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Expression of CLDN18.2 in Invasive Mucinous Adenocarcinomas of the Lung

KUNIO MOCHIZUKI, IPPEI TAHARA, TOMOHIRO INOUE, KAZUNARI KASAI and TETSUO KONDO

Department of Pathology, University of Yamanashi, Yamanashi, Japan

Abstract

Background/Aim: Invasive mucinous adenocarcinoma (IMA) of the lung is a rare adenocarcinoma subtype with abundant intracytoplasmic mucin and poor response to standard non-small cell lung cancer chemotherapy. Molecularly targeted therapies may be useful in IMA. Zolbetuximab is a chimeric IgG1 monoclonal antibody against Claudin-18.2 (CLDN18.2) that induces cell death in CLDN18.2-positive gastric and gastro-esophageal junction adenocarcinomas. If lung IMAs also highly express CLDN18.2, they may be candidates for zolbetuximab treatment. This study evaluated CLDN18.2 expression in lung IMAs by immunohistochemistry.

Patients and Methods: We analyzed surgically resected samples comprising 30 IMAs, 10 non-mucinous adenocarcinomas (NMAs), 10 squamous cell carcinomas (SqCCs), 10 small cell carcinomas (SmCCs), 9 large cell carcinomas (LCCs), and 10 large cell neuroendocrine carcinomas (LCNECs) of the lung.

Results: We confirmed CLDN18.2 expression (H-score ≥50 points) in 77% of IMA samples. Its expression was rarely observed in other histological types of lung cancer.

Conclusion: CLDN18.2 is frequently expressed in IMAs but rarely in other major lung cancer subtypes, suggesting that zolbetuximab may represent a promising targeted therapy for IMA.

Keywords: CLDN18.2, invasive mucinous adenocarcinoma, lung, zolbetuximab.

Introduction

Invasive mucinous adenocarcinoma (IMA) of the lung is a rare variant of adenocarcinoma characterized by abundant intracytoplasmic mucin within the tumor, and it has poor sensitivity to conventional chemotherapy regimens used for non-small cell lung cancer (1). However, IMA has an oncogenic profile distinct from that of other invasive adenocarcinomas, with frequent *Kirsten rat sarcoma virus* (*KRAS*) mutations and infrequent oncogenic mutations, including *epidermal growth factor receptor* (*EGFR*) mutations (2). Meanwhile, several reports of molecularly targeted therapies for IMA can be found. Seven cases of advanced or recurrent IMA treated

Kunio Mochizuki, MD, PhD, Department of Pathology, University of Yamanashi, 1110 Shimokato, Chuo, Yamanashi, 409-3898, Japan. Tel: +81 552739529, Fax: +81 552739534, e-mail: kuniom@yamanashi.ac.jp

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with bevacizumab (BEV)-combined chemotherapy showed a favorable overall response rate (ORR) (1). A study presenting the first case of *Mesenchymal-epithelial transition* exon 14-skipping mutation (*MET*ex14)-positive IMA treated with a MET-tyrosine kinase inhibitor (TKI) (3) was reported, and Tepotinib demonstrated therapeutic effectiveness; however, the patient died of exacerbated interstitial lung disease (ILD), possibly associated with this treatment (3). In a case of IMA with *EGFR* mutation, gefitinib therapy resulted in a rapid reduction of bronchorrhea, associated with a dramatic improvement in dyspnea, hypoxia, and radiographic abnormalities (4).

Claudin-18 isoform 2 (CLDN18.2) is a tight junction protein that is normally expressed exclusively in gastric mucosa cells, its expression is retained in most gastric and gastro-esophageal junction adenocarcinoma cells, and is the dominant CLDN18 isoform expressed in both normal and malignant gastric cells (5). Zolbetuximab is a first-in-class chimeric immuno-globulin G1 monoclonal antibody that targets and binds to CLDN18.2 that mediates cell death of CLDN18.2-positive gastric and gastro-esophageal junction adenocarcinoma cells *via* antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity. Targeting CLDN18.2 with zolbetuximab significantly prolonged progression-free survival and overall survival when combined with mFOLFOX6 [modified folinic acid (or levofolinate), fluorouracil, and oxaliplatin regimen] *versus* placebo combined with mFOLFOX6 in patients with CLDN18.2-positive, HER2-negative, locally advanced unresectable or metastatic gastric or gastro-esophageal junction adenocarcinoma (6).

Arpa *et al.* reported that gastric surface type mucin MUC5AC and MUC6 are expressed in IMA, which may indicate that IMA has a differentiation similar to that of the gastric mucosa cells (7). If IMA is also found to have high expression of CLDN18.2, it may also be eligible for treatment with zolbetuximab. We found a few studies of CLDN18.2 expression in IMAs of the lung (8-12). This study aimed to clarify its expression in IMAs using immunohistochemistry. For comparison, we also included samples of non-mucinous adenocarcinoma (NMA), squamous cell carcinoma (SqCC), small cell carcinoma (SmCC), large cell carcinoma (LCC) and large cell neuroendocrine carcinoma (LCNEC) of the lung.

Table I. *Expression of CLDN18.2 in lung cancers.*

Tumor type	H-score (Classification)[a]			
	0	1	2	3
IMA (n=30)	7	1	19	3
NMA (n=10)	10	0	0	0
SqCC (n=10)	10	0	0	0
SmCC (n=10)	10	0	0	0
LCC (n=9)	9	0	0	0
LCNEC (n=10)	8	2	0	0

IMA: Invasive mucinous adenocarcinoma; NMA: non-mucinous adenocarcinoma; SqCC: squamous cell carcinoma; SmCC: small cell carcinoma; LCC: large cell carcinoma; LCNEC: large cell neuroendocrine carcinoma. [a]0=0 to 49 points; 1=50 to 99 points; 2=100 to 199 points; 3=200 to 300 points.

Patients and Methods

Patients. We collected 30 IMA, 10 NMA, 10 SqCC, 10 SmCC, 9 LCC and 10 LCNEC samples of the lung obtained surgically at the University of Yamanashi Hospital. The pathological diagnosis of these tumors followed the *WHO Classification of Thoracic Tumours* (13). Two pathologists (K.M. and I.T.) independently reviewed hematoxylin and eosin (HE) stained slides blinded to the original pathological diagnosis. The Research Ethics Committee of the Faculty of Medicine, University of Yamanashi approved this study (approval number: 2859).

Immunohistochemistry. In our previous report, we described the immunohistochemical method, review and H-score (14). Claudin18.2 (EPR19202; Abcam, Cambridge, UK; dilution 1:500) was used as the primary antibody. We used a positive control (stomach) to perform the primary antibody reaction. We performed antigen retrieval through heat treatment by autoclaving at 121°C for 10 min in Antigen Retrieval solution pH 9 (Nichirei Biosciences, Tokyo, Japan). We inhibited endogenous peroxidase by using 3% H_2O_2.

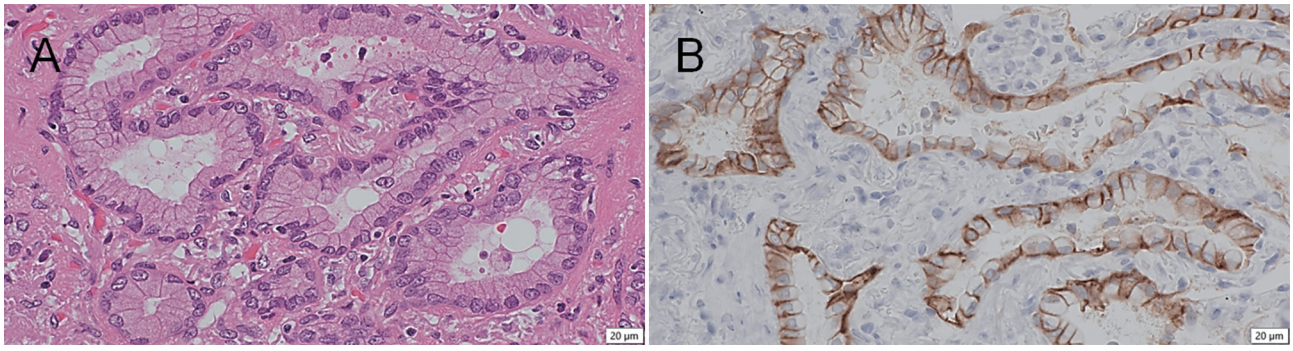

Figure 1. *Representative invasive mucinous adenocarcinoma (IMA). (A) Hematoxylin-Eosin staining. (B) IMA exhibiting CLDN18.2 immunoreactivity in the cell membrane (staining intensity 3+). Original magnification ×400.*

Results

Table I shows the summarized immunohistochemical results. We used the H-score as an immunohistochemical evaluation system, which is calculated by adding the products obtained by multiplying each of four staining intensity gradations with its percentage of positive cells. We classified the H-score as 0=0 to 49 points, 1=50 to 99 points, 2=100 to 199 points, and 3=200 to 300 points with the classifications of 1, 2 or 3 defined as positive and 0 as negative. In 30 IMAs, CLDN18.2 had the following immunostaining patterns: 23% classified 0, 3% classified 1, 63% classified 2, and 10% classified 3 (Figure 1). In 10 NMAs, CLDN18.2 had the following immunostaining patterns: 100% classified 0, 0% classified 1, 0% classified 2, and 0% classified 3. In 10 SqCCs, CLDN18.2 had the following immunostaining patterns: 100% classified 0, 0% classified 1, 0% classified 2, and 0% classified 3. In 10 SmCC, CLDN18.2 had the following immunostaining patterns: 100% classified 0, 0% classified 1, 0% classified 2, and 0% classified 3. In nine LCCs, CLDN18.2 had the following immunostaining patterns: 80% classified 0, 20% classified 1, 0% classified 2, and 0% classified 3. In 10 LCNECs, CLDN18.2 had the following immunostaining patterns: 100% classified 0, 0% classified 1, 0% classified 2, and 0% classified 3.

Discussion

The CLDN family is capable of forming tight-junction strands and thereby represents the backbone of tight junctions in vertebrate epithelial and endothelial cells, as well as in other types of cells; it is composed of 27 members in mammals, and a specific combination of CLDNs is expressed in a given cell/tissue type (15).

Treatment with zolbetuximab plus mFOLFOX6 led to a clinically meaningful and significant benefit in progression-free survival and overall survival compared with placebo plus mFOLFOX6 in patients with previously untreated, CLDN18.2-positive, HER2-negative, locally advanced unresectable or metastatic gastric or gastro-esophageal junction adenocarcinoma (6).

Furthermore, Hong *et al.* reported that CLDN18.2 expression was evaluated in 414 patients using immunohistochemistry; in total, 4.1% (17/414) of the patients were CLDN18.2-positive, including patients with pancreatic (16.7%, 1/6), gastric (14.1%, 12/85), biliary tract (6.3%, 1/16), genitourinary/miscellaneous (2.2%, 1/46), and colorectal (0.9%, 2/203) cancers (16).

Sahin *et al.* showed that CLDN18.2 expression in gastric (77%, 51/66), pancreatic (80%, 8/10), esophageal (77%, 17/22), ovarian (10%, 4/42) adenocarcinomas by immunohistochemistry (17). Cha *et al.* demonstrated that high CLDN18.2 expression correlates with favorable clinical-pathological features and improved survival outcomes in pancreatic ductal adenocarcinoma, particularly in patients receiving adjuvant chemotherapy, suggesting its potential utility as a therapeutic and prognostic marker (18). They found frequent ectopic activation of CLDN18.2 in cancers in various organs. These

data suggest its therapeutic application outside of gastric cancer.

Our immunohistochemical results showed 77% of the IMA samples had a high rate of CLDN18.2 expression (H-score ≥50 points). Therefore, zolbetuximab that targets and binds to CLDN18.2 may have a therapeutic effect on advanced IMAs. We also confirmed CLDN18.2 immunoexpression (H-score ≥50 points) in 0% of NMA, SqCC, SmCC and LCC samples and 20% of LCNECs.

In support, Micke *et al.* reported that the expression of CLDN18.2 in lung adenocarcinomas is very low (12/195, 6.2%) (8). However, Liu *et al.* showed strong cellular expression (25/68, 36.8%) in adenocarcinomas with mucous or hyper columnar epithelium (9). Furthermore, Kim *et al.* showed that CLDN18.2 positivity was more frequent in invasive mucinous lung adenocarcinomas (4/5, 80%) (10). Wang *et al.* reported CLDN18.2 expression in 76.2% (64/84) of patients with IMA (11). Yan *et al.* showed CLDN18.2 expression in 67.5% (27/40) of lung mucinous adenocarcinoma (12).

However, these reports did not adequately demonstrate the extent of CLDN18 expression in other histological types of lung cancer. Our results support these data.

In conclusion, our results showed that CLDN18.2 is expressed at a high rate in IMAs of the lung. Future studies, including clinical trials, are needed to evaluate the therapeutic utility of zolbetuximab in IMA and to further elucidate the relationship between CLDN18.2 expression and the biological characteristics of IMA in larger patient cohorts.

Conflicts of Interest

The Authors declare no competing interests in relation to this study.

Authors' Contributions

KM collected and analyzed the data and drafted the manuscript. IT, TI, KK and TK analyzed the data and contributed to the final draft of the manuscript. All Authors read and approved the final manuscript.

Artificial Intelligence (AI) Disclosure

No artificial intelligence (AI) tools, including large language models or machine learning software, were used in the preparation, analysis, or presentation of this manuscript.

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