In Vivo Endoluminal Ultrasound Biomicroscopic Imaging in a Mouse Model of Colorectal Cancer

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Rationale and Objectives: The gold-standard tool for colorectal cancer detection is colonoscopy, but it provides only mucosal surface visualization. Ultrasound biomicroscopy allows a clear delineation of the epithelium and adjacent colonic layers. The aim of this study was to design a system to generate endoluminal ultrasound biomicroscopic images of the mouse colon, in vivo, in an animal model of inflammation-associated colon cancer.

Materials and Methods: Thirteen mice (*Mus musculus*) were used. A 40-MHz miniprobe catheter was inserted into the accessory channel of a pediatric flexible bronchofiberscope. Control mice ($n = 3$) and mice treated with azoxymethane and dextran sulfate sodium ($n = 10$) were subjected to simultaneous endoluminal ultrasound biomicroscopy and white-light colonoscopy. The diagnosis obtained with endoluminal ultrasound biomicroscopy and colonoscopy was compared and confirmed by postmortem histopathology.

Results: Endoluminal ultrasound biomicroscopic images showed all layers of the normal colon and revealed lesions such as lymphoid hyperplasias and colon tumors. Additionally, endoluminal ultrasound biomicroscopy was able to detect two cases of mucosa layer thickening, confirmed by histology. Compared to histologic results, the sensitivities of endoluminal ultrasound biomicroscopy and colonoscopy were 0.95 and 0.83, respectively, and both methods achieved specificities of 1.0.

Conclusions: Endoluminal ultrasound biomicroscopy can be used, in addition to colonoscopy, as a diagnostic method for colonic lesions. Moreover, experimental endoluminal ultrasound biomicroscopy in mouse models is feasible and might be used to further develop research on the differentiation between benign and malignant colonic diseases.

Key Words: Ultrasound biomicroscopy; animal model; diagnostic imaging; colonic neoplasm.

Colorectal cancer (CRC) has a high incidence in the world, being the third most common cancer and the third leading cause of cancer-related mortality in the United States, irrespective of gender (1). Ninety percent of malignant tumors can be cured if diagnosed in the early stages of localized disease (1), and this motivates great interest in the development and design of new tools for the early detection and staging of CRC. The gold-standard tool for CRC detection as well as for neoplastic alterations such as polyps and flat lesions in the mucosa is colonoscopy (1). However, it provides only mucosal surface visualization.

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Polyps and flat lesions in the mucosa are mostly benign and can often be adequately resected endoscopically (2,3). Nevertheless, differentiation from carcinomatous lesions that invade the muscularis mucosa is paramount to provide the correct approach (4). Regarding malignant tumors, the determination of their penetration depth through the colonic layers is also important for accurate lesion staging and treatment strategy (5,6). Therefore, some cases may require the colonoscopic results to be complemented with additional information obtained with a diagnostic technique able to determine tumor penetration depth through the colonic wall. In this context, the use of endoscopic ultrasonography in the diagnosis and determination of the malignant potential and depth of colonic lesions has been proposed by some authors (7–11). For the rectum, endoscopic ultrasound staging has already been a standard for several years, along with magnetic resonance imaging (MRI) (12,13).

Imaging the gastrointestinal tract with 20-MHz ultrasound (7,14) provides data on the correct depth of lesions through the intestinal layers and accurately determines if tumors are restricted to the mucosa and submucosa, with clear delineation of the epithelium and muscularis. Such results were compared to those obtained with magnifying colonoscopy (15) and optical coherence tomography (OCT)

(16) and demonstrated advantages regarding lesion staging, such as a better determination of small CRC invasion depth. Ultrasound with higher frequencies (40–50 MHz), usually denominated ultrasound biomicroscopy, provides images of living biologic tissues with near microscopic resolution (17). The benefits of ultrasound biomicroscopy include resolution with typical values of 30 μm (axial) and 72 μm (lateral) at 40 MHz (18).

The field of knowledge of endoscopic high-frequency ultrasonography is yet to be fully developed, and a reproducible and feasible animal model has the power to further develop the potential for the technique. CRC mouse models (19,20) can be used to understand the pathogenic mechanisms and to establish therapeutic and preventive measures related to human chronic intestinal inflammation and colon cancer. Additionally, important advantages of mouse models include relatively easy breeding and the possibility of using syngeneic mouse strains.

Despite the great number of investigations related to high-frequency ultrasonic imaging in small animal models (17,21–24), very few ultrasound biomicroscopic (UBM) images acquired in vivo from the murine colon have been presented in the literature. Chiou et al (25) used a 20-MHz ultrasound intravascular miniprobe, connected to a standard echocardiographic system, for transrectal assessment of the mouse aorta and iliac arteries. The ultrasonic images presented by those investigators do not clearly elucidate a detailed colon wall, and the reason seems to involve the low ultrasonic frequency and corresponding insufficient resolution to image the colon. Alternatively, our research group has used sector-scan UBM instrumentation, operating at 45 MHz, for in vitro UBM imaging of the dissected mouse colon (26). The results obtained by Alves et al (26) demonstrated the feasibility of ultrasound biomicroscopy to identify the layers of the colon from mice with adequate contrast among them and with enough resolution.

The present work was motivated by the previous investigation carried out by Alves et al (26) and includes in vivo mouse colon imaging with endoluminal examination as the main novelty. Endoluminal ultrasound biomicroscopy, based on an ultrasound miniprobe catheter inserted into the accessory channel of a bronchofiberscope, was associated with colonoscopy to generate simultaneous colon images from a mouse model of CRC in vivo.

MATERIALS AND METHODS

Animals

The animals were maintained at room temperature with appropriate circadian cycle and diet. The *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health was also considered. The procedure to induce colon tumor was conducted under a protocol (DAHEICB 042) approved by the Animal Care and Use Committee of the Biological Science Institute/Federal University of Rio de Janeiro. Studies involving colon imaging, with endoluminal ultrasound biomicroscopy associated with colonoscopy, were conducted under a protocol (71/08) approved by the Ethics Committee for Laboratory Animal Research/Federal University of Rio de Janeiro.

Thirteen mice (*Mus musculus*), three females and 10 males, $p53^{+/+}$ and $p53^{+/-}$ (heterozygous for tumor suppressor gene *TrP53*), with an average age of 51.19 $\pm$ 9.24 weeks and an average weight of 24 $\pm$ 4 g, were used. The animals were originally purchased from The Jackson Laboratory (Bar Harbor, ME), kept in 129/SvJ background, and genotyped as described in Jacks et al (27).

Ten animals were treated for tumor induction, and the other three (untreated males) were used as negative controls.

Mouse *TrP53* mutations, in combination with specific gene mutations, accelerate tumorigenesis in several tissues (28), including colon cancers (29). In addition, $p53^{+/-}$ mice have increased susceptibility, relative to control strains, to the rapid development of neoplasia by mutagenic carcinogens (30).

Azoxymethane (AOM) and Dextran Sulfate Sodium (DSS) Carcinogenesis Protocol

AOM and DSS were used to induce colon tumors in the mice (31). AOM, a colon-specific carcinogen, associated with DSS, a mucosal-irritant agent, mimics an inflammation-associated colon carcinogenesis (31). The animals were subjected to a single intraperitoneal injection of AOM (A5486; Sigma Aldrich, St Louis, MO) at a concentration of 12.5 mg/kg. One week following AOM administration, the mice were fed with water containing 3% DSS salt, 36,000 to 50,000 Da (02160110; Sigma Aldrich), during 1 week. All animals received solid food and water ad libitum, with regular water given after DSS intake. Water consumption was monitored and found to be similar among all mice.

Endoluminal UBM (eUBM) System

The eUBM imaging system used in the present work functions as a conventional B-mode imaging instrument used for medical diagnosis. The main difference is the higher ultrasound frequency used with the eUBM system.

A 3.6-F, 40-MHz miniprobe catheter (Atlantis SR Pro Coronary Imaging Catheter; Boston Scientific Corporation, Natick, MA), designed for intravascular imaging, was used in the present work to transmit and receive ultrasonic pulses. The miniprobe consists of two main assemblies: the imaging core and the catheter body. The imaging core contains a radial-looking 40-MHz ultrasonic transducer at the distal tip. The catheter body is formed by the telescoping, the proximal single, and the distal luminal sections. These luminal sections constitute the catheter's working length (135 cm), with outer diameters of 1.18 mm (3.6 F) and 0.83 mm (2.5 F) for the proximal and distal single luminal sections, respectively.

The miniprobe imaging core was mechanically driven by a motor-drive unit (MD5; Boston Scientific Corporation) and rotated 360° around its axis, inside the catheter body, to