Label-free detection of Chondroitin Sulphate Proteoglycan 4 by a Polyaniline/graphene Nanocomposite Functionalized Impedimetric Immunosensor

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Supplementary information

Direct ELISA and sandwich ELISA for measuring the CSPG4 in cell culture medium and cell membrane protein

Standard ELISA protocol was conducted to detect cell samples. The supernatant of cell growth medium and cell membrane protein were prepared following the experiment condition detailed in ***section 2.3 preparation of cell sample***.

Direct ELISA: 100 μL cell growth medium supernatant or cell membrane protein was pipetted into a 96-well microplate (Fisher Scientific, USA) and incubated at 37°C for 2 hour. The cell sample was discarded and the micro-well was filled with 150 μL 2% bovine serum albumin (BSA). After the BSA blocking (37°C for 2 hour), the micro-wells were gently washed by TBS-T (pH 7.4 0.05 M tris buffer saline- 0.5% tween 20). Next, 100 μL anti-CSPG4 specific mAb D2.8.5 (0.1 mg/mL), isotype control mAb Mk2-23 (0.1 mg/mL) was added into the cell samples immobilized micro-well and incubated for 1 hour at 37°C. The wells were thoroughly washed by TBS-T buffer. Then, 100 μL HRP-lablled rabbit anti-mouse IgG Abs (1:10000, Sigma) was added and incubated at 37°C for 45 minute. After five-times TBS-T washing, 100 μL TMB solution was pipetted into wells. After 15 minute reaction, 50 μL H_2SO_4 was added into each well. The absorbance value at 450 nm was measured using a spectrophotometric microplate reader (ELx800t, Gene Company).

Sandwich ELISA: 100 μL anti-CSPG4 specific mAb D2.8.5 (0.1 mg/mL) was added into 96-well microplate and incubated at 37°C for 2 hour. After the BSA blocking (37°C for 2 hour), 100 μL cell growth medium supernatant or cell membrane protein was pipetted into a 96-well microplate and incubated at 37°C for 2 hour. The wells were washed 3 times with TBS-T washing buffer. 100 μL anti-CSPG4 specific mAb 763.74 (0.05 mg/mL) was added into each well for 2 hour. After 3 times TBS-T washing, 100 μL peroxidase-conjugated rabbit anti-mouse IgG Abs was added. Following washing step, peroxidase substrate TMB solution was added. Finally, the absorbance value at 450 nm was measured by a microplate reader.

All experiments were repeated three times in triplicates.