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Clinicopathological and prognostic significance of MUC13 and AGR2 expression in intraductal papillary mucinous neoplasms of the pancreas

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ABSTRACT

Background: Intraductal papillary mucinous neoplasm (IPMN) of the pancreas is a primary pancreatic ductal epithelial neoplasm with the potential to develop into an invasive adenocarcinoma. This study aimed to investigate the clinicopathologic and prognostic significance of four potential biomarkers for the preoperative evaluation of patients with IPMN.

Materials and methods: Clinicopathologic materials from 104 patients with IPMN who underwent surgical resection at Jichi Medical University Hospital were analyzed. IPMNs (110 lesions in total) were histologically classified into low-grade IPMN (Group 1; n = 68), high-grade IPMN (Group 2; n = 16), or IPMN with an associated invasive carcinoma (Group 3; n = 26). We evaluated the immunohistochemical expression of MUC13, AGR2, FUT8, and FXYD3, which were previously reported to be overexpressed in pancreatic ductal adenocarcinoma.

Results: The expression of MUC13 was more common in Group 3 compared with groups 1 and 2 ($p < 0.001$) and was associated with poor prognosis ($p = 0.004$). The expression of MUC13 was not associated with age, sex, tumor location, histological subtype, lymphatic or vascular invasion, or neural invasion. In most cases of IPMN, the loss of expression of AGR2 appeared to show an association with tumor recurrence and poorly differentiated histology of invasive carcinoma; however, this association was not statistically significant. The expressions of FUT8 and FXYD3were not associated with the clinicopathological features of IPMNs.

Conclusions: The results suggest that MUC13 overexpression and loss of expression of AGR2 may predict the progression of IPMN and an unfavorable prognosis in patients with IPMN.

Introduction

Intraductal papillary mucinous neoplasms (IPMNs) are commonly diagnosed as cystic neoplasms of the pancreas and account for approximately 21%–33% of all clinically encountered pancreatic cystic lesions [1]. In recent years, they have been characterized by their clinicopathologic features and the associated risk of malignant transformation [2,3]. IPMNs may arise from the main pancreatic duct (main-duct type, MD-IPMN) or its side branches (branch-duct type, BD-IPMN), or may involve both the main pancreatic duct (MPD) and the side branches (combined-type IPMN) [4,5]. MD-IPMN is associated with a significantly higher mean risk of malignancy than BD-IPMN (61.6% vs. 25.5%) [2]. IPMN with MPD involvement warrants surgical resection due to the high risk of malignancy. However, it has been reported that some MD-IPMN has almost unchanged for a long time [6,7].

International consensus guidelines for the management of IPMN were established in 2006, recommending observation for asymptomatic IPMN through the presence of a cyst with a maximum size of 3 cm, as well as a non-dilated MPD, negative cytology, and the

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absence of intramural nodules [8]. As cases accumulated, questions appeared regarding the role of cyst size [9,10] and symptoms [11]. The guidelines were revised in 2012 in Fukuoka, Japan, to include the classifications: "high-risk stigmata" and "worrisome features" [2]. In the revised 2012 guidelines, the threshold of MPD dilation was lowered to ≥5 mm [1]. According to these guidelines, a main duct diameter of 5–9 mm must be considered a "worrisome feature" [2]. However, in this subgroup, resection is only recommended if additional criteria such as obstructive jaundice or a solid nodule component are observed as these criteria are regarded as "high-risk stigmata" [2]. However, reports show that invasive carcinoma may also be found in patients with MPD of a smaller diameter without nodules or symptoms [4,12,13]. This often causes frustration, as surgery could have been performed.

The guidelines suggest a therapeutic strategy based on imaging studies. In addition, biomarkers to distinguish low-grade and high-grade lesions using pancreatic juice cytology or biopsy specimens would be very useful in decision making. This study aimed to investigate the clinicopathologic and prognostic significance of several potential biomarkers in patients with IPMN. We retrieved previous reports and analyzed data on four biomarkers that were reported to be overexpressed in cases of pancreatic ductal adenocarcinoma (PDAC), including mucin 13 (MUC13), anterior gradient protein 2 (AGR2), fucosyltransferase 8 (FUT8), and FXYD domain containing ion transport regulator 3 (FXYD3). MUC13 is a transmembrane mucin aberrantly expressed in ovarian and gastrointestinal cancers [14], AGR2 is a protein disulfide isomerase overexpressed in several adenocarcinomas [16–21], and FUT8 is α1,6-fucosyltransferase associated with fucosylation, one of the most important types of glycosylation for malignant transformation and metastasis [22]. Watanabe et al. reported that fucosylation, particularly α-1,6-fucosylation as indicated by FUT8 expression, is upregulated in IPMNs and may be associated with malignant transformation [22]. FXYD3, a chloride channel or chloride channel regulator and a member of the FXYD family of single membrane span proteins, was found to be expressed differentially in PDAC [23].

Materials and methods

Patients

We retrospectively evaluated tissue samples from 104 patients (63 men and 41 women; mean age, 66.9 years; range, 32–82 years) who underwent surgical resection of IPMN (110 lesions in total) at Jichi Medical University Hospital (Shimotsuke, Japan) between January 1, 2000 and May 31, 2016. The study protocol was approved by the Ethics Committee of the Jichi Medical University. Medical information, such as age, sex, history of diseases and recurrences, and outcomes of the participants, was obtained from clinical records.

Histopathological examination

The resected pancreas samples were fixed in 15% formalin, and paraffin blocks were prepared. IPMNs were classified into one of the three histological groups based on the Baltimore Consensus Meeting for Neoplastic Precursor Lesions in the Pancreas [24]: low-grade IPMN (Group 1), high-grade IPMN (Group 2), or IPMN with an associated invasive carcinoma (Group 3). Furthermore, based on the immunohistochemical and histologic profiles of the proliferating epithelial cells, IPMNs were classified into one of the four subtypes: gastric, intestinal, pancreatobiliary (PB), or oncocytic. If two or more subtypes coexisted in the same lesion, the dominant subtype was used for analysis. To assess the tumor size, we traced the outline of IPMN and the associated invasive carcinoma on each slide and added together the tumor areas on the gross photographs. Subsequently, we measured the largest dimension of the tumor. One patient, for whom a detailed report on the tumor volume was missing, was excluded from the tumor size analysis. IPMNs were classified into three groups based on distribution predominance: MPD type, branch-duct type, or combined type. In addition, we classified the histology of the invasion as colloid carcinoma or tubular adenocarcinoma. When two different invasive features coexisted in the same lesion, the dominant component of the invasive carcinoma was used for the analysis. In patients with IPMN with an associated invasive carcinoma, we also evaluated the lymphatic invasion, vascular invasion, and neural invasion. Each section was reviewed by two authors (KM and NF), and a consensus was reached in all the cases.

Immunohistochemistry

We evaluated the expression of MUC13, AGR2, FUT8, and FXYD3 by immunohistochemistry because the expressions of these genes were previously reported in cases of PDAC. A representative section of each lesion was selected for the analysis. The immunohistochemical analysis was performed on 4-μm sections of paraffin-embedded, formalin-fixed tissues. All procedures were performed using a BenchMark ULTRA fully automated staining instrument (Ventana Medical Systems Inc., Oro Valley, AZ, USA). Each section was deparaffinized and incubated in Cell Conditioning Solution 1 (pH 8.5; Ventana Medical Systems Inc.) for 64 min: AGR2 and FXYD3 or 36 min: MUC13 and FUT8 at 95 °C for antigen retrieval. Then, the sections were incubated with primary antibodies against the following molecules (all obtained from Novocastra Laboratories Ltd., Newcastle Upon Tyne, UK) for 32 min: AGR2 (rabbit polyclonal, NBP2-27393, 1:100 dilution; Novus Biologicals, Littleton, CO), FUT8 (rabbit polyclonal, HPA043410; 1:500 dilution; SIGMA-ALDRICH, St. Louis, MO), and FXYD3 (rabbit polyclonal, HPA010856; 1:100 dilution; SIGMA-ALDRICH, St. Louis, MO) or 16 min: MUC13 (mouse monoclonal, MABC209, clone 2E11.1; 1:1000 dilution, Darmstadt, DE). Signals were visualized using an iView DAB Universal Kit (Ventana Medical Systems Inc.). Finally, the sections were counterstained with Hematoxylin II (Ventana Medical Systems Inc.) for 8 min and post-stained with Bluing Reagent (Ventana Medical Systems Inc.) for 4 min.

The proportion of positive cancer cell staining was graded as follows: 0 (negative), <25% (1+), 25%–50% (2+), 50%–70% (3+), and >75% (4+) [26]. For simplicity, the cells were considered positive if at least 25% of the cytoplasm was stained based on the results of the immunohistochemistry.

In a previous report the expression of MUC13 in colorectal carcinomas [25] was evaluated by localization: membrane, cytoplasm, and nucleus. Furthermore, MUC13 is expressed weakly in the apical membrane of the normal pancreatic duct. The expression of MUC13 tends to gradually increase toward the basal laminae. Therefore, the expression of MUC13 was classified into three grades (Fig. 1) [23,24]. We defined the pattern seen in Fig. 1-A as negative and the patterns seen in Fig. 1-B and 1-C as positive. The intensity of the expression was also evaluated.

Statistical analysis

All statistical analyses were performed using EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [27]. Differences between groups in age were compared using the Kruskal–Wallis test or Mann–Whitney *U* test, while all other features were compared using a chi-square test or Fisher exact test. The difference in the expression of MUC13 among cases of IPMN

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