

47-mG_{2a}: A Mouse IgG_{2a}-Type of PcMab-47 Useful for Detecting Podocalyxin in Esophageal Cancers by Immunohistochemistry

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Esophageal cancer is one of the highly malignant cancers. It comprises two of the most common histological tumor types: squamous cell carcinoma (SCC) and adenocarcinoma. SCC accounts for about 90% of esophageal cancers. Despite developments in treatment strategies, the prognosis and survival rate remain poor. Podocalyxin (PODXL) is a highly glycosylated type-I transmembrane protein. It is expressed in normal tissues such as kidney, heart, breast, and pancreas. Upregulation of PODXL correlates with tumor progression, invasion, and metastasis. Therefore, this glycoprotein could be a potential biomarker for predicting the prognosis of some cancers, for instance, brain, colorectal, oral, lung, bladder, prostate, and ovarian cancers. We previously developed a specific and sensitive anti-PODXL monoclonal antibody (mAb), PcMab-47 (mouse IgG₁, kappa) and its mouse IgG_{2a}-type (47-mG_{2a}). We showed their utility in immunohistochemical analysis of oral cancers. Herein, we demonstrate that PcMab-47 and 47-mG_{2a} can also be used to detect esophageal squamous cell carcinoma (ESCC) with this technique. These two antibodies, respectively, stained 123/130 (94.6%) and 127/130 (97.7%) ESCC cases, indicating that they can detect PODXL with high sensitivity in this carcinoma. Of more than 3+ cases, 47-mG_{2a} was more effective than PcMab-47, respectively, staining 56/127 (44.1%) and 41/123 (33.3%). Therefore, 47-mG_{2a} can be used for the detection of PODXL in ESCC using immunohistochemical analysis.

Keywords: podocalyxin, PODXL, monoclonal antibody, esophageal cancer

Introduction

ESOPHAGEAL CANCER IS one of the highly malignant tumors with respect to prognosis and mortality rate. It is the eighth most common cancer and most common cause of cancer-related death. More than 450,000 new cases and 400,000 deaths have been reported worldwide.^(1,2) Esophageal cancer comprises two common histological types: squamous cell carcinoma (SCC) and adenocarcinoma (AC). SCC accounts for about 90% of esophageal cancers.^(3,4) Despite improvement in treatment strategies, prognosis remains poor with overall 5-year survival rates ranging from 15% to 25%.⁽⁵⁾ This cancer is treated by surgery that can be complemented by radiotherapy with or without chemotherapy. Preoperative neoadjuvant chemotherapy with cisplatin accompanied by 5-fluorouracil can be effective and is regarded as standard treatment.^(6,7) In contrast, adjuvant therapy is ineffective in many cases.^(8,9) Several clinical trials

testing the treatment potential of monoclonal antibodies (mAbs) against esophageal cancer are ongoing.⁽¹⁰⁻¹²⁾ However, few molecular targeting drugs against this disease have been approved.

Podocalyxin (PODXL) is a type-I transmembrane protein highly glycosylated with *N*-glycan or *O*-glycan⁽¹²⁾ belonging to the CD34 family of sialomucins. It is expressed in normal tissues, such as kidney, heart, breast, and pancreas. PODXL upregulation has been correlated with tumor progression, invasion, and metastasis.⁽¹³⁾ It is used as a diagnostic marker in numerous cancers, including brain,⁽¹⁴⁾ colorectal,⁽¹⁵⁾ oral,⁽¹⁶⁾ lung,^(17,18) bladder,⁽¹⁹⁾ prostate,⁽²⁰⁾ and ovarian cancers.⁽²¹⁾ Indeed, Borg et al. reported that PODXL expression in esophageal AC is an independent prognostic biomarker for poor overall survival.⁽²²⁾ Therefore, specific and sensitive anti-PODXL mAbs are required.

We previously established anti-PODXL mAbs PcMab-47 (mouse IgG₁, kappa)⁽²³⁾ and mouse IgG_{2a}-type (47-mG_{2a}).⁽²⁴⁾ In

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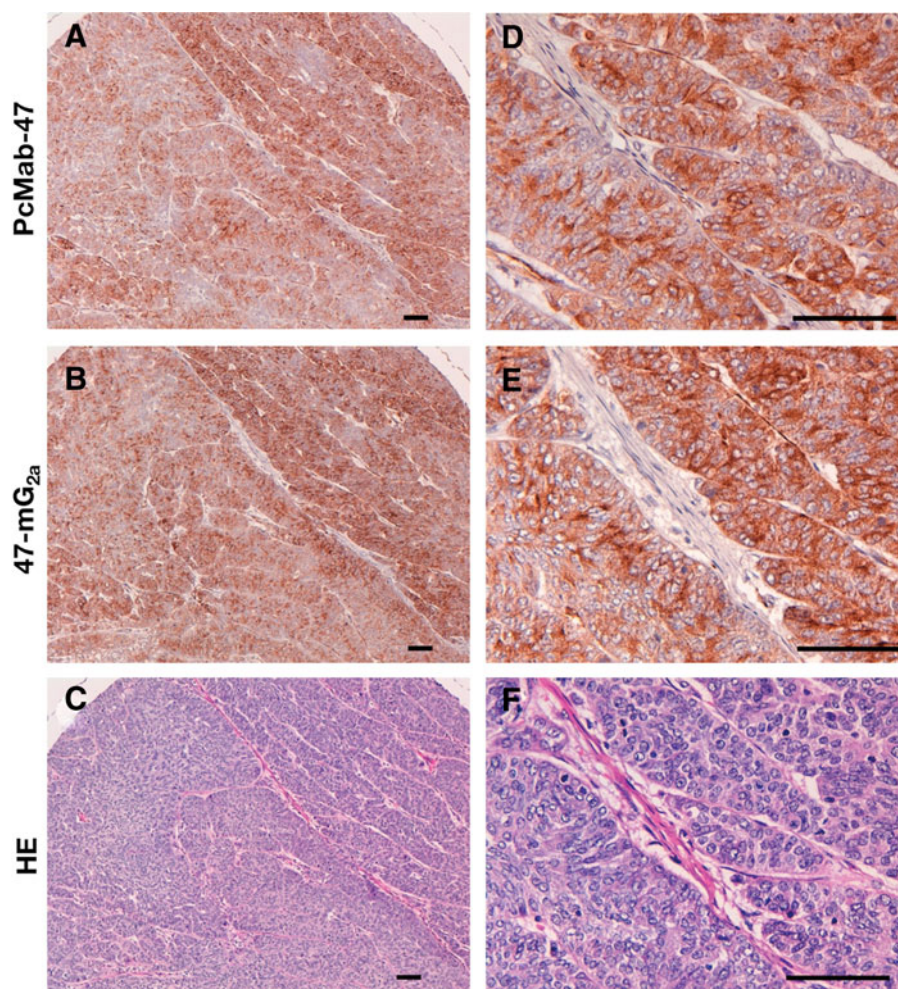


FIG. 1. Immunohistochemical analysis by anti-PODXL antibodies against esophageal squamous cell carcinoma (Case-26). Sections were incubated with 5 $\mu\text{g}/\text{mL}$ of PcMab-47 (A, D) and 0.5 $\mu\text{g}/\text{mL}$ 47-mG_{2a} (B, E) for 1 hour at room temperature followed by treatment with Envision+ kit for 30 minutes. Color was developed using 3,3-diaminobenzidine tetrahydrochloride (DAB) for 2 minutes, and sections were then counterstained with hematoxylin. (C, F) HE staining; scale bar = 100 μm . HE, hematoxylin and eosin; PODXL, podocalyxin.

this study, we investigated their utility for immunohistochemical analysis of esophageal squamous cell carcinoma (ESCC).

Materials and Methods

Tissues

Cancer tissue microarrays of esophageal cancers were purchased from Cybrdi, Inc. (Frederick, MD; Cat. No. CC02-01-001): Case-1–62; and, US Biomax, Inc. (Rockville, MD; Cat. No. BC02011): Case-63–130.

Antibodies

PcMab-47, a mouse anti-PODXL mAb (IgG₁, kappa), was developed as previously described.⁽²³⁾ Appropriate V_H and V_L complementary DNAs (cDNAs) of mouse PcMab-47 and C_H and C_L of mouse IgG_{2a} were subcloned into pCAG-Ble and pCAG-Neo vectors (FUJIFILM Wako Pure Chemical Industries Ltd., Osaka, Japan), respectively, to generate 47-mG_{2a}.⁽²⁴⁾ Antibody expression vectors were transfected into ExpiCHO-S cells using the ExpiCHO Expression System

(Thermo Fisher Scientific, Inc., Waltham, MA). 47-mG_{2a} was purified using Protein G-Sepharose (GE Healthcare Bio-Sciences, Pittsburgh, PA).

Immunohistochemical analyses

Histologic sections (4 μm thick) were deparaffinized in xylene and then rehydrated and autoclaved in citrate buffer (pH 6.0; Agilent Technologies, Inc., Santa Clara, CA) for 20 minutes. Sections were then incubated with 0.5 or 5 $\mu\text{g}/\text{mL}$

TABLE 1. SUMMARY OF IMMUNOSTAINING USING PCMAB-47 AND 47-MG_{2A}

	No. of cases	Immunostaining				No. of positive cases
		–	1+	2+	3+	
PcMab-47	130	7	50	32	41	123/130 (94.6%)
47-mG _{2a}	130	3	31	40	56	127/130 (97.7%)

The intensity of staining was evaluated as –, 1+, 2+, 3+.

primary mAbs for 1 hour at room temperature and treated using an Envision+ kit (Agilent Technologies, Inc.) for 30 minutes. Color was developed using 3,3-diaminobenzidine tetrahydrochloride (Agilent Technologies, Inc.) for 2 minutes, and sections were counterstained with hematoxylin (FUJIFILM Wako Pure Chemical Industries Ltd.). The intensity of staining was evaluated as –, 1+, 2+, or 3+.

Results and Discussion

Previously, we immunized mice with recombinant PODXL and produced PcMab-47 (mouse IgG₁, kappa).⁽²³⁾ This antibody was then engineered into a mouse IgG_{2a}-type mAb (47-mG_{2a}).⁽²⁴⁾ 47-mG_{2a} exhibited a much higher PODXL-binding affinity compared with PcMab-47. Immunohistochemical analysis of oral cancer tissues revealed that those mAbs sensitively stain cells in a cytoplasmic pattern. In this study, we performed immunohistochemical analysis using PcMab-47 and 47-mG_{2a} against ESCC.

Representative immunohistochemistry results of PcMab-47 and 47-mG_{2a} against ESCC are shown in Figure 1. Both antibodies stained cancer cells in a cytoplasmic pattern (Fig. 1). As shown in Table 1 and Supplementary Table S1, PcMab-47 stained 123/130 (94.6%) and 47-mG_{2a} 127/130 (97.7%) of ESCCs, indicating that both antibodies can be used to detect PODXL in ESCC using this method. Among more than 3+ cases, 47-mG_{2a} was more sensitive than PcMab-47 with 56/127 (44.1%) and 41/123 (33.3%) occurrences, respectively (Table 1). In conclusion, 47-mG_{2a} can be used to detect PODXL in ESCC by immunohistochemical analysis.

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Author Disclosure Statement

The authors have no conflict of interest.

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