Methods

Reagents and Cell culture

The reagents used in the study are as follows: Dual-Luciferase® Reporter Assay (Promega, #E1901), LipofectamineTM 3000 (Invitrogen, Carlsbad, CA). 293T human embryonic kidney fibroblasts(ATCC, Manassas, VA, USA) and H4 human glioma cells(ATCC, Manassas, VA, USA) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO, USA), 100 U penicillin, and 0.1 mg streptomycin (Sigma) per milliliter, at 37°C and 5% CO_2 in a humidified atmosphere.

Luciferase reporter assay

Based on the binding sequence of has-miR-155-5p, the full-length of CTB-89H12.4 and the 3' UTR of ERBB3 containing the has-miR-155-5p binding site was amplified by PCR. The human cDNA was used as a template for PCR amplification and inserted into the pGL3 vector (Promega, WI) and named ERBB3-WT and ERBB3-mut reporter. The PCR product of CTB-89H12.4 was cloned into pcDNA3.1(+). Then, 1×10^6 cells were cotransfected with hsa-miR-155-5p mimics or 155-5p inhibitor (final concentration of 50 nM), 1 μg of ERBB3-WT (or ERBB3-mut) plasmid using Lipofectamine 3000. After 48 h transfection, luciferase activity was measured using a luciferase assay kit. The cells were collected, and quantitative detection was performed subsequently.

RNA extraction and Real-time quantitative Reverse Transcription PCR (qRT-PCR)

Total RNA was extracted using Trizol reagent. The ABI reverse transcription kit was used for reverse transcription of the target mRNA, and the miRNA reverse transcription and quantitative detection kit of Guangzhou Ruibo was used for detection. The primer sequences used are shown in Table S1.