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MicroRNAs in Solid Tumors

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Abstract

MicroRNAs (miRNAs or miRs) are small, noncoding RNAs (~20–22 nucleotides) that have critical functions in cell proliferation, apoptosis, and differentiation. These evolutionarily conserved RNA sequences are the result of a complex sequence of processing steps, which can regulate the expression of tens, and even hundreds, of genes. Their regulatory effect is based upon the degree of complementarity between the mature miRNA and the 3' untranslated region region of the target mRNA resulting in either complete degradation or translational inhibition of the target mRNA. In vertebrates they are often tissue specific in their expression patterns and dysregulated in malignancies. Thus, miRNA profiling has been used to create signatures for many solid malignancies. These profiles have been used to not only classify tumors, but also to help predict survival and outcome. Herein, we review the role of miRNAs in the development and progression of solid tumors.

Keywords

microRNA; miRNA; solid tumors; pancreatic cancer; lung cancer; colon cancer; hepatocellular cancer; papillary thyroid cancer

Introduction

Cancer is the second leading cause of death in the United States, following closely behind heart disease, accounting for over 500,000 deaths every year [1]. With the initiation and completion of the Human Genome Project, researchers and the lay public have learned of the great importance of genetics in the initiation and progression of cancer. Yet even now, we are just learning of the importance of smaller noncoding regions of the genome and their impact on normal cellular function and, when altered, dysfunction. Dysregulation of miRNAs have been shown to be involved in tumor initiation and progression. The explosion of data on miRNAs and cancer has put them in the spotlight over the past few years.

MicroRNAs (miRNAs or miRs) are small (~20–22 nucleotides), noncoding RNAs, which have critical functions in various biological processes [2]. They were first described in 1993 in the nematode *Caenorhabditis elegans*. Ambrose *et al.* found that a small RNA interacted with the 3' untranslated region (UTR) of the *lin-14* mRNA to inhibit its expression instead

of coding for a protein [3]. To date, over 450 miRNAs have been reported and a number of them have been shown to play a role in cell proliferation, apoptosis, and differentiation. These naturally occurring miRNAs function by binding to target mRNAs, resulting in their degradation or translational inhibition based upon the degree of complementarity with their target mRNA. Such flexibility in binding allows a single miRNA to potentially bind with and prevent hundreds of mRNA messages from being translated. As such, miRNAs have been proposed to contribute to oncogenesis by functioning as either oncogenes or tumor suppressors through their interactions with target genes critical to tumorigenesis and progression. These dysregulated miRNAs are often referred to as oncomiRs.

The initial connection of miRNAs and cancer was elucidated in leukemia and hematological malignancies, later spurring interest in solid malignancies. MiRNAs are one of the largest classes of gene regulators, comprising 1% to 4% of all expressed genes [4]. One of the first connections made between cancer and miRNAs was established when miR-15 and miR-16 were found to be located on chromosome 13q14 in a 30kb region of loss in chronic lymphocytic leukemia (CLL). Both of these genes are lost or down-regulated in 68% of cases of CLL [5]. It has also been shown that E (mu)-mmu-miR-155 transgenic mice exhibit preleukemic pre-B-cell proliferation in spleen and bone marrow [6], and overexpression of mir-155 was found in Hodgkin's lymphoma [7], human B-cell lymphomas [8], and Burkitt's lymphoma [9]. More recently, miR-21 and miR-155 were found to be significantly overexpressed in CLL patients [10].

Abnormal expression of these oncomiRs has been found in a variety of solid tumors, including colon, breast, lung, thyroid, liver, and pancreas. By understanding the intricacies of miRNA processing, expression, and interaction with specific targets, the role of miRNAs in cancer diagnosis, prognosis, and treatment seems limitless. Herein, we describe the known mechanisms of miRNA expression and apparent function in solid tumors.

MicroRNA Processing

MiRNA genes are often located in clusters and transcribed as polycistrons [11]. The biogenesis and processing of miRNAs involves multiple steps. First, the miRNA gene is transcribed by RNA polymerase II into a double stranded hairpin, sized from several hundred to over a thousand nucleotides, called the primary transcript (pri-miRNA) [12]. The primary transcript is later modified by polyadenylation or capping. Subsequently, the pri-miRNA forms a specific hairpin shaped secondary structure cleaved by Drosha, an RNase III endonuclease, along with the essential cofactor DGCR8/Pasha in the nucleus, to form the miRNA precursor (pre-miRNA) [13, 14]. The pre-miRNA is a 60 to 70 nucleotide sequence with a 5' phosphate and a 3' two nucleotide overhang. Exportin 5 recognizes the two nucleotide 3' overhang end structure of the microRNA precursor, allowing it to be transported to the cytoplasm [15, 16]. This brings the double-stranded miRNA precursor into contact with Dicer and undergoes further processing into a single-stranded mature miRNA [13, 17, 18]. Mature miRNA are then incorporated into the RNA induced silencing complex, which is able to negatively regulate targets by inhibition of protein synthesis or direct cleavage [19, 20], (Fig. 1). If the miRNA has perfect or near perfect complementarity to the 3' untranslated region of the target mRNA, it induces the RNA induced silencing

complex to cleave the mRNA. On the other hand, if the miRNA and the 3' UTR do not match perfectly, it results in translational inhibition [2, 21].

“OncomiRs”

Multiple reports delineate the role of miRNAs in apoptosis, cell proliferation, and tumorigenesis. The evidence for deregulation of miRNAs in human cancers is growing rapidly. MiRNA genes are often located at fragile sites as well as in minimal regions of loss of heterozygosity, minimal regions of amplification, and in common breakpoint regions, thus leading to either loss or amplification and, subsequently, a role in tumorigenesis. Copy number abnormalities of miRNAs and their regulatory genes are widespread in cancer. This may serve to explain why miRNA genes are frequently deregulated and reported in several tumor types [22]. MiRNA overexpression or underexpression can both lead to cancer because of their roles as either a tumor suppressor or oncogene. Overexpression of a miRNA could result in down-regulation of a tumor suppressor, or underexpression of a miRNA could lead to up-regulation of an oncogene, thus both leading to cancer. Let-7 is one such example; it is down-regulated in lung cancer and suppresses Ras, thus acting as a tumor suppressor [23].

MiRNAs have also been shown to have a role in apoptosis in several studies. In 2005, Cimmino *et al.* found that miR-15 and miR-16 induce apoptosis by targeting Bcl2 mRNA [24]. More recently, all transretinoic acid-induced differentiation and down-regulation of both Bcl2 and Ras in promyelocytic leukemia was found to correlate with the activation of miR-15 and miR-16 [25]. Additionally, Scott and colleagues have been able to reduce invasion and migration in breast cancer cells using miR-125 [26]. Response to chemotherapy has also been proposed to be altered by expression of miRNA in a recent study by Meng and colleagues. Inhibiting miR-21 and miR-200b increased the sensitivity of malignant cholangiocytes to gemcitabine, and inhibiting miR-141 decreased cell growth [27].

MiRNA has quickly come to the forefront of research in epigenetics. Global genomic DNA hypomethylation, aberrant DNA hypermethylation of tumor suppressor genes, and disruption of histone modification are all well studied epigenetic mechanisms. This led to the study of miRNA silencing by hypermethylation. Recently, Iorio and colleagues found that epigenetic mechanisms may play a role in miRNA expression in ovarian cancer. They proposed that DNA hypomethylation was possibly responsible for the overexpression of miR-21, miR-203, and miR-205 [28]. In the future, drugs such as histone deacetylase inhibitors and DNA demethylating agents may be used to restore the appropriate expression of miRNAs and thus revert to normal the tumoral phenotype [29].

MicroRNAs and Solid Tumors

The first reports of expression and down-regulation of miRNA in solid tumors was miR-143 and -145 in colorectal cancer by Michael *et al.* and Bandres *et al.* [30, 31], (Table 1). Bandres also found that miR-31, miR-96, miR-133b, miR-145, and miR-183 were all significantly down-regulated in 16 colorectal cancer cell lines and 12 matched pairs of tumor and normal tissues. Up-regulation of miR-31 was also found to be associated with the stage

of cancer [31]. They further demonstrated that miR-143 had stronger signals using *in situ* hybridization in the ascending glandular cells than in the proliferating cells at the base of the crypt. This may be because miR-143 is down-regulated in rapidly proliferating cells while increased in the ascending glandular cells, which are soon to undergo apoptosis [32]. Most recently, Schetter *et al.* found 37 differentially expressed miRNA in a set of 84 matched pairs of colorectal adenocarcinomas and nontumorous tissues. Overexpression of miR-21 was also associated with poor survival, regardless of tumor-node-metastasis stage [33]. In a study of multiple types of solid tumors, Volinia and colleagues found a miRNA signature for solid tumors by analysis of 540 samples, including lung, breast, stomach, prostate, colon, and pancreatic tumors. They also found that protein coding tumor suppressor genes and oncogenes were the targets for the differentially expressed miRNAs [34].

Microarray analysis has been invaluable in evaluating lung cancers. Patients with lung cancers expressing reduced levels of let-7 were found to have significantly shorter survival. This study also found that overexpression of let-7 in an adenocarcinoma cell line led to decreased cell growth [17]. In 2006, Yanaihara and colleagues confirmed a unique miRNA expression profile in lung cancers using microarray technology. Forty-three miRNAs were found to be down-regulated and could accurately distinguish normal *versus* malignant tissues, even in Stage I cancer. Increased levels of miR-155 and decreased levels of let-7a-2 were associated with poorer survival in these patients. Interestingly, they also found a molecular signature for subsets of lung cancer. Six miRNAs were identified to have differential expression in adenocarcinoma and squamous cell cancer. For example, the expression of miR-99b and miR-102 was higher in adenocarcinoma [35]. These data from the microarray were also confirmed by real-time polymerase chain reaction.

More recently, expression profiles were identified in pancreatic cancers. Real-time polymerase chain reaction of miRNA precursors was used to evaluate 28 tumors, 15 adjacent benign, 4 chronic pancreatitis, and 6 normal samples, and found an expression signature for pancreatic ductal adenocarcinoma. These data could correctly identify 28 of 28 tumors, 6 of 6 normal pancreas, and 11 of 15 adjacent benign tissues [36]. Using miRNA microarrays, Bloomston *et al.* identified global expression patterns in 65 resected pancreatic ductal adenocarcinomas with matched benign adjacent pancreas and 42 chronic pancreatitis specimens. Twenty-one miRNAs were differentially overexpressed and four underexpressed in the pancreatic cancers relative to chronic pancreatitis and adjacent benign pancreas. A subset of six miRNAs was shown to be predictive of long-term survival in node-positive patients, while high expression of miR-196a2 was associated with a poorer survival [37]. In another study, miR-196a and miR-196b expression was not found in normal pancreas or chronic pancreatitis; however, it was overexpressed in pancreatic ductal adenocarcinoma [38]. MiRNAs were, similarly, found to be predictive of tumor aggression by Roldo *et al.*, who showed that miR-21 expression was associated with liver metastasis and a high proliferation index in pancreatic endocrine and acinar tumors [39].

Like other solid tumors, miRNAs were also found to be aberrantly expressed in breast cancers. MiR-10b, miR-125b, and miR-145 were most significantly down-regulated, whereas miR-21 and miR-155 were most significantly up-regulated in breast tumors. A set of 15 miRNAs established a profile that could distinguish malignancies from normal tissue

in 100% of the samples tested. Additionally, there was underexpression of let-7 in samples with lymph node metastasis or a higher proliferation index [40]. This may potentially be linked to a poorer prognosis in breast cancer, as it is in lung cancer [24, 28]. In a similar study with ovarian tumors, the miRNA expression profile was able to clearly define tumor from normal ovarian tissue. Mir-199a, miR-140, miR-145, and mir-125b1 were all found to be underexpressed, whereas miR-200a, miR-141, miR-200c, and miR-200b were all overexpressed. There was also a correlation between the miRNA expression profile and certain pathologic features of the tumors [28].

In hepatocellular carcinoma, three miRNAs were overexpressed and five were underexpressed compared with normal adjacent tissue [41]. They were able to distinguish hepatocellular carcinoma from normal tissue with 97.8% accuracy. Interestingly, they also found that the expression level was correlated with differentiation of the tumors. While able to distinguish cirrhosis from cancer, they were unable to differentiate samples infected with hepatitis B or hepatitis C.

In papillary thyroid carcinomas (PTC), an expression profile was delineated from 30 PTC patient samples and 10 normal thyroid samples. This profile clearly distinguishes normal tissue from tumor with miR-221, miR-222, and miR-181b overexpressed in papillary cancer [42]. In a similar study, five miRNAs were found to be able to distinguish PTC from normal tissue with miR-221, miR-222, and miR-146 being most up-regulated. In some of the patients with PTC, miR-21 was also found to be up-regulated in the unaffected thyroid tissue, thus indicating that miR-21 dysregulation may be an early event in carcinogenesis [43].

Diagnostic Capabilities, Therapeutic Targets, and Manipulation of miRNA

Based upon the intricacies of miRNA interaction with multiple targets and, hence, multiple pathways, it stands to reason that miRNAs serve as potential targets for novel therapeutics or to aid in the diagnosis of disease. Expression profiling gives the prospect of developing biomarkers for the diagnosis, treatment, and risk stratification of cancer patients. In today's age of personalized healthcare, miRNA profiles may have a significant impact on particular therapies available to individual patients in the future. Lung cancer patients with reduced expression of let-7 and increased miR-155 were found to have poorer survival; knowing this early in the disease process may allow for these patients to undergo more aggressive therapy and follow-up [44]. Due to the differential expression of miRNA in cancer, they could be used to aid in the diagnosis and may distinguish between malignant and reactive lesions when other traditional methods have failed [45]. They could also be used to help distinguish subgroups of each tumor type.

The manipulation of miRNAs by inducing their expression was the first method used to elucidate their function in cell lines. This was achieved by transfection of double stranded RNA molecules, which are similar to the miRNA duplex formed after cleavage of Dicer [46]. This is an excellent method for transient overexpression, but if longer term overexpression is required, DNA plasmids can be used. Plasmids can continuously generate miRNAs via either endogenous or viral promoters. Both of these methods can yield low

transfection efficiency. To overcome this, adenoviruses or retroviruses have been used as vectors to deliver the miRNA of interest *in vitro* and *in vivo* [46].

There are also several methods used to silence miRNA. Anti-miRNAs conjugated to cholesterol, called antagomiRs, have been found to be able to inhibit miRNA *in vivo* in a mouse model. Injection of this antisense oligonucleotide effectively inhibited mature miRNA in the liver for up to 23 days [47]. In another study, injection of antogmiR-16, which is ubiquitously expressed, led to complete absence of miR-16 everywhere except the brain in a mouse model. Genetic alterations can also be used to knock out miRNA genes directly or via conditional alleles, leading to a nonfunctional Dicer and the resultant silencing of miRNAs [48]. While the use of miRNA alteration has not made the leap to the clinical setting, such advancements are forthcoming.

Conclusions

The field of miRNA research has exploded in the past several years. The data and discoveries made are very promising in the area of cancer research and treatment. However, much more needs to be done to fully elucidate the role of miRNA in molecular pathways and cancer, and to further discover novel therapeutics that can be instituted in clinical practice.

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Fig. 1. MiRNA processing. Reprinted with permission from Macmillan Publishers Ltd.: Nature Reviews Cancer, Oncomirs-microRNAs with a role in cancer. 2006;6:259–269, Copyright 2006.

Table 1
Overview of miRNA Studies in Solid Tumors

Year	Author	Tumor type	No. of samples	Significant findings
2005	He <i>et al.</i> [43]	PTC	20T, 20N (matched)	miRNA expression pattern (17 over-expressed, 6 underexpressed)
2005	Iorio <i>et al.</i> [40]	Breast	76T, 9N	miRNA expression pattern, let-7 underexpression correlated with lymph node metastasis and higher proliferation index
2006	Pallante <i>et al.</i> [42]	PTC	30T, 10N	5 miRNA overexpressed, miR-21 described in carcinogenesis
2006	Roldo <i>et al.</i> [39]	PET	44T, 12N	miRNA expression pattern, miR-21 correlation with stage and proliferation index
2006	Yanaihara <i>et al.</i> [35]	Lung	104T, 104N (matched)	miRNA expression pattern (43 underexpressed), miR-155 and let-7-a2 associated with poorer survival
2006	Volinia <i>et al.</i> [34]	Mixed	363T, 177N	Tumor-specific miRNA expression patterns, oncogenic target genes described
2006	Bandres <i>et al.</i> [31]	CRC	12T, 12N (matched)	miRNA expression pattern, miR-31 correlation with stage
2006	Murakami <i>et al.</i> [41]	HCC	25T, 25N (matched)	miRNA expression pattern, correlation with tumor grade
2007	Lee <i>et al.</i> [36]	PDA	28T, 15N (matched)	miRNA expression pattern
2007	Bloomston <i>et al.</i> [37]	PDA	65T, 65N (matched)	miRNA expression pattern (21 overexpressed), miR-196a2 associated with poorer survival
2007	Iorio <i>et al.</i> [28]	Ovarian	69T, 15N	miRNA expression pattern, correlation with pathologic features
2008	Schetter <i>et al.</i> [33]	CRC	84T, 84N (matched-US) 113T, 113N (matched-Chinese)	miRNA expression pattern in US samples and verified in Chinese samples, miR-21 correlated with TNM stage

PTC = papillary thyroid cancer; PET = pancreatic endocrine tumor; CRC = colorectal cancer; HCC = hepatocellular carcinoma; PDA = pancreatic ductal adenocarcinoma; T = tumor; N = normal; “matched” refers to adjacent benign tissue; TNM = tumor-node-metastasis.