Int J Clin Exp Pathol 2016;9(2):2044-2048
www.ijcep.com /ISSN:1936-2625/IJCEP0013138

Original Article

Down-regulation of microRNA-143 contributes to unfavorable prognosis for patients with nasopharyngeal carcinoma

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Received July 20, 2015; Accepted August 25, 2015; Epub February 1, 2016; Published February 15, 2016

Abstract: Background: MicroRNAs (miRNAs) can serve as biomarker for prognosis in many cancers. However, little is known about the role of miR-143 for prognosis in nasopharyngeal carcinoma (NPC). Methods: Quantitative real time polymerase chain reaction (qRT-PCR) was used to quantify miR-143 expression in NPC tissues. The relationship of miR-143 expression with clinicopathological parameters of NPC patients was analyzed using Chi-square test. Kaplan-Meier curves were used to estimate the association between miR-143 expression and overall survival. A Cox proportional hazards modeling was performed to identify the prognostic value of miR-143 expression in NPC patients. Results: MiR-143 was significantly down-regulated in NPC clinical tissues ($P < 0.05$). There were significantly relationship between low miR-143 expression and clinicopathological features, such as clinical stages ($P = 0.000$), T classification ($P = 0.002$), N classification ($P = 0.014$), locoregional recurrence ($P = 0.001$), and distant metastasis ($P = 0.032$). In addition, patients with low expression of miR-143 had poorer overall survival than that with high expression ($P = 0.000$). Furthermore, miR-143 was an independent prognostic factor in NPC in multivariate Cox regression analysis ($P = 0.001$, HR: 2.847, 95% CI: 1.513-5.360). Conclusion: Our study presents that miR-143 may serve as an important tumor suppressor and indicates that down-regulated expression of miR-143 in NPC could be used as a potential prognostic marker.

Keywords: MicroRNA-143, nasopharyngeal carcinoma, prognosis

Introduction

Nasopharyngeal carcinoma (NPC) is a non-lymphomatous, squamous cell malignancy arising from the epithelial cells lining of the nasopharynx. Compared to other malignant tumors of the upper aero digestive tract, NPC is a special type of head and neck cancer in terms of epidemiology, pathology and clinical presentation. Although there is a rare occurrence in the majority of countries [1], the incidence and death rates of NPC are markedly high among Southern Chinese populations [2]. Despite of advances made in clinical treatment, the prognosis of NPC patients, especially with advanced disease, is still very poor due to the recurrence and distant metastasis [3, 4]. Therefore, it is necessary to identify novel molecular markers of NPC for facilitating prognosis prediction of NPC patients.

MicroRNAs (miRNAs) are a class of small non-coding RNA molecules with 19-25 nucleotides in length, which negatively regulate gene expression through binding the 3'-untranslated region (3'-UTR) of targeted transcripts, resulting in mRNA cleavage or translation repression [5, 6]. Through regulating their target genes, miRNAs play essential roles in diverse biological processes of various human cancers including cell proliferation, development, differentiation, apoptosis, and others [7]. Recent evidence also indicated that miRNAs may function as tumor suppressors or oncogenes, and play critical roles in carcinogenesis [8, 9]. In particular, previous studies have found the abnormal expression of miRNAs in many types of cancers [10-12], including NPC [13-15]. Sun et al. indicated that miR-143 was significantly down regulated in NPC cell lines and clinical samples [16].

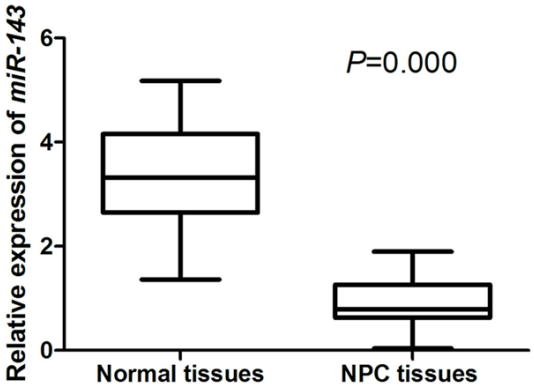

Figure 1. miR-143 expression in 136 NPC tissues 54 normal tissues were respectively detected by qRT-PCR. After normalization to RNU6B, the expression level of miR-143 in NPC tissues was significantly lower than that in normal tissues. Results are expressed as mean ± SD for three replicate determination. All data analyzed using Student's t test.

However, the role of miR-143 for prognosis in NPC is still unknown. Thus, the present study attempted to detect the expression of miR-143 in NPC patients and evaluate the prognostic value of miR-143.

Methods and materials

Patients and specimens

A total of 136 fresh tumor specimens used for qRT-PCR analyses were collected from NPC patients who had undergone biopsies at Affiliated Hospital of Xi'an Medical University. The clinical data of all patients were complete and no patients had received radiotherapy or chemotherapy prior to biopsy. In addition, 54 non-neoplastic nasopharyngeal tissue specimens served as the normal control group, which were all samples of nasopharyngeal chronic mucosal inflammation, with or without the lymphoid hyperplasia. Tissue samples were frozen immediately in liquid nitrogen after resection and stored at -80°C. This study was conducted in accordance with the Declaration of Helsinki and with approval from the ethics committee of the Affiliated Hospital of Xi'an Medical University. Written informed consent was obtained from all participants.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from all the 136 NPC and 54 normal specimens using the Trizol reagent (Invitrogen, Carlsbad, CA, USA). Only those total RNA samples with an OD A260/A280 ratio close to a value of 2.0, which indicates that the RNA is pure, were subsequently analyzed. The *miR-143* and RNU6B internal control-specific cDNAs were synthesized using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems) according to the protocol. The reverse transcription products were then amplified and detected by real-time PCR using a Taqman MicroRNA Assay (Applied Biosystems). Each sample was examined in triplicate, miR-143 expression was normalized with respect to RNU6B. The mean normalized miR-143 expression ± the standard deviation (SD) was calculated from triplicate analyses. The real-time PCR was performed with an ABI 7500 system (Applied Biosystems), and the $2^{-\Delta\Delta ct}$ equation was used to calculate relative expression levels.

Statistical analysis

The difference in the expression of miR-143 between NPC and the normal specimen was analyzed with Student's t test. Associations between miR-143 expression and clinicopathological characteristics were analyzed via Chi-square test. The survival curves were estimated using the Kaplan-Meier method, and the log-rank test was used to analyze the differences between clinicopathological characteristics and survival in NPC patients. A Cox proportional hazards modeling of the factors potentially related to survival was performed to identify those factors that might have a significant influence on survival. Differences with a *P* value of 0.05 or less were considered to be statistically significant.

Results

MiR-143 is downregulated in NPC tissues and associated with clinical pathological factors of NPC patients

We detected *miR-143* expression level in 136 NPC tissues and 54 normal tissues by qRT-PCR. The relative expression of *miR-143* was in NPC tissues and in the normal tissues. As shown in **Figure 1**, after normalization to RNU6B expression levels, the expression level of miR-143 in NPC tissues was significantly lower than that in normal tissues ($P < 0.05$). The data indicated that abnormal miR-143

Table 1. Associations between miR-143 expression and clinical characteristics in patients with NPC

Characteristics	NO. (n = 136)	*miR-143* expression		x^2	*P* values
		Low (n = 86)	High (n = 50)		
Age					
< 45 years	70	40	30	2.303	0.129
≥ 45 years	66	46	20		
Gender					
Male	93	63	30	2.570	0.109
Female	43	23	20		
Histology					
WHO type 1	30	19	11	0.684	0.408
WHO type 2.1 ± 2.2	106	47	39		
Clinical stage					
I-II	41	15	26	17.931	0.000
III-IV	95	71	24		
T classification					
T1-T2	53	25	28	9.641	0.002
T3-T4	83	61	22		
N classification					
N0-N1	81	58	23	6.035	0.014
N2-N3	55	28	27		
Locoregional recurrence					
Yes	21	20	1	10.941	0.001
No	115	66	49		
Distant metastasis					
Yes	16	14	2	4.592	0.032
No	120	72	48		

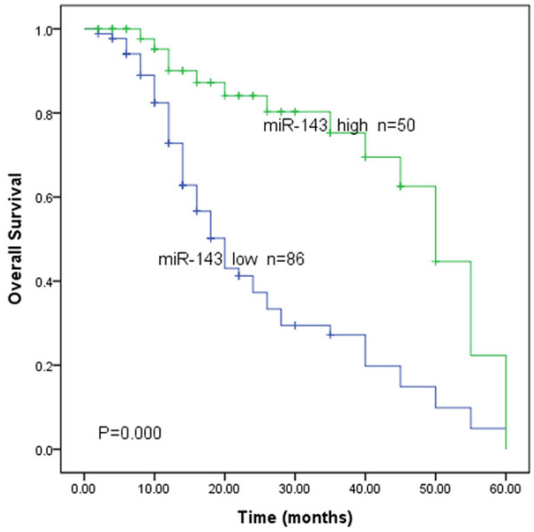

Figure 2. The overall survival of patients with NPC was estimated by Kaplan-Meier survival analysis. Patients with low miR-143 expression have significantly shorter survival times than those with high miR-143 expression (log rank test, P < 0.001).

expression may be related to NPC pathogenesis.

We manually grouped the 136 specimens into two groups according to the miR-143 expression. Relative expression of miR-143 more than belonged to the high miR-143 expression group (n = 50), and the rest were to the low miR-143 expression group (n = 86). The relationship between miR-143 expression and different clinicopathological features was shown in **Table 1**. Down-regulation of *miR-143* was significantly correlated with clinical stages (*P* = 0.000), T classification (*P* = 0.002), N classification (*P* = 0.014), locoregional recurrence (*P* = 0.001), and distant metastasis (*P* = 0.032). There was no significant association between miR-143 expression and other clinicopathological features, such as patient age, gender, and WHO classification (*P* > 0.05, **Table 1**).

Down-regulation of miR-143 is associated with poorer survival in NPC

We investigated the correlation between the miR-143 expression levels and the survival of NPC patients after surgery. The association between miR-143 expression and survival of NPC patients was analyzed based on Kaplan-Meier analysis and log-rank test. The results showed that NPC patients with low miR-143 expression had significantly shorter overall survival than those with high miR-143 expression (log-rank test: P = 0.000, **Figure 2**).

In addition, variables with a value of *P* < 0.05 were selected for multivariate analysis. Multivariate analysis showed that *miR-143* expression was an independent prognostic indicator for overall survival in patients with NPC (*P* = 0.001, HR: 2.847, 95% CI: 1.513-5.360) (**Table 2**).

Discussion

Previous studies have shown miRNAs are associated with patients' prognosis in several human cancers. MicroRNA-143 (miR-143) located at chromosome 5q32, were first identi-

Table 2. Multivariate Cox regression analysis of different prognostic variables in NPC patients

Variables	HR	95% CI	*P* values
MiR-143 expression	2.847	1.513-5.360	0.001
Clinical stage	1.103	0.602-2.022	0.751
T classification	1.136	0.645-2.000	0.660
N classification	1.010	0.623-1.637	0.968
Locoregional recurrence	1.046	0.504-2.174	0.903
Distant metastasis	1.116	0.530-2.349	0.773

fied in mouse [17]. Abnormal expression of miR-143 is observed in several types of tumors [18]. Some studies indicate that miR-143 is up-regulated in tumor tissues [19, 20], such as hepatocellular carcinoma and prostate cancer [21, 22]. However, most of the studies demonstrate that *miR-143* is frequently down-regulated in several types of tumors, such as colon cancer, non small cell lung cancer, cervical squamous cell cancer, endometrial cancers, glioblastomas, etc [17, 18, 23-25]. The potential effects of oncogene or anti-tumor make miRNAs expected to be molecular markers in the diagnosis and prognosis of cancers so that provide new therapy strategies.

Recent studies have demonstrated that miR-143 was significantly down regulated in NPC cell lines and clinical samples based on microRNA expression profiling [16]. In this study, we also found that *miR-143* was down-regulated in NPC tissues compared to in normal tissues by qRT-PCR, which indicated that miR-143 might be a tumor suppressor in NPC. To best of our knowledge, the prognostic value of miR-143 in NPC has never been studied. Then we studied the prognostic value of miR-143 in NPC by statistical analysis. Through Chi-square test, our results further demonstrated that the relative expression level of miR-143 was closely associated with clinical stages, T classification, N classification, locoregional recurrence, and distant metastasis. However, miR-143 expression was not associated with patient's age, gender, and WHO classification. Notably, we found that the down-regulation of miR-143 was significantly associated with worse survival in patients with NPC via Kaplan-Meier methods and log-rank test. Multivariate Cox regression analysis further demonstrated that low expression of miR-143 was an independent prognostic indicator for NPC patients. These results indicated that miR-143 could be a useful prognostic biomarker to stratify NPC patients into different risk groups and further guide the personalized therapy for NPC patients.

In conclusion, this study indicated that miR-143 was down-regulation in NPC tissues, and low expression of miR-143 associated with poorer survival in patients with NPC. In addition, miR-143 was identified as an independent marker for predicting the clinical outcome of NPC patients.

Disclosure of conflict of interest

None.

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References

[1] Lo KW, To KF and Huang DP. Focus on nasopharyngeal carcinoma. Cancer Cell 2004; 5: 423-428.

[2] Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[3] Lai SZ, Li WF, Chen L, Luo W, Chen YY, Liu LZ, Sun Y, Lin AH, Liu MZ and Ma J. How does intensity-modulated radiotherapy versus conventional two-dimensional radiotherapy influence the treatment results in nasopharyngeal carcinoma patients? Int J Radiat Oncol Biol Phys 2011; 80: 661-668.

[4] Siddique MA, Sabur MA, Kundu SC, Mostafa MG, Khan JA, Ahmed S, Karim MA and Hanif MA. Difficulty in diagnosis of nasopharyngeal carcinoma. Mymensingh Med J 2012; 21: 158-161.

[5] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.

[6] Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136: 215-233.

[7] Ambros V. microRNAs: tiny regulators with great potential. Cell 2001; 107: 823-826.

[8] Calin GA and Croce CM. MicroRNA-cancer connection: the beginning of a new tale. Cancer Res 2006; 66: 7390-7394.

[9] He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, Powers S, Cordon-Cardo C, Lowe SW, Hannon GJ and Hammond

SM. A microRNA polycistron as a potential human oncogene. Nature 2005; 435: 828-833.
[10] Calin GA and Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006; 6: 857-866.
[11] Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR and Golub TR. MicroRNA expression profiles classify human cancers. Nature 2005; 435: 834-838.
[12] Liu C, Iqbal J, Teruya-Feldstein J, Shen Y, Dabrowska MJ, Dybkaer K, Lim MS, Piva R, Barreca A, Pellegrino E, Spaccarotella E, Lachel CM, Kucuk C, Jiang CS, Hu X, Bhagavathi S, Greiner TC, Weisenburger DD, Aoun P, Perkins SL, McKeithan TW, Inghirami G and Chan WC. MicroRNA expression profiling identifies molecular signatures associated with anaplastic large cell lymphoma. Blood 2013; 122: 2083-2092.
[13] Chen HC, Chen GH, Chen YH, Liao WL, Liu CY, Chang KP, Chang YS and Chen SJ. MicroRNA deregulation and pathway alterations in nasopharyngeal carcinoma. Br J Cancer 2009; 100: 1002-1011.
[14] Sengupta S, den Boon JA, Chen IH, Newton MA, Stanhope SA, Cheng YJ, Chen CJ, Hildesheim A, Sugden B and Ahlquist P. MicroRNA 29c is down-regulated in nasopharyngeal carcinomas, up-regulating mRNAs encoding extracellular matrix proteins. Proc Natl Acad Sci U S A 2008; 105: 5874-5878.
[15] Liu N, Chen NY, Cui RX, Li WF, Li Y, Wei RR, Zhang MY, Sun Y, Huang BJ, Chen M, He QM, Jiang N, Chen L, Cho WC, Yun JP, Zeng J, Liu LZ, Li L, Guo Y, Wang HY and Ma J. Prognostic value of a microRNA signature in nasopharyngeal carcinoma: a microRNA expression analysis. Lancet Oncol 2012; 13: 633-641.
[16] Xu YF, Li YQ, Guo R, He QM, Ren XY, Tang XR, Jia WH, Kang TB, Zeng MS, Sun Y, Ma J and Liu N. Identification of miR-143 as a tumour suppressor in nasopharyngeal carcinoma based on microRNA expression profiling. Int J Biochem Cell Biol 2015; 61: 120-128.
[17] Wang CJ, Zhou ZG, Wang L, Yang L, Zhou B, Gu J, Chen HY and Sun XF. Clinicopathological significance of microRNA-31, -143 and -145 expression in colorectal cancer. Dis Markers 2009; 26: 27-34.
[18] Chen Y, Ma C, Zhang W, Chen Z and Ma L. Down regulation of miR-143 is related with tumor size, lymph node metastasis and HPV16 infection in cervical squamous cancer. Diagn Pathol 2014; 9: 88.
[19] Szafranska AE, Davison TS, John J, Cannon T, Sipos B, Maghnouj A, Labourier E and Hahn SA. MicroRNA expression alterations are linked to tumorigenesis and non-neoplastic processes in pancreatic ductal adenocarcinoma. Oncogene 2007; 26: 4442-4452.
[20] Akagi I, Miyashita M, Ishibashi O, Mishima T, Kikuchi K, Makino H, Nomura T, Hagiwara N, Uchida E and Takizawa T. Relationship between altered expression levels of MIR21, MIR143, MIR145, and MIR205 and clinicopathologic features of esophageal squamous cell carcinoma. Dis Esophagus 2011; 24: 523-530.
[21] Zhang X, Liu S, Hu T, He Y and Sun S. Up-regulated microRNA-143 transcribed by nuclear factor kappa B enhances hepatocarcinoma metastasis by repressing fibronectin expression. Hepatology 2009; 50: 490-499.
[22] Fan X, Chen X, Deng W, Zhong G, Cai Q and Lin T. Up-regulated microRNA-143 in cancer stem cells differentiation promotes prostate cancer cells metastasis by modulating FNDC3B expression. BMC Cancer 2013; 13: 61.
[23] Gao W, Yu Y, Cao H, Shen H, Li X, Pan S and Shu Y. Deregulated expression of miR-21, miR-143 and miR-181a in non small cell lung cancer is related to clinicopathologic characteristics or patient prognosis. Biomed Pharmacother 2010; 64: 399-408.
[24] Zhang X, Dong Y, Ti H, Zhao J, Wang Y, Li T and Zhang B. Down-regulation of miR-145 and miR-143 might be associated with DNA methyltransferase 3B overexpression and worse prognosis in endometrioid carcinomas. Hum Pathol 2013; 44: 2571-2580.
[25] Lee HK, Bier A, Cazacu S, Finniss S, Xiang C, Twito H, Poisson LM, Mikkelsen T, Slavin S, Jacoby E, Yalon M, Toren A, Rempel SA and Brodie C. MicroRNA-145 is downregulated in glial tumors and regulates glioma cell migration by targeting connective tissue growth factor. PLoS One 2013; 8: e54652.