Trends in Cancer

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MicroRNAs Regulating MicroRNAs in Cancer

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MicroRNAs (miRNA) are capable of self-regulation, termed miRNA to miRNA interaction. Very little is known about these interactions and their impact on the cellular milieu. We discuss known miRNA to miRNA interactions, potential mechanisms, and their role in cancer.

The Function of MiRNAs

MiRNAs are classified as noncoding RNAs that regulate the expression of messenger RNA (mRNA) by binding to complementary sequences within the 3′ untranslated region (3′ UTR) [1]. The transcribed primary RNA transcript undergoes cleavage by the enzymes Drosha and Dicer to produce precursor and mature miRNA, respectively [1]. Mature miRNAs are bound to Argonaute (Ago) to form the RNA induced silencing complex (RISC) for gene regulation. Due to their role, alterations in miRNA expression can disrupt mRNA expression, including that of oncogenes and tumour suppressor genes, leading to potential oncogenic changes [1,2]. Thus, it is important that more is known about the regulation of miRNAs to further understand their influence in a cellular system. The purpose of this article is to highlight an emerging area of miRNA regulation, whereby one miRNA controls the expression of another.

MiRNA to MiRNA Regulation

Several processes are involved in regulating the biogenesis of miRNAs and their endogenous levels. However, recent studies have shown that miRNAs can bind to and control other noncoding RNAs, including miRNAs. This process is known as a miRNA:miRNA interaction. The overall result of this interaction is the regulation of a miRNA's abundance and biogenesis by another miRNA, with subsequent effects on mRNA regulation.

This phenomenon was first observed by Lai *et al.,* who found two miRNA pairs in *Drosophila*, miR-5:miR-6 and miR-9:miR-79, based on nucleotide sequence complementarity [3]. This suggested that miRNAs could bind and regulate both mRNA and noncoding RNA. However, since these miRNA pairs were identified through sequencing and were not experimentally validated, it is uncertain whether these miRNA complexes are formed in biological systems. It has been proposed that miRNA pairing between similar miRNAs increases their individual stability [3]. Additionally, the binding of miRNA pairs prevents the control of their target mRNA. This results in a decrease in target regulation, and thus increases target abundance. This creates a feedback mechanism between miRNA and mRNA, and alters downstream cellular function [3].

Proceeding studies further investigated the direct regulation of a miRNA by another miRNA. For example, miR-107 can regulate the abundance of let-7 through binding to complementary sequences within its stem loop [4]. Since let-7 targets several oncogenes such as RAS, destabilisation and degradation by miR-107 has consequences on tumorigenesis [4]. Matkovich *et al*. found that, in cardiac cells, miR-499 overexpression lead to the upregulation of 11 miRNAs and the downregulation of 6 miRNAs [5]. These studies hinted at the potential contribution of miRNA:miRNA interactions to oncogenesis, highlighting why understanding these interactions is important to explore new modalities of gene regulation.

Several models and mechanisms have been proposed to explain miRNA:miRNA interactions. Multiple studies have introduced the concept of complementary binding between miRNAs in their mature form [3,4]. This is a feasible concept, but poses the question of how RISC-bound miRNAs can bind to each other, and how this results in miRNA regulation. Findings by Flamand *et al*. showed that the amino acid residues within the Ago2 complex interact to promote the binding of miRNAs to noncanonical sites [6]. Given this observation, it is possible that Ago2 can force the binding of two different miRNAs in a similar manner to noncanonical binding, and promote miRNA:miRNA interactions.

Also suggested is RISC stabilisation from miRNA binding, however there is still no mechanistic evidence for this action [3,4,7]. Park *et al*. demonstrated that the RISC complex is destabilised when it binds to noncanonical sites [8]. Complementary to this, the binding of RISC to canonical sites results in stabilisation [8]. From these observations, it is suggested that the direct binding of two miRNAs results in stabilisation, preventing miRNA degradation, and altering their turnover. These studies only provide potential models as to how these interactions may occur, and more research needs to be performed to elucidate the binding mechanism and exact biochemical pathway behind miRNA:miRNA interactions.

miRNAs can regulate miRNA biogenesis by targeting primary or precursor miRNAs. For example, in mice miR-709 regulates miR-15a/16-1 production by binding to its primary transcript [9]. This introduced the concept of a miRNA hierarchy, whereby a miRNA regulates a group of specific miRNAs at the post-transcriptional level [9]. There is also the potential for self-regulation via a positive feedback mechanism [7]. This was first shown in *Caenorhabditis elegans*, where the mature form of let-7 binds to a

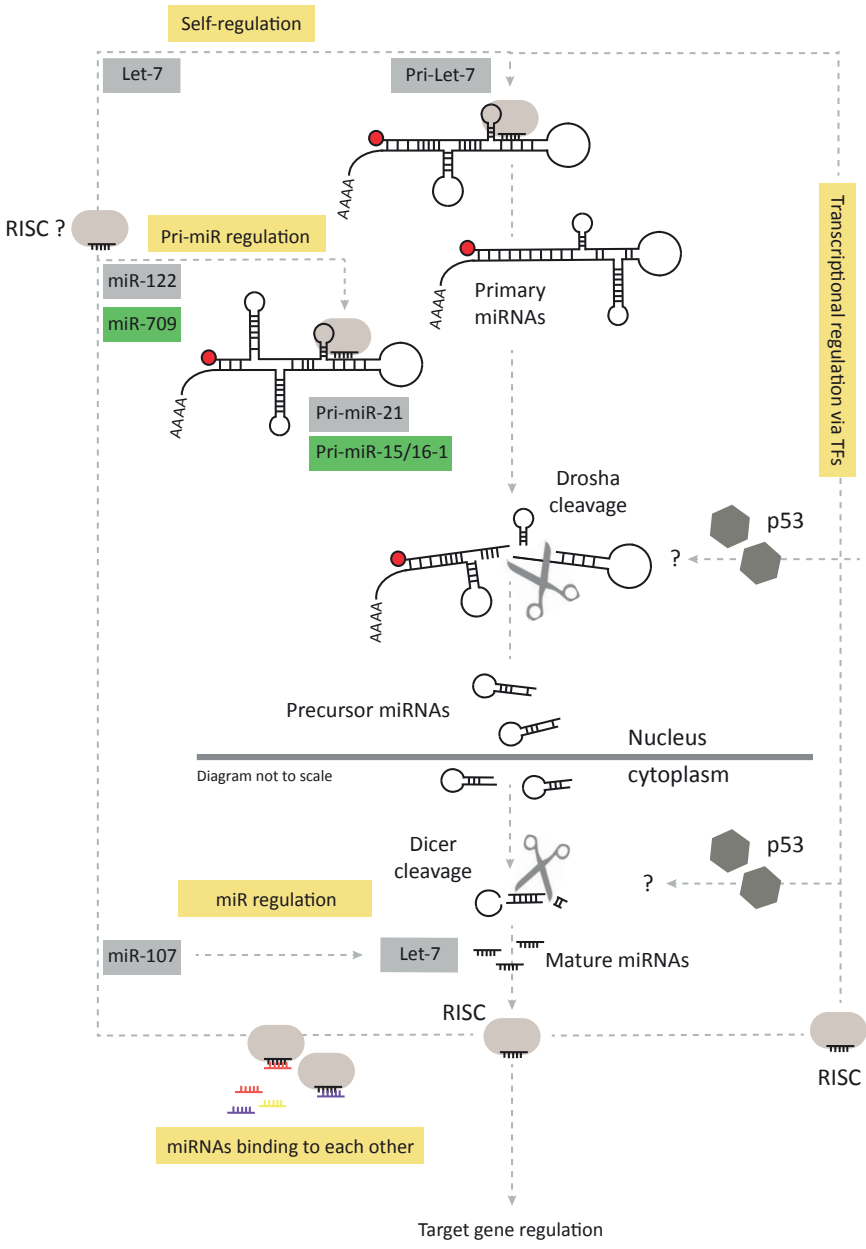

Figure 1. Potential miRNA:miRNA Regulatory Pathways. In the centre is the canonical miRNA biogenesis pathway, whereby pri-miRNA is cleaved by Drosha to produce pre-miRNA. Pre-miRNA is exported to the cytoplasm and cleaved by Dicer to form mature miRNA, which is bound by Ago to form RISC, which performs target regulation. Direct miRNA:miRNA regulatory pathways are included to the left of the canonical pathway, and are depicted in yellow. This includes the self-regulation of miRNA via RISC at the pri-miRNA stage of biogenesis (e.g., Let-7). Pri-miRNA regulation occurs through the binding of mature miRNAs to the primary transcript of another (e.g., miR-122 and pri-miR-21 shown in grey, and miR-709 and pri-miR-15a/16-1 as shown in green). At the mature level, RISC-bound miRNAs can interact directly for regulation (e.g., miR-107 and Let-7). To the right, the indirect mechanism for miRNA:miRNA interactions, the modulation of transcription factors (TFs), is seen in yellow. p53 is also included on the right at both the Drosha and Dicer processing steps, as an indirect pathway for miR-486-5p regulation. The arrow heads depict the direction of the interaction.

recognition site within pri-let-7 to promote further let-7 production [7]. This study demonstrated that mature miRNAs may perform their regulatory role inside the nucleus and is the first to show that miRNAs have the capacity for self-regulation.

Recent studies in this area have focused on the role of miRNA:miRNA interactions in tumorigenesis. A new study found that the oncogenic miR-21, which is frequently overexpressed in most cancers, is negatively regulated by miR-122 [10]. This occurs through the binding of miR-122 to the miR-21 primary transcript to block Drosha cleavage, leading to a decrease in the overall abundance of mature miR-21 [10]. There is a suggestion that the targeting of primary or precursor miRNA transcripts for miRNA self-regulation is not limited to the miR-122 and miR-21 pair. It is estimated that 79% of mature miRNAs contain sequences similar to motifs found in primary miRNA transcripts [11], therefore this mechanism may represent a wider mode of miRNA regulation [10]. This discovery provides great impetus for more research into the self-regulation of miRNAs, how these interactions affect biogenesis, and their impact on tumorigenesis.

The Role of Transcription Factors and Regulators

Although miRNA:miRNA interactions occur through direct binding, several studies have described an indirect pathway involving transcription factors and repressors. For instance, miR-660-5p can alter the expression of miR-486-5p via its regulation of Mouse Double Minute 2 (MDM2) and p53 [12]. In this model, a decrease in MDM2, initiated by miR-660-5p, stabilises p53, and promotes the production of miR-486-5p [12]. This study sets forth the ideal that the p53 pathway may be a key player in the regulation of miRNAs by other miRNAs. However, more studies are needed to determine its exact role. Given this pathway is often dysregulated in cancers, its network with

miRNAs may also be affected, producing downstream effects on gene regulation and tumorigenesis [12].

The effect of transcription factors on the production of miRNAs and their total abundance in a cellular system has been less explored, but is vitally important in understanding this mode of regulation. The study by Matkovich *et al*. found that secondary miRNA changes were observed in response to miR-499-mediated regulation of miRNAs [5]. These changes were primarily through the modulation of transcription factors and the subsequent alterations in transcriptional activity [5]. Although this is an important study, there is little work discussing biologically validated global miRNA:miRNA interactions, specifically in cancer cells. These findings broaden our understanding of the mechanisms behind miRNA:miRNA interactions, their transcriptional impact, and the subsequent indirect regulation of miRNA and mRNA. The direct and indirect mechanisms for miRNA:miRNA interactions are summarised in Figure 1.

Concluding Remarks and Remaining Questions

The self-regulation of miRNAs is an important emerging field. These interactions, especially on a cellular scale, have a direct influence on mRNA expression and are vital in understanding gene regulation. However, we still do not fully understand the mechanism behind miRNA:miRNA interactions, or whether multiple processes are involved. Additionally, questions remain regarding how these interactions affect the miRNA milieu, mRNA levels, transcription factors, and their cellular implication. Since the expression of miRNA is commonly altered in diseases, including cancer, this could have a direct effect on the expression of other miRNAs and mRNAs, contributing to the disease phenotype [2]. Hence, miRNA:miRNA interactions and their influence on the cellular system and functioning is of significance, and further research in this field is greatly encouraged.

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Disclaimer Statement

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References

1. Daugaard, I. and Hansen, T.B. (2017) Biogenesis and function of Ago-associated RNAs. *Trends Genet.* 33, 208–219
2. Kalfert, D. *et al.* (2015) MicroRNA profile in site-specific head and neck squamous cell cancer. *Anticancer Res.* 35, 2455–2463
3. Lai, E.C. *et al.* (2004) Complementary miRNA pairs suggest a regulatory role for miRNA:miRNA duplexes. *RNA* 10, 171–175
4. Chen, P.S. *et al.* (2011) miR-107 promotes tumor progression by targeting the let-7 microRNA in mice and humans. *J. Clin. Invest.* 121, 3442–3455
5. Matkovich, S.J. *et al.* (2013) Regulation of cardiac microRNAs by cardiac microRNAs. *Circ. Res.* 113, 62–71
6. Flamand, M.N. *et al.* (2017) A non-canonical site reveals the cooperative mechanisms of microRNA-mediated silencing. *Nucleic Acids Res.* 45, 7212–7225
7. Zisoulis, D.G. *et al.* (2012) Autoregulation of microRNA biogenesis by let-7 and Argonaute. *Nature* 486, 541–544
8. Park, J.H. *et al.* (2017) Non-canonical targets destabilize microRNAs in human Argonautes. *Nucleic Acids Res.* 45, 1569–1583
9. Tang, R. *et al.* (2012) Mouse miRNA-709 directly regulates miRNA-15a/16-1 biogenesis at the posttranscriptional level in the nucleus: evidence for a microRNA hierarchy system. *Cell Res.* 22, 504–515
10. Wang, D. *et al.* (2017) Nuclear miR-122 directly regulates the biogenesis of cell survival oncomiR miR-21 at the posttranscriptional level. *Nucleic Acids Res.* 46, 2012–2029
11. Auyeung, V.C. *et al.* (2013) Beyond secondary structure: primary-sequence determinants license pri-miRNA hairpins for processing. *Cell* 152, 844–858
12. Borzi, C. *et al.* (2017) mir-660-p53-mir-486 network: a new key regulatory pathway in lung tumorigenesis. *Int. J. Mol. Sci.* 18, E222