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Mechanism of the development of nonalcoholic steatohepatitis after pancreaticoduodenectomy

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ABSTRACT

Background and aim: It is recognized that nonalcoholic fatty liver disease (NAFLD), including nonalcoholic steatohepatitis (NASH), may develop after pancreaticoduodenectomy (PD). However, the mechanism of NASH development remains unclear. This study aimed to examine the changes in gene expression associated with NASH occurrence following PD.
Methods: The expression of genes related to fatty acid/triglyceride (FA/TG) metabolism and inflammatory signaling was examined using liver samples obtained from 7 post-PD NASH patients and compared with 6 healthy individuals and 32 conventional NASH patients.
Results: The livers of post-PD NASH patients demonstrated significant up-regulation of the genes encoding CD36, FA-binding proteins 1 and 4, acetyl-coenzyme A carboxylase α, diacylglycerol acyltransferase 2, and peroxisome proliferator-activated receptor (PPAR) γ compared with normal and conventional NASH livers. Although serum apolipoprotein B (ApoB) and TG were decreased in post-PD NASH patients, the mRNAs of ApoB and microsomal TG transfer protein were robustly increased, indicating impaired TG export from the liver as very-low-density lipoprotein (VLDL). Additionally, elevated mRNA levels of myeloid differentiation primary response 88 and superoxide dismutases in post-PD NASH livers suggested significant activation of innate immune response and augmentation of oxidative stress generation.
Conclusions: Enhanced FA uptake into hepatocytes and lipogenesis, up-regulation of PPARγ, and disruption of VLDL excretion into the circulation are possible mechanisms of steatogenesis after PD.
General significance: These results provide a basis for understanding the pathogenesis of NAFLD/NASH following PD.

Abbreviations: ACACA, acetyl-CoA carboxylase α; ACACB, acetyl-CoA carboxylase β; ACADM, medium-chain acyl-CoA dehydrogenase; ACOX1, acyl-CoA oxidase 1; ALT, alanine aminotransferase; ApoB, apolipoprotein B; AST, aspartate aminotransferase; BMI, body mass index; CAT, catalase; CoA, coenzyme A; CPT1A, carnitine palmitoyl-CoA transferase 1α; CT, computed tomography; CYBB, cytochrome b-245 β polypeptide; CYP, cytochrome P450; DGAT, diacylglycerol acyltransferase; FA, fatty acid; FABP, fatty acid-binding protein; FASN, fatty acid synthase; γGT, gamma-glutamyltransferase; HADHA, hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase α; HBV, hepatitis B virus; HCV, hepatitis C virus; HOMA-IR, homeostasis model assessment for insulin resistance; LPS, lipopolysaccharide; LXR, liver X receptor; MCD, methionine- and choline-deficient diet; MTTP, microsomal triglyceride transfer protein; MYD88, myeloid differentiation primary response 88; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; PD, pancreaticoduodenectomy; PPAR, peroxisome proliferator-activated receptor; PPARGC, PPARγ co-activator; qPCR, quantitative polymerase chain reaction; ROS, reactive oxygen species; RXR, retinoid X receptor; SCD, stearoyl-CoA desaturase; SOD, superoxide dismutase; SREBF1, sterol regulatory element-binding transcription factor 1; TG, triglyceride; TGFB1, transforming growth factor β1; TLR, Toll-like receptor; TNF, tumor necrosis factor α; US, ultrasonography; VLDL, very-low-density lipoprotein.

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1. Introduction

The prevalence of nonalcoholic fatty liver disease (NAFLD) is increasing worldwide. In general, NAFLD is closely linked with overnutrition, visceral fat accumulation, and obesity. Nonalcoholic steatohepatitis (NASH) is a serious subtype of NAFLD that may progress to cirrhosis, hepatocellular carcinoma, and hepatic failure [1–3]. Therefore, understanding the pathogenesis of NASH is important for the development of proper preventive and therapeutic strategies. The initial step of NASH development is accumulation of triglycerides (TGs) into hepatocytes. Sources of intrahepatic TG are non-esterified fatty acids (FAs) released from white adipose tissue and absorbed from the small intestine, as well as those newly synthesized from citrate. FAs are further metabolized to acetyl-coenzyme A (CoA) mainly through mitochondrial β-oxidation or esterified to TG, which is either stored in hepatocytes or incorporated into very-low-density lipoprotein (VLDL) and released into the circulation. Therefore, disruption of these metabolic pathways causes hepatosteatosis. Enhanced inflammatory signaling and cellular stress injure steatotic hepatocytes and activate Kupffer cells and stellate cells, resulting in steatohepatitis [4–6].

The pancreas plays a central role in the absorption of essential nutrients, such as fat, amino acids, and fat-soluble vitamin. It is well known that NAFLD/NASH may develop after pancreatic resection [7–9]. We previously reported clinical characteristics of NAFLD developed after pancreaticoduodenectomy (PD) [7]. Most of these patients were diagnosed as having steatohepatitis by liver biopsy, but were lean and had lower levels of serum albumin, total cholesterol, apolipoprotein B (ApoB), and insulin compared with conventional NASH patients [7]. Hepatic steatosis following PD was ameliorated by intensifying oral supplementation of pancreatic enzymes [7], revealing a close link between steatogenesis, pancreatic exocrine insufficiency, and malabsorption/maldigestion. These results are in agreement with the recent reports from the other groups [8,9] and suggest that the mechanism of steatogenesis after PD is different from that of conventional NAFLD/NASH accompanying obesity and insulin resistance. However, the mechanism of post-PD NAFLD/NASH occurrence has not been evaluated.

In the present study, the expression of genes associated with FA/TG metabolism, inflammation, and oxidative stress, which are key contributors of NASH development, was examined using liver samples obtained from post-PD NASH patients and compared with healthy individuals and conventional NASH patients. The livers of post-PD NASH exhibited significant increases in the mRNAs related to intrahepatic FA uptake and FA/TG synthesis. The mRNAs encoding ApoB and microsomal TG transfer protein (MTTP) were increased regardless of reduced circulating ApoB and TG, suggesting impairment of TG excretion from the liver. Additionally, hepatic mRNAs of myeloid differentiation primary response 88 (MyD88, encoded by *MYD88*) and superoxide dismutase (SOD) 1 and 2 (encoded by *SOD1* and *SOD2*, respectively), which are associated with innate immunity and oxidative stress, respectively, were augmented. These results propose possible mechanisms of post-PD NASH development caused by pancreatic exocrine insufficiency and malabsorption/malnutrition.

2. Material and methods

2.1. Patients

2.1.1. Post-PD NASH patients

The detailed patients' selection criteria were described previously [7]. Briefly, 80 patients who underwent PD (Whipple's procedure) between January 2001 and December 2006 at Showa Inan General Hospital and Iida Municipal Hospital without regular alcohol consumption were examined. These patients were all negative for hepatitis B virus (HBV) surface antigen and anti-hepatitis C virus (HCV) antibody and did not have detectable hepatic steatosis before PD. Eight patients died within 6 months after PD and 12 were unavailable for repeated abdominal computed tomography (CT) examinations for more than 6 months afterwards. The presence of newly appearing hepatic steatosis was judged as a liver-to-spleen attenuation ratio of less than 0.9 in unenhanced abdominal CT. In 13 patients developing NAFLD after PD, 8 patients received percutaneous liver biopsy and were diagnosed as having steatohepatitis [7]. Liver samples from 7 patients were available for mRNA analysis.

2.1.2. Conventional NASH patients

Liver samples were obtained from 32 NASH patients who underwent a liver biopsy at Shinshu University or its affiliated hospitals between April 2006 and March 2008. NASH was suspected by the following criteria: (1) the detection of steatosis by abdominal ultrasonography (US); (2) the absence of regular intake of alcohol or drugs; (3) negative results for HBV surface antigen and anti-HBV core and anti-HCV antibodies; and (4) the absence of other types of chronic liver disease, such as autoimmune liver disease, hereditary hemochromatosis, Wilson's disease, α1-antitrypsin deficiency, and citrin deficiency. The diagnosis of NASH was confirmed by liver histology.

2.1.3. Normal controls

Normal livers were obtained from 6 healthy liver transplantation donors at the time of pre-operative liver biopsy who satisfied the following criteria: (1) the absence of past history of liver disease and regular intake of alcohol and drugs; (2) the absence of obesity, diabetes, hypertension, and hyperlipidemia; (3) normal liver function tests; and (4) normal liver histology [10,11].

2.1.4. Clinical data collection

Body height and weight were determined by nursing staff unaware of the subjects' medical information. The presence of obesity was defined as having a body mass index (BMI) of more than 25 kg/m^2 based on criteria released by the Japan Society for the Study of Obesity. The diagnosis of the presence of hypertension, diabetes, and hyperlipidemia is made based on the criteria described previously [10–12]. Blood samples were obtained at the time of liver biopsy following overnight fasting for 8–10 h. Laboratory data, such as aspartate and alanine aminotransferase (AST and ALT, respectively) and γ-glutamyltransferase (γGT), were measured by standard methods using automated analyzers. The homeostasis model assessment for insulin resistance (HOMA-IR) value was calculated as described elsewhere [10–12].

2.2. Liver biopsy and histological evaluation

Liver samples were obtained from 2 different sites in the same lobe using a 14-gauge needle by percutaneous US-guided biopsy [7,10,11]. Fragments of liver tissue (5–7 mm) were immediately frozen with a RNA stabilization solution (RNAlater® solution, Life Technologies, Grand Island, NY, USA) in liquid nitrogen and stored at −80 °C until RNA extraction. The remaining specimens were fixed in 10% neutral formalin, cut in 4-μm thickness, and stained using the hematoxylin and eosin or Azan–Mallory method. Histological findings were assessed in a blinded fashion by an independent pathologist and scored according to the staging/grading system proposed by Kleiner et al. [13]. As a minor modification, Mallory bodies were scored as none to rare (0), few (1), or many (2). The NAFLD histological activity score (NAS) was calculated as the unweighted sum of the scores for steatosis (0–3), lobular inflammation (0–3), and ballooning (0–2). The histological diagnosis of NASH was made by the presence of macrovesicular steatosis and hepatocyte ballooning.

2.3. mRNA analysis

Total RNA was extracted from frozen liver samples of healthy individuals (n = 6), conventional NASH (n = 32), and post-PD NASH