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Original contribution

# Loss of PAR-3 protein expression is associated with invasion, lymph node metastasis, and poor survival in esophageal squamous cell carcinoma☆,☆☆

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**Summary** Disrupted cell polarity is a feature of epithelial cancers. The partitioning defective 3 (PAR-3) protein, a key component of the PAR complex that regulates the polarization of cells, is involved in tight junction formation at epithelial cell–cell contacts. Our previous study detected a homozygous deletion of the PAR-3 gene in esophageal squamous cell carcinoma (ESCC) cell lines and frequent copy number loss of the PAR-3 gene in primary ESCC. Here, we aimed to investigate the clinicopathological relevance of altered expression of the PAR-3 protein in primary ESCC. We immunohistochemically analyzed expression of the PAR-3 protein, as well as that of other tight junction proteins, ZO-1 and claudin-1, in 74 primary ESCCs. While the PAR-3 protein was expressed in the cytoplasm of basal cells, it was localized on the plasma membrane of suprabasal cells of normal squamous epithelium of the esophagus. Of the 74 ESCC tumors, 20 (27%), 11 (15%), and 13 (18%) were negative for PAR-3, ZO-1, and claudin-1 proteins, respectively. Negative PAR-3 protein expression, but not negative ZO-1 or claudin-1 expression, was significantly associated with deeper tumor invasion ($P < .01$), positive lymph node metastasis ($P = .03$), and advanced tumor stage ($P = .01$). Patients with PAR-3–negative tumors showed marginally significantly shorter overall survival after surgery than those with PAR-3–positive tumors ($P = .053$). In conclusion, these results suggest that PAR-3 protein expression is frequently lost in primary ESCC and that loss of the PAR-3 protein is associated with aggressive clinicopathological features of ESCC.


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## 1. Introduction

Esophageal cancer is the eighth most common cancer and the sixth leading cause of cancer mortality worldwide [1]. Esophageal squamous cell carcinoma (ESCC) is the predominant histological subtype in Asia, Africa, and South America [2].

Cell polarity proteins regulate tight junction (TJ) formation and directional migration in epithelial cells. Three conserved protein complexes—the partitioning defective (PAR), Crumbs and Scribble complexes—control many polarization processes and function to specify and maintain apical and basolateral membrane domains [3,4]. The PAR complex consists of PAR-3, PAR-6, and atypical protein kinase C (aPKC) and assembles at TJs. PAR-3 is a multidomain scaffolding protein that consists of one self-oligomerization domain, three PDZ protein interaction domains, and one aPKC-binding domain. These domains enable PAR-3 to form a PAR complex with PAR-6 and aPKC [5].

TJs constitute a barrier both to the passage of ions and molecules through the paracellular pathway and to the movement of proteins and lipids between the apical and the basolateral domains of the plasma membrane. TJs are composed of transmembrane and cytoplasmic proteins [6]. The former include claudins, occludin, and junction adhesion molecule (JAM). The latter include zonula occludens (ZO)-1, which has been used as a marker of TJs [7], ZO-2, and ZO-3. JAM binds directly to ZO-1 and PAR-3, which enable the recruitment of ZO-1 and PAR-3 to TJs [8]. Claudins consist of four transmembrane domains and two extracellular loops through which these proteins bind to corresponding claudins in cell–cell contact. Claudins also bind to ZO-1 via their carboxyl terminal domain in the cytoplasm [6,9]. The claudin family consists of over twenty members, which show tissue-specific expression. Several studies have shown the expression of claudin-1 in the esophagus [10-12].

Disrupted cell polarity is a feature of epithelial cancers. Loss of apical-basolateral polarity is an early event in epithelial cancers. Recent studies, including ours [13], have shown that the expression of several polarity proteins is altered by gene amplification, deletion, or epigenetic regulation in many cancers [4].

We previously detected a homozygous deletion of the PAR-3 gene (*PARD3*) in ESCC cell lines by using a high-density oligonucleotide microarray approach [13]. We detected a copy number loss of *PARD3* in 15% of primary ESCC tumors and found that expression of *PARD3* mRNA was significantly reduced in 70% of ESCC tumors compared with their nontumorous counterparts [13]. Furthermore, our preliminary study suggested that reduced expression of *PARD3* mRNA may be associated with positive lymph node metastasis and poor differentiation of ESCC [13]. Thereafter, deletions of *PARD3* have been found in cancer cell lines and in primary tumors from head and neck squamous cell carcinoma, lung squamous cell carcinoma, and glioblastoma [14], and mutations of *PARD3* have been detected in lung squamous cell carcinoma [15]. However, PAR-3 protein expression has never been investigated in primary ESCC, and the clinical relevance of altered expression of the PAR-3 protein in ESCC remains to be elucidated.

In the present study, we studied the expression status of the PAR-3 protein, as well as that of other TJ proteins, ZO-1 and claudin-1, in the normal esophagus and in primary ESCC using immunohistochemistry. We show that PAR-3 protein expression is frequently lost in primary ESCC tumors and that loss of the PAR-3 protein is associated with aggressive clinicopathological features of ESCC.

## 2. Materials and methods

### 2.1. Patients and tumor samples

Primary ESCCs were obtained from 74 patients who underwent curative surgical resection for ESCC at the Hospital of Kyoto Prefectural University of Medicine between 2004 and 2007. This study received approval from the ethics committees of Kyoto Prefectural University of Medicine and was conducted in accordance with the Declaration of Helsinki.

Patients and tumor characteristics are summarized in Table 1. The series consisted of 56 male and 18 female patients with a mean age of 63 years (range, 42–82 years). Twelve patients (16%) received preoperative radiotherapy or chemotherapy. Fifteen tumors (20%) were in the upper thoracic esophagus, 29 (39%) in the middle thoracic esophagus, and 30 (41%) in the lower thoracic or abdominal esophagus. Median tumor size was 37 mm (range, 10–140 mm). The tumors were classified according to the tumor-node-metastasis (TNM) *Classification of Malignant Tumuors*, 7th edition [16], published by the Union for International Center Control (UICC). The depth of tumor invasion (T) was T1 (ie, tumor invades lamina propria, muscularis mucosae, or submucosa) in 30 patients (41%), T2 (tumor invades muscularis propria) in 12 patients (16%), T3 (tumor invades adventitia) in 27 patients (36%), and T4 (tumor invades adjacent structures) in 5 patients (7%). Thirty-three patients (45%) had lymph node metastasis. There was no distant metastasis. Histologically, 54 (73%) cases were well- or moderately differentiated ESCC, and 20 (27%) cases were poorly differentiated ESCC.

### 2.2. Immunohistochemical staining

All samples were fixed in 10% buffered formalin and embedded in paraffin. Deparaffinized sections were treated with 0.3% hydrogen peroxide to block endogenous peroxidase activity and were then autoclaved in 10 mmol/L citrate buffer (pH 6.0) for 15 min for antigen retrieval. After incubation in Protein Block Solution (Dako Cytomation, Glostrup, Denmark), sections were incubated overnight at 4°C with primary antibody. The following rabbit polyclonal antibodies were used: anti–PAR-3 antibody (1:100 dilution; #HPA030443, Atlas Antibodies, Stockholm, Sweden), anti–ZO-1 antibody