Applications of MicroRNA in Cancer: Exploring the Advantages of miRNA

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MicroRNAs (miRNAs) are short nucleotide noncoding RNA molecules that impose transcriptional and translational regulation of gene expression.[1] Evolutionarily conserved, miRNAs modulate vital processes, including cell cycle, metabolism, differentiation and development, tissue patterning, and aging. The human genome contains about 1,000 miRNAs, and each has been suggested to regulate hundreds of transcripts, totaling about a third of all genes. These regulatory nucleic acids arise through transcription of sequences in the non-coding regions of chromosomes as pri-miRNAs, precursors transcribed by RNA polymerase II.[2,3] Pri-miRNA are then cleaved by the ribonuclease III, Drosha, and DGCR8/Pasha, a double-stranded DNA binding protein, which generates hairpin pre-miRNAs. These intermediates are translocated into the cytosol by the nuclear export factor exportin 5/Ran GTP, where the ribonuclease III, Dicer, processes them into 19- to 25-nucleotide miRNA duplexes. Processed duplexes then become the targeting core of RNA-induced silencing complexes (RISC), inhibiting translational inhibition by cleavage and degradation of messenger RNA, in the case of perfect complimentarity, or by blocking translation, in the case of imperfect complimentarity, defining the pool of available genes.[1,3,4] MicroRNAs are the most recent addition to an increasingly complex system controlling the flow of information from the nucleus to the cytoplasm. Importantly, their function has provided novel insight into the genetic mechanisms dictating cell fate.

MicroRNAs are encoded in genomic regions related to cancer, including regions of amplification, loss of heterozygosity, fragile sites, and common breakpoint regions associated with oncogenes or tumor suppressor genes.[1,3,4] Indeed, miRNAs are abnormally expressed in human tumors, functioning themselves as oncogenes or tumor suppressors (*Figure 1*).[2,4,5] Additionally, different tumor types express unique patterns of miRNAs, referable to their tissues of origin. In this regard, patterns of miRNA expression may be an ontogenetically richer source of tumor information than expression profiling that uses messenger RNA microarrays.[6] Thereby, unique patterns of expression of miRNAs offer information-rich signatures for cancer diagnosis, prognosis of disease-specific survival, and prediction of responses to therapy. Beyond their value as diagnostic molecular biomarkers, a role for miRNAs in the pathogenesis of cancer highlights their potential as mechanism-based targets for cancer therapy.[1–4]

Driving Clinical Innovation

MicroRNAs as prognostic and predictive molecular biomarkers highlight the future for the practice of medicine, reflecting the spectrum linking fundamental discovery, clinical development, governmental regulation, and the evidence base of clinical practice essential to translating the new biology into health care applications.[7–9] In that context, biomarker discovery has driven clinical innovation, requiring systematic validation to define assay performance metrics, as essential elements to support broad application of molecular diagnostics. Moreover, analytes can be assessed using heterogeneous technical platforms whose performance and compatibility have not been verified. Lack of analytical and clinical validation and performance metrics reflecting absence of standardization represents a critical obstacle to broader clinical application of molecular markers.[7–9] Historically, miRNAs have been assessed on microarray or bead-based platforms.[1,2] More recently, quantitative reverse transcription-polymerase chain reaction, northern analysis, and *in situ* hybridization have been applied to the analysis of miRNAs.[10,11] From the perspective of analytical validation, one challenge to wide utility of miRNAs as prognostic and predictive biomarkers remains cross-validating performance metrics of different platforms to achieve uniform analytical results.[7–9] From a disease-management perspective, associations between miRNAs and patient outcomes await clinical qualification in appropriately designed and powered prospective, blinded, randomized clinical trials.[7–9,12]

Beyond utility as prognostic and predictive biomarkers, the contribution of miRNAs in processes underlying transformation and lineage-dependent tumorigenesis suggest that these molecules might serve as targets to prevent and treat cancer.[13] Indeed, therapy could be directed to re-establishing expression of silenced miRNA tumor suppressors, while antisense oligonucleotides could silence overexpressed oncogenic miRNAs.[1,2,13,14] Antisense oligonucleotides targeted to miRNA sequences with modified RNA backbone chemistry resistant to nuclease degradation irreversibly eliminates overexpression of oncogenic miRNAs.[13] Similarly, locked nucleic acid analogs resist degradation and stabilize the miRNA target-antisense duplex required for silencing.[15] Further, single-stranded RNA molecules complimentary to oncogenic miRNAs, known as antagomirs, silence miRNApression in mouse models *in vivo*.[16] The specificity of targeting encoded in nucleic acid base complimentarity, associated with their mechanistic role in tumorigenesis, suggests miRNAs as attractive therapeutic targets for cancer prevention and control.

Regulation by miRNAs is a new essential element in the integrated organization of gene expression and information flow within the cell. Corruption of miRNA-dependent programming constitutes one mechanism contributing to genetic abnormalities that characterize neoplastic transformation. Unique patterns of miRNA expression in tumors offer the opportunity to develop biomarkers for diagnosis, prognosis, and prediction, as well as targeted therapies for cancer prevention and control. These considerations underscore the unprecedented opportunity represented by miRNAs for accelerating the discovery-to-translation paradigm leading to early adoption and clinical application.

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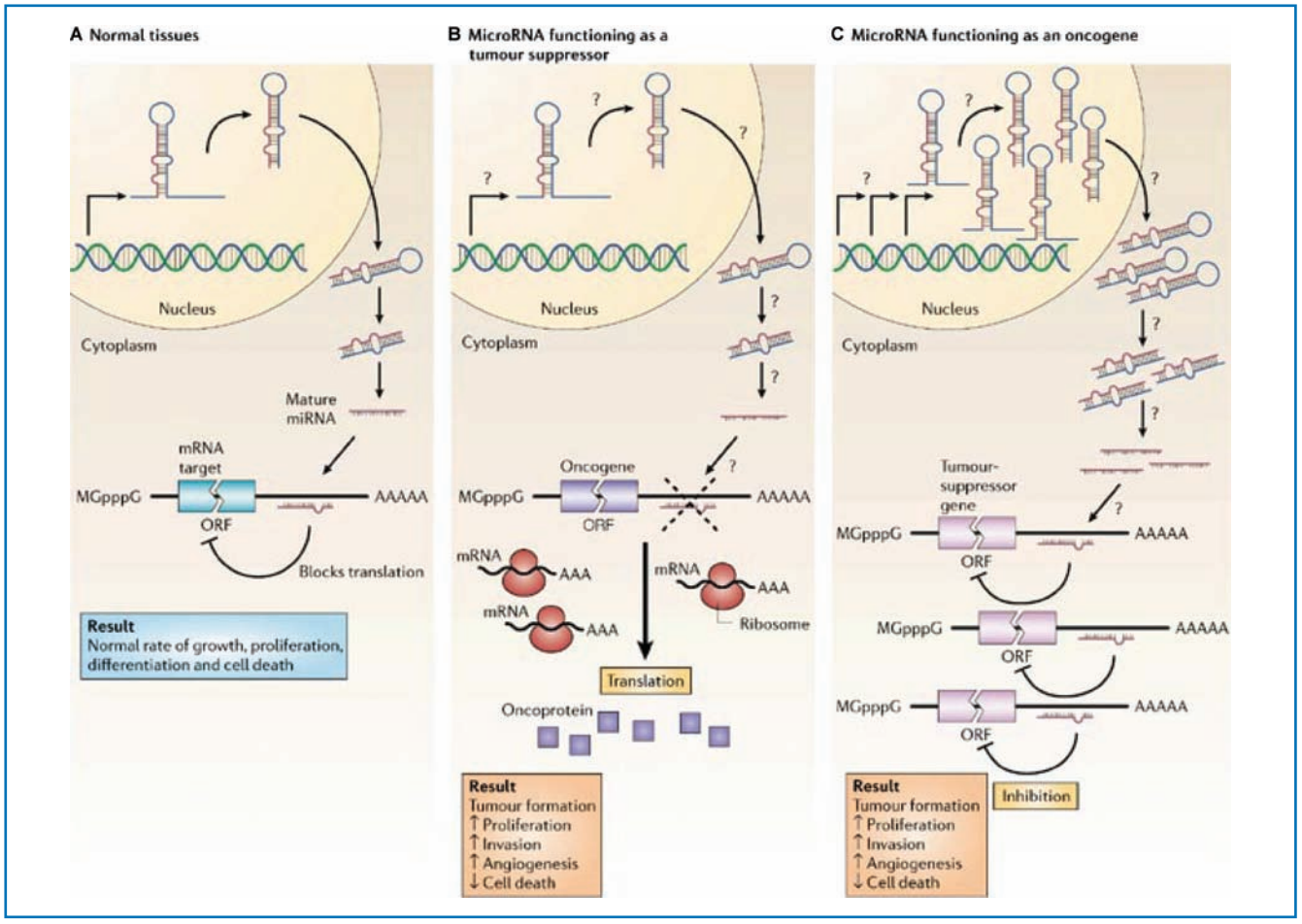

Figure 1. MicroRNA oncogenes and tumor suppressors. (**A**) Normally, microRNA (miRNA) binding to target mRNA represses gene expression by blocking protein translation or inducing miRNA degradation, contributing to homeostasis of growth, proliferation, differentiation, and apoptosis. (**B**) Reduced miRNA levels, reflecting defects at any stage of miRNA biogenesis (indicated by question marks), produce inappropriate expression of target oncoproteins (purple squares). The resulting defects in homeostasis increase proliferation, invasiveness or angiogenesis, or decrease levels of apoptosis or differentiation, potentiating tumor formation. (**C**) Conversely, overexpression of an oncogenic miRNA eliminates the expression of tumor-suppressor genes (pink), leading to cancer progression. Increased levels of mature miRNA could reflect amplification of the miRNA gene, a constitutively active promoter, increased efficiency in miRNA processing or increased stability of the miRNA (indicated by question marks). ORF, open reading frame. Figure reproduced from Esquela-Kerscher and Slack.[4]

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