

## A Novel Upregulated circ\_0032746 on Sponging with MIR4270 Promotes the Proliferation and Migration of Esophageal Squamous Cell Carcinoma

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**Abstract :** Background: Esophageal squamous cell carcinoma (ESCC) is a tumor arising from esophageal epithelial cells and is one of the major disease subtype in Asian countries, including China. Esophageal cancer is the 7th highest incidence based on the 2020 data of GLOBOCAN. The pathogenesis of cancer is still not well understood as many molecular and genetic basis of esophageal carcinogenesis has yet to be clearly elucidated. Circular RNAs are RNA molecules that are formed by back-splicing covalently joined 3'- and 5'-ends rather than canonical splicing, and recent data suggest circular RNAs could sponge miRNAs and are enriched with functional miRNA binding sites. Hence, we studied the mechanism of circular RNA, its biological function, and the relationship between microRNA in the carcinogenesis of ESCC. Methods: 4 pairs of normal and esophageal cancer tissues were collected in Zhongda hospital, affiliated to Southeast University, and high-throughput RNA sequencing was done. The result revealed that circ\_0032746 was upregulated, and thus we selected circ\_0032746 for further study. The backsplice junction of circRNA was validated by sanger sequence, and stability was determined by RNase R assay. The binding site of circRNA and microRNA was predicted by circinteractome, miranda and RNAhybrid database. Furthermore, circRNA was silenced by siRNA and then by lentivirus. The regulatory axis of circ0032746/miR4270 was validated by shRNA, mimic, and inhibitor transfection. Then, in vitro experiments were performed to assess the role of circ0032746 on proliferation (CCK-8 assay and colon formation assay), migration and invasion (Transwell assay), and apoptosis of ESCC. Results: The upregulated circ0032746 was validated in 9 pairs of tissues and 5 types of cell lines by qPCR, which showed high expression and was statistically significant ( $P < 0.005$ ). Upregulated circ0032746 was silenced by shRNA, which showed significant knockdown in KYSE 30 and TE-1 cell lines expression compared to control. Nuclear and cytoplasmic mRNA fraction experiment displayed the cytoplasmic location of circ0032746. The sponging of miR4270 was validated by co-transfection of sh-circ0032746 and mimic or inhibitor. Transfection with mimic showed the decreased expression of circ\_0032746, whereas inhibitor inhibited the result. In vitro experiments showed that silencing of circ\_0032746 inhibited the proliferation, migration, and invasion compared to the negative control group. The apoptosis was seen higher in a knockdown group than in the control group. Furthermore, 11 common microRNA target mRNAs were predicted by Targetscan, MirTarbase, and miRanda database, which may further play role in the pathogenesis. Conclusion: Our results showed that novel circ\_0032746 is upregulated in ESCC and plays role in its oncogenicity. Silencing of circ\_0032746 inhibits the proliferation and migration of ESCC whereas increases the apoptosis of cancer cells. Hence, circ0032746 acts as an oncogene in ESCC by sponging with miR4270 and could be a potential biomarker in the diagnosis of ESCC in the future.

**Keywords :** circRNA, esophageal squamous cell carcinoma, microRNA, upregulated

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