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MiR-34c-3p targets Notch2 to inhibit cell invasion and epithelial-mesenchymal transition in nasopharyngeal carcinoma

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Abstract

This study was to investigate role of miR-34c-3p/Notch2-EMT signal axis in Nasopharyngeal Carcinoma (NPC). Ten samples of NPC tissues and chronic inflammatory tissues of nasopharynx (CITN) were collected. 133 differentially expressed miRNAs, including 31 down-regulated and 102 up-regulated were identified in NPC tissues. MiR-34c-3p was down-regulated and Notch2 was up-regulated in NPC tissues compared with CITN tissues. MiR-34c-3p was overexpressed in 6-10B cells after transfected with miR-34c-3p mimic, while reduced in 5-8F cells after transfected with miR-34c-3p inhibitor. Notch2 was confirmed as a target gene of miR-34c-3p. miR-34c-3p overexpression in 6-10B cells suppressed, while knockdown in 5-8F cells promoted cell invasion ability. In molecular level, the expression of E-cadherin was increased, while N-cadherin was decreased after miR-34c-3p overexpression in 6-10B cells. Knockdown of miR-34c-3p obviously decreased E-cadherin, but increased N-cadherin expression in 5-8F cells. Targeting Notch2 by miR-34c-3p might be a promising target for NPS treatment.

Keywords: nasopharyngeal carcinoma; miR-34c-3p; Notch2; epithelial-mesenchymal transition.

Practical Application: Targeting Notch2 by miR-34c-3p might be a promising target for treatment of nasopharyngeal carcinoma.

1 Introduction

Now there have been over 2,000 types of miRNAs being identified in human genomes that could regulate the expression of one third of total mRNA in human bodies (Kuang et al., 2017). MiRNAs mainly regulate one or several groups of target genes via its core sequence and multiple miRNAs could act jointly on the same target gene and constitute a complex regulation network with miRNA-miRNA interactions, thus regulating numerous cellular functions (Slattery et al., 2016; Tang et al., 2016). Abnormal miRNAs, such as miR-148b and miR-139-3p, play an important role in tumor carcinogenesis and development; these sequences are closely related to proliferation, differentiation, migration, invasion of tumor cells and formation of tumor blood vessels (Vahidian et al., 2019; Li et al., 2019; Tian et al., 2019). miRNA chip assay serves as a rapid and effective approach to analyze differential miRNA expression. This approach has advantages such as good reproductivity, fast result-generation, and convenience (Zhang et al., 2018). In 2004, Liu et al. (2004) first adopted miRNA chip and identified 245 miRNAs in mammals; their results are highly reproductive.

In this study we measured the differential expression of miRNAs in NPC tissues through U.S. Affymetrix gene chip technology, then examined the key miRNA molecules to further analyze their functions. We found that miR-34c-3p was most down-regulated, so we conducted RT-PCR (quantitative Real Time Polymerase Chain Reaction) on miR-34c-3p to confirm the result, and expression level of miR-34c-3p appeared to be the same as results in chip assay. Bioinformatic analysis were further performed and indicated that miR-34c-3p could target Notch2, while Notch2 gene participated in Epithelial Mesenchymal Transformation (EMT) formation which might be related to NPC invasion and metastasis. This study mainly examined the relationship between miR-34c-3p-Notch2-EMT signal axis and NPC invasion and metastasis, aiming to provide experimental evidence and theoretical support for molecule-targeted treatment of NPC.

2 Materials and methods

2.1 Clinical tissue specimens

This study was approved by the ethical committee of the First Affiliated Hospital of Nanhua University. The tissue specimens were collected from NPC patients (9 males and 1 female, age

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range: 21-67, median age 51.9) and CITN participants (7 males and 3 females, age range: 19-60, median age 39.1) under Electronic Nasopharyngoscope in E.N.T Outpatient Department of the First Affiliated Hospital of Nanhua University from January 2015 to April 2015. Neither of all participants received radio-therapy or chemical therapy before diagnosis. Their conditions were pathological categorized into non-keratinizing squamous cell carcinoma according to WHO's pathological categorization of NPC in 1991, and the results were confirmed by pathologists.

2.2 GeneChip investigation

The GeneChip containing a total of 938 miRNA probes used in this study was made by U.S. Affymetrix Gene Company. Chip hybridization, scanning, and data analysis were all performed by Technical Service Department of Shanghai Genechem Genetic Biology Corporation. The RNA samples were first analyzed via Agilent 2100, tagged with Poly(A) tails and biotin via FlashTag Biotin HSR Labeling Kit. After hybridization, the chip was washed and stained with GeneChip Hybridization Wash and Stain Kit, then scanned to generate images and primary data. The main steps were listed as follows: 1) Conducting fluorescent labeling on sample RNAs with biotin labeling kit; adding Poly(A) tails on samples with Poly(A) polymerase, then binding modified RNAs to biotin-labeled signal molecules with ligase, and connecting the products with chips to perform hybridization; 2) Collecting the images on chips and analyzing the data: drying chips and put into a scanner to scan and save images, then using Affymetrix GeneChip Command Consoles 1.1 to analyze the data; 3) Applying Significance Analysis of Microarrays (SAM) to sort out the differential expression of miRNAs in data of the two chips with the criteria of p-value less than 0.05 and change fold more than 2.0.

2.3 Cell culture

NPC cell lines, including NP69, 6-10B and 5-8F were donated by the Cancer Center of Sun Yat-sen University and incubated in upgraded RPMI1640 culture medium with 10% of FBS at 37 °C in a thermostat incubator containing 5% CO_2. Cells were growing in a single layer and passaged at 80% degree of confluence. Cell passaged was conducted after cells grew fully in the medium, then cells in log-phase growth were collected for further investigation.

2.4 Cell transfection

MiR-34c-3p mimic (5′-AAUCACUAACCACACGGCCAGG-3′), miR-34c-3p inhibitor (5′-UUAGUGAUUGGUGUGCCGGUCC-3′) and control group (NC) (5′-CACAAAAUUUCUUAACAC-3′) were synthesized by Guangzhou Ruibo Biotech Co., Ltd. For cell transfection, 6-10B cells were transfected with miR-34c-3p mimic or mimic NC, while 5-8F cells were transfected with miR-34c-3p inhibitor or inhibitor NC using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instructions.

2.5 RT-PCR

Total RNA was isolated using Trizol reagent and complementary DNA was synthesized using High-Capacity cDNA Reverse Transcription Kit (ThermoFisher Scientific, USA) according to the manufacturer's instructions. RT-PCR was performed in a Bio-Rad cycler using the 25 μL reaction system (2.5μL of dNTP(2.5 mM), 2.5 μL of 10 × PCR buffer solution, 1.5 μL of MgCl2 solution, 1 unit of Taq polymerase, 0.25× final concentration of Sybergreen I solution, 1 μL of 10uM specific PCR primer F, 1 μL of 10 uM specific PCR primer R, 1 μL of cDNA or DNA and water). The PCR reaction conditions were as follows: 95 °C, 15min, 40 cycles (denaturing: 94 °C, 15 sec; extending: 55 °C, 30 sec; annealing: 70 °C, 30 sec). All primers used in this study were designed and synthesized by Guangzhou Ruibo Biotech Co., Ltd, including hsa-miR-205 (forward primer sequence: 5'-AAAGAUCCUCAGACAAUCCA-3', reverse primer sequence: 5'-CAGGAGGCAUGGAGCUGACA-3'), hsa-miR-34c-3p (forward primer sequence: 5'-AGUCUAGUUACUAGGCAGUG-3', reverse primer sequence:5'-CACGGCCAGGUAAAAAGAUU-3'), and U6 (forward primer sequence: 5'-GCTTCGGCAGCACATATACTAAA-3', reverse primer sequence: 5'-CGCTTCACGAATTTGCGTGTCAT-3'). The relative gene expression in the samples was normalized to U6 using the comparative threshold cycle (2–ΔΔCT) method. All independent experiments were repeated three times in triplicates.

2.6 Bioinformatic analysis

Bioinformatic analysis was conducted with the following devices and database: (1) dChip2004 (DNA-Chip Analyzer, 2021), a professional software designed by Affymetrix Company to analyze microarray data; (2) Hierarchical Clustering Explorer (HCE)3.0; (3) Starbase (2021); (4) Microrna (2021); (5) Targetscan (2021).

2.7 Dual-luciferase reporter assay

We prepared the wild type (WT) and mutant (MUT) pmiR-RB-REPORT™/Notch2 3′UTR primers of miR-43c-3p according to the following primer sequences: WT Primer: h_Notch2_3_UTR_F: 5`-GCTACAGTTGTCGCTGCTTG-3`, h_Notch2_3_UTR_R:5`- GGCACACTGGCAGGAGTAAT-3`; MUT Primer: h_Notch2_mut_F:5`-GCTGGTGTTGTCGCTGCTTG-3`; h_Notch2_mut_R:5`-GGGTTACTGGCAGGAGTAAT-3`. Then, 6-10B cells were co-transfected with miR-34c-3p mimic or NC together with WT or MUT Notch2 plasmids using riboFECTTMCP Reagent synthesized by Ruibo Biotech Co., Ltd. After 48h of transfection, luciferase activity was measured using a dual-luciferase assay kit (Promega). Renilla luciferase activity was measured and normalized to firefly luciferase activity using the dual-luciferase assay kit (Promega). All independent experiments were repeated three times in triplicates.

2.8 Transwell invasion assay

Cell invasion was evaluated using Transwell chamber precoated with 60 μL of diluted Matrigel. In brief, transfected 6-10B or 5-8F cells were adjusted to the cellular concentration to 2×10^5/mL and resuspended in serum free medium, which were then added into the upper chambers. Meanwhile, 600-800 μL of medium containing 10% FBS was added into the lower chambers. After 24 h incubation, the invaded cells to the lower chambers were stained with Giemsa

at room temperature for 15-30 min and washed with PBS for twice. After air drying, the number of invasive cells were counted by selecting random ten view of the microscope.

2.9 Western blot

Total protein was extracted using RIPA lysis buffer (Abcam, USA). After protein quantification with BCA assay, equal amount of protein sample was separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto PVDF membranes. The membranes were blocked for 1 h with a 5% non-fat dried milk in PBS at room temperature and incubated with primary antibodies against Notch 2 (ab8926, Abcam), E-cadherin (ab244084, Abcam), N-cadherin (ab256744, Abcam) and β-actin (ab8226, Abcam) overnight at 4 °C, followed by incubated with appropriate secondary antibody (ab150077, Abcam) for 1 h at room temperature. After washed for 5 min twice, the membranes were detected for protein bands with enhanced chemiluminescence reagent (Pierce, USA).

2.10 Statistical analysis

All independent experiments were repeated three times in triplicates. Statistical analysis was performed with SPSS 21.0 and data were expressed as mean ± SD. The Student's t-test or ANVOA analysis of variance was used to evaluate statistical differences with p-value less than 0.05 as a cut-off.

3 Results

3.1 Chip scanning result

The miRNA expression of each sample was represented as the fluorescent signals (Supplementary Figure 1) Strength of fluorescence corresponds to the level of miRNA expression. No fluorescence indicates no miRNA expression of this sample at that spot, as examined by the probe.

3.2 Cluster analysis

Cluster Analysis was conducted upon differential miRNA chip data. Different types of miRNAs were found to express at different levels in CITN and NPC, which means that the cluster result of miRNA expression profiling is specific and used to differentiate CITN from NPC (Figure 1). MiRNA GeneChip was adopted to measure the difference of miRNA expression between NPC and CITN tissues. A total of 133 miRNAs were found to differentially expressed between NPC and CITN tissues, 31 of which were down-down-regulated in NPC tissues, and

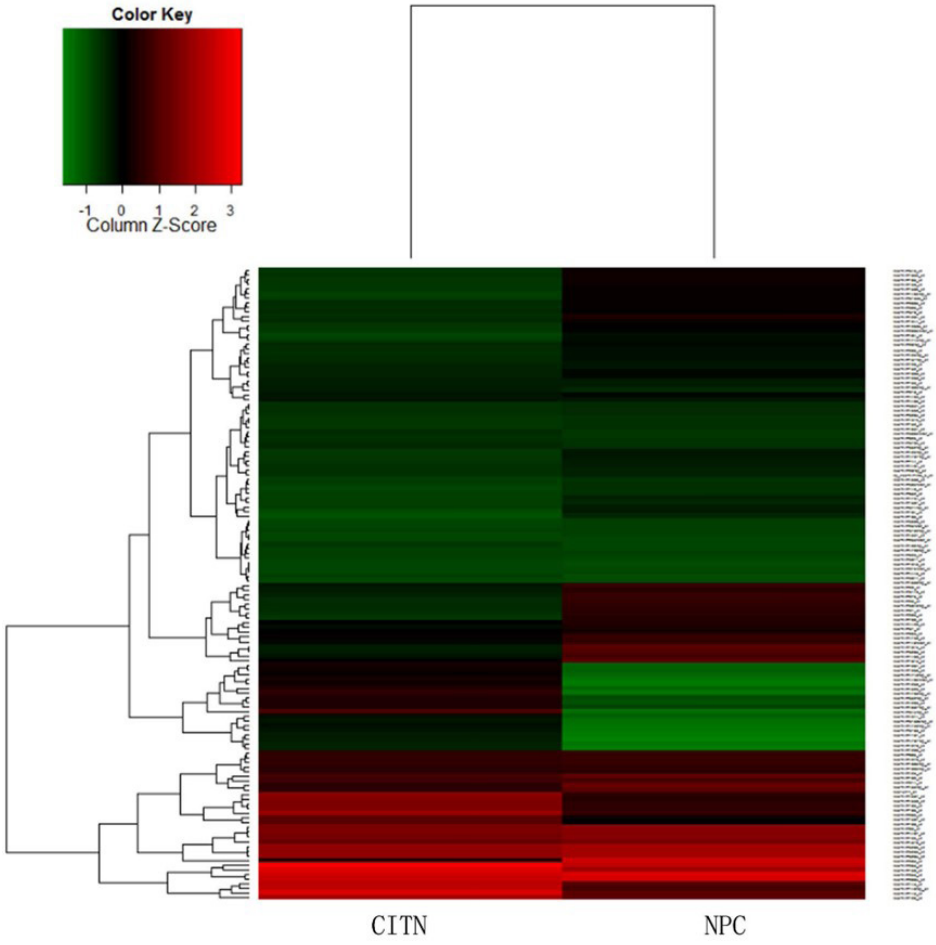

Figure 1. Result of Stratified Cluster Analysis of Different Genes.

the remaining 102 were up-regulated in the same kind of tissue (Supplementary Table 1).

3.3 Predication and validation of the target gene of miR-34c-3p

Starbase, an online bioinformatic software, was adopted to predict the target gene of miR-34c-3p. As a result, five software (TargetScan, PICTAR, RNA22, PITA and miRanda) all predicted thirteen target genes of miR-34c-3p, including ACSL4, DAAM1, NRN1, ARID4B, BRPF3, E2F3, C14orf43, MET, NOTCH2, LIMD2, MLLT3, FOXP1 and JAG1. Furthermore, thermodynamic and sequence conservancy analysis performed with bioinformatic software identified six genes, respectively, ACSL4, NRN1, MET, NOTCH2, LIMD2 and FOXP1 that basically fitted the requirements (mirSVR score<-0.1, PhastCons score:0.5-0.7, data now shown). We then analyzed with Starbase the relevancy between miR-34c-3p and the six predicted target genes in tumors, and found that miR-34c-3p was significantly related to Notch2 and FOXP1 in head and neck tumors, while Notch2 was closely related to EMT, invasion and metastasis of tumors (data now shown). Targetscan anticipated that miR-34c-3p could bind with Notch2 (Figure 2A). Results of dual luciferase reporter gene test revealed that miR-34c-3p mimic could significantly inhibit the strength of reporter fluorescence of Notch2 in wild type carriers. After mutating the predicted binding site, miR-34c-3p mimic did not produce remarkable down-regulative effect on the strength of reporter fluorescence of Notch2 mutate carriers; reporter fluorescence in mutated carriers recovered, suggesting that miR-34c-3p might regulate expression of that gene via Notch2-corresponding site on 3'UTR (Figure 2B).

3.4 Expression of miR-34c and Notch2 in NPC tissues and cell lines

miR-34c was down-regulated in NPC but up-regulated in PT ($p < 0.05$); Notch2 was up-regulated in NPC while down-regulated in PT ($p < 0.05$, Figure 3). Expression of miR-34c elevated in NP69 and declined in 6-10B and 5-8F ($p < 0.05$). In highly transfected 6-10B cells, expression of miR-34c was inferior to that in low transfected 5-8F ($p < 0.05$); expression of Notch2 was down-regulated in NP69 while up-regulated in 6-10B and 5-8F ($p < 0.05$). Expression of Notch2 was higher in highly transfected 6-10B than in low transfected 5-8F ($p < 0.05$, Figure 4).

3.5 Overexpression of miR-34c-5p suppressed the cell invasion

Test results indicated that the number of invasion cell was significantly lower in NPC cell line 6-10B-miR-34c-3p mimic than that in mimic NC or 6-10B group; in NPC cell line

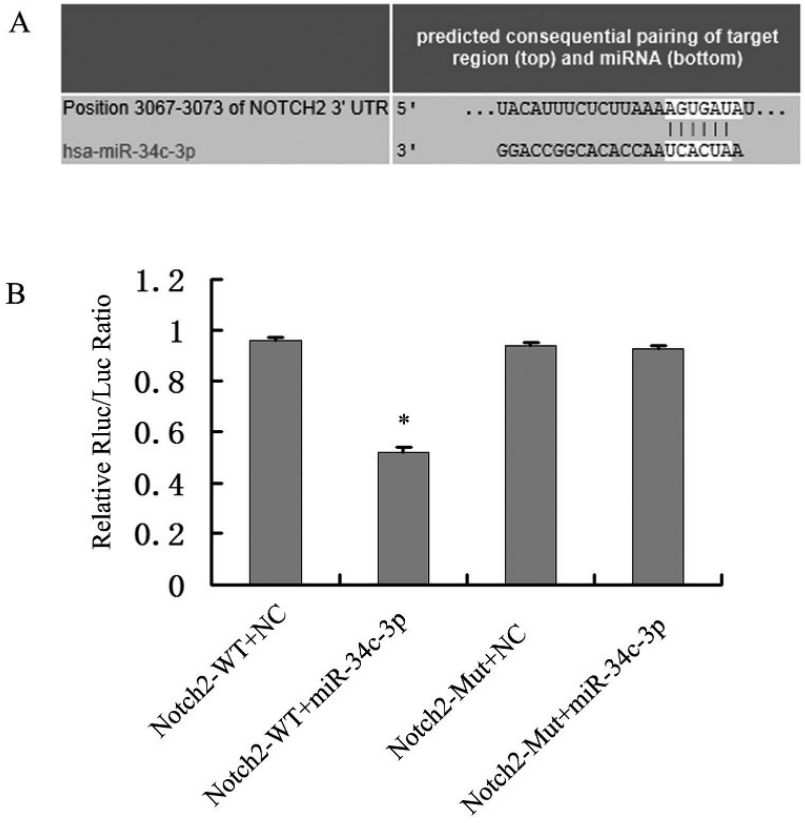

Figure 2. Notch 2 was identified as a target gene of miR-34c-3p. (A) The predicted binding sites between miR-34c-3p and Notch 2; (B) Luciferase reporter assay was performed in 6-10B cells after co-transfected with WT OR MUT Notch 2 together with miR-34c-3p mimic or NC. *P<0.05, compared with WT + NC.

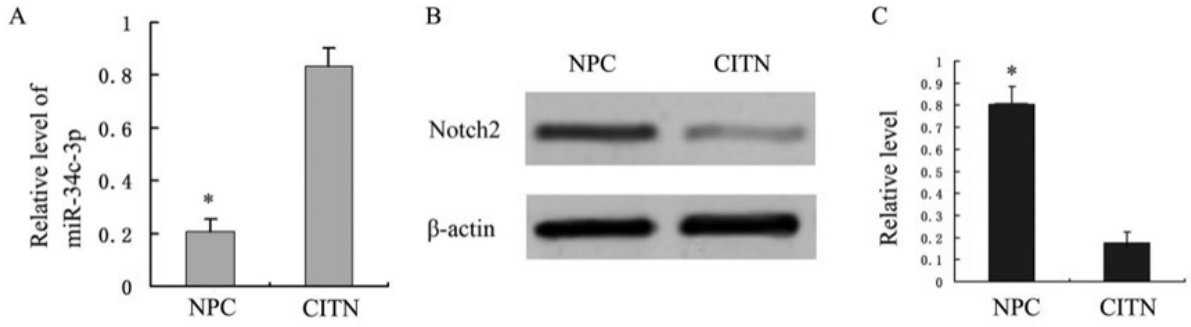

Figure 3. Expression of miR-34c and Notch2 in NPC and PT; (A) Relative expression of miR-34c; (B) Western blot result; (C) relative grayness; *p<0.05, NPC vs. PT.

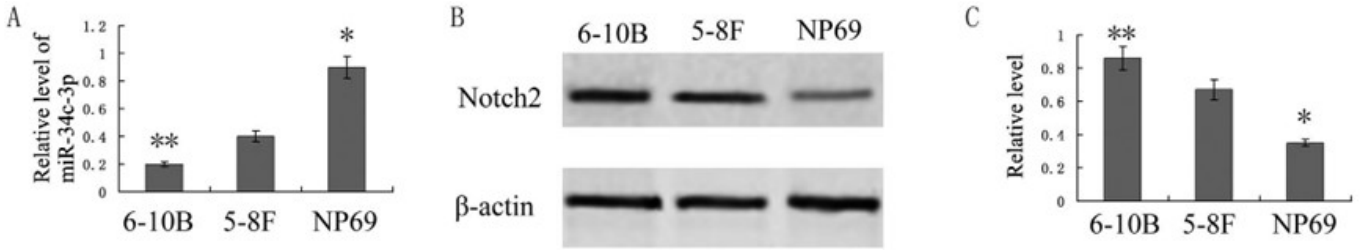

Figure 4. Expression of miR-34c and Notch2 in Different Cell Lines; (A) Relative expression of miR-34c; (B) Western blot result; (C) relative grayness; *p<0.05, NP69 vs. 6-10B or 5-8F; **p<0.05, 6-10B vs. 5-8F.

5-8F-miR-34c-3p inhibitor, the number of invasion cell was considerably higher than that in inhibitor NC or 5-8F group (p<0.05, Figure 5).

3.6 Overexpression of miR-34c-5p suppressed the EMT markers

As shown in Figure 6A, the expression of miR-34c-3p was significantly up-regulated in 6-10B cells after miR-34c-3p mimic transfection compared with mimic NC transfection, while it was down-regulated after miR-34c-3p inhibitor transfection in 5-8F cells. Using western blot analysis (Figure 6B, C), we further found overexpression of miR-34c-3p obviously decreased the expression of Notch 2 and N-cadherin, but increased E-cadherin expression in 6-10B cells. Knockdown of miR-34c-3p obtained the opposite results in regulating EMT markers.

4 Discussion

Abnormally expressed miRNA may act as a sort of oncogene or anti-oncogene in tumor pathology (Baranwal & Alahari, 2010). Up to now, there have been a lot of study reporting the aberrant expression of miRNAs in NPC (Barker et al., 2009; Chen et al., 2009). Researchers have discovered that miR-216b (Deng et al., 2011), miR-218 (Alajez et al., 2011), miR-26a (Lu et al., 2011), miR-10b (Li et al., 2010), miR-let-7 (Wong et al., 2011), miR-141 (Zhang et al., 2010) and miR-200a (Xia et al., 2010) all exert some tumor-inhibitive functions in NPC. However, the prognosis of NPC has not been significantly improved. Here, this study identified total 133 differentially expressed miRNAs between NPC and CITN tissues via miRNA GeneChip assay, including 102 up-regulated while the other 31 down-regulated or missing. We also confirmed two miRNAs (miR-205 and miR-34c-3p) that were most down- or up-regulated in NPC tissues through RT-PCR analysis.

Human miR-34c-3p gene locates in 11q23.1 region in chromosomes and is highly conservative. Studies in recent years reveal that expression of miR-34c-3p is down-regulated in lung cancer, colorectal cancer, liver cancer, breast cancer, prostate cancer, ovarian cancer, and is related to tumor invasion and metastasis (Russo et al., 2018; Du et al., 2018; Xiao et al., 2017; Wu et al., 2017). This study indicated that miR-34c was down-regulated in NPC and up-regulated in PT, while Notch2 was up-regulated in NPC and down-regulated in PT, which suggested that miR-34c-3p might be related to the carcinogenesis of NPC. MiR-34c was first discovered in nemathelminth. It is coded by a conservative miRNA sequence and is homologous in some invertebrates. In vertebrate, miR-34 has three homologous genes, namely, miR-34a, miR-34b and miR-34c (Engkvist et al., 2017). It is reported that miR-34c-3p is expressed in a low level in non-small cell lung cancer (NSCLC) tissues and cell lines, whereas over-expression of it could inhibit proliferation, invasion and migration of A549 cells. Moreover, miR-34c-3p recognizes and bind the 3'-UTR of PAC1 on a specific binding site, thus producing a crucial effect in pathology of NSCLC (Zhou et al., 2015). It is also reported that miR-34c is expressed low in basal-type breast cancer cells and could inhibit breast cancer cell proliferation and promote apoptosis primary by inducing cell growth to stagnate at G2/M phase (Achari et al., 2014).

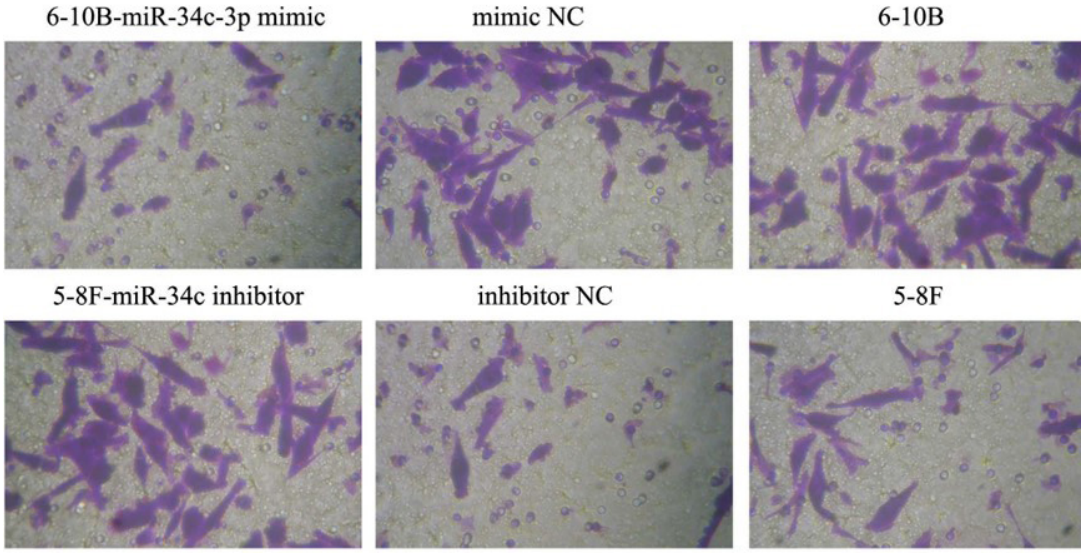

Figure 5. Change in Invasion Capacity of NPC Cell Lines Measured by Transwell.

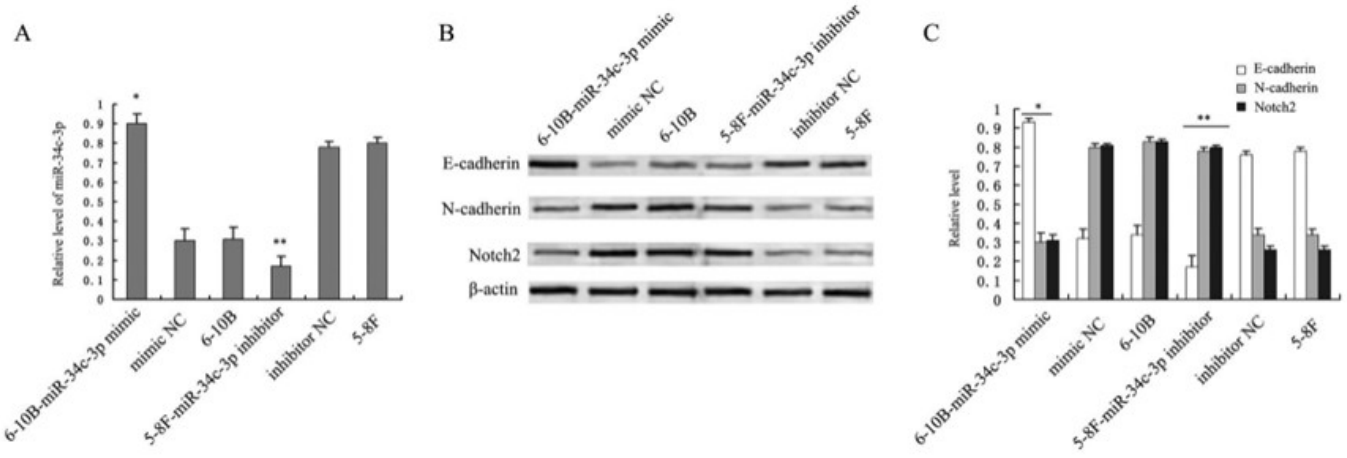

Figure 6. Expression of miR-34c-3p, Notch2, N-cadherin and E-cadherin in NPC cell lines. (A) Relative expression of miR-34c; (B) Western blot result; (C) relative grayness; *p<0.05, 6-10B-miR-34c mimics, compared with other two groups; **p<0.05, 5-8F-miR-34c inhibitor, compared with other two groups.

This study found that the expression of miR-34c-3p was significantly lower in NPC than that in NP69 cells; expression of miR-34c-3p is also significantly different in highly transfected cell line 5-8F transfected with mimic-miR-34c-3p and low cell line 6-10B transfected with miR-34c-3p inhibitor, which suggested that it might participate in NPC carcinogenesis and play an curtail role in NPC metastasis. MicroRNA mainly functions by inhibiting the expression of downstream genes. At present, no many downstream genes of miR-34c has been identified, while it has been discovered that miR-34c regulates several cancer-related target genes such as FRA-1 (Yang et al., 2013), KITLG (Yang et al., 2014), MET (Li et al., 2015), c-Met (Cai et al., 2010) and Notch signal pathway (Liu et al., 2015; Bae et al., 2012). Li et al. (2015) found that miR-34c is down-regulated in NPC and inhibits growth and migration of NPC cells through targeted down-regulation of Met oncogene. Yu et al. (2012) found that miR-34c inhibits self-renewal, EMT and metastasis of initial breast cancer cells through targeting Notch4. Wu et al. (2013) discovers that miR-34c-3p and miR-34c-5p are both down-regulated in glioma tissues and cell lines; in addition, their expression is negatively correlated to WHO stages, and both of them could inhibit proliferation and invasion of glioma cells. In addition, overexpression of miR-34c-3p could inhibit Notch2 expression, while over-expressed miR-34c-5p does not have the function. This study conducted a dual-luciferase reporter gene test and confirmed that Notch2 is the target of miR-34c-3p and further explored the role of Notch2-targeted miR-34c-3p in NPC.

EMT refers to the phenomenon that epithelial cells transform into mesenchymal cells under specific physiological

and pathological conditions, mainly expressing as the loss of polarity of epithelial cells, reduced affinity of epithelial cells to surrounding cells and matrix, enhanced cell invasion and migration capacity that induces to metastasis, down-regulation of epithelial cell tags, and up-regulation of mesenchymal cell tags (Gao et al., 2019). More and more studies are showing that EMT acts a critical role in invasion and metastasis of cancer, which has made it a hot topic in tumor study. Abnormal expression of microRNA and several signal transduction pathways, such as Wnt and Notch signal pathway participate in EMT progress of tumor cells (Li et al., 2017, 2018).

Notch signal pathway is closely related to tumorigenesis and development of many kinds of tumors, and might act as oncogene or anti-oncogene in different types of tumors. Research has found that miR-1 (Liu et al., 2018), miR-184 (Zhu et al., 2018), miR-598 (Chen, et al., 2017) and miR-146a-5p (Wang et al., 2016) could all target Notch2 to mediate EMT, then regulate tumor cell invasion and migration. Liu et al. (2018) found that overexpression of miR-1 in ESCC cells reduced Notch2 protein, whereas suppression of miR-1 led to an increase in Notch2 protein. A dual-luciferase experiment validated that Notch2 was a direct target of miR-1. Further studies verified that miR-1 regulates EMT signaling pathways directly through Notch2. Zhu et al. (2018) found that miR-184 functions as a tumor-suppressive miRNA targeting Notch2 and inhibits the invasion, migration and metastasis of NPC. Chen et al. (2017) found that overexpression of JAG1 induces epithelial mesenchymal transition (EMT) and promotes the metastasis of CRC cells. Decreased Notch2expression suppresses CRC cells metastasis and EMT. Together, these results indicate that miR-598 is a novel regulator of colorectal cancer metastasis. miR-598 is implicated in regulating EMT by directly suppressing its downstream target gene JAG1 to inactivate Notch signaling pathway. Wang et al. (2016) found that miRNA target gene prediction databases indicated the potential of Notch2 as a direct target gene of miR-146a-5p. miR-146a-5p functions as a tumor-suppressive miRNA targeting Notch2 and inhibits the EMT progression of ESCC. It has also been reported that miRNA could affect tumorigenesis, EMT, invasion and metastasis by regulating Notch2, which suggests that Notch2 must act importantly in tumor EMT. However, targeting Notch2 by miR-34c-3p on in NPC has not been reported yet.

This study confirmed that miR-34c-3p targeted the 3`-UTR end of Notch2 which appeared to be a downstream gene of miR-34c-3p. In NPC cell line 6-10B-miR-34c-3p mimic, after miR-34c targeted and bound to Notch2, expression of miR-34c-3p remarkably exceeded that in NC or 6-10B group; Notch2 and N-cadherin were down-regulated, while E-cadherin was up-regulated. Transwell test indicated a decline in NPC's invasion ability. In NPC cell line 5-8F-miR-34c-3p inhibitor, expression of miR-34c-3p was remarkably inferior to that in NC or 5-8F group; expression of Notch2 and N-cadherin was up-regulated, while E-cadherin was down-regulated, and Transwell test revealed an incline in NPC's invasion ability, which suggested that miR-34c-3p, after targeting Notch2, could regulate expression of EMT-related molecules (down-regulating N-cadherin and up-regulating E-cadherin), inhibit EMT of NPC, and suppress invasion and metastasis of NPC cells. On the opposite, down-regulation of miR-34c-3p expression, due to release of targeted inhibitive effect on Notch2, might induce to elevated N-cadherin expression, attenuated E-cadherin expression, promote EMT of NPC, and aggravate invasion and metastasis of NPC cells. miR-34c-3p acts as an anti-oncogene of NPC and could inhibit NPC invasion and metastasis.

In conclusion, we first utilized GeneChip technology to obtain a differential miRNA expression profiling of NPC, from which we noticed an obvious decline in miR-34c-3p expression in NPC. We further demonstrated that Notch2 was a downstream gene of miR-34c-3p. At the same time, we also discovered that miR-34c-3p could negatively regulate Notch2 to suppress EMT, causing inhibition of invasion in NPC cells. We hope this study could provide some theoretical basis and experimental support for clinical miRNA target-treatment of NPC.

Abbreviations

NPC, Nasopharyngeal Carcinoma; EMT, Epithelial Mesenchymal Transformation; CITN, Chronic inflammatory tissues of nasopharynx; NC, Negative control; qRT-PCR, quantitative Real Time Polymerase Chain Reaction.

Conflict of interest

The authors declare that they have no competing interests.

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Author contributions

Guarantor of integrity of the entire study: Sanyuan Tang; study concepts: Weiguo Huang; study design: Weiguo Huang; definition of intellectual content: Sanyuan Tang; literature research: Yuan Zhu; clinical studies: Ju Su, Yini Mao; experimental studies: Xiangqi Zhou, Yuan Zhu, Ju Su; data acquisition: Ju Su; data analysis: Chengchuan Jiang; statistical analysis: Yuan Zhu; manuscript preparation: Xiangqi Zhou; manuscript editing: Ling Wang, Yuqing Kuang; manuscript review: Sanyuan Tang.

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Supplementary Material

Supplementary material accompanies this paper.

Supplementary Table 1 Differential microRNAs expression profile in NPC and CITN

Supplementary Figure 1 GeneChip Images of NPC and CITN Tissues (A) CITN GeneChip. (B) NPC GeneChip

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