Medical Hypotheses 117 (2018) 21–27

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

miR-146a induces apoptosis in neuroblastoma cells by targeting BCL11A

Sheng-Hua Li[a,1], Jin-Pin Li[a,1], Lan Chen[b], Jing-Li Liu[a,*]

[a] Department of Neurology, The First Affiliated Hospital of Guangxi Medical University in Nanning, China
[b] Department of Internal Medicine, The Second Affiliated Hospital of Guangxi Medical University in Nanning, China

ARTICLE INFO

Keywords:
miR-146a
BCL11A
Neuroblastoma
Apoptosis

ABSTRACT

Aberrant expression of miR-146a has been reported to be involved in the progression and metastasis of various types of human cancers; however, its potential role in human neuroblastoma is still poorly understood. The purpose of our study was to investigate the molecular mechanism and possible role of miR-146a in human neuroblastoma. In this study, targeted genes were predicted by bioinformatic analysis and confirmed by dual-Luciferase reporter assay. The expression level of miR-146a in the human neuroblastoma SK-N-SH cell line was detected by quantitative RT-PCR. We used flow cytometric analysis to determine apoptosis and necrosis of SK-N-SH cells after transfection with miR-146a inhibitor, miR-146a mimic, and negative controls. The expression level of target genes was detected by RT-PCR and Western blotting. We identified BCL11A as a target of miR-146a. Thus, miR-146a targets the 3′UTR of BCL11A and inhibits its mRNA and protein expression. Overexpression of miR-146a can inhibit the growth and promote the apoptosis of human neuroblastoma SK-N-SH cells through inhibiting the expression of BCL11A. Furthermore, we found that upregulation of BCL11A by miR-146a inhibitor can promote SK-N-SH cells growth and protect SK-N-SH cells against apoptosis. Our results showed that miR-146a is a potential tumor suppressor gene in human neuroblastoma via directly targeting BCL11A. These findings suggest that miR-146a might be a new candidate target for treatment of human neuroblastoma.

Introduction

Neuroblastoma is one of the most common extracranial tumors of neuroectodermal cell origin in children, accounting for seven percent of childhood malignancies and more than fifteen percent of all childhood cancer deaths [1]. Human neuroblastoma is a rare solid tumor in children and originates from the neural crest cells, which are the precursor cells of the sympathetic nervous system, and its clinical features are heterogeneous, ranging from spontaneous remission to rapid progression with a fatal outcome [2]. Despite recent advances in neuroblastoma therapy, the overall five-year survival rate is still poor. More than seventy percent of patients with neuroblastoma have progressed to malignant lesions at the time of initial diagnosis [3]. Although multiple genes have been found to be associated with the ability of neuroblastoma cells to metastasize and invade normal tissue [4], the molecular mechanisms, particularly the role of microRNAs, in neuroblastoma metastasis are still poorly understood. Further elucidation of the potential molecular mechanisms of human neuroblastoma will contribute to a more comprehensive understanding of the response to treatment and outcome, potentially resulting in identification of optimal therapeutic agents for neuroblastoma therapy.

MicroRNAs have attracted greater attention due to their potential regulatory roles in a wide range of cellular and biological processes, including cell proliferation, apoptosis, and differentiation, as well as in human carcinogenesis [5,6]. MicroRNAs are a group of small non-coding RNAs that modulate gene expression through translational repression or degradation of mRNAs by directly binding to the 3′-untranslated regions (3′-UTRs) of target mRNAs. Abnormal expression of microRNAs has been found in many kinds of tumors, where specific miRNA can behave either as an oncogenic or a cancer suppressor gene [7,8]. Some studies have found that miRNAs are associated with clinical features and poor clinical outcome in patients with neuroblastoma [9,10], and individual miRNAs have been verified to regulate crucial processes such as cell migration, proliferation, and apoptosis in neuroblastoma [11–14].

miR-146a is located on chromosome 5q34, a region that is frequently deleted in many types of human tumors [15], and has been found to be aberrantly expressed in multiple cancer types, including gastriccancer [16], prostate cancer [17], breast cancer [18], pancreatic cancer [19] and non-small cell lung cancer [20]. Functionally, miR-146a inhibits cell growth, migration, and invasion in a variety of cancers [19–21]. However, there is little knowledge about the functional

* Corresponding author at: No. 6, Shuangyong Road, Nanning, Guangxi, China.
E-mail addresses: lilicomet@163.com, 1020802185@qq.com (J.-L. Liu).
[1] Designated as co-first authors, these authors contributed equally to this work.

https://doi.org/10.1016/j.mehy.2018.05.019
Received 21 March 2018; Accepted 25 May 2018

role of miR-146a in human neuroblastoma tumorigenesis and metastasis, and the potential role of miR-146a in human neuroblastoma is still unclear.

Mechanistically, we examined an as yet undescribed miR-146a target, BCL11A, transcript predicted to be miR-146a target gene by TargetScan, miRDB, miRWalk, miRBase and PicTar, which is involved in anti-apoptosis.

BCL11A, a BCL11 gene family member, has been identified as a transcriptional repressor essential for lymphoid development [22,23]. A previous study has confirmed the crucial role of BCL11A in the proliferation and survival of B cells, and also proved that down-regulation of BCL11A expression can promote apoptosis in SUDHL6 cell line [24]. Moreover, gene expression profiling found that large numbers of genes associated with proliferation and apoptosis are changed during BCL11A siRNA-induced apoptosis in SUDHL6 cell [25]. Additionally, studies have shown that BCL11A deficient B cells exhibit reduced expression of MDM2 [23,26]. Furthermore, a recent study showed that FOXQ1 inhibition constrains MDM2 by controlling BCL11A, resulting in the suppression of proliferation and invasion, and the induction of apoptosis in prostate cancer cells [26], but the role of BCL11A in human neuroblastoma remains unclear.

In particular, bioinformatics analyses predict that BCL11A is a potential target of miR-146a. Therefore, we proposed that miR-146a might also regulate BCL11A. Thus, down-regulation of BCL11A by miR-146a may be an efficient treatment strategy in human neuroblastoma and other tumors, but novel and optimal treatment strategies remain to be elucidated.

In this study, we address the following questions: [1] Does miR-146a overexpression inhibit cell growth and promote apoptosis in human neuroblastoma cells? [2] Is BCL11A a direct target gene of miR-146a? [3] What functional target of miR-146a is involved in human neuroblastoma cell growth and apoptosis? 4) Does miR-146a inhibit cell growth and induce apoptosis via targeting BCL11A? The answers to the above questions could provide greater understanding of the potential functional role of miR-146a in human neuroblastoma development and provide a novel treatment option for patients with human neuroblastoma.

Materials and methods

Cell culture

The human neuroblastoma cell line SK-N-SH and 293T cells were obtained from the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). SK-N-SH cells were maintained in DMEM/F-12-L-Glutamine medium (Life Technologies, Gibco, USA) with 10% FBS (Gibco, USA) and 100U penicillin/streptomycin (Thermo-life, USA) in a humidified atmosphere containing 5% CO2 at 37 °C. 293 T cells were cultured in DMEM media (Thermo-life, USA) containing 10% FBS (Gibco, USA), and 100 U penicillin-streptomycin (Thermo-life, USA) at 37 °C with 5% CO2.

Cell transfection

For the transfection of miR-146a inhibitor, miR-146a mimic, and miR-146a mimic/inhibitor negative, human neuroblastoma SK-N-SH cells were inoculated into six-well plates until 50–60% confluent. MiR-146a inhibitor, miR-146a mimic, and miR-146a mimic/inhibitor negative control were diluted with riboFECT™CP Buffer (RiboBio Co. LTD, China), incubated at 25 °C for 15 min at a final concentration of 100 nM, and then added to each well. miR-146a mimic, miR-146a inhibitor, and miR-146a mimic/inhibitor negative controls were transfected into the cells via Lipofectamine 2000 (Invitrogen, USA), following the manufacturer's instructions. miR-146a inhibitor, miR-146a mimic and miR-146a mimic/inhibitor negative control were synthesized by Guangzhou RiboBio Co. LTD (http://ribobio.cn.china.cn) with the following sequences: miR-146a inhibitor (5′-AACCCAUGGAAUUCAGUUCUCA-3′), miR-146a mimic (5′-UGAGAACUGAAUUCCAUGGGUU-3′), and mimic/inhibitor negative (5′-UUGUACUACACAAAAGUACUG-3′).

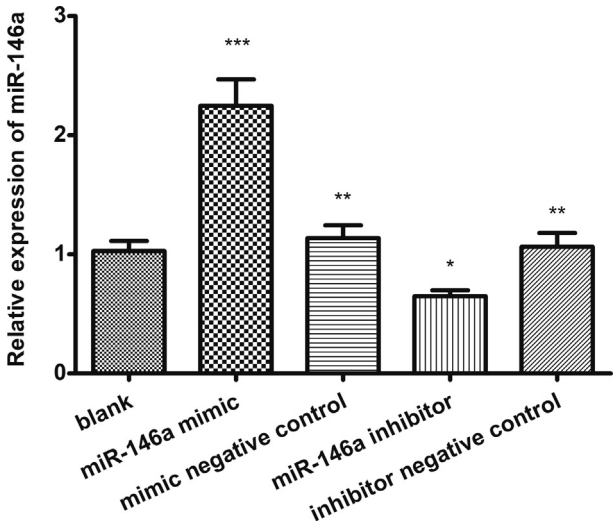

Fig. 1. miR-146a expression in human neuroblastoma SK-N-SH cells. $^{***}P < 0.05$, miR-146a mimic compared with miR-146a mimic negative control; $^{*}P < 0.05$, miR-146a inhibitor compared with miR-146a inhibitor negative control. $^{**}P > 0.05$, miR-146a mimic or inhibitor negative control compared with blank group.

Analysis of cell apoptosis by flow cytometry

Cells apoptosis was detected by flow cytometry and using the Annexin V- FITC and PI double staining kit (Invitrogen, USA). After treatment, cells were washed twice with ice-cold phosphate-buffered saline (PBS), then SK-N-SH cells were stained in 100 μl binding buffer containing 1 μl PI and 5 μl annexin V-FITC. After incubation in the dark at room temperature for 15–20 min, the samples were analyzed by flow cytometry (BD Biosciences, USA). Late apoptotic and necrotic cells show both PI and annexin V-FITC-positive staining, while early apoptotic cells show only annexin V-FITC-positive staining. Cells that are considered viable are both PI and FITC Annexin V-negative.

MicroRNA target prediction

The potential mRNA targets of miR-146a were predicted by miRDB (http://www.mirdb.org/), PicTar (http://pictar.mdc-berlin.de/), miRWalk (http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/), TargetScan (http://www.targetscan.org/), and miRBase (http://www.mirbase.org/). We predicted that the 3′UTR of BCL11A as a potential target of miR-146a.

Plasmid construction

Plasmid construction was performed by a service provider (Guangzhou RiboBio CO., LTD, China). A 724-bp segment from the 3′UTR of the BCL11A gene containing miR-146a binding sites was amplified by RT-PCR from human genomic DNA. The following primer sets were used to generate specific fragments: BCL11A-UTR forward, 5′-GGCGGCTCGAGCTCACTCCCACCTGACAC-3′, BCL11A-UTR reverse, 5′-AATGCGGCCGCGTTTTAATAGATCCAAGGCACTCAT-3′. Mutants of the BCL11A 3′-UTR were generated on miR-146a target recognition sites by site mutation. The sequence of mutant BCL11A-3′UTR segments contained BCL11A_mut_forward, 5′-TCCTTTATCAAGAGACCGTTTGAATGCATGAT-3′; BCL11A_mut_reverse, 5′-CAAACGGTCTCTTGATAAAGGAAAAAAAAAAA-3′.