SCiRS-7 Enhances the Resistance of Endometrial Cancer Cells to Endoplasmic Reticulum Stress by Inhibiting the Expression of miR-7

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Abstract: Objective: To investigate the relationship between circular RNA ciRS-7 and endoplasmic reticulum (ER) stress in endometrial cancer (EC) cells. Methods: Stable ciRS-7-overexpressing Ishikawa EC cells were constructed via lentiviral-transfection. Proliferation, migration, and invasion were assessed via colony formation, scratch, and Transwell assays. MiR-7 levels and ER stress/apoptosis-related proteins were analyzed by qRT-PCR and Western blot. Overexpression/knockdown models were used to validate mechanisms. Results: ciRS-7 overexpression enhanced proliferation, migration, invasion, and ER stress resistance by suppressing miR-7, reducing pro-apoptotic proteins. Dual ciRS-7/miR-7 overexpression or ciRS-7 knockdown reversed these effects, increasing apoptosis and ER stress sensitivity. Conclusion: ciRS-7 promotes ECprogression and ER stress resistance via miR-7 inhibition.

***Keywords*:** SCiRS-7, endometrial cancer, miR-7

1. Introduction

Endometrial cancer (EC) is one of the most common malignant tumors in women, with its global incidence and mortality rates increasing annually [1]. EC originates from the malignant transformation of endometrial epithelial cells and is clinically classified into type I and type II. EC is often diagnosed at an advanced age, leading to suboptimal treatment efficacy and poor prognoses in patients with advanced-stage disease [2]. In recent years, immunotherapy has been applied to EC treatment, but highly specific and effective therapeutic targets remain lacking [3].

Thus, identifying suitable therapeutic targets is critical for EC management. Circular RNAs (circRNAs), a class of non-coding RNAs, often regulate tumorigenesis and progression by targeting microRNAs (miRNAs) [4]. CircRNA ciRS-7 was first identified in 2011 by Hansen et al. as originating from the cerebellar degeneration-related protein 1 antisense transcript (CDR1AS) [5]. Further studies revealed that ciRS-7, due to its structural specificity, typically targets miR-7 and acts as an oncogene in the development and progression of various cancers [6]. However, the role of ciRS-7 in tumor cell endoplasmic reticulum (ER) stress responses remains poorly understood. This study preliminarily explores the function and mechanisms of ciRS-7 in ER stress regulation in EC cells.

2. Materials and Methods

7 knockdown, and their respective controls were purchased from Shanghai GenePharma Co., Ltd. Following the manufacturer's protocol, cells were seeded into 24-well plates and cultured in medium containing lentiviral particles. After 24 hours of infection, the medium was replaced with fresh medium. At 48 hours post-infection, cells were selected using 10 μg/mL puromycin to obtain stably transfected cell lines.Cells were lysed with RIPA buffer to extract total protein. Protein concentration was quantified using the BCA assay. Denatured proteins were separated by SDS-PAGE and transferred to methanol-activated PVDF membranes.

Membranes were blocked with 5% skim milk for 1 hour, followed by overnight incubation with primary antibodies at 4 °C. Subsequently, membranes were incubated with secondary antibodies at room temperature for 1 hour. Protein bands were visualized using ECL chemiluminescence. All primary antibodies were diluted at 1:2000, and secondary antibodies at 1:10, 000. Some antibodies were also applicable for subsequent immunofluorescence assays.

3. Result and Discussion

First, lentiviral transfection efficiency was validated via qRT-PCR (Figure 1A). Cells were divided into ciRS-7 overexp ression (ciRS-7-OE) and ciRS-7 overexpression negative control (ciRS-7-OENC) groups. Subsequently, colony formation, s cratch, and Transwell assays were performed to evaluate the impact of ciRS-7 overexpression on the malignant biological be havior of Ishikawa cells. Colony formation assay results demonstrated that ciRS-7-OE cells exhibited significantly higher pr oliferation activity compared to ciRS-7-OENC cells (Figure 1B). Scratch assay results at 48 hours revealed that ciRS-7 overe

xpression markedly enhanced Ishikawa cell migration (Figure 1C). Finally, Transwell assay results showed that ciRS-7 over expression promoted Ishikawa cell invasion (Figure 1D). These findings collectively indicate that ciRS-7 overexpression enh ances the tumorigenic activity of Ishikawa cells.

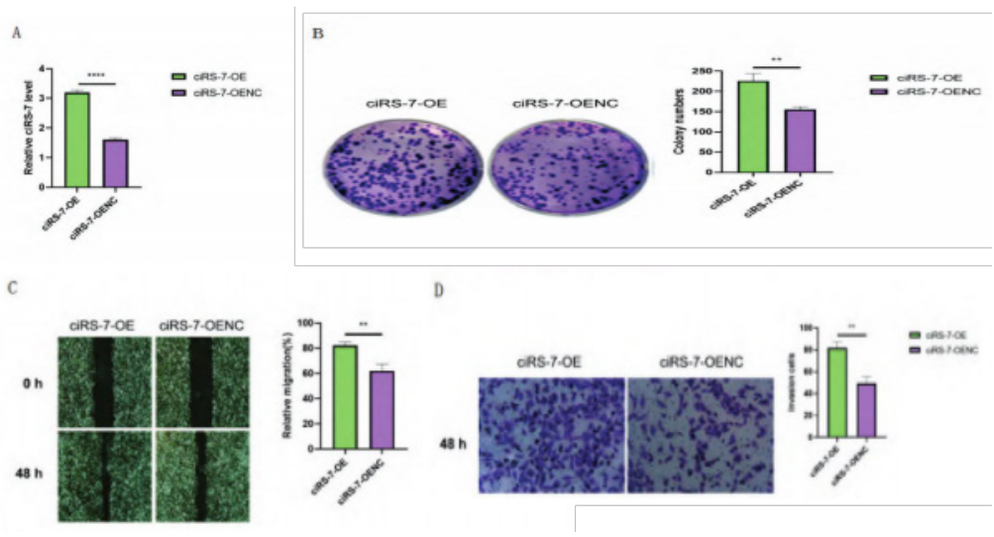

Figure 1. CiRS-7 overexpression enhances Ishikawa cell proliferation, migration, and invasion.

A: Overexpression efficiency of ciRS-7 was validated by qRT-PCR. $P < 0.0001$.

B: Colony formation assay demonstrating the effect of ciRS-7 overexpression on Ishikawa cell proliferative activity. $P < 0.01$.

C: Scratch assay comparing migration ability between ciRS-7-overexpressing cells and negative control (NC) cells. $P < 0.01$

D: Transwell assay evaluating changes in Ishikawa cell invasion capability. $P < 0.01$.

CiRS-7 Reverses ER Stress-Induced Apoptosis by Suppressing miR-7 Expression: Previous studies have shown that ciRS-7 primarily functions in cancer cells by targeting miR-7 [6]. To investigate whether the ciRS-7/miR-7 axis is involved in Ishikawa cell resistance to ER stress, miR-7 expression levels in cells subjected to 24-hour serum starvation were measured by qRT-PCR. Results revealed that miR-7 levels in ciRS-7-OE cells were significantly lower than those in ciRS-7-OENC cells (Figure 2A). Ishikawa cells were further co-transfected via dual lentiviral transfection to generate stable ciRS-7/ miR-7 co-overexpressing cells (ciRS-7/miR-7-OE) (Figure 2B). Following 24-hour starvation to induce ER stress, Western blot analysis showed no significant difference in ER stress levels between ciRS-7/miR-7-OE and ciRS-7-OE groups. However, pro-apoptotic proteins Bax and Bad were significantly upregulated in ciRS-7/miR-7-OE cells compared to ciRS-7-OE cells (Figure 2C). These results suggest that ciRS-7 suppresses ER stress-induced apoptosis by inhibiting miR-7 expression.

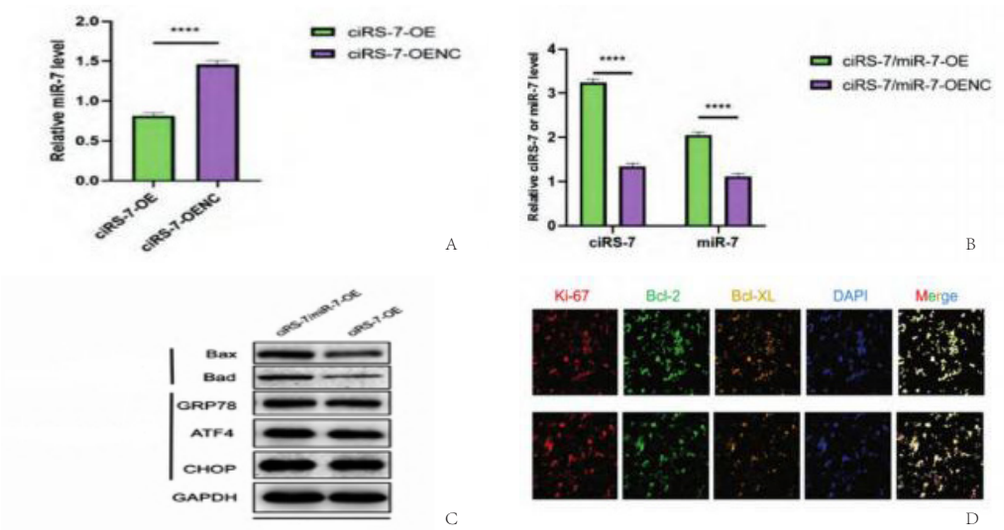

Figure 2. CiRS-7 reverses ER stress-induced apoptosis by suppressing miR-7 expression

A: miR-7 expression levels in cells after 24-hour serum starvation were detected by qRT-PCR. $P < 0.0001$.

B: Dual overexpression efficiency of ciRS-7 and miR-7 was validated by qRT-PCR. $P < 0.0001$.

C: Western blot analysis of ER stress-related proteins and pro-apoptotic protein

expression levels in ciRS-7/miR-7 co-overexpressing and ciRS-7 single-overexpressing cells after 24-hour serum starvation.

D: Untreated cells and ciRS-7-overexpressing cells (ciRS-7-OE) subjected to 24-hour serum starvation were analyzed for expression of Ki-67, Bcl-2, and Bcl-XL. Fluorescence labeling: Red: Ki-67 (proliferation marker). Green: Bcl-2 (anti-apoptotic protein). Yellow: Bcl-XL (anti-apoptotic protein). Blue: DAPI (nuclear counterstain).

Endometrial cancer (EC) is one of the most prevalent malignancies in women, posing a serious threat to their health. However, current clinical strategies for EC treatment remain limited, necessitating a focus on molecular-level therapeutictargets. Endoplasmic reticulum (ER) stress plays a critical role in cellular homeostasis and biological processes. Environmental stressors such as osmotic pressure, nutrient deprivation, and hypoxia can induce ER stress to varying degrees [7]. ER stress often triggers significant apoptosis, which can be exploited for tumor cell elimination [8]. For example,surfactin-induced ER stress enhances apoptosis in osteosarcoma cells [9]. Additionally, recent studies demonstrate that dihydroartemisinin elevates ER stress by modulating reactive oxygen species (ROS) production, promoting apoptosis in human tongue squamous cell carcinoma [10]. Thus, targeting ER stress in combination with clinical therapeutics may offer an effective strategy for cancer treatment.

In this study, we constructed EC cell lines stably overexpressing ciRS-7 and/or miR-7 or with ciRS-7 knockdown to explore the role of the ciRS-7/miR-7 axis in ER stress regulation. Our findings reveal that ciRS-7 enhances EC cell resistance to ER stress and reduces apoptosis by suppressing miR-7 expression. These results highlight the potential of the ciRS-7/miR-7 axis as a therapeutic target in EC. Future studies should validate these findings in in vivo models to further elucidate the clinical relevance of this axis in EC management. Targeting the ciRS-7/miR-7 axis presents a dual therapeutic opportunity: (1) silencing ciRS-7 to restore miR-7's tumor-suppressive functions or (2) directly enhancing miR-7 activity using mimics or nanoparticle-based delivery systems. Preclinical studies have shown promise in targeting circRNAs with antisense oligonucleotides (ASOs) or smallinterfering RNAs (siRNAs) in other cancers, suggesting similar strategies could be adapted for EC.

While this study establishes a link between ciRS-7 and ER stress resistance in vitro, its translational relevance requires validation in in vivo models. Orthotopic EC mouse models or patient-derived xenografts (PDXs) could replicate the tumor microenvironment's complexity, including hypoxia and nutrient gradients, to assess how ciRS-7 influences ER stress adaptation under physiologically relevant conditions. Additionally, clinical correlation studies analyzing ciRS-7 and miR-7 expression levels in EC patient tissues could reveal prognostic or predictive biomarkers for ER stress-targeted therapies. However, its role in ER stress regulation appears context-dependent. For example, in glioblastoma, miR-7 overexpression exacerbates ER stress-induced apoptosis, whereas in EC, our data suggest ciRS-7's suppression of miR-7 promotes survival. This dichotomy highlights the need for tissue-specific studies to refine therapeutic strategies. Comparative analyses across cancer types could uncover conserved versus unique mechanisms of circRNA-mediated stress adaptation.

4. Conclusion

In summary, this study positions ciRS-7 as a key regulator of ER stress tolerance in EC via miR-7 suppression, offering a novel avenue for therapeutic intervention. Future work should integrate multi-omics approaches, in vivo validation, and preclinical drug testing to translate these findings into actionable therapies. By unraveling the circRNA-miRNA networks governing stress responses, we move closer to overcoming the clinical limitations of current EC treatments and improving patient outcomes.

Acknowledgments

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