MicroRNAs are involved in cervical cancer development, progression, clinical outcome and improvement treatment response (Review)

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Received March 10, 2015; Accepted July 29, 2015

DOI: 10.3892/or.2015.4369

Abstract. Cervical cancer (CC) is the third most diagnosed cancer among females worldwide and the fourth cause of cancer-related mortality. Prophylactic HPV vaccines and traditional pap-smear screening are undoubtedly capable of decreasing the incidence and mortality of CC. However, a large number of females succumb to the disease each year due to late diagnosis and resistance to conventional treatments. Thus, it is necessary to identify new molecular markers to predict the clinical outcome and to design powerful treatments. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression and are involved in the modulation of several cell pathways associated with progression from pre-malignant to invasive and metastatic disease, increasing tumor malignancy. The aim of this review was to summarize the recent data that describe the important role of miRNAS involved in CC in order to determine their potential as prognostic biomarkers and as therapy targets. Studies of >40 miRNAs with roles in cancer regulation were identified. We also identified 17 miRNAs associated with progression, 12 involved with clinical outcome and 7 that improved CC treatment response. The present review is expected to broaden understanding of the functional role and potential clinical uses of miRNAs in CC.

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Key words: microRNAs, cervical cancer, cancer progression, clinical outcome

Contents

1. Introduction

Cervical cancer (CC) is the third most common malignancy among females worldwide, with a global incidence of >500,000 diagnosed new cases and ~260,000 mortalities each year (1). Over 85% of the CC global burden occurs in developing countries, where it represents ~13% of all types of cancer of female patients. Higher incidence regions include Eastern and Western Africa (>30 cases/100,000 inhabitants), Southern Africa (26.8/100,000 inhabitants), South-Central Asia (24.6/100,000 inhabitants), and South America and Middle Africa (23.9 and 23.0/100,000 inhabitants, respectively). Cervical cancer remains the most common cancer among females only in Eastern Africa, South-Central Asia and Melanesia (1,2). In Latin America, the mortality rates in the region are seven-fold greater than those in North America, with an estimated 72,000 new cases of cervical cancer and 33,000 mortalities that occur annually (3).

Conventional treatment of CC patients involves surgery and chemoradiotherapy, although radical hysterectomy is employed in early stages. Patients with locally advanced cervical cancer (LACC) are usually treated with radiotherapy in combination with cis-platinum (4,5). Nevertheless, ~50% of patients present with recurrent or persistent disease, which may be explained by the presence of therapy-resistant cells inside the tumor mass.

Despite advances in our understanding of this neoplasm, the absence of predictive conventional treatment response markers and the lack of alternative treatments hamper CC

personalized treatment. Thus, it is imperative to understand the biology of this tumor, by analyzing its molecular dynamics as well as the clinical characteristics of CC patients in order to improve the outcome.

MicroRNAs or miRNAs are small (21-23 nucleotide long) endogenous, non-coding, single-stranded RNAs that control gene expression by binding to the 3' untranslated region (3'UTR) of messenger RNA (mRNA), leading to mRNA degradation or protein translation inhibition (6,7). Over 1,000 miRNAs are present in the human genome, each of which potentially regulates hundreds of mRNAs. Approximately 60% of all protein-coding genes are potentially regulated by miRNAs, which confers them a fundamental role in the modulation of numerous cell processes (8,9). MicroRNAs with altered expression patterns have been found to have oncogenic (oncomirs) or tumor-suppressing (anti-oncomirs) functions present in the pathogenesis of most malignancies. In the canonical model, oncomirs are upregulated and anti-oncomirs are downregulated, which has been attributed to amplification, deletion and/or mutation of miRNA loci, dysregulation of transcription factors and epigenetic silencing. Globally, miRNA expression alterations lead to the disturbance of several oncogenic or tumor-suppressor protein levels, which in turn alter cell growth by favoring tumor malignancy (10,11).

Over the last decade, the widespread deregulation of miRNAs in virtually all types of cancer has been clearly established, as have their implications during each stage of disease initiation, progression and development (12,13). Consequently, miRNAs are solid diagnostic and prognostic biomarker candidates and viable CC therapeutic targets (14,15).

2. miRNAs are associated with cervical carcinogenesis

Several factors are required for CC development, including the interaction of viral, environmental and host-dependent factors, which trigger tumor growth, invasion and metastasis. In addition, evidence of the importance of epigenetic regulation mechanisms has steadily increased over the last two decades, and has focused on the dysregulation of oncogenes and tumor-suppressor genes as the main generator of the malignant phenotype. In this regard, miRNAs have an important role as regulators of cell processes such as apoptosis, cell cycle progression, metastases and both chemo- and radioresistance (16). In an effort to understand the important role of several miRNAs during cervical carcinogenesis, we conducted an extensive literature review and found 60 research articles published between 2009 and 2014. These studies describe >40 deregulated miRNAs that target genes and are likely to be involved in the development and progression of CC, as well as in the chemoradiotherapy resistance mechanism (Table I).

Evidence supporting the correlation between miRNA expression and CC-related processes was initially described in 2009 (17). Findings of that study demonstrated that the expression of miR-21 promoted cell proliferation in HeLa CC cells, while its inhibition suppressed cell proliferation by overexpression of the tumor-suppressor gene PDCD4, a related programmed cell death protein. It also provided direct evidence that PDCD4 3'UTR is a functional target of this miRNA. Subsequently, it was demonstrated that miR-21 is a major oncomir, overexpressed in a wide variety of cancers including CC (15).

miR-34a, a small molecule, has been described in studies on cervical carcinogenesis. Pang *et al*, demonstrated that it is downregulated in different CC cell lines such as HeLa, SiHa, C4I, C33a and CaSki, while the induction of its expression in HeLa cells reduced the invasiveness, affecting Notch and Jagged1 proteins as well as Notch downstream signaling by regulating urokinase plasminogen activator (uPA) expression. The binding of miR-34a to the 3'UTR of Notch and Jagged1 was determined using siRNA functional assays (18).

MicroRNA expression profiling in squamous CC and adjacent non-tumor tissues showed that miR-886-5p was overexpressed in CC tissues. Subsequent *in vitro* assays in human H8 cervical squamous epithelial cell line revealed that this miRNA depressed BAX protein levels, reducing apoptosis and promoting cell proliferation. Conversely, knocking down miR-886-5p increased its pro-apoptotic protein target, BAX, inducing apoptotic cell death (19).

The same expression profile revealed miR-143 downregulation. Functional characterization of this miRNA through its overexpression in HeLa cells significantly inhibited cell proliferation and promoted apoptosis, while the co-expression of anti-miR-143 reestablished the tumor phenotype. Additionally, HeLa cells transfected with pre-miR-143 were injected subcutaneously into the flanks of female athymic mice and miR-143 upregulation suppressed tumor formation. The functional target of this miRNA is Bcl-2, as shown through functional assays in which miR-143 suppressed the activity of a luciferase reporter carrying the 3'UTR of the Bcl-2 mRNA. Bcl-2 expression levels in CC tissues were inversely proportional to the levels of miR-143 (20).

By contrast, miR-203 was consistently downregulated in both biopsies and tumor cell lines due to hypermethylation of its promoter region. Functional assays revealed that this miRNA suppresses proliferation, tumor growth and angiogenesis (21). Lao *et al* (22) recently demonstrated that miR-155 was upregulated in CC tissues compared to adjacent non-neoplasic tissues, and its overexpression in HeLa and SiHa cells promoted proliferation. By contrast, its downregulation, inhibited cell cycle progression, promoted apoptosis and induced cell cycle arrest in those cell lines by directly targeting the *LKB1* gene, which codes for a primary upstream kinase involved in AMPK activation, cell growth and proliferation suppression. Moreover, LKB1 was significantly downregulated in CC tissues, suggesting that this miRNA promoted CC cell proliferation by regulating LKB1 expression (22).

The aforementioned findings demonstrate that miRNAs bear an important role in CC tumorigenesis. The main miRNA-regulated targets participate in mechanisms such as cell growth, apoptosis, cell cycle arrest and angiogenesis, while it is widely accepted that deregulation of these cell processes are the major hallmarks of CC.

3. miRNAs involved in cervical cancer progression

A key process during CC progression is the proliferation of differentiating epithelial cells, and the participation of several miRNAs therein has been investigated. We conducted an extensive search of the literature, which yielded informa-

Table I. Differential expression of miRNAS in CC vs. normal samples.

miRNA	Status	Target gene	Refs.	miRNA	Status	Target gene	Refs.
miR-155	U	LKB1	(22)	miR-944	U	HECW2/S100PB	(44)
miR-196a	U	HOXC8	(45)	miR-497	D	IGF-1R	(46)
miR-31	U	ARID1A	(33)	miR-214	D	BCL2L2	(37)
miR-130a	U	Dicer	(47)	miR-155	D	EGF	(48)
miR-215	U	Not identified	(49)	miR-303-367	D	AKT1	(50)
miR-99a/99b	D	mTOR	(51)	miR-424	D	CHK1	(52)
miR-506	D	GLI3	(53)	miR-205	U	CYR61/CGF	(54)
miR-135a	U	SIAH1	(55)	miR-17-5p	D	TP53INP1	(56)
miR-129-5p	D	SP1	(57)	miR-10a	U	CHL1	(58)
miR-590	U	CHL1	(59)	miR-19a/19b	U	CUL5	(60)
miR-181a	U	PRKCD	(40)	miR-125b	D	PIK3CD	(61)
miR-99	D	TRIB2	(62)	miR-20a	U	TNKS2	(63)
miR-196a	U	NTN4	(64)	miR-214	D	GALNT7	(65)
miR-125b	U	BAK1	(66)	miR-143	D	BCL2	(20)
miR-203	D	VEGFA	(21)	miR-133b	U	MST2/CDC42/RHOA	(67)
miR-196b	D	HOXB7	(68)	miR-21	U	CCL20	(17)
miR-7	D	XIAP	(69)	miR-1	D	PLK1	(24)
miR-218	D	Not identified	(70)	miR-375	D	SP1	(15)
miR-886-5p	U	BAX	(19)	miR-372	D	CDK2/cyclin A1	(71)
miR-29	D	YY1/CDK6	(25)	miR-34a	D	Notch	(18)
miR-23b	D	uPA	(72)	miR-519	D	HUR	(73)
miR-214	D	Plexin-B1	(74)	miR-21	U	PDCD4	(75)

D, downregulated; U, upregulated; CC, cervical carcinoma.

tion concerning 17 miRNAs associated with the progression of premalignant lesions to invasive cancer, as identified in 20 articles (Table II).

The differential miRNA expression pattern associated with CC progression using normal tissues, moderate/severe dysplasia and invasive squamous cell carcinoma infected with HPV16 sequences has been identified. The deregulated expression of 9 miRNAs was detected in cancer and dysplasia compared with normal cervical tissues. Significantly over-expressed miRNAs were miR-148a, miR-10a, miR-196a and miR-132; while significantly underexpressed miRNAs were miR143, miR145, miR-99a, miR-513 and miR-29a. The findings suggest that these miRNAs can potentially be used to recognize cervical cancer dysplasia from normal tissue (23).

An interesting study performed by Li *et al* (24) evidenced that, miR-100 downregulation plays an important role through loss of inhibition of its target gene PLK1, a kinase involved in the G2/M transition during CC development. Authors of that study examined the expression of miR-100 by RT-qPCR and the PLK1 mRNA and protein by RT-qPCR and immunoblotting, respectively, in 125 cervical tissues including normal cervical epithelia, cervical intraepithelial neoplasia (CIN) and cervical cancer, as well as in five CC cell lines. miR-100 expression gradually decreased from low-grade to high-grade CIN and cervical cancer tissues, which correlated with the upregulated expression of PLK1 in CIN3 and cervical tissues. Findings of that study also showed that modulating the expression of miR-100 increased cell proliferation, deregulated the cell cycle and decreased apoptosis (24).

To determine the relationship of HPV 16-infection and miRNA expression in CC progression, Li *et al* (25), employed samples from 18 tissues obtained from normal, CIN 2-3 and squamous cell carcinoma biopsies to perform miRNAs microarray analysis; they covered 875 human miRNAs. Notably, the findings showed 31 unique miRNAs with a significantly deregulated expression from normal to CC (17 up- and 14 downregulated). Among these, miR-218 was the most significantly downregulated in the course from normal tissue to CC, while miR-29 was the most overexpressed. Furthermore, miR-29 showed an important negative correlation between YY1 and CDK6 expression. The findings of that suggested that the expression level of miR-29 was regulated by HR-HPV E6/E7 (25).

Wang *et al* carried out *in vitro* assays that revealed a specific seed match between miR-34a and the 5'UTR of p18Ink4c, an important modulator of cell cycle progression, which suppresses its expression. Cervical pre-cancer lesions and cervical cancer showed an increase of p18Ink4c protein. Consistently, the immunohistochemical staining of cervical tissue arrays showed an increased p18Ink4c expression of 4.8% in normal cervical tissues, of 8.3% in chronic cervical inflammation and of 68% in cervical cancer. Those findings suggested that miR-34a was downregulated in infected cervical tissues, which is critical to persistent p18Ink4c activation (26).

Table II. miRNAs involved in cervical cancer progression.

miRNA	Status	Cellular process	Target gene	Clinical background	Refs.
miR-34a	D	p53-dependent pathway (cell cycle progression, cellular senescence)	NOTCH, P18Ink4c, CDK4, CDK6, cyclin A, E2, E2F1, BCL2, BIRC3	↓CIN I, ↓↓CIN II, ↓↓↓CIN III	(26,76)
miR-218	D	Focal adhesion	LAMB3	↓CIN III, ↓↓↓CaCu	(77)
miR-200a, miR-205	NC	Metastases (inhibit the EMT)	ZEB1, ZEB2 and Sip1	CaCu → CaCu metastasis	(78)
miR-372	D	Cell growth (induces arrest in the S/G2 phases of cell cycle	CDK2, cyclin A1	Cervical normal tissue → cervical cancer tissue	(71)
miR-203	D	Keratinocyte differentiation/ maintenance HPV episomes	p-63-family	Normal epithelia → HPV-infected epithelia	(79)
miR-143	D	Cell growth and proliferation	PPAR signaling	↑Normal, ↓↓CIN, ↓↓CIN III, ↓↓carcinoma	(23)
miR-145	D	Cell motility	IGF-1	↑Normal, ↓CIN, ↓CIN III, ↓carcinoma	(23)
miR-99a, miR-513, miR-29a	D	Cell death, tissue development	IGF-1, BCL2L2, VEGFA and CDK6	↑Normal, ↓CIN, ↓CIN III, ↓carcinoma	(23)
miR-148a	U	Tumor suppressor genes	PTEN, P53INP1 and TP53INP2	↑Normal, ↑↑CIN, ↑↑CIN III, ↑↑↑carcinoma	(23)
miR-10a, miR-96a, miR-132	U	Cell transformation and progression	HOX genes	↑Normal, ↑↑↑CIN, ↑↑↑CIN III, ↑↑↑carcinoma	(23)
miR-886-5p	U	Cell transformation and progression	BAX	↑ANTT, ↑↑↑CSCC	(19)
miR-100	D	Growth, cell cycle and apoptosis	PLK1	↑Normal, ↓CIN, ↓↓carcinoma	(24)

D, downregulated; U, upregulated; NC, no changes in expression level.

Concerning invasion and metastasis, Lee *et al* (27) analyzed the expression level of 157 human mature miRNAs by means of TaqMan quantitative PCR array. In that study, 10 tumor biopsies staged as primary invasive squamous cell carcinomas (ISCC) and 10 normal tissues were used. The results identified significant evidence of 70 miRNAs being differentially expressed, 68 of which were upregulated and two downregulated. Of these, 10 miRNAs were significantly overexpressed, i.e., miR-199-s, miR-9, miR199a*, miR-199a, miR-199b, miR-145, miR-133a, miR-133b, miR-214 and miR-127 and only two were underexpressed, i.e., miR-149 and miR-203. Of note, the expression of miR-127 was significantly associated with lymph node metastasis. *In vitro* assays showed that blocking miR-199a expression exhibited an important reduction in cell growth (27). Fig. 1 summarizes the principal miRNAs involved in cervical carcinogenesis.

miRNAs associated with complex CC progression have been investigated. However, known CC-associated miRNAs target important, well-studied regulators of cell metabolism, strengthening the significance of their participation in the evolution of this pathology.

4. miRNAs involved in cervical cancer clinical outcome

The conventional treatment for LACC patients is radiotherapy concomitant with cisplatin. However, ~50% of LACC patients that receive radiotherapy exhibit recurrence or persistent disease, which may be explained by the presence of radio-resistant cells within the tumor mass. Therefore, radio- and chemoresistance are major obstacles for an efficient cervical cancer treatment (4,5). Nonetheless, a meta-analysis showed that CC patients, treated with radiotherapy alone or in combination

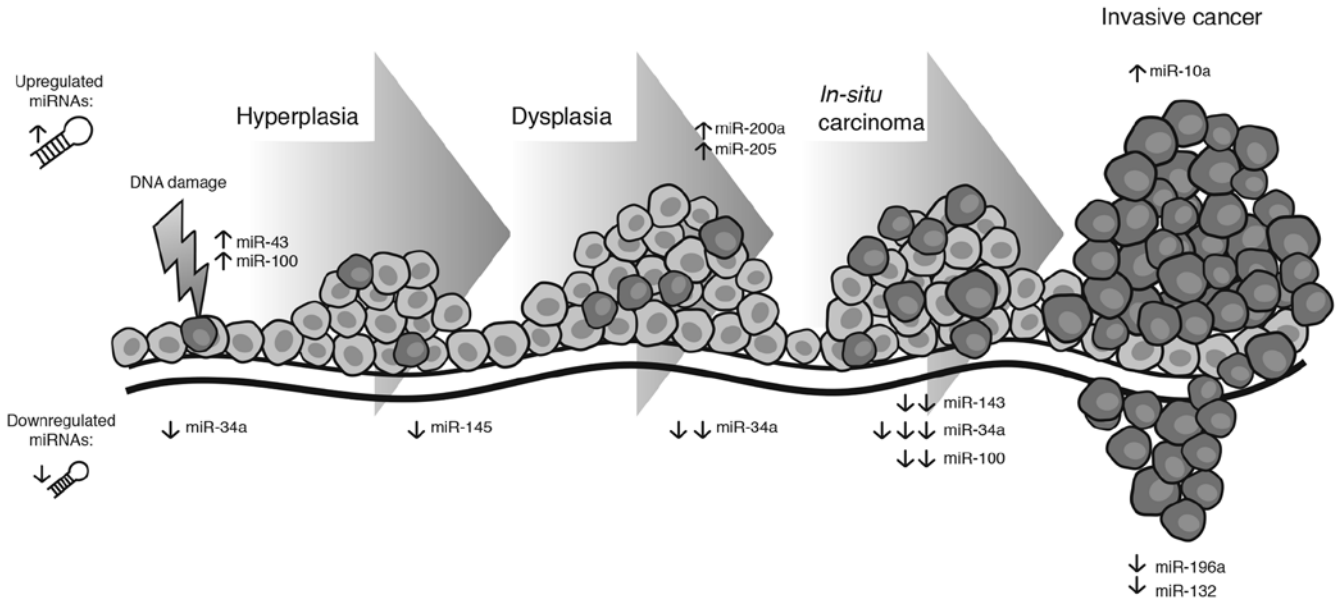

Figure 1. Expression of principal miRNAs associated with different clinical stages, from normal tissue, hyperplasia and *in situ* cancer to invasive cancer. There is evidence of a number of miRNAs that reduce their expression from normal tissue to *in situ* cancer, such as miR-143 and miR-100, while miR-10a, miR-196a and miR-132 are overexpressed in invasive cancer. Notably, miR-34 maintains its level through the evolution of CC. The result of this deregulation alters cell mechanisms such as cell cycle progression, focal adhesion, metastasis, cell growth, apoptosis and cell motility. CC, cervical carcinoma.

with different chemotherapeutic agents had a 40-70% 5-year survival rate (28).

In the last years, several miRNAs have been associated with survival and prognosis of CC patients. Thus, the expression and regulation of their targets have become molecular markers with clinical relevance prediction. In this section, we summarized data from an extensive bibliographical investigation regarding miRNAs associated with clinical outcome (Table III).

For instance, the expression profile of 96 cancer-related miRNAs from 102 CC tumor biopsies was analyzed (29). Through a mathematical algorithm, the authors identified that miR-200a and miR-9 were significantly associated with overall survival (OS). These miRNAS were individually transfected into HeLa cells and the expression profile of transfected cells was analyzed. Gene set enrichment and gene ontology-based analyses showed that miR-200a regulated *ZEB1*, *ZEB2*, *TGFB2* and *EXOC5* genes involved in the metastatic potential of cancer cells. Genes regulated by miR-9 were involved in metabolic processes, explaining the maintenance of a high metabolic rate by tumor cells, an important trait of the rapid proliferation of cervical cancer cells (29).

A similar study published in 2012, analyzed the expression profiles of 30 miRNAs associated with tumor metastasis from the formalin-fixed paraffin-embedded samples of 44 SCCC patients who underwent radical hysterectomy. Seven miRNAs, i.e., let-7c, miR-10b, miR-100, miR-125b, miR-143, miR-145 and miR-199a-5p, were significantly downregulated in the advanced stage SCCC patients (FIGO IB2-IV) compared to the early stage SCCC patients (FIGO IB1). Downregulation of these miRNAS, with the exception of miR-10b, were significantly associated with lymph node metastasis and with reduced survival in SCCC. Through a survival analysis, the authors identified that SCCC patients with a low expression of miR-100 and miR-125b projected a significant tendency towards a poorer prognosis (30).

Another study reported that the expression of miR-224 was significantly upregulated in cancer tissues of advanced FIGO stage cervical cancer patients, in lymph node metastasis-positive patients and in less-differentiated tumors (31). Kaplan-Meier analysis showed that patients with a higher miR-224 expression exhibited a shorter OS. Additionally, it was associated with aggressive progression and poor prognosis and was employed as an independent marker for predicting the clinical outcome (31).

Subsequently, the expression of miR-93 and miR-200a was retrospectively evaluated in 116 patients with invasive CC and 100 patients undergoing hysterectomy for benign lesions in order to determine their clinical significance. The levels of miR-93 and miR-200a were measured by RT-qPCR, and the proteins RECK, MMP2 and MMP9 were assessed by immunohistochemical staining. Results showed the upregulation of miR-93, miR-200a, and MMP2 and MMP9 proteins, but the downregulation of RECK in CC tissues compared to benign lesion tissues. Notably, patients with a strong RECK expression had a 5-year survival rate, significantly higher than that of patients with lower RECK-expressing tumors. In addition, a significant inverse correlation was identified between RECK downregulation with invasion and lymphatic metastases. Thus, the expression of miRNAs and RECK, served as potential prognostic markers for the long-term survival of CC patients (32).

The role of miR-31 as an independent prognosis factor was determined and it was demonstrated that it is upregulated in CC cell lines as well as clinical samples. The high miR-31 level was significantly correlated with higher FIGO stages, node metastases, vascular involvement and deep stromal invasion and with poorer OS. Those results showed that, the

Table III. miRNAs involved in cervical cancer clinical outcome.

miRNA	Status	Target gen	Outcome	Function	CC cell type	Refs.
miR-9	D	Not defined	Poor survival	Invasion and cell motility	Squamous and adenocarcinoma cells	(29)
miR-93, miR-200a	U	RECK	Poor survival	Invasion and lymphatic metastases	Invasive carcinoma	(32)
miR-31	U	ARID1A	Poor survival	Node metastases, stromal invasion	Invasive carcinoma	(33)
miR-26a	D	PRL-1	Poor survival	Inhibits cell proliferation and invasion	Invasive carcinoma	(80)
miR-224	U	Not defined	Poor survival	Aggressive progression	Invasive carcinoma	(31)
Let-7c	D	HMGA2	Poor survival	Lymph node metastases	Small cell carcinoma	(30)
miR-100	D	RSP3, PLK1	Poor survival	Lymph node metastases	Small cell carcinoma	(30)
miR-125b	D	BAK1	Poor survival	Lymph node metastases	Small cell carcinoma	(30)
miR-143	D	BCL2, KRAS, DNMT3A	Poor survival	Lymph node metastases	Small cell carcinoma	(30)
miR-145	D	BNIP3, IRS, STAT1, C-MYC	Poor survival	Lymph node metastases and poor survival	Small cell carcinoma	(30)
miR-199a-5p	D	SWI, SNF, PAK4	Poor survival	Lymph node metastases and poor survival	Small cell carcinoma	(30)

D, downregulated; U, upregulated.

downregulation of miR-31 impaired cell proliferation, colony formation, *in vitro* cell migration and invasion, and inhibited *in vivo* xenograft tumor growth. The authors verified that ARID1A was a direct target of miR-31, which was further confirmed by the inversely correlated expression of miR-31 and ARID1A in patient specimens. The authors of that study concluded that the miR-31/ARID1A pathway provides insight into the progression and clinical outcome of CC (33).

The abovementioned findings suggested that miRNAs may be used as biomarkers of OS, as they regulate genes involved in cell processes such as invasion, migration, growth and metastases. In this sense and to attempt to integrate and comprehend the complexity of the deregulation and interaction of miRNAs and the cell processes involved in the clinical outcome, we sketched a network with the miRNAs and their targets that shows miRNA-mRNA interaction using 7 of the 12 miRNAs involved in the clinical outcome, i.e., miR-199a-5p, miR125b, miR-143, miR-145, miR-9, miR93 and miR-200a (Fig. 2). These 7 miRNAs have a direct or indirect association with the regulation of their target. For example, miR-9 and miR-199a-5p have an indirect interaction with SOX9, a transcription factor associated with epithelial-mesenchymal transition (EMT), proliferation and regulation of apoptosis, while miR-199a-5p and miR-125b putatively target ERBB, a tyrosine kinase that activated the AKT/PI3K pathway, indirectly. Another instance is miR-125b, which interacts indirectly with miR-143 through the regulation of AKT1 and its migration and cell proliferation pathway. Furthermore, miR-143 and miR-145 inhibit HRAS secondarily, leading to activation of the MAPK cascade, progression of the cell cycle and suppression of apoptosis. VEGF-A, in turn, is downregulated by miR-145 and miR-9, while miR-9 also acts together with miR-93 and deregulates the expression of CCND1, a cyclin that activates the cell cycle. Notably, miR-93 interrelates with another 3 miRNAs: with miR-200a through the regulation of KLF11, a transcription factor that participates in cell cycle progression, proliferation and the inhibition of apoptosis; miR-125b, which possibly targets STAT3, a transcription factor involved in proliferation and suppression of apoptosis; and miR-199a-5p, which activates apoptosis through the underexpression of the tumor suppressor SMAD4.

5. miRNAs involved in the improvement of cervical cancer treatment response

miRNAs have become important regulators of treatment response and the understanding of their mechanisms represent potentially personalized molecular markers for the prediction of individual clinical outcomes. In addition, miRNAs were employed as novel therapeutic targets (34,35). Although they have been studied in different types of cancer, it has been established that 7 miRNAs were involved in CC treatment response, two of which were associated with chemoresistance, one with chemosistance and radioresistance, and the remaining miRNAs only with radioresistance.

The first study that established the association between miRNAs with treatment response was published by Shi *et al* (36). The aim of their study was to demonstrate a novel mechanism

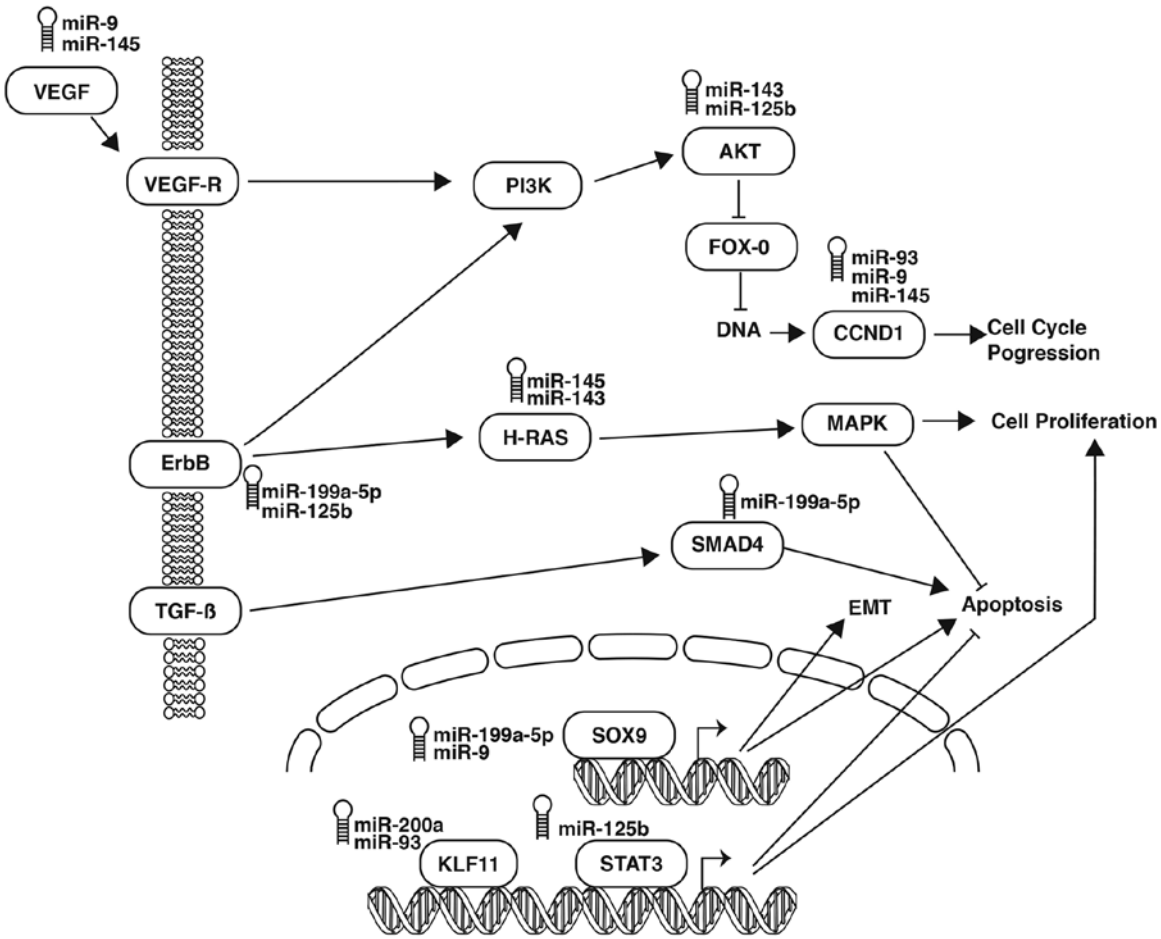

Figure 2. Possible interactions of 7 miRNAs involved in CC clinical outcome. Selecting only a few miRNA and their targets yields a fairly complex scenario that includes important cell processes. Active miRNA studies are poised to widen this perspective and provide data regarding how tightly miRNA-mRNA interactions control gene expression and consequently, cell fate. CC, cervical carcinoma.

by which glucocorticoids modulate p53-dependent miR-145 expression in HPV-positive CC cells through the induction of E6 proteins. miR-145 expression was reduced in CC tissues by the action of cortisol, which simultaneously induced HPV-E6 expression and p53 suppression in CC cells. Expression of miR-145 in CC cells was wild-type and p53-dependent, and the cortisol-induced downregulation of miR-145 prevented chemotherapy-induced apoptosis, whereas its overexpression enhanced sensitivity to mitomycin and reversed the chemo-resistance induced by glucocorticoids. Furthermore, miR-145 enhanced the effects of p53 by suppressing its inhibitors in CC cells, suggesting that miR-145 plays a role in p53 tumor suppression and inhibition in the motility and invasion of CC cells. These findings identified a novel pathway by which the neuroendocrine macro-environment affected cervical tumor growth, invasion and therapy resistance, while showing that miR-145 served as a target for CC therapy (36).

miR-214 is another miRNA that inhibits cell growth, migration and invasion. Wang *et al* (37) analyzed its role and function focusing on demonstrating that increasing levels of miR-214 reduced cell survival and enhanced cisplatin-induced cytotoxicity in CC cells. Concordantly, the authors of that study showed that the ectopic expression of this miRNA reduced the cell survival rate, induced apoptosis and enhanced sensitivity to cisplatin by directly inhibiting BCL2L2 expression in HeLa and C-33A cervical cancer cells. Further analysis revealed that apoptosis was correlated with increased expression of Bax, caspase-3, -8 and -9. Collectively, those findings suggested that miR-214 is a potential target for the development of novel therapeutic strategies (37).

The mechanisms responsible for CC radioresistance regulated by miRNAs are largely unexplored, nevertheless, Zhang *et al* aimed to identify specific miRNAs involved (38). In order to find a specific miRNA signature, they established radioresistant CC cell variants by repeated radiation selection. The miRNA profiles of radioresistant cells and their corresponding controls were analyzed and compared using microarrays. Among the differentially expressed profiles, 20 miRNAs showed similar patterns of alteration: 14 miRNAs were overexpressed, while 6 were underexpressed in all three radioresistant CC cell variants compared to their controls. miR-630, miR-1246, miR-1290 and miR-3138, exhibited a >5-fold increase in radioresistant cells. Subsequent analysis revealed that the four miRNAs could be upregulated in CC cells by radiation treatment in time- and dose-dependent manners. Ectopic expression of each miRNA was demonstrated to markedly increase the survival fraction of irradiated CC cells. Notably, inhibition of miR-630, a miRNA of the specific signature reversed radioresistance of CC cells (38).

Ke *et al* demonstrated that miR-181a had an important role in radiation therapy (39). The aim of that study was to describe the roles of miR-181a as a regulator in CC-radioresistant phenotype, to explore the underlying mechanism and to evaluate the potential of miR-181a as a radio-sensitivity biomarker. miRNA profiles of CC tumor specimens were analyzed. The specimens were sourced from 18 patients with a histological diagnosis of squamous CC (clinical stage IIIB). The patients had not received any chemotherapy prior to radiation therapy. A higher expression of miR-181a was observed in radiation-insensitive CC specimens and cell lines, when compared to sensitive cancer specimens. Furthermore, miR-181a negatively regulated the expression of PRKCD, a pro-apoptotic protein kinase, through interaction with its 3'UTR messenger, thereby resulting in the inhibition of irradiation-induced apoptosis and decreasing G2/M blockade. The role of miR-181a in radioresistance was validated in cell culture and mouse tumor xenograft models. Cells bearing anti-miRNA were unable to resist radiation therapy, in contrast to cells expressing a miR181a mimic. Thus, the expression of miR-181a in CC constitutes a potential biomarker of sensitivity or response to radiation therapy and target miR-181a represented a new approach to sensitize CC to radiation treatment (39).

In addition to its role in radioresistance, miR-181 also confers chemoresistance in CC, according to studies of its significant upregulation in clinical biopsies from patients that do not respond to conventional cisplatin treatment (40). To clarify the role of miR-181a in regulating the chemoresistance of CC, its overexpression was induced in human cervical squamous cancer cell lines, SiHa and Me180, which resulted in enhanced chemoresistance to cisplatin through apoptosis reversion. In a nude mouse xenograft model, the overexpression of miR-181a markedly inhibited the therapeutic response to cisplatin in a PRKCD-mediated manner. Additionally, PRKCD silencing yielded a similar effect to that of miR-181a upregulation, inhibiting apoptosis in CC cells. Thus, miR-181a may be used as a biomarker to predict chemosensitivity to cisplatin in patients with cervical squamous cancer (40).

The studies associated with miRNAs involved clinical outcome in CC patients. However, few may be applied as biomarkers of therapy response and as co-adjuvant therapeutic targets. As therapeutic agents, miRNAs were employed to enhance chemoradiosensitivity with the downregulation of its anti-apoptotic, DNA damage repair and cell cycle progression targets (41-43).

5. Conclusions

Complex diseases such as CC require the conjunction of different factors. Thus, there are a number of events that lead an HPV-infected cell to form an invading, chemo- and radioresistant tumor mass that eventually takes the life of its host. It has been long established that many of these events can be genetic mutations that arise due to genome instability and persist due to selection. In this regard, many deregulated genes have been studied, the majority of which are associated with apoptosis, cell cycle control, migration, genetic instability, cell adhesion and metastasis.

Since miRNA profiles can be used to describe the chemo-radioresistance of tumors prior to treatment delivery and to monitor the response through the treatment, miRNA profiles can be useful in the selection of intensification strategies and predict final response to therapy and risks of recurrence or metastases. By contrast, individual miRNAs present an important opportunity to enhance treatment efficacy, as is the case of miR-181a. This miRNA was proven to be a chemosensitizer and radiosensitizer, and in both cases, it downregulates PRKCD, a kinase involved in the regulation of apoptosis and inhibition of cell growth, making it a reasonable candidate for miRNA-based therapy enhancement.

The evidence reviewed in the present study showed that miRNAs affect various biological pathways associated with the development, progression (CIN 1, 2, 3 and cancer), clinical outcome and treatment response improvement in CC and reinforce their relatively recent role as key players in carcinogenesis. Consequently, their use in cervical cancer for diagnosis as well as clinical outcome prediction and therapy improvement may be utilized.

Acknowledgements

The present study was submitted by V. G.-Q. in partial fulfillment of the requirements for the degree of Doctor of Philosophy (Doctorado en Ciencias Biomedicas), Universidad Nacional Autonoma de Mexico. It was financially supported in part by the Universidad Nacional Autonoma de Mexico (UNAM; grant PAPCA-2014-6) and by Consejo Nacional de Ciencia y Tecnologia (CONACyT; grant SALUD-2010-01-141907). V. G.-Q. received a CONACyT fellowship.

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