Available online at www.sciencedirect.com
ScienceDirect
Annals of Diagnostic Pathology 14 (2010) 408–412
Annals of DIAGNOSTIC PATHOLOGY

Muscularis mucosae versus muscularis propria in gallbladder, cystic duct, and common bile duct: smoothelin and desmin immunohistochemical study☆

Kirtee Raparia, MD, Qihui J. Zhai, MD, Mary R. Schwartz, MD, Steven S. Shen, MD, Alberto G. Ayala, MD, Jae Y. Ro, MD*

Department of Pathology, The Methodist Hospital, Weill Medical College, Cornell University, Houston, TX 77030, USA

Abstract The muscle layer in the cystic duct and common bile duct is not well defined, and it is unresolved whether it represents muscularis mucosae or muscularis propria. Smoothelin is a novel smooth muscle–specific contractile protein expressed only in fully differentiated smooth muscle cells of the muscularis propria and not in proliferative or noncontractile smooth muscle cells of the muscularis mucosae. In this study, we characterize the histologic aspects of the muscle layer in gallbladder, cystic duct, and common bile duct by evaluation of routine histologic sections and the utilization of immunohistochemistry using desmin and smoothelin. Formalin-fixed, paraffin-embedded sections of the gallbladder (15 cases), cystic duct (11 cases), and common bile duct (10 cases) were stained for smoothelin and desmin. Staining intensity was evaluated as weak or strong. The staining pattern score was evaluated as follows: 0 or negative = less than or equal to 5% positivity, +1 or focal = 6% to 10% positivity, +2 or moderate = 11% to 50% positivity, and +3 = greater than 50% muscle cells positivity. With desmin, strong and diffuse (+3) staining was observed in all gallbladder cases (15/15, 100%), highlighting one continuous muscle layer. The muscle layer was discontinuous and interrupted in all cystic duct cases and in most common bile ducts, highlighted by the desmin stain. Smoothelin intensely stained (at least +2) muscle fibers in the gallbladder in 11 (73%) of 15 cases similar to that observed with desmin staining. In contrast, common bile ducts predominantly had absent or weak and focal immunostaining (0 or +1 staining) with smoothelin (7/10, 70%), with only a few cases (3/10, 30%) having +2 staining (no cases with +3). Cystic ducts also showed absent or weak and focal immunostaining with smoothelin, with 5 (44%) of 11 cases showing 2+ immunostaining with smoothelin (no cases with 3+). Based on our findings, we conclude that, in the gallbladder wall, the muscle layer is muscularis propria and there is no muscularis mucosae present. In the cystic duct and common bile duct, only an attenuated and incomplete muscle layer of muscularis mucosae is present; because there is no muscularis propria, there probably is limited contractile function. Differentiating these anatomical muscle structures may be important for the pathologic staging of carcinoma in these organs.

Keywords: Muscularis propria; Muscularis mucosae; Smoothelin; Desmin; Cancer staging

1. Introduction

The organs of the gastrointestinal tract and urinary system have 2 distinct muscle layers: muscularis mucosae (MM) and muscularis propria (MP). The MM in the urinary bladder is usually incomplete, and the muscle bundles are usually thin and different from those of the MP. Thickened hyperplastic MM can occur occasionally in the wall of the urinary bladder and create difficulty in diagnosis and staging, particularly in transurethral resections of bladder tumors [1].

The normal histology of the gallbladder and biliary tree is different from the remainder of the gastrointestinal tract, with the presence of only one muscle layer and lack of MM in gallbladder and a single discontinuous layer in the cystic

☆ This work was presented at the 98th Annual Meeting of the United States and Canadian Academy of Pathology, Boston, MA, March 2009.

* Corresponding author. Tel.: +1 713 441 2263; fax: +1 713 793 1603.
E-mail address: jaero@tmhs.org (J.Y. Ro).

1092-9134/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.anndiagpath.2010.05.013

duct and common bile duct (CBD). The scattered muscle fibers in cystic duct and CBD are not well characterized. Smoothelin is a novel smooth muscle–specific contractile protein expressed only in fully/terminally differentiated smooth muscle cells. Its expression is absent or weak in proliferative or noncontractile smooth muscle cells or myofibroblasts [2,3]. In a recent study, the immunohistochemical staining pattern of smoothelin in MP and MM (including its hyperplastic forms) in urinary bladder proved to be an attractive marker to be incorporated in the evaluation of transurethral resection specimens when distinguishing between the 2 layers was difficult [4,5].

To resolve unanswered questions and to determine the potential clinical significance about the characterization of the muscle layers in gallbladder, cystic duct, and CBD, we studied the muscle layers of these organs (36 cases in total) by immunohistochemistry using desmin and smoothelin. To the best of our knowledge, this is the first study in the literature that tries to delineate the basic histologic characteristics of muscle layers of these organs, which may be important for the pathologic staging of carcinoma in these organs.

2. Materials and methods

2.1. Specimens

Archival formalin-fixed, paraffin-embedded tissue blocks of the gallbladder (15 cases), cystic duct (11 cases), and CBD (10 cases) from The Methodist Hospital, Houston, TX, were randomly retrieved for the immunohistochemical study. The gallbladder and cystic duct specimens were selected from chronic cholecystitis cases of the same patients. The CBD specimens were from Whipple specimens removed for pancreatic cancer. Because the muscle layer is different depending on the level of CBD [6], the terminal portion of CBD was selected. These pancreatic cancer cases showed no carcinomatous involvement of the CBD.

2.2. Immunohistochemistry

After deparaffinization and rehydration of tissue sections, heat-induced epitope retrieval was performed in 10-mmol/L citrate buffer (pH 6.0); and heating for 3 times was performed before immunostaining. The following antibodies were used: smoothelin (R4A; 1:100 dilution; Abcam Inc, Cambridge, MA) and desmin (1:200; Dako, Carpinteria, CA). Tissue sections were incubated with primary antibody for 30 minutes at room temperature, washed with phosphate-buffered saline, and incubated with a secondary antibody conjugated to horseradish peroxidase (Benchmark IHC/ISH module; Ventana, Tucson, AZ). Hematoxylin was used as a counter stain. The interpretation of immunoreactivity was performed in a semiquantitative manner by analyzing the extent of the staining positivity of the muscle cells. Staining intensity was evaluated as weak

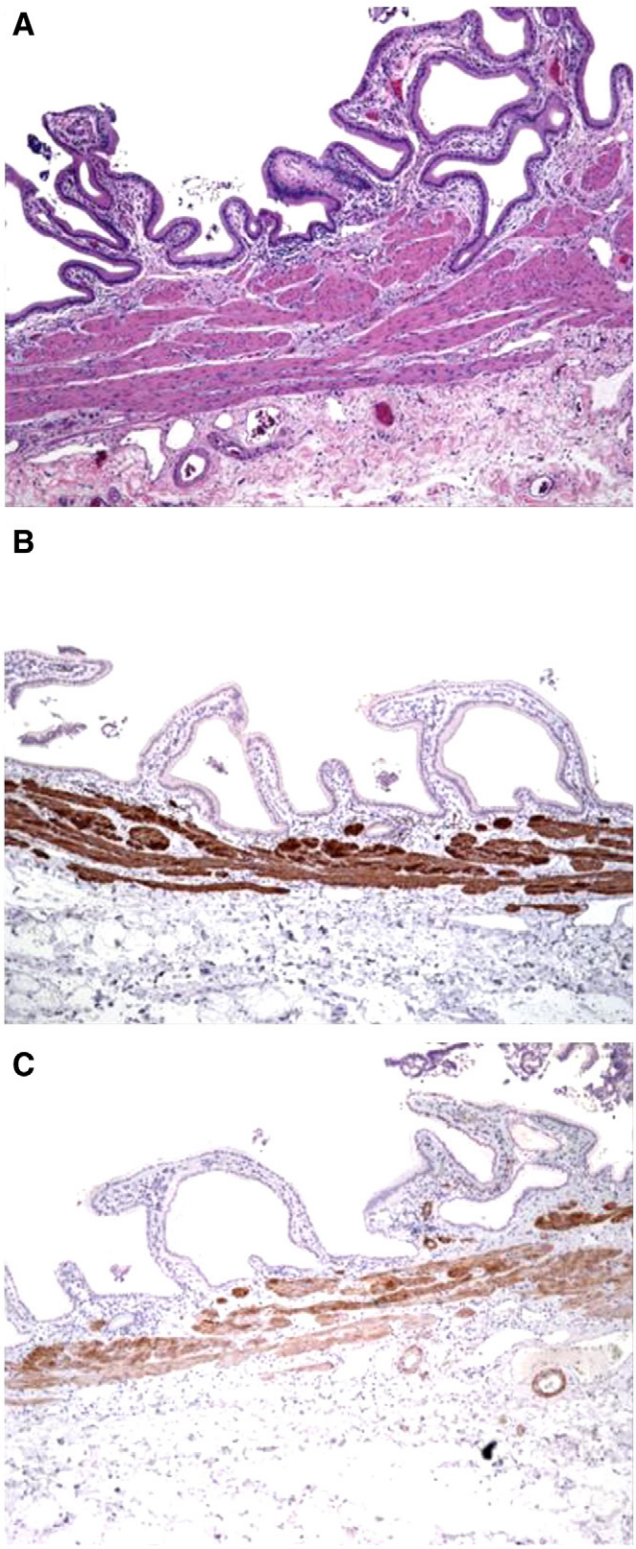

Fig. 1. (A) Smooth muscle layer of gallbladder showing one layer of smooth muscle fibers without any interruptions. (B) Staining of muscle layer of the gallbladder. Desmin highlights the single discontinuous muscle layer with strong and diffuse immunostaining. (C) Smoothelin also intensely stains the muscle layer of the gallbladder, suggesting it to be MP-type muscle.