Pathology – Research and Practice 211 (2015) 797–800

Contents lists available at ScienceDirect

Pathology – Research and Practice

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

Short Communicaiton

Squamous morula formation in colorectal adenoma: Immunohistochemical and molecular analyses

Kunio Mochizuki*, Tetsuo Kondo, Naoki Oishi, Ippei Tahara, Tomohiro Inoue, Kazunari Kasai, Tadao Nakazawa, Ryohei Katoh

Department of Pathology, School of Medicine, University of Yamanashi, Yamanashi, Japan

ARTICLE INFO

Article history:
Received 5 February 2015
Received in revised form 23 April 2015
Accepted 5 May 2015

Keywords:
Squamous morula
Colorectal adenoma
Cytokeratin 5/6
β-Catenin
Ki-67

ABSTRACT

Little is known about the squamous morular component (SMC) in colorectal neoplasms because of its rarity. The aim of the present study is to elucidate the morphological, immunohistochemical and genetic characteristics of SMCs in colorectal adenomas. Five colorectal adenomas having SMCs were resected from five patients endoscopically. On immunohistochemical examination (four cases), all SMCs were positive for cytokeratin 5/6 in their cytoplasm and positive for β-catenin in their cytoplasm and nuclei. A nuclear positivity of p63 was detected in one SMC. All SMCs were negative for p53, chromogranin A, synaptophysin and NCAM. There was no Ki-67 expression in any of the SMCs. We detected none of mutations of *β-catenin*, *KRAS* and *BRAF* by microdissection and polymerase chain reaction-direct sequence in any of the four examined SMCs. SMCs are a rare but problematic finding in colorectal adenomas. Using immunohistochemistry for β-catenin, cytokeratin 5/6, Ki-67, p53, chromogranin A, synaptophysin and NCAM can facilitate the diagnosis of these peculiar cell nests.

Introduction

Five thousand seven hundred seventy-eight adenomas or adenomas containing carcinoma from 3215 patients were examined by routine histologic methods for the presence of epithelial metaplasias (squamous cell metaplasia: 0.44%; Paneth cell metaplasia: 0.20%; melanocytic metaplasia: 0.017%); the areas of squamous differentiation usually had the appearance of discrete nests of immature squamous epithelial cells without the formation of identifiable keratin or keratin pearls [1]. Sarlin and Mori first used the term 'squamous morula' in colorectal neoplasms in 1984 [2]. Apart from colonic adenoma and adenocarcinoma, squamous morula formation has been reported in other benign and malignant neoplasms, including well differentiated fetal adenocarcinoma of the lung, endometrioid adenocarcinoma of the uterus and ovary, cribriform-morular variant of thyroid carcinoma, pyloric gland-type adenoma of the gallbladder and pancreatoblastoma [2–12].

Aberrant nuclear expression of β-catenin has recently been detected in most of the aforementioned squamous morula-associated neoplasms; it is reportedly a common denominator in squamous morulae [3–10]. β-catenin acts as a transcriptional activator of the Wnt signaling pathway and plays an important role in determining tissue morphology [13,14]. The tumor suppressor gene *APC* is the major component of the Wnt signaling pathway and controls the intracytoplasmic expression of β-catenin. Although genetic alterations of *APC* are much more frequent in colorectal adenoma/adenocarcinomas than in other neoplasms previously reported as squamous morula-associated neoplasms [15], we rarely see squamous morula formations in colorectal neoplasms.

Information is scarce on squamous morula formation in colorectal neoplasms because of its rarity. The aim of the present study is to elucidate the morphological and immunohistochemical characteristics of squamous morular components (SMCs) in colorectal adenomas and to clarify the gene mutations of *β-catenin*, *KRAS* and *BRAF*.

Materials and methods

We collected five colorectal adenomas having SMCs obtained by endoscopic mucosal resection between July 2009 and August 2014 at the University of Yamanashi and Fujiyoshida Municipal Hospitals. In the current study, we defined squamous morulae as having discrete nests or uniformly demarcated sheets of

* Corresponding author at: Department of Pathology, School of Medicine, University of Yamanashi, 1110 Shimokato, Chuo, Yamanashi, 409-3898, Japan. Tel.: +81 55 2739529; fax: +81 55 2739534.
E-mail address: kuniom@yamanashi.ac.jp (K. Mochizuki).

http://dx.doi.org/10.1016/j.prp.2015.05.002
0344-0338/

Table 1
Clinicopathologic findings of five cases with squamous morular component in colonic adenomas.

Case	Age	Sex	Location	Tumor size (mm)	Gross feature
1	81	M	Transverse colon	13	Pedunculated
2	84	M	Rectum	15	Semi-pedunculated
3	72	F	Ascending colon	18	Semi-pedunculated
4	80	F	Rectum	9	Superficial elevated
5	74	M	Sigmoid colon	13	Pedunculated

M: male; F: female.

nonkeratinizing (immature) squamous cells that are oval or spindle-shaped, bland, uniform and lacking prominent nucleoli [12]. Two pathologists (K.M. and R.K.) independently reviewed hematoxylin-eosin stained slides blinded to the original pathological diagnosis.

Sections, 4-μm thick, were cut from formalin-fixed, paraffin-embedded tissue blocks which were dewaxed and rehydrated. Cytokeratin 5/6 (D5/16 B4, 1:100; Dako, Glostrup, Denmark), p63 (4A4, diluted; Nichirei Biosciences, Tokyo, Japan), β-catenin (17C2, 1:200; Novocastra Laboratories, Newcastle upon Tyne, UK), p53 (DO-7, 1:50; Dako, Glostrup, Denmark), chromogranin A (polyclonal, diluted; Nichirei Biosciences, Tokyo, Japan), synaptophysin (27G12, diluted; Nichirei Biosciences, Tokyo, Japan), NCAM (1BS, diluted; Nichirei Biosciences, Tokyo, Japan) and Ki-67 (MIB-1, 1:100; Dako, Glostrup, Denmark) were used as the primary antibodies. For antigen retrieval, heat treatments were applied (120 °C, 10 min in citrate buffer) by autoclaving before each primary antibody reaction. After inhibiting endogenous peroxidase, we used positive controls to perform the primary antibody reactions. We used the N-Histofine Simple Stain MAX PO (MULTI) (Nichirei Biosciences, Tokyo, Japan) with diaminobenzidine as a chromogen and a light counterstain with hematoxylin to perform immunohistochemistry. Two pathologists (K.M. and R.K.) simultaneously reviewed immunostained sections using a double-headed light microscope. We defined specimens that contained >10% immunostained cells as immunopositive and sections that contained ≤10% immunostained cells as immunonegative. The Ki-67 labeling index was determined from a count of 100 cells.

Five 5-μm thick serial sections were cut from routinely processed, formalin-fixed and paraffin-embedded tissue blocks and subsequently stained with hematoxylin after deparaffinization. The tumor tissue was microdissected with Applied Biosystems® Arcturus$^{XT™}$ Microdissection System (Life Technologies, CA) and the nucleic acids extracted in standard procedures. The squamous morula and adenoma components were separately microdissected and examined at the same time. For DNA extraction, we treated the microdissected tissue samples with RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE (Life Technologies, CA, USA). We amplified DNA by polymerase chain reaction (PCR) with HotStarTaq DNA Polymerase (QIAGEN, Tokyo, Japan) using pairs of primers encompassing *β-catenin* exon 3 (5′-ATGGAACCAGACAGAAAAGCG-3′ and 5′-CAGGATTGCCTTTACCACTCA-3′), *KRAS* codons 12/13 (5′-TTAACCTTATGTGTGACATGTTCTAA-3′ and 5′-AGAATGGTCCTGCACCAGTAA-3′), *KRAS* codon 61 (5′-CCAGACTGTGTTTCTCCCTTCT-3′ and 5′-AAACCCACCTATAATGGTGAATATC-3′) and *BRAF* exon 15 (5′-TCATAATGCTTGCTCTGATAGGA-3′ and 5′-GGCCAAAAATTTAATCAGTGGA-3′). Samples were denatured at 95 °C for 15 minutes, followed by 40 three-step cycles (95 °C for 30 seconds, 58 °C for 30 seconds and 72 °C for 60 s) and then at 72 °C for 10 min in the MyCycler Thermal Cycler (BIO-RAD, Tokyo, Japan). Amplified fragments were separated on a 3% agarose gel and visualized by Midori Green Advance DNA Stain (Nippon Gene, Tokyo, Japan). BEX Co., Ltd. (Tokyo, Japan) performed analyses by direct sequence of *β-catenin* exon 3, *KRAS* codons 12/13, *KRAS* codon 61 and *BRAF* exon 15.

Results

Clinical findings of the five patients with colorectal adenoma having squamous morular formation are summarized in Table 1. There were three males and two females ranging in age from 72 to 84 years with a mean age of 78.2 years. SMC was detected in five colorectal adenoma samples obtained during endoscopic mucosal resection for colorectal polyp. There were two lesions from the rectum, one in the ascending colon, one in the transverse colon and one in the sigmoid colon. Two lesions were the pedunculated type, two lesions were semi-pedunculated and one lesion was the superficial elevated type. The maximum diameter of tumors ranged from 9 to 18 mm (average 13.6 mm).

Histological examination revealed solitary or multiple SMCs that occupied less than 10% of tumor tissue. They replaced the adenomatous glands partially or entirely, were primarily well-defined forming solid epithelial nests without keratinization or intercellular bridges (Fig. 1a). The constituent cells were relatively small, round to short-spindled and slightly eosinophilic with round to oval, bland nuclei (Fig. 1b).

Table 2 contains a summary of the expressions of cytokeratin 5/6, p63, β-catenin, p53, chromogranin A, synaptophysin, NCAM and Ki-67 seen in 4 of the 5 SMCs. Because there was only a very small quantity of SMC in the colonic adenoma (case 5), this case could not be examined by immunohistochemical analysis. Cytokeratin 5/6 expressed in all 4 SMCs (Fig. 1c), and p63 expressed in 1/4 SMCs (Fig. 1d). In all 4 SMCs, β-catenin expressed intensely in the nuclei and faintly in the cytoplasm (Fig. 1e), whereas none of the SMCs had any expression of p53, chromogranin A, synaptophysin, NCAM or Ki-67 (Fig. 1f).

We did not detect *β-catenin*, *KRAS* or *BRAF* mutations in either the squamous morula or adenoma components from any of the four

Table 2
Immunohistochemical results of squamous morular component in each colorectal adenoma.

	CK 5/6	p63	β-Catenin[a]	p53	Chromogranin A	Synaptophysin	NCAM	Ki-67 labeling index (%)
Case 1	+	–	+	–	–	–	–	0
Case 2	+	–	+	–	–	–	–	0
Case 3	+	+	+	–	–	–	–	0
Case 4	+	–	+	–	–	–	–	0
Case 5	NI	NI	NI	NI	NI	NI	NI	NI

CK: cytokeratin; NI: not informative.
[a] Staining cells were expressed intensely in the nuclei and faintly in the cytoplasm.