Cell cultures, transfection and treatments

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ABSTRACT

Human glioblastoma U251 cells and rat brain glioma C6 cells were grown to confluence in Dulbecco´s Minimal Essential Medium (DMEM) supplemented with 10% (v/v)fetal bovine serum (FBS),1%(v/v)Penicillin-Streptomycin and 1% (v/v) L-glutamine, and maintained at 37ºC in a humidified atmosphere containing 5% CO_2. Medium was changed every other day and cells were passed once a week by trypsinization. Briefly, cells were incubated with TrypLE Express dissociation reagent for 5 min at 37ºC for cell detachment, followed by a centrifugation step at 300 g for 5 min at room temperature and sub-cultured according to the desired dilution. For fluorescent microscopy and single-molecule tracking experiments, 3×10^5 cells were grown on 35 mm glass-bottom dishes (21 mm glass surface diameter, Ibidi GmbH, Gräfelfing, Germany). For flow cytometry, 3×10^5 cells were seeded on 6-well plates (35 mm diameter, Corning, NY,USA). For Western blotting, 8×10^5 cells were seeded on 60 mm dishes (Orange Scientific, Braine-L'Alleud,Belgium). Twenty-four hours after seeding, cells were transiently transfected with 1 µg of the corresponding DNA constructs using jetPRIME transfection reagent (Polyplus transfection, Illkirch, France) in a proportion of 3:1 (µL of transfection reagent: µg of DNA).
All drugs and HaloTag ligands were diluted in DMSO (100% v/v). To test the neuroprotective activity of CNB-001 (0.1, 1 or 10 µM), cells were treated for 20 min before transfection and incubated for 24 h at 37ºC. For single-molecule experiments, 24 h after transfection, serum was removed for 2 h, and cells were treated with LIF (100 ng/ml), Nocodazole (10 µM) or Latrunculin B (10 µM) for 2 h at 37ºC in serum-free medium. Cells were then incubated with JF647 (100nM), JF549 (100 nM) or PA-JF549 (1nM) HaloTag ligands in serum-free medium for 20 min at 37ºC.Subsequently, cells were washed twice with sterile PBS. At the end of the final wash, the medium was changed to Leibovitz's L-15 without phenol red.

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MATERIALS TEXT

Cell culture media (Dulbecco's Modified Eagle Medium -DMEM- and Leibovitz's L-15 medium), Fetal Bovine Serum (FBS), penicillin-streptomycin commercial antibiotic mixture (Pen-Strep), L-glutamine, TrypLE Express dissociation reagent, Ca^{2+}- and Mg^{2+}-free phosphate buffer saline (PBS) 1X and Leukemia inhibitory factor (LIF) were obtained from Gibco (Waltham, MA, USA). Janelia Fluor 64 7nm (JF647) and Janelia Fluor 549 nm (JF549) HaloTag ligands were obtained from Promega Corporation (Madison, WI, USA), and the photoactivatable Janelia Fluor 549 nm (PA-JF549) HaloTag ligand was a kind gift from Dr.Luke Lavis (HHMI's Janelia research campus, Ashburn, VA, USA). Nocodazole was acquired from Target Mol (Wellesley Hills, MA, USA) and Latrunculin B from Focus Biomolecules (Plymouth Meeting, PA, USA). J147 and CNB-001 drugs were a kind gift from Dr. David Schubert (The Salk Institute for Biological Studies, LA Jolla, CA, United States). Human U251 and rat C6 glioma cells were obtained from Public Health England (Salisbury, UK).

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