Mol Cell Biochem (2013) 384:105–111
DOI 10.1007/s11010-013-1786-4

miR-335 suppresses migration and invasion by targeting ROCK1 in osteosarcoma cells

Yong Wang · Wei Zhao · Qin Fu

Received: 19 June 2013 / Accepted: 9 August 2013 / Published online: 22 August 2013
© Springer Science+Business Media New York 2013

Abstract Accumulating evidence has shown that microRNAs are involved in multiple processes in cancer development and progression. Recently, miR-335 has been identified as a tumor-suppressing microRNA in many human cancers. However, the specific function of miR-335 in osteosarcoma is unclear at this point. In this study, we found that the expression of miR-335 in osteosarcoma tissues and cell lines was much lower than that in normal control, respectively, and the downregulated miR-335 was significantly associated with lymph-node metastasis. Transfection of miR-335 mimics could significantly inhibit the cell migration and invasion in MG-63 and U2OS osteosarcoma cell lines. Moreover, we also showed that ROCK1 was negatively regulated by miR-335 at the posttranscriptional level, via a specific target site within the 3′UTR by luciferase reporter assay. The expression of ROCK1 was inversely correlated with miR-335 expression in osteosarcoma tissues, and knockdown of ROCK1 by siRNA-inhibited osteosarcoma cells migration and invasion resembling that of miR-335 overexpression. Thus, our findings suggest that miR-335 acts as tumor suppressor by targeting the ROCK1 gene and inhibiting osteosarcoma cells migration and invasion. The findings of this study contribute to current understanding of the functions of miR-335 in osteosarcoma.

Y. Wang · Q. Fu (✉)
Department of Orthopaedics, Sheng Jing Hospital of China Medical University, Shenyang 110004, Liaoning, People's Republic of China
e-mail: fuqin7812@163.com

W. Zhao
Department of Orthopaedics, The Affiliated Central Hospital, Shenyang Medical College, Shenyang 110024, Liaoning, People's Republic of China

Keywords miR-335 · Osteosarcoma · Migration and invasion · ROCK1

Introduction

Osteosarcoma is the most common primary malignancy of bone in children and adolescents. The estimated worldwide incidence rate is 4 million per year, with a peak incidence at the age of 15–19 years [1]. Despite the recent advance in therapeutic strategies, such as wide tumor excision, adjuvant chemotherapy, and radiotherapy, the prognosis of osteosarcoma patients remains poor. Because, approximately 80 % of patients eventually developing metastatic disease following surgical treatment [2], pulmonary metastasis of osteosarcoma patients is the major cause of fatal outcome [3]. Therefore, it is essential to identify metastasis-associated molecules and to better understand the mechanisms behind the lung metastasis of osteosarcoma.

MicroRNAs (miRNAs) are endogenous small RNAs averaging 20–24 nucleotides, transcribed from non-protein-coding genes or introns, which mediate translational suppression or cleavage of their target mRNAs by binding to complementary sites in their 3′-UTR. Aberrant expression of miRNAs occurs in many types of cancers, some of which function as tumor suppressor genes or oncogenes [4, 5]. MicroRNA-335 (miR-335), as a transcript of genomic region chromosome 7q32.2 [6], acts as a tumor suppressor or tumor promoter in various human malignancies. Recently, miR-335 was found to function as tumor suppressor genes in various cancers including breast cancer [7, 8], ovarian cancer [9], prostate cancer, [10] and gastric cancer [11]. While in astrocytoma [12] and meningiomas [13], miR-335 functions as tumor oncogenes. However, the function of miR-335 and