Induced senescence and calcification in anaplastic meningioma

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Supplementary Methods

Immunofluorescence

Meningioma cells were seeded at a density of 1×10^4 cells per well on Matsunami glass in 96-well plates overnight. After fixation with ice-cold methanol, cells were permeabilized with 0.1% phosphate-buffered saline/Tween, and blocked with 1% bovine serum albumin. The cells were then stained with primary antibodies overnight at 4˚C. After incubation, Alexa Fluor 488 or Alexa Fluor 555 conjugated with goat anti-rabbit or goat anti-mouse immunoglobulin G secondary antibody at a ratio of 1:500 was added and incubated for 1 hour at room temperature in the dark. Cells were mounted with 4',6-diamidino-2-phenylindole-containing encapsulant and then photographed under a fluorescence microscope (ZEISS Axio Imager.A1; ZEISS Japan, Tokyo, Japan).

Histological Examination

Hematoxylin and eosin staining was performed with a standard method. Immunoperoxidase staining was performed with an Envision kit (Dako, Tokyo, Japan) according to the manufacturer's instructions with minor modifications. Briefly, the sections were placed in 0.01 M citrate buffer and heated in a microwave oven (700 W) for 10 minutes, and endogenous peroxidase activity was blocked with 0.5% hydrogen peroxidase in methanol for 30 minutes, followed by incubation with normal goat serum for 1 hour at room temperature. Thereafter, the sections were incubated with primary antibody for 12 hours at 4°C. The sections were then washed with phosphate buffered saline (PBS) and incubated with secondary antibody for 10 minutes at room temperature. The reaction was revealed with 0.02% 3.30-diaminobenzidine.

Antibody

The primary antibodies were KI-67 (M7240, 1:50 dilution) and vimentin (M0725, 1:1 dilution) purchased from Agilent Technology (Santa Clara, CA, USA), GREM2 (13892-1-AP, 1:100 dilution) purchased from Proteintech (Rosemont, IL, USA), and BMPR1A (38-6000, 1:100 dilution) purchased from Thermo

Fisher Scientific (Waltham, MA, USA).

Water-Soluble Tetrazolium Salt (WST-8) Assay

After initial seeding and culture of the tumor cells, the culture medium was removed and replaced with various concentrations of K02288-containing medium. After 3 days or 7 days of incubation, 20 µl WST-8 dye (Cell Counting Kit-8; Dojindo Corporation, Kumamoto, Japan) was added to each well. After 3 hours, the plates were read at 450 nm/655 nm. For half maximal inhibitory concentration (IC_{50}) calculation, cells were incubated with various concentrations of K02288 for 3 days. IC_{50} values were calculated by linear approximation regression of the percentage survival versus the drug concentration using GraphPad PRISM9 software.

Gene Expression Analysis

Gene expression was analyzed with a GeneChip™ System with a Human Genome Clariom™ D Array (Thermo Fisher Scientific) according to the manufacturer's instructions. mRNA expression analysis used amplification and biotin labeling of fragmented cDNA with the biotin labeling system (GeneChip™ WT PLUS Reagent Kit; Thermo Fisher Scientific) in duplicate. Labeled probes were hybridized to the Human Genome Clariom™ D Array (Thermo Fisher Scientific) with the GeneChip™ Scanner 3000 7G (Affymetrix®; Thermo Fisher Scientific). Expression data of mRNA were extracted from image files using GeneSpring GX 14.9.1 (Agilent Technologies, Santa Clara, CA, USA). Normalization and expression value calculations were performed using the GeneSpring system.