Role of microRNAs in the molecular diagnosis of cancer

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Abstract

MicroRNAs (miRNAs) are evolutionarily conserved, endogenous, small non-coding RNA molecules of about 22 nucleotides in length that function as posttranscriptional gene regulators. They are involved in numerous cellular processes including development, cell differentiation, cell cycle regulation and apoptosis. There is increasing evidence to show that miRNAs are mutated or differentially expressed in many types of cancer and specific functions of the miRNAs are now becoming apparent. Here we discuss the current literature on potential usefulness of miRNAs as diagnostic markers, emphasizing the involvement of specific miRNAs in particular tumor types, highlighting their potential role in distinguishing benign from malignant tissues and/or the different subtypes of the same tumor and/or in diagnosis and classification of tumor of unknown origin.

Introduction

Up to 1,000 miRNAs have been estimated in the humane genome, but only 200-300 miRNAs have been currently identified in humans.[1] Some miRNAs are highly conserved from species to species in animals and plants.[2-3] MiRNAs are involved in numerous physiological cellular processes including development, differentiation, proliferation, apoptosis, stress response, and cancer.[4-11] Estimates based on bioinformatics as well as on microarrays analyses suggest that ~30% of all genes are subject to regulation by multiple miRNAs.[12] Most importantly, there is growing evidence showing that numerous microRNAs are aberrantly expressed in human cancers.[11,13] This is due to the fact that most of them are located within genomic regions that frequently result amplified or lost in cancer.[14] Genomic alterations represent one of the main mechanisms that result in deregulation of miRNAs and their precursor molecule expression that drives opposite effects depending on their targets. Indeed, excessive accumulation of miRNAs that normally regulate synthesis of a tumor suppressor gene may promote tumorigenesis by causing a decrease of a tumor suppressor; conversely, a decrease of miRNAs that control a proto-oncogene leads to its activation resulting in tumor formation.[15]

Some miRNAs are deregulated in several types of cancer indicating that they may contribute in the generation of a common tumor phenotype; otherwise, alteration of specific miRNAs are unique in a particular type of cancer. The identification of differently expressed miRNAs may be useful in the diagnosis and classification of cancer.

Biogenesis and function of miRNAs

MiRNAs are a family of 21-25 nucleotide, non-coding small RNAs that act as gene regulators.

Biogenesis of miRNAs has been largely elucidated and consists of two distinct phases that occur in separate cellular compartments.[16]

Briefly, miRNAs are transcribed in the nucleus as long primary transcripts, named pri-miRNAs, by a RNA polymeraseII.[17,18] Here, the pri-miRNAs are processed by the RNAseIII enzyme Drosha and the double-stranded RNA-binding protein Pasha (DGCR8) into a stem-loop precursors ~70nt, called pre-miRNA.[19-20]

The pre-miRNA is exported by the transporter exportin-5 from the nucleus to the cytoplasm in a RanGTP-dependent manner.[21-23]

Dicer, a member of the RNAseIII superfamily of bidentate-nuclease, and TRBP, a double-stranded RNAbinding-domain protein, process, in the cytoplasm, the pre-miRNA to generate a transient miRNA duplex of ~22nt, referred to as the miRNA:miRNA*duplex.[24-26]

Finally, the miRNA:miRNA*duplex is incorporated into the RNA-induced silencing complex (RISC) where the mature miRNA strand negatively regulates its target genes.[27] MiRNAs control gene expression in a sequence-specific manner. The mature 22-nt strand recognizes complementary sequences in the 3'UTR region of target mRNAs, particularly in the so called seed sequence at the 5'end (2-8 nucleotides), and triggers the miRNA-RISC complex to induce gene repression by either inhibiting translation and/or causing mRNA degradation.[16] In fact, different studies showed that the degree of sequence complement determines whether the mRNA target is degraded or its translation into protein is repressed.[28-29]

Implication of MiRNAs in cancer

The importance of miRNAs in tumorigenesis is associated with the interplay of multiple mechanisms, such as genomic abnormalities, epigenetic silencing, transcriptional regulation and miRNAs processing alteration, that have the potential to deregulate their repression.[30]

Thus, it has been reported that miRNAs are frequently located in cancer hotspot chromosomal regions, such as fragile sites, regions of loss of heterozygosity, amplification or common breakpoint regions, where their expression can potentially be disrupted by chromosomal abnormalities.[14]

A modification of miRNAs expression has been observed, or has been predicted to affect the activities of targeted mRNA encoding proteins that have oncogenic or anti-oncogenic functions. Many miRNA-transcription factors relationships have been well documentated in cancers. Particularly, it has been demonstrated that one mechanism of miR-17-92 cluster upregulation is transcriptional activation by *c-Myc* and *E2Fs*.[31] Also, several papers reported that all miR-34 family members are directly activated by p53 and its effects could be mediated through transcriptional activation of miRNAs.[32]

In the past few years several studies demonstrated that miRNAs expression is predictive of the outcome in solid tumors and hematologic malignancies, highlighting their potential diagnostic utility in cancer.[11-13]

Currently, the main approach for studying the role of miRNAs in cancer is represented by the analysis of miRNAs expression profiling. Particularly, studies of differentially expressed microRNAs appear to be a promising diagnostic approach for distinguishing benign from malignant tissues and/or the different subtypes of the same tumor and/or in the classification of tumor of unknown origin.

MiRNAs in the diagnosing of cancer

Several studies proved that miRNAs might

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represent novel diagnostic tools for cancer diagnosis. Indeed, miRNAs possess several features that make them attractive diagnostic biomarkers. Especially, their smaller size and the resistance to RNase degradation render them superior molecular marker as compared to mRNAs.[33]

For example, it has been demonstrated that miRNAs can be efficiently extracted and evaluated from formalin-fixed paraffin-embedded (FFPE) tissues. MiRNAs from FFPE showed improved stability and maintained the same expression profiles when compared with those from frozen samples.[34-36] The opportunity to evaluate the levels of miRNA directly in FFPE tissues represents a suitable method to uniform the collection and storage of the specimen to be analyzed and allows a comparison of the interpretation of results obtained from different studies.

Furthermore, the search for non-invasive sensitive markers for tumor diagnosis is currently one of the most rapidly growing areas in cancer research. Very recently, miRNAs usefulness as diagnostic markers has been further expanded by studies performed in human plasma or serum.[37-38] Table 1 summarizes MiRNAs detectable in serum that are diagnostically relevant.

The first serum-miRNA biomarker was reported by Lawrie *et al.* that showed that sera levels of miR-21 are specific for diffuse large B-cell lymphoma (DLBCL) and are associated with relapse-free survival.[39]

Mitchell *et al.* showed that serum levels of miR-141 might distinguish patients with prostate cancer from healthy controls.[40]

MiRNAs expression profiles from serum showed significant differences especially in lung and colorectal cancers.

Chen *et al.* showed that there are some "common" tumor-related miRNAs in the serum from lung and colorectal cancer patients. However, among them they identify a unique expression profile of 8 serum miRNAs in lung cancer patients, that included specific miRNAs such as miR-25 and miR-223 previously reported to be involved in tumor formation; and 14 serum miRNAs, including miR-485-5p, miR-361-3p, miR-326, miR-487b, in colorectal cancer patients.[37]

More recently, Ng *et al.* showed that miR-92 was significantly upregulated in plasma samples from colorectal cancer patients and may represent a significative marker for this type of cancer.[41] The ratio of miR-92a/miR-638 in plasma has been shown to be useful for distinguishing leukemia patients from healthy individuals.[42]

However, although these findings reveal great impact representing a promising non-invasive method in the clinical practice for early cancer diagnosis, further independent studies on a larger cohort of patients will be necessary before any such markers can be proven to be useful in a clinical setting.

Table 1. MicroRNAs potential diagnostic application serum.

Specific miRNA	Diagnostic application	Cancer type	Refs.
	8 serum miRNAs classify lung 14 serum miRNAs classify colorectal cancer	Lung and colorectal cancer	37
miR-21	Significant deregulation in serum	DLBC	39
miR-141	Serum levels differentiate cancer from healthy controls	Prostate cancer	40
miR-92	Significantly upregulated in plasma cancer samples	Colorectal cancer	41
miR 92a/ miR638	The ratio between this two miRNAs can distinguish these from healthy controls	Leukemia	42

Table 2. Example of microRNAs with potential diagnostic application in different malignancies.

Specific miRNA	Diagnostic application	Cancer type	Refs.
	miRNAs expression profiles classify solid tumors	Different cancer types	11
	miRNAs expression profiles classify poorly differentiated tumors	Different cancer types	13
	48 miRNAs identify tissue in cancer with unknown primary origin	Different cancer types	44
	32 miRNAs differentially expressed in primary tumors vs metastatic samples	Different cancer types	45
miR92b miR9/9*	Can distinguish primary from metastasis	Brain tumors	46
miR-205	Can distinguish squamous subtypes	Lung tumor	47
	29 miRNAs deregulated in cancer vs. normal	Breast	48
	miRNAs deregulated in cancer vs. normal unique sets of miRNAs correlates with ER PR expression	Breast	49
miR-30d, miR-125b, miR-26a miR-30a-5p	Can distinguish anaplastic thyroid carcinomas (ATC) from normal thyroid tissue and from papillary thyroid carcinomas (PTC).	Thyroid cancer	50
miR-146b	Can distinguish papillary thyroid subtypes	Thyroid cancer	51
	7 miRNAs thyroid can distinguish tumors vs. hyperplastic nodules	Thyroid cancer	52

miRNAs may improve diagnosis of tumor of unknown origin

Metastatic cancer of unknown primary origin accounts for 3-5% of all new cancer cases and is usually a very aggressive disease with poor prognosis.[43] Clinical management of these tumors shows a high degree of variation and although many protocols have been evaluated, they show relatively little benefit, suggesting that a more accurate definition of this lesion is necessary for their treatment.

The development of technologies to study the expression levels of hundreds of miRNAs at one time demonstrated that miRNAs are an invaluable tool to distinguish tumors from normal tissue, indicating miRNAs as highly tissues specific biomarkers, as summarized in Table 2.

Two earlier studies reported that unlike with a mRNA detection method, only a modest number of miRNAs might be sufficient to classify accurately human cancers according to their developmental lineage and differentiation state.[11,13] Lu *et al.* showed that 129 of 217 miRNAs analyzed are downmodulated in cancer compared with normal tissues, demonstrating that an miRNA-based classifier can establish the correct diagnosis of poorly differentiated cancers with far greater accuracy than an mRNA based classifier.[13]

In the last years numerous groups focused on identifying distinctive cancer-specific miRNA signatures that improve molecular classification of cancer.

Rosenfeld *et al.* have demonstrated that miRNAs can accurately identify the tissue of origin for a given cancer. They measured miRNA expression levels from 22 different tumor tissues and metastases and constructed a classifier based on 48 miRNAs with an accuracy of 90%. These results showed that a restricted number of miRNAs are sufficient to classify a cancer tissue and correlates more

accurately with cancer-stage and clinicopathological features.[44]

Baffa *et al.* performed a microarray miRNA analysis on 43 matched primary tumors and corresponding lymph node metastases. This analysis identified 32 miRNAs that were differentially expressed between the 43 primary tumors and the related metastatic samples. Among them, they identified miRNAs related with the processes of tumor invasion and metastasis such as miR-200 family, miR-205 and miR 10b.[45]

Recently, Nass *et al.* showed that miR MiR-92b and miR-9/9* are specifically expressed in brain primary tumors and can be used to differentiate primary from metastatic tumors.[46]

These works significantly highlight that the use of miRNAs profiling outperformed mRNA in the classification of unknown primary tumor.

miRNAs may improve diagnosis of tumor subtype

A further aspect where miRNAs may play an advantageous role is the classification of a tumor subtype, instrumental for an appropriate treatment. The unique patterns of aberrant miRNAs expression in a specific type of cancer may improve this classification.

For example, a number of classifiers have been developed for human lung cancer and miRNAs such as miR21 and miR-205, that are located in a chromosomic region frequently amplified in this type of tumor was overexpressed.[53]

It has been demonstrated that miRNAs have high specificity to distinguish between non-small cell lung cancer (NSCLC), small cell lung carcinoma (SCLC), adenocarcinoma and squamous cell carcinoma.

Very recently, Lebanony showed that unlike the current diagnostic tools available, miR-205 may be highly specific to distinguish squamous from non-squamous NSCLC.[47]

Iorio *et al.* identified 29 miRNAs that may potentially act as a diagnostic molecular marker in breast cancer.[48] A recent study found that a number of miRNAs were differentially expressed in breast tumor versus normal breast tissue and that unique sets of miRNA expression correlated with their ErbB2 (let-7f, let-7g, miR-107, mir-10b, miR-126, miR-154, miR-195) or ER/PR status (*miR-142-5p, miR-200a, miR-205 and miR-25*).[49]

Several studies, reviewed recently by Pallante, explored the utility of miRNA profiling for the pre-operative diagnosis of thyroid nodules.[54]

Particularly, Visone *et al*. identified a significant decrease in miR-30d, miR-125b, miR-26a and miR-30a-5p in anaplastic thyroid carcinomas (ATC) in comparison to normal thyroid tissue and from papillary thyroid carcinomas (PTC).[50]

Chen *et al.* indicated miR-146b as a potential marker to improve diagnosis of papillary thyroid carcinoma.[51]

Recently, Nikiforova *et al.* identified a set of seven miRNAs (miR-187, miR-221, miR-222, miR-146b, miR-155, miR-224, and miR-197) that were most differentially over-expressed in thyroid tumors versus hyperplastic nodules, showing high accuracy of thyroid cancer detection.[52]

Conclusions

In the past few years, there has been much evidence indicating that the miRNAs play an important role in cancer.

It has been demonstrated that there are miRNAs involved directly in cancer development controlling different cellular pathways, including cell growth, cell differentiation or apoptosis; and miRNAs that are involved indirectly in cancer development acting by targeting oncogenes and/or tumor suppressor genes.

Curently, miRNA profiling is considered the main method of investigation because it is able to show miRNAs that are differentially expressed in certain cancer tissues as compared to normal adjacent tissues and these differences may be used as biomarkers for a specific tumor.[13] Moreover, an increasing number of studies highlighted significant correlations between miRNAs expression with tumor subtypes or clinical parameters. The usefulness of miRNAs in sensitively identifying the tissue of origin of metastatic cancer is a significant advance in clinical practice.

It has been demonstrated that each miRNAs may down-regulate a large number of target mRNAs, therefore the role of each miRNA may depend on the cellular context and on the pathway that they impair.

A further elucidation of the miRNA function may represent a new prospective for the identification of biomarkers in cancer.

However most of the published studies have been conducted in small and limited patient populations. To ensure the introduction of miRNAs in clinical practice as diagnostic molecular markers it will be necessary to perform further validation in multiple cohorts.

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