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REVIEW

A possible link between specific transfer RNA methylation and tumorigenic phenotype of breast cancer

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The human RNA methyltransferase BCDIN3D is overexpressed in breast cancer cells and involved in cellular invasion and poor prognosis of breast cancer. Several years ago, BCDIN3D was reported to dimethylate the 5'-monophosphate of specific precursor miRNAs (pre-miRNAs), such as the tumor suppressor miR145. Dimethylation of the 5'-monophosphate of the pre-miRNA negatively regulates the subsequent processing by Dicer *in vitro*, and results in the downregulated expression of the mature form of the miRNA. The depletion of BCDIN3D also reportedly results in the suppression of the tumorigenic phenotype of breast cancer cells. Thus, these findings suggested that BCDIN3D promotes the cellular invasion of breast cancer cells, by downregulating the expression of tumor suppressor miRNAs via the dimethylation of the 5'-monophosphate of the corresponding pre-miRNAs. Recently, we found that cytoplasmic $tRNA^{His}$ is actually the primary target of human BCDIN3D, rather than pre-miR145. BCDIN3D monomethylates the 5'-phosphate of cytoplasmic $tRNA^{His}$ much more efficiently than that of pre-miRNA *in vitro*, and is responsible for the monomethylation of the 5'-phosphate of cytoplasmic $tRNA^{His}$ *in vivo*. BCDIN3D recognizes the eight-nucleotide long extended acceptor helix with the G_{-1}-A_{73} mis-pair at the top of the acceptor stem of $tRNA^{His}$, which are exceptional features among cytoplasmic tRNA species. These results not only reveal the primary target of BCDIN3D, which is overexpressed in breast cancer cells, but also highlight the possible involvement of the 5'-phosphomethylation of tRNA and/or tRNA in the tumorigenesis of breast cancer cells, beyond its established function in protein synthesis.

Keywords: cytoplasmic $tRNA^{His}$; methylation; breast cancer; BCDIN3D

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BCDIN3D, the BCDIN3 (bicoid-interacting protein 3) Domain containing protein, contains an S-(5'-adenosyl)-L-methionine (AdoMet) binding motif, and is homologous to a conserved family of eukaryotic protein methyltransferases acting on RNA-binding proteins [1]. BCDIN3D is conserved from worm to human [2]. The BCDIN3D mRNA is overexpressed in human breast cancer cells, and the elevated expression of BCDIN3D is related to poor prognosis in breast cancer [3]. The biological roles and functions of BCDIN3D are poorly understood, and the molecular basis of the involvement of BCDIN3D in the tumorigenic phenotype of breast cancer has remained enigmatic.

Several years ago, it was reported that BCDIN3D dimethylates the 5'-monophosphate of specific precursor micro RNAs (pre-miRNAs), such as the tumor suppressors miR145 and miR23b [2, 4-7]. Dimethylation of the 5'-monophosphate of the pre-miRNA negatively regulates the subsequent processing by Dicer *in vitro*, and results in the downregulated expression of the mature miRNA. The downregulation of tumor suppressor miRNAs could be related to the tumorigenic phenotype of breast cancer cells. Indeed, it was also reported that the depletion of BCDIN3D resulted in the suppression of the tumorigenic phenotype of MDA-MB231 breast cancer cells [2]. Therefore, it was suggested that BCDIN3D promotes the cellular invasion of breast cancer cells, by negatively regulating the expression of tumor suppressor miRNAs. However, the mechanisms by which BCDIN3D recognizes only a specific group of pre-miRNAs and negatively regulates the expression of mature miRNAs in breast cancer cells are not well understood.

With an aim to identify other possible RNA substrates that are methylated by BCDIN3D, and to clarify the mechanism by which BCDIN3D recognizes specific RNAs and controls their expression, we analyzed BCDIN3D-binding RNAs in human HEK293T cells [8]. When BCDIN3D, expressed in HEK293T cells, was purified from the cell extract, we noticed that a distinct, seventy-eighty nucleotide RNA molecule was co-purified with the BCDIN3D protein. Since the nucleotide sequences of cytoplasmic $tRNA^{His}$ from human and fruit fly were reported to have a 5'-monomethylphosphate [9, 10], we assumed that the RNA might be cytoplasmic $tRNA^{His}$. The analysis of the RNA co-purified with BCDIN3D, by RT-PCR and DNA sequencing of RT-PCR products, confirmed that cytoplasmic $tRNA^{His}$ co-purified with BCDIN3D from the cell extracts, but other tRNAs, such as $tRNA^{Phe}$, did not. A subsequent detailed analysis of the $RNaseT_1$ and RNaseA-digested fragments of the RNA by LC-mass spectrometry [11-13] clearly demonstrated that the RNA is cytoplasmic $tRNA^{His}$. More importantly, the 5'-monophosphate of cytoplasmic $tRNA^{His}$ was monomethylated, but not dimethylated. The 5'-monophosphate of cytoplasmic $tRNA^{His}$, co-purified with BCDIN3D from extracts of HEK293T cells overexpressing BCDIN3D, is fully methylated. Thus, we asked whether the 5'-phosphate of cytoplasmic $tRNA^{His}$ is methylated under normal physiological conditions. Cytoplasmic $tRNA^{His}$ was purified from HEK293T cells and analyzed by LC-mass spectrometry, which revealed that the 5'-monophosphate of $tRNA^{His}$ is fully monomethylated under normal physiological conditions in HEK293T cells.

These results prompted us to examine whether BCDIN3D could methylate the 5'-monophosphate of cytoplasmic $tRNA^{His}$. The recombinant human BCDIN3D protein was expressed in *Escherichia coli*, and its enzymatic activity was examined using the human cytoplasmic $tRNA^{His}$ transcript as the substrate and SAM [S-(5'-adenosyl)-L-methionine] as the methyl-group donor *in vitro*. The results showed that the cytoplasmic $tRNA^{His}$ transcript is efficiently methylated by BCDIN3D *in vitro*. However, unexpectedly, the human miR145 precursor transcript, which was previously shown to be dimethylated by BCDIN3D, is hardly methylated under the same conditions. The reaction products were further analyzed by LC-mass spectrometry, and the results confirmed that BCDIN3D monomethylates the 5'-monophosphate of cytoplasmic $tRNA^{His}$ much more efficiently than the pre-miR145 transcript. Moreover, BCDIN3D could not dimethylate either $tRNA^{His}$ or pre-miR145 *in vitro*. The steady state kinetics of the methylation of these RNA substrates also confirmed that cytoplasmic $tRNA^{His}$ is a better substrate than pre-miR145, by over two to three orders of magnitude.

To explore whether BCDIN3D is responsible for the monomethylation of the 5'-monophosphate of cytoplasmic $tRNA^{His}$ *in vivo*, BCDIN3D-knockdown cells were established from HEK293T cells by CRISPR/Cas9 editing [14-16]. The growth rate of the BCDIN3D knockdown cells was slightly slower than that of the parental HEK293T cells. The cytoplasmic $tRNA^{His}$ isolated from the BCDIN3D knockdown cells lacked the methyl moiety at its 5'-monophosphate. The exogenous expression of BCDIN3D in the BCDIN3D knockdown cells restored the 5'-monomethylphosphate modification of the cytoplasmic $tRNA^{His}$. Thus, BCDIN3D catalyzes the monomethylation of the 5'-monophosphate of cytoplasmic $tRNA^{His}$ in HEK293T cells, under normal physiological conditions. Moreover, except for cytoplasmic $tRNA^{His}$, no other RNAs in BCDIN3D knockdown cells are significantly methylated by recombinant BCDIN3D *in vitro*. Together with the results obtained in the *in vitro* methylation assay, using recombinant BCDIN3D and the $tRNA^{His}$ transcript, it is most likely that the primary target of BCDIN3D is cytoplasmic $tRNA^{His}$ rather than pre-miRNAs, and BCDIN3D has a monomethylation activity acting on the 5'-phosphate of RNA [8]. Under specific conditions or in certain biological processes, the pre-miRNA might be methylated efficiently, and unknown regulatory factors specific to breast cancer cells might enhance the efficient pre-miRNA (di)methylation process *in vivo*. The *in vivo* mechanism of the dimethylation of the 5'-monophosphate of specific pre-miRNAs, such as pre-miR145, in breast cancer cells awaits further studies.

How does BCDIN3D specifically recognize cytoplasmic $tRNA^{His}$? Human cytoplasmic $tRNA^{His}$ is matured through unique processes [17, 18]. After transcription by RNA

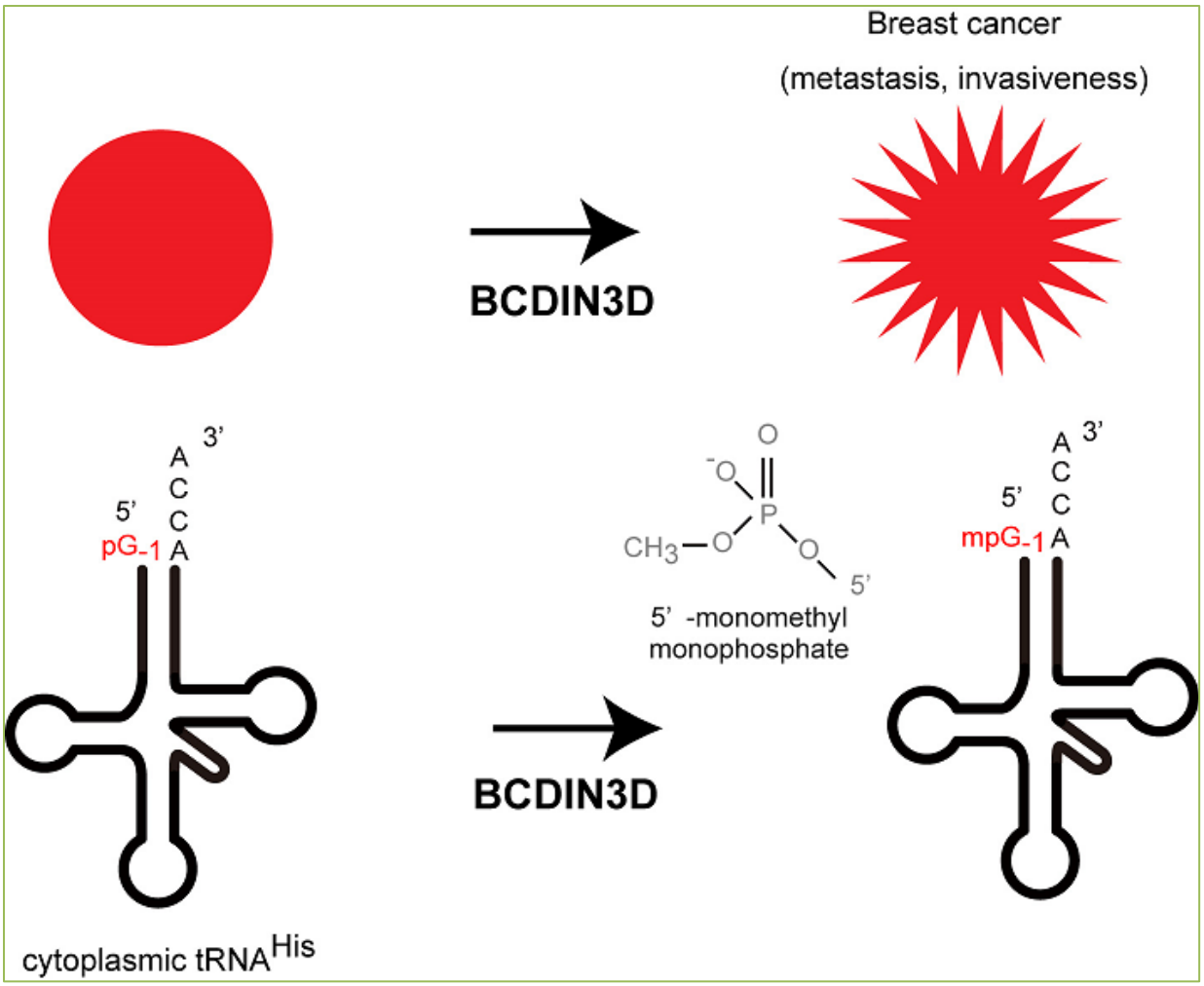

Figure 1. A possible link between tRNA methylation and the tumorigenic phenotype of breast cancer. BCDIN3D is overexpressed in breast cancer cells, and the elevated expression is related to the tumorigenic phenotype and poor prognosis of breast cancer. Cytoplasmic $tRNA^{His}$ is now found to be the primary target of BCDIN3D. The 5'-monophosphate of cytoplasmic $tRNA^{His}$ is monomethylated by BCDIN3D. The modification of $tRNA^{His}$ and/or $tRNA^{His}$ itself might be involved in the tumorigenic phenotype of breast cancer, beyond its established function in protein synthesis.

polymerase-III, the 5'-leader and 3'-tail sequences of the precursor $tRNA^{His}$ are removed. Subsequently, a single guanosine residue (G) is attached to the 5'-end (at position -1; G_{-1}) by a $tRNA^{His}$-specific guanylyltransferase (Thg1) [17, 19, 20] and the CCA is added at the 3'-end (positions 74-76) [21]. As a result, the mature cytoplasmic $tRNA^{His}$ has an eight-nucleotide long acceptor helix with G_{-1}-A_{73} mis-pairing at the top of the helix, while other cytoplasmic tRNAs have seven-nucleotide long acceptor helices. Analyses of the *in vitro* steady state kinetics of methylation of mutant cytoplasmic $tRNA^{His}$ transcripts by recombinant BCDIN3D revealed that BCIDN3D recognizes G_{-1}, and the eight-nucleotide long extended acceptor helix with G_{-1}-A_{73} mis-pairing at the top of the acceptor stem of cytoplasmic $tRNA^{His}$. Thus, BCDIN3D recognizes the unique features of cytoplasmic $tRNA^{His}$, and discriminates cytoplasmic $tRNA^{His}$ from other tRNA species [8].

The function of the 5'-monomethylphosphate of cytoplasmic $tRNA^{His}$ has remained elusive. While the 5'-monomethylphosphate of cytoplasmic $tRNA^{His}$ lowers the affinity of $tRNA^{His}$ toward histidyl-tRNA synthetase (HRS), as expected from the complex structure of bacterial HRS with $tRNA^{His}$ [22], the overall aminoacylation efficiency is not affected by the modification. The steady-state levels of cytoplasmic $tRNA^{His}$ in parental HEK293T and BCDIN3D-knockdown cells are not significantly different. Furthermore, the stabilities of cytoplasmic $tRNA^{His}$ from HEK293T and BCDIN3D-knockdown cells after the treatment of the cells with actinomycin-D were not significantly different. However, the 5'-monomethylmonophosphate protects cytoplasmic $tRNA^{His}$ from degradation *in vitro*, in cytoplasmic cell extracts. Thus, BCDIN3D could act as a cytoplasmic $tRNA^{His}$-specific 5'-methylphosphate capping enzyme, and the methylation of the 5'-monophosphate of cytoplasmic $tRNA^{His}$ might be

involved in increasing its stability under specific conditions or in certain biological processes.

The elucidation of the involvement of the 5'-monophosphate methylation of cytoplasmic $tRNA^{His}$ in the tumorigenic phenotype of breast cancer is challenging (Figure 1). tRNAs are classical non-coding RNAs, and their established functions, as adaptor molecules between genetic codes and amino acids, have been well studied for more than sixty years. However, besides their roles as adaptor molecules in protein synthesis, tRNAs are involved in various biological processes in cells [23-25]. Recent studies have shown that the expression level of initiator $tRNA^{Met}$ is elevated in breast cancer cells [26]. The upregulation of specific tRNAs, such as $tRNA^{Glu}UUC$ and $tRNA^{Arg}CCG$, reportedly stabilizes mRNAs containing the corresponding codons and enhances translation in highly metastatic breast cancer cells [27]. However, the steady state level of cytoplasmic $tRNA^{His}$ is not affected by the knockdown of BCDIN3D in HEK293T cells. Thus, it is unlikely that the methylation of the 5'-monophosphate of cytoplasmic $tRNA^{His}$ increases the translation of specific mRNAs, although it should be examined in the future. Recent studies also have provided evidence that small RNA fragments derived from tRNAs - tRNA fragments (tRFs) - participate in various cellular functions [23-25]. These tRFs are often produced under stress conditions [28-32]. In breast and prostate cancers, specific tRNAs, such as cytoplasmic $tRNA^{Lys}$ and $tRNA^{His}$, are cleaved by angiogenin, and the tRNA halves are abundantly expressed in a sex hormone-dependent manner [33]. These tRNA halves have also been shown to promote the proliferation of breast and prostate cancer cells, by an as yet unknown mechanism. In human and mouse cells, 3'- or 5'-terminal tRFs (3'-tRF or 5'-tRF) reportedly accumulate in cells in an asymmetric manner, and these tRFs associate with Ago2 [34]. These tRFs probably act as typical miRNAs. tRNA modifications, such as 5-methyl cytidine, are associated with the stress induced cleavage of tRNA molecules and the production of tRFs in cells [31]. The 5'-monomethylation of the 5'-monophosphate of $tRNA^{His}$ may control the expression of the tRNA halves and/or tRFs derived from $tRNA^{His}$ in breast cancer cells or under specific biological or stress conditions. Future work will clarify whether the methylation of the 5'-phosphate of $tRNA^{His}$ by BCDIN3D is involved in the tumorigenic phenotype of breast cancer and other cancers.

Conflicting interests

The authors have declared that no conflict of interests exist.

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References

1. Zhu W, Hanes SD. Identification of drosophila bicoid-interacting proteins using a custom two-hybrid selection. Gene 2000; 245: 329-339.
2. Xhemalce B, Robson SC, Kouzarides T. Human RNA methyltransferase BCDIN3D regulates microRNA processing. Cell 2012; 151: 278-288.
3. Yao L, Chi Y, Hu X, Li S, Qiao F, Wu J, *et al.* Elevated expression of RNA methyltransferase BCDIN3D predicts poor prognosis in breast cancer. Oncotarget 2016; DOI: 10.18632/oncotarget.9656.
4. Shi B, Sepp-Lorenzino L, Prisco M, Linsley P, deAngelis T, Baserga R. Micro RNA 145 targets the insulin receptor substrate-1 and inhibits the growth of colon cancer cells. J Biol Chem 2007; 282: 32582-32590.
5. He L, He X, Lowe SW, Hannon GJ. microRNAs join the p53 network - another piece in the tumour-suppression puzzle. Nat Rev Cancer 2007; 7: 819-822.
6. Spizzo R, Nicoloso MS, Lupini L, Lu Y, Fogarty J, Rossi S, *et al.* miR-145 participates with TP53 in a death-promoting regulatory loop and targets estrogen receptor-[alpha] in human breast cancer cells. Cell Death Differ 2009; 17: 246-254.
7. Sachdeva M, Zhu S, Wu F, Wu H, Walia V, Kumar S. p53 represses c-Myc through induction of the tumor suppressor miR-145. Proc Natl Acad Sci USA 2009; 106: 3207-3212.
8. Martinez A, Yamashita S, Nagaike T, Sakaguchi Y, Suzuki T, Tomita K. Human BCDIN3D monomethylates cytoplasmic histidine transfer RNA. Nucleic Acids Res 2017; DOI: 10.1093/nar/gkx051.
9. Rosa MD, Hendrick JP, Jr., Lerner MR, Steitz JA, Reichlin M. A mammalian tRNAHis-containing antigen is recognized by the polymyositis-specific antibody anti-Jo-1. Nucleic Acids Res 1983; 11: 853-870.
10. Cooley L, Appel B, Soll D. Post-transcriptional nucleotide addition is responsible for the formation of the 5' terminus of histidine tRNA. Proc Natl Acad Sci USA 1982; 79: 6475-6479.
11. Suzuki T, Ikeuchi Y, Noma A, Suzuki T, Sakaguchi Y. Mass spectrometric identification and characterization of RNA-modifying enzymes. Methods Enzymol 2007; 425: 211-229.
12. Soma A, Ikeuchi Y, Kanemasa S, Kobayashi K, Ogasawara N, Ote T, *et al.* An RNA-modifying enzyme that governs both the codon and amino acid specificities of isoleucine tRNA. Mol Cell 2003; 12: 689-698.

13. Ohira T, Suzuki T. Precursors of tRNAs are stabilized by methylguanosine cap structures. Nat Chem Biol 2016; 12: 648-655.

14. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, *et al*. Multiplex genome engineering using CRISPR/Cas systems. Science 2013; 339: 819-823.

15. Pyzocha NK, Ran FA, Hsu PD, Zhang F. RNA-guided genome editing of mammalian cells. Methods Mol Biol 2014; 1114: 269-277.

16. Sander JD, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes. Nat Biotechnol 2014; 32: 347-355.

17. Jackman JE, Gott JM, Gray MW. Doing it in reverse: 3'-to-5' polymerization by the Thg1 superfamily. RNA 2012; 18: 886-899.

18. Betat H, Long Y, Jackman JE, Morl M. From end to end: tRNA editing at 5'- and 3'-terminal positions. Int J Mol Sci 2014; 15: 23975-23998.

19. Gu W, Jackman JE, Lohan AJ, Gray MW, Phizicky EM. tRNAHis maturation: an essential yeast protein catalyzes addition of a guanine nucleotide to the 5' end of tRNAHis. Genes Dev 2003; 17: 2889-2901.

20. Jackman JE, Phizicky EM. tRNAHis guanylyltransferase adds G-1 to the 5' end of tRNAHis by recognition of the anticodon, one of several features unexpectedly shared with tRNA synthetases. RNA 2006; 12: 1007-1014.

21. Tomita K, Yamashita S. Molecular mechanisms of template-independent RNA polymerization by tRNA nucleotidyltransferases. Front Genet 2014; 5: 36.

22. Tian Q, Wang C, Liu Y, Xie W. Structural basis for recognition of G-1-containing tRNA by histidyl-tRNA synthetase. Nucleic Acids Res 2015; 43: 2980-2990.

23. Sobala A, Hutvagner G. Transfer RNA-derived fragments: origins, processing, and functions. Wiley Interdiscip Rev RNA 2011; 2: 853-862.

24. Raina M, Ibba M. tRNAs as regulators of biological processes. Front Genet 2014; 5: 171.

25. Kumar P, Kuscu C, Dutta A. Biogenesis and Function of Transfer RNA-Related Fragments (tRFs). Trends Biochem Sci 2016; 41: 679-689.

26. Pavon-Eternod M, Gomes S, Rosner MR, Pan T. Overexpression of initiator methionine tRNA leads to global reprogramming of tRNA expression and increased proliferation in human epithelial cells. RNA 2013; 19: 461-466.

27. Goodarzi H, Nguyen HC, Zhang S, Dill BD, Molina H, Tavazoie SF. Modulated Expression of Specific tRNAs Drives Gene Expression and Cancer Progression. Cell 2016; 165: 1416-1427.

28. Ivanov P, Emara MM, Villen J, Gygi SP, Anderson P. Angiogenin-induced tRNA fragments inhibit translation initiation. Mol Cell 2011; 43: 613-623.

29. Gebetsberger J, Polacek N. Slicing tRNAs to boost functional ncRNA diversity. RNA Biol 2013; 10: 1798-1806.

30. Durdevic Z, Schaefer M. tRNA modifications: necessary for correct tRNA-derived fragments during the recovery from stress? BioEssays 2013; 35: 323-327.

31. Durdevic Z, Mobin MB, Hanna K, Lyko F, Schaefer M. The RNA methyltransferase Dnmt2 is required for efficient Dicer-2-dependent siRNA pathway activity in Drosophila. Cell Rep 2013; 4: 931-937.

32. Ivanov P, O'Day E, Emara MM, Wagner G, Lieberman J, Anderson P. G-quadruplex structures contribute to the neuroprotective effects of angiogenin-induced tRNA fragments. Proc Natl Acad Sci USA 2014; 111: 18201-18206.

33. Honda S, Loher P, Shigematsu M, Palazzo JP, Suzuki R, Imoto I, *et al*. Sex hormone-dependent tRNA halves enhance cell proliferation in breast and prostate cancers. Proc Natl Acad Sci USA 2015; 112: 3816-3825.

34. Li Z, Ender C, Meister G, Moore PS, Chang Y, John B. Extensive terminal and asymmetric processing of small RNAs from rRNAs, snoRNAs, snRNAs, and tRNAs. Nucleic Acids Res 2012; 40: 6787-6799.