Cancer Letters 438 (2018) 126–132

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Emerging microRNAs in cancer diagnosis, progression, and immune surveillance

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ARTICLE INFO

Keywords:
Cancer immunity
miR-21
miR-375
oncomiR
Prognostic biomarker
RAS pathway

ABSTRACT

MicroRNAs (miRNAs) are small noncoding RNAs that function in post-transcriptional regulation of gene expression. Dysregulation of miRNAs has been reported in different stages of cancer development and progression. This dysregulation results in different miRNA profiles between cancer and normal tissues. Many studies have shown a significant correlation between miRNA profile and cancer diagnosis and prognosis. Additionally, since a single miRNA regulates multiple mRNA targets, miRNAs dysregulation can affect several pathways involved in cancer development. Finally, due to their regulatory role in immune cell development, many recent studies have reported that certain miRNAs play key roles in cancer immunology. In this brief review, we discuss the role of miR-21 and miR-375 in the RAS pathway as well as their role in cancer diagnosis and progression, along with the role of other select miRNAs in cancer immune surveillance.

1. Introduction

MicroRNAs (miRNAs) are small, approximately 22-nucleotide-long, non-coding RNAs. MiRNAs are encoded in single miRNA gene loci, miRNA gene clusters, or within introns of protein-coding genes. These small RNAs post-transcriptionally regulate cellular genes that are responsible for fundamental cellular processes, including cell differentiation and development [1,2]. MiRNAs regulate gene expression by interacting primarily with the 3′-UTR of their target mRNA, but may also interact with the 5′-UTR and/or coding region [3,4]. The interaction usually results in either repression of translation or mRNA degradation, but stimulation of mRNA translation has also been reported.

Among non-coding RNAs, miRNAs are the most studied in normal and many disease conditions [5–7]. According to the miRNA database miRBase (http://www.mirbase.org, Release 22), the human genome encodes 2693 mature miRNAs. The biogenesis of miRNAs starts in the nucleus. Long primary miRNAs (pri-miRNAs) are transcribed from miRNA gene loci via RNA polymerase II. The pri-miRNAs are first processed by Drosha and associated protein DGCR8 to ~65 nucleotide-long hairpin structures called precursor miRNAs (pre-miRNAs). The pre-miRNAs exit the nucleus via exportin 5 into the cytoplasm. Each pre-miRNA is cleaved by Dicer into a small RNA duplex. Ultimately one of the functional strands is loaded and remains bound to Argonaute protein. This RNA-protein complex along with members in the GW182/TNRC6 family proteins, constitute the RNA-induced silencing complex (RISC) [8,9]. The position 2–7 nucleotide of the miRNA, commonly known as the “seed sequence”, directs the RISC complex to bind to target mRNAs and leads to translational inhibition or mRNA degradation. The miRNA-mRNA interaction relies primarily on seed sequence matching and imperfect complementarity at 3’ end of each strand. This gives each miRNA the potential to target hundreds of endogenous mRNAs [10]. GW182/TNRC6 proteins are critical in this miRNA-Ago mediated process as demonstrated in early studies [11–13]. According to recent analyses using purified subcellular foci and a newly developed technique called fluorescence activated particle sorting, GW/P bodies may play a key role in translational repression [14].

The first miRNA discovered was lin-4 in *C. elegans*. Lin-4 regulates the expression of developmental genes in *C. elegans* [15]. Later, the discovery that miRNA let-7 is conserved among diverse animal species, led many investigators to pursue research on miRNAs in humans [16].

Abbreviations: AKT, serine/threonine-protein kinase; CIP2A, cancerous inhibitor of PP2A; ERK, extracellular-signal-regulated kinase; FFPE, formalin-fixed, paraffin-embedded; HNC, head and neck cancer; IFN-ɣ, interferon gamma; JAK2, Janus kinase 2; MEK, mitogen-activated ERK kinases; MHC, major histocompatibility complex; miRNA, microRNA; MMPs, matrix metalloproteinases; MTDH, metadherin; OSCC, oral squamous cell carcinoma; PDCD4, programmed cell death 4; PDK1, phosphoinositide-dependent kinase 1; PDL1, programmed death-ligand 1; PI3K, phosphoinositide-3 kinase; pre-miRNAs, precursor miRNAs; pri-miRNAs, primary miRNAs; PTEN, phosphatase and tensin homolog; RECK, reversion-inducing cysteine-rich protein with Kazal motifs; RHOB, Ras homolog gene family member B; SPRY, sprouty homolog; TCGA, The Cancer Genome Atlas; UTR, untranslated region; ZEB1, zinc finger E-box binding homeobox 1

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https://doi.org/10.1016/j.canlet.2018.09.019
Received 1 August 2018; Received in revised form 6 September 2018; Accepted 7 September 2018

In this review, we will focus on the role of miRNAs in cancer development and predication of cancer prognosis, along with the role of miR-21 and miR-375 in the RAS pathway, a well-known pathway involved in carcinogenesis.

2. Dysregulation of miRNAs in cancer

In 2002, Calin et al. showed that miR-15 and miR-16, transcribed from loci on chromosome 13q14, were predominantly either downregulated or absent in patients with B-cell chronic lymphocytic leukemia [17]. This was the first evidence that aberrant expression of miRNA genes was associated with cancer development [17]. In 2005 and 2006, two groups showed that tumors from patients with different types of cancer had significantly different miRNA profiles [18,19]. More importantly, miRNA expression profiles were able to classify poorly differentiated tumors more accurately than mRNA profiles when applied to the same samples [18]. These studies provide key evidence that miRNA profiling can be important in cancer diagnosis and that select miRNAs can be involved in cancer cell biology and pathogenesis. Dysregulation of miRNAs in cancer can result from defects in miRNA biogenesis and maturation. Furthermore, regulation of miRNAs through competitive interaction with other non-coding RNAs has also been reported in cancer [5].

3. Critical role of oncogenic miR-21 and tumor suppressor miR-375 in cancer

Cancer is generally the result of an accumulation of gene mutations [20]. It is widely accepted that the dysregulation of two sets of genes is commonly associated with cancer. These genes known as oncogenes and tumor suppressor genes work to promote and suppress tumor growth, respectively [21]. Dysregulation of oncogenes and tumor suppressor genes can lead to stimulation of cell growth and inhibition of cell death, both characteristic features of carcinogenesis [22]. Similarly, there are subsets of miRNAs with oncogenic or tumor suppressor phenotypes. Elevated expression of oncogenic miRNAs can repress the expression of some tumor suppressor genes, while reduced expression of tumor suppressive miRNAs can lead to increase expression of oncogenes [23]. Although some miRNAs appear to have a distinct role in cancer development, it can be difficult to predict their oncogenic or tumor-suppressive role. For example, the miR-29 family act as tumor-suppressor miRNAs in mantle-cell lymphoma, lung cancer, diffuse large B cell lymphoma, and Burkitt lymphoma. However, the same miRNA family can act as oncogenic miRNAs in indolent human B cell chronic lymphocytic leukemia and metastatic breast cancer [24].

Among oncogenic miRNAs, as shown in Table 1, miR-21 is highly expressed in various types of cancer including prostate [25], breast [26], esophageal [27], pancreatic [28], and gastric cancer [29]. MiR-21 is also known to target many cancer related genes and it has been associated with poor patient survival and response to chemotherapy [30]. On the other hand, miR-375 is a prominent tumor suppressor miRNA that was first identified in a murine pancreatic cell line, as a regulator of insulin secretion [31,32]. This tumor suppressor miRNA is underexpressed in several types of cancer, including esophageal [33], cervical [34], and oral cancer [35] (Table 1). Clearly there are other well-known tumor suppressor miRNAs, such as miR-34a, which is underexpressed in many cancer types including breast, colon, and lung cancer and many hematologic malignancies. Overexpression of miR-34a in cancer cells can induce apoptosis and cell cycle arrest [36].

4. Expression of miRNA-21 and miRNA-375 in cancer

Our previous microarray analysis on oral squamous cell carcinoma (OSCCs) compared with normal tongue tissues, showed that miR-21 is one of the most significantly overexpressed miRNAs in oral cancer ($p < 0.05$) [37]. From the same microarray, we found that miR-375 was the most underexpressed miRNA in oral cancer ($p < 0.01$) [35,37]. Data from The Cancer Genome Atlas (TCGA), showed overexpression of miR-21 (average 15-fold increase) and underexpression of miR-375 (average 5-fold decrease) in 488 patients with head and neck cancer (HNC) compared to 44 controls. In addition, the same database showed that miR-21 is 2.5-fold overexpressed and miR-375 is 150-fold underexpressed in 43 paired HNC tumors compared to controls tissues [38]. Interestingly, in our recent follow-up study examining these miRNAs during progression from premalignant lesion to cancer, 31 paired tissues of progressive premalignant lesions and OSCC samples, showed that miR-375 was underexpressed in both oral progressive premalignant lesions and OSCCs, but not in similar lesions that did not progress to cancer [38]. Thus, in the case of miR-375, its expression level may become an important diagnostic biomarker to determine whether early lesions will develop into oral cancer.

In oral cancer, we showed that miR-21 targets reversion-inducing cysteine-rich protein with kazal motifs (RECK). RECK proteins are membrane-anchored glycoproteins that target at least three cancer-associated Matrix Metalloproteinases (MMP-2, MMP-9, and MT1-MMP) [37]. Our further studies have demonstrated the potential importance of miR-375 as a master regulator in both HPV+ and HPV- oral cancers. As a tumor suppressor miRNA, miR-375 downregulates Cancerous inhibitor of PP2A (CIP2A) protein which leads to proteolytic degradation of MYC, and eventually inhibition of cell proliferation in oral cancer cell lines [35]. In HPV + oral cancers, miR-375 targets *E6* and *E7* oncogenes, which leads to increased expression of tumor suppressor proteins p53, p21, and RB, and ultimately cell cycle arrest [39].

Complementary to our findings, He et al. found that compared to the control group, miR-21 was overexpressed by ~4.5 folds, while miR-375 was underexpressed by ~1000 folds in 19 patients with OSCC [40]. These investigators proposed that using the expression of both miR-21 and miR-375 can help researchers discriminate between oral

Table 1
Upregulation of miR-21 and downregulation of miR-375 in different cancer types.

Cancer type	Upregulation of miR-21 expression		Downregulation of miR-375 expression	
	Number of patients and type of sample	Fold change/P value	Site and number of patients	fold Change/P value
Oral Cancer	17 patients Tongue (fresh frozen)	~2.5 folds P < 0.05 [37]	31 patients Multiple sites of the oral cavity (FFPE)	8 folds P < 0.0001 [38]
Esophageal Cancer	71 patients Serum	~6 folds P < 0.001 [67]	60 patients (Fresh frozen)	~15 folds P < 0.001 [43]
Gastric Cancer	37 patients (Fresh frozen)	> 2 folds P < 0.005 [45]	22 patients (Fresh frozen)	~8 folds P < 0.001 [46]
Hepatocellular Carcinoma	60 patients (FFPE)	3 folds [49]	60 patients (Fresh frozen)	25.6 folds [48]
Cervical Cancer	142 patients (FFPE)	> 5 folds P < 0.0001 [51]	170 patients (Fresh frozen)	~4.5 fold P < 0.05 [34]

FFPE, formalin-fixed, paraffin-embedded.