Gene Technology
ISSN: 2329-6682

Kolari, et al., Gene Technology 2015, 4:2
DOI: 10.4172/2329-6682.1000120

Short Communication Open Access

Novel Nuclear Biology of Small Non-Coding RNAs

Ida-Liisa Kolari[1#], Pia Laitinen[1*], Mikko P. Turunen[1] and Seppo Ylä-Herttuala[1,2*]

[1]*Department of Biotechnology and Molecular Medicine, A.I.Virtanen Institute, University of Eastern Finland, Kuopio, Finland*

[2]*Science Service Center and Gene Therapy Unit, Kuopio University Hospital, Kuopio, Finland*
[#]*Authors with equal contribution*

[*]**Corresponding author**: Seppo Ylä-Herttuala, A. I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, P.O. Box 1627, FIN-70211 Kuopio, Finland, Tel: +358 40 355 2075; Fax: +358 17 163751; E-mail: seppo.ylaherttuala@uef.fi

Rec date: March 03, 2015; **Acc date:** April 01, 2015; **Pub date:** April 06, 2015

Description

When ENCODE project [1] was recently published it became evident that there is unexpectedly large amount of non-coding RNAs (ncRNAs) in mammalian cells and these findings have boosted a wide interest in the role of ncRNAs in both basic cellular processes and their functions in pathological conditions. The first big boom in ncRNA research was fueled by findings of Fire and Mello [2] after they had found RNA interference (RNAi), which discovery was awarded Nobel prize in 2006. Small interfering RNAs (siRNA) and microRNAs (miRNAs) have since been developed both as important tools for molecular biology research and also as therapeutics for gene therapy applications. These ncRNAs have been thought to function mostly in cytoplasm of target cells mediating post transcriptional gene silencing (PTGS). However, Morris et al. [3] demonstrated for the first time that siRNAs, that are complementary to the promoter region of a gene, can regulate gene activity by mediating transcriptional gene silencing (TGS). Same year, it was published that RNAi effector complex termed RITS (RNA-induced initiation of transcriptional gene silencing) is required for heterochromatin assembly in fission yeast and that work suggested a mechanistical role of of RNAi factors and small RNAs in regulation of heterochromatin [4]. These findings have revealed that these short ncRNAs have important regulatory functions also in the nucleus of target cells. Also opposite effect of promoter targeted siRNAs was soon described, when Li et al. [5] showed that they can also mediate transcriptional gene activation (TGA) of targeted genes.

We have shown that small hairpin RNAs (shRNA) can mediate both TGA and TGS on the same promoter, depending on the targeted locus [6]. When we delivered these shRNAs to ischemic mouse hindlimbs in vivo using lentiviral vectors, we observed increased vascularity in muscles where expression of Vascular Endothelial Growth Factor A (VEGF-A) was upregulated by TGA [6]. In a later study, we analyzed therapeutic potential of shRNA mediated TGA of VEGF-A in a murine myocardial infarction model [7]. In the treated group infarction size was siqnificantly reduced after two weeks of lentiviral injection as analyzed by MRI and histology. This surprisingly good therapeutic efficiency led us to consider following possibilities for the observed effects: Since we showed that all isoforms of VEGF-A are upregulated by upregulating activity of endogenous promoter, could this lead to better therapeutical outcome as compared to traditional gene therapy where usually only one isoform is delivered? Since it has been recently shown that miRNAs can be transferred from endothelial cells to smooth muscle cells [8], could these shRNAs also be secreted in vivo and therefore spread the therapeutic effect in transduced heart? Our preliminary unpublished data seems to indicate that this is the case.

The mechanism of action of these TGA/TGS mediating shRNAs has been studied by us and others [6,7,9-13]. For example, it is known that pre-existing epigenetic status of the promoter determines if it is susceptible to TGS/TGA and that this can be affected treating cells with 5-Azacytidine, a DNMT inhibitor [7]. The detailed model of action has not yet been established and different mechanisms likely exist between different promoters, but there are several examples suggesting that these small RNAs target non-coding transcripts present at promoters and recruite chromatin modifying proteins which then mediate epigenetic modifications. Also, depending on whether small RNA targets sense or antisense transcript could determine the outcome, since it is possible that the mass balance of non-coding transcripts in the promoter affects gene expression [14]. Interestingly, it has been shown that also endogenous miRNAs can mediate TGS or TGA of a gene by targeting promoter region in chromatin [15,16]. Therefore, for finding potential sites for promoter targeted small RNAs one can use bioinformatic tools, such as RegRNA (http://regrna2.mbc.nctu.edu.tw), to predict natural miRNA targets site.

Traditionally miRNAs have been considered to function in cytoplasm mediating PTGS by targeting 3´-UTRs of mRNAs. In recent years it has become evident that miRNAs are also present in nucleus [17]. A specific hexanucleotide element has been suggested as a nuclear localization signal for miRNAs [18], but it seems to be the case only for miR-29b and not other miRNAs [17]. It is also interesting that different arms of the same miRNA can have either cytoplasmic or nuclear preference, as in the case of miR-373-3p which is able to penetrate into nucleus whereas miR-373-5p is not [19]. It might be that the presence of available miRNA targets determines the sub-cullular distribution of the miRNA [20].

These recently discovered new nuclear roles for small RNAs have great impact on the use of small RNAs as therapeutics [21]. On one hand, new safety considerations for clinical use of RNAi therapeutics must be done, since the apparent nuclear-cytoplasmic trafficking of small RNAs must be taken into consideration when estimating possible off-target effects. On the other hand, manipulation of epigenetic code of target genes by non-coding small RNAs might offer new, efficient ways for regulating gene expression.

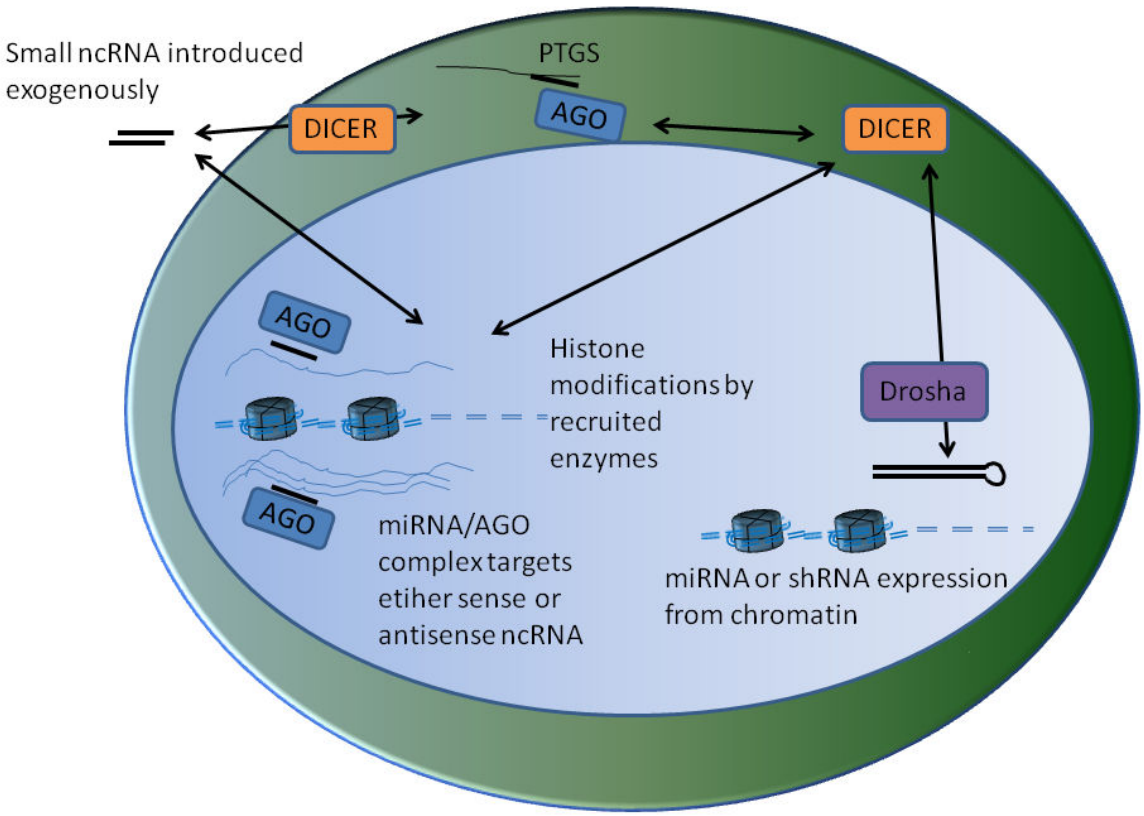

Figure 1: Small RNAs (such as siRNAs, shRNAs and miRNAs), can have functions both in cytoplasma (PTGS) and in nucleus (TGS/TGA). Small RNAs likely suffle between nucleus and cytoplasm. Pre-existing epigenetic status of the promoter and balance between sense and antisense transcripts could determine whether gene is activated (TGA) or downregulated (TGS).

References

1. Yavartanoo M, Choi JK (2013) ENCODE: A Sourcebook of Epigenomes and Chromatin Language. Genomics Inform 11: 2-6.
2. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, et al. (1998) Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 19: 806-11.
3. Morris KV, Chan SW-L, Jacobsen SE, Looney DJ (2004) Small interfering RNA-induced transcriptional gene silencing in human cells. Science 305: 1289-1292.
4. Verdel A, Jia S, Gerber S, Sugiyama T, Gygi S, et al. (2004) RNAi-mediated targeting of heterochromatin by the RITS complex. Science 303: 672-676.
5. Li L-C, Okino ST, Zhao H, Pookot D, Place RF, et al. (2006) Small dsRNAs induce transcriptional activation in human cells. Proc Natl Acad Sci 103: 17337-17342.
6. Turunen MP, Lehtola T, Heinonen SE, Assefa GS, Korpisalo P, et al. (2009) Efficient regulation of VEGF expression by promoter-targeted lentiviral shRNAs based on epigenetic mechanism: a novel example of epigenetherapy. Circ Res Sep 105: 604-609.
7. Turunen MP, Husso T, Musthafa H, Laidinen S, Dragneva G, et al. (2014) Epigenetic upregulation of endogenous VEGF-A reduces myocardial infarct size in mice. PLoS One 9: e89979.
8. Hergenreider E, Heydt S, Tréguer K, Boettger T, Horrevoets AJG, et al. (2012) Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. Nat Cell Biol. 14: 249-256
9. Schwartz JC, Younger ST, Nguyen N-B, Hardy DB, Monia BP, et al. (2008) Antisense transcripts are targets for activating small RNAs. Nat Struct Mol Biol. 15: 842-848.
10. Janowski B, Huffman KE, Schwartz JC, Ram R, Nordsell R, et al. (2006) Involvement of AGO1 and AGO2 in mammalian transcriptional silencing. Nat Struct Mol Biol 13: 787-792.
11. Han J, Kim D, Morris KV (2007) Promoter-associated RNA is required for RNA-directed transcriptional gene silencing in human cells. Proc Natl Acad Sci 104: 12422-12427.
12. Weinberg MS, Villeneuve LM, Ehsani ALI, Amarzguioui M, Aagaard L, et al. (2006) The antisense strand of small interfering RNAs directs histone methylation and transcriptional gene silencing in human cells. RNA 12: 256-562.
13. Khalil AM, Guttman M, Huarte M, Garber M, Raj A, et al. (2009) Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. Proc Natl Acad Sci USA 106: 11667-11672.
14. Ylä-Herttuala S, Kaikkonen MU (2014) Does mass balance between sense and antisense transcripts fine-tune the outcome of gene expression? EMBO Reports 15: 125-126.

15. Place RF, Li L, Pookot D, Noonan EJ, Dahiya R (2008) MicroRNA-373 induces expression of genes with complementary promoter sequences. Proc Natl Acad Sci USA 105: 1608-1613.
16. Younger ST, Corey DR (2011) Transcriptional gene silencing in mammalian cells by miRNA mimics that target gene promoters. Nucleic Acids Res 39: 5682-5691.
17. Liao JY, Ma LM, Guo YH, Zhang YC, Zhou H, et al. (2010) Deep sequencing of human nuclear and cytoplasmic small RNAS reveals an unexpectedly complex subcellular distribution of mirnas and tRNA 3′ trailers. PLoS One 5: e10563.
18. Hwang H-W, Wentzel EA, Mendell JT (2007) A Hexanucleotide Element Directs MicroRNA Nuclear Import Science 315: 97-100.
19. Jeffries CD, Fried HM, Perkins DO (2010) Additional layers of gene regulatory complexity from recently discovered microRNA mechanisms. Int J Biochem Cell Biol 42: 1236-1242.
20. Berezhna SY, Supekova L, Supek F, Schultz PG, Deniz A (2006) siRNA in human cells selectively localizes to target RNA sites. Proc Natl Acad Sci U S A 103: 7682-7687.
21. Husso T, Ylä-herttuala S, Turunen MP (2014) A New Gene Therapy Approach for Cardiovascular Disease by Non-coding RNAs Acting in the Nucleus. Mol Ther Nucleic Acids 18: e197.