Trends in Cell Biology

CellPress

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Global miRNA to miRNA Interactions: Impacts for miR-21

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miRNAs inherently alter the cellular environment by regulating target genes. miRNAs may also regulate other miRNAs, with far-reaching influence on miRNA and mRNA expression. We explore this realm of small RNA regulation with a focus on the role of the oncogenic miR-21 and its impact on other miRNA species.

Canonical miRNA Function

miRNA, a form of noncoding RNA (ncRNA), negatively regulates target mRNA via the identification of miRNA recognition elements (MREs) within the 3′ untranslated region (UTR) [1]. Up to 60% of known mRNAs are regulated in this manner, and each miRNA family has been estimated to target 534 different mRNAs [2].

The canonical biogenesis of these small regulators consists of a series of cleavage steps. In the nucleus, Drosha and Di George Critical Region 8 (DGCR8) cleave primary (pri)-miRNA to produce precursor (pre)-miRNA, which is followed by Dicer cleavage in the cytoplasm to form double-stranded miRNA [1]. One strand of this complex is incorporated into the RNA-induced silencing complex (RISC), which facilitates miRNA-driven gene suppression (Figure 1) [1].

Regulation of miRNAs by miRNAs

miRNAs are well established in the literature as negative regulators of gene expression [1]. However, an unconventional aspect of miRNA functioning is miRNAs' role in controlling the expression of other miRNAs (Figure 1). This is called a miRNA–miRNA interaction [3]. Current evidence suggests that these interactions may occur directly between miRNAs at the pri-miRNA or mature miRNA stages of their biogenesis or via indirect means, such as the targeting of transcription factors or regulators of miRNAs [3].

Cellular Impact of miRNA–miRNA Interactions

The regulatory impact of a miRNA or set of miRNAs on mRNA expression has only recently been considered. In the cardiac cells of transgenic mice overexpressing miR-499, it was shown that miR-499 was able to regulate target genes but also the expression of 11 miRNAs, including miR-34c-5p, miR-208b-3p, and miR-214-5p [4]. Of the 969 downregulated target genes that were identified, only 7.8% were verified miR-499 targets, whereas 38.8% were targets of the 11 upregulated miRNAs [4]. This indicates that hundreds of genes may be altered in expression as a result of secondary miRNA and mRNA changes. What directs these alterations is currently unknown, but it is evident that miRNA–miRNA interactions have substantial compounding effects on miRNA and mRNA expression.

A similar study in ovarian cancer found that only 14% and 11.9% of differentially expressed genes were targets of the transfected miR-7 and miR-128, respectively [5]. These miRNAs were chosen because they are highly upregulated in ovarian cancer tissue and are related to cancer progression through their control of the epidermal growth factor receptor (EGFR) [5]. This indicates that the majority of dysregulated target genes are the result of changes in other miRNAs, exhibiting the widening impact of miRNA–miRNA interactions on the molecular milieu.

Groups of miRNAs, rather than individual miRNAs, also participate in miRNA–miRNA regulation. Ooi *et al.*, in their study of cardiac pathology, discovered that the three members of the miR-34 family (miR-34a, b, and c) co-operate to positively control 60 pathological miRNAs and 57 cardioprotective miRNAs. The balance of these two miRNA groups influences cardiac hypertrophy [6]. The created network demonstrated the suppression of transcription factors, such as Sirt1 and CTCF, via the miR-34 family members, which altered pri-miRNA transcription of 'pathological' or 'cardioprotective' miRNAs [6]. Another miRNA family acting together to alter miRNA expression is the miR-130 family (miR-130b, miR-301a, and miR-301b) [7]. Within a mouse model, this miRNA family suppressed peroxisome proliferator-activated receptor-γ (PPAR) and signal transducer and activator of transcription 3 (STAT3), resulting in a decrease of miR-204, miR-424, and miR-503 and amplification of the signals for increased cell proliferation [7]. The decrease in these three miRNAs may have contributed to the maintenance and progression of pulmonary hypertension observed in these mice [7]. This finding highlights that miRNA homeostasis has the potential to increase the pathogenicity of a single miRNA or miRNA family, which in turn would have profound phenotypic consequences.

miRNA–miRNA Interactions of miR-21

The most ubiquitously upregulated miRNA in solid human malignancies is the oncogenic miRNA, miR-21 [8]. For this reason, it is important to examine the implications of increased miR-21 expression, with a focus on miRNA–miRNA regulation and its cascading effect on cell function. Through these currently unexplored interactions, the notion is that miR-21 may facilitate and coordinate changes in the miRNA profile, altering cancer-related pathways and accumulating deleterious molecular alterations.

Previously, miR-21 has been described to participate in several miRNA–miRNA

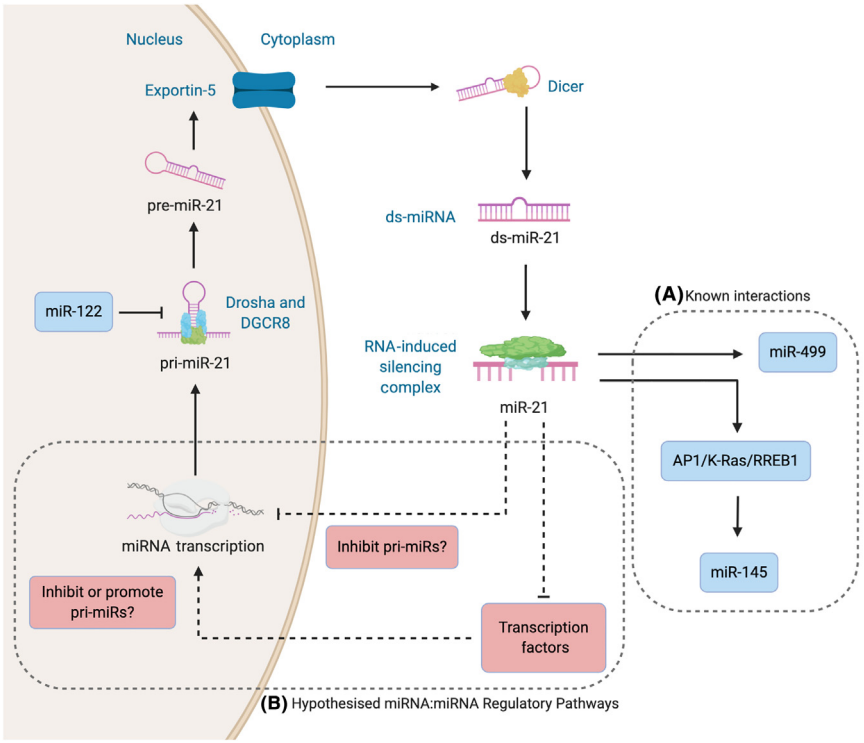

Figure 1. Known and Hypothesised Examples of miRNA-21–Mediated miRNA Regulation. The canonical miRNA biogenesis of miR-21 is shown, whereby it is cleaved by Drosha and Di George Critical Region 8 (DGCR8), exported into the cytoplasm by exportin-5, and cleaved again by Dicer before incorporation into the RNA-induced silencing complex (RISC). (A) miRNAs known to interact with miR-21 are highlighted and shown in blue. (B) Hypothesised pathways for miRNA–miRNA regulation are indicated by broken lines, with descriptors in red. The arrowheads or blunt ends depict the direction of the interaction. The figure was created using BioRender.com. Abbreviations: AP1, activator protein 1; ds, double stranded; pre-, precursor; pri, primary; RREB1, Ras-responsive element–binding protein 1.

interactions. However, these investigations only considered its direct impact on miRNA or mRNA expression. A recent study showed that miR-21 stabilised mature miR-499 in a post-transcriptional manner, which aided in the suppression of programmed cell death 4 (PDCD4) in head and neck squamous cell carcinoma (HNSCC), resulting in increased cancer growth and invasion [8]. Another example is the feedback loop between miR-21 and miR-145 via K-Ras, activator protein 1 (AP1), and Ras-responsive element–binding protein 1 (RREB1) in colorectal cancer [9]. This in turn aided the growth of colorectal cancer stem cells and contributed to chemoresistance [9].

miR-21 is also controlled through miRNA–miRNA regulation. In human liver cells, it was found that the primary form of miR-21 is targeted by miR-122 in the nucleus [10]. Loss of the homeostatic suppression of miR-21 by miR-122 in hepatocellular carcinoma resulted in elevated miR-21 levels and a subsequent decrease in its targets, such as PDCD4, resulting in increased cancer cell growth [10].

Current evidence also supports the regulation of pri-miRNAs by mature miRNAs within the nucleus [10]. Approximately 20% of cellular miR-21 is located within the nucleus [11]. Therefore, it is reasonable to hypothesise that miR-21 may target several pri-miRNAs. This phenomenon is yet to be fully explored and may apply to several miRNAs, further expanding and complicating their regulatory role.

A visual summary of the miRNA–miRNA interactions of miR-21 is shown in Figure 1. This diagram encompasses the regulatory pathways involving both the nucleus and cytoplasm and indicates potential pathways for the investigation of miRNA–miRNA interactions of miR-21.

Concluding Remarks

Unveiling the underlying control of miRNAs by miR-21 has important implications in the future design and implementation of miRNA-directed therapeutics. For therapies of this kind, not only the direct action of the therapeutic agent with the targeted miRNA but also the downstream alterations in miRNA and gene expression that may alter the effect of the therapeutic need to be considered. So far, the question of miR-21's role in globally regulating miRNAs has yet to be addressed. The introduction of miRNA mimics and antisense inhibitors inherently alters the miRNA profile, and this approach is limited in its capacity to identify dysregulated miRNAs [12]. The reduction or deletion of miR-21 using methods such as knockout mouse models or Crispr-Cas9 is similar to that of antisense inhibitors in terms of the impact on miRNA and mRNA expression, and thus it also presents challenges in the identification of miRNA–miRNA interactions [12].

Moving forward, there is an urgent need to establish more appropriate methods to measure the interactions between miRNAs and their downstream effect to fully understand this form of regulation. Currently, comparison with a scramble oligonucleotide provides the most accurate option for mimicking the effect of miRNA saturation as in the case of transfection, but it has the disadvantage of also altering miRNA and mRNA levels. The origin of

studied cells and experimental conditions also need to be carefully considered to ensure that any conclusions concerning miRNA–miRNA interactions are scientifically sound. There is a new world of miRNA regulation, and the exploration of these interactions is vital to gain a full understanding of cellular processes in healthy and diseased cells.

Acknowledgments

M.H. is supported by an Australian Research Training Program Scholarship and a Translational Cancer Research Network PhD Scholarship Top-Up Award, supported by the Cancer Institute NSW.

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https://doi.org/10.1016/j.tcb.2020.10.005

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