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MiR-21 is involved in cervical squamous cell tumorigenesis and regulates CCL20

Tingting Yao, Zhongqiu Lin *

Department of Gynecological Oncology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, 107 Yan Jiang West Road, Guangzhou 510120, People's Republic of China
Key laboratory of malignant tumor gene regulation and target therapy of Guangdong Higher Education Institutes, Sun Yat-sen University, People's Republic of China

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ABSTRACT

MicroRNA 21 (miR-21) has been implicated in various aspects of carcinogenesis. However, its function and molecular mechanism in cervical squamous carcinoma have not been studied. Using TaqMan quantitative real-time PCR and Northern blot, we confirmed that miR-21 is significantly overexpressed in human cervical squamous cancer tissues and cell lines. Remarkably, we showed that the level of miR-21 correlates with the tumor differentiation and nodal status by ISH. Furthermore, we demonstrated that miR-21 regulates proliferation, apoptosis, and migration of HPV16-positive cervical squamous cells. In order to identify candidate target genes for miR-21, we used gene expression profiling. By luciferase reporter assays, we confirmed that CCL20 is one of its target genes, which is related to the HPV16 E6 and E7 oncogenes. Our results suggest that miR-21 may be involved in cervical squamous cell tumorigenesis.

1. Introduction

Cervical carcinoma is second only to breast cancer in female malignancies worldwide [1,2]. Hence, it poses an important public health problem. Approximately 80% of primary cervical cancers arise from pre-existing squamous dysplasia. The most important etiologic agent in the pathogenesis is human papillomavirus (HPV). However, not all women infected with high-risk HPV develop cervical carcinoma. This suggests that other cofactors must be present in the pathogenic pathway between cervical dysplasia and carcinoma.

MicroRNAs (miRNAs) are a class of endogenous small, non-coding RNA molecules of about 21 to 23 nucleotides that have the capacity to specifically inhibit translation or induce mRNA degradation, predominantly through targeting the 3′ untranslated regions (UTRs) of mRNA. Abnormal expression levels of miRNAs are associated with a variety of human cancers [3]. One of these microRNAs, miR-21, is a key player in human cancers, including breast [4], prostate [5], pancreatic [6] and colon cancer [7]. This miRNA has been linked to tumor aggression and carcinogenesis as an oncogene. Inhibition of miR-21 in tongue squamous cell increases expression of Tropomyosin1 (TPM1), suppresses tumor growth and induces apoptosis [8]. Knockdown of miR-21 upregulates programmed cell death 4 (PDCD4), causing significant reduction in invasion and metastasis [9–11]. It has been reported that miR-21 is overexpressed and promotes cell proliferation and down-regulates the expression of programmed cell death 4 (PDCD4) in HeLa cervical carcinoma cells [12,13]. However, the expression of miR-21 and its target gene has not been reported in squamous cervical carcinoma. Whether miR-21 has relationship with HPV remains elusive. Furthermore, miRNAs share only partial complementarity to their targets, and the conditions required for miRNA targeting have not been fully established. Identification and validation of the key targets that function in a specific cell setting or process are still a challenge. In this study, we aimed to examine miR-21 expression in HPV16 positive squamous cervical carcinomas. Next, we correlated miR-21 expression with the clinical status. We transfected anti-miR-21 in squamous cervical carcinoma cell lines, Caski and Siha, and investigated its contribution to tumor cell growth, apoptosis and migration. We also evaluated the role of miR-21 in tumor formation in immunocompromised mice inoculated subcutaneously. Bioinformatic analysis was used to screen and identify various genes with miR-21 target sites. Luciferase activity assays indicated that CCL20 contains putative miR-21 binding sites. Furthermore, introduction of miR-21 could reduce the expression of CCL20 protein and CCL20 mRNA in SCC cell lines.

2. Materials and methods

2.1. Patients and tissue

Paired cervical tumors (intra-epithelial neoplasia or primary cervical squamous carcinomas) and adjacent normal tissues were obtained from 126 patients (Table 1), who were admitted to the Department of Gynecologic Oncology of Sun Yat-sen Memorial Hospital, Sun Yat-sen University, from January 2002 to June 2011. None of the patients recruited into the present study received radiotherapy or chemotherapy

* Corresponding author at: Department of Gynecological Oncology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, 107 Yan Jiang West Road, Guangzhou 510120, People's Republic of China. Tel.: +86 13802921545; fax: +86 20 81332853.
E-mail address: linzhongqiu@hotmail.com (Z. Lin).

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Table 1
Summary of demographic and clinicopathologic features in human cervical tumors.

No.	Age	Cell type	FIGO Stage	HPV	Tumor diameter	Lymph nodal metastasis	Parametrial invasion	Vessel invasion	Differentiation	MiR-21
1	34	SCC	Ib1	16	3 cm	−	−	+	Poor	+
2	50	SCC	Ib1	16	Erosion	−	−	−	Poor	+
3	56	SCC	Ib1	16	1.5 cm	−	−	−	Well	−
4	39	SCC	Ib1	16, 18	2 cm	−	−	+	Mediate	+
5	48	SCC	Ib1	16, 31	Erosion	−	−	−	Poor	+
6	40	SCC	Ib2	16	4 cm	−	−	−	Well	+
7	61	SCC	Ib1	16	3 cm	−	−	+	Poor	+
8	42	SCC	Ib1	16, 33	2.3 cm	−	−	+	Poor	++
9	33	SCC	Ib1	16	Erosion	−	−	+	Poor	++
10	42	SCC	Ib1	16	3 cm	−	−	−	Well	−
11	42	SCC	Ib1	16	Erosion	−	−	+	Poor	++
12	50	SCC	Ib1	16	2.5 cm	−	−	−	Poor	+
13	53	SCC	Ib1	16	1 cm	−	−	+	Poor	+
14	52	SCC	Ib2	16, 35	5 cm	−	−	−	Mediate	−
15	40	SCC	Ib1	16	3.5 cm	−	−	−	Poor	+
16	37	SCC	Ib1	16	1 cm	−	−	−	Mediate	++
17	28	SCC	Ib2	16	5 cm	−	−	+	Poor	+
18	52	SCC	IIa	16	2 cm	+	−	−	Poor	++
19	47	SCC	Ib2	16	7 cm	−	−	+	Poor	+
20	40	SCC	Ib1	16	Erosion	+	−	−	Poor	++
21	38	SCC	Ib1	16	1 cm	−	−	−	Mediate	−
22	53	SCC	Ib1	16	3 cm	−	−	+	Poor	+
23	44	SCC	Ib1	16, 39	Erosion	+	−	+	Poor	+
24	50	SCC	IIa	16, 33	4 cm	−	+	−	Well	+
25	38	SCC	Ib1	16	3 cm	+	−	−	Poor	++
26	58	SCC	Ib1	16	Erosion	+	+	+	Poor	++
27	34	SCC	Ib1	16	Erosion	+	+	−	Poor	+
28	31	SCC	Ib2	16	4.5 cm	−	−	+	Poor	−
29	60	SCC	IIa	16	Erosion	−	−	−	Well	+
30	50	SCC	IIa	16	4.7 cm	+	−	+	Poor	+
31	48	SCC	IIa	16	3 cm	+	+	−	Poor	+
32	58	SCC	IIa	16	Erosion	−	−	−	Poor	+
33	50	SCC	IIa	16	5 cm	+	+	+	Poor	++
34	57	SCC	IIa	16	2 cm	+	−	+	Mediate	+
35	37	SCC	Ib2	16	4 cm	+	−	−	Poor	++
36	30	SCC	Ib1	16	2.5 cm	−	−	−	Well	−
37	41	SCC	Ib1	16	Erosion	−	−	−	Poor	−
38	30	SCC	Ib1	16	3 cm	−	−	−	Poor	++
39	40	SCC	Ib1	16	2 cm	−	−	−	Mediate	++
40	40	SCC	Ib1	16	Erosion	−	−	−	Mediate	+
41	53	SCC	Ib1	16, 18	3 cm	−	−	+	Poor	+
42	50	SCC	Ib1	16, 18	2 cm	−	−	−	Mediate	++
43	50	SCC	Ib1	16	1.5 cm	−	−	−	Well	+
44	47	SCC	Ib1	16	4 cm	−	−	+	Poor	++
45	52	SCC	Ib1	16	Erosion	+	−	−	Poor	+
46	38	SCC	Ib1	16	3 cm	+	−	−	Mediate	+
47	44	SCC	Ib1	16	3 cm	−	−	+	Poor	++
48	55	SCC	Ib1	16	2.5 cm	−	−	−	Poor	+
49	43	SCC	Ib1	16	Erosion	−	−	−	Well	+
50	38	SCC	Ib1	16	Erosion	−	−	+	Poor	+
51	53	SCC	Ib2	16	5 cm	+	−	+	Poor	+
52	49	SCC	Ib2	16	4 cm	+	−	+	Poor	++
53	53	SCC	Ib2	16	4 cm	−	−	−	Poor	++
54	30	SCC	Ib2	16	4 cm	+	−	+	Poor	+
55	44	SCC	Ib2	16, 45	5 cm	−	−	+	Well	+
56	38	SCC	Ib2	16, 66	6 cm	−	−	−	Mediate	++
57	44	SCC	Ib1	16	2 cm	+	−	+	Poor	+
58	38	SCC	Ib2	16	Erosion	+	−	+	Poor	++
59	28	SCC	Ib1	16	2 cm	−	−	−	Well	−
60	38	SCC	Ib1	16	3 cm	−	−	+	Poor	++
61	53	SCC	Ib2	16	5 cm	+	+	+	Poor	++
62	42	SCC	IIa	16	2 cm	−	−	−	Well	+
63	47	SCC	IIa	16	4 cm	−	−	−	Mediate	−
64	49	SCC	IIa	16	5 cm	+	−	−	Mediate	++
65	55	SCC	Ib1	16	Erosion	+	−	−	Mediate	+
66	46	SCC	IIa	16	4 cm	−	−	+	Mediate	+
67	53	SCC	IIa	16	5 cm	−	−	+	Well	−
68	63	SCC	Ib2	16, 31	4.8 cm	+	+	−	Poor	+
69	52	SCC	IIa	16	5 cm	+	−	+	Poor	++
70	43	SCC	IIa	16	1.5 cm	−	+	+	Well	−
71	30	SCC	Ib1	16	2.5 cm	−	−	−	Well	−
72	38	SCC	IIa	16	2.5 cm	−	−	+	Well	−
73	45	SCC	IIa	16	Erosion	+	−	+	Mediate	+
74	37	SCC	IIa	16	5 cm	−	−	−	Well	−

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