

RT-qPCR assay: The total RNA from A431 cell line were extracted by TRIzol reagent (Invitrogen).

The RNA were reverse-transcribed into complementary DNA by PrimeScript® RT reagent Kit (Takara Bio Inc., Kusatsu, Shiga, Japan) on a S1000™ Thermal Cycler (BioRad Laboratories, Inc., Hercules, CA, USA) at 37 °C for 10 minutes and 85 °C for 6 seconds. The SERPINB1 mRNA level was measured using the SYBR® Premix Ex Taq™ (Takara Bio Inc.,) on a QuantStudio™ 6 Flex Real-Time PCR System (Thermo Fisher Scientific, Inc.).

The initial denaturation was performed at 90°C for 30 s, and the subsequent PCR reaction was conducted at 90°C for 5 s and at 60°C for 30 s for 40 cycles. The primers of SERPINB1 and GAPDH were as follows: SERPINB1, Forward: GCCAAGGTCCTGGAAATACCA, Reverse: TTCCATCAATTTCTCAGCAGTGA; GAPDH, Forward: CTGGCCGGGACCTGACT, Reverse: GCAGCCGTGGCCATCTC. The experiment were analyzed in triplicate.

The relative fold changes were calculated by the $2^{-\Delta\Delta C_t}$ method, and GAPDH was used as an internal reference.

Western Blot assay: The cells were lysed in RIPA lysis buffer (Beyotime Biotechnology, Inc., Jiangsu, China) with PMSF (1 mmol/L) (Beyotime Biotechnology, Inc., Jiangsu, China) for 10 minutes on ice with intermittent vortex, followed by centrifuging at 12,000 g at 4°C for 15 minutes. The supernatants were collected immediately. The protein concentrations were analyzed using BCA Protein Assay kit (Beyotime Biotechnology, Inc., Jiangsu, China). Protein lysates separated by sulfate-polyacrylamide gel electrophoresis were then transferred onto polyvinylidene difluoride membrane (Millipore, MA, USA). The anti-SERPINB1 antibody (Abcam, Inc., USA, catalogue number: Ab181084, with antibody dilutions of 1: 2000) was added as primary antibody and anti-GAPDH was used as an internal reference control. The bands were detected by enhanced chemiluminescence using Pierce™ SuperSignal West Femto Chemiluminescent substrate (Thermo Fisher Scientific, Inc.). The experiment were analyzed in triplicate.

Wound healing assay: Cells were seeded into six-well plates and then cultured for 24 h to 90% confluence. A linear wound was performed by scratching the sub-confluent cell monolayer using a 200 µl pipette tip, and the debris was removed by washing with phosphate-buffered saline. After incubation at 37 °C for 48 h, cells were photographed and wound width were obtained at 0 h and 24 h. The experiment were analyzed in triplicate.

Trans-well assays: The cells were harvested and re-suspended in 200 µl serum-free medium at a density of 10⁶/mL. The re-suspended cells were seeded into the upper chamber of 24-well trans-well chambers with Matrigel (BD Biosciences, NJ, USA) for the invasion assay. About 600 µl 20% fetal bovine serum medium was added to the lower chamber. After 24 h, cells on the lower surface of the inserts were stained with 0.1% crystal violet. Cells were viewed under a microscope and counted in five random microscopic fields. The experiment were analyzed in triplicate.