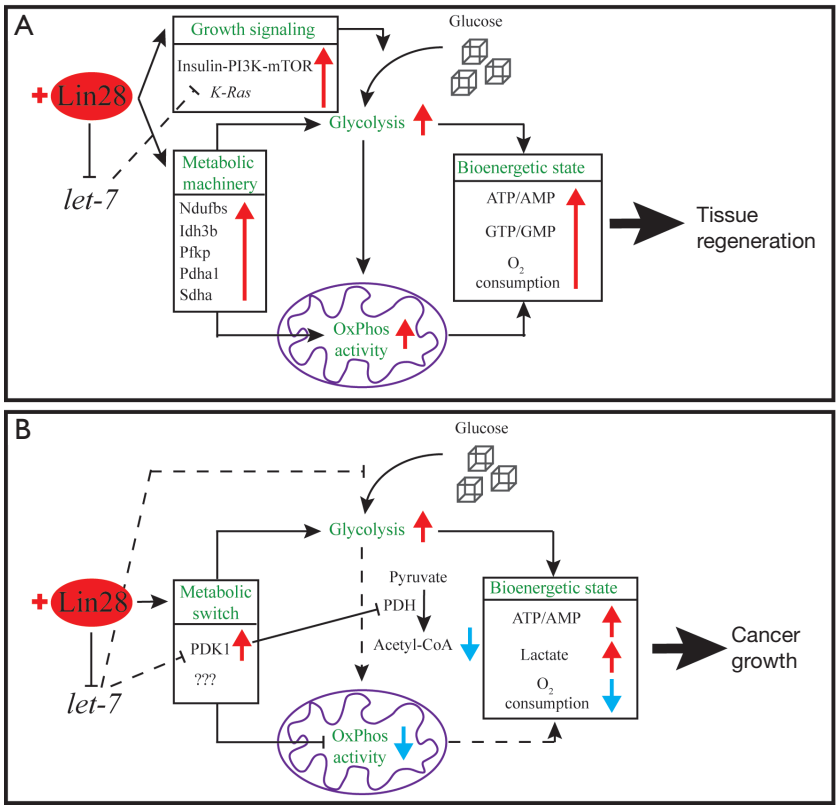

Figure 1 Reactivation of *Lin28* reprograms cell metabolism to enhance tissue regeneration and promote cancer growth. (A) Reactivation of *Lin28* in physiological condition in mouse tissues enhances regeneration by suppressing *let*-7, upregulating *K-Ras* expression and the insulin-PI3K-mTOR signaling, and more importantly upregulating the expression of metabolic machinery. These changes lead to enhanced glucose uptake, increased activity of both glycolysis and OxPhos, resulting in *Lin28*-positive cells having a much higher bioenergetic state; (B) Reactivation of *Lin28* promotes growth of multiple cancer cell lines in part by suppressing *let*-7 and upregulating PDK1 protein expression. When *let*-7 expression is reduced, glucose uptake is enhanced. PDK1 is a negative regulator of OxPhos activity since it inhibits the conversion of pyruvate to acetyl-CoA. When PDK1 expression is enhanced by *Lin28* overexpression and *let*-7 suppression, it promotes glycolysis and blocks OxPhos activity. Consequently, *Lin28*-positive cancer cells switch to aerobic glycolysis for glucose metabolism.

and Irs2) (*Figure 1A*). In terms of signaling output, Akt and S6 phosphorylation is increased in a *let*-7-dependent manner, which increases the insulin-sensitivity and glucose uptake of myoblasts (6). Furthermore, when muscle specific *Tsc1* deficient mice, whose mTOR signaling is increased, were crossed with *Lin28b* deficient mice, *Lin28b*'s dwarfism phenotype was rescued. This further confirmed that mTOR signaling genetically interacts with the *Lin28* program (65). Another line of evidence that further corroborates this concept is a recent report showing that under nutrient deprivation, *let*-7 prevents mTORC1 activation to induce autophagy in primary cortical neurons, muscle, and white fat (69). These studies show that the *Lin28/let*-7 axis strongly influences a known controller of organismal and cancer metabolism, but the following studies demonstrated more interesting mechanisms.

Overexpressing just a modest amount of *Lin28a* led to increased body size and delayed mouse puberty (62). Even more striking is the superior tissue repair observed in *Lin28a* Tg mice (7). We and the Daley Lab showed that *Lin28a* Tg mice exhibited enhanced hair regeneration after shaving, digit repair after amputation, and ear wound healing after hole punch (7). We found that this superior regenerative phenotype in *Lin28a* Tg mice was not caused by suppression of *let*-7 alone, since *let*-7 antimiR delivered to wild-type (WT) mice failed to phenocopy the enhanced regeneration caused by *Lin28a* overexpression (7). By profiling metabolism during tissue repair, we demonstrated

that Lin28a enhances both glycolysis and mitochondrial OxPhos activity through direct binding and translational enhancement of mRNAs that encode several major metabolic enzymes such as phosphofructokinase, pyruvate dehydrogenase (PDH), and isocitrate dehydrogenase (7) (*Figure 1A*). However, enhancement of OxPhos activity by Lin28a turned out to be required for better regeneration in all examined tissues, whereas enhancement of glycolysis was only required in some contexts (*Figure 1A*). To further understand how this metabolic enhancement influences tissue regeneration, Shyh-Chang *et al*. treated *Lin28a* Tg mouse embryonic fibroblasts (MEFs), which migrate significantly faster than WT MEFs, with an OxPhos inhibitor and found that the pro-migration phenotype was preferentially suppressed in *Lin28a* overexpressing cells (7). This suggested that *Lin28a*-mediated metabolic enhancements are sufficient to promote cell migration (7). Consistent with the fact that suppression of *let*-7 alone was not sufficient to recapitulate the regenerative phenotype, suppression of *let*-7 in MEFs had no effect on cell migration (7). Taken together, these lines of evidence demonstrated that Lin28 regulates metabolism through direct impact on the translation of core metabolic enzymes.

Metabolic reprogramming by the *Lin28/let-7* axis in cancer

Recent evidence shows that these oncogenic effects have a metabolic basis. Ma *et al*. showed that overexpression of either *LIN28A* or *LIN28B* in Hep3B, a human liver cancer cell line, promotes the Warburg effect in the form of enhanced glucose uptake, lactate production, and O_2 consumption rate (70) (*Figure 1B*). Treating these cells with *let*-7 mimics, however, resulted in the opposite effects (70) (*Figure 1B*). Unexpectedly, they found that *LIN28*-overexpressing cell lines under normoxic condition showed only marginally activated AKT-mTOR signaling when *LIN28A* or *LIN28B* is expressed (70). However, when they examined protein expression of metabolic enzymes, they found that PDH kinase 1 (PDK1) was highly upregulated in multiple *LIN28*-overexpressing cancer cell lines (70) (*Figure 1B*). PDK1 is a well-known metabolic regulator whose role is to inhibit the conversion of pyruvate to acetyl-CoA, which is used as a starting material for the Krebs cycle. PDK1 does so by phosphorylating PDH and inhibiting its activity (71) (*Figure 1B*).

The regulation of PDK1 by LIN28 most likely occurs post-transcriptionally since the *PDK1* mRNA level was unchanged (70). Consistent with the fact that *let*-7 mimics block glucose uptake in the examined cancer cell lines, *let*-7 was shown to specifically suppress *PDK1* expression but no other OxPhos enzymes (70). Luciferase experiments with *PDK1* 3' UTR demonstrated that *PDK1* is a direct target of *let*-7 (70) (*Figure 1B*). More importantly, Ma *et al*. showed that knocking down *PDK1* in cell lines and xenografts impaired the growth-promoting effects of *LIN28* overexpression (70). Together, this study made two important findings: first, when expressed in multiple cancer cell lines, Lin28 actively promotes aerobic glycolysis while inhibiting mitochondrial OxPhos, distinct from what we reported in the context of tissue repair. Second, blocking a metabolic effector of the *Lin28* program in this context can disrupt cancer cell growth. Recently, we also showed that conditionally overexpressing human *LIN28B* in the liver resulted in the development of liver cancer, with histological features of both hepatoblastoma and HCC (12). Based on 2-deoxy-2-(^{18}F) fluoro-D-glucose positron emission tomography imaging, human *LIN28B*-driven liver tumors are more glucose-avid than surrounding normal tissues, a feature that is seen only in a subset of aggressive human HCC (12). In light of the Lin28 and PDK1 connection, it would be interesting to determine if LIN28B preferentially promotes aerobic glycolysis in this endogenous cancer setting, and what role this metabolic mechanism has on tumor initiation and progression. If inhibition of aerobic glycolysis or glycolytic enzymes such as PDK1 can abrogate the oncogenic effects of LIN28B, it would not only support the idea that Lin28 promotes aerobic glycolysis, but also identifies a downstream effector of the *Lin28* program that is potentially druggable. As molecules that effectively block Lin28 activity have not yet been developed, identifying a more readily actionable target could be beneficial in treating *Lin28*-expressing cancers.

Conclusions

Since the initial discovery of *Lin28* and *let*-7 by Ruvkun and Ambros 30 years ago, we are closer to understanding the full spectrum of mammalian functions for this heterochronic pathway. Furthermore, these studies on *Lin28* and *let*-7 have provided insights into the possible phenotypic outputs of post-transcriptional regulation. We have only recently identified their novel roles in metabolic regulation and great strides have been made in understanding how this translates to organismal homeostasis, regeneration, and disease. There are still many open questions. Do *Lin28*-expressing tumors

have a distinctive metabolic signature when compared to those that are *Lin28*-negative? If Lin28 does promote the Warburg effect, what impact do these effects have on tumor initiation versus progression? If *let*-7 is to be used as an anti-cancer agent to target *Lin28*-positive tumors, will it alone be able to reverse the metabolic reprogramming events caused by Lin28? Since Lin28 interacts and enhances translation of thousands of genes, would targeting a subset of these genes be sufficient to abrogate Lin28's oncogenic effects? We hope that future studies on *Lin28* and *let*-7 can shed light on some of these questions.

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Footnote

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