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Experimental Small Animal Colonoscopy

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1. Introduction

Diseases of the colon and rectum produce a large burden of morbidity and mortality. Inflammatory bowel disease (IBD), such as Crohn's disease and ulcerative colitis, affects as many as 1.4 million people in the United States and 2.2 million people in Europe and leads to significant utilization of healthcare resources (Loftus, 2004). Crohn's disease (CD) is a chronic inflammatory disease that can affect the entire gastrointestinal tract from mouth to anus, and typically manifests itself with abdominal pain, diarrhea, and weight loss, as well as intestinal strictures, obstruction, perforation, or fistulae formation. CD most often manifests in the second to third decade of life. The usual disease course is periods of abdominal pain and diarrhea alternating with relatively asymptomatic periods. Over time, the symptomatic periods become longer, more frequent, and more severe. Ulcerative colitis (UC) is limited to the colon and occasionally the terminal ileum, and typically presents with diarrhea, passage of mucus, and rectal bleeding. UC is also most commonly diagnosed in patients younger than 30 years of age. IBD and colorectal cancer (CRC) are intimately linked, as long-standing IBD leads to an increasing risk for CRC over time. In patients with UC, the risk for CRC begins to increase after approximately 10 years of disease. The estimated risk of CRC is 25% after 25 years with the disease, 35% at 30 years, 45% at 35 years, and 65% at 40 years (reviewed in Townsend, 2008).

CRC is the third most commonly diagnosed cancer and the third-leading cause of cancer-related death in the United States in both men and women (American Cancer Society, 2011). In 2010, the American Cancer Society estimated that there were 142,579 new cases of CRC and 51,370 deaths attributed to this disease in the US (National Cancer Institute, 2011). The incidence of CRC is rising in many countries, in part because a western-style diet is being widely adopted (Center et al., 2009). CRC is currently the fourth leading cause of cancer deaths in the world (World Health Organization, 2011).

IBD and CRC are active areas of research, and a number of useful animal models of these diseases have been generated. Some of the most widely studied are rodent models, including various rat and mouse models. Mouse models are particularly attractive. Mouse genetics have been extensively studied, and there is a detailed knowledge base describing hundreds of inbred mouse strains as well as a variety of transgenic, knockout, and knockin models (reviewed in Rosenberg et al., 2009). Both genetic and chemically induced models of IBD and CRC have been validated (reviewed in Kanneganti et al., 2011; Rosenberg et al., 2009). These models are continually used to more fully understand the natural history of colonic diseases as well as test strategies for prevention and treatment. See Table 1 for an overview of genetic and chemically induced mouse models of IBD and CRC.

Models of Inflammatory Bowel Disease			
Genetic			
Model	**Human Disease**	**Mechanism**	**Phenotype**
SAMP-Yit	CD	High IFN-γ production	Spontaneous terminal ileitis, occasional perianal ulcers and fistulae
C3H/HejBir	UC	Increased IFN-γ and IL-2 production	Spontaneous ileocecal and right-sided colonic ulcers and crypt abscesses
$TNF^{\Delta ARE}$/TNF 3′ $UTR^{-/-}$	CD	Increased constitutive and inducible TNF	Polyarthritis and transmural intestinal inflammation
T-cell receptor-$\alpha^{-/-}$	UC	Increased aberrant T_H2-type T-cells producing IL-4	Pancolitis with soft stools
STAT4 transgenic	UC	Overproduction of STAT-4, leading to CD4+ T-cell production of TNF-α and IFN-γ	Severe transmural colitis
STAT3 deficient	CD	Disruption of STAT3 in macrophages and neutrophils, decreased IL-10	Enterocolitis with high incidence of colorectal adenocarcinomas
IL-$10^{-/-}$/CRF2-4 deficient	CD	Decreased IL-10 with increased IL-12 and TNF-α, loss of downregulation of T_H1-type T cells, NK cells, macrophages	Chronic enterocolitis with lesions in duodenum, proximal jejunum, and ascending colon primarily
IL-$2^{-/-}$/IL-2 receptor $\alpha^{-/-}$	UC	Decreased IL-2 (key regulatory immune cytokine)	Pancolitis with ulcers and wall thickening, crypt abscesses, mucin depletion, and epithelial dysplasia
IL-7 transgenic	UC	Initial IL-7 increase, then IL-7 deficiency	Initial acute colitis, followed by chronic colitis with proctoptosis and anal bleeding
$CD4^+CD45RB^{Hi}$ T-cell transfer	UC	Decreased IL-10, increased IFN-γ	Diarrhea, weight loss, transmural colonic inflammation, and death
Heat shock protein 60-specific $CD8^+$ T-cell transfer	CD	Presentation of bacterial protein on MHC class 1 with action of TNF-α	Severe small intestinal inflammation, death

Model	Human Disease	Mechanism	Phenotype
A20 deficient	CD	Lack of inhibition of TNF-induced NFκB activity	Intestinal inflammation, cachexia, death
IKK-γ (NEMO)/IKKαβ deficient	UC	Complete shutdown of NFκB signaling, leading to massive inflammatory cell infiltration	Severe chronic pancolitis
MDR1 deficient	UC	Spontaneous inflammatory response triggered by intestinal bacterial flora	Intestinal inflammation
Keratin $8^{-/-}$	UC	Primary intestinal epithelial cell defect leading to inflammation from intestinal bacterial flora	Colitis and colonic hyperplasia
Double negative N-cadherin transgenic/chimeric	IBD	Disrupted intestinal mucosal barrier leading to inflammation from contact with intestinal bacterial flora	Chronic inflammation in chimeric regions of intestinal epithelium
Chemical			
Model	**Human Disease**	**Mechanism**	**Phenotype**
Acetic acid	UC	Enema; epithelial inflammation and damage	Epithelial necrosis and edema extending from lamina propria to as deep as muscularis layer
Iodoacetamide	UC	Enema; sulfhydryl blocker that decreased amount/action of protective sulfhydryl groups	Diarrhea, dilation, adhesions, mucosal erosions to deep ulcerations, inhibited weight gain
Indomethacin	CD	In diet; inhibition of protective prostaglandin synthesis (PGE1, PGE2, prostacyclin)	Ulceration and transmural inflammation of mid-small intestine
Trinitrobenzene sulfonic acid (TNBS)	UC	Enema; haptenization of colonic autologous or microbial proteins making them immunogenic – delayed hypersensitivity response	Acute and chronic colitis

Model	Human Disease	Mechanism	Phenotype
Oxazolone	UC	Enema; haptenization of colonic autologous or microbial proteins making them immunogenic – delayed hypersensitivity response	Distal colitis
Dextran sodium sulfate (DSS)	UC	Drinking water; directly toxic to epithelial cells in basal crypts	Colitis with bloody diarrhea, ulcerations, granulocytic infiltration, weight loss, shortening of intestines
Models of Colorectal Cancer			
Genetic			
Model	**Human Disease**	**Mechanism**	**Phenotype**
Apc$^{Min/+}$ mutations	FAP	Truncating mutations of *Apc* (codons 850, 716, 1638, and others)	Multiple small intestinal adenomas
Mismatch repair gene mutations (*Msh2, Msh3, Msh6, Mlh1, Mlh3*)	HNPCC	Mutations in various mismatch repair genes	Adenomas and adenocarcinomas of entire GI tract, some mutations prone to lymphomas, squamous or basal cell carcinomas
β-catenin stabilizing mutations	FAP	Stabilization of β-catenin leading to activation of *c-Myc* and c*yclin D*	Hundreds of small intestinal adenomas
Smad3$^{-/-}$	CRC	Loss of cellular signaling protein in TGF-β pathway	CRC with occasional metastasizes to regional lymph nodes
K-ras^{V12G}	CRC	Activation of mutated *K-ras*	Colorectal tumors ranging from microadenomas to invasive adenocarcinomas without metastasis
Muc2$^{-/-}$	IBD-related CRC	Mutation of *Muc2*, which controls gastrointestinal mucin	Adenomas and adenocarcinomas in the intestines without distant metastasis, rectal cancers
IL-2$^{-/-}$/ β_2-microglobulin$^{-/-}$	UC-related CRC	Chronic inflammation from decreased IL-2 and β2-microglobulin	Adenocarcinoma of colon and rectum

Model	Human Disease	Mechanism	Phenotype
IL-10$^{-/-}$	CD-related CRC	Chronic inflammation with decreased IL-10 in setting of colonic bacterial infection	Adenocarcinomas without metastasis or mutations in *K-ras*, *p53*, *Apc*, and *Msh* genes
RAG2$^{-/-}$	Inflammation-related CRC	Induced with *Helicobacter hepaticus* infection	Intestinal dysplasia, tubular adenomas, and adenocarcinomas of cecum and colon
RAG2$^{-/-}$/*Tgfβ1*$^{-/-}$	Colitis-related CRC	Downregulation of TGF-β signaling pathway	Locally invasive adenocarcinomas in cecum and colon
TCRβ$^{-/-}$/*p53*$^{-/-}$	UC-related CRC	Dysregulation of T-cell function with lack of p53 tumor suppression	Dysplasia and adenocarcinoma of ileum and cecum
Gpx1$^{-/-}$/Gpx2$^{-/-}$	Ileocolitis-related CRC	Loss of glutathione peroxidase 1 and 2 leading to peroxidative stress with bacteria-associated inflammation	Dysplasia, adenocarcinomas, signet ring cell carcinoma seen in ileum and colon
$G_{\alpha i2}^{-/-}$	UC-related CRC	Loss of G protein function	Colonic ulcerations, atypical colonic glands
Conditional *Apc*$^{-/-}$	Metastatic CRC	Floxed *Apc* mutation activated by adenovirus-delivered cre recombinase	Invasive colorectal cancers with metastases to liver
Xenografts	Metastatic CRC	Implantation of human CRC tumor cells in immunocompromised mice	Invasive CRC with metastases
Chemical			
Model	**Human Disease**	**Mechanism**	**Disease/Pathology**
1,2-dimethylhydrazine (DMH)/ Azoxymethane (AOM)/methyl-azoxymethanol (MAM)	CRC	Intraperitoneal injection; procarcinogens activated to DNA-reactive products which alkylate molecules in liver and colon	Distal colon tumors
Heterocyclic amines (PhIP, IQ, etc.)	CRC	In diet; mutagenic and tumorigenic agents in broiled food	Colon cancers, mammary tumors, prostate tumors

Model	Human Disease	Mechanism	Phenotype
Aromatic amines (DMAB)	CRC	Subcutaneous injection; tumorigenic agent	Adenomas, invasive adenocarcinomas of colon and mammary glands, salivary gland sarcomas, skin and ear squamous cell carcinomas, stomach squamous cell papillomas, sarcomas/lymphomas/ urothelial carcinomas of bladder
Alkylnitrosamides (MNNG, MNU)	CRC	Enema; direct alkylating agent	Sessile and polypoid adenomas and adenocarcinomas, rare metastases

Table 1. Mouse models of inflammatory bowel disease (IBD) and colorectal cancer (CRC). This table provides a summary of currently utilized mouse models of IBD and CRC. In addition to these singly listed models, various models are often combined, e.g. DSS and AOM or DSS in *Apc*$^{Min/+}$ mice. (Reviewed in Hung, 2010; Jurjus et al., 2004; Kanneganti et al., 2011; McCart et al., 2008; Rosenberg et al., 2009; Taketo, 2006; Taketo & Edelmann, 2009; Wirtz & Neurath, 2007)
Abbreviations: CD: Crohn's disease; IFN: interferon; UC: ulcerative colitis; IL: interleukin; TNF: tumor necrosis factor; UTR: untranslated region; STAT: signal transducer and activating transcription; MHC: major histocompatibility complex; IBD: inflammatory bowel disease; *Apc*: *Adenomatous polyposis coli*; *Min: Multiple intestinal neoplasia*; FAP: familial adenomatous polyposis; HNPCC: hereditary nonpolyposis colon cancer; CRC: colorectal cancer; TGF: transforming growth factor; PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; IQ: 2-amino-33-methylimidazo[4,5-*f*]quinoline; DMAB: 3,2′-dimethyl-4-aminobiphenyl; MNNG: *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; MNU: methylnitrosourea

Although murine models of colonic diseases are powerful, one limitation has been the large number of animals needed to complete an adequately powered study. Traditionally, experiments had a cross-sectional design in which mice from different groups were sacrificed at a set point in time, and the intestinal tract was examined at necropsy. This limitation has been overcome with recent advances in technology. A variety of imaging tools have been developed, allowing longitudinal assessment of the large intestine in animal models. Currently utilized methods include computed tomography (CT), magnetic resonance imaging (MRI), and direct visualization with colonoscopy. The ability to serially assess changes in the large intestine allows more detailed information about diseases to be gathered with a smaller cohort of experimental animals (Durkee et al., 2009).
This chapter will discuss the use of colonoscopy in rodent models of IBD and CRC. The method of performing colonoscopy in experimental animals will be described as well as the

various adjuncts that can be combined with colonoscopy to enhance the amount of data that can be gathered. The strengths and limitations of colonoscopy will be discussed. Finally, colonoscopy will be compared and contrasted with alternative methods of imaging the large intestine, such as CT and MRI.

2. Colonoscopic technique

Colonoscopy is the gold standard for screening, evaluating, and potentially treating diseases of the colon, including IBD and CRC. In humans, this procedure in its current manifestation involves inserting a flexible endoscope through the anus, using compressed gas to insufflate the colon, and carefully advancing to the cecum and terminal ileum. The camera at the tip of the colonoscope displays images on a monitor. As the colonoscope is withdrawn, the colonic mucosa is carefully inspected. The colonoscope has channels allowing insertion of various tools (biopsy forceps, snare cautery, needles for injection, etc.), allowing collection of biopsies, removal or destruction of potentially neoplastic lesions, or other interventions.

The power of this tool was recognized by researchers, and various groups have attempted to adapt it for use in animal models of IBD and CRC. Colonoscopy has been successfully adapted for use in rat models. Using modified bronchoscopes (Hull et al., 1990) or other small-caliber flexible endoscopes (Haughn et al., 2006), total colonoscopy of the rat has been performed successfully, as well as other variations of this procedure (Zhang et al., 1994). Colonoscopy in mice was first attempted with a pediatric cystoscope with good results, although because of anatomic and instrumental limitations, the entire colon to the cecum could not be visualized (Huang et al., 2002). High resolution endoscopy can now be performed with colonoscopes designed specifically for work with rat and mouse models of colonic disease.

Becker, Fantini, and Neurath published a description of high-resolution colonoscopy in live mice (Becker et al., 2006). This procedure is followed by our lab with modifications. We use the Coloview miniendoscope system (Karl Storz, Tuttlingen, Germany), which includes an colonoscope (0 degree, 1.5 mm, rigid) with operating sheath and light source, video monitor, and camera with the capability to record video as well as obtain still images during colonoscopy, and a small air pump. See Figure 1 for an illustration of the set-up that we use. The mouse is anesthetized with inhaled isoflurane, and is placed ventral-side down on an operating platform. An oral gavage needle on a syringe is used to introduce Dulbecco's phosphate buffered saline (PBS) via the anus as a pre-procedure enema for bowel preparation to ensure adequate visualization. This step can be repeated as needed to ensure adequate clearing of colonic contents prior to colonoscopy. We do not fast the mice prior or provide any additional bowel preparation other than the PBS enema, and we are nearly always able to obtain adequate visualization of the colonic mucosa. Note that we initially did fast the mice and provide oral NuLytely (an osmotic bowel preparatory agent), but we found that this step was unnecessary to obtain excellent visualization of the colon. The PBS enema also serves as lubrication prior to insertion of the colonoscope. The air pump is attached to the colonoscope to provide insufflation of the colon for adequate visualization throughout the procedure. Figure 2 shows an experimental mouse undergoing colonoscopy while under general anesthesia.

The mouse colon has relatively simple geometry, unlike the tortuosity that is associated with the human colon. The mouse colon extends in a fairly straight line for approximately 4 cm cranially from the anus toward the left kidney, where it turns approximately 90 degrees and

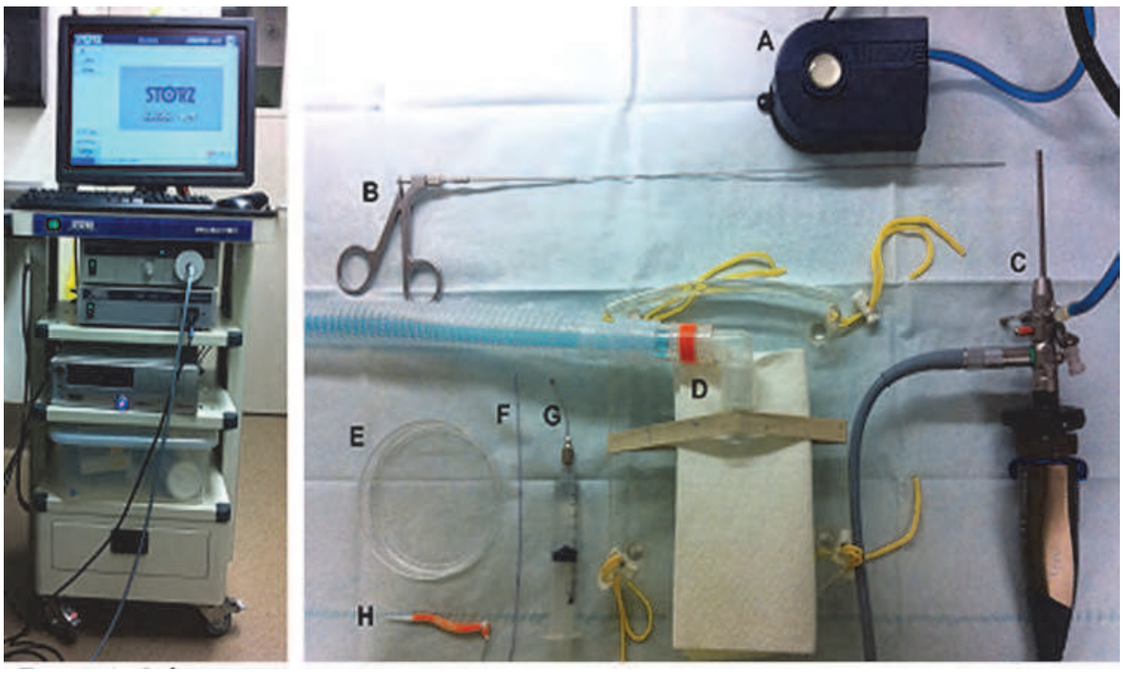

Fig. 1. Colonoscopy set-up.
On the left is the portable tower containing the monitor, light source, camera, and computer used for recording and saving videos and still images. On the right is the benchtop set-up for colonoscopy in mice. A: air pump for insufflation of the colon. B: biopsy forceps. C: colonoscope with working sheath in place, connected to air pump (blue tube) and light source (gray cable). D: nose cone for administration of inhaled anesthetic attached to operating platform. E: PBS for pre-operative enemas. F: flexible catheter to be inserted through working channel of colonoscope with scale markings for standardization of images and in situ measurments. G: gavage needle attached to syringe with PBS for administration of pre-procedure enemas. H: soft brush for cleaning lens of colonoscope.

extends across the upper abdominal cavity, connecting with the generous mouse cecum near the right kidney. The colonoscope is carefully introduced into the anus and advanced about 4 cm, or until the first area of curvature of the colon (corresponding to the splenic flexure) while observing progress on the monitor. We typically record video and obtain still images as the colonoscope is slowly withdrawn. The entire procedure takes approximately 5 minutes or less. The mouse is then allowed to awake from anesthesia. Figure 3 demonstrates the appearance of normal mucosa on colonoscopic examination.

This procedure is very well tolerated by experimental animals. However, some complications should be mentioned. Mice that are ill or moribund may not tolerate general anesthesia, so careful assessment of the animal's overall state of health should be made prior to the procedure. The wall of the colon is quite thin, so care must be taken not to cause perforation. Perforation can occur at the time of enema with the gavage needle or with the colonoscope. This injury is most likely to happen at the time of insertion, as inadvertent trauma to the colon becomes less likely under direct visualization. Care needs to be taken during insufflation as two potential complications could occur. Over-insufflation can cause air to travel throughout the length of the gastrointestinal tract to the point that gastric

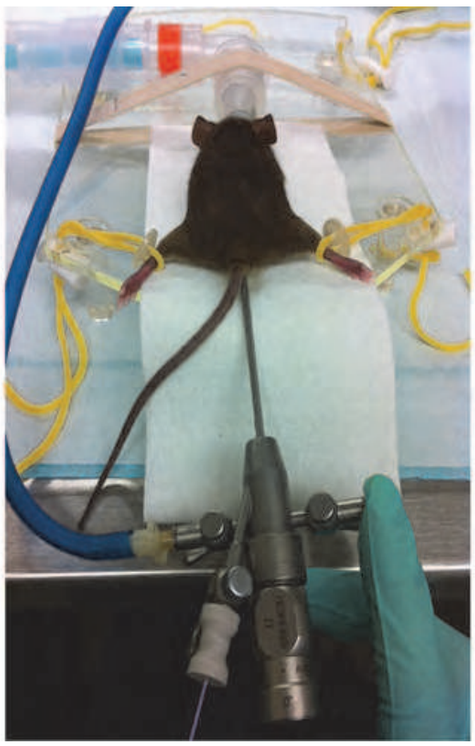

Fig. 2. Mouse colonoscopy. Image of colonoscopy being performed in a living mouse under general anesthetic. Insufflation is provided by air via the blue tube shown above. The amount of insufflation is controlled by placing a finger over the opposite opening of the working sheath. The graduated flexible measuring catheter is seen protruding from the working channel of the colonoscope.

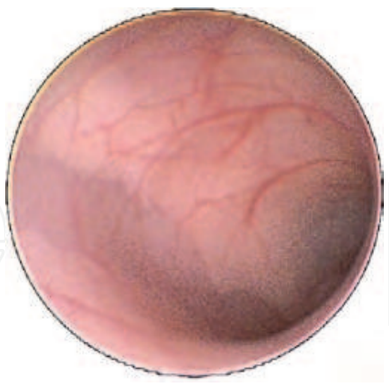

Fig. 3. Normal intestinal muscosa. Colonoscopy image showing normal intestinal mucosa.

contents are forced up the esophagus and lead to aspiration. In addition, over-distention of the intestines can lead to respiratory compromise if the abdomen becomes sufficiently distended to affect diaphragmatic function. Such complications are rare for experienced operators. Incorporation of additional procedures, such as biopsies, during the colonoscopy has the potential to increase complications. Reported mortality rates range from <1% to 2.9% (Becker, 2005; Hensley, 2009).

2.1 Experimental uses and adjuncts

Colonoscopy allows visual grading of colitis and repeat assessment over time. Both video and still images can be obtained and stored for analysis. Scoring systems of colitis have been developed and published. The criteria that can be easily visualized include the thickness of the colon, changes in vascular pattern, presence of fibrin, mucosal surface granularity, and stool consistency (Becker et al., 2005, 2006). In order to better visualize crypt patterns and detect aberrant crypt foci, which some consider early neoplastic lesions, the colonic epithelium can be stained with a 1% solution of methylene blue and then examined with the colonoscope. By performing this procedure, termed chromoendoscopy, according to previously published protocols, aberrant crypt foci can be identified that would be undetectable without staining, potentially enabling early recognition of pre-neoplastic lesions prior to tumor formation (Becker et al., 2005, 2006).

Colonic tumors that are at or distal to the splenic flexure (approximately the distal 3-4 cm of colon) can be followed serially by colonoscopy. This allows study of the natural history of tumors. Figure 4 demonstrates the progression of a single tumor in one mouse over the course of four months. Several groups have published methods of visually grading the size of tumors assessed on colonoscopy. Becker and colleagues have published a method of scoring tumor size relative to the lumen of the insufflated colon (Becker et al., 2005). However, the still images must be taken at a consistent distance from the tumor and the colon must be consistently insufflated in order for meaningful comparisons to be made between time-points. Hung and colleagues describe using the relative lumen size of a fully insufflated colon as a normalization factor when obtaining images (Hung et al., 2010). Various tools can be inserted through the working channel of the colonoscope to provide a visual frame of reference and guide for standardization of view. A tool that has a known diameter and has markings

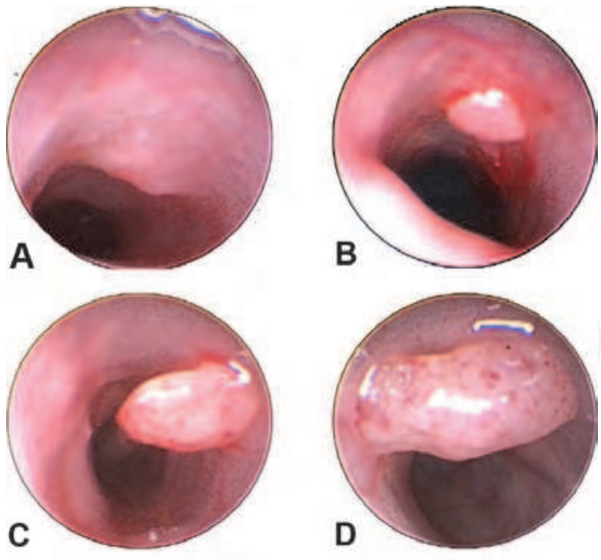

Fig. 4. Intestinal tumor development. Colonoscopy images showing the development of an intestinal tumor in a single mouse over time. Panel A: Early neoplastic lesion. Panel B: Small flat tumor approximately 1 month after image in A. Panel C: The tumor has continued to increase in size and is now pedunculated after 10 weeks. Panel D: The same tumor after an additional 4 weeks, with noticeable increase in size.

at set intervals can be used to quantify the size of tumors seen on endoscopy. Hensley and collegues have described a method using biopsy forceps. The 1-mm-diameter flexible metal biopsy forceps was inserted through the working channel of the colonoscope and advanced until it was visible in the field of view adjacent to the tumor. Still images were taken in this configuration, and the images were analyzed using a specifically written software program that allowed estimation of the tumor size by performing geometric reconstruction based on the position of the cylindrical forceps relative to the adenoma (Hensley et al., 2009). We use a flexible embolectomy catheter that has been marked at 1 mm intervals to standardize our images (see item F in Figure 1).

Biopsy forceps are available from Karl Storz which can be passed through the instrument channel of the operating sheath. Using these small, flexible forceps, tissue can be taken from tumors or areas of colon wall thickened by colitis. Figure 5 demonstrates the endoscopic biopsy of a single tumor in an experimental mouse. Care must be taken, however, not to biopsy normal colonic mucosa as the colon wall in the mouse is quite thin, and biopsies of this tissue would have an unacceptably high rate of perforation. Biopsies can be snap frozen with liquid nitrogen, placed in stabilizing media, or fixed in formalin for immunohistochemistry, molecular analysis, hematoxylin-eosin staining, or other biomolecular studies (Becker et al., 2005, 2006). In addition, Becker and colleagues have described directly injecting individual tumors with reagents via a small-gauge needle under endoscopic guidance. They inserted a 26-gauge needle mounted on a small tube through the working channel of their endoscope and injected fluorescein isothiocyanate into a tumor under direct visualization. On necropsy, the tumor was dissected free from the mouse colon and cryosections were analyzed by immunofluorescence, which showed fluorescein isothiocyanate throughout the tumor (Becker et al., 2005).

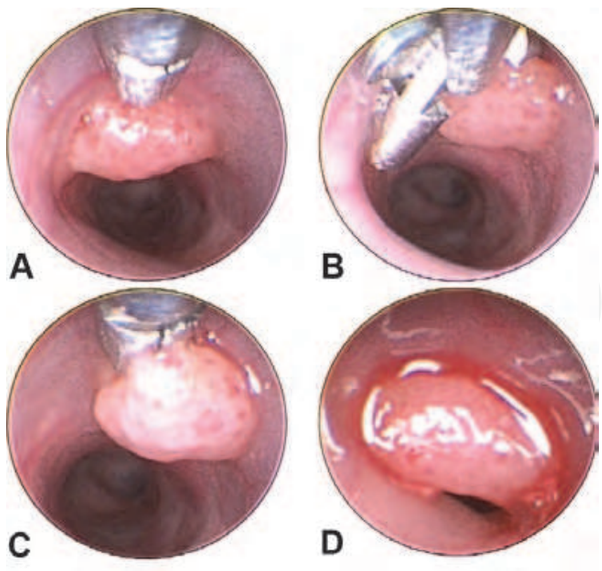

Fig. 5. Colonoscopic tumor biopsy. This series of images shows the steps in obtaining a biopsy of a tumor during colonoscopy. Panel A: insertion of biopsy forceps. Panel B: opening biopsy forceps. Panel C: grasping tumor with forceps. Panel D: bleeding from the tumor after biopsy ensures that adequate tissue was obtained.

An exciting recent development in small animal research is the use of bioluminescent and fluorescent molecules to image diverse cellular, molecular, and tissue processes. Fluorescent probes have been developed for specific antibodies, protein ligands, and other substrates (see Citrin & Camphausen, 2004, and Luker & Luker, 2008, for reviews of these techniques). These technologies can be combined with endoscopy to provide real-time imaging of fluorescent probes to detect perfusion and protease activity, as was demonstrated by Funovics and colleagues with their miniaturized multichannel near-infrared endoscope. They designed an endoscope that also allowed simultaneous fluorescent imaging of murine colonic tumors, allowing them to superimpose fluorescent perfusion and protease activity over white-light images (Funovics et al., 2003). This method has been shown to be useful for imaging adenomas as well as adenocarcinomas (Funovics et al., 2006). Hung and colleagues have reported using protease-activated synthetic probes to identify colonic lesions with near-infrared colonoscopy (Hung et al., 2010). Fluorescent probes such as this may prove useful in identifying early neoplastic lesions that are not easily visible on white-light endoscopy or in monitoring action of novel preventive or therapeutic agents.

3. Strengths and limitations

3.1 Strengths

Utilizing colonoscopy in mice has a number of distinct advantages. One of the most striking is the ability to monitor changes in colonic pathology over time. Within the same mouse, the initiation and evolution of colitis as well as response to treatment can be followed. Similarly, tumors can also be followed longitudinally, potentially from their earliest manifestation and as they progress from a benign to invasive state over time. This allows the study of a single tumor in a single mouse over time. Researchers are able to gain valuable information about the morphology of the tumor, i.e. flat vs. pedunculated, smooth vs. lobulated, as well as visualize vascular patterns in and around the tumor mucosa. Small sessile lesions that are missed on other *in vivo* imaging modalities can be easily identified on colonoscopy. Additionally, tissue can be obtained by biopsy at multiple time points for histology as well as for molecular analysis. Prior to use of colonoscopy, in order to study the natural history of colonic disease, multiple mice would have to be sacrificed at various time points, and the results of the examinations aggregated. With colonoscopy, diseases such as colitis or colon neoplasm can be studied at multiple time points in the same mouse, allowing the mouse to serve as its own control, thereby eliminating variation owing to genetic and environmental effects. In addition, being able to serially study the same mouse greatly reduces the number of mice needed in order to design an adequately powered study (Becker et al., 2005, 2006; Durkee et al., 2009; Hensley et al., 2009).

Colonoscopy in mice is a relatively simple and cost-effective procedure that is well-tolerated by experimental animals. Colonoscopy is portable and relatively inexpensive, although there is an initial cost to purchase the equipment. Other methods of imaging murine colonic disease, such as microCT colonography or MRI, require more expensive scanners as well as a dedicated space for the necessary equipment.

3.2 Limitations

There are significant limitations of colonoscopy that deserve discussion. Although colonoscopy is a relatively safe procedure, there is still an associated morbidity and mortality, as discussed earlier in the section on colonoscopic technique**.** There are also

limitations inherent in the procedure itself. The quality of the data gathered by colonoscopy is operator-dependent, and there is a learning curve before the scope can be safely and effectively used. The images that are obtained are two-dimensional, so estimating the volume of a tumor is difficult. Most significantly, current technology only allows visualization of the distal half (3-4 cm) of the mouse colon. A flexible colonoscope that can be used safely in mice is unavailable, which means that any lesions proximal to the splenic flexure are inaccessible *in vivo*.

4. Comparison to other imaging modalities

4.1 Micro-computed tomography colonography

A number of methods for imaging the colons of experimental mice are becoming available. Micro-computed tomography (mCT) colonography is a useful tool for examining colonic disease, in particular neoplasia. Also known as virtual colonoscopy, this modality uses x-rays to image the colon and surrounding tissues, and can be used to generate either two-dimensional or three-dimensional reconstructions of individual tumors. Virtual colonoscopy has been shown to be an accurate screening tool for detecting colon polyps in humans (Kim et al., 2007), making its utilization in murine experiments very clinically relevant. Several groups have modified this technology for use in the mouse, allowing longitudinal study of mouse models of colonic disease (Durkee et al., 2010). Choquet and colleagues have used mCT with luminal and intraperitoneal contrast to detect azoxymethane-induced cecal heterotypia and colon tumors. They identified 9 of 9 areas of heterotypic thickened cecal wall and 11 of 11 colon tumors with no false positives (Choquet et al., 2007). Pickhardt and colleagues showed that by modifying feed and providing bowel preparation with an osmotic agent, mCT could detect colonic tumors ≥2mm in maximum diameter with 93.3% sensitivity and 92% specificity (Pickhardt et al., 2005). These investigators went on to demonstrate tumor volume measurements by mCT were accurate predictors of actual tumor size (Durkee et al., 2008). Good quality mCT scans had a mean standard deviation in tumor volume measurements of 8%, meaning that changes in tumor volume of >16% are detectable with a 95% confidence interval. Thus, colon tumors can be reliably identified and followed over time by mCT in order to determine if they grow, regress, or remain static either spontaneously or in response to therapy (Durkee et al., 2009). This imaging platform is very powerful when testing therapeutic interventions.

mCT colonography offers several advantages over colonoscopy. This is a non-invasive procedure, so there is minimal risk of morbidity or mortality to the experimental animals, although general anesthesia and colonic insufflation are not without risk. Current technology allows three-dimensional rendering of tumors, allowing accurate measurement of volume, as opposed to the size estimates that can be made using flat two-dimensional images from colonoscopy (Durkee et al., 2008). Importantly, the entire colon from cecum to anus can be assessed by mCT colonography, rather only the distal 4 cm as is currently visible on colonoscopy. In addition, extracolonic manifestation of disease, specifically metastatic lesions, can be seen on mCT *in vivo*.

There are advantages offered by colonoscopy, however. Compared to mCT colonography, colonoscopy is faster (an average of 5 minutes versus 20 minutes), requiring less time under anesthesia for experimental animals (Durkee et al., 2009). The equipment and set-up for

colonoscopy are also less expensive than mCT, both in terms of actual hardware required as well as the dedicated space needed for a CT scanner. Colonoscopy is also able to detect small or sessile tumors that are not visible on mCT colonography, which relies on the contrast between the appearance of colonic contents and tissue structures, and is thus unable to detect lesions <2mm or flat lesions. Colonoscopy can also be used to monitor colonic inflammation, which is not easily appreciated on mCT colonography. mCT colonography does employ ionizing radiation, and the length of the scan currently exposes experimental animals to approximately 0.25 Gray, which is to up to 10 times the amount used on humans. The effects of this level of radiation on mice are unknown. Finally, colonoscopy offers the opportunity to perform additional procedures concurrently under direct visualization. mCT colonography does not offer the opportunity to obtain biopsies, perform *in vivo* staining as with chromoendoscopy, or add any of the other adjunctive procedures discussed above.

4.2 Magnetic resonance imaging

Another imaging modality used to study colonic disease is magnetic resonance imaging (MRI). Hensley and colleagues described a protocol whereby the entire colon is visualized by MRI. Using this imaging platform, polypoid tumors 1.5 mm in largest dimension could be reliably identified, with 17 correctly identified tumors, 2 false negatives, and 2 false positives. Volumes of the tumors were estimated from MRI and correlated well with tumor weight (Hensley et al., 2004). Young and colleagues were able to accurately measure the volume of polypoid colonic tumors at multiple time points (Young et al., 2009). Estimates of cross-sectional area made on colonoscopy, MRI, and necropsy were compared by Hensley and colleagues, and were found to have a strong correlation (Hensley et al., 2009). In addition to colon tumors, numerous investigators have demonstrated that colitis can be visualized on MRI. A number of groups have been able to appreciate acute experimentally induced colonic inflammation when imaging mice after treatment with dextran sodium sulfate, a commonly used agent to model acute colon inflammation in the mouse. Acute inflammation seen on MRI was confirmed on histological examination of the colon after sacrifice (Larsson et al., 2006; Melger et al., 2007; Mustafi et al., 2010; Young et al., 2009). The colon wall thickness, T2-weighted imaging, and quantitative analysis of contrast uptake and wash-out were all significantly different in inflamed colons. These studies show that MRI colonography is a viable option for longitudinal study of experimentally induced colitis, allowing the longitudinal study of inflammatory colon diseases (Larsson et al., 2006; Melger et al., 2007; Mustafi et al., 2010; Young et al., 2009).

MRI colonography offers the same advantages over colonoscopy that mCT colonography does with the additional advantage that no ionizing radiation is used. However, there are reasons to choose colonoscopy over MRI. MRI colonography requires the use of contrast agents, either intravenously, intramuscularly, or rectally; in addition to being difficult to administer, the exact effects of these agents on experimental mice are unknown. As with mCT, set-up and maintenance of an imaging facility are expensive. MRI is also less than ideal for identifying flat tumors when compared to colonoscopy as these are not as readily apparent as polypoid tumors. Table 2 compares and contrasts these modalities for imaging colon tumors *in vivo* in mice.

Imaging Platform	Advantages	Disadvantages
Colonoscopy	Quick Relatively safe for experimental animals Relatively inexpensive Serial examinations Direct visualization of colonic mucosa Visualization of flat or polypoid lesions Ability to perform *in vivo* staining Ability to obtain tissue biopsies Ability to combine with fluorescent probes for protease or vascular imaging	Occasional mortality in experimental animals Initial cost of equipment Unable to visualize proximal to splenic flexure Unable to visualize extra-luminal disease 2-dimensional images only, so difficult to accurately assess tumor size and volume Quality of images operator-dependent
mCT colonography	Non-invasive with minimal risk of mortality in experimental animals Ability to measure tumors in 3 dimensions, resulting in accurate and precise measurements of tumor volume Ability to image entire length of colon Visualization of extracolonic lesions, i.e. metastases	More time required for procedure Ionizing radiation with unknown effects Expensive Inability to identify sessile lesions or lesions <2 mm Unable to perform additional procedures (biopsy, staining, etc.)
MRI colonography	Noninvasive with minimal risk of mortality No ionizing radiation Ability to visualize inflammation Ability to measure tumors in 3 dimensions Ability to visualize extracolonic lesions	Contrast required More time required Expensive Inability to identify small or sessile lesions Unable to perform additional procedures

Table 2. Comparison of imaging modalities.
This table compares and contrasts colonoscopy, mCT colonography, and MRI colonography for colorectal cancer in mouse models.

5. Conclusion

Colonoscopy is a powerful tool for studying pathology in mouse models of colonic disease. This is a safe, relatively quick procedure that enables researchers to study the natural history of colonic diseases, to visually assess response to therapeutics or interventions, and to obtain tissue from living animals. By allowing serial examinations of the colon, it decreases the number of mice needed to adequately power a study. Colonoscopy can be combined with staining techniques or fluorescent probes to gather data about a variety of cellular, molecular, or tissue processes simultaneously. Colonoscopy is comparable to other imaging modalities available to study the murine colon, such as mCT or MRI colonography.

6. Acknowledgements

The authors would like to thank Linda Clipson for critical review of the manuscript and Brian Olson for technical help and critical review.

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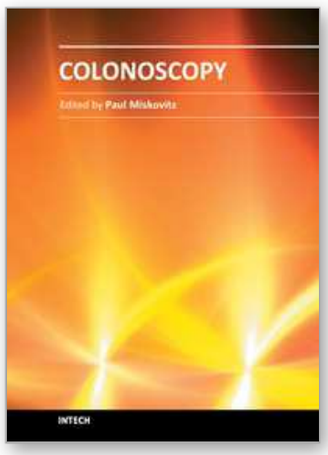

Colonoscopy

Edited by Prof. Paul Miskovitz

ISBN 978-953-307-568-6
Hard cover, 326 pages
Publisher InTech
Published online 29, August, 2011
Published in print edition August, 2011

To publish a book on colonoscopy suitable for an international medical audience, drawing upon the expertise and talents of many outstanding world-wide clinicians, is a daunting task. New developments in videocolonoscope instruments, procedural technique, patient selection and preparation, and moderate sedation and monitoring are being made and reported daily in both the medical and the lay press. Just as over the last several decades colonoscopy has largely supplanted the use of barium enema x-ray study of the colon, new developments in gastrointestinal imaging such as computerized tomographic colonography and video transmitted capsule study of the colonic lumen and new discoveries in cellular and molecular biology that may facilitate the early detection of colon cancer, colon polyps and other gastrointestinal pathology threaten to relegate the role of screening colonoscopy to the side lines of medical practice. This book draws on the talents of renowned physicians who convey a sense of the history, the present state-of-the art and ongoing confronting issues, and the predicted future of this discipline.

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