Experimental and Toxicologic Pathology 67 (2015) 349–353

Contents lists available at ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

Induction of duodenal mucosal tumors of intestinal epithelial cell origin showing frequent nuclear β-catenin accumulation similar to the concurrently induced colorectal tumors in rats after treatment with azoxymethane

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ARTICLE INFO

Article history:
Received 24 January 2015
Accepted 26 March 2015

Keywords:
Rat
Azoxymethane
Duodenal tumors
Histogenesis
Cytokeratin
Mucin

ABSTRACT

Azoxymethane (AOM) is a potent carcinogen used for induction of colon tumors in rats and mice. It is also known that AOM treatment induces small bowel tumors in addition to colorectal tumors in rats. The present study examined the histogenesis of AOM-induced rat duodenal tumors in comparison with concurrently induced colorectal tumors by histochemical and immunohistochemical approaches. Duodenal and colorectal tumors were positive for both periodic acid-Schiff reaction and Alcian blue staining. Immunohistochemically, duodenal tumors were positive for intestinal epithelial markers such as cytokeratin (CK) 20 (100%) and mucin (MUC) 2 (91.7%) but negative for pancreaticobiliary markers such as CK7 (100%) and MUC1 (100%). All colorectal tumors were also negative for CK7 and MUC1 but positive for CK20. Eighty percent of colorectal tumors were positive for MUC2. In addition, nuclear accumulation of β-catenin was found in duodenal tumors (70.8%), which was similar to colorectal tumors (90.0%). These results indicate that duodenal tumors induced by AOM treatment of rats were derived from intestinal epithelium. Similar to colorectal tumors, nuclear accumulation of β-catenin indicates activation of Wnt signaling as a driving force for tumor progression in AOM-induced duodenal tumors.

1. Introduction

In humans, small bowel tumors are rarely observed (Hancock, 1970; Treadwell and White, 1975) and their histogenesis is different between tumors generated at the papilla of Vater in the duodenum. The papilla of Vater consists of four parts: the ampulloduodenum, ampullopancreatobiliary common duct, ampullopancreatic duct, and ampullobiliary duct (Fischer and Zhou, 2004; Matsubayashi et al., 1999). The papilla is formed by the union of 2 distinct types of mucosa. The ampulloduodenum is covered with intestinal epithelia, and the other parts are covered with pancreatobiliary-type ductal epithelia (Fischer and Zhou, 2004). Ampullopancreatobiliary common duct tumors are classified into two histological subtypes, intestinal and pancreaticobiliary, depending on the origin of the transformed epithelial cells (Albores-Saavedra, 2000; Kimura et al., 1994). Most ampullopancreatobiliary common duct tumors of the intestinal type are positive for cytokeratin (CK) 20 and mucin (MUC) 2 but negative for CK7 (Fischer and Zhou, 2004). In contrast, most tumors of the pancreaticobiliary type are positive for CK7 but negative for CK20 and MUC2 (Fischer and Zhou, 2004).

Azoxymethane (AOM) is a potent carcinogen used for induction of colorectal tumors in rats and mice (Takahashi and Wakabayashi, 2004). It has been used in studies to evaluate the efficacy of preventative treatments for AOM-induced colorectal carcinogenesis (Escribano et al., 2004; Marotta et al., 2003; Orii et al., 2003). It has been also reported that treatment with AOM induces duodenal tumors as well as colorectal tumors in rats (Simmen et al., 2009). While the histogenesis of these tumors has not been investigated until now, it is possible that the papilla of Vater is their origin.

β-Catenin plays a key role in inflammation and tumor development in the colon (Keerthivasan et al., 2014). Aberrant activation of the Wnt/β-catenin pathway has been implicated in the progression of colorectal tumors in humans (Clevers and Nusse, 2012). Upon

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http://dx.doi.org/10.1016/j.etp.2015.03.002
0940-2993/

activation, β-catenin in the cytoplasm is stabilized and translocated into the nucleus to bind to Tcf4 and serve as a transcriptional regulator of growth-promoting genes (Clevers and Nusse, 2012; He et al., 1998; Tetsu and McCormick, 1999). Because the tumor suppressor adenomatous polyposis coli (APC) destabilizes β-catenin to allow ubiquitin-proteasomal protein degradation (Willert and Nusse, 1998), loss of APC function due to mutation facilitates nuclear translocation of β-catenin (Anderson et al., 2002). In experimental colon carcinogenesis, AOM-induced colorectal tumors also show an increase in nuclear accumulation of β-catenin (Sheng et al., 1998). However, it is unknown whether experimentally induced small bowel tumors also show induction of nuclear β-catenin accumulation.

Because of frequent induction of duodenal tumors (Simmen et al., 2009), AOM treatment of rats may provide a versatile model for investigation of rare duodenal tumors in man, in addition to the well-established human colorectal tumor model. In the present study, we examined the histogenesis of AOM-induced duodenal tumors by applying histochemical and immunohistochemical approaches. For comparison, colorectal tumors that were concurrently induced by AOM treatment were also examined.

2. Materials and methods

2.1. Animals and experimental design

Twenty-seven 5-week-old male F344/NSlc rats, purchased from Japan SLC, Inc. (Hamamatsu, Japan), were acclimated to laboratory conditions for 1 week. Animals were given free access to powdered diets and were kept under standard conditions (room temperature: 22 ± 3 °C; relative humidity: 56 ± 11%; 12-h light/dark cycle). After 1 week of acclimation, all animals were subjected to three subcutaneous injections each of azoxymethane (AOM; 15 mg/kg body weight) (Sigma–Aldrich Co., St Louis, MO, USA) at 1 week intervals and then maintained for 29–35 weeks. The animals were euthanized by exsanguination under deep isoflurane anesthesia, and all 24 duodenal tumors developed were removed, fixed in 10% neutral buffered formalin, and embedded in paraffin. Ten concurrently induced colorectal tumors in AOM-treated animals were also similarly processed for comparison.

All procedures were conducted in compliance with the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, June 1, 2006) and according to a protocol approved by the Animal Care and Use Committee of the Tokyo University of Agriculture and Technology.

2.2. Histology and immunohistochemistry

Sections were cut and stained with hematoxylin and eosin (HE) and Alcian blue, and subjected to periodic acid-Schiff (PAS) reaction.

For immunohistochemistry, primary antibodies against the following markers were used: CK7 (mouse monoclonal, clone OV-TL12/30, prediluted; BioGenex, Fremont, CA, USA), expressed in broad types of epithelial cells except for intestinal epithelial cells (Chu et al., 2000; Ramaekers et al., 1990), CK20 (mouse monoclonal, clone Ks20.8, prediluted, Dako, Carpinteria, CA, USA), expressed in the gastric and intestinal epithelium, urothelium, and Merkel cells (Chu et al., 2000; Moll et al., 1992), MUC1 (mouse monoclonal, clone VU4H5, 1:50; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), a membrane-associated mucin expressed on the apical surfaces of most epithelial cells (Ho et al., 1993), including those of the pancreatic ductal as well as centroacinar cells (Jonckheere et al., 2010), but is sparsely expressed in gastric surface cells, and small intestinal and colonic epithelium (Ho et al., 1993), MUC2 (mouse monoclonal, clone CCP58, 1:500; Dako), a secreted mucin expressed in intestinal epithelia (Ho et al., 1993), and β-catenin (rabbit monoclonal, clone H-102, 1:100; Santa Cruz Biotechnology) that is expressed in the cell membrane of broad types of epithelial cells, and its nuclear translocation indicates acquisition of the malignant phenotype of epithelial tumors such as colorectal tumors (Willert and Nusse, 1998). The sections were subjected to antigen retrieval by autoclaving in citrate buffer (pH 6.0) at 121 °C for 10 min. To quench endogenous peroxidase, the sections were incubated in 0.3% (vol/vol) hydrogen peroxide in absolute methanol for 30 min, followed by incubation with the primary antibodies. Immunodetection was carried out using a Vectastain® Elite ABC kit (Vector Laboratories Inc., Burlingame, CA, USA) with 3,3′-diaminobenzidine/hydrogen peroxide as the chromogen as described previously (Shibutani et al., 2007). The sections were then counterstained with hematoxylin and coverslipped for microscopic examination.

As positive controls, rat skin epidermal tissue was used for CK7, rat colorectal mucosal tissue for CK20 and MUC2, rat pancreatic tissue for MUC1, and an AOM-induced rat colorectal tumor for β-catenin. For negative controls, the primary antibody was replaced with non-immunized sera.

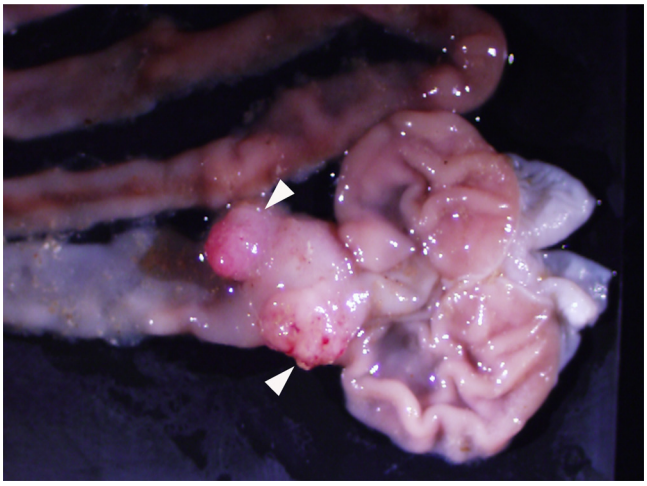

Fig. 1. Macroscopic view of a duodenal tumor that developed at the location just posterior to the pyloric ring of the glandular stomach. The tumor is separated into two parts, as indicated by arrowheads, after cutting through the duodenum.

3. Results

All 24 duodenal tumors had developed at the location just posterior to the pyloric ring of the glandular stomach at a total incidence of 55.6% and multiplicity of 0.89/animal. Five duodenal tumors were histopathologically diagnosed as adenomas (20.8% of total duodenal tumors) and 19 as adenocarcinomas (79.2%). Among 10 colorectal tumors examined, seven colorectal tumors were diagnosed as adenomas (70.0% of total colorectal tumors) and three as adenocarcinomas (30.0%). All of these tumors displayed macroscopical protrusion (Fig. 1). Adenomas were histologically classified as tubular or papillary types, and adenocarcinomas as the tubular type (Fig. 2A) accompanied by papillary growth in some parts with goblet cell differentiation at various degrees (Fig. 2B) in HE-stained sections. Similar to all of the examined colorectal tumors, goblet cells in all duodenal tumors were positive for both PAS reaction (Fig. 3A) and Alcian blue staining (Fig. 3B).

Immunohistochemically, all duodenal tumors were exclusively negative for CK7 (Fig. 4A) and MUC1 (Fig. 4C) but positive for CK20 (Fig. 4B). Twenty-two out of 24 tumors (91.7%) were also positive for MUC2 (Fig. 4D). All 10 colorectal tumors were negative for CK7