Bioorganic & Medicinal Chemistry Letters 26 (2016) 4942–4946

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

A druggable target for rescuing microRNA defects

Ken Asada [a], Emanuele Canestrari [a], Zain Paroo [a,b,*]

[a] Center for Pharmaceutical Biotechnology, University of Illinois at Chicago, Chicago, IL 60612, USA
[b] Department of Pharmacology, University of Illinois at Chicago, Chicago, IL 60612, USA

ARTICLE INFO

Article history:
Received 9 June 2016
Revised 15 August 2016
Accepted 6 September 2016
Available online 13 September 2016

Keywords:
Dicer
High-throughput screen
miRNA therapeutics
Small molecule
Translin
Trax

ABSTRACT

Despite immense promise, development of microRNA (miRNA) therapeutics remains limited by pharmacodynamic challenges that have hindered progress of related oligonucleotide-based technologies. Recent discovery of enzymes that mediate miRNA metabolism represent potential pharmacological targets for directing miRNA function, circumventing barriers associated with oligonucleotides. We previously identified the Translin/Trax (TN/TX) ribonuclease complex as a pre-miRNA degrading enzyme that competes with pre-miRNA processing by Dicer. Here, we establish a high-throughput TN/TX assay and screened 2320 drug and natural product compounds for inhibitors of TN/TX. Secondary analyses demonstrate small molecule mediated inhibition of pre-miRNA degradation by TN/TX and enhanced miRNA processing by Dicer. This application of traditional enzyme-inhibitor pharmacology to the miRNA pathway establishes a druggable target for rescuing global miRNA defects, providing an important complement to current approaches towards miRNA therapeutics. More broadly, demonstrating feasibility of pharmacological targeting of the '*ribonucleome*' is particularly important given emerging classes of regulatory RNA and growing understanding of their importance in health and disease.

Substantial investments have been made towards development of miRNA therapeutics. Current approaches are based on oligonucleotides that function as miRNA mimics or anti-miRNA inhibitors. A significant limitation of these nucleic acids-based platforms is the requirement of formulations for effective distribution and absorption in vivo. Such pharmacodynamic challenges have, for decades, thwarted development of related technologies including antisense, ribozymes and siRNA.[1–4] Developing approaches that circumvent challenges associated with oligonucleotides would be of significant value for realizing miRNA-based therapeutics.

Recent discovery of enzymes that mediate mammalian miRNA metabolism represent potential pharmacological targets for directing miRNA function.[5–7] Given that small molecules comprise the vast majority of clinically approved drugs and that enzymes represent a substantial proportion of drug targets, this is an important, yet underexplored avenue for miRNA therapeutics.

Genetic defects in the miRNA-generating enzyme, Dicer, are increasingly linked to disease. Notably, Dicer functions as a haploin sufficient tumor suppressor.[8–11] Genetic deletion of *dicer* is observed in up to 40% of human tumors and loss of Dicer function is associated with poor patient prognoses.[7,9,12] Wholesale miRNA depletion resulting from Dicer dysfunction promotes tumor development through reactivation of proliferative and embryonic cellular programming.[13–15] In addition, inherited *dicer* mutations predispose to a variety of pediatric cancers collectively termed *dicer1* syndrome.[16–18] Further, preclinical models indicate that genetic *dicer* defects promote ageing, diabetes, neurodegeneration and obesity.[19–23] However, there are currently no therapeutic approaches for correcting *dicer* defects.

Loss of miRNA in *dicer* deficiency was thought to be due to loss of miRNA-generating activity. However, we demonstrated that the Translin/Trax (TN/TX) ribonuclease complex drives miRNA depletion in *dicer* deficiency.[7] TN/TX was identified as a pre-miRNA degrading enzyme that competes with pre-miRNA processing by Dicer (Fig. 1A). These studies indicate that miRNA depletion in *dicer* deficiency is due to the combined loss of miRNA-generating activity and catabolic function of TN/TX. Importantly, genetic inhibition of TN/TX mitigated loss of miRNA and loss of tumor suppression with *dicer* haploinsufficiency. These studies revealed a potentially druggable target for restoring global miRNA function in cancers and emerging *dicer* deficiencies. Here, we demonstrate feasibility of pharmacological inhibition of a ribonuclease. Small molecule mediated inhibition of pre-miRNA degradation by TN/TX enhanced miRNA processing by Dicer. These studies establish a druggable target for rescuing global miRNA function in *dicer* deficiency.

We reconstituted the competing effect of pre-miRNA degradation by TN/TX on pre-miRNA processing by Dicer in a recombinant system. We generated wild-type and catalytic mutant recombinant

* Corresponding author.
E-mail address: zain.paroo@gmail.com (Z. Paroo).

http://dx.doi.org/10.1016/j.bmcl.2016.09.019
0960-894X/

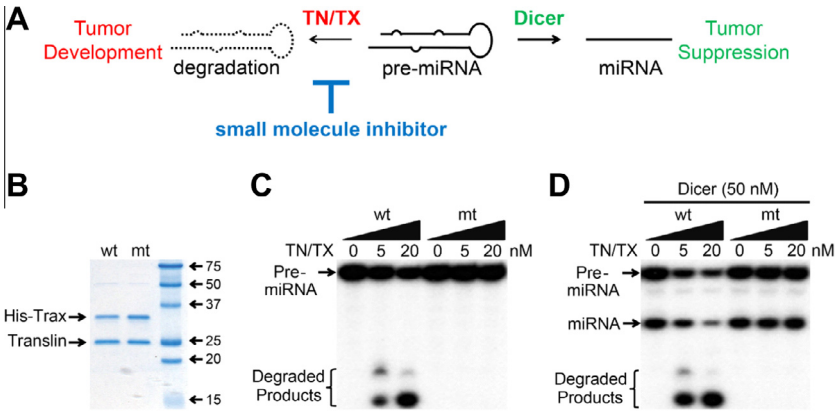

Figure 1. Pre-miRNA degradation by the TN/TX ribonuclease competes with pre-miRNA processing by Dicer. (A) Graphical summary of genetic studies. Dicer processes pre-miRNA to mature miRNA and functions as a tumor suppressor. The TN/TX ribonuclease complex functions as a pre-miRNA degrading enzyme that competes with Dicer. Genetic depletion of TN/TX mitigated loss of miRNA and loss of tumor suppression in *dicer* deficiency. (B) Colloidal blue-stained polyacrylamide gel depicting wild-type (wt) and catalytic mutant (mt; E126A) recombinant TN/TX complexes. Molecular weight standards are indicated (kDa). (C) Pre-miRNA degradation assays using indicated amounts of wild-type or catalytic mutant TN/TX. (D) Pre-miRNA processing reactions performed with 50 nM recombinant Dicer and indicated amounts of wild-type or mutant TN/TX.

TN/TX complexes using a dual expression system in *Escherichia coli* (Fig. 1B). Previous structural studies established that TN serves as a scaffold for the TX subunit which contains the catalytic site, including E126.[24–26] Wild-type, but not mutant TN/TX, yielded dose dependent pre-miRNA degrading activity (Fig. 1C). Previous cleavage mapping studies indicated that TN/TX cleaves single-strand bulges in the stem region of pre-miRNA.[7] miRNA-generating assays were performed with a constant level of Dicer and titrations of wild-type or catalytic mutant TN/TX. miRNA production was inhibited with wild-type, but not mutant enzyme (Fig. 1D). These findings demonstrate that pre-miRNA degradation by TN/TX limits available substrate for productive pre-miRNA processing by Dicer.

We developed a high throughput TN/TX assay based on the principle of fluorescence resonance energy transfer (Fig. 2A). TN/TX functions as a single-strand endonuclease.[7,24–26] A short, single-strand RNA substrate was 5′-labeled with carboxyfluorescein and 3′-labeled with Iowa black quencher FQ, with overlapping emission and absorption spectra, respectively. Following endonucleolytic cleavage of the probe, quenching of the fluorophore is relieved, resulting in fluorescence emission. Assay optimization yielded excellent signal to noise ratios between wild-type and mutant TN/TX (Fig. 2B), with *z*-factors consistently at ~0.8.

We screened the Microsource Spectrum Collection of 2320 structurally diverse drug and natural product compounds. Screening was done in 384-well plates, each containing 32 wells with 40 nM wild-type TN/TX (plus DMSO) and 32 wells with 40 nM mutant TN/TX (plus DMSO). *z*-Factors were calculated for each of the nine plates used in the screen, ranging from a low of 0.833 to a high of 0.895. Test wells contained 40 nM wild type TN/TX plus 30 μM of test compounds (Fig. 2C).

Hits were determined using an initial threshold of 50% inhibition. Hits were filtered on the basis of strong compound color (non-specifically interfering with assay fluorescence) and promiscuity (hits in the current screen were cross referenced with those from other screens performed at our high-throughput screening facility). This yielded a filtered hit rate of <1% (19 compounds; Table S2). Among these was a group of structurally related derivatives of gallic acid (Fig. 3). These included ellagic acid, a dimer of gallic acid and esters of gallic acid, including epigallocatechin, epigallocatechin-3-monogallate and epigallocatechin 3,5-digallate.

We then performed secondary analyses using our traditional gel electrophoresis assays. To confirm purity and structure, these studies were performed with compound purchased from an independent source. Ellagic acid inhibited pre-miRNA degradation by TN/TX (Fig. 4A; IC_{50} 3.5 ± 0.3 μM), without interfering with miRNA processing by Dicer (Fig. 4B). Importantly, ellagic acid eliminated the competing effect of pre-miRNA degradation by TN/TX on pre-miRNA processing by Dicer (Fig. 4C). That is, inhibition of TN/TX enhanced Dicer-mediated miRNA-generating activity. These findings are consistent with loss-of-function genetic studies.[7] This demonstration of small molecule mediated inhibition of TN/TX establishes a druggable target for rescuing defects in the miRNA biogenesis machinery.

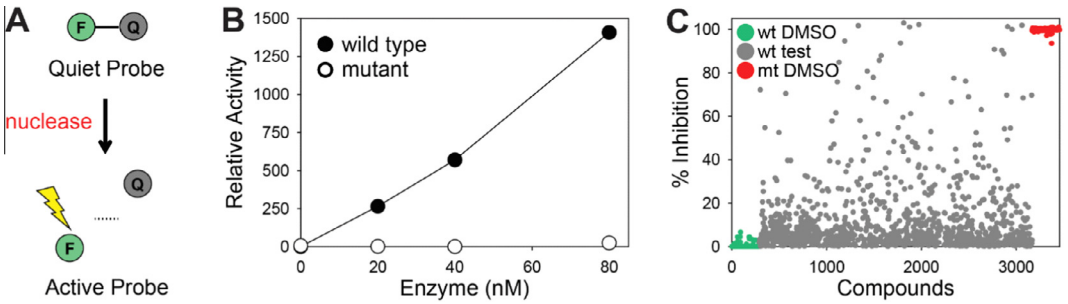

Figure 2. A high throughput screen for small molecule inhibitors of TN/TX. (A) Assay design. An RNA substrate was labeled with a 5′ fluorescein and 3′ quencher. Following endonucleolytic cleavage of the probe, quenching of the fluorophore is relieved resulting in fluorescence emission. (B) Assays were performed with indicated amounts of wild-type or catalytic mutant TN/TX. (C) Summary of screening studies. Experiments were performed with 40 nM wild-type (green circles) or mutant (red circles) TN/TX in the presence of DMSO. Test compounds were assayed for inhibition of wild-type TN/TX (grey circles).

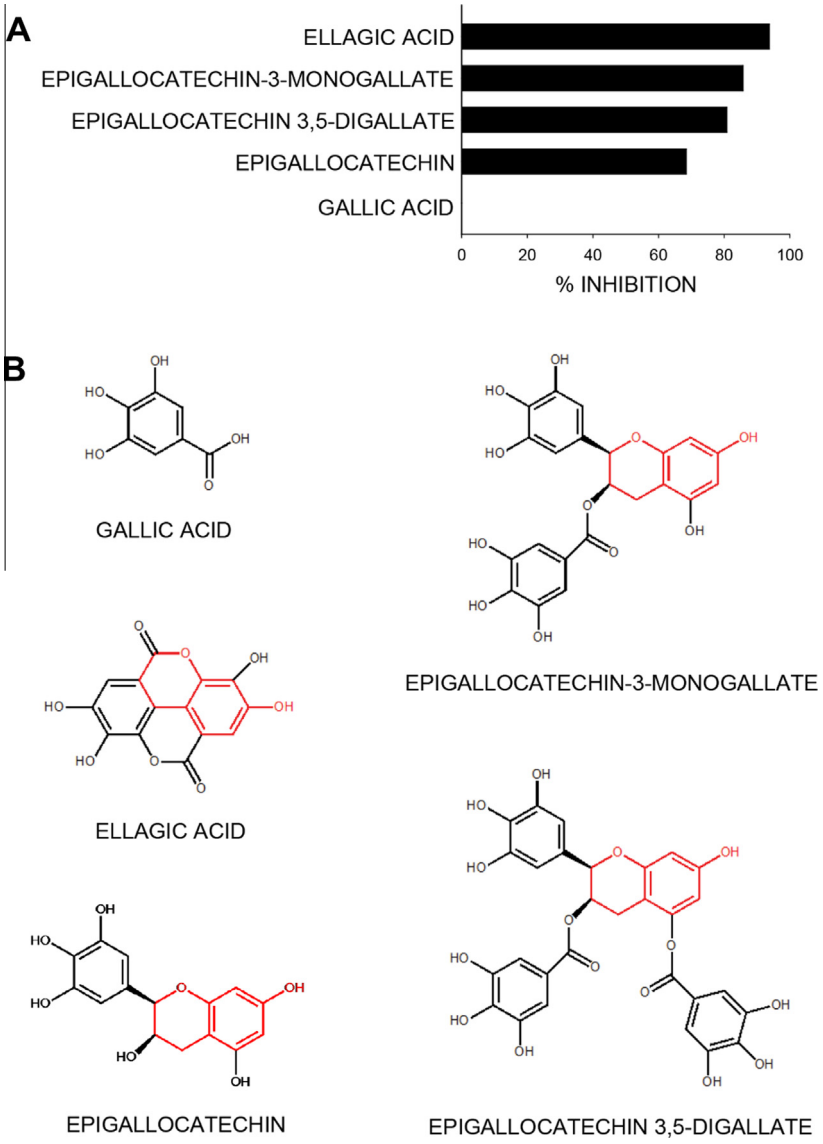

Figure 3. Structurally related hits. (A) High-throughput screening yielded inhibition of TN/TX by derivatives of gallic acid (30 μM). (B) Chemical structures with a core chromane motif illustrated in red.

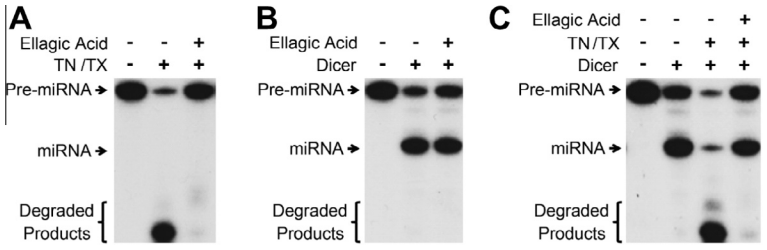

Figure 4. Ellagic acid selectively inhibits TN/TX and enhances miRNA processing by Dicer. (A) Pre-miRNA degradation assays performed with TN/TX in the presence of DMSO or ellagic acid (5 μM). (B) miRNA-generating assays performed with Dicer in the presence of DMSO or ellagic acid (5 μM). (C) Pre-miRNA processing assays performed with Dicer and TN/TX in the presence of DMSO or ellagic acid (5 μM).

Genetic depletion of TN/TX mitigated loss of miRNA and loss of tumor suppression with *dicer* haploinsufficiency.[7] It is tempting to speculate that part of the anti-cancer effects of ellagic acid and epigallocatechins, which are found in fruits, vegetables and green tea,[27] may be mediated through TN/TX, particularly in *dicer* deficient tumors. Notably, genetic depletion of TN/TX yielded modest miRNA changes in wild-type *dicer* contexts.[7] Thus, TN/TX inhibitors may act selectively in *dicer* impaired cells. Although TN/TX has been studied in varied aspects of nucleic acids and developmental biology,[28] examination of post-neonatal *translin*−/− mice suggests limited potential toxicities of systemic TN/TX inhibition.[29–31] These mice are most frequently studied for their neurological deficits, which may be averted through restrictions of the blood–brain barrier. Clinical applications of TN/TX inhibitors

include non-cytotoxic targeting and chemosensitization of *dicer* deficient tumors and chemoprevention in *dicer1* syndrome. As the scope of *dicer* disorders continues to expand, TN/TX inhibitors may be applied for these including ageing, diabetes and obesity.

In addition to its constitutive function as a molecular and cellular antagonist of Dicer, the importance of TN/TX in global miRNA metabolism may be heightened in specific clinical contexts. In a large transcriptome profiling study of hepatocellular carcinomas, expression of both *tn* and *tx* were elevated relative to normal livers (Fig. S1).[32] Similar associations were reported in other studies, with expression of *tn*/*tx* positively correlating with tumor stage.[32–35] Transcriptome profiling of pre-cancerous colorectal adenomas revealed elevated *tn* expression relative to patient matched non-tumor tissue.[36] Collectively, these findings suggest that elevated expression of *tn*/*tx* may promote early and ongoing events in tumor development.

The importance of miRNA in mediating and mitigating molecular pathology offers immense promise for miRNA therapeutics. Although oligonucleotide formulations with improved efficacy and reduced toxicity are in development, the long history of challenges for nucleic acids therapeutics suggests continued limitations. Further, oligonucleotide cocktails are unlikely to be employed in correcting global miRNA loss in *dicer* deficiencies. As small molecules comprise the vast majority of clinically approved drugs, early efforts were made towards identifying miRNA modulating compounds.[37–39] More targeted approaches have recently emerged. For example, a number of methods have been employed to identify compounds for direct recognition of miRNA (for excellent reviews please see Refs. 40–42). These efforts aim to incorporate the transcriptome as a new cellular constituency in the druggable realm. Further, inhibitors of a pre-let-7 modifying enzyme that tags the substrate for destruction have been demonstrated.[43,44] As enzymes represent traditional pharmacological targets, regulatory ribonucleases such as TN/TX offer attractive opportunities for therapeutic modulation of miRNA, circumventing barriers associated with oligonucleotides. More broadly, this concept of drugging the '*ribonucleome*' is particularly important given rapidly emerging classes of non-coding RNA and growing understanding of their importance in health and disease.

Acknowledgements

We thank Drs. Kiira Ratia and Hyun Lee for high-throughput screening support. We thank Dr. Jay Baraban for critical review of this manuscript. This work was supported by the American Cancer Society (279336 to Z.P.). Z.P. is a Junior Investigator of the Chicago Biomedical Consortium with support from The Searle Funds at The Chicago Community Trust (R-005).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.09.019.

References and notes

1. Braasch, D. A.; Paroo, Z.; Constantinescu, A.; Ren, G.; Oz, O. K.; Mason, R. P.; Corey, D. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1139.
2. Paroo, Z.; Corey, D. R. *Trends. Biotechnol.* **2004**, *22*, 390.
3. Burnett, J. C.; Rossi, J. J. *Chem. Biol.* **2012**, *19*, 60.
4. Cheng, C. J.; Saltzman, W. M.; Slack, F. J. *Curr. Med. Chem.* **2013**, *20*, 3582.
5. Suzuki, H. I.; Arase, M.; Matsuyama, H.; Choi, Y. L.; Ueno, T.; Mano, H.; Sugimoto, K.; Miyazono, K. *Mol. Cell* **2011**, *44*, 424.
6. Upton, J. P.; Wang, L.; Han, D.; Wang, E. S.; Huskey, N. E.; Lim, L.; Truitt, M.; McManus, M. T.; Ruggero, D.; Goga, A.; Papa, F. R.; Oakes, S. A. *Science* **2012**, *338*, 818.
7. Asada, K.; Canestrari, E.; Fu, X.; Li, Z.; Makowski, E.; Wu, Y. C.; Mito, J. K.; Kirsch, D. G.; Baraban, J.; Paroo, Z. *Cell Rep.* **2014**, *9*, 1471.
8. Kumar, M. S.; Lu, J.; Mercer, K. L.; Golub, T. R.; Jacks, T. *Nat. Genet.* **2007**, *39*, 673.
9. Kumar, M. S.; Pester, R. E.; Chen, C. Y.; Lane, K.; Chin, C.; Lu, J.; Kirsch, D. G.; Golub, T. R.; Jacks, T. *Genes Dev.* **2009**, *23*, 2700.
10. Lambertz, I.; Nittner, D.; Mestdagh, P.; Denecker, G.; Vandesompele, J.; Dyer, M. A.; Marine, J. C. *Cell Death Differ.* **2010**, *17*, 633.
11. Heravi-Moussavi, A.; Anglesio, M. S.; Cheng, S. W.; Senz, J.; Yang, W.; Prentice, L.; Fejes, A. P.; Chow, C.; Tone, A.; Kalloger, S. E.; Hamel, N.; Roth, A.; Ha, G.; Wan, A. N.; Maines-Bandiera, S.; Salamanca, C.; Pasini, B.; Clarke, B. A.; Lee, A. F.; Lee, C. H.; Zhao, C.; Young, R. H.; Aparicio, S. A.; Sorensen, P. H.; Woo, M. M.; Boyd, N.; Jones, S. J.; Hirst, M.; Marra, M. A.; Gilks, B.; Shah, S. P.; Foulkes, W. D.; Morin, G. B.; Huntsman, D. G. *N. Eng. J. Med.* **2012**, *366*, 234.
12. Merritt, W. M.; Lin, Y. G.; Han, L. Y.; Kamat, A. A.; Spannuth, W. A.; Schmandt, R.; Urbauer, D.; Pennacchio, L. A.; Cheng, J. F.; Nick, A. M.; Deavers, M. T.; Mourad-Zeidan, A.; Wang, H.; Mueller, P.; Lenburg, M. E.; Gray, J. W.; Mok, S.; Birrer, M. J.; Lopez-Berestein, G.; Coleman, R. L.; Bar-Eli, M.; Sood, A. K. *N. Eng. J. Med.* **2008**, *359*, 2641.
13. Bernstein, E.; Kim, S. Y.; Carmell, M. A.; Murchison, E. P.; Alcorn, H.; Li, M. Z.; Mills, A. A.; Elledge, S. J.; Anderson, K. V.; Hannon, G. J. *Nat. Genet.* **2003**, *35*, 215.
14. Kanellopoulou, C.; Muljo, S. A.; Kung, A. L.; Ganesan, S.; Drapkin, R.; Jenuwein, T.; Livingston, D. M.; Rajewsky, K. *Genes Dev.* **2005**, *19*, 489.
15. Thomson, J. M.; Newman, M.; Parker, J. S.; Morin-Kensicki, E. M.; Wright, T.; Hammond, S. M. *Genes Dev.* **2006**, *20*, 2202.
16. Hill, D. A.; Ivanovich, J.; Priest, J. R.; Gurnett, C. A.; Dehner, L. P.; Desruisseau, D.; Jarzembowski, J. A.; Wikenheiser-Brokamp, K. A.; Suarez, B. K.; Whelan, A. J.; Williams, G.; Bracamontes, D.; Messinger, Y.; Goodfellow, P. J. *Science* **2009**, *325*, 965.
17. Rio Frio, T.; Bahubeshi, A.; Kanellopoulou, C.; Hamel, N.; Niedziela, M.; Sabbaghian, N.; Pouchet, C.; Gilbert, L.; O'Brien, P. K.; Serfas, K.; Broderick, P.; Houlston, R. S.; Lesueur, F.; Bonora, E.; Muljo, S.; Schimke, R. N.; Bouron-Dal Soglio, D.; Arseneau, J.; Schultz, K. A.; Priest, J. R.; Nguyen, V. H.; Harach, H. R.; Livingston, D. M.; Foulkes, W. D.; Tischkowitz, M. *JAMA* **2011**, *305*, 68.
18. Slade, I.; Bacchelli, C.; Davies, H.; Murray, A.; Abbaszadeh, F.; Hanks, S.; Barfoot, R.; Burke, A.; Chisholm, J.; Hewitt, M.; Jenkinson, H.; King, D.; Morland, B.; Pizer, B.; Prescott, K.; Saggar, A.; Side, L.; Traunecker, H.; Vaidya, S.; Ward, P.; Futreal, P. A.; Vujanic, G.; Nicholson, A. G.; Sebire, N.; Turnbull, C.; Priest, J. R.; Pritchard-Jones, K.; Houlston, R.; Stiller, C.; Stratton, M. R.; Douglas, J.; Rahman, N. *J. Med. Genet.* **2011**, *48*, 273.
19. Lynn, F. C.; Skewes-Cox, P.; Kosaka, Y.; McManus, M. T.; Harfe, B. D.; German, M. S. *Diabetes* **2007**, *56*, 2938.
20. Schaefer, A.; O'Carroll, D.; Tan, C. L.; Hillman, D.; Sugimori, M.; Llinas, R.; Greengard, P. *J. Exp. Med.* **2007**, *204*, 1553.
21. Haramati, S.; Chapnik, E.; Sztainberg, Y.; Eilam, R.; Zwang, R.; Gershoni, N.; McGlinn, E.; Heiser, P. W.; Wills, A. M.; Wirguin, I.; Rubin, L. L.; Misawa, H.; Tabin, C. J.; Brown, R.; Chen, A.; Hornstein, E. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 13111.
22. Schneeberger, M.; Altirriba, J.; Garcia, A.; Esteban, Y.; Castano, C.; Garcia-Lavandeira, M.; Alvarez, C. V.; Gomis, R.; Claret, M. *Mol. Metab.* **2012**, *2*, 74.
23. Mori, M. A.; Raghavan, P.; Thomou, T.; Boucher, J.; Robida-Stubbs, S.; Macotela, Y.; Russell, S. J.; Kirkland, J. L.; Blackwell, T. K.; Kahn, C. R. *Cell Metab.* **2012**, *16*, 336.
24. Liu, Y.; Ye, X.; Jiang, F.; Liang, C.; Chen, D.; Peng, J.; Kinch, L. N.; Grishin, N. V.; Liu, Q. *Science* **2009**, *325*, 750.
25. Tian, Y.; Simanshu, D. K.; Ascano, M.; Diaz-Avalos, R.; Park, A. Y.; Juranek, S. A.; Rice, W. J.; Yin, Q.; Robinson, C. V.; Tuschl, T.; Patel, D. J. *Nat. Struct. Mol. Biol.* **2011**, *18*, 658.
26. Ye, X.; Huang, N.; Liu, Y.; Paroo, Z.; Huerta, C.; Li, P.; Chen, S.; Liu, Q.; Zhang, H. *Nat. Struct. Mol. Biol.* **2011**, *18*, 650.
27. Saiko, P.; Steinmann, M. T.; Schuster, H.; Graser, G.; Bressler, S.; Giessrigl, B.; Lackner, A.; Grusch, M.; Krupitza, G.; Bago-Horvath, Z.; Jaeger, W.; Fritzer-Szekeres, M.; Szekeres, T. *Phytomedicine* **2015**, *22*, 213.
28. Jaendling, A.; McFarlane, R. J. *Biochem. J.* **2010**, *429*, 225.
29. Chennathukuzhi, V.; Stein, J. M.; Abel, T.; Donlon, S.; Yang, S.; Miller, J. P.; Allman, D. M.; Simmons, R. A.; Hecht, N. B. *Mol. Cell. Biol.* **2003**, *23*, 6419.
30. Stein, J. M.; Bergman, W.; Fang, Y.; Davison, L.; Brensinger, C.; Robinson, M. B.; Hecht, N. B.; Abel, T. *J. Neurosci.* **2006**, *26*, 2184.
31. Wu, Y. C.; Williamson, R.; Li, Z.; Vicario, A.; Xu, J.; Kasai, M.; Chern, Y.; Tongiorgi, E.; Baraban, J. M. *J. Neurochem.* **2011**, *116*, 1112.
32. Roessler, S.; Jia, H. L.; Budhu, A.; Forgues, M.; Ye, Q. H.; Lee, J. S.; Thorgeirsson, S. S.; Sun, Z.; Tang, Z. Y.; Qin, L. X.; Wang, X. W. *Cancer Res.* **2010**, *70*, 10202.
33. Chen, X.; Cheung, S. T.; So, S.; Fan, S. T.; Barry, C.; Higgins, J.; Lai, K. M.; Ji, J.; Dudoit, S.; Ng, I. O.; Van De Rijn, M.; Botstein, D.; Brown, P. O. *Mol. Biol. Cell.* **2002**, *13*, 1929.
34. Wurmbach, E.; Chen, Y. B.; Khitrov, G.; Zhang, W.; Roayaie, S.; Schwartz, M.; Fiel, I.; Thung, S.; Mazzaferro, V.; Bruix, J.; Bottinger, E.; Friedman, S.; Waxman, S.; Llovet, J. M. *Hepatology* **2007**, *45*, 938.
35. Mas, V. R.; Maluf, D. G.; Archer, K. J.; Yanek, K.; Kong, X.; Kulik, L.; Freise, C. E.; Olthoff, K. M.; Ghobrial, R. M.; McIver, P.; Fisher, R. *Mol. Med.* **2009**, *15*, 85.
36. Sabates-Bellver, J.; Van der Flier, L. G.; de Palo, M.; Cattaneo, E.; Maake, C.; Rehrauer, H.; Laczko, E.; Kurowski, M. A.; Bujnicki, J. M.; Menigatti, M.; Luz, J.; Ranalli, T. V.; Gomes, V.; Pastorelli, A.; Faggiani, R.; Anti, M.; Jiricny, J.; Clevers, H.; Marra, G. *Mol. Cancer Res.* **2007**, *5*, 1263.
37. Shan, G.; Li, Y.; Zhang, J.; Li, W.; Szulwach, K. E.; Duan, R.; Faghihi, M. A.; Khalil, A. M.; Lu, L.; Paroo, Z.; Chan, A. W.; Shi, Z.; Liu, Q.; Wahlestedt, C.; He, C.; Jin, P. *Nat. Biotechnol.* **2008**, *26*, 933.
38. Paroo, Z.; Ye, X.; Chen, S.; Liu, Q. *Cell* **2009**, *139*, 112.

39. Melo, S.; Villanueva, A.; Moutinho, C.; Davalos, V.; Spizzo, R.; Ivan, C.; Rossi, S.; Setien, F.; Casanovas, O.; Simo-Riudalbas, L.; Carmona, J.; Carrere, J.; Vidal, A.; Aytes, A.; Puertas, S.; Ropero, S.; Kalluri, R.; Croce, C. M.; Calin, G. A.; Esteller, M. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 4394.
40. Connelly, C. M.; Deiters, A. In *MicroRNA in Cancer*; Alahari, S., Ed.; Springer, 2013; pp 119–145.
41. Monroig, P. C.; Chen, L.; Zhang, S.; Calin, G. A. *Adv. Drug Deliv. Rev.* **2015**, *81*, 104.
42. Velagapudi, S. P.; Vummidi, B. R.; Disney, M. D. *Curr. Opin. Chem. Biol.* **2015**, *24*, 97.
43. Lin, S.; Gregory, R. I. *RNA Biol.* **2015**, *12*, 792.
44. Cording, A.; Gormally, M.; Bond, P. J.; Carrington, M.; Balasubramanian, S.; Miska, E. A.; Thomas, B. *RNA Biol.* **2016**. http://dx.doi.org/10.1080/15476286.2015.1137422.