2. Materials and methods

2.6. RT-qPCR (Real-time quantitative reverse transcription polymerase chain reaction)

The reaction steps were as follows: (1) pre-denaturation at 95℃ for 5 min. (2) 95℃ for 10 s, 60℃ for 20 s, 72℃ for 20 s, the above 3 steps for a cycle, repeat 40 cycles. (3) 95℃ for 15 s, 60℃ for 60 s, 95℃ for 15 s. The relative expression levels of the mRNA were normalized to β-actin and then calculated by the formula: $2^{-\triangle\triangle Ct}$, then compared by GraphPad Prism 8.0 software (GraphPad Software, San Diego, CA, USA). The primer sequences were as follows:

Mouse Beta-actin forward, 5'-AACAGTCCGCCTAGAAGCAC-3';

Mouse Beta-actin reverse, 5'-CGTTGACATCCGTAAAGACC-3';

Mouse RELMβ forward, 5'-ATGAAGCCTACACTGTGTTTCC-3';

Mouse RELMβ reverse, 5'-CTGCCAGAAGACGTGACACT-3';

Mouse E-cadherin forward, 5'-CAGTTCCGAGGTCTACACCTT-3';

Mouse E-cadherin reverse, 5'-TGAATCGGGAGTCTTCCGAAAA-3';

Mouse α-SMA forward, 5'-CCCAGACATCAGGGAGTAATGG-3';

Mouse α-SMA reverse, 5'-TCTATCGGATACTTCAGCGTCA-3';

Mouse Vimentin forward, 5'-CGTCCACACGCACCTACAG-3';

Mouse Vimentin reverse, 5'-GGGGGATGAGGAATAGAGGCT-3';

Human Beta-actin forward, 5'-CTCCATCCTGGCCTCGCTGT-3';

Human Beta-actin reverse, 5'-GCTGTCACCTTCACCGTTCC-3';

Human RELMβ forward, 5'-CCGTCCTCTTGCCTCCTTC-3';

Human RELMβ reverse, 5'-CTTTTGACACTAGCACACGAGA-3';

Human E-cadherin forward, 5'-CGAGAGCTACACGTTCACGG-3';

Human E-cadherin reverse, 5'-GGGTGTCGAGGGAAAAATAGG-3';

Human Vimentin forward, 5'-GACGCCATCAACACCGAGTT-3';

Human Vimentin reverse, 5'-CTTTGTCGTTGGTTAGCTGGT-3';

2.7. Immunoblotting

The incubated primary antibodies were as follows: anti-RELM-β (ab11429, Abcam, Cambridge, MA), which can only react with mouse; anti-RELM-β (ab84224, Abcam, Cambridge, MA), which can only react with human; anti-E-cadherin (3195T, CST, Danvers, MA, USA), anti-vimentin (ab92547, Abcam, Cambridge, MA), anti-α-SMA (19245T, CST, Danvers, MA, USA) and anti-β-tubulin (2128T, CST, Danvers, MA, USA), which can react with both mouse and human. The incubated secondary antibodies were peroxidase affinipure goat anti-mouse IgG (H+L) (Jackson ImmunoResearch, 115-035-003) and peroxidase affinipure goat anti-rabbit IgG (H+L) (Jackson ImmunoResearch, 111-035-003).