ON-LINE APPENDIX: METHODS

Quantitative qRT-PCR

TRIzol reagent (Invitrogen) was used to extract total RNA from cell lines and clinical samples, and the RNA samples were reverse-transcribed using a ReverTra Ace qPCR RT kit (Toyobo). Piezo1 expression was monitored by real-time PCR applying Biosystems SYBR Green Master Mix (Toyobo). The qRT-PCR results were
AQ: O normalized to the stable housekeeping gene *β-Actin*. PCR primers were designed using Primer Express Version 2.0 software (Applied Biosystems), and the sequences of the primers used were as follows: Actin-F: 5'-TCAAGATCATTGCTCCTCCTGAG-3', Actin-R: 5'-ACATCTGCTGGAAGGTGGACA-3.

Western Blot Analysis

The protein obtained from cell lysates and tumor tissue was lysed with RIPA lysis buffer (Beyotime) supplemented with protease inhibitors. The protein concentrations were measured using the Bio-Rad Protein Assay kit (Life Science Research). Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and then transferred to polyvinylidene difluoride membranes. After transfer of the membrane, the polyvinylidene difluoride membrane was placed in 1% Bis(trimethylsilyl)acetamide in Tris-buffered saline/0.1% Tween (TBST; Millipore Sigma) for 1 hour at room temperature and then incubated with the primary antibodies overnight at 4°C. After three 10-minute washes in TBST, the membranes were incubated with the corresponding secondary antibodies for 1 hour at room temperature, followed by three 10-minute washes in TBST. Finally, protein visualization was performed using an automatic chemiluminescence imaging analysis system.

IHC

All tumor tissues were examined by histopathology and IHC after surgical resection. The tumor tissue and normal brain tissue were cut into 5-mm-thick sections and affixed to (3-Aminopropyl) triethoxysilane glass slides. The paraffin slices were baked in a 60°C incubator for 120 minutes. Then the paraffin sections were treated with dewaxing, hydration, and an antigen repair solution. After being blocked by endogenous peroxidase, the paraffin sections were incubated with corresponding primary antibodies overnight at 4°C and then treated with anti-mouse/rabbit biotinylated antibody (DAKO) at room temperature for 30 minutes. The paraffin sections were developed with diaminobenzidine. All paraffin sections were restained with Mayer hematoxylin. The proliferation degree of the tumor cells was estimated on the basis of the number of cells that stained positive for the Ki-67 antigen. The primary antibody used was an anti-Ki-67 rabbit monoclonal antibody (catalog No. GB13030-2; Wuhan Servicebio Technology Co). According to the median value of the Ki-67 proliferation index, Ki-67 expression was divided into low (Ki-67 $<$ median value) and high (Ki-67 $\geq$ median value) expression.

The primary Piezo1 antibody (catalog No. 15939-1-AP; dilution, 1:200) was purchased from the Proteintech Group. The immunostaining steps were performed as described previously. The evaluation of Piezo1 expression levels mainly depended on both the cell staining intensity and the proportion of positively stained cells. The proportion and staining intensity of positive cells under a microscope were scored by 2 individuals. Five high-power visual fields (400 $\times$ 100 cells) were randomly collected from each slice for observation. Staining intensity was scored as follows: 0 (unstained), 1 (weakly stained), 2 (moderately stained), and 3 (strongly stained). The positive staining tumor cell score (PSTCS) was classified as follows: 0 (PSTCS $\leq$ 5%), 1 (5% $<$ PSTCS $\leq$ 25%), 2 (26% $<$ PSTCS $\leq$ 50%), and 3 (50% $<$ PSTCS). The IRS is equal to the product of the staining intensity score and the PSTCS. According to the IRS value, Piezo1 expression was divided into low (IRS $<$ 4) and high (IRS $\geq$ 4) expression.

IHC was performed on specimens using an *IDH1* R132H antibody (catalog No. DIA-H09; dilution, 1:50) purchased from Dianova and a p53 antibody (catalog No. M7001; dilution, 1:50) purchased from DAKO. The *IDH1* status was based on *IDH1* R132H staining, which was divided into positive reactions (strong cytoplasmic immunoreaction) and negative reactions (weak diffuse staining and unstained cells). The p53 status was based on the nuclear staining cell rate. Specimens with a nuclear staining cell rate of at least 10% were considered positive for p53, and those with $<$10% were considered negative for p53.