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How does Lin28 let-7 control development and disease?

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Abstract

One of the most ancient and highly conserved microRNAs (miRNAs), the let-7 family, has gained notoriety owing to its regulation of stem cell differentiation, essential role in normal development, as well as its tumor suppressor function. Mechanisms controlling let-7 expression have recently been uncovered, specifically the role of the RNA-binding protein Lin28 – a key developmental regulator - in blocking let-7 biogenesis. This review focuses on our current understanding of the Lin28-mediated control of let-7 maturation and highlights the central role of Lin28 in stem cell biology, development, control of glucose metabolism, and dysregulation in human disease. Manipulating the Lin28 pathway for the precise control of let-7 expression may therefore provide novel therapeutic opportunities for cancer and other diseases.

Keywords

Lin28; let-7; microRNA; TUTase; Stem Cells; Development; Cancer

Regulating the regulators: Lin28 blocks miRNA expression

Post-transcriptional gene regulation by microRNAs (miRNAs) is an increasingly well-appreciated regulatory mechanism involved in nearly every biological process of multicellular life. Typically miRNAs are transcribed by RNA polymerase II and undergo sequential endonucleolytic cleavages by the RNase-III enzymes Drosha and Dicer to generate a 22-nucleotide double-stranded RNA [1, 2]. One strand of the mature miRNA duplex is selectively retained in an Argonaute protein (Ago1–4), the core component of the miRNA-Induced Silencing Complex (miRISC), which targets messenger RNAs (mRNAs) with partial sequence complementarity in their 3' untranslated region (UTR) for repression by translation inhibition, and/or mRNA deadenylation and decay, or, in the rare event of perfect complementarity between the miRNA and its target, endonucleolytic cleavage by Ago2 (Figure 1a) [3, 4]. The miRNA pathway is evolutionarily ancient and plays vital roles in diverse aspects of biology. First identified in the regulation of larval developmental timing in the nematode worm *Caenorhabditis elegans*, miRNAs make up part of a larger class of heterochronic genes that either promote or retard the progression of worms throughout stereotypical developmental phases [5–9]. This initial class of miRNAs—including the highly conserved miRNA let-7—functions by directly targeting the translation

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of other heterochronic genes such as the small RNA-binding protein Lin28, and ongoing work has uncovered extensive co-regulation between these heterochronic genes.

Identified as the first mammalian miRNAs to have functional and sequence conservation to a *C. elegans* counterpart, the mammalian let-7 family comprises twelve members expressed from eight distinct loci (let-7a-1, -2, -3; let-7b; let-7c; let-7d; let-7e; let-7f-1, -2; let-7g; let-7i; miR-98)[10, 11]. Although distributed throughout the genome, many members of the let-7 family are coordinately regulated during development with let-7 expressed at high levels in differentiated cell types. During differentiation of mouse embryonic stem cells (ESCs), mature let-7 miRNAs accumulate but the corresponding primary let-7 transcript (pri-let-7) remains constant[12, 13]. The discrepancy between let-7 transcriptional activity and the delay in mature let-7 accumulation implies the existence of a negative regulatory factor inhibiting let-7 processing in undifferentiated cells. To determine proteins that potentially mediate this block several groups performed biochemical purifications of complexes associating with pre-let-7. Mass spectrometric analyses revealed two predominant interacting proteins to be Lin28 and Lin28B. Lin28 family members are small (<30kDa) proteins containing two CCHC-type zinc fingers and a Cold-Shock Domain (CSD), both implicated in RNA binding and specifically expressed in embryonic cells (Figure 1b). Purified Lin28A blocks the processing of let-7 at both the pri- and pre-miRNA steps, as Lin28-bound let-7 is resistant to cleavage *in vitro* using either purified Microprocessor or Dicer complexes. Furthermore, overexpression of Lin28A or Lin28B in differentiated cells potently and specifically represses mature let-7 levels, while RNAi-mediated knockdown of Lin28A in undifferentiated cells or Lin28B in cancer cells is sufficient to specifically alleviate let-7 repression[14–17]. Thus, regulation of let-7 expression during embryonic development, stem cell differentiation, and various cancers is controlled primarily through the post-transcriptional blockade of let-7 biogenesis by Lin28 proteins (Figure 1a).

Because Lin28A and Lin28B mRNAs are themselves let-7 targets, this Lin28/let-7 axis establishes a double-negative feedback loop whereby either let-7 or Lin28 is expressed at high levels, promoting either a differentiated or embryonic cell fate, respectively[17]. Aside from their role as developmental regulators, Lin28 proteins are oncogenes reactivated in approximately 15% of all cancers analyzed, functioning largely through their repression of let-7 miRNAs[18]. In accordance with reestablishing a gene expression signature reminiscent of ESCs, Lin28 overexpression in conjunction with other defined factors is sufficient to reprogram somatic cells to an induced pluripotent state[19]. Antagonizing let-7 function with antisense oligonucleotides similarly enhances the reprogramming of fibroblasts to induced pluripotent stem cells (iPSCs)[20]. This review focuses on the regulation of let-7 miRNAs by Lin28 and the manifold biological processes in which this pathway plays a role. Ranging from embryonic development to cancer, inflammation and metabolic processes, the Lin28/let-7 axis is now recognized as central to maintaining proper cell fate and coordinating proliferation, growth, and energy utilization at the cellular level as well as growth, developmental timing, tissue homeostasis, and metabolism in whole organisms. This primal pathway contributes to human disease when dysregulated as exemplified by the oncogenic role of Lin28 in a wide variety of cancers.

Lin28 selectively inhibits let-7 biogenesis

The initial identification of Lin28 and its inverse expression with let-7 was carried out in *C. elegans*, but the molecular mechanism tying these two heterochronic genes remained unknown until quite recently. The Lin28 pathway predominantly regulates expression of the let-7 family whereas expression of other miRNAs is largely unaffected by Lin28. Lin28 binds to the terminal loop or pre-element (preE) of pre- and pri-let-7 *in vitro* and fails to

repress the expression of chimeric let-7 miRNAs bearing the preE of divergent miRNAs[21]. Indeed, the preE of let-7 alone is sufficient for Lin28 binding. These *in vitro* assays correspond well with the global effects on miRNA expression where Lin28A depletion leads to the specific accumulation of multiple let-7 miRNAs in ESCs; similarly, Lin28A or Lin28B overexpression leads to the selective repression of let-7 miRNAs with the levels of other miRNAs unchanged[15, 22, 23]. This binding and repression of let-7 requires both the CSD and CCHC zinc fingers of Lin28 protein[21, 23]. A more refined picture of the Lin28/let-7 interaction is provided by high-resolution crystal structures of a minimal Lin28 protein bound to several let-7 precursor RNAs[24, 25]. Confirming earlier results, these studies revealed that both the CSD and CCHC zinc fingers of Lin28 interact with conserved residues in preE. Specifically, the Lin28 CSD is inserted into the apical point of the precursor loop, while the CCHC zinc fingers dimerize on a GGAG motif adjacent to the Dicer cleavage site[24]. A flexible linker peptide between these domains allows for Lin28 to recognize any let-7 isoform, explaining the ability of Lin28 proteins to repress all let-7 family members despite differences in their loop sequence and length. The CCHC zinc fingers disrupt the double-stranded nature of the miRNA stem near the base of the loop and render the miRNA refractory to Dicer cleavage (Figure 1c). Lin28 represents one of the first of a growing family of RNA-binding proteins that specifically recognizes the terminal loop of miRNAs to control expression[26–28]. This novel regulatory process revealed a potentially powerful strategy for cells to regulate specific subset(s) of miRNAs. Indeed, a few non-let-7 miRNAs have been verified as Lin28 substrates after the identification of conserved sequences in their terminal loops, yet the extent of regulation appears quite subtle compared to the repression of let-7[23].

Uncovering the regulatory mechanism of Lin28 towards let-7

The recognition and binding of Lin28 to the preE of let-7 precursors *in vitro* is sufficient to inhibit enzymatic activity of miRNA-processing enzymes, yet the physiological relevance of these *in vitro* observations and how this binding leads to the loss of mature miRNA levels initially remained unresolved. Careful examination of Lin28-associated RNAs revealed a high molecular weight let-7 isoform containing a stretch of 3' untemplated uridines[14]. This modified pre-let-7 is a poor substrate for Dicer processing, is difficult to detect in total RNA, and has a shortened half-life in cellular extracts, implying that pre-let-7 uridylation is a signal for pre-let-7 degradation[14]. Biochemical purifications of Lin28-containing ribonucleoprotein complexes identified Zcchc11 (also known as TUT4), a non-canonical poly(A) polymerase, as the enzyme responsible for pre-let-7 uridylation[22, 23, 29]. Previously identified as a regulator of Toll-Like Receptor signaling in macrophages and supporting polyuridylation activity *in vitro*[29, 30], Zcchc11 was the first Terminal Uridyl Transferase (TUTase) identified to uridylylate miRNAs[22, 23, 31]. Lin28-binding to pre-let-7 recruits Zcchc11/TUT4 to catalyze the addition of an oligo-uridine 3' tail *in vitro*. Upon Zcchc11 depletion in ESCs, let-7 miRNAs are specifically de-repressed in a manner similar to Lin28 knockdown, albeit more modestly[22, 23]. A possible Zcchc11 ortholog, PUP-2, was implicated in control of let-7 biogenesis in *C. elegans* indicating possible conservation of this regulatory mechanism[32]. A more recent study however indicated the Lin28-mediated control of let-7 expression in worms does not involve PUP-2 or pre-let-7 uridylation but rather the blockade occurs in the nucleus[33]. Nevertheless in both models Lin28 represses the accumulation of the functional let-7 miRNAs in worm embryos as in mammals.

Lin28 is a stem cell pluripotency factor

Lin28 is highly expressed in undifferentiated ESCs and represents part of a pluripotency network in these cells with the Lin28A promoter region occupied by key ESC transcription

factors including Oct-4, Sox2 and Nanog[34]. During embryonic development Lin28 levels decline allowing an accumulation of let-7 miRNAs. let-7 induction inhibits the self-renewal of undifferentiated cells and promotes differentiation. Introduction of synthetic let-7 is sufficient to rescue the compromised differentiation phenotype of miRNA-deficient ESCs[20]. let-7 directly targets several 'stemness' factors including c-Myc, Sall4 and Lin28, all of which have let-7 binding sites in their 3'UTR[20]. Reprogramming differentiated cells to induced pluripotent stem cells (iPSCs) can be achieved by the introduction of key pluripotency transcription factors into somatic cells[35, 36]. One of the first studies reporting the derivation of human iPSCs utilized ectopic expression of Oct4, Sox2, Nanog, and Lin28 as reprogramming factors[19]. This observation, together with the report that antagonizing let-7 using antisense oligonucleotide inhibitors enhances reprogramming of mouse fibroblasts to iPSCs, indicates that Lin28-mediated inhibition of let-7 expression promotes cell de-differentiation during reprogramming[20]. Accordingly, it was found that Lin28 accelerates reprogramming by promoting cell proliferation[37]. Together, these results indicate that the Lin28/let-7 axis operates as a bistable switch functioning to maintain either a differentiated or embryonic cell fate and that this pathway can be exploited to manipulate cellular pluripotency.

Lin28 regulates development

Lin28 is dynamically regulated during development but is stably expressed in several embryonic cell types. While the molecular role of Lin28 in the regulation of let-7 expression is now quite well established, studies in various cellular and developmental contexts raise the possibility that Lin28 may have additional regulatory roles and bind certain mRNAs to promote their translation. The relative contribution of Lin28-mediated repression of let-7 and the miRNA-independent functions of Lin28 in most of these contexts remains to be determined. A recent report indicates that Lin28 has let-7-independent roles also in the worm heterochronic pathway[38, 39]. Lin28 promotes the rapid proliferation of mouse and human ESCs and directly binds several mRNAs including those encoding certain cell cycle regulatory factors controlling G2/S to M transition, Histone H2a, as well as Oct4[39, 40]. Indeed genome-wide analysis of Lin28-associated mRNAs in hESCs revealed an enrichment of metabolic genes and ribosomal protein mRNAs, connecting metabolic regulation to ESC proliferation[41]. Lin28-mediated translational enhancement requires RNA Helicase A and relies on sequence-specific binding elements within the coding region of these mRNAs. The mechanism underlying the interaction between Lin28, mRNA, and the translation machinery remains poorly understood[42]. Lin28 resides in polysomes of muscle progenitors and can enhance the translation of IGF-2 mRNA[43]. During neurogenesis Lin28 levels decrease rapidly upon retinoic acid-induced differentiation of mouse embryonal carcinoma cells[44]. Forced expression of Lin28 in differentiation conditions allows neural differentiation but inhibits the transition to mature glia. Interestingly, with constitutive expression of Lin28 a significant shift in gene expression occurs before let-7 accumulates, and the overexpression of a Lin28 mutant permitting let-7 accumulation remains inhibitory to glial differentiation implying let-7-independent functions of Lin28 in glial development[45]. Studies of intrinsic neural signaling in the developing mouse brain have linked Lin28 to the specific temporal regulation of a critical subset of neuronal mRNAs[46]. Brain-Derived Neurotrophic Factor (BDNF) promotes the differentiation of excitatory neurons by selectively promoting the translation of a small subset of genes. Rather than directly promoting translational efficiency, BDNF instead stimulates Lin28 expression, which blocks let-7 biogenesis leading to lower levels of let-7 and a corresponding de-repression of let-7 target genes including Dicer. Increased Dicer expression leads to an overall increase in miRNA levels (other than let-7 family members) that further repress their target mRNAs through canonical miRNA silencing activity. Thus, let-7 target mRNAs are elevated and proportionally overrepresented in differentiated neurons following BDNF stimulation[46]. Through this

Lin28-mediated mechanism developing neurons specifically increase the translation of a minor subset of mRNAs in response to BDNF.

Lin28 is also highly expressed in mouse and human primordial germ cells (PGC). Using an *in vitro* ESC differentiation and RNAi-based strategy, Lin28 was found to be an essential regulator of PGC formation in this system through inhibition of let-7 maturation and consequential induction of the let-7 target gene Blimp1[47]. Lin28 proteins also have an important role in hematopoiesis. Lin28B is abundantly expressed in fetal hematopoietic stem cells (HSCs) whereas it is absent in terminally differentiated blood cells and adult HSCs. The specific expression of Lin28B in these cells leads to reduced levels of let-7 miRNAs and is responsible for the embryonic-specific lineage commitment of fetal HSC-derived blood cells[48]. Overexpression of Lin28A in adult HSCs reprograms these cells to repopulate donor animals with fetal-specific cell types of all lymphoid lineages[48]. The ability for Lin28 to enhance the reprogramming of differentiated cells to an embryonic state, whether iPSCs or fetal HSCs, is further supported by its ability to mediate wound healing in certain contexts. Unlike mammals, ray-finned fish can repair retinal damage by a program of dedifferentiation to retinal progenitors followed by selective differentiation to replace damaged tissues. Upon injury several pluripotency factors including c-Myc and Lin28 are rapidly upregulated in response to the dedifferentiation transcription factor Ascl1[49]. The rapid increase in Lin28 represses let-7 miRNAs, which normally target dedifferentiation genes including c-Myc, Pax6, Ascl1, and Lin28 itself, and therefore Lin28 is required for cell dedifferentiation. The upregulation of Lin28 and subsequent repression of let-7 miRNAs in response to injury provide telling insight into how cells manage their own reprogramming in response to transitional phases and times of stress. The emerging diversity of multiple developmental pathways involving Lin28 shows the importance and breadth of miRNA and mRNA regulation in normal development.

Oncogenic role of Lin28A and Lin28B

After the elucidation of the Lin28/let-7 connection, several lines of evidence supported the potential role for Lin28 as an oncogene, including the direct targeting of oncogenes by let-7 miRNAs, the embryonic-specific expression of Lin28, and the ability for Lin28 to synergize in somatic cell reprogramming with several known oncogenes[11, 37, 50, 51]. Indeed, Lin28 overexpression is sufficient to transform NIH/3T3 cells[18]. Furthermore, depletion of Lin28B in human leukemia cells increases let-7 levels, reduces proliferation, and leads to the decrease of let-7 targets including Myc. Importantly, an array of human hepatocellular carcinoma lines shows an inverse correlation between the expression of Lin28B and all let-7 family members, while high levels of Lin28B are strongly correlated with early tumor recurrence[18]. Recent studies have implicated Lin28A or Lin28B upregulation in a growing list of various different cancers and in some cases expression correlates with advanced tumor-stage and poor prognosis (Table 1). Also an interesting emerging concept is that Lin28A/B expression may characterize distinct tumorigenic subpopulations of cells within the tumor, so-called tumor initiating or cancer stem cells (CSCs)[52]. This is intriguing given the parallels of the role of Lin28 in maintaining ESC properties and has important implications for possible therapeutic intervention. Together, these studies underscore the importance of Lin28 proteins in promoting and characterizing various human malignancies.

The clinical relevance of Lin28A/B is now readily appreciated and recent work is beginning to uncover the mechanism underlying the Lin28 mis-expression that is observed in human cancers. Lin28B is potentially upregulated in an inducible Myc-mediated lymphoma model and leads to the dramatic reduction of let-7 levels[53]. Myc binds directly to the Lin28B promoter and drives its expression, while loss of Lin28B in this model disrupts transformation, partly due to an increase in let-7 and subsequent repression of Myc. The

establishment, therefore, of a Myc/Lin28B/let-7 oncogenic circuit is necessary and sufficient to transform cells in at least one context. Lin28B expression is also increased by the Src oncoprotein in an inducible breast cancer model[54]. Transient activation of Src leads to an inflammatory expression pattern, which ultimately leads to complete cellular transformation. This regulatory switch relies on increased NF- κ B signaling; expression of IL-6, a pro-inflammatory cytokine; decreased let-7 levels; and the activation of Lin28B. Intriguingly, IL-6 mRNA is targeted by let-7 miRNAs, and NF- κ B binds and enhances expression at the Lin28B promoter. Antagonizing this circuit is sufficient to inhibit transformation and ameliorate the inflammatory gene signature. Similarly, transient introduction of any of these perturbations is sufficient to drive transformation and establish this feedback loop, which persists for many generations in culture[54]. Together these studies provide insight into the direct activation of oncogenic Lin28B and connect miRNA dysregulation to well-known inflammatory and oncogenic expression patterns. How exactly expression of Lin28A is regulated remains largely unknown but a recent study has unveiled a role for Tristetraprolin (TTP), an RNA binding protein, which recognizes sequence elements in the 3' UTR and facilitates degradation of Lin28A mRNA[55]. TTP expression is positively correlated with let-7 expression.

Certain types of cancer appear to predominantly express either Lin28A or Lin28B (Table 1). This raises the important question as to the functional equivalence of these paralogous genes. While both proteins selectively repress let-7 expression recent work indicates the mechanism by which this is accomplished is different[56]. The role of the TUTase Zcchc11 in the Lin28 pathway was established in mouse ESCs and embryonal carcinoma cells that predominantly express Lin28A[22, 23]. Because both Lin28A and Lin28B can enhance the activity of the TUTase in biochemical assays *in vitro*, it was surprising that Zcchc11 depletion by shRNA in Lin28B-expressing human cancer cells does not lead to let-7 elevation. Lin28A-expressing human cancer cells are, however, sensitive to Zcchc11 depletion, as knockdown of Zcchc11 increases let-7 levels to a similar extent as observed in ESCs. An explanation for these initially perplexing data came from an analysis of the subcellular localization of Lin28A and Lin28B proteins. Lin28A was detected predominantly in the cell cytoplasm where it interacts with Zcchc11 to repress pre-let-7, while Lin28B resides in the nucleolus and represses pri-let-7 through a TUTase-independent mechanism. Lin28B contains a nuclear localization signal (NLS) and a nucleolar localization signal (NoLS), each of which is sufficient to localize a fluorescent transgene to its appropriate cellular compartment (Figure 1b). Although both proteins act identically in biochemical assays, their non-overlapping localization explains their differential reliance on pre-let-7 uridylation by Zcchc11. In support of this finding Zcchc11 depletion inhibited growth of Lin28A- but not Lin28B-expressing human breast tumors in mouse xenograft assays[56]. Initially thought to be functionally identical and distinguished primarily by expression pattern, the distinct mechanisms of Lin28A and Lin28B in human cancers further refine the view of disease-specific miRNA regulation.

Role of Lin28 proteins in coordinating growth, development, and metabolism

The Lin28/let-7 axis is highly conserved across the animal kingdom in organisms as evolutionarily distant as nematode worms and humans. The first clues that Lin28 family members have retained their heterochronic function in humans came from genome-wide association studies (GWAS). Lin28B was found to be one of several novel loci associated with human height, age of puberty onset, age of menopause, and body-mass index (BMI), [57–61]. A transgenic mouse line overexpressing Lin28A recapitulated several of these developmental timing phenotypes[62]. In these animals low levels of ectopic Lin28A delayed signs of aging including later cessation of growth, later time to first estrus and first

litter, and delayed vaginal opening[62]. Interestingly, Lin28A and Lin28B transgenic animals are more sensitive to insulin and have reduced peripheral glucose levels. Let-7 overexpressing mice have the opposite phenotype of lower insulin sensitivity and higher glucose levels [63, 64]. The connection between the Lin28/let-7 axis and metabolism seems therefore to be largely controlled through let-7-dependent regulation. Numerous metabolic genes are direct let-7 targets, notably those in the Insulin-PI3K-mTOR pathway, including INSR, IGF1R, and IRS2[63, 64]. The increased size and metabolic differences observed in Lin28A overexpressing animals is rescued by pharmacologically inhibiting the mTOR pathway or overexpressing a let-7 transgene that bypasses Lin28 regulation. Furthermore, meta-analyses of published data confirmed that metabolic genes targeted by let-7 are associated with Type 2 Diabetes (T2D) in humans[63, 64]. The significant effect of Lin28 and let-7 regulation on metabolism is a surprising one, considering the initial discovery of this pathway in regulating development. However, it poses the intriguing possibility that embryonic development and metabolism are co-regulated over time such that the Lin28/let-7 axis functions early in development to integrate cell proliferation with the high metabolic demands of cells in a rapidly growing embryo, while in the adult, the pathway coordinates systemic metabolism at the organismal level (Figure 2).

The future of Lin28 and let-7 – Concluding remarks

The regulation of let-7 miRNAs by Lin28 is a rapidly growing field and points to the importance of small RNA metabolism in disparate fields of mammalian biology. Ongoing studies will develop our understanding of the molecular and cellular mechanisms of this pathway. A more complete understanding of how Lin28A and Lin28B are regulated and how these proteins help maintain precise levels of let-7 miRNA in the context of development, tissue homeostasis/regeneration, aging, and oncogenesis will facilitate the development of ways to exploit this regulatory pathway by manipulating Lin28/let-7 axis for novel treatments of human diseases including cancer and diabetes. One complication arising from the understanding of Lin28/let-7 regulation is the dual effect of Lin28 in being both protective and causal in different types of human disease. The animal models of Lin28A/B overexpression indicate the protective effect let-7 inhibition has in T2D development [62–64]. However, Lin28 functions as an oncogene in cancer raising the likelihood that persistent Lin28 expression (or let-7 repression) will likely lead to hyperplastic disorders and in certain cases cancer. However, a recent proof-of-principle is encouraging in this regard; inhibition of let-7 with an antimiR (an miRNA inhibitor) improved insulin sensitivity and glucose tolerance, raising the intriguing possibility that let-7 antimiR treatment could provide a new approach to treating T2D. It is also noteworthy that no cancer developed in antimiR-treated mice, although the 10-week time course was relatively short[64]. Nevertheless, evidence supports that Lin28 expression levels must be tightly regulated both during developmental stage and cellular context. Indeed, the GWAS findings linking Lin28B to human development underscore this point. Although it may be useful to repress let-7 levels in inflammatory circumstances, doing so to a significant extent may metabolically reprogram cells to an embryonic-like state and lead to the initiation of cancer. This so-called Warburg Effect [65], where a switch occurs to glycolytic metabolism, will increase glucose consumption in peripheral tissues, which could protect against diabetes, but this metabolic transformation is a hallmark of cancer.

Fine-tuning of gene expression by miRNAs is known to have significant clinical and biological impacts, but now it seems that subtle modulation of miRNA-regulating factors may have similarly dramatic effects. The elucidation of Lin28 and let-7 in mammalian development and human disease has uncovered many exciting new aspects in various realms of biology. Bridging the gap from embryonic development to metabolism and the way that these pathways can activate or ameliorate chronic inflammation and cancer, these studies

have done a great deal in tying together formerly disparate fields (Figure 2). Further work on uncovering mechanistic details of this pathway, especially finding inhibitors and identifying novel regulators, will go a long way in understanding how best to treat and control human diseases.

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Glossary

Heterochronic Gene	Any gene that either positively or negatively regulates developmental timing in multicellular organisms. Mutations promoting an adult fate are precocious, while those promoting the embryonic cell fate are retarded
Pluripotency	The ability for a cell to differentiate into any tissue of the adult organism
Self-Renewal	The ability for a cell to divide and make a daughter cell of the same type and with the same differentiation capacity
Pre-element (PreE)	The hairpin loop structure of miRNA precursors (pre-miRNAs) that is removed by Dicer to yield the double-stranded 22-nucleotide mature miRNA
Terminal Uridylyl Transferase (TUTase)	A ribonucleotidyl transferase that belongs to the non-canonical poly(A) polymerase family of enzymes. Catalyzes the transfer of UMP residues to the 3'-ends of preexisting RNA
Cellular Reprogramming	The process of stably changing a terminally differentiated cell to a distinct cell type. Often used to describe the process of Induced Pluripotent Stem (iPS) cell generation where adult cells are reverted to a stem cell-like state by discrete genetic factors
Hematopoiesis	The differentiation process of cellular components in the blood
Insulin Resistance	The condition where insulin becomes less effective at clearing circulating glucose, leading to increased glucose levels and, over time, to Type 2 diabetes
Malignant Transformation	A multistage process where normal cells or tissues acquire genetic or epigenetic perturbations and are converted to the cancerous state
Glycolytic Metabolism	A process in glucose metabolism leading to the production of lactate and pyruvate. Cancer cells and embryonic cells typically employ glycolysis at rates much higher than healthy differentiated cells

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Box 1**Outstanding questions**

- How does Lin28 enhance Zcchc11 TUTase activity towards pre-let-7?
- What enzyme(s) is responsible for the rapid decay of oligo-uridylated pre-let-7?
- Through what mechanism does Lin28B inhibit pri-let-7 processing in the nucleolus?
- Where and when are Lin28A and Lin28B expressed during development and in adults?
- Are Lin28A and Lin28B relevant in adult stem cell populations? During tissue repair and regeneration?
- What are the phenotypic consequences of Lin28A/B knockout mice?
- What signaling pathways and transcription factors control Lin28A and Lin28B expression?
- Are Lin28 proteins dynamically regulated post-translationally in different cellular contexts?
- To what extent can the Lin28 pathway be modulated pharmacologically?
- How large of an impact do let-7-independent roles of Lin28A/B play in mammalian biology?

Figure 1. Lin28 selectively blocks let-7 biogenesis

(a) Model for Lin28-mediated control of let-7 biogenesis. (left) In the absence of Lin28 family members, let-7 miRNAs are processed through the canonical miRNA biogenesis pathway. Transcribed by RNA polymerase II, pri-let-7 is cleaved by the microprocessor complex of DGCR8 and Drosha, yielding pre-let-7, which is exported to the cytoplasm. Pre-let-7 is then cleaved by Dicer, resulting in a mature let-7 duplex, which is asymmetrically loaded into one of several Argonaute proteins within the RNA-induced Silencing Complex (RISC). RISC-mediated repression of target mRNAs can occur through translational inhibition and deadenylation, or, if the miRNA shares perfect sequence complementarity with its target, mRNA cleavage. (right) Expression of Lin28A in the cytoplasm blocks let-7 processing by Dicer through direct RNA-protein interactions with the terminal loop of pre-let-7 and recruits the terminal uridylyl transferase (TUTase) Zcchc11/TUT4 to catalyze the addition of 3' oligouridine tail, marking pre-let-7 for degradation by unknown nucleases. Lin28B is primarily expressed in the nucleolus and binds to pri-let-7, thereby blocking the activity of the microprocessor complex through an unknown TUTase-independent mechanism. **(b)** Domains of Lin28A and Lin28B proteins. Lin28A and Lin28B share significant protein sequence identity and are composed of several common domains including a Cold Shock Domain (CSD, blue) and CCHC zinc fingers (green). Lin28B contains both a nuclear localization signal (NLS) and a nucleolar localization signal (NoLS), explaining its nucleolar localization. **(c)** Molecular basis for Lin28 interaction with pre-let-7.

A cartoon representation of Lin28A binding to the terminal loop of a generic pre-let-7 miRNA based on high-resolution crystal structures. The CSD (blue) is inserted into the terminal loop, while the CCHC zinc fingers (green) dimerize around a conserved sequence motif (GGAG) proximal to the Dicer cleavage site. Binding of the zinc fingers partially unwinds the RNA duplex of pre-let-7, perhaps explaining the resistance of Lin28-bound pre-let-7 to Dicer cleavage. Figure reproduced from [24].

Cellular Phenotype:*Differentiation**Decreased
Proliferation**Increased
Proliferation**Stem cells
Reprogramming
Regeneration**Transformation***Cancer:***Tumor Suppression**Tissue homeostasis**Chronic
Inflammation**Oncogenesis***Development and Physiology:***Growth
retardation**Germ cell loss –
Infertility**Normal stature**Delayed puberty**Overgrowth***Metabolism:***Diabetes**Insulin
Resistance**Glucose homeostasis**Resistance to
obesity and
diabetes**Glycolytic
metabolism*

Figure 2.
Balancing the Lin28/let-7 axis in development and disease.

Table 1

Oncogenic role of Lin28A and Lin28B

Tissue	Gene	Primary Tumor/Tissue	Cell Line	Let-7 level	Comments	Refs
Embryonic	<i>Lin28A</i>	Embryonic carcinoma cells	P19	Low	Lin28A knockdown leads to elevated let-7 expression	[1–6]
	<i>Lin28B</i>	Chronic Myelogenous Leukemia	K562	Low	Lin28B expression correlates with Blast crisis; Lin28B knockdown leads to elevated let-7 expression	[7, 8]
	<i>Lin28B</i>	B cell lymphoma	P493–6	Low	Lin28B is transactivated by Myc; Lin28B knockdown elevates let-7 and inhibits cell proliferation	[9]
	<i>Lin28B</i>	Medulloblastoma Primary tumors	ND	ND	Lin28B expression detected in most tumors	[10]
Breast	<i>Lin28A</i>	Her2+ tumors	T47D	Low	Lin28A expression in Her2+ tumors; Lin28A knockdown elevated let-7 and inhibited tumor growth	[8]
	<i>Lin28B</i>	Triple negative tumors	MDA-MD-231	Low	Lin28B expression in triple negative tumors; Lin28B knockdown elevated let-7 and inhibited tumor growth	[8]
	<i>Lin28A</i>	Primary tumors	ND	Low	Positively correlated with estrogen receptor- α . Progesterone receptor negatively correlated with HER2	[11]
Colon	<i>Lin28A</i> or <i>Lin28B</i>	Primary human colon adenocarcinoma	Lin28A: HCT116 Lin28B: CaCO2	Low	Lin28A expressed in ~30% tumors; Lin28B expressed in ~30% tumors; Lin28B depletion led to elevated let-7 and inhibition of tumorigenesis	[8, 12]
	<i>Lin28B</i>	Primary human colon adenocarcinoma	LoVo and DLD-1	Low	Lin28B correlates with poor patient survival and increased tumor recurrence; Lin28B overexpression represses let-7 expression and promotes metastasis	[13, 14]
Kidney	<i>Lin28B</i>		HEK293	Low	ND	[2]
	<i>Lin28A</i> or <i>Lin28B</i>	Primary human Wilms' Tumors	ND	ND	Lin28A/Lin28B are overexpressed in late stage Wilms' tumors; A t(6;15) translocation associated with Lin28B expression	[7]
Liver	<i>Lin28B</i>	Hepatocellular Carcinoma	HepG2, Huh7, Hep3B	Low	Lin28B represses let-7	[2]
	<i>Lin28B</i>	Hepatocellular Carcinoma primary tumors and cell lines	HepG2	Low	Lin28B correlated with poor prognosis	[7]
	<i>Lin28B</i>	Hepatocellular Carcinoma cell lines samples, cell lines, and mouse models	HCC36, HA22T	Low	Lin28B expression induces Lin28B correlates with high-grade tumors; Lin28B knockdown inhibits growth and tumorigenicity; Lin28B promotes epithelial-mesenchymal transition	[15]
		Primary Hepatocellular Carcinoma tumors	ND	ND	Lin28B expression in high-grade tumors; Lin28 mRNA is a let-7 target	[16]
	<i>Lin28B</i> (<i>Lin28A</i>)	Primary Hepatoblastoma	ND	Low	High Lin28B correlates with low let-7 in aggressive subtypes of hepatoblastoma	[17]
Lung	<i>Lin28A</i>	Small Cell Lung Cancer Cell Lines	NCI-H446	Low	Lin28A knockdown elevates let-7 and inhibits cell cycle	[18]
	<i>Lin28A</i> or <i>Lin28B</i>	Primary tumors	ND	ND	Lin28B detected in primary human lung tumors	[7]

Tissue	Gene	Primary Tumor/Tissue	Cell Line	Let-7 level	Comments	Refs
	<i>Lin28B</i>	Sorted CD133+ cells Non-small cell lung cancer	ND	Low	Lin28B is overexpressed in purified tumor initiating cells; Lin28B knockdown inhibits tumorigenicity	[19]
	<i>Lin28B</i>	Non-small cell lung cancer cells	H1299	Low	Lin28B knockdown elevated let-7 and inhibited tumor growth	[7, 8]
Oesophagus	<i>Lin28A</i> or <i>Lin28B</i>	Primary human tumor samples	ND	Low	Lin28 expression correlates with metastasis, poor prognosis, and low let-7; Lin28B knockdown inhibits cell proliferation and invasion	[20]
Ovary	<i>Lin28A</i> or <i>Lin28B</i>	Primary ovarian tumors	IGROV1(Lin28A)	ND/Low	Lin28A knockdown causes elevated let-7 expression in IGROV1 cells	[7, 8]
	<i>Lin28A</i>	Primary patient tumor samples and cancer cell lines	Igrov1, A2780	ND	Correlate with Oct4+ subpopulation of cells; Lin28A knockdown in IGROV1 cells inhibits growth	[21]
	<i>Lin28A</i> or <i>Lin28B</i>	Primary human tumors	ND	Low	Lin28 expression correlates with poor survival; Lin28B expression is correlated with IGF-II levels and cancer grade; Lin28B but not Lin28A is negatively correlated with let-7 levels	[22]
Prostate	<i>Lin28A</i> or <i>Lin28B</i>	Primary human tumors and adjacent normal tissue	Lin28A: LNCaP, PC-3, Lin28B: C4-2B Undetermined: DU145	Low	Lin28A/B is upregulated in tumors compared to adjacent tissue	[23]
Skin	<i>Lin28B</i>		SK_MEL_28		Lin28B knockdown inhibited tumor growth in xenografts	[8]
Testes	<i>Lin28A</i> or <i>Lin28B</i>	Patient samples and cell lines including primordial germ cell tumors, teratomas, extragonadal tumors and yolk sac tumors	ND	ND	Primary malignant germ cell tumors of multiple histologies express high levels of Lin28A and Lin28B	[24]
	<i>Lin28A</i>	Patient samples of various extragonadal tumors	ND	ND	Identified Lin28 as a sensitive marker for extragonadal tumor identification	[25]
	<i>Lin28A</i>	Patient samples including primordial germ cell tumors, teratomas, extragonadal tumors and yolk sac tumors	ND	ND	LIN28 is a marker of testicular intratubular germ cell neoplasias, classic seminomas, embryonal carcinomas, and yolk sac tumors	[26]

ND = Not determined