**Materials and Methods**

*Patients*

The study included 30 patients recruited from the Children’s Hospital of Fudan University (Shanghai, China) between June 2008 and June 2016 (KHE, 15; TA, 5; IH, 5; and LM, 5). The study was approved by the Local Research Ethics Committee of our hospital, and written informed consent was obtained from the parents of each patient. Each tissue sample was fixed with 10% formaldehyde, embedded in paraffin, and sectioned. All the sections were examined by one pathologist.

*Immunohistochemistry Staining*

Between 18 and 24 h after fixation, the tissue blocks were embedded in paraffin, then cut into 4-μm-thick sections and affixed onto slides. Antigen retrieval was needed to break the methylene bridges and expose the antigenic sites after being deparaffinized and rehydrated. The antigens were retrieved in boiled sodium citrate buffer (10 mM sodium citrate and 0.05% Tween 20 [pH 6.0]) for 15 min and cooled naturally to room temperature. The reaction was blocked by immersing the sections in 10% goat serum with 1% bovine serum albumin (BSA) in Tris-buffered saline (TBS) for 2 h at room temperature, then incubating the sections with primary antibody diluted in TBS with 1% BSA (anti-TSC2 antibody, 1:200, ab32554 Abcam; cytoplasm, membrane; anti-p-mTOR antibody, 1:200, ab84400; Abcam; cytoplasm, nucleus; anti-p-S6K1 antibody, 1:200, ab131436; Abcam; cytoplasm, nucleus; anti-p-4EBP1 antibody, 1:200, 2855T; CST; cytoplasm, nucleus; and anti-PTEN antibody, 1:200, 9188S; CST; cytoplasm, nucleus) at 4°C overnight. The next day, the slides were incubated in 0.3% H2O2 in TBS for 20 min. An enzyme-conjugated secondary antibody was applied (Dako EnVision+ System-HRP labeled polymer, K4003; Dako) to the slide and incubated for 2 h at room temperature. The DAB polymer detection kit was used as a developing reagent (Gene Tech) according to the manufacturer’s instructions. Finally, the slides were counterstained with hematoxylin. Omission and substitution of primary antibody with antibody diluent were used as the reagent blank to ensure the validity of the staining.

*Image Analysis*

The immunostaining images were selected from at least 3 separate microscope fields of view and were semiquantified with Image-Pro Plus software (version 7.0.1.658; Media Cybernetics, Rockville, MD, USA) [19]. The measurement parameters included integral optical density (IOD) and area sum. The optical density was calibrated and the area of interest was set as follows: hue, 0–35; saturation, 20–255; and intensity, 0–255. The image was then converted to grayscale, and the values were measured. We used the stained area IOD/area to represent the degree of protein expression.

*Statistical Analyses*

Data analysis was performed using Stata 10.1 statistical software (StataCorp, 4905 Lakeway Drive). Data are expressed as the mean ± SD, and statistical analyses were carried out using a *t* test. A *p* < 0.05 was defined as statistically significant.