International Immunopharmacology 60 (2018) 211–220

Contents lists available at ScienceDirect

International Immunopharmacology

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

Orally administered brown seaweed-derived β-glucan effectively restrained development of gastric dysplasia in *A4gnt* KO mice that spontaneously develop gastric adenocarcinoma

Mark Joseph Desamero[a,b], Shigeru Kakuta[a,*], James Kenn Chambers[c], Kazuyuki Uchida[c], Satoshi Hachimura[d], Masaya Takamoto[e], Jun Nakayama[f], Hiroyuki Nakayama[c], Shigeru Kyuwa[a]

[a] Laboratory of Biomedical Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
[b] Department of Basic Veterinary Sciences, College of Veterinary Medicine, University of the Philippines Los Baños, Laguna 4031, Philippines
[c] Laboratory of Veterinary Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
[d] Research Center for Food Safety, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
[e] Department of Infection and Host Defense, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan
[f] Department of Molecular Pathology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan

ARTICLE INFO

Keywords:
Anti-inflammatory
β-Glucan
Brown seaweed
Dysplasia
Precancerous lesion
Disease model

ABSTRACT

β-Glucan refers to a heterogeneous group of chemically defined storage polysaccharides containing β-(1,3)-D-linked glucose polymers with branches connected by either β-(1,4) or β-(1,6) glycosidic linkage. To date, an extensive amount of scientific evidence supports their multifunctional biological activities, but their potential involvement in the progression of premalignant lesions remains to be clarified. *A4gnt* KO mice that lack α1,4-*N*-acetylglucosamine-capped *O*-glycans in gastric gland mucin are a unique animal model for gastric cancer because the mutant mice spontaneously develop gastric cancer through hyperplasia-dysplasia-adenocarcinoma sequence. In particular, *A4gnt* KO mice show gastric dysplasia during 10–20 weeks of age. Here we investigated the putative gastro-protective activity of brown seaweed-derived β-glucan (Laminaran) against development of gastric dysplasia, precancerous lesion for gastric cancer in *A4gnt* KO mice. The mutant mice at 12 weeks of age were randomly assigned into three treatment groups namely, wildtype control + distilled water (normal control), *A4gnt* KO mice + distilled water (untreated control), and *A4gnt* KO mice + 100 mg/kg Laminaran. After 3 weeks, the stomach was removed and examined for morphology and gene expression patterns. In contrast to the untreated control group, administration of Laminaran substantially attenuated gastric dysplasia development and counterbalanced the increased induction in cell proliferation and angiogenesis. Furthermore, Laminaran treatment effectively overcame the *A4gnt* KO-induced alteration in the gene expression profile of selected cytokines as revealed by real-time PCR analysis. Collectively, our present findings indicate that β-glucan can potentially restrain the development of gastric dysplasia to mediate their tissue-preserving activity.

1. Introduction

Safeguarding health over a wide spectrum of diseases still remains to be a daunting task for life scientists and researchers as current treatment approaches and control measures have only afforded limited therapeutic value. This has prompted scientific investigations to be focused on disease prevention and early disease management leading to intensified explorative studies on the potential role of food and nutrition. The term *functional food* was eventually conceptualized and is generically defined as any food or food components with health-promoting activities in addition to their essential nutritional benefits [1]. To date, a broad range of examples has been extensively characterized, among which includes eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), probiotics, phytochemicals, lycopene and β-glucans, etc. [2].

β-Glucans are heterogeneous group of chemically-defined polysaccharides containing β-(1,3)-D-glucopyranosyl backbone with β-(1,4)- or β-(1,6)-linked side chains and acting as structural cell wall components or storage carbohydrates in plants, cereals, fungi, algae and some bacterial species [3]. Depending on the source, they may be referred to by multiple terms such as Pleuran (*Pleurotus ostreatus*), Lentinan (*Lentinus edodes*), Schizophyllan (*Schizophyllum commune*), Grifolan (*Grifola*

* Corresponding author.
E-mail address: akakuta@mail.ecc.u-tokyo.ac.jp (S. Kakuta).

https://doi.org/10.1016/j.intimp.2018.05.002
Received 7 March 2018; Received in revised form 22 April 2018; Accepted 6 May 2018
1567-5769/ © 2018 Elsevier B.V. All rights reserved.

frondosa), Scleroglucan (*Sclerotium rolfsii*), Sparan (*Sparassis crispa*) and Laminaran (*Laminaria* spp., *Eisenia* spp.) [4]. As humans and vertebrate animals are inherently devoid of the machinery to synthesize β-glucans, these polysaccharide compounds are traditionally regarded as biological response modifiers [5], which can mediate a broad range of health-enhancing properties in conjunction to their relative safety and compatibility with current chemotherapeutic and radiation treatment modalities [6–8]. However, in spite of the overwhelming amount of experimental data supporting the anti-cancer property of β-glucans as shown by various in-vitro, in-vivo and human clinical studies [9–11], their potential role in the progression of precancerous lesions still requires further investigation.

α1,4-*N*-acetylglucosaminyltransferase (α4GnT) is a glycosyltransferase responsible for the biosynthesis of α1,4-*N*-acetylglucosamine-capped *O*-glycans (αGlcNAc) in gastric gland mucin [12]. *A4gnt* KO mice deficient in this particular enzyme is a unique animal model for gastric cancer, because the mutant mice spontaneously develop gastric cancer through hyperplasia-dysplasia-adenocarcinoma sequence [13]. In particular, *A4gnt* KO mice show gastric dysplasia, precancerous lesion of gastric cancer, during 10–20 weeks of age.

Here in the present study, we determined the potential merit of oral administration of soluble β-glucan from the brown algae, *Eisenia bicyclis*, on the development of gastric dysplasia in *A4gnt* KO mice during 15–18 weeks of age. The findings of this study shall allow better substantiation of the efficacy of β-glucan supplementation in the development of gastric dysplasia.

2. Materials and methods

2.1. Chemical and dosage

Laminaran was purchased from Tokyo Chemical Industrial Co., Ltd. (Tokyo, Japan). As previously described by Ermakova et al. [14], Laminaran from the brown algae, *Eisenia bicyclis*, is a low molecular weight (~5 kDa), soluble polysaccharide defined by the presence of β-(1–3)-linked glucose polymers and contains branches connected by β-(1–6) glycosidic bonds. The ratio of β-D-1,3 and β-D-1,6 glycosidic bonds is approximately 1.5:1 whereas the degree of polymerization (DP) falls within the range of about 20–25 [15]. The dosage adopted in the present study essentially conforms to the previous data on β-glucans as published elsewhere [16,17].

2.2. Animals

Deletion of *A4gnt* gene encoding α1,4-*N*-acetylglucosaminyltransferase in mice led to loss of αGlcNAc in the gastric gland mucin [13]. *A4gnt* KO mice spontaneously developed differentiated-type gastric adenocarcinoma in a hyperplasia-dysplasia-carcinoma sequence that is restrictively distributed in the antrum of the stomach. In this study, we preferentially used 12-week old *A4gnt* KO animals, which exhibited a low-grade gastric dysplasia, and age-matched wildtype C57BL/6J mice obtained from Nihon SLC as control animals.

Animals were housed in standard polycarbonate cages and maintained under specific pathogen-free condition with 12 h light/dark cycle (8:00 am:8:00 pm). Rodent chow (CRF-1, Oriental Yeast Co., Ltd., Japan) and water were given ad libitum. All experimental procedures were performed in accordance with the guidelines and approval of the Institutional Animal Care and Use Committee, Graduate School of Agriculture and Life Sciences, The University of Tokyo (Approval No. P17-005H02).

2.3. Treatment group

Twelve week-old wildtype (C57BL/6J) and *A4gnt* KO mice of both sexes (18–25 g) were randomly assigned into the following treatment groups consisting of two replicates as follows: Group I: wild-type + distilled water (n = 8) (normal control); Group II: *A4gnt* KO + distilled water (n = 12) (untreated control); and Group III: *A4gnt* KO + 100 mg/kg BW/day Laminaran (n = 12). All treatments were administered daily via oral gavage for 21 consecutive days.

2.4. Gross assessment of pyloric mucosa

To analyze the effect of Laminaran treatment on pyloric mucosa in *A4gnt* KO mice, the following semi-quantitative scoring was used: 0-healthy mucosa/none, 1-mildly, 2-moderately and 3-markedly elevated.

2.5. Histopathology

One hour prior to sacrifice, animals were injected intraperitoneally with Bromodeoxyuridine (5-bromo-2-deoxyuridine, BrdU) solution (10 mg/kg) to label rapidly proliferating cells in the S-phase of the cell cycle [13]. Stomach along with a small portion of the duodenum was harvested and divided into half for subsequent immunohistochemical and gene expression analyses. Stomach was cut longitudinally along the greater curvature, washed with 1× PBS, fixed in 10% buffered formaldehyde for 48 h and subjected to routine paraffin technique. Four-micrometer sections were then prepared and stained with standard H&E. Pyloric mucosal thickness was measured from the base of the gastric mucosal layer up to the highest tip of a properly oriented epithelium at three different areas of the pyloric region. Mean of triplicate measurements for each animals was obtained. For determining the number of infiltrating granulocytes, a defined 100 μm area with the highest cell density along the pyloric region was counted and a mean of triplicate measurements was then obtained.

2.6. CD3, IL-10, BrdU and CD31 immunohistochemistry

Immunohistochemical analysis was performed to further ascertain the effect of Laminaran treatment on the inflammatory process as well as on the proliferative and angiogenic processes through determination of CD3, IL-10, BrdU and CD31 marker expressions, respectively. Briefly, Formalin-fixed and paraffin-embedded (FFPE) tissue sections were deparaffinized, rehydrated with increasing grades of alcohol, and washed with Tris-buffered saline (TBS; 0.1 M, pH 7.4). Prior to incubation with primary antibodies, antigen retrieval was carried out using 4 N HCl followed by digestion with 0.5% trypsin (Gibco®, Life Technologies Corp., USA) for 30 min at 37 °C for anti-BrdU whereas incubation in 0.1 M sodium citrate buffer, pH 6 for 10 min was done for anti-IL-10, anti-CD3 and anti-CD31. After this, blocking of endogenous peroxidase and non-specific background staining were accomplished using 10% H_2O_2-methanol solution and 8% skimmed milk, respectively. Tissue sections were subsequently incubated overnight in a humidified chamber at 4 °C using the following primary antibodies: anti-CD3 (DakoCytomation, Denmark; polyclonal), anti-IL-10 (BD Biosciences, USA; clone JES-2A5), anti-BrdU (DakoCytomation, Denmark; clone Bu2a) and anti-CD31 (NeoMarkers, USA; polyclonal). Immunolabeling of tissue sections was performed using Histofine Mousestain Kit (Nicherei Biosciences, Japan) for anti-BrdU antibody, streptavidin-labeled secondary antibody for anti-IL-10 antibody, and ready-to-use secondary antibody for anti-CD3 and CD31 antibodies. Immunoreaction was finally visualized using diaminobenzidine tetrahydrochloride-H_2O_2 solution. Tissue sections in lieu of primary antibody served as internal control showing absence of any immunoreaction.

The number of BrdU-positive cells was assessed using a defined 50 μm area in the pyloric region with the highest cell density and the mean of three measurements was obtained. CD3- and CD31-positive cells were also determined in a defined 100 μm area along the pyloric region with the highest cell density and the average of triplicate measurements was achieved. IL-10 immunoreaction, on the other hand, was

Download English Version:

https://daneshyari.com/en/article/8531139

Download Persian Version:

https://daneshyari.com/article/8531139

Daneshyari.com