Suppression of microRNA-629 enhances sensitivity of cervical cancer cells to 1′S-1′-acetoxychavicol acetate via regulating RSU1

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Background: Cervical cancer is the fourth most frequent malignancy affecting women worldwide, but drug resistance and toxicities remain a major challenge in chemotherapy. The use of natural compounds is promising because they are less toxic and able to target multiple signaling pathways. The 1′S-1′-acetoxychavicol acetate (ACA), a natural compound isolated from wild ginger Alpinia conchigera, induced cytotoxicity on various cancer cells including cervical cancer. MicroRNAs (miRNAs) are short noncoding RNAs that regulate numerous biological processes, such as apoptosis and chemosensitivity. Past studies reported that miR-629 is upregulated in many cancers, and its expression was altered in ACA-treated cervical cancer cells. However, the role of miR-629 in regulating sensitivity toward ACA or other anticancer agents has not been reported. Hence, this study aims to investigate the role of miR-629 in regulating response toward ACA on cervical cancer cells.

Methods: The miR-629 expression following transfection with miR-629 hairpin inhibitor and hairpin inhibitor negative control was measured using quantitative real-time polymerase chain reaction (RT-qPCR). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to investigate sensitivity toward ACA. Apoptosis was detected using Annexin V/propidium iodide and Caspase 3/7 assays. The gene target for miR-629 was identified using miRNA target prediction programs, luciferase reporter assay and Western blots. Gene overexpression studies were performed to evaluate its role in regulating response toward ACA.

Results: Transfection with miR-629 hairpin inhibitor downregulated its expression in both cervical cancer cell lines. Suppression of miR-629 increased sensitivity toward ACA by reducing cell proliferation and inducing apoptosis. Luciferase reporter assay confirmed RSU1 as a direct target of miR-629. Overexpression of miR-629 decreased RSU1 protein expression, while inhibition of miR-629 increased RSU1 protein expression. Overexpression of RSU1 augmented antiproliferative and apoptosis-inducing effects of ACA.

Conclusion: Our findings showed that combination of ACA with miR-629 and RSU1 may provide a potential strategy in treating cervical cancer.

Keywords: miR-629, 1′-acetoxychavicol acetate, cervical cancer, Ras suppressor-1, apoptosis, cell proliferation

Introduction

Cancer of the cervix is the fourth most common malignancy affecting women worldwide after breast cancer. In 2012, there were an estimated 527,600 new cases diagnosed and 265,700 mortalities worldwide, with the highest incidences happening in less developed countries. Chemotherapy is often used together with surgery and radiotherapy to treat cervical cancer in combinatorial approach. However, poor clinical outcomes