Downregulation of miR-138 Sustains NF-κB Activation and Promotes Lipid Raft Formation in Esophageal Squamous Cell Carcinoma

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Abstract

Purpose: Constitutive activation of NF-κB signaling plays vital roles in esophageal squamous cell carcinoma (ESCC) progression. The aim of this study was to evaluate the effect of miR-138 on NF-κB activation and ESCC progression.

Experimental Design: Expression of miR-138 in ESCC cell lines, ESCC tissues, and 205 archived ESSC specimens was determined using real-time PCR analysis. Anchorage-independent growth, chicken chorioallantoic membrane, Transwell matrix invasion and Annexin V–binding assays, and a xenograft tumor model were used to determine the role of miR-138 in ESCC progression. The effect of miR-138 on NF-κB activation was investigated using IKK in vitro kinase, electrophoretic mobility shift, lipid raft isolation, and luciferase reporter assays.

Results: miR-138 was downregulated and inversely correlated with tumor progression and patient survival in ESCCs. Downregulation of miR-138 enhanced, whereas upregulation of miR-138 reduced, the aggressive phenotype of ESCC cells both in vitro and in vivo. Silencing miR-138 promoted K63-linked polyubiquitination of the NF-κB signaling intermediaries TRAF2 and RIP1 and sustained NF-κB activation. Furthermore, downregulation of miR-138 induced lipid raft formation via upregulating multiple components of lipid rafts, including FLOT1, FLOT2, and caveolin-1. Importantly, the in vitro analysis was consistent with a significant inverse correlation between miR-138 expression and NF-κB hyperactivation in a cohort of human ESCC specimens.

Conclusion: Our results show that miR-138 functions as a tumor-suppressive miRNA and that downregulation of miR-138 contributes to constitutive NF-κB activation and ESCC progression. Clin Cancer Res; 19(5); 1083–93. ©2013 AACR.

Introduction

Esophageal squamous cell carcinoma (ESCC), one of the most aggressive malignancies of the gastrointestinal tract, is the sixth most common cause of cancer-related deaths worldwide (1, 2). Numerous studies have revealed that constitutive activation of NF-κB signaling plays vital roles in the development and progression of ESCCs, and blockade of the NF-κB pathway can inhibit ESCC proliferation, sensitize ESCCs to chemotherapeutic drugs and suppress angiogenesis and metastasis in ESCCs (3, 4). However, the precise molecular mechanisms which regulate the NF-κB pathway in ESCCs are still not completely understood.

Lipid rafts are defined as small (10–200 nm), highly dynamic liquid-ordered domains that are enriched with cholesterol and glycosphingolipids and are physically characterized by their insolubility in Triton X-100 at 4°C (5, 6). Functioning as a physical platform, lipid rafts have been reported to be essential for NF-κB signaling transduction via inducing signaling molecules in close proximity (7–11). In response to TNF-α, TNF receptor (TNFR) translocates to lipid rafts and forms a signaling complex through association with the adaptor proteins, TRADD, TRAF2, and RIP1. Meanwhile, TRAF2 and RIP1 in lipid rafts are rapidly K63-polyubiquitylated, which facilitates the recruitment and activation of TAK1 and IKK complexes, resulting in phosphorylation and ubiquitin–proteasomal degradation of IκBs and consequently leading to NF-κB activation. It is of note that interfering with lipid raft composition not only inhibits TNF-α–mediated NF-κB activation but also provokes TNF-α-induced apoptosis, which indicates that lipid raft formation is associated with the outcomes of TNF-α.

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**Translational Relevance**

Numerous studies have shown that constitutive activation of NF-κB signaling plays vital roles in the development and progression of esophageal squamous cell carcinoma (ESCC). Functioning as physical platform, lipid rafts have been reported to be essential for NF-κB signaling transduction via inducing signaling molecules in close proximity. Herein, we found that downregulation of miR-138 enhanced, whereas upregulation of miR-138 reduced, the aggressive phenotype of ESCC cells both in vitro and in vivo. Silencing of miR-138 promoted K63-linked polyubiquitination of the NF-κB signaling intermediaries TRAF2 and RIP1 and sustained NF-κB activation. Furthermore, downregulation of miR-138 induced lipid raft formation via upregulation of multiple constitutive components of lipid rafts, including FLOT1, FLOT2, and caveolin-1. Therefore, our study highlights an important tumor-suppressive role for miR-138 in ESCC progression; miR-138 may possibly be used as a novel prognostic marker and/or as an effective therapeutic target for ESCCs.

**Vectors, retroviral infection, and transfection**

The human miR-138 gene was PCR-amplified from gDNA and cloned into a pMSCV-puro retroviral vector. The miR-138 anti-sense was cloned into miRZip plasmid purchased from System Biosciences and used according to previous report (24). The 3'-untranslated regions (3'-UTR) of human FLOT1, FLOT2, and caveolin-1, generated by PCR amplification from gDNA isolated from NEECs, were cloned into the pGL3- luciferase reporter plasmid (Promega.). The point mutations in the tentative miR-138–binding seed regions were created using the Stratagene QuickChange Mutagenesis Kit (Stratagene). pNF-κB-luc and control plasmids (Clontech) were used to examine NF-κB activity. pBabe-Puro-IκBα-mut (plasmid 15291) expressing IκBα dominant-negative mutant (IκBα-mut) was purchased from Addgene. Transfection of siRNA or plasmids was conducted using the Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer’s instruction. Stable cell lines expressing miR-138 or miR-Zip-138 were generated via retroviral infection using HEK293T cells and selected with 0.5 μg/mL puromycin for 10 days.

**Xenografted tumor model, immunohistochemistry, and hematoxylin and eosin staining**

BALB/c-nu mice (4–5 weeks of age, 18–20 g) were purchased from the Center of Experimental Animal of Guangzhou University of Chinese Medicine (Guangzhou, China). All experimental procedures were approved by the Institutional Animal Care and Use Committee of Sun Yat-sen University. The BALB/c nude mice were randomly divided into 2 groups (n = 6/group). One group of mice was inoculated subcutaneously with Kyse30/Vector

**Materials and Methods**

**Cells**

Primary cultures of normal esophageal epithelial cells (NEEC) were established from fresh specimens of the adjacent noncancerous esophageal tissue, which is over 5 cm from the cancerous tissue, according to previous report (22). The esophageal cancer cell lines EC18, Eca109, and HKES1 were kindly provided by Professors S.W. Tsao and G. Srivastava (The University of Hong Kong, Hong Kong). ESCC cell lines Kyse30, Kyse140, Kyse180, Kyse410, Kyse510, and Kyse520 were obtained from DSMZ, the German Resource Centre for Biological Material (23). All cell lines were authenticated by short tandem repeat (STR) fingerprinting at Medicine Lab of Forensic Medicine Department of Sun Yat-sen University (Guangzhou, China).

**Patient information and tissue specimens**

This study was conducted on a total of 205 archived ESCC samples, which were histopathologically and clinically diagnosed at the Sun Yat-sen University Cancer Center from 2001 to 2006. For the use of these clinical materials for research purposes, prior patient consent and approval from the Institutional Research Ethics Committee were obtained. Clinical information on the samples is summarized in Supplementary Table S1. Twenty ESCC tissues and the matched adjacent noncancerous esophageal tissues were frozen and stored in liquid nitrogen until further use.
cells \( (5 \times 10^6) \) in the left dorsal flank and with Kyse30/miR-138 cells \( (5 \times 10^6) \) in the right dorsal flank per mouse. Another group was inoculated subcutaneously with Kyse30/miRZip-Vector cells \( (5 \times 10^6) \) in the left dorsal flank and with Kyse30/miRZip-138 cells \( (5 \times 10^6) \) in the right dorsal flank. Tumor volume was calculated using the equation \( (L \times W^2)/2 \). On day 43, tumors were detected by an IVIS imagining system (Caliper), then animals were euthanized, tumors were excised, weighed, and paraffin-embedded. Serial 6.0-μm sections were cut and subjected to immunohistochemistry (IHC) analyzed using an anti-Ki67 and anti-CD31 antibodies (Dako) or hematoxulin and eosin (H&E) stained with Mayer’s hematoxylin solution. Proliferation index was quantized by counting proportion of Ki67-positive cells. Apoptotic index was measured by percentage of terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL)-positive cells. Macrophages index was measured by assessing the percentage of F4/80-positive cells.

Lipid raft isolation

Lipid rafts were isolated by sucrose density gradient centrifugation essentially as described (25). In brief, subconfluent cells from two 15-cm culture dishes were lysed on ice for 20 minutes in 1 mL of MNX buffer (1% Triton X-100 in 25 mmol/L MES, 150 mmol/L NaCl, pH 6.5, 1 mmol/L Na3VO4) supplemented with 10 μg/mL benzamidine, 2 μg/mL antipain, and 1 μg/mL leupeptin and homogenized with a loose-fitting glass dounce homogenizer (Polylabo). The homogenates were mixed with equal amount 85% sucrose made with MNX buffer and placed on the bottom of a centrifuge tube. The samples were then overlaid with 6 mL of 35% sucrose and 3 mL of 5% sucrose and centrifuged at 200,000 \( \times g \) in a Hitachi CP80WX centrifuge (Hitachi) for 20 hours at 4°C. One milliliter fractions were collected from the top of the gradient and analyzed by Western blotting. The pellet present at the bottom of the gradient was sonicated in 1 mL of MNX.

Statistical analysis

Statistical tests for data analysis included Fisher exact test, log-rank test, \( \chi^2 \) test, and the Student 2-tailed t test. Multivariate statistical analysis was conducted using a Cox regression model. Statistical analyses were conducted using the SPSS 11.0 statistical software package. Data represent mean ± SD. \( P < 0.05 \) was considered statistically significant.

Results

Reduced expression of miR-138 correlates with ESCC progression and poor prognosis

By analyzing published miRNA expression profiles obtained from 153 primary ESCC tissues and 104 normal esophageal tissues (NCBI/GEO/GSE6188), miR-138 was identified to be significantly downregulated in ESCC tissues compared with normal tissues (Fig. 1A). Furthermore, real-time PCR analysis revealed that miR-138 was downregulated in all 9 tested ESCC cell lines compared with 2 NEECs and in 20 ESCC samples compared with the matched adjacent nontumor tissues (Fig. 1B and C). Collectively, these results indicate that miR-138 expression is reduced in ESCCs.

To evaluate whether downregulation of miR-138 correlates with clinical ESCC progression, we examined miR-138 expression in 205 archived human ESCC specimens. Statistical analyses revealed that miR-138 expression inversely correlated with the clinical stage \((P < 0.001)\), tumor–node–metastasis (TNM) classification \((P < 0.001)\), and histologic differentiation \((P = 0.024)\) in patients with ESCCs (Supplementary Fig. S1 and Supplementary Table S2). Importantly, patients with lower miR-138 expression had a shorter survival time, whereas patients with higher miR-138 expression had a longer survival time \((P < 0.001);\) Fig. 1D). Univariate and multivariate analyses revealed that clinical stage and miR-138 expression were each recognized as independent prognostic factors in ESCCs (Supplementary Table S3). Taken together, these results suggest a possible link between reduced expression of miR-138 and the progression of human ESCCs.

Downregulation of miR-138 promotes the aggressiveness of ESCC cells in vitro

To investigate the biologic role of miR-138 downregulation in ESCC progression, we established miR-138 knockdown Kyse30 and Kyse410 ESCC cell lines (Supplementary Fig. 2A). We found that silencing miR-138 augmented the anchorage-independent growth ability and the invasive ability of ESCC cells, provoked their ability to induce human umbilical vein endothelial cell (HUVEC) tube formation, and chicken chorioallantoic membrane (CAM) neovascularization, and enhanced their resistance to apoptosis when treated with the chemotherapeutic reagent cisplatin (Supplementary Fig. S2B–S2F). In contrast, overexpression of miR-138 in Kyse30 and Kyse510 ESCC cells dramatically inhibited the anchorage-independent growth ability and invasive ability of ESCCs, decreased the ability of ESCCs to induce HUVEC tube formation and CAM neovascularization, and enhanced the sensitivity of ESCCs to cisplatin (Supplementary Fig. S3A–S3F). These results suggest that downregulation of miR-138 promotes ESCC aggressiveness.

Downregulation of miR-138 contributes to ESCC progression in vivo

The biologic effect of miR-138 reduction on ESCC progression was further examined using an in vivo tumor
As shown in Fig. 2A–C, the tumors formed by miR-138–silenced cells were larger, in both size and weight, than the tumors formed by control cells. Conversely, the tumors formed by miR-138–transduced ESCC cells were smaller and had lower tumor weights than control tumors. IHC analysis revealed that miR-138–silenced tumors showed increased percentages of Ki67-positive cells, microvascular density (MVD) and decreased TUNEL-positive cells, whereas miR-138–overexpressing tumors displayed lower Ki67 proliferation index and MVD and a higher percentage of TUNEL-positive apoptotic cells (Fig. 2D). Taken together, our results show that miR-138 downregulation contributes to ESCC progression in vivo.

Figure 1. Expression of miR-138 inversely correlates with ESCC progression and poor prognosis. A, expression profiling of miRNAs in 153 primary ESCC tissues and 104 normal esophageal tissues (NCBI/GEO/GSE6188). B and C, real-time PCR analysis of miR-138 expression in 2 NEECs and 9 ESCC cell lines (B) as well as 20 paired ESCC samples (T) and tumor-adjacent tissues (TAT; C). Transcript levels were normalized to U6 expression. Each bar represents the mean ± SD of 3 independent experiments. * P < 0.05. D, Kaplan–Meier curves of patients with ESCCs with low versus high expression of miR-138 (n = 205; P < 0.001, log-rank test).

miR-138 downregulation sustains NF-κB activity

Notably, we observed that the number of F4/80-positive cells, the infiltrating macrophages, was significantly increased in miR-138–downregulated tumors and reduced...
in miR-138–overexpressing tumors compared with the respective control tumors (Fig. 2D), suggesting that miR-138 might play a role in the regulation of NF-κB signaling. Indeed, downregulation of miR-138 significantly enhanced, whereas overexpression of miR-138 inhibited, the aggressive phenotype of ESCCs in vivo, as indicated by the percentages of Ki67−, CD31−, TUNEL−, and F4/80-positive cells. Each bar represents the mean ± SD of 3 independent experiments. *, P < 0.05.

Figure 2. Downregulation of miR-138 contributes to ESCC progression in vivo. A–C, xenograft model in nude mice. The miR-138/Kyse30, miRZip-138/Kyse30, and control/Kyse30 ESCCs were injected into the dorsal flank of the mice (n = 6/group). A, representative images of tumor-bearing mice (left) and images of the tumors from all of the mice in each group (right). B, tumor volumes were measured on the indicated days. C, mean tumor weights. D, H&E and IHC staining displayed that downregulation of miR-138 induced, whereas overexpression of miR-138 inhibited, the aggressive phenotype of ESCCs in vivo, as indicated by the percentages of Ki67−, CD31−, TUNEL−, and F4/80-positive cells. Each bar represents the mean ± SD of 3 independent experiments. *, P < 0.05.

miR-138 Functions as a Tumor Suppressor
overexpression of miR-138 restrained, the decreased IκBα level induced by TNF-α treatment (Supplementary Fig. S6). Comparable results were also obtained using an in vitro kinase assay, as chronic activation of the endogenous IKK kinase complex was persistent in miR-138-silenced cells but rapidly decreased in miR-138-transduced cells (Fig. 3D). These results suggest that silencing miR-138 sustains NF-κB activation in ESCCs.

Lipid rafts contribute to miR-138 downregulation-mediated NF-κB activation

The levels of K63 polyubiquitination of TRAF2 and RIP1 were dramatically increased in miR-138-silenced cells and decreased in miR-138-transduced cells compared with the respective control cells (Fig. 4A). Indicating that miR-138 is involved in modulating the ubiquitination of NF-κB signaling. Interestingly, the expression levels of TNFR, TRAF2,
RIP1, and IKK-β were lower in lipid rafts isolated from control cells than in miR-138-silenced ESCCs but higher than the levels in lipid rafts isolated from miR-138–transduced cells (Fig. 4B), suggesting that miR-138 plays a role in recruiting NF-κB signaling intermediaries to lipid rafts. Furthermore, immunofluorescent (IF) staining revealed that the quantity of ganglioside GM1, a marker of lipid rafts (26), drastically increased in miR-138-silenced cells and decreased in miR-138–transduced cells compared with the respective vector cells (Fig. 4C), indicating that miR-138 downregulation also induces lipid raft formation. Moreover, disrupting lipid rafts using methyl-β-cyclodextrin (MβCD) significantly inhibited NF-κB activity in miR-138-silenced cells (Fig. 4D), further showing that lipid rafts contribute to silencing miR-138-mediated NF-κB activation.

miR-138 directly suppresses multiple constitutive components of lipid rafts

Using publicly available algorithms (PITA and TargetScan5.2), we found that FLOT1, FLOT2, and caveolin-1, the constitutive components of lipid rafts (15–23), might be potential targets of miR-138 (Fig. 5A). Western blotting revealed that the expression of FLOT1, FLOT2, and caveolin-1 increased in miR-138–silenced cells but decreased in miR-138–transduced cells (Fig. 5B). Luciferase assay showed that overexpression of miR-138 attenuated, but downregulation of miR-138 elevated, the reporter activities driven by the 3'-UTRs of these transcripts. However, dysregulation of miR-138 did not result in the alteration of the reporter activities driven by the mutated 3'-UTRs of these transcripts within miR-138–binding seed regions (Fig. 5C). Moreover, miRNPs immunoprecipitation assay revealed a
selective association of miR-138 with FLOT1, FLOT2, and caveolin-1 but not with glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Supplementary Fig. S7), indicating that miR-138 negatively regulates these proteins via directly binding to their 3'-UTRs. In addition, individual silencing of these target genes potently inhibited NF-κB activity in miR-138–silenced cells (Fig. 5D and Supplementary Fig. S8), further showing that FLOT1, FLOT2, and caveolin-1 are functional effectors of miR-138–regulated NF-κB activation.

**Clinical relevance of miR-138 downregulation–mediated NF-κB activation in ESCC**

Finally, we examined whether miR-138 downregulation–mediated NF-κB activation in ESCCs was clinically relevant. As shown in Fig. 6A and B, correlation studies in 205 ESCC specimens showed that miR-138 levels were inversely correlated with the expression of p-IKK-β, Ki67, and CD31 \( (P = 0.004, P < 0.001, P < 0.001) \). In addition, miR-138 expression in 10 freshly collected ESCC samples was also inversely correlated with the mRNA levels of multiple NF-κB downstream targets, including Cyclin D1 \( (r = -0.754, P = 0.012) \), VEGFC \( (r = -0.738, P = 0.015) \), Bcl-xL \( (r = -0.815, P = 0.004) \), MMP9 \( (r = -0.857, P = 0.002) \), as well as the DNA-binding activity of NF-κB \( (r = -0.764, P = 0.006; \text{Fig. 6C}) \). Collectively, our results show that miR-138 downregulation activates NF-κB signaling through promoting lipid raft formation, resulting in ESCC aggressiveness and poorer clinical outcomes (Fig. 6D).

**Discussion**

The key findings of the current study provide new insights into the important role of miR-138 in the inhibition of ESCC progression and NF-κB signaling pathway. We found that miR-138 is downregulated in ESCCs, which induces the formation of lipid rafts, resulting in NF-κB activation and promotion of ESCC aggressiveness in vitro and in vivo. These findings uncover a novel mechanism for constitutive NF-κB activation in ESCCs and may represent a new target for clinical intervention in human ESCCs.
miR-138 Functions as a Tumor Suppressor

Figure 6. Clinical relevance of miR-138 downregulation–induced NF-κB activation in human ESCCs. 

A, miR-138 levels were inversely associated with p-IKK-β (S181), Ki67, and CD31 in 205 primary human ESCC specimens. Two representative cases are shown. Original magnification, ×200. B, percentage of ESCC tissues showing low or high miR-138 expression, relative to the level of p-IKK-β (S181), Ki67, and CD31. C, expression analysis (left) and correlation (right) of miR-138 with Cyclin D1, VEGF-C, Bcl-xL, and MMP9 mRNA expression and NF-κB DNA-binding activity in 10 freshly collected human ESCC samples. Each bar represents the mean ± SD of 3 independent experiments. *, P < 0.05. D, proposed model: downregulation of miR-138 promotes lipid raft formation and facilitates recruitment of the TNFR and IKK signalosome into lipid rafts, activates the NF-κB signaling pathway, consequently leading to progression and poorer clinical outcomes in human ESCCs.
Given their vital role in various signaling transduction pathways, lipid rafts have been shown to contribute to cancer progression through involvement in multiple biologic processes, including cell proliferation, apoptosis, angiogenesis, and metastasis (10, 11). Consistently, FLOT1, FLOT2, and caveolin-1, the markers of lipid rafts, are overexpressed in multiple cancer types and strongly associated with cancer mortality (13–20). FLOT1 is upregulated in prostate, breast, infiltrative colorectal, and renal cell carcinomas, suggesting that FLOT1 plays an oncogenic role in various cancers (15–17, 27, 28). Overexpression of FLOT2 not only transforms nontumorigenic and nonmetastatic melanoma cells into highly tumorigenic and metastatic cells but also promotes breast cancer progression through the stabilization of the receptor tyrosine kinase ErbB2 (17, 29). Interestingly, downregulation of caveolin-1 is reported to be important for cell transformation and the induction of breast cancer, whereas its overexpression contributes to breast cancer cell metastasis, suggesting that caveolin-1 may exert distinct functions during the development and progression of cancer (5, 6, 19–21). Similarly to FLOT1, caveolin-1 is reported to be overexpressed in ESCCs and positively correlated with patient survival (18, 30). However, the regulatory mechanisms by which FLOT1, FLOT2, or caveolin-1 are upregulated in ESCCs remain unclear. In the current study, combined analysis of publicly available algorithms and the results of in vitro and in vivo experiments showed that miR-138 suppresses the translation of FLOT1, FLOT2, and caveolin-1 by directly targeting their 3′-UTRs. Consistent with the oncogenic effects of FLOT1, FLOT2, and caveolin-1 in ESCCs, miR-138 downregulation dramatically promoted aggressiveness of ESCCs both in vitro and in vivo. Thus, our results represent a novel mechanism leading to overexpression of FLOT1, FLOT2, and caveolin-1 in ESCCs and a functionally and clinically relevant epigenetic mechanism of ESCC pathogenesis.

Lipid rafts have been shown to play important roles in proximal NF-κB signaling (7–9). In response to various stimuli, such as TNF-α, IL-1β, LPS, and CD40L, engaged receptors translocate to lipid rafts to form complexes with proximal signaling intermediates, which induce inflammation and cell survival through IkB kinase (IKK) dependent-activation of NF-κB (7–9). Disruption of lipid rafts or interfering with their composition could block TNF-α–induced translocation of TNFR to lipid rafts and reduce the recruitment of TRAF2 and RIP1 to the engaged receptor (7). Herein, we found that downregulation of miR-138 in ESCCs not only promoted the formation of lipid rafts but also facilitated the recruitment of TNFR and the signaling intermediates, TRAF2 and RIP1, to lipid rafts, suggesting that miR-138 downregulation activates NF-κB at the proximal receptor step. On the other hand, lipid rafts are also required for the ubiquitination of NF-κB signaling intermediaries, which has emerged as an important regulatory mechanism for NF-κB signaling (31, 32). In agreement with previous reports, silencing miR-138 also promoted the K63 polyubiquitination levels of TRAF2 and RIP1. Thus, our results suggest that miR-138 plays a regulatory role in receptor proximal signaling–mediated NF-κB activation.

miR-138 has been found to be downregulated in multiple human cancers, and downregulation of miR-138 contributes to cancer cell proliferation and invasion and inhibits apoptosis via different mechanisms (33–40). However, the expression of miR-138 has also been shown to be upregulated in tumor-initiating glioma stem cells (GSC) compared with nonneoplastic tissues, and upregulation of miR-138 is associated with tumor recurrence and survival (41). These findings indicate that miR-138 functions as both an oncogenic and tumor-suppressive miRNA depending on the tumor type. To investigate the clinical significance and the precise mechanism of action of miR-138 in ESCC pathogenesis, we examined the miR-138 expression in ESCCs and found that miR-138 is downregulated in ESCC, and miR-138 expression inversely correlated with the clinicopathologic features and overall survival of patients with ESCCs, suggesting that miR-138 may be associated with the progression of ESCCs. Consistently, upregulation of miR-138 dramatically inhibited the aggressiveness of ESCCs by inhibiting NF-κB signaling, further showing the tumor-suppressive role of miR-138 in ESCCs. Meanwhile, Ye and colleagues recently reported that overexpression of miR-138 results in p53 downregulation in mouse embryonic fibroblasts (MEF; ref. 42). However, we did not observe an obvious alteration in p53 expression in miR-138–transduced NEECs compared with vector control cells (data not shown). These results further support the notion that a single miRNA may have distinct functions in different cell types (43, 44).

In summary, given the tumor-suppressive effects of miR-138 and the oncogenic roles of NF-κB signaling in human cancers, our study improves our understanding of ESCC cell biology and tumor progression but may also allow the development of a novel diagnostic marker and therapeutic strategy for ESCCs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: L. Song, J. Li
Development of methodology: H. Gong, C. Lin, A. Liu
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H. Gong, C. Lin
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H. Gong, L. Song, C. Lin, A. Liu, X. Lin, J. Wu, M. Li
Writing, review, and/or revision of the manuscript: J. Li
Study supervision: L. Song, M. Li, J. Li

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