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A soluble form of human nectin-2 impairs exocrine secretion of pancreas and formation of zymogen granules in transgenic mice

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ABSTRACT

Transgenic mouse lines expressing a soluble form of human nectin-2 (hNectin-2Ig Tg) exhibited distinctive elevation of amylase and lipase levels in the sera. In this study, we aimed to clarify the histopathology and to propose the transgenic mouse lines as new animal model for characteristic pancreatic exocrine defects. The significant increase of amylase and lipase levels in sera of the transgenic lines approximately peaked at 8 weeks old and thereafter, plateaued or gradually decreased. The histopathology in transgenic acinar cells was characterized by intracytoplasmic accumulation of abnormal proteins with decrease of normal zymogen granules. The hNectin-2Ig expression was observed in the cytoplasm of pancreatic acinar cells, which was consistent with zymogen granules. However, signals of hNectin-2Ig were very weak in the transgenic acinar cells with the abnormal cytoplasmic accumulaion. The PCNA-positive cells increased in the transgenic pancreas, which suggested the affected acinar cells were regenerated. Acinar cells of hNectin-2Ig Tg had markedly small number of zymogen granules with remarkable dilation of the endoplasmic reticulum (ER) lumen containing abundant abnormal proteins. In conclusion, hNectin-2Ig Tg is proposed as a new animal model for characteristic pancreatic exocrine defects, which are due to the ER stress induced by expression of mutated cell adhesion molecule that is a soluble form of human nectin-2.

1. Introduction

Nectins are Ca^{2+}-independent immunoglobulin (Ig)-like cell–cell adhesion molecules [1]. The nectin family is composed of four members, nectin-1, -2, -3 and -4, all of which, except nectin-4, have two or three splicing variants: nectin-1α, -1β, -1γ, -2α, -2δ, -3α, -3β, and -3γ [2]. Nectin members, except nectin-1γ, consist of an extracellular region with three Ig-like loops, a single transmembrane region, and a cytoplasmic tail. The extracellular regions form homo-cis-dimers and promote homophilic or heterophilic trans-interactions. Extracellular Ig-like domains of each member of the nectin family consisted of a single V-like domain (first Ig-like domain) and two C-like extracellular domains. Nectin-2 forms a variety of cell–cell junctions, including cadherin-based adherens junctions in epithelial cells and fibroblasts in culture, synaptic junctions in neurons [3,4], Sertoli cell-spermatid junctions in the testis [5], and also known to be needed for maintenance of cardiac structure and function [6]. In addition, nectin-2 functions as a receptor for some herpesviruses [7–9].

Recently, we generated transgenic mouse lines expressing a soluble form of human nectin-2 (hNectin-2Ig Tg), to examine the interaction of nectin-2 with herpesviruses. In the process of phenotypic examination of hNectin-2Ig Tg, we incidentally found that the transgenic lines exhibited distinctive elevation of serum amylase and lipase. It has been reported that nectin-2 express in human pancreatic tissue and pancreatic cancer [10,11], though the functions in the pancreas have yet to be determined. Then we did not suppose why the serum pancreatic enzyme levels were

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elevated in hNectin-2Ig Tg. In the present study, we analyzed blood biochemical and cytoarchitectonic abnormalities and cellular expression of hNectin-2Ig Tg to understand the phenotype and pathology in the transgenic mouse. We aimed to propose the transgenic mouse lines as new animal model for characteristic pancreatic exocrine defects.

2. Methods and materials

2.1. Generation of transgenic animals

To construct plasmid expressing a soluble form of human nectin-2 (hNectin-2Ig) that consist of the extracellular domain and the Fc portion of human IgG1, synthesized cDNA of the extracellular domain was amplified by PCR using pENTR221 plasmid containing a complete human nectin-2 gene (pENTR221/nectin-2; GenBank accession number: DQ891829, GeneCopoeia) as a template was cloned into a plasmid carrying IgG1-Fc DNA [12] and then, the chimeric gene fragment encoding each fusion protein was inserted into pCXN2 vector as described previously [13]. The PCR primer set used for the construction of plasmid is as follows: 5′-GGACCCCTCGAGCCACCATGGCCCGGGCCGC-3′ and 5′-CTGAGCGGGATCCTCTCGGACAAAGATGACC-3′. Transgenic mice expressing hNectin-2Ig were generated as described previously [14], by microinjection method of the transgenic fragment containing the CAG promoter (cytomegalovirus IE enhancer and chicken ß-actin promoter), the hNectin-2Ig gene, and the rabbit ß-globin polyA signal. To identify transgenic founders, genomic DNA was prepared by using the FTA elute micro cards (Whatman) and PCR was performed using specific primers (5′-CTCTGCATCTCCAAAGAGGG-3′ and 5′-CTGTGCAGACGAAGGTGGTA-3′ for hNectin-2Ig). All mice were kept with free access to food and water in a standard light/dark cycle and were maintained in the animal facility at our institutes and treated according to Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology in Japan. The ethics committees of our institutes approved all experiment protocols.

2.2. Blood biochemical examination

Under deep combination anesthesia (0.3 mg/kg medetomidine, 4.0 mg/kg midazolam, 5.0 mg/kg butorphanol), blood samples (200 μL per sample) were collected from malar venous plexus of the 4-, 6-, 8-, 12-, 16-, and 24-week-old mice; hNectin-2Ig Tg line 2216 male ($n=6$) and female ($n=5$), hNectin-2Ig Tg line 2246 male ($n=6$) and female ($n=6$), non-Tg male ($n=12$) and female ($n=10$). Blood biochemical examination about items including Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), amylase, lipase, total bilirubin, glucose, blood urea nitrogen, total cholesterol, triglyceride, Glutamic Pyruvic Transaminase (GPT), and C-reactive protein (CRP) were performed by using the dry-chemistry system (Fuji DRI-CHEM 7000VZ) and appropriate FUJI DRI-CHEM slides (FUJIFILM).

2.3. Antibodies

For Western blot analysis, we used an anti-human IgG (Fc specific)-alkaline phosphatase antibody (from rabbit, Sigma, 1:3000) for detection of hNectin-2Ig. For immunohistochemistry, we used antibodies against human IgG (from goat, Sigma, 1:2000), nectin-2 (from rabbit, GeneTex, 1:3000, it was not reacted with hNectin-2Ig), proliferating cell nuclear antigen (PCNA) (from mouse, DAKO, 1:1000), carboxypeptidase A (CPA) (from rabbit, Abcam, 1:1000), cleaved caspase-3 (from rabbit, Cell Signaling Technology, 1: 1000), and Grp78 (from rabbit, GeneTex, 1:3000) as primary antibodies.

2.4. Western blot analysis

To confirm the expression of hNectin-2Ig in the transgenic sera, Western blot analysis was performed by the method of Towbin et al. [15]. 10 μL of each serum sample was added protein G-Sepharose 4B (Sigma) to absorb fusion proteins for 3 h at 4 °C. After the beads were washed with phosphate-buffered saline, the bound proteins were fractionated by 10% SDS-polyacrylamide gel. Then the separated proteins were electrophoretically transferred to a Sequi-Blot PVDF membrane (Bio-Rad). Blotted membrane was treated sequentially with blocking solution (5% skim milk in phosphate buffered saline with 0.05% Tween 20), primary antibody, and finally the detection was performed using Western Blue Stabilized Substrate for Alkaline Phosphatase (Promega) according to the manufacturer's instructions.

2.5. Histopathological analyses

Under deep combination anesthesia mentioned above, mice were perfused transcardially with 4% paraformaldehyde (PFA) in 0.1 M sodium phosphate buffer (PB). The pancreases and other organs were excised and further immersed overnight in the same fixative or Bouin's fixative (Wako). The fixed organs were dehydrated and then embedded in paraffin-wax in the usual manner. Paraffin sections (2–4 μm in thick) were cut and stained with haematoxylin and eosin (H. E.). Immunohistochemistry was performed using a Histofine SAB-PO(G) kit for human IgG, Simple Stain MAX-PO(M) kit for PCNA, or Simple Stain MAX-PO(R) kit for other rabbit antibodies, according to the manufacturer's instructions (Nichirei). Briefly, the rehydrated sections were incubated with 0.1% H_2O_2 in methanol for endogenous peroxidase blocking and were subjected to heat-induced antigen retrieval by using ImmunoSaver (Nisshin EM) for PCNA, cleaved caspase-3, CPA, followed by primary antibody incubation overnight 4 °C. Then sections were treated with immunohistochemical detection system mentioned above, followed by a detection with 3,3′-Diaminobenzidine tetrahydrochloride solution (ImmPACT DAB Substrate, Vector Laboratories).

2.6. Transmission electron microscope

Ultrastructural analyses of pancreatic tissue from 28-week-old hNectin-2Ig Tg and non-Tg were performed using transmission electron microscopy (TEM). For TEM, sections were fixed with 2% PFA-2.5% glutaraldehyde (GA) in 0.1 M PB and ultrathin sections were prepared and stained with 2% uranyl acetate according to standard procedures. The ultrathin sections on the grids were observed by a TEM (JEM-1200EX; JEOL) at an acceleration voltage of 80 kV.

2.7. Statistics

The values were expressed as the averages of at least three times ± standard deviations. Statistical analysis was performed by Student's *t*-test.

3. Results

3.1. Significant increase in serum amylase and lipase in hNectin-2Ig Tg

Two transgenic mouse lines (2216 and 2246) expressing hNectin-2Ig were generated. Western blot analysis of their sera