miR-564 negatively regulates RAB35 to inhibit epithelial-mesenchymal transition and malignant progression in non-small cell lung cancer

Yingyu Yang[1, a,*], Aimin Ge[2,a], Yaru Xu[1], Jianbo Li[1], Wenwen Shi[1], Junling Wang[1], Zhipeng Zhao[1]

[1]The First People's Hospital of Qujing/The Qujing Affiliated Hospital of Kunming Medical University, Qujing, Yunnan, 655000, China.

[2]Department of Pathology, Heze Municipal Hospital, Heze, Shandong, 274031, China.

[a]These authors contributed to the work equally and should be regarded as co-first authors.

*Correspondence should be addressed to Yingyu Yang (yyy13988962179@163.com).

Department of Pathology, The First People's Hospital of Qujing/The Qujing Affiliated Hospital of Kunming Medical University, No.1 Yuanlin Road, Qilin District, Qujing, Yunnan, 655000, China.

Abstract

Objective: This study aimed to investigate the expression of miR-564 in non-small cell lung cancer (NSCLC) and its effects on malignant biological behaviours such as cell proliferation, migration, and apoptosis, whilst further analysing its potential mechanisms of action.

Methods: In vitro cell experiments were conducted by transfecting miR-564 mimics into NSCLC cell lines to establish overexpression models. Cell proliferation capacity was assessed using the CCK-8 assay; cell invasion and migration capabilities were evaluated via Transwell and scratch assays; apoptosis rates were determined by flow cytometry; qPCR measured miR-564 and RAB35 expression levels; and Western blotting examined the expression of RAB35, Rac1, E-cadherin, N-cadherin, and apoptosis-related proteins Bcl-2 and Bax. The dual luciferase reporter assay and AGO2 immunoprecipitation (RIP) experiment validated the interaction between miR-564 and RAB35. A xenograft tumour mouse model was established for further validation.

Results: Overexpression of miR-564 significantly suppressed the proliferative capacity of NSCLC cells and effectively inhibited cell invasion and migration. Concurrently, miR-564 overexpression markedly induced apoptosis. Overexpression of miR-564 led to a significant downregulation in the protein expression levels of RAB35, Rac1, and N-cadherin, while E-cadherin expression increased. miR-564 negatively regulates RAB35 expression. Overexpression of RAB35 reversed the effects of miR-564 overexpression, further promoting NSCLC cell proliferation, invasion, and migration. It inhibited apoptosis, upregulated Rac1 and N-cadherin protein expression, and downregulated E-cadherin expression. Overexpression of miR-564 significantly inhibited tumour growth in vivo while downregulating N-cadherin, Bcl-2, RAB35, and Rac1 expression. It concurrently upregulated miR-564, E-cadherin, and Bax expression.

Conclusion: miR-564 exerts tumour-suppressing effects in NSCLC by negatively regulating RAB35, thereby inhibiting cancer cell proliferation and migration, suppressing the epithelial-mesenchymal transition (EMT) process, and promoting apoptosis.

Keywords: non-small cell lung cancer (NSCLC); miR-564; RAB35; epithelial-mesenchymal transition (EMT)