The Relationship Between miR-106b Expression and Esophageal Squamous Cell Carcinoma (Post-print)

Authors: Xiao Changyan, Guo Zijian, Cao Lei, Ding Xiaowen, Liu Qiang, Wang Xiaochun

Date: 2017-12-21T00:00:00+00:00

Abstract

Objective To investigate the expression of microRNA-106b (mir-106b) in esophageal squamous cell carcinoma (ESCC) tissues and further analyze its correlation with clinicopathological features and prognosis. Methods Tumor tissues and corresponding adjacent non-cancerous tissues were collected from 200 ESCC patients who underwent surgical resection at the Chinese Academy of Medical Sciences between 2001 and 2007. The relative expression level of mir-106b in tissues was detected by qRT-PCR and further validated by Northern blot. Statistical methods were applied to analyze the correlation between mir-106b expression and clinicopathological features and prognosis. Results Compared with adjacent non-cancerous tissues, the expression level of mir-106b was significantly elevated in ESCC tumor tissues. Statistical data showed that mir-106b expression was correlated with lymph node metastasis, tumor stage, and smoking ($P<0.05$). Moreover, the median survival time of patients with low mir-106b expression (60 months) was significantly higher than that of patients with high mir-106b expression (37 months, $P=0.024$). Additionally, Cox regression analysis revealed that mir-106b expression, regional lymph node metastasis, and smoking were all independent prognostic factors for ESCC patients ($P<0.05$). Conclusion mir-106b is highly expressed in tumor tissues of ESCC patients and is closely associated with lymph node metastasis and poor prognosis, which can be used as a diagnostic and prognostic biomarker for ESCC.

Full Text

Preamble

DOI: 10.3969/j.issn.1673-4254.2016.12.13
Journal: J South Med Univ, 2016, 36(12): 1667-1671

Authors: Xiao Changyan[1], Guo Zijian[3], Cao Lei[2], Ding Xiaowen[2], Liu Qiang[1], Wang Xiaochun[2]

Affiliations:
[1]Chamber of Radiation Damage Effect, Institute of Radiation Medicine, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin 300192, China
[2]Department of Radiation Protection, Beijing Chemical Occupational Disease Prevention and Treatment Hospital, Beijing 100093, China
[3]Department of Interventional Therapy, First Affiliated Hospital of Guangxi University of Chinese Medicine, Nanning 530023, China

---

Abstract

Objective: To investigate the expression of microRNA-106b (mir-106b) in esophageal squamous cell carcinoma (ESCC) tissues and analyze its correlation with clinicopathological features and prognosis.

Methods: Tumor tissues and corresponding adjacent non-cancerous tissues were collected from 200 ESCC patients who underwent surgical resection at the Chinese Academy of Medical Sciences between 2001 and 2007. The relative expression level of mir-106b was detected using quantitative real-time PCR (qRT-PCR) and further validated by Northern blot analysis. Statistical methods were applied to analyze the relationship between mir-106b expression and clinicopathological characteristics and prognosis.

Results: Mir-106b expression was significantly upregulated in ESCC tumor tissues compared with adjacent non-cancerous tissues. Statistical analysis revealed that mir-106b expression correlated significantly with lymph node metastasis, tumor stage, and smoking status ($P<0.05$). Moreover, patients with low mir-106b expression had significantly higher 5-year survival rates (60 months) compared with those with high expression (37 months, $P=0.024$). Cox regression analysis demonstrated that mir-106b expression, regional lymph node metastasis, and smoking were independent prognostic factors for ESCC patients ($P<0.05$).

Conclusion: Mir-106b is overexpressed in ESCC tissues and closely associated with lymph node metastasis and poor prognosis, suggesting its potential as a diagnostic and prognostic biomarker for ESCC.

Keywords: mir-106b; overexpression; prognosis; esophageal squamous cell carcinoma

Introduction

Esophageal cancer is a common malignant tumor, ranking 7th in incidence and 6th in mortality worldwide [1-2]. The disease comprises two major histological subtypes: esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) [3]. In Asia and China, ESCC accounts for approximately 90% of esophageal cancer cases and represents one of the most aggressive gastrointestinal malignancies with poor prognosis [4]. Despite significant advances in clinical treatment, the 5-year survival rate for ESCC patients remains low (<30%) due to delayed diagnosis and high recurrence rates [1]. Postoperative recurrence and metastasis are the leading causes of mortality in ESCC patients, primarily resulting from activation of oncogenes, inactivation of tumor suppressor genes, and abnormal expression of various proteins [5]. Therefore, effective biomarkers for early diagnosis and prognostic evaluation are urgently needed for clinical prevention and treatment of esophageal cancer.

MicroRNA expression differs significantly between tumor cells and adjacent normal cells, and microRNA expression profiles can better classify poorly differentiated tumors compared with microRNA expression sequence tag (EST) classification [6]. Consequently, increasing numbers of studies have reported abnormal microRNA expression in tumor tissues. By investigating minimal regions of amplification, loss of heterozygosity (LOH), or breakpoint cluster regions where microRNA-encoding genes may be located, researchers can explore the potential roles of microRNAs in tumorigenesis. Amplification or overexpression of oncogenic microRNAs can suppress or eliminate expression of tumor suppressor genes targeted by these microRNAs, ultimately leading to carcinogenesis [7]. Given these characteristics, microRNAs can serve as effective biomarkers for tumor diagnosis and monitoring.

Previous studies have identified mir-106b as a member of the mir-106b-25 cluster that is highly expressed in various tumors, including laryngeal cancer [8], colorectal cancer [9], gastric cancer [10], hepatocellular carcinoma [11], glioma [12], renal cancer [13], breast cancer [14], and head and neck squamous cell carcinoma [15]. Mir-106b regulates critical cellular functions such as proliferation, invasion, migration, and transformation. For instance, Yau et al. [16] demonstrated that mir-106b promotes tumor invasion and metastasis by facilitating epithelial-mesenchymal transition (EMT). Additionally, Dai et al. [17] showed that mir-106b promotes proliferation, migration, invasion, and EMT in ESCC KYSE150 cells and identified its significant upregulation in ESCC tissues via microarray analysis. However, research on the relationship between mir-106b and ESCC remains limited. To further investigate its role in ESCC, our research group conducted an in-depth study using qRT-PCR and Northern blot

techniques on a large sample of ESCC specimens.

Materials and Methods

1.1 Materials

Fresh tissue specimens were obtained from ESCC patients who underwent surgical resection at the Chinese Academy of Medical Sciences between 2001 and 2007. Primary tumor tissues and corresponding adjacent non-cancerous tissues were isolated from the same patients by experienced pathologists and immediately stored. All patients had not received any preoperative treatment, had no other medical history, and provided informed consent for sample collection. The use of patient tumor and adjacent tissue samples was approved by the PUMC/CAMS Ethics Committee (No. 12-097/631).

1.2 Methods

1.2.1 Total RNA Extraction Tissue specimens were processed by retrieving ESCC tumor tissues and corresponding adjacent tissues, thawing them on ice, and cutting pea-sized tissue pieces into 1.5 mL centrifuge tubes. One milliliter of RNAiso Plus was added to each tube, and tissues were homogenized using an electric grinder. Total RNA was then extracted from ESCC and adjacent tissues using the Stratagene RNA extraction kit according to the manufacturer' s protocol. RNA quantity was measured using a Beckman Coulter DUVR 800 UV/Vis spectrophotometer (Beckman Coulter, Fullerton, CA), requiring A260/A280 ratios between 1.8-2.0. Samples were immediately reverse-transcribed or stored at -80°C.

1.2.2 Reverse Transcription PCR (RT-PCR) Mir-106b expression was detected using the TaqMan MicroRNA Assay (ABI PRISM) with a stem-loop method [18], using RNU6B as the internal reference gene. For reverse transcription, 10 ng of total RNA was mixed with reverse transcription primers per reaction. Reverse transcription conditions were: 16°C for 30 minutes, followed by 42°C for 30 minutes, 85°C for 5 minutes, and final maintenance at 4°C. For PCR amplification, 1.5 L of the resulting cDNA was mixed with 2 L of TaqMan primers and analyzed using the ABI 7500 Real-Time PCR System. PCR parameters were: 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. Results were analyzed using the comparative Ct method, with mir-106b expression presented relative to the quantification cycle (Cq) values. Reverse transcription and PCR primers for mir-106b were purchased from ABI PRISM. Relative expression was calculated using the 2^-ΔΔCt method, where -ΔΔCt = [(Ct_target - Ct_reference)_experimental_group - (Ct_target - Ct_reference)_control_group].

1.2.3 MicroRNA Northern Blot Total RNA samples were separated by denaturing polyacrylamide gel electrophoresis and transferred to membranes (PerkinElmer). Hybridization was performed using Ambion' s UltraHyb-Oligo buffer. Oligonucleotides complementary to mature mir-106b, end-labeled with T4 kinase (Invitrogen), were used as probes. Membranes were hybridized overnight at 42°C, then washed twice with 0.1×SSPE and 0.1% SDS for 15 minutes each. Membranes were placed on storage phosphor screens (GE Healthcare) for 8 hours and imaged using a Typhoon 9410 multimodal imager (GE Healthcare). Images were saved and cropped using Photoshop CS6 software.

1.2.4 Statistical Analysis ESCC clinicopathological parameters were classified according to the 6th edition of the AJCC Cancer Staging Manual. SPSS 16.0 software was used to evaluate differences in mir-106b expression between tumor and adjacent tissues and associations with pathological parameters. Data were verified to be non-normally distributed, so Mann-Whitney U test and Kruskal-Wallis test were used for two-group and multi-group comparisons, respectively. Survival curves were calculated using the Kaplan-Meier method. Multivariate Cox proportional hazards regression analysis was performed to evaluate relative risk of mir-106b expression and clinicopathological parameters. Measurement data are presented as mean ± standard deviation. Mir-106b expression in tumor tissues below the mean was defined as low expression, while expression equal to or above the mean was defined as high expression. $P<0.05$ was considered statistically significant.

Results

2.1 Mir-106b Expression in ESCC Tissues

Using RT-qPCR with RNU6B as an internal reference, we detected mir-106b expression levels in tumor and adjacent tissues from 200 ESCC patients. The results demonstrated that mir-106b expression was significantly elevated in ESCC tumor tissues compared with adjacent non-cancerous tissues. Agarose gel electrophoresis of RT-qPCR products further confirmed this finding [Figure 1: see original paper]A, B.

To further validate these results, Northern blot analysis was performed on four pairs of samples using 5S rRNA as an internal reference. Consistent with RT-qPCR findings, mir-106b expression was also elevated in ESCC tumor tissues compared with adjacent tissues [Figure 1: see original paper]C.

2.2 Relationship Between Mir-106b Expression and Clinicopathological Features

2.2.1 Mann-Whitney Analysis Compared with adjacent tissues, mir-106b expression in ESCC tumor tissues showed significant correlations with smoking status, lymph node metastasis, and age (all $P<0.050$) .

2.2.2 Kruskal-Wallis Analysis Mir-106b expression in ESCC tumor tissues was significantly associated with clinical stage ($P=0.005$), but showed no significant correlation with T-stage or tumor differentiation grade (both $P>0.05$).

2.2.3 Kaplan-Meier Survival Analysis To verify the relationship between mir-106b expression and poor prognosis, Kaplan-Meier analysis was used to calculate survival rates of ESCC patients. Significant differences in survival were observed based on mir-106b expression levels in tumor tissues. Patients with low mir-106b expression had higher survival rates (60 months) compared with those with high expression (37 months) ($P=0.024$) [Figure 2: see original paper].

Multivariate Cox proportional hazards regression analysis revealed relative risk (RR) values of 4.375, 3.273, and 3.125 for mir-106b expression, smoking, and lymph node metastasis, respectively, indicating a decreasing impact on mortality. Mir-106b expression ($P=0.017$), smoking ($P=0.019$), and lymph node metastasis ($P=0.021$) were identified as independent prognostic factors affecting survival duration in ESCC patients . However, tumor stage showed no statistically significant relationship with survival duration.

Discussion

Mir-106b is a member of the mir-106b-25 cluster located on human chromosome 7q22. It regulates gene expression by binding to the 3' -untranslated region of target mRNAs, thereby inducing mRNA degradation or inhibiting translation [19]. Mir-106b shares high homology with mir-106a in the mir-17 family; while mir-106a functions as a tumor suppressor and is downregulated in ESCC [20], mir-106b exhibits opposite expression patterns and functions. MicroRNA sequencing studies have demonstrated that mir-106b is highly expressed in various tumors and acts as an oncogene associated with tumor growth, cell survival, and angiogenesis [10], attracting considerable research attention.

Numerous studies have confirmed elevated mir-106b expression across multiple cancer types. Liu et al. [12] found that mir-106b-5p overexpression in glioma cells significantly promoted proliferation and inhibited apoptosis. Mechanistic studies revealed that mir-106b-5p enhanced proliferation by targeting tumor suppressor genes RBL1 and RBL2, while inhibiting apoptosis by downregulating caspase-8. Ying et al. [8] observed high mir-106b expression in laryngeal

carcinoma, which promoted cell proliferation and invasion by suppressing tumor suppressor genes RUNX3 and Rb. Urinary cell-free mir-106b expression correlated with tumor stage in bladder cancer patients [21]. Similarly, mir-106b expression was significantly elevated in renal cell carcinoma tissues and cell lines, with correlation analysis indicating that its expression level was associated only with histological type, being significantly higher in clear cell renal carcinoma than in other types, suggesting its importance for pathological classification and prognostic assessment [22]. Additionally, mir-106b is commonly overexpressed in colorectal cancer [9], gastric cancer [10], hepatocellular carcinoma [11], breast cancer [14], and head and neck squamous cell carcinoma [15]. Plasma mir-106b expression has been linked to patient prognosis, with higher expression indicating greater risk of cancer recurrence in prostate cancer patients [23] and increased risk of systemic metastasis and poor prognosis in osteosarcoma patients [24].

Extensive research has also demonstrated mir-106b involvement in multiple critical signaling pathways during tumorigenesis and progression. For example, in the transforming growth factor- (TGF-) pathway, mir-106b promotes tumor cell proliferation, migration, invasion, apoptosis, and cell cycle progression by downregulating p21 [25] or upregulating EMT-related signaling pathways [23], thereby enabling tumor cells to escape TGF- -induced growth inhibition. Poliseno et al. [26] identified mir-106b as part of the insulin/IGF pathway, affecting cell cycle and tumor microenvironment by downregulating PTEN expression. In gastric cancer, mir-106b promoted cancer cell migration and invasion by regulating PTEN [27]. Smith et al. [28] found that mir-106b activated the TGF- signaling pathway and promoted EMT in breast cancer by targeting Smad7. Recently, Dai et al. [17] reported that mir-106b was also highly expressed in ESCC and promoted proliferation, invasion, migration, and EMT in KYSE150 cells. Based on these findings, our study expanded the sample size to analyze 200 ESCC specimens to investigate the relationship between mir-106b expression and clinicopathological features. Statistical analysis demonstrated that mir-106b overexpression significantly correlated with lymph node metastasis, smoking, and age, consistent with previous studies.

In summary, our study confirmed that mir-106b is highly expressed in ESCC tumor tissues and, for the first time, identified that mir-106b expression significantly correlates with lymph node metastasis, smoking, and poor prognosis. Furthermore, mir-106b expression serves as an independent prognostic factor for ESCC. Combined with previous research findings, mir-106b shows promise as a novel diagnostic and prognostic biomarker for ESCC and a potential target for future personalized interventional therapy. However, given the limited current research on the relationship between mir-106b and ESCC, extensive follow-up studies are required before clinical application.

References

[1] Enzinger PC, Mayer RJ. Esophageal cancer [J]. N Engl J Med, 2003, 349(23): 2241-2252.

[2] Kamangar F, Dores GM, Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world [J]. J Clin Oncol, 2006, 24(14): 2137-2150.

[3] Rustgi AK, El-Serag HB. Esophageal carcinoma [J]. N Engl J Med, 2014, 371(26): 2499-2509.

[4] Song Y, Li L, Ou Y, et al. Identification of genomic alterations in esophageal squamous cell cancer [J]. Nature, 2014, 509(7498): 91-95.

[5] Li S, Su H, Zhao D, et al. Plasma miRNA-506 as a prognostic biomarker for esophageal squamous cell carcinoma [J]. Med Sci Monit, 2016, 22: 2195-2201.

[6] Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers [J]. Nature, 2005, 435(7043): 834-838.

[7] Calin GA, Liu CG, Sevignani C, et al. MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias [J]. Proc Natl Acad Sci USA, 2004, 101(32): 11755-11760.

[8] Xu Y, Wang K, Gao W, et al. MicroRNA-106b regulates the tumor suppressor RUNX3 in laryngeal carcinoma cells [J]. FEBS Lett, 2013, 587(19): 3166-3174.

[9] Wang X, Lang F, Liu X, et al. In situ hybridization analysis of the expression of miR-106b in colonic cancer [J]. Int J Clin Exp Pathol, 2015, 8(1): 786-792.

[10] Li F, Liu J, Li S. MicroRNA 106b-25 cluster and gastric cancer [J]. Surg Oncol, 2013, 22(2): e7-e10.

[11] Li Y, Tan W, Neo TW, et al. Role of the miR-106b-25 microRNA cluster in hepatocellular carcinoma [J]. Cancer Sci, 2009, 100(7): 1234-1242.

[12] Liu F, Gong J, Huang W, et al. MicroRNA-106b-5p boosts glioma tumorigenesis by targeting multiple tumor suppressor genes [J]. Oncogene, 2014, 33(40): 4813-4822.

[13] Slaby O, Jancovicova J, Lakomy R, et al. Expression of miRNA-106b in conventional renal cell carcinoma is a potential marker for prediction of early metastasis after nephrectomy [J]. J Exp Clin Cancer Res, 2010, 29: 90.

[14] Zhou Y, Hu Y, Yang M, et al. The miR-106b-25 cluster promotes bypass of doxorubicin-induced senescence and increase in motility and invasion by targeting the E-cadherin transcriptional activator EP300 [J]. Cell Death Differ, 2014, 21(3): 462-474.

[15] Hui AB, Lenarduzzi M, Krushel T, et al. Comprehensive microRNA profiling for head and neck squamous cell carcinomas [J]. Clin Cancer Res, 2010,

16(4): 1129-1139.

[16] Yau WL, Lam CS, Ng L, et al. Over-expression of miR-106b promotes cell migration and metastasis in hepatocellular carcinoma by activating epithelial-mesenchymal transition process [J]. PLoS One, 2013, 8(3): e57882.

[17] Dai F, Liu T, Zheng S, et al. Effects of miR-106b on proliferation and epithelial-mesenchymal transition in esophageal squamous carcinoma KYSE150 cells [J]. J Xinjiang Med Univ, 2015, 38(12): 1528-1533.

[18] Schmittgen TD, Lee EJ, Jiang J. High-throughput real-time PCR [J]. Methods Mol Biol, 2008, 429: 89-98.

[19] Maher ER. Genomics and epigenomics of renal cell carcinoma [J]. Semin Cancer Biol, 2013, 23(1): 10-17.

[20] Yuan J, Mao M, Luo W, et al. Impact of miRNA-106a expression level on prognosis of esophageal squamous cell carcinoma patients [J]. Chin J Oncol, 2013, 35(8): 590-594.

[21] Zhou X, Zhang X, Yang Y, et al. Urinary cell-free microRNA-106b as a novel biomarker for detection of bladder cancer [J]. Med Oncol, 2014, 31(10): 197.

[22] Tao L, Li Y, Jin L, et al. Expression and clinical significance of MicroRNA-106b in renal cell carcinoma [J]. J Clin Urol, 2015, 30(10): 888-892.

[23] Hudson RS, Yi M, Esposito D, et al. MicroRNA-106b-25 cluster expression is associated with early disease recurrence and targets caspase-7 and focal adhesion in human prostate cancer [J]. Oncogene, 2013, 32(35): 4139-4147.

[24] Arabi L, Gsponer JR, Smida J, et al. Upregulation of the miR-17-92 cluster and its two paralogs in osteosarcoma-reasons and consequences [J]. Genes Cancer, 2014, 5(1-2): 56-63.

[25] Zhao Z, Bai J, Zhou Q, et al. TSA suppresses miR-106b-93-25 cluster expression through downregulation of MYC and inhibits proliferation and induces apoptosis in human EMC [J]. PLoS One, 2012, 7(9): e45133.

[26] Poliseno L, Salmena L, Riccardi L, et al. Identification of the miR-106b-25 microRNA cluster as a proto-oncogenic PTEN-targeting intron that cooperates with its host gene MCM7 in transformation [J]. Sci Signal, 2010, 3(117): ra29.

[27] Yang S, Yang H, Chen X, et al. MicroRNA-106b in cancer-associated fibroblasts from gastric cancer promotes cell migration and invasion by targeting PTEN [J]. FEBS Lett, 2014, 588(13): 2196-2207.

[28] Smith AL, Iwanaga R, Drasin DJ, et al. The miR-106b-25 cluster targets Smad7, activates TGF- signaling, and induces EMT and tumor initiating cell characteristics downstream of Six1 in human breast cancer [J]. Oncogene, 2012, 31(50): 5162-5171.