Novel Nuclear Biology of Small Non-Coding RNAs

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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 significantly 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 therapeutic 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 recruit chromatin modifying proteins which then mediate epigenetic modications. 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 subcellular 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 cytoplasm (PTGS) and in nucleus (TGS/TGA). Small RNAs likely shuttle 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


