5-Aza-CdR Enhances ZO-1 Tight Junction Protein Expression by Upregulating miR-126 Through Promoter Hypomethylation in HMEC-1 Cells

Yuzhi Shao[1], Wei Xie[2], Rengui Bade[2, *]

[1]School of Life Science, Guangzhou University, Guangzhou, China

[2]Center for Translational Medicine, Baotou Medical College, Baotou, China

Email address:

shao_yuzhi_china@163.com (Yuzhi Shao), xiewei@tmu.edu.cn (Wei Xie), bdrg119410343@163.com (Rengui Bade)

[*]Corresponding author

Abstract

Cellular junctions are crucial for the structure and function of endothelial cells (ECs). Zonula occludens-1 (ZO-1), a tight junction protein, is a key component of these junctions and is regulated by miR-126. Previous studies have shown that miR-126 expression can be influenced by DNA methylation. 5-Aza-2′-deoxycytidine (5-Aza-CdR), a DNA methyltransferase (DNMT) inhibitor, can alter DNA methylation levels. In this study, we examined the effects of 5-Aza-CdR on cellular junctions by analyzing ZO-1 and miR-126 expression in HMEC-1 cells in vitro. HMEC-1 cells were treated with 5-Aza-CdR, and ZO-1 expression was assessed using real-time PCR and Western blotting. miR-126 expression and its promoter's DNA methylation levels were evaluated by real-time PCR and methylation-specific PCR (MS-PCR). Additionally, DNMT mRNA and protein levels were measured using real-time PCR and Western blotting. Global methylation levels were analyzed via 5-mC-positive signals using laser confocal microscopy, and Alu and Long Interspersed Element-1 (LINE-1) methylation patterns were assessed by combined bisulfite restriction analysis (COBRA). Cell apoptosis and cell cycle phases were analyzed via flow cytometry. Our results showed that ZO-1 expression increased with the upregulation of miR-126, which was associated with promoter DNA hypomethylation. Treatment with 5-Aza-CdR reduced DNMT1 and DNMT3A levels, as well as global methylation, while inducing S-phase cell cycle arrest in HMEC-1 cells. This study suggests that 5-Aza-CdR induces ZO-1 expression by upregulating miR-126 through a DNA methylation-dependent mechanism in endothelial cells.

Keywords

5-Aza-CdR, Tight Junction, Zonula Occludens-1, miR-126, DNMTs