Electronic supplementary materials

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Comparative analysis of a panel of biomarkers related to protein phosphatase 2A between laryngeal squamous cell carcinoma tissues and adjacent normal tissues*#

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Data S1 Materials and methods

Materials

The primary antibodies used in western blot are as follows: anti-phospho-Y307 PP2A/C rabbit mAb (Abcam), anti-α4 mouse mAb (Upstate), anti-CIP2A rabbit mAb (Cell Signaling Technology), anti-phospho-Akt (Thr308) rabbit mAb (Cell Signaling Technology), anti-Akt rabbit Ab (Cell Signaling Technology), anti-phospho-Ezrin (Thr567) rabbit mAb (Cell Signaling Technology), anti-Ezrin rabbit mAb (Cell Signaling Technology), anti-14-3-3 rabbit Ab (Abcam), anti-FAK rabbit mAb (Cell Signaling Technology), anti-β-actin mouse mAb (Abcam), anti-α-tubulin mouse mAb (Abcam) and anti-GAPDH mouse mAb (Kang Chen). The secondary antibodies used in western blot are as follows: IRDye 800CW goat anti-rabbit IgG, IRDye 680LT goat anti-mouse IgG (LI-COR), HRP-linked anti-rabbit/mouse IgG (Cell Signaling Technology). ECL western blotting substrate was from ThermoFisher Scientific. Other chemicals and reagents were purchased from commercial sources.

Clinical specimens

28 pairs of pathologically confirmed laryngeal squamous cell carcinoma samples and matched adjacent normal samples were collected from patients with primary laryngeal carcinoma who underwent surgical laryngectomy at the Department of Otorhinolaryngology, Second Affiliated Hospital, Zhejiang University School of Medicine between 2012 and 2015. Sample acquisition was approved by the institutional review board of Second Affiliated Hospital, Zhejiang University School of Medicine in accordance with the Helsinki Declaration, and written informed consent

was signed by each patient. All of the patients were cigarette smokers and most of them admitted frequent consumption of strong alcohols. The fresh specimens were immediately taken after the surgery, and were stored in liquid nitrogen for western blot assay.

Western blot

For patient tissues, proteins were extracted by homogenization with lysis buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 15 mM EDTA, 0.1% TritonX-100, 1 mM PMSF, 1 mM NaF, and 1 mM Na_3VO_4) on ice for 15 min. After centrifugation at 16000*g* for 20 min to precipitate the pellet, the protein in supernatant was quantified using the BCA assay. Next, the proteins were denatured and loaded onto SDS-polyacrylamide gels for electrophoresis (50 V for 30 min in an 8% stacking gel and then 90 V for 70 min in a 12% separation gel). The proteins were then transferred to nitrocellulose membranes (100 V for 90 min). After the membranes were blocked with TBS (50 mM Tris-HCl, pH 7.6, 150 mM NaCl) containing 5% non-fat milk for 3 h at room temperature, primary antibodies were applied for incubation at 4°C overnight and then appropriate secondary antibodies for 1 h at room temperature on the following day. These procedures utilized the dilutions of the antibodies recommended by manufacturers. The target bands were visualized using an infrared imaging system (LI-COR Biosciences) or a chemiluminescence imaging system (OmegaLum C, Aplegen). Representative figures for several patients were shown. α-tubulin, β-actin or GAPDH was used as the internal control according to the molecular weight. The densitometric data were collected using Image J software (National Institutes of Health) for further statistical analysis. All data were expressed as the means ± SEM from more than three representative patients (n=3). Pairwise comparisons between the means were tested using a two-tailed Student's t test. * P<0.05; ** P<0.01.