Pancreatology 15 (2015) 432–438

Contents lists available at ScienceDirect

Pancreatology

journal homepage: www.elsevier.com/locate/pan

Original article

Serum *N*-glycan profiles in patients with intraductal papillary mucinous neoplasms of the pancreas

Yutaka Akimoto [a, *], Kazuhiro Nouso [a], Hironari Kato [a], Koji Miyahara [a], Chihiro Dohi [a], Yuki Morimoto [a], Hideaki Kinugasa [a], Takeshi Tomoda [a], Naoki Yamamoto [a], Koichiro Tsutsumi [a], Kenji Kuwaki [a], Hideki Onishi [a], Fusao Ikeda [a], Shinichiro Nakamura [a], Hidenori Shiraha [a], Akinobu Takaki [a], Hiroyuki Okada [a, b], Maho Amano [c, d], Shin-Ichiro Nishimura [c, d], Kazuhide Yamamoto [a]

[a] Department of Gastroenterology & Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan
[b] Departments of Endoscopy, Okayama University Hospital, Okayama, Japan
[c] Field of Drug Discovery Research, Faculty of Advanced Life Science & Graduate School of Life Science, Hokkaido University, Sapporo, Hokkaido, Japan
[d] Medicinal Chemistry Pharmaceuticals, Co., Ltd., Sapporo, Hokkaido, Japan

ARTICLE INFO

Article history:
Available online 27 May 2015

Keywords:
Biological markers
Fucosylation
Glycomics
Intraductal papillary mucinous neoplasm
Pancreas
Malignancy

ABSTRACT

Background/objectives: Diagnosing the invasiveness of intraductal papillary mucinous neoplasms (IPMNs) is difficult, especially by blood test. Alterations in serum glycan profiles have been reported for several cancers, but changes in serum glycan profiles have not been investigated in patients with IPMNs. The objectives of this study were to determine the serum *N*-glycan profile and to investigate its clinical utility in patients with IPMNs.

Methods: We measured serum *N*-glycan profiles in 79 patients with IPMNs, including 13 invasive IPMNs, by performing comprehensive glycome analysis and assessed the relationship between *N*-glycan changes and clinical parameters.

Results: Seventy glycans were identified and their expression profiles were significantly different depending on the cyst size, the presence of an enhancing solid component, and the histological grade of the IPMN. Nine glycans were highly expressed in patients with invasive IPMNs. The glycan m/z 3195, which is a fucosylated tri-antennary glycan, had the highest diagnostic value for distinguishing invasive IPMNs from non-invasive IPMNs (area under the receiver operating characteristic curve = 0.803). Multivariate analyses revealed high levels of m/z 3195 [odds ratio (OR), 20.5; 95% confidence interval (CI) 2.60–486.4] and the presence of enhancing solid components (OR, 35.8; 95% CI, 5.39–409.6) were significant risk factors for invasive IPMNs.

Conclusions: We performed a comprehensive evaluation of the changes in serum *N*-glycan profiles in patients with IPMNs for the first time. We determined that increased expression of fucosylated complex-type glycans, especially m/z 3195, is a potential marker for invasive IPMNs.

Introduction

Intraductal papillary mucinous neoplasms (IPMNs) of the pancreas are potentially malignant mucin-producing intraductal epithelial neoplasms. The true incidence of IPMNs is unknown because many IPMNs are small and asymptomatic. Recent studies have reported that the prevalence of unsuspected pancreatic cysts, including IPMNs identified on computed tomography (CT) or magnetic resonance imaging (MRI), is ~2.6–20% in adults [1–3].

Clinical guidelines recommend that main duct-type IPMNs (MD-IPMNs) and branch duct-type IPMNs (BD-IPMNs) with high-risk stigmata (obstructive jaundice with cystic lesion of the head of the pancreas, enhancing solid component within the cyst, and main pancreatic duct cyst size ≥ 10 mm) be treated by resection

* Corresponding author. Department of Gastroenterology & Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Kita-ku, Okayama-city, Okayama 700-8558, Japan. Tel.: +81 86 235 7219; fax: +81 86 225 5991.
E-mail address: qttsp974@gmail.com (Y. Akimoto).

http://dx.doi.org/10.1016/j.pan.2015.05.470

because of the high risk of malignancy [4,5]. Pancreatic resection is associated with high rates of perioperative morbidity and mortality. Several reports indicate that malignancy rates of resected MD-IPMNs and BD-IPMNs are not sufficiently high (36–100% and 6–47%, respectively), to justify performing high-risk pancreatic resection for certain cases [5]. The new International consensus guidelines 2012 and the European experts consensus statement on cystic tumors of the pancreas 2013 attempt to improve the specificity of the recommendations, but their value for patient outcomes is still controversial [5,6]. Therefore, a new serum biomarker for invasive IPMNs is required to improve the accuracy of preoperative diagnosis.

Glycan-based serological assays are useful to detect serum biomarkers for cancer. Carbohydrate antigen 19-9 (CA19-9) and lens culinaris agglutinin-reactive fraction of alpha-fetoprotein (AFP-L3) are commonly used as tumor markers [7,8]. Aberrant glycosylation of serum proteins is observed in sera of patients with various types of cancer, including colon cancer, ovarian cancer, and pancreatic cancer [9–12]. We reported previously that multi-branch antennary and fucosylated glycan was elevated in pancreatic cancer and hepatocellular carcinoma [13,14]. Although these changes might occur in patients with invasive IPMNs, the serum glycome profile of these patients has not been investigated.

In the present study, we analyzed changes in serum *N*-glycan profiles in patients with various stages of IPMNs and evaluated the potential use of glycans as new clinical markers for invasive IPMNs.

Methods

Patients and diagnosis

We enrolled 146 consecutive patients who were diagnosed with IPMNs via imaging modalities and were admitted to Okayama University Hospital between May 2004 and August 2013. The diagnostic criteria were as follows: (i) dilation of the main pancreatic duct (MPD) and/or a cystic dilation of the branch duct and (ii) secretion of mucin from the major or minor papilla identified by endoscopic retrograde cholangiopancreatography or duodenoscopy. We excluded 67 patients from which serum samples were not obtained or histocytological examinations were not performed. The study subjects included 79 patients with IPMNs who were diagnosed using both radiographic imaging and histocytological examination. All patients were evaluated with CT or MRI and determined to have the maximum diameter of MPD and cyst size, the presence of mural nodules, and an enhancing solid component. In this study, we expressed the size of MD-IPMNs as 0 mm, and we defined mural nodules that were enhanced by contrast-enhanced CT or MRI as “enhancing solid component”. Serum tumor markers, including carcinoembryonic antigen (CEA), CA 19-9, s-pancreas-1 antigen (Span-1), and duke pancreatic monoclonal antigen type 2 (DUPAN-2) were measured at initial diagnosis. CEA and CA19-9 were analyzed by an electrochemiluminescence immunoassay. Span-1 was analyzed by an immunoradiometric assay. DUPAN-2 was analyzed by an enzyme immunoassay. A total of 40 patients underwent surgery. We confirmed the pathological diagnosis of the resected tissues according to the following World Health Organization 2010 IPMN grade classification [15]: low-grade dysplasia ($n = 13$), intermediate-grade dysplasia ($n = 8$), high-grade dysplasia ($n = 9$), and invasive carcinoma ($n = 10$) including minimal invasion ($n = 2$). Thirty-nine patients did not require surgery. These patients were evaluated by radiological, cytological, or histological examination of specimens obtained by fine needle aspiration (FNA) or brushing, and classified into the following three subgroups: low-intermediate grade dysplasia ($n = 35$; cytology = class II–III, without radiographic invasion, without progression during a median observation period of 5 years; range 1–10 years), high-grade dysplasia ($n = 1$; cytology = class V; without radiographic invasion, without progression during 3 years), and invasive carcinoma ($n = 3$; cytology = class V; with radiographic invasion and/or metastasis). We excluded the patients whose diagnoses were changed from dysplasia to invasive carcinoma within a year. Classification into MD-IPMNs, BD-IPMNs, and mixed-type was performed according to the 2012 international consensus guidelines [5].

Written informed consent for collecting serum and using clinical data was obtained from all patients. The study protocol conformed to the ethical guidelines of the World Medical Association Declaration of Helsinki and was approved by the institutional review board (authorization number #667).

Glycoblotting

We collected serum samples from all patients at the time of hospital admission. The blood samples were centrifuged for 10 min at 15,000 × *g*, the supernatant was removed and immediately frozen, and the samples were stored below −30 °C until use. Glycoblotting was performed according to a procedure described previously [16]. In brief, 10 μL serum samples were applied to an automated instrument (Sweetblot prototype 7, System Instruments Co.) for pre-treatment and for glycoblotting. Glycans were enzymatically cleaved from proteins, captured on BlotGlyco H beads (Sumitomo Bakelite Co.), and sialic acids were methyl-esterified. The processed glycans were tagged with benzyloxyamine (BOA), released from the beads, and analyzed using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Ultraflex 3, Bruker, Germany). Sweetblot takes 11 h to analyze 96 serum samples.

Statistical analysis

Fisher's exact test was used to compare categorical data. Wilcoxon's rank sum test was used to compare continuous data. The diagnostic value of potential markers was assessed using an area under the receiver operating characteristic curve (AUROC). A multivariate logistic regression model was used to identify risk factors for invasive IPMNs. The cutoff values for the glycans were determined by receiver operating characteristic (ROC) analysis, with the best combination of sensitivity/specificity values used to assign the patients into invasive IPMN and non-invasive IPMN groups. For statistical analysis, $p < 0.05$ was considered significant. JMP (version 10.0.2) software packages (SAS Institute, Cary, North Carolina, USA) were used for statistical analyses.

Results

Patient population characteristics

This study included 79 patients. The median age of the patients was 69 years (range, 43–83). Forty-six (58%) patients were male and 33 (42%) patients were female (Table 1). Thirteen patients had invasive carcinoma. Of these, ten (77%) patients were confirmed by surgical histology, and three (23%) patients were diagnosed by histocytological malignancy and imaging of tumor invasion or metastasis. Forty-three (54%) patients were classified as having BD-IPMNs, including four patients with invasive IPMNs. Thirty-six (46%) patients were classified as MD-IPMNs or mixed-type IPMNs, including nine patients with invasive IPMNs. Enhancing solid components were observed in 10 out of 13 (77%) patients with invasive IPMNs and 7 out of 66 (11%) patients with non-invasive IPMNs.