miR-577 suppressed the metastasis, EMT and viability via NF-κB pathway by targeting CXCL5 through in hepatocellular carcinoma

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miR-577, viability, invasion, EMT, hepatocellular carcinoma
Abstract

Background

Hepatocellular carcinoma (HCC) is a common malignant cancer worldwide. miR-577 have a role in inhibiting cell viability, metastasis in many tumors. This research was to explore the great role of miR-577 in hepatocellular carcinoma.

Methods

RT-qPCR and western blot were performed to evaluate the the miR-577 and genes mRNA and protein levels. Transwell assay and CCK-8 were applied to measure the viable and invasive abilities. Meanwhile, Kaplan-Meier method was used to assess the survival of HCC patients.

Results

miR-577 was downregulated in HCC tissues, which predicted a worse overall survival in HCC. miR-577 targeted to CXCL5 and mediated its expression in HCC. miR-577 suppressed cell invasion and EMT in HuH-7 cells. miR-577 inhibited cell viability via NF-κB pathway. In addition, miR-577 overexpression impaired the xenograft growth of HuH-7 cells.

Conclusion

miR-577 inhibited cell invasion, EMT and viability via NF-κB pathway by targeting to CXCL5 in HCC. The newly identified miR-577/CXCL5 axis provides novel insight into the pathogenesis of hepatocellular carcinoma.

Introduction

Hepatocellular carcinoma (HCC) is a major cause of cancer death especially in Africa and Asia [1, 2]. Due to the hepatitis C virus epidemic, the incidence of HCC is increasing in western countries [3]. The current treatment for hepatocellular carcinoma is limited to surgical resection, but resection results in a recurrence rate of more than 70% within 5 years, while 80% of present are not suitable to surgery [4]. Therefore, it is urgent to explore biomarkers for the treatment.

The discovery of MicroRNAs (miRNAs) opened up a new generation of cognition of in hepatocellular carcinoma [5, 6]. miRNAs negatively mediated gene expression through translational repression or mRNA degradation to be involved in the development of tumors [7]. Several miRNAs, including miR-122, miR-325, miR-206, miR-122 and miR-224 were played great roles in hepatocellular carcinoma [8-11]. miR-577 acted tumor suppressor to suppress tumor growth and enhances chemosensitivity in colorectal cancer [12]. miR-577 regulated cell proliferation and promoted G1-S phase transition in esophageal squamous cell carcinoma [13]. Similarly, miR-577 inhibited pancreatic β-cell function and survival in pediatric diabetes [14]. In non-small cell lung cancer, miR-577 suppressed cell growth and EMT in regulating WNT2B via Wnt/β-catenin pathway [15]. However, there was little studies elucidated the roles of miR-577 in HCC, thus, the experiments were performed to explore the vital functions of miR-577 in HCC.

C-X-C motif chemokine ligand 5 (CXCL5), known as ENA-78 or SCYB5 was a member of CXC subfamily of chemokines, binds the G-protein coupled receptor chemokine (C-X-C motif) receptor 2 to recruit neutrophils, to promote angiogenesis and to remodel connective tissues [16]. CXCL5 was thought to play roles in cell proliferation, migration, and invasion of cancer [17, 18]. CXCL5 citrullination may exert inflammatory properties by recruiting monocytes to inflamed joint tissue in a mouse model of inflammatory arthritis [19]. CXCL5 acted as an important angiogenic factor in idiopathic pulmonary fibrosis and non-small cell lung cancer [20, 21]. CXCL5 was involved in the interaction between cholangiocarcinoma cells and cancer-associated fibroblasts and inhibition of tumor-stromal interactions [18]. In our study, we discovered that miR-577 enhanced cell viability
and invasion through binding to CXCL5 in HCC. miR-577 promoted invasion EMT and viability through PI3K/AKT pathway in HCC.

**Material And Methods**

**Clinical specimens**

Pairs of HCC tissues and peritumoral normal tissues were gathered from 48 hepatocellular carcinoma patients in Shandong Provinical Hospital affiliated to Shandong University during January 2016 to December 2018. Specimens was immediately frozen in liquid nitrogen and then stored at -80°C after surgery. We obtained the written informed consent and the Ethics Committees of Shandong Provincial Hospital affiliated to Shandong University approved for this study.

**Cell culture**

We purchased HCC cells HuH-7 and a normal hepatocyte cell L-02 from American Type Culture Collection (ATCC; Rockville, MD, USA). All the cells were incubated in DMEM medium (Invitrogen, Carlsbad, CA, USA) with 10% FBS (Sigma-Aldrich, Louis, MO, USA) at 37°C in a humidified chamber with 5 % CO₂.

**Transfection**

The specific plasmids of miR-577 mimic or miR-577 inhibitor as well as their negative control were designed and synthetized from Gene-Pharma (Shanghai, China). The transfection was carried out using HuH-7 cells that were incubated in 6-well plate. The Lipofectamine 2000 Reagent (Invitrogen, USA) diluted using Opti-MEM/Reduced serum medium (Thermo Scientific, Shanghai, China) was used to perform the transfection. Geneticin (G418; Thermo Scientific, Shanghai, China) was used to select the stable transfection cells, while we harvest the transient transfection cells after transfected 48 h.

**Quantitative real-time PCR**

TRIzol Reagent (Invitrogen) and miRNeasy Mini Kit (Qiagen, Hilden, Germany) were employed to extract total mRNAs and miRNAs from tissues or cells. Omniscript Reverse Transcription Kit (Qiagen) and TaqMan miRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) were used to synthesize the first CDNA chain; followed QuantiTect SYBR Green PCR Kit (Qiagen) and miRNA-specific TaqMan MiRNA Assay Kit (Applied Biosystems) were conducted to carry out the qPCR in a Quantitect SYBR green PCR system (Qiagen). The relative levels of mRNA and miRNA were derived using 2-ΔΔCt method, the GAPDH and U6 small nuclear RNA utilized as normalization. The primers used for RT-qPCR were as follows: miR-577 forward 5'-TGGGTTGATAAAATATTGG-3', reverse 5'-GTGCAGGGTCCGAGGT-3'; U6 forward 5'-GCTTCGGGCAGCACATATAAAAT-3', reverse 5'-CGCTTCACGAAATTGCGTGTCAT-3'; CXCL5 forward 5'-AGCTGCCGGTACCCGATTAC-3', reverse 5'-TGCGAACACTTGCAGATTAC; GAPDH forward 5'-AAGGTGAAGGTCGGAGTCAA-3', reverse 5'-AATGAAGGGGTCATTGATGG-3'.

**Western blot analysis**

The total proteins were lysed by RIPA Lysis Buffer (Sigma, USA) containing 10% PMSF (Sigma). The SDS-PAGE was applied to separate the protein and then the blots were electro-transferred to PVDF membranes (Millipore, USA). After being blocked by 5% fat-free milk at ream temperature for 1 h, the membranes were incubated with primary antibodies. The primary antibodies were against CXCL5 (1:1000; Abcam, Cambridge, USA), E-cadherin (1:1000; Abcam), N-cadherin (1:1000; Abcam), Vimentin (1:1000; Abcam), c-Myc (1:1000, Abcam ), TRAF6 (1:1000, Abcam). Next, the blots were incubated by secondary anti-rabbit HRP-conjugated antibody (Cell Signaling). The protein signals were captured using Enhanced Chemiluminescence Detection Kit (ECL, Pharmacia Biotech, Arlington, USA).

**MTT assay**
The HuH-7 cells were plated into 96-well plates and cultivated for 24h, 48h, 72h and 96h. We added 20 μl of MTT (5 mg/ml, Sigma) into each well and followed cultured for 6 h. Next, we discarded the supernatant and added 100 μl of DMSO (Sigma) to each well. After agitating for 10 min, the absorbance at a wavelength of 570 nm was evaluated using an ELISA reader (Bio-Rad, Hercules, CA, USA).

Transwell assay

The transwell insert (8 μm membrane, Corning, Cambridge, MA) were placed in 24-well plate to evaluate the cell invasive ability. The HuH-7 cells were suspended by FBS free RPMI-1640 medium and we added 200 μl in the upper chamber, whereas the lower chamber was filled with 500 μl medium containing 15% FBS, which acted as inducer. After the cells were incubated for 24 h at 37°C, the non-invasive cells, which still on the upper surface, were removed by cotton swabs. We fixed and then stained the invasive cells using 4% paraformaldehyde and 10% crystal violet respectively; and followed counted the cells under a microscope (Olympus Corporation, Tokyo, Japan).

miRNA targets prediction and dual-luciferase reporter assay

TargetScan was conducted to perform the prediction of target genes of miR-577 and we discovered that CXCL5 was one of potential target gene. We mutated the binding sequences from UUUUUCU to AAAUUAGA to confirm miR-577 binding to CXCL5 in HCC cells. Followed, we inserted the wild type and the mutational 3'-UTR of CXCL5 into the dual luciferase reporter vectors, which were designated as WT or MUT. We utilized Lipofectamine 2000 Reagent (Invitrogen, USA) to co-transfect miR-577 mimic and WT or MUT vector into HuH-7 cells. Finally, the luciferase activity was measured using dual luciferase reporter assay system (Promega, USA).

Statistical analysis

All the statistical analysis was performed to use SPSS 16.0 software (IBM, Armonk, NY, USA) and the data were presented as mean ± SD. Student’s t test was performed to compare the differences between two groups, besides, one-way ANOVA was utilized to compare the differences between three or more groups. The association between miR-577 expression and the overall survival for HCC patients were assessed by Kaplan-Meier curve and log-rank test. P< 0.05 was considered to be statistical significant.

Results

miR-577 downregulation predicted poor prognosis of HCC

The miR-577 level was assessed in 48 pairs of HCC and peritumoral normal tissues and we found that miR-577 expression was overexpression in HCC tissues versus corresponding peritumoral normal tissues (P<0.05) (Figure 1A). Kaplan-Meier method elucidated the miR-577 expression has association with poor overall survival of HCC patients (P<0.05) (Figure 1B).

miR-577 inhibited cell viability and invasion in HuH-7 cells

miR-577 expression were evaluated in HCC cells HuH-7 and a hepatocyte cell L-02. miR-577 expression was higher in L-02 cells than HuH-7 (P < 0.01) cells (Figure 2A). To assess the roles of miR-577, miR-577 mimic and miR-577 inhibitor were employed to up- (P < 0.01) or down-regulate (P < 0.05) miR-577 in HuH-7 cells calculated by RT-qPCR (Figure 2B).

MTT assay illuminated that miR-577 mimic suppressed (P < 0.05) cell viability, while miR-577 inhibitor promoted (P < 0.05) the proliferative ability in HuH-7 cells (Figure 2C). Transwell assay indicated that miR-577 mimic inhibitor (P < 0.05) cell invasive ability whereas miR-577 enhanced (P < 0.05) (Figure 2D). All the results revealed miR-577 inhibited the viability and invasion in HCC cell HuH-7.

miR-577 regulated CXCL5 expression through binding to CXCL5 mRNA 3'-UTR
CXCL5 was predicted as a target gene of miR-577 using TargetScan, and the binding site was located at 249 to 255 on CXCL5 mRNA 3'-UTR. To validate miR-577 binding to the potential binding site of CXCL5, we mutated the potential binding sites, and then calculated the luciferase activity (Figure 3A). The luciferase reporter assay proved that miR-577 reduced (P < 0.05) the luciferased activity of HuH-7 cells that transfected wild type CXCL5 3'-UTR, however, it make no difference (P > 0.05) on the luciferase activity of cells transfected mutated CXCL5 3'-UTR (Figure 3B). Moreover, we evaluated CXCL5 mRNA levels after transfected miR-577 mimic or miR-577 inhibitor in HuH-7 cells, and miR-577 overexpression inhibited CXCL5 mRNA level (P < 0.05), while knockdown miR-577 promoted CXCL5 expression in HuH-7 cells (P < 0.05) (Figure 3B). All the results indicated that miR-577 mediated CXCL5 expression by targeting to its mRNA 3'-UTR in HCC cells HuH-7.

**miR-577 suppressed cell invasion, EMT and viability through PI3K/AKT signal pathway**

RT-qPCR was employed to assess the CXCL5 expression in tissues and cells. The CXCL5 expression in HCC tissues was higher than that in peritumoral normal tissues (P < 0.05) (Figure 4A). As expected, the CXCL5 expression was lower in hepatocyte cell L-02 than HCC cells HuH-7 (P < 0.05) (Figure 4B). Moreover, the proteins levels of EMT and pathway associated were assessed by western blot in HuH-7 cells. Research found that miR-577 mimic suppressed CXCL5 and E-cadherin expression, while improved N-cadherin and Vimentin expression in HuH-7 cells (Figure 4C), which suggested that miR-577 suppressed cell EMT through CXCL5. In addition, miR-577 inhibited CXCL5, c-Myc and TRAF6 expression in HuH-7 cells (Figure 4D), which proved that miR-577 inhibited cell proliferation through NF-κB pathway. All the results revealed that miR-577 inhibited cell invasion, EMT and viability through NF-κB signal pathway.

**miR-577 impaired the xenograft growth in vivo**

The nude mice were inject the HuH-7 cells stably transfected miR-577 mimic or control plasmid at subcutaneous. The volumes of xenograft tumors were measured every 3 days and the group of transfecting miR-577 mimic had a slower growth rate than control group, which indicated that miR-577 inhibited the HCC growth in vivo (Figure 5A). After 26 days of training, the nude mice were sacrificed. The volumes were calculated and the tumor volume of cells overexpressed miR-577 was smaller than the control group (P < 0.05) (Figure 5B).

**Discussion**

Hepatocellular carcinoma is one of the most common causes of cancer-related death worldwide with a lower 5-year survival rate [22, 23]. However, it is remain incompletely understood of the molecular mechanisms of hepatocellular carcinoma.

miRNAs were associated with translational repression and mRNA degradation at post transcriptional level [24, 25]. miR-577 acted as tumor suppressor to inhibit cell proliferation, migration and invasion in papillary thyroid carcinoma [26]. miR-577 suppressed metastasis and EMT of breast cancer [27]. Consistent with all the findings, we proposed that miR-577 was downregulated and miR-577 inhibited cell viability and invasion in HCC. We also revealed that miR-577 low expression predicted worse outcome of HCC patients. miR-577 suppressed tumor growth of hepatocellular carcinoma, which was consistent with the findings in glioblastoma [28].

CXCL5 acted as oncogene and enhanced cell growth and metastasis in several tumors, including bladder cancer, pancreatic cancer, cervical cancer and cutaneous melanoma [29-32]. CXCL5 was overexpressed in intestinal epithelium in inflammatory bowel disease and also in malignant pancreatic diseases [33, 34]. CXCL5 directly enhance tumor cell survival and proliferation in gastric cancer [35]. Consistent with all the findings, we discovered CXCL5 was upregulated in HCC tissues and cells. CXCL5 overexpression was associated with poor prognosis of HCC patients. In colorectal cancer, CXCL5 promoted tumor angiogenesis via AKT/NF-κB pathway [36]. It's the first time to propose that CXCL5 was a target gene of miR-577 in HCC. miR-577 regulated cell invasion, EMT and viability through NF-κB signaling pathway by regulating CXCL5 in HuH-7 cells.
miR-577 was low expressed in HCC tissues and miR-577 downregulation predicted poor prognosis of HCC patients. CXCL5 was a target gene of miR-577 and its expression was regulated by miR-577 in HCC. miR-577 impaired cell invasion, EMT and through NF-κB pathway in HuH-7 cells by targeting to CXCL5. miR-577 overexpression inhibited xenograft growth of HuH-7 cells.

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**Author contribution**

LT and JL are responsible for the conception or design of the work. WL contributes the acquisition, analysis, or interpretation of data for the work. XS provides the tissue samples. HX helps in the follow-up of the patients. JL helps in reviewing the histopathology slides. All authors finally approved the manuscript version to be published.

**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Conflict of interest**

The authors declare that they have no conflict of interest.

**Ethical approval and consent to participate**

The study was approved by Ethical Committee of Jinan Infectious Diseases Hospital affiliated to Shandong University and conducted in accordance with the ethical standards. Signed written informed consents were obtained from the patients and/or guardians.

**Consent for publication**

Not applicable.

**References**

1. Bosch FX, Ribes J, Diaz M, Cléries R. Primary liver cancer: worldwide incidence and trends. Gastroenterology. 2004; 127: S5-S16.
2. Yang JD, Roberts LR. Epidemiology and management of hepatocellular carcinoma. Infect Dis Clin North Am. 2010; 24: 899-919.
3. El-Serag HB, Davila JA, Petersen NJ, McGlynn KA. The continuing increase in the incidence of hepatocellular carcinoma in the United States: an update. Ann Intern Med. 2003; 139: 817-23.
4. Imamura H, Matsuyama Y, Tanaka E, Ohkubo T, et al. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. J Hepatol. 2003; 38: 200-7.
5. Mao B, Wang G. MicroRNAs involved with hepatocellular carcinoma (Review). Oncol Rep. 2015; 34: 2811-20.

6. Chen E, Xu X, Liu R, Liu T. Small but Heavy Role: MicroRNAs in Hepatocellular Carcinoma Progression. Biomed Res Int. 2018; 2018: 6784607.

7. Meister G. miRNAs get an early start on translational silencing. Cell. 2007; 131: 25-8.

8. Turato C, Fornari F, Pollutri D, Fassan M, et al. MiR-122 Targets SerpinB3 and Is Involved in Sorafenib Resistance in Hepatocellular Carcinoma. J Clin Med. 2019; 8.

9. Zhang Z, Han Y, Sun G, Liu X, Jia X, Yu X. MicroRNA-325-3p inhibits cell proliferation and induces apoptosis in hepatitis B virus-related hepatocellular carcinoma by down-regulation of aquaporin 5. Cell Mol Biol Lett. 2019; 24: 13.

10. Wang Y, Tai Q, Zhang J, Kang J, et al. MiRNA-206 inhibits hepatocellular carcinoma cell proliferation and migration but promotes apoptosis by modulating cMET expression. Acta Biochim Biophys Sin (Shanghai). 2019; 51: 243-253.

11. Amr KS, Elmawgoud Atia HA, Elazeem Elbnhawy RA, Ezzat WM. Early diagnostic evaluation of miR-122 and miR-224 as biomarkers for hepatocellular carcinoma. Genes Dis. 2017; 4: 215-221.

12. Jiang H, Ju H, Zhang L, Lu H, Jie K. microRNA-577 suppresses tumor growth and enhances chemosensitivity in colorectal cancer. J Biochem Mol Toxicol. 2017; 31.

13. Yuan X, He J, Sun F, Gu J. Effects and interactions of MiR-577 and TSGA10 in regulating esophageal squamous cell carcinoma. Int J Clin Exp Pathol. 2013; 6: 2651-67.

14. Chen XY, Li GM, Dong Q, Peng H. MiR-577 inhibits pancreatic beta-cell function and survival by targeting fibroblast growth factor 21 (FGF-21) in pediatric diabetes. Genet Mol Res. 2015; 14: 15462-70.

15. Wang B, Sun L, Li J, Jiang R. miR-577 suppresses cell proliferation and epithelial-mesenchymal transition by regulating the WNT2B mediated Wnt/beta-catenin pathway in non-small cell lung cancer. Mol Med Rep. 2018; 18: 2753-2761.

Lee BC, Lee J. Cellular and molecular players in adipose tissue inflammation in the development of obesity-induced insulin resistance. Biochim Biophys Acta. 2014; 1842: 446-62.

17. Kawamura M, Toiymama Y, Tanaka K, Saigusa S, et al. CXCL5, a promoter of cell proliferation, migration and invasion, is a novel serum prognostic marker in patients with colorectal cancer. Eur J Cancer. 2012; 48: 2244-51.

18. Okabe H, Beppu T, Ueda M, Hayashi H, et al. Identification of CXCL5/ENA-78 as a factor involved in the interaction between cholangiocarcinoma cells and cancer-associated fibroblasts. Int J Cancer. 2012; 131: 2234-41.

19. Yoshida K, Korchynskyi O, Tak PP, Isozaki T, et al. Citrullination of epithelial neutrophil-activating peptide 78/CXCL5 results in conversion from a non-monocyte-recruiting chemokine to a monocyte-recruiting chemokine. Arthritis Rheumatol. 2014; 66: 2716-27.

20. Keane MP, Belperio JA, Burdick MD, Lynch JP, et al. ENA-78 is an important angiogenic factor in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2001; 164: 2239-42.

21. Arenberg DA, Keane MP, DiGiovine B, Kunkel SL, et al. Epithelial-neutrophil activating peptide (ENA-78) is an important angiogenic factor in non-small cell lung cancer. J Clin Invest 1998; 102: 465-72.

22. Yezaz Ahmed Ghouri, Idrees Mian, Boris Blechacz. Cancer review: Cholangiocarcinoma. J Carcinog. 2015; 14: 1.

23. Forner A, Reig M, Varela M, Burrel M, Feliu J, et al. Diagnosis and treatment of hepatocellular carcinoma: An update. World J Hepatol. 2015; 7: 362-76.

24. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009; 136: 215-33.

25. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004; 116: 281-97.

26. Xue KC, Hu DD, Zhao L, Li N, Shen HY. MiR-577 inhibits papillary thyroid carcinoma cell proliferation, migration and invasion by targeting SphK2. Eur Rev Med Pharmacol Sci. 2017; 21: 3794-3800.

27. Yin C, Mou Q, Pan X, Zhang G, Li H, Sun Y. MiR-577 suppresses epithelial-mesenchymal transition and metastasis of breast cancer by targeting Rab25. Thorac Cancer. 2018; 9: 472-479.

28. Zhang W, Shen C, Li C, Yang G, et al. miR-577 inhibits glioblastoma tumor growth via the Wnt signaling pathway. Mol Carcinog. 2016; 55: 575-85.

29. Gao Y, Guan Z, Chen J, Xie H, et al. CXCL5/CXCR2 axis promotes bladder cancer cell migration and invasion by activating PI3K/AKT-induced upregulation of MMP2/MMP9. Int J Oncol. 2015; 47: 690-700.

30. Li A, King J, Moro A, Sugi MD, Dawson DW, et al. Overexpression of CXCL5 is associated with poor
survival in patients with pancreatic cancer. Am J Pathol. 2011; 178: 1340-9.

31. Feng X, Zhang D, Li X, Ma S, et al. CXCL5, the upregulated chemokine in patients with uterine cervix cancer, in vivo and in vitro contributes to oncogenic potential of Hela uterine cervix cancer cells. Biomed Pharmacother. 2018; 107: 1496-1504.

32. Forsthuber A, Lipp K, Andersen L, Ebersberger S, et al. CXCL5 as Regulator of Neutrophil Function in Cutaneous Melanoma. J Invest Dermatol. 2019; 139: 186-194.

33. Z'Graggen K, Walz A, Mazzucchelli L, Strieter RM, Mueller C. The C-X-C chemokine ENA-78 is preferentially expressed in intestinal epithelium in inflammatory bowel disease. Gastroenterology. 1997; 113: 808-16.

34. Frick VO, Rubie C, Wagner M, Graeber S, et al. Enhanced ENA-78 and IL-8 expression in patients with malignant pancreatic diseases. Pancreatology. 2008; 8: 488-97.

35. Verbeke H, Geboes K, Van Damme J, Struyf S. The role of CXC chemokines in the transition of chronic inflammation to esophageal and gastric cancer. Biochim Biophys Acta. 2012; 1825: 117-29.

36. Chen C, Xu ZQ, Zong YP, Ou BC, et al. CXCL5 induces tumor angiogenesis via enhancing the expression of FOXD1 mediated by the AKT/NF-kappaB pathway in colorectal cancer. Cell Death Dis. 2019; 10: 178.
Figure 1

Downregulation of miR-577 predicted poor prognosis of HCC (A) miR-577 expression was downregulated in HCC tissues versus corresponding peritumoral normal tissues. (B) miR-577 downregulation predicted poor 5-year survival in HCC.
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hsa-miR-577 3'-...GUCCAG
3'UTR MUT 5'-...GCUAUUU

B

HUH-7

|                | WT       | MUT     |
|----------------|----------|---------|
| NC             | 1.0      | 1.0     |
| miR-577 mimic  | 0.5      | *       |
|                | #        |         |
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