Review Article

Perspectives on MicroRNA Study in Oncogenesis: Where Are We? ☆

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MicroRNA (miR) was initially discovered as a regulator of developmental timing in *C. elegans* by the laboratory team of Dr. Victor R. Ambros in 1993 [1]. Since then, our growing knowledge about this type of small non-coding RNA has redefined the global picture of gene regulation at the molecular level [2]. Studies have reported that miRs play crucial roles in various biological processes, including cancer, immunity, metabolism, and cell development, and are of great interest in the fields of neuroscience, stem cell research, and microbiology [3]. Moreover, miR dysregulation has been linked to different types of pathological diseases, including autoimmune diseases, metabolic disorders, and human cancer [4]. Recent evidence indicates that miRs remain a hot topic in the fields of RNA and cancer biology research [5–7].

Cancer is a complex disease involving altered expressions of various genes, including oncogenes and tumor suppressors. While research attention was traditionally focused on protein-coding genes, it is now well-known that miRs play essential roles in different aspects of cancer progression [8–11]. High-throughput technologies based on miR profiling have brought advancements in cancer diagnosis and prognosis [12]. The latest technology for high-throughput miR biopsy, termed inCell-Biopsy, provides a commercially available method for miR profiling *in situ* in living cells, which could reveal cellular heterogeneity based on miR expression [13]. Moreover, the improvement of conventional miR amplification and detection technologies, such as RT-qPCR, Northern blot, microarray, and RNA-seq, has opened new possibilities for improved clinical diagnosis of cancer patients. Further research is needed to continue to enhance the stability, sensitivity, and specificity of these technologies for future cancer diagnosis and prognosis.

Differentially expressed circulating miRs have been used as potential biomarkers for initial cancer screening [14]. Circulating miRs play critical roles in cell–cell communications. Exosomes export key oncoproteins and miRs, thus facilitating intercellular communications in the tumor microenvironment [15]. Therefore, cancer cell-secreted miRs might be useful as universal cancer diagnosis markers, and as potential therapeutic targets. An improved understanding of the roles and molecular mechanisms of exosome-mediated miRs during oncogenesis could guide future clinical applications of engineered exosome miRs.

Modulation of miR bioprocessing genes (e.g., XPO5, RISC complex, Dicer, and Drosha) through genomic alteration, or in other ways, could significantly affect cancer initiation, progression, and metastasis [16–19]. Recent investigations in mouse models and colon cancer clinical samples have shown that XPO5 knockdown can hinder cancer progression [20]. It was also recently demonstrated that Drosha mutation enhances lung cancer cell metastasis, and Dicer 1 dysregulation predisposes humans to various solid cancers [21,22]. It would be interesting to examine other key miR bioprocessing proteins (e.g., DGCR8 and TRBP) in the setting of cancer. In future studies, engineered mouse models could be a valuable tool for assessing the pathological roles of these miR bioprocessing proteins in different types of human cancers.

A mature miR strand acting as a gene silencer is typically viewed as the guide strand (5p), and the other strand as the miR star (miR*) strand (3p), which usually behaves as a degraded strand [23]. Among both 5p and 3p strands, there may be many potential targets relating to oncogenesis. For example, miR-34a/b-3p blocks cervical cancer cell progression and metastasis [24]. However, many unanswered questions remain. How do miRs select both strands in different types of cancer cells? What molecular mechanisms underlie this selection? Which types of modifications might contribute to these molecular mechanisms? What are the overlapping targets of both strands in certain types of cancer cells? Uncovering the answers to all of these questions could guide the development of miR-targeting cancer therapies. Future research is also needed to further elucidate additional functional roles of more 3p strands.

Several helpful bioinformatics algorithms are available for miR research, such as TargetScan [25], miR-TV [26], CAMIRADA [27], and miRDB [28], which are used to predict potential miR target genes in cancer studies. However, these tools each have strengths and weaknesses. To minimize the weakness of the programs, it is better to refer to the combined predictions. In the future, it is worthy to create a new "miR-Cancer" database, which can include all key features—such as the predicted miR targetome according to multiple key principals, validated targets, and associated diseases, along with all the related publications, and the tissue culture and engineered animal models used in specific cancer studies. With the continuing development of IT technology, a novel database of this nature will likely be developed in the near future.

At this time, miRbase serves as a helpful database for published miRs across different species [29]. After nearly 30 years of research efforts, the latest version of miRbase includes ~38,589 entries. To date, miRs have
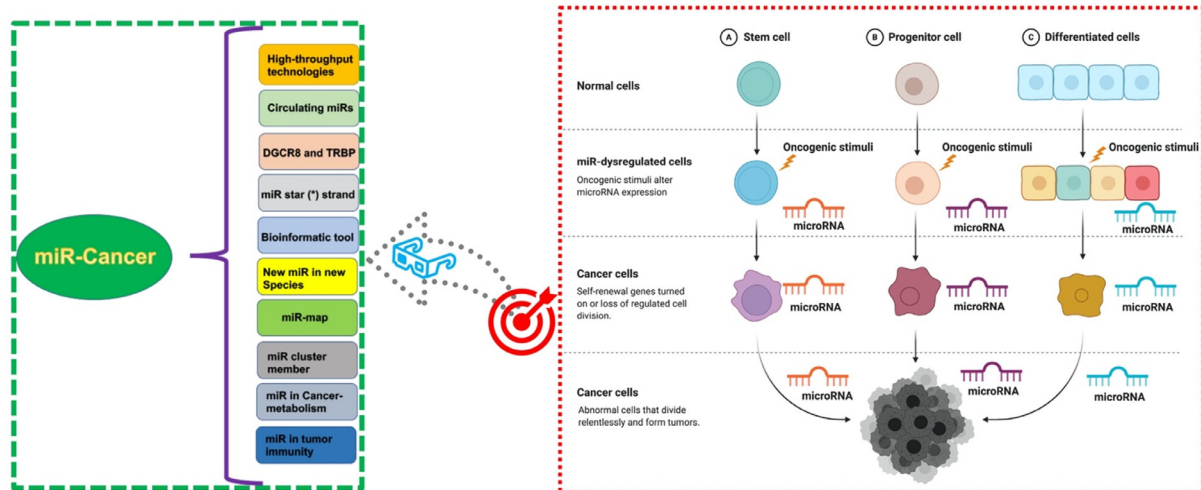
Figure 1. Overview of microRNA (miR) research in the miR-cancer field.

been detected in about 270 species, including vertebrate species and plants. The identification of new miRs in other species, and the characterization of commonalities and differences among new miR pathways, are worthy goals for future studies.

Excitingly, several FDA-approved miR drugs have entered clinical testing. Some candidate miRs drugs, such as Miravirsen for miR-122 [30], are reportedly being tested in phase 3 clinical trials. MRG-106 for miR-155 has been used to treat patients with T-cell lymphoma in a phase 2 clinical trial [31]. Many miR companies have been working towards developing new antigomiR and miR mimics for clinical use in cancer patients. However, such research is still in infancy, and the miR therapeutic bottleneck persists. Current challenges include specific miR *in vivo* delivery efficiency, off-target effects, cellular toxicity, and high cost. Another issue is to identify the best single miR candidate or miR combination for use in specific cancer types, which we hope to resolve in the near future. This issue will be further complicated by the heterogeneity of miR expression during different stages of cancer progression, which may be addressed by careful analysis of tumor biopsy samples from cancer patients during different stages. In the future, a miR "map" for individual cancer patients may be a promising therapeutic strategy.

Some miRs have cluster members, such as the miR-143/145 cluster, let-7 cluster, and miR-17~92 cluster in the human and mouse genome, which show a high level of conservation across species. Further investigations are warranted to determine whether single members of miR clusters play individual roles or redundant roles as either onco-miRs or tumor suppressor miRs in different types of cancer cells. MiR knock-out (KO) mice combined with spontaneous tumor models, such as the MMTV mouse model and EL-KRAS^{G12D} (EK) mice, would be useful for dissecting the pathological roles of miR single members or clusters in cancer.

Interestingly, there are some different mechanisms of gene modulations by other non-coding RNAs (ncRNAs), including long ncRNA (lncRNA), circular RNA (circRNA), and tRNA-derived small RNA (tsRNA), acting in oncogenesis as either tumor-suppressor or oncogenic ncRNAs. For instance, circHIPK3, a tumor-suppressive circRNA, downregulates bladder cancer progression and metastasis by interacting with microRNA-558 [32]. Also, some lncRNAs interact with the same microRNA family members, or one lncRNA interacts with different microRNAs by "sponge" or "decoy" the microRNA's activities in various cancers. For example, CCAT1, H19, and HOST2 enhance the progression of different types of tumors, including liver cancer, breast cancer, and epithelial ovarian cancer, by interacting with different microRNA let-7 members, respectively [33–35]. The lncRNA

TUG1 functions as an oncogene by decoying other microRNAs (e.g., miR-145, miR-299, miR-300, and miR-9-5p) in various types of human cancers, including bladder cancer, glioblastoma, gallbladder cancer, and osteosarcoma [36–39]. These studies indicate that the gene regulations by ncRNAs are critical but complicated in oncogenesis. It will be interesting to identify more other subtypes of ncRNAs such as piRNAs and tsRNAs, contributing to microRNA-mediated oncogenesis in future research. Combination cancer therapy based on microRNA and its interacting ncRNAs might be a promising strategy in future clinical trials.

The functional role of miRs in oncogenesis remains unclear, but miRs could be involved in several aspects [40]. We know that dysregulation of metabolic reprogramming influences cancer progression [41]. It is possible that some miRs could be involved in cancer metabolism. Further studies investigating metabolic alterations by miRs in the setting of cancer may hold great potential for future cancer-metabolic therapy. Additionally, several miRs are involved in tumor immunity, including miR-155 and miR-146 [42]. The identification of more miRs in the tumor microenvironment will provide further support for miR cancer therapy.

I hope that this short perspective article will be interesting and thought-provoking for biologists studying cancer and RNA, and will provide an idea of where we presently are in the "miR-cancer" field (Figure 1).

Acknowledgments

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