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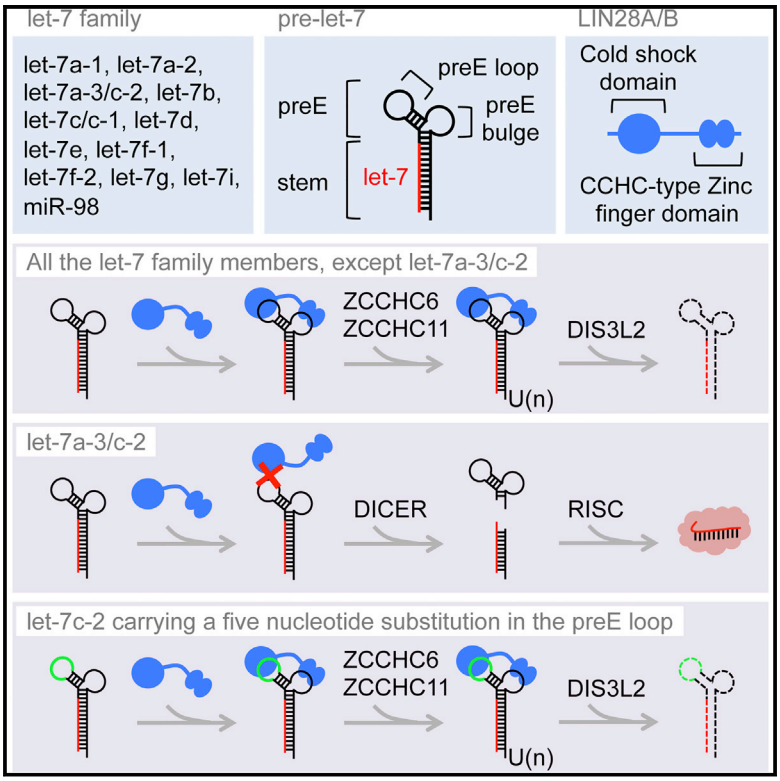
Report

A Single Let-7 MicroRNA Bypasses LIN28-Mediated Repression

Graphical Abstract



Highlights

- Human let-7a-3 and mouse let-7c-2 miRNAs escape LIN28 regulation
- Human let-7a-3 and mouse let-7c-2 pre-miRNAs are refractory to LIN28 binding
- LIN28 cold-shock domain interaction with let-7c-2 pre-miRNA loop is compromised
- A five-nucleotide substitution in pre-let-7c-2 loop restores LIN28 repression

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In Brief

LIN28 proteins are negative regulators of let-7 miRNA biogenesis. Triboulet et al. show that LIN28 inhibits processing of all but one of the 12 let-7 family members. These findings refine the current model of let-7 regulation by LIN28 and have important implications for understanding this pathway in development and disease.

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A Single Let-7 MicroRNA Bypasses LIN28-Mediated Repression

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SUMMARY

Let-7 microRNAs (miRNAs) are critical regulators of animal development, stem cell differentiation, glucose metabolism, and tumorigenesis. Mammalian genomes contain 12 let-7 isoforms that suppress expression of a common set of target mRNAs. LIN28 proteins selectively block let-7 biogenesis in undifferentiated cells and in cancer. The current model for coordinate let-7 repression involves the LIN28 cold-shock domain (CSD) binding the terminal loop and the two CCHC-type zinc fingers recognizing a GGAG sequence motif in precursor let-7 (pre-let-7) RNAs. Here, we perform a systematic analysis of all let-7 miRNAs and find that a single let-7 family member, human let-7a-3 (and its murine ortholog let-7c-2), escapes LIN28-mediated regulation. Mechanistically, we find that the pre-let-7c-2 loop precludes LIN28A binding and regulation. These findings refine the current model of let-7 regulation by LIN28 proteins and have important implications for understanding the LIN28/let-7 axis in development and disease.

INTRODUCTION

Let-7 is one of the most highly conserved microRNAs (miRNAs) in animals (Pasquinelli et al., 2000; Reinhart et al., 2000). The human let-7 family comprises 12 members that are expressed from eight different loci (let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, and miR-98) (Roush and Slack, 2008). Each member is embedded in a let-7 primary miRNA hairpin (let-7 pri-miRNA or pri-let-7) that is processed by the DROSHA-DGCR8-containing Microprocessor complex (Denli et al., 2004; Gregory et al., 2004). This processing generates 67- to 80-nt-long let-7 precursor miRNA (let-7 pre-miRNAs or pre-let-7) that can be classified into two groups: group I let-7 pre-miRNAs (let-7a-2, let-7c, and let-7e), which are directly processed by the Dicer complex in the cytoplasm; and group II let-7 pre-miRNAs (all the other let-7 members), which undergo 3′ mono-uridylation by terminal uridyl transferases (TUTases) ZCCHC6, ZCCHC11, and GLD2 in order to be efficiently matured by DICER (Heo et al., 2012). Nearly identical 22-nt-long mature let-7 miRNAs (namely, let-7-5p) are generated by DICER processing and associate with Argonaute proteins in the miRNA-induced silencing complex (miRISC), where they function by repressing a broad array of genes involved in the control of development, cell proliferation, cell growth, metabolism, and inflammation (Büssing et al., 2008). These functions of let-7 miRNAs primarily are accomplished in differentiated cells where they are expressed abundantly.

Let-7 pri- and pre-miRNAs harbor a typical hairpin structure with a stem containing the let-7-5p miRNA sequence base paired extensively with the partially complementary let-7-3p miRNA sequence, connected by a so-called terminal loop region of variable lengths and structures among different let-7 family members, a region referred to as pre-element (preE) (Nam et al., 2011). Let-7 preE serves as a platform to recruit RNA-binding proteins, such as LIN28, KHSRP (also known as KSRP), hnRNPA1, and TRIM25, in order to posttranscriptionally regulate let-7 biogenesis (Heo et al., 2008; Michlewski and Cáceres, 2010; Newman et al., 2008; Rybak et al., 2008; Trabucchi et al., 2009; Viswanathan et al., 2008; Zhang et al., 2015). LIN28 proteins play pervasive roles during animal development and are often dysregulated in cancer (Ambros and Horvitz, 1984; Moss et al., 1997; Moss and Tang, 2003; Shyh-Chang and Daley, 2013; Thornton and Gregory, 2012). The paralogous *LIN28A and LIN28B* genes are expressed predominantly in undifferentiated cells, such as embryonic stem cells (ESCs), and primarily function to repress let-7 miRNA expression thereby relieving repression of let-7 target mRNAs, and possibly also function to regulate mRNA translation and/or splicing by unknown mechanisms (Polesskaya et al., 2007; Wilbert et al., 2012). LIN28 proteins contain a cold-shock domain (CSD) and two CCHC-type zinc-finger domains that bind respectively to the GNGAY consensus sequence (Y, pyrimidine; N, any base) in the let-7 preE loop and a conserved GGAG motif in the let-7 preE bulge (Ali et al., 2012; Heo et al., 2009; Loughlin et al., 2012; Mayr et al., 2012; Nam et al., 2011; Piskounova et al., 2008). LIN28A and LIN28B are predominantly cytoplasmic and nuclear, respectively, and repress let-7 biogenesis by two distinct mechanisms. LIN28A recruits ZCCHC6 and ZCCHC11, which catalyze 3′ oligo-uridylation of pre-let-7 miRNA (Hagan et al., 2009; Heo et al., 2009; Thornton et al., 2012). This