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A study of micro RNAs in silico and in vivo: Diagnostic and therapeutic applications in cancer

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Abstract

There is emerging evidence of abnormal expression in human tumors of microRNAs (miRNAs) which have been assigned oncogenic and/or tumor suppressor functions. While some miRNAs commonly exhibit altered expression across tumors, more often, different tumor types express unique patterns of miRNAs, referable to their tissues of origin. The role of miRNAs in tumorigenesis underscores their value as mechanism-based therapeutic targets in cancer. Similarly, unique patterns of altered microRNA expression provide fingerprints that may serve as molecular biomarkers for tumor diagnosis, classification, prognosis of disease-specific outcomes, and prediction of therapeutic responses.

Keywords

Micro RNA; biomarkers; cancer; diagnosis; prognosis; prediction; individualized therapy

Cancer is a leading cause of mortality in the United States, with ~25% of deaths attributable to neoplasia [1, 2]. Worldwide, cancer-related global mortality follows only cardiovascular and infectious diseases [3]. In this context of expanded incidence and growing prevalence, clinical oncology is poised for unprecedented innovation. Harnessing discoveries in disease pathobiology, increasingly propelled by the development of high-throughput technologies including genomics, proteomics and metabolomics, modern cancer biology offers previously unavailable diagnostic and therapeutic paradigms tailored to meet the needs of individuals and populations [4]. Transforming clinical management is predicated on translation of the new science into application of advanced markers and targets for personalized cancer prediction, prevention, diagnosis, and treatment [4–6].

Indeed, the epigenetic, genetic, and post-genetic circuits restricting cell destiny are increasingly decoded, and their dysfunction linked to lineage-dependence underlying tumorigenesis [2, 7]. Critical in cell fate specification is post-transcriptional regulation of gene expression by microRNAs (Fig. 1) [8]. These molecules arise as transcripts from cognate genes in non-coding regions of chromosomes. MicroRNA undergo nuclear and cytoplasmic processing [8, 9] producing the targeting core of a multimeric complex hybridizing with mRNA molecules resulting in their sequestration or degradation, and thereby define the genes available for lineage commitment [10, 11]. This is the most recent

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addition to the hierarchical spectrum of molecular mechanisms defining nuclear-cytoplasmic information exchange [12], and forms the interface between transcriptional, translational, and post-translational regulation [13]. Significantly, microRNAs represent a regulatory, rather than structural, mechanism that coordinates normal gene expression and whose dysregulation underlies neoplastic transformation [8, 10, 11].

MicroRNAs and cancer

The essential nature of this novel mechanism indelibly patterning gene expression in cell lineage specification [8], in the context of the established model of cancer as a genetic disease in which pathobiology recapitulates cell and tissue ontogeny [14, 15], naturally implicates microRNAs in neoplastic transformation. In fact, altered microRNA expression is a defining trait of tumorigenesis [16, 17]. While the expression of some microRNAs are universally altered in tumors, more often unique patterns of microRNA expression reflect the lineage-dependence of tumors, referable to their tissues of origin [16–22]. Similarly, fundamental processes underlying tumorigenesis, including genomic instability, epigenetic dysregulation, and alterations in expression or function of regulatory proteins directly alter the complement of microRNAs expressed by cancer cells [8]. Additionally, microRNAs regulate key components integral to tumor initiation and progression, including tumor suppressors and oncogenes [8, 17, 23]. Further, microRNA signatures are a more informative source for classification of tumor taxonomy than genomic profiling [16]. Moreover, microRNAs can serve as unique targets for diagnostic imaging *in vivo* for taxonomic classification of tumors [24]. The emerging role of microRNAs in neoplasia highlights their potential value as mechanism-based therapeutic targets and biomarkers for diagnosis, prognosis of disease-specific outcomes, and prediction of therapeutic responses [25]. While there are numerous detailed reviews of this field, the purpose of this minireview is to provide, in overview, a summary of the potential application of microRNAs as diagnostic and therapeutic targets in cancer.

MicroRNAs as mechanism-based therapeutic targets in cancer

The case for microRNAs as tumor suppressors and oncogenes reflects their loss or gain, respectively, as a function of neoplastic transformation, their dysregulation in different tumors, the relevance of their mRNA targets to mechanisms underlying tumorigenesis, and their ability to directly alter tumorigenesis in model cells and organisms (Fig. 2; Table 1) [8, 26, 27]. Typically, microRNAs that serve as oncogenes are characterized by a gain of expression which inhibits levels of genes encoding tumor suppressors. Conversely, tumor suppressor microRNAs exhibit a loss of expression in cancer producing over-expression of transcripts encoded by oncogenes.

MicroRNA Tumor Suppressors (Fig. 2, Table 1)

The best characterized tumor suppressor microRNAs are miR-15a and miR-16-1. B cell chronic lymphocytic leukemia (CLL) is the most common adult leukemia in developed countries and universally associated with loss of chromosomal region 13q14 [8, 27, 28]. Within this deletion is a ~30 kb region in which resides miR-15a and miR-16-1, which are lost in ~70% of patients with CLL [29]. Similarly, loss of chromosomal region 13q14, including miR-15a and miR-16-1, occurs in prostate cancer, mantle cell lymphoma, and multiple myeloma [29, 30]. Tumor suppression by miR-15a and miR-16-1, in part, reflects inhibition of the expression of the anti-apoptotic oncogenic protein Bcl-2, which is characteristically over-expressed in CLL, promoting survival of leukemia cells [31]. Indeed, there is a reciprocal relationship between expression of miR-15a and miR-16-1 and Bcl-2, and heterologous expression of these microRNAs suppresses Bcl-2 levels [32]. Suppression is specifically mediated by complimentary binding sites for those microRNAs in the 3'

untranslated region of the Bcl-2 transcript [32]. Further, heterologous expression of miR-15a and miR-16-1 produces apoptosis in leukemia cell lines [32]. Moreover, mouse models of spontaneous CLL possess a mutation in the 3' untranslated region of miR-16-1, identical to mutations in patients with CLL, associated with decreased expression of that microRNA [33]. Heterologous expression of miR-16-1 in CLL cells derived from those mice alters the cell cycle, proliferation and apoptosis of these tumor cells [33].

The microRNA let-7, a phylogenetically conserved gene product that regulates the transition of cells from proliferation to differentiation in invertebrates [34], also serves as a tumor suppressor [27]. There are twelve let-7 homologs in humans forming eight distinct clusters of which four are localized to chromosomal regions lost in many malignancies [35]. In that context, downregulation of let-7 family members in lung cancer is associated with poor prognosis [22]. A role for these microRNAs in growth regulation and the expression of the tumorigenic phenotype is highlighted by the ability of heterologous let-7 expression in lung cancer cells in vitro to inhibit colony formation [36]. Key downstream targets for let-7 include the human Ras family of proteins, oncogenes that are commonly mutated in many human tumors [23]. Indeed, KRas and NRas expression in human cells is regulated by let-7 family members [27]. Moreover, loss of let-7 expression in human tumors correlates with over-expression of Ras proteins [23].

MicroRNA Oncogenes (Fig. 2, Table 1)

The miR-17 cluster comprises a group of six miRNAs (miR-17-5p, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92) at 13q31-32, a chromosomal region amplified in large B-cell lymphoma, follicular lymphoma, mantle cell lymphoma and primary cutaneous B-cell lymphoma [37]. Consistent with their functions as oncogenes, over-expression of this microRNA cluster is associated with amplification of the 13q31-32 genomic region in lymphoma cells in vitro [37, 38]. These miRNAs are over-expressed in many types of tumors, including lymphoma, colon, lung, breast, pancreas and prostate [17, 38, 39]. Interestingly, expression of the miR-17 cluster is induced by c-Myc, an oncogene over-expressed in many tumors. Heterologous expression of c-Myc up-regulates expression of the miR-17 cluster, mediated by direct binding of that transcription factor to the chromosomal region harboring those microRNAs [40]. In turn, the miR-17 cluster appears to regulate several downstream oncogene targets. Thus, miR-19a and miR-19b may regulate phosphatase and tensin homolog (PTEN), a tumor suppressor with a broad mechanistic role in human tumorigenesis, through interactions with complimentary sites in the 3' untranslated region of this transcript [41]. Similarly, miR-20a may reduce the expression of transforming growth factor (TGF)- β receptor II (TGFBR2), a tumor suppressor frequently mutated in cancer cells which regulates the cell cycle, imposing growth inhibition [17]. The best characterized target of the miR-17 cluster is the E2F1 transcription factor whose expression is regulated by miR-17-5p and miR-20a [42]. In turn, E2F1 regulates cell cycle progression by inducing genes mediating DNA replication and cell cycle control [43]. Beyond the regulation of key targets contributing to transformation, the miR-17 cluster directly induces the tumorigenic phenotype. Heterologous expression of the miR-17 cluster increased proliferation in lung cancer cells in vitro [39]. Moreover, components of this cluster accelerate the process of lymphomagenesis in mice [44].

The microRNA miR-21 is over-expressed in many solid tumors including breast, colon, lung, prostate, stomach, and endocrine pancreas tumors, glioblastomas, and uterine leiomyomas [17, 45-47]. This microRNA is encoded at chromosome 17q23.2, a genetic locus which is frequently amplified in many tumors. The tumorigenic effects of miR-21 are mediated, in part, by targeting a number of mediators in critical cell survival pathways. Thus, in glioblastoma cells in vitro miR-21 modulates the expression of the common tumor suppressor PTEN, a central regulator of cell growth, proliferation, and survival mediated by

the PI3 kinase-Akt pathway [48]. Also, miR-21 regulates breast cancer cell growth by reciprocally regulating apoptosis and proliferation, in part reflecting regulation of the anti-apoptotic protein Bcl-2 [49]. Moreover, miR-21 controls expression of the tumor suppressor tropomyosin 1 whose over-expression in breast cancer cells suppresses anchorage-independent growth [50]. Beyond signaling analyses, elimination of miR-21 expression in glioblastoma cells induces caspase-dependent apoptosis, underscoring the importance of this microRNA in mediating the survival phenotype [51]. Similarly, antisense oligonucleotides to miR-21 suppressed the growth of breast cancer cells in vitro and in xenografts in mice [48].

MicroRNAs as biomarkers in cancer

Their fundamental role in development and differentiation, and their pervasive corruption in lineage-dependent mechanisms underlying tumorigenesis suggest that microRNAs may be a particularly rich source of diagnostic, prognostic and predictive information as biomarkers in cancer [8, 26, 52]. Differential expression of microRNAs compared to their normal adjacent tissue counterparts is a characteristic of every type of tumor examined to date [8, 52], a feature that could be particularly useful in diagnosing incident cancers in otherwise normal tissues. Indeed, this approach discriminates normal and neoplastic tissues in various cancer types, including CLL, breast cancer, glioblastoma, thyroid papillary carcinoma, hepatocellular carcinoma, lung cancer, colon cancer and endocrine pancreatic tumors [8, 17–22, 26, 45, 52–54]. Similarly, microRNA expression profiles provide a powerful source of molecular taxonomic information, with an accuracy for classifying tumors according to their developmental lineage and differentiation state that surpasses mRNA expression profiling [16, 17]. These observations suggest the utility of microRNA expression profiling for identifying metastatic tumors of unknown origin, which represent ~5% of all malignancies worldwide [16, 17, 52]. Also, differential microRNA expression patterns are associated with disease prognosis [8, 52]. Specific patterns of microRNA expression identified patients with pancreatic cancer who survived >24 months, compared to those who survived <24 months [53]. In addition, the expression of specific microRNAs predicted overall poor survival in patients with pancreatic cancer [53]. Similarly, over-expression of specific microRNAs was an independent prognostic variable associated with advanced disease stage and decreased survival in patients with colon cancer [54]. Beyond diagnosis and prognosis, microRNA expression patterns predict responses to therapy, and over-expression of oncogenic microRNAs was associated with improved survival following adjuvant chemotherapy in patients with colon cancer [54]. These observations highlight the potential of microRNAs as biomarkers for diagnosis, taxonomic classification, prognosis, risk stratification, and prediction of therapeutic responses in patients with cancer.

Corruption of microRNA expression in cancer

The genetic basis of cancer, in part, reflects chromosomal rearrangements encompassing translocations, deletions, amplifications, and exogenous episomal integrations which alter gene expression. The essential role of microRNAs in tumorigenesis predicts coincidence between the location of their encoding genes and those cancer-associated chromosomal regions. Indeed, more than half of microRNA genes are located in cancer-associated genomic regions in a wide array of tumors including lung, breast, ovarian, colon, gastric, liver, leukemia, and lymphoma [28, 35]. Conversely, chromosomal regions harboring microRNAs are sites of frequent genomic alterations involved in cancer [28, 55]. Additionally, the impact of chromosomal remodeling on gene copy number directly translates to altered microRNA expression [19, 28, 55]. Beyond structural reorganization, epigenetic remodeling of chromosomal regions harboring microRNA loci contributes to transformation, and tumor-suppressing microRNAs silenced by CpG island

hypermethylation result in dysregulation of essential proteins accelerating the cell cycle, including cyclin D and retinoblastoma [56, 57]. Moreover, alterations in the machinery responsible for processing microRNA contributes to tumorigenesis, and impairment of Dicer enhances lung tumor development in experimental mouse models and is associated with poor prognosis in patients with lung cancer [58–60].

Therapeutic targeting of microRNAs

The causal role of microRNAs in molecular mechanisms underlying transformation and the contribution of specific microRNA species to lineage-dependent tumorigenesis suggest that these molecules could serve as therapeutic targets to prevent and treat cancer [61]. In the context of established therapeutic paradigms in medical oncology, individualized therapy with microRNAs could re-establish the expression of silenced microRNA tumor suppressors, while antisense oligonucleotides could silence over-expressed oncogenic microRNAs [8, 28, 52, 61]. Indeed, antisense oligonucleotides targeted to microRNA sequences (AMOs) with modified RNA backbone chemistry resistant to nuclease degradation irreversibly eliminates over-expression of oncogenic microRNAs [61]. Similarly, locked nucleic acid analogs resist degradation and stabilize the microRNA target-antisense duplex required for silencing [62]. Moreover, single stranded RNA molecules complementary to oncogenic microRNAs, termed antagomirs, silence microRNA expression in mouse models in vivo [63]. The specificity of targeting inherent in nucleic acid base complementarity coupled with their mechanistic role in neoplastic transformation make microRNAs attractive therapeutic targets for future translation.

Summary

MicroRNAs represent one fundamental element of the integrated regulation of gene expression underlying nuclear-cytoplasmic communication. Disruption of these regulatory components in processes underlying tumor initiation and promotion contributes to the genetic basis of neoplasia. Beyond molecular mechanisms underlying pathophysiology that constitute therapeutic targets, unique patterns of microRNA expression characterizing lineage dependent tumorigenesis offer unique opportunities to develop biomarkers for diagnostic, prognostic, and predictive management of cancer. These novel discoveries are positioned to launch a transformative continuum linking innovation to patient management. Advancement of these novel paradigm-shifting concepts into patient application will proceed through development and regulatory approval to establish the evidence basis for integration of microRNA-based diagnostics and therapeutics into clinical practice

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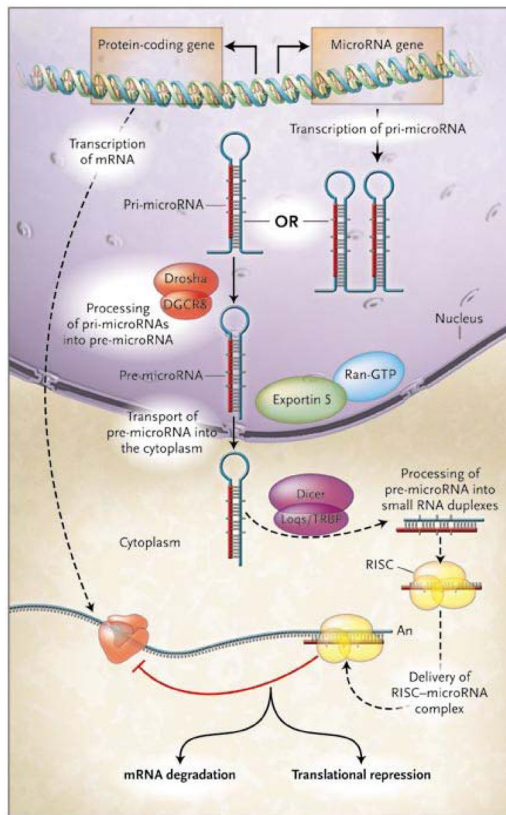


Figure 1. MicroRNA generation and gene regulation [9]

Mature microRNAs of about 22 nucleotides originate from primary microRNA (pri-microRNA) transcripts. Nuclear pri-microRNAs of hundreds to thousands of base pairs are converted into stem-loop precursors (pre-microRNA) of about 70 nucleotides by Drosha, an RNase III endonuclease, and Pasha, a homologue of the human DiGeorge syndrome critical region gene 8 (DGCR8). Pre-microRNAs undergo cytoplasmic translocation mediated by exportin 5 in conjunction with Ran-GTP and subsequently processed into RNA duplexes of about 22 nucleotides by Dicer, an RNase III enzyme, and Loqacious (Loqs), a double-stranded RNA-binding-domain protein that is a homologue of the HIV transactivating response RNA-binding protein (TRBP). The functional strand of the microRNA duplex guides the RNA-induced silencing complex (RISC) to the mRNA target for translational repression or degradation. Figure reproduced from (9).

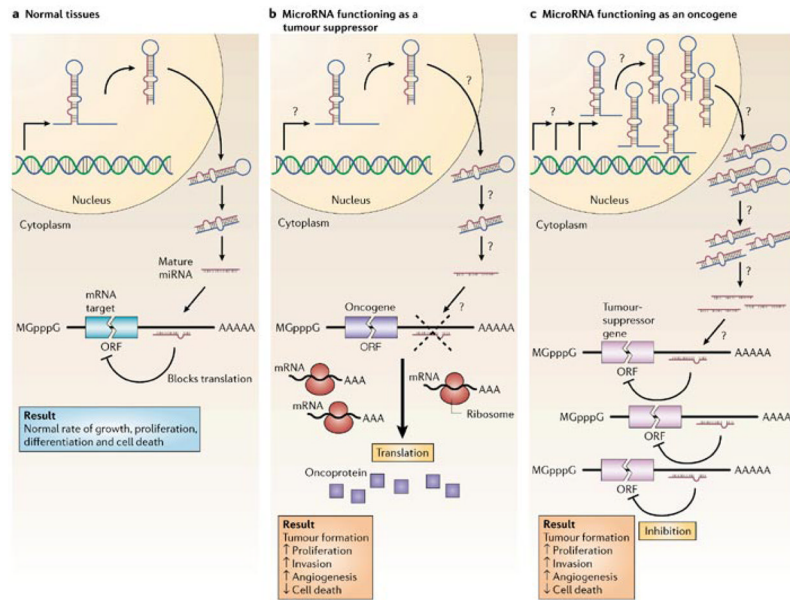


Figure 2. MicroRNA oncogenes and tumor suppressors [26]

a. Normally, microRNA (miRNA) binding to target mRNA represses gene expression by blocking protein translation or inducing mRNA 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 (26).

Table 1

MicroRNAs in tumorigenesis

MicroRNA	Gene Locus	Tumor Types	Gene Targets	Ref
Suppressors				
mir-15a, 16-1	13q14	CLL, prostate, mantle cell lymphoma, multiple myeloma	BCL-2	28–32
let-7	8 clusters	lung, gastric	RAS	22, 23, 26, 34, 35
Oncogenes				
mir-17 cluster	13q31–32	B-CLL, follicular lymphoma, mantle cell lymphoma, cutaneous B cell lymphoma, colon, lung, breast, pancreas, prostate	PTEN TGF β RII E2F1	17, 36–38, 40–43
mir-21	17q23.2	breast, colon, lung, prostate, gastric, endocrine pancreas, glioblastomas, leiomyomas	PTEN BCL-2 Tropomyosin I	17, 44–50, 54