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An overview of chemically induced rodent models for sporadic colorectal cancer: histopathological and translational perspectives

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Summary

Globally, colorectal cancer (CRC) is one of the most frequently diagnosed human gastrointestinal neoplasia and the second leading cause of cancer-related death in both men and women. Despite considerable efforts currently devoted to the study of the biology and treatment of CRC, patient prognosis and survival are still poor. Sporadic CRC is a complex multistep disease and usually emerges in the setting of lifestyle and dietary changes mainly observed in industrialized countries with high human development index (HDI) (westernized style). The

molecular pathogenesis of sporadic CRC presents genetic heterogeneity with *APC*, *RAS*, *PIK3CA*, *TGFBR*, *SMAD4,* and *TP53* mutations usually detected during the progression of this malignancy. The establishment of sporadic CRC models has become essential for both basic and translational research to improve our understanding of the pathophysiology, unravel new molecular drivers, and preventive/therapeutic improvement of this malignancy. Chemically induced rodent models of sporadic CRC recapitulate most key morphological and genetic/epigenetic events observed during the promotion and progression of this malignancy, establishing effective diagnostic and prevention strategies to be translated into clinical practice. The present review gathers the main features of the state-of-the-art evidence on chemically induced rodent models, widely applied for translational modelling of sporadic CRC with a specific focus on histopathology and prevention perspectives. Our narrative review reinforces the persistent value of these bioassays and encourages the use of multimodel strategies for further investigations.

Key Words: Sporadic colorectal cancer; chemically induced rodent models, preneoplastic and neoplastic lesions; chemoprevention and preventive strategies;

Introduction

Colorectal cancer (CRC) is one of the most frequent neoplasia and is the second cause of death in both men and women among cancers worldwide, with 1.9 million new cases and 935,000 deaths estimated in 2020 (Keum and Giovannucci, 2019; Sung et al., 2021). Most CRCs are considered sporadic diseases (55–70%) or bear a hereditary familial basis (20-30%), while fewer than 5% of cases occur in individuals with inherited predisposition syndromes, including familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC) (Keum and Giovannucci, 2019; Sung et al., 2021). Moreover, CRC incidence and mortality are higher in very high or high-human development index (IDH) countries, which may be linked to the "Westernized" lifestyle (Keum and Giovannucci, 2019; Sung et al., 2021). This lifestyle includes excessive alcohol consumption, sedentarism, cigarette smoking, high fat, sugar, and red/processed meat intake, all factors associated with the risk of CRC occurrence. Conversely, high physical activity, and an adequate intake of whole grains, soluble and insoluble fiber, fruits, and vegetables seem to reduce CRC risk (Johnson et al., 2013; Keum and Giovannucci, 2019)

(**Fig. 1**). Thus, given the relevance of CRC as a global health issue, the search for novel preventive/therapeutic strategies in public health against this disease is crucial.

The natural history of CRC development comprises a complex and multistep process across progressive clinical, morphological, biochemical, genetic, and epigenetic alterations. When this malignancy is diagnosed in the wall of the large intestine (stages I and II) it is potentially curable by surgical and therapeutic interventions. However, most colon cancer and rectal cancer are diagnosed at advanced stages and the overall survival rates for patients with regional-stage (nodal stage III) or distant-stage (metastatic phase, stage IV) are reduced by 50-70% and 10%-14%, respectively (Nawa et al., 2008; Conteduca et al., 2013). In addition, CRC is aetiologically heterogeneous depending on the anatomical site. For instance, right-side (proximal) colon cancer (RSCC) features are different compared with left-side (distal) colorectal cancer (LSCRC) due to the distinct blood supply, macroscopic appearance of the lesions and clinicopathological parameters (4,5). RSCCs are typically bulky, exophytic, polypoid macroscopic lesions that project into the lumen **(Fig. 2)** and cause significant anemia. In contrast, LSCRCs are typically infiltrating, constricting lesions that encircle the lumen, often leading to obstruction **(Fig. 2)**. Right-sided cancers are more incident in women and older individuals and commonly diagnosed at an advanced stage, whereas left-sided cancers are prevalent in men and younger individuals (Nawa et al., 2008; Conteduca et al., 2013). Concerning molecular features in both regions, RSCCs contain microsatellite instability (MSI)-high, CpG island methylator, phenotype (CIMP)-high, or BRAF mutations, whereas LSCRCs are often associated with chromosomal instability (CIN)-positive (Roth et al., 2010; Missiaglia et al., 2014; Carethers and Jung, 2015). Additionally, one or more cellular pathways become deregulated in CRC, including Wnt-β-catenin, transforming growth factor beta (TGF-β), epidermal growth factor receptor (EGFR), Ras/Raf/mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (P13K) and p53 signalling (Roth et al., 2010; Missiaglia et al., 2014; Carethers and Jung, 2015; Aljama et al., 2023). Recent evidence postulates the division of CRC into colon cancer (right-sided and left-sided) and rectal cancer as self-standing tumoral entities, as their differences are not restricted to the molecular and morphological aspects, but also encompass distinct responses to immunotherapy and chemotherapy (Baran et al., 2018; Guo et al., 2022).

Concerning the morphological evolution, most human CRC evolves through conventional (tubular or villous) adenomas via the adenoma–carcinoma pathway, involving *APC*, *RAS*, *PIK3CA*, *TGFBR*, *SMAD4*, *DCC,* and *TP53* mutations (Nawa et al., 2008; Conteduca et al., 2013) but serrated colorectal polyps (30% of CRCs) have emerged as an alternative pathway for

colorectal carcinogenesis in humans. Based on epidemiologic and clinical evidence, adenomatous polyps are considered surrogate endpoints in CRC development, since it has been shown that their removal reduces the risk of developing colon cancer (Nawa et al., 2008; Conteduca et al., 2013; Missiaglia et al., 2014). Macroscopically, adenomas are pedunculated (polyp) or sessile lesions, classified into major histological types: tubular, villous, and tubulovillous (Nawa et al., 2008; Conteduca et al., 2013; Missiaglia et al., 2014). Most tubular adenomas remain benign and asymptomatic lesions (Nawa et al., 2008; Conteduca et al., 2013; Missiaglia et al., 2014). Adenocarcinomas/carcinomas are defined as lesions with high-grade dysplasia able to invade the submucosa, and potentially metastasize to other organs, such as the liver and lung, or spread to the peritoneal cavity (Nawa et al., 2008; Aljama et al., 2023). In chemically-induced rodent colon carcinogenesis models (see below), most colon tumors are diagnosed as pedunculated/protruding or flat/sessile lesions classified as well-differentiated tubular/tubulovillous adenomas or adenocarcinomas, and few cases are signet-ring cell/mucinous adenocarcinomas or serrated lesions (Rubio, 2017a,b).

Rodent models of sporadic CRC

Laboratory animals are commonly employed as experimental models for the study of biology, progression, or intervention of sporadic CRC, since the histopathology and some molecular biomarkers in rodents are very similar to the corresponding human disease (Perše and Cera, 2011; Johnson and Fleet, 2013; Ward and Treuting, 2014). The large intestine of rats and mice is divided into proximal, middle, and distal parts followed by an indistinct short rectum segment (Walthall et al., 2005). In comparison to the human colon, rodent proximal and middle-distal anatomical sites correspond to the right-side and left-side colons, respectively (Walthall et al., 2005) (**Fig. 1**). In contrast to human CRC, spontaneous colonic tumors are rarely detected in older rats and mice, indicating that chemically induced models of colon carcinogenesis in rodents are suitable for the study of risk factors, cancer prevention, or tumoral development biology (Johnson and Fleet, 2013; Ward and Treuting, 2014). To understand the different morphological and genetic/epigenetic aspects of CRC tumorigenesis, chemically induced murine models were established, including mainly those induced by carcinogen 1,2-dimethylhydrazine (DMH) and its metabolite, azoxymethane (AOM) (Perše and Cera, 2011; Suzui et al., 2013). In the liver, DMH is oxidized to AOM followed by the formation of ultimate carcinogen methylazoxymethanol (MAM) that reaches the colon and, in this organ, MAM is converted into alkylating reactive metabolites, such as methyldiazonium ion by β-glucuronidase, β-glucosidase, and sulfatase bacterial

enzymes, thus leading to initiation of colon carcinogenesis by the formation of aberrant crypt foci (ACF) (Bird and Good, 2000; Perše and Cera, 2011; Suzui et al., 2013).

Single or multiple doses of DMH/AOM induce the development of both colonic preneoplastic (non-dysplastic and dysplastic ACF) and neoplastic lesions such as adenomas and adenocarcinomas, more frequently detected in the middle and distal colon/rectum than in the proximal segment (Bird and Good, 2000; Perše and Cera, 2011; Suzui et al., 2013). ACF, composed of one or more aberrant crypts (AC), is considered the earliest lesion during both human and rodent colorectal carcinogenesis (Di Gregorio et al., 1997; Bird and Good, 2000; Seike et al., 2006). This putative preneoplastic lesion has been also identified in human post-mortem and colonoscopy screening studies and is considered a suitable biomarker for adenoma recurrence in high-risk patients (Di Gregorio et al., 1997; Seike et al., 2006). In rodents, ACF detection and quantification have been performed in whole-mount preparation of the colon stained with 1-2% methylene blue (MB) or in histological sections (swiss-rolling or *en face* sections) stained with hematoxylin-eosin (H&E) (**Fig. 3**) (Bird and Good, 2000; Suzui et al., 2013). In colonic whole-mount stained with MB, the mean number of AC and ACF and the multiplicity of ACF (AC/ACF) are the main parameters evaluated in proximal, medial, or distal/rectum segments (Bird and Good, 2000; Suzui et al., 2013). Mucin-depleted foci (MDF) and β-catenin accumulated crypts (BCAC) foci have been described as hallmarks of malignant potential due to their different dysplasia-grade patterns and increasing size with time after carcinogen exposure (Yamada et al., 2000; Suzui et al., 2013). In most rodent models, colon tumors possibly evolve from dysplastic ACF to adenoma-carcinoma, whereas some adenocarcinomas can arise from de novo flat lesions rather than from adenomas (Rubio, 2017b). The earliest cellular events after single or multiple DMH/AOM administrations include impaired antioxidant defense and further oxidative stress increase, which causes DNA adduct formation in colonic epithelial cells. As a result, the initiation step occurs, represented by early AC appearance (Bird and Good, 2000). Early ACs may evolve to ACF with multiple ACs (> 2, > 4, or >10 ACs/ACF), and some premalignant lesions with high AC/ACF multiplicity may progress toward neoplastic lesions (Bird and Good, 2000; Perše and Cera, 2011; Suzui et al., 2013). As part of animal modelling nature, the classical chemically induced CRC rodent models bear advantages and disadvantages, since most ACFs do not evolve into tumors and metastasis is usually rare in medium-term models.

We have reviewed the histologic phenotype of DMH-induced colonic tumors in male Wistar rats in all studies performed at our laboratory in the last two decades. The tumors were

obtained from animals who received four DMH subcutaneous injections (40 × 40 mg/kg, twice a week) and were euthanized between 22-25 weeks of protocol. This DMH regimen was previously established and reviewed by our research group (Rodrigues et al., 2002; Ramos Caetano et al., 2020). The animals developed a total of 251 colonic tumors: 116 adenomas and 135 invasive adenocarcinomas. Of the 116 adenomas, 68 (59.5%) were exophytic adenomas **(Fig. 4A-A1)** and the remaining 48 (41.40%) were flat/sessile adenomas. Of the 135 adenocarcinomas analysed, 69 (48.1%) were exophytic lesions, 61 (49.7%) were flat/sessile adenocarcinomas (**Fig. 4 B-B1**), and the remaining 6 lesions (29.2%) were mucinous/signet-ring cell type adenocarcinomas (**Fig. 4 C-C1**). Twenty-three tumors (9.1%) emerged around the lymphoid tissue-associated colonic mucosa (**Fig. 4 D-D1**). As exophytic lesions were the most frequent lesion type detected in DMH-treated rats, total RNA was isolated from these lesions (1 tumor/animal, n = 5, all showing similar histological grade and size) (**Fig. 5 A-B**) and non-tumor surrounding colonic mucosa of the same animals (n=5). The mRNA expression profiles were analysed using 96-well Low-Density TaqMan Array Cards (TLDA, Life Technologies, Carlsbad, USA)-based real-time polymerase chain reaction (RT-PCR) (see Ramos Caetano et al., 2020 for further methodological details). The custom TLDA consisted of 96 genes (91 target genes and 5 reference genes) involved in the regulation of cell proliferation, cell death, and DNA repair. The expression of the reference control genes *Actb*, *Gapdh*, and *Gusb* were used to normalize mRNA data based on geNorm calculations while relative quantitation was calculated based on the 2-(ΔΔCt) method using Expression Suite Software v1.1 (Life Technologies, USA) (Ramos Caetano et al., 2020), considering fold change≥1.5 and p≤0.05.

Among the different genes detected in samples of exophytic colon tumors (**Fig. 5**), the main findings showed that there is a downregulation of *Mgmt*, *Ogg1, Xrcc6* and *Bax* genes and an upregulation of *Bcl2* gene in comparison to the non-tumor colonic mucosa in DMH-treated animals. The *Mgmt* (O^6-methylguanine-DNA methyltransferase) gene encodes a specific repair enzyme that acts against toxicity from alkylating agents, removing the O^6-mG adducts and other methylated moieties of the DNA to its molecule and restoring the normal structure of guanine (Bai et al., 2023). The Ogg1 (8-oxoguanine DNA glycosylase) gene encodes a DNA glycosylase/AP lyase that removes 8-OH-G lesions, a mutagenic base byproduct, from genomic DNA (Li et al., 2022), while the *Xrcc6* (X-ray repair cross complementing 6) gene is associated with DNA recombination and repair events (*i.e.,* repair of nonhomologous DNA ends such as that required for double-strand break repair) (Jia et al., 2015). Apoptosis is a fundamental regulatory process for the protection against tumor initiation and progression by removing cells with genomic

instability and by deleting cells with DNA damage induced by genotoxic agents such as food carcinogens (Roos et al., 2016). The regulators of apoptosis are divided into intrinsic and extrinsic apoptotic programs. Both involve the activation and/or suppression of several gene families, including *Bcl-2* and *Bax* protein-coding genes that inhibit or potentiate cell death, respectively (Senichkin et al., 2020; Hafezi and Rahmani, 2021). The bcl2/bax protein ratio intrinsically controls the relative susceptibility of target cells to survival or death after an apoptotic stimulus (Senichkin et al., 2020; Hafezi and Rahmani, 2021). In light of these findings at the mRNA level: 1) a reduced DNA repair capacity and resistance to cell death induction were detected in the colon tumors analysed; 2) These alterations could result in a higher susceptibility to DNA damage induced by cooking mutagens (heterocyclic amines and polycyclic aromatic hydrocarbons), oxidative stress associated with higher resistance to apoptosis in tumor cells, which could contribute to the progression these lesions induced by the DMH carcinogen.

Use of chemically induced sporadic CRC models for prevention strategies

Although there are promising treatments for advanced CRC, the available regimens do not often produce fully satisfactory results, considering the current and growing epidemiological data for CRC-related mortality (Keum and Giovannucci, 2019; Sung et al., 2021). Epidemiological studies postulate an inverse association between the regular consumption of fruits, vegetables, fish, dairy products, and whole grains and CRC (Thanikachalam and Khan, 2019; Veettil et al., 2021). Thus, CRC prevention could be achieved through screening programs and intake of food consumption rich in bioactive compounds with the potential to prevent, inhibit, or reverse the risk of developing this malignancy (Stoner et al., 1997; Steward and Brown, 2013). Originally defined by Michael B. Sporn in 1976, the concept of chemoprevention of cancer addresses the use of natural and synthetic agents to suppress, prevent, or delay tumorigenesis by limiting exposure, biotransformation or DNA damage due to carcinogens exposure (*e.g.*, blocking agents, administered before or during carcinogen exposure) or by reduction of tumor promotion/progression stage (*e.g.,* suppressing agents, administered after carcinogen exposure) before the establishment of the malignant phenotype stage (Stoner et al., 1997; Steward and Brown, 2013). Various food bioactive compounds (FBCs), derived from fruits, vegetables, vegetal and fish oils, or whole grains may protect against CRC initiation and/or progression because of their antimutagenic, antioxidant, anti-inflammatory, and other biological properties (Pan et al., 2011; Costea et al., 2018; Amintas et al., 2023). Using colonic ACF and tumors as biomarkers, several potential preventive agents (blocking and suppressing agents) have been evaluated in

chemically induced sporadic CRC rodent bioassays with potential translational value for human cancer prevention (Wargovich et al., 2000; Corpet and Pierre, 2005; Clapper et al., 2020). Cumulating preclinical animal evidence has demonstrated that various FBCs such as polyphenols/flavonoids/anthocyanins, carotenoids, isothiocyanates, and prebiotics have preventive potential against the rodent chemically induced colon carcinogenesis **(Table 1).** FBCs have been administered before and/or during the initiation stage (I), in the post-initiation (promoting) stage (PI), or during all experiment periods (EP= I+PI) (**Table 1**). The attenuating effects of some FBCs on the total number of putative preneoplastic lesions (ACF, MDF, and BCAC) or neoplastic colorectal lesions (adenomas and adenocarcinomas) have been demonstrated in different rodent models. Blocking and suppressing the effects of FBCs include reduction of cell proliferation, pro-apoptotic properties on colonic crypts bearing initiated colonocytes, preneoplastic and neoplastic lesions, and increase in short-chain fatty acids (SCFAs, end products of fermentation of dietary fibers by the anaerobic gut microbiota) formation as well as decreased cecal bacterial β-glucuronidase and β-galactosidase enzyme activities, demonstrating that some FBCs could also modulate colon microbiota components related to colon carcinogenesis (Temple and Basu, 1987; Rao et al., 1998; Kim et al., 1998; Kawamori et al., 1999; Tessitore et al., 2000; Wargovich et al., 2000; Chung et al., 2000; Verghese et al., 2002; Nomoto et al., 2004; Raju et al., 2005; Sengottuvelan and Nalini, 2006; Bauer-Marinovic et al., 2006; Plate and Gallaher, 2006; Kuno et al., 2010; Xu et a, 2010; Reynoso-Camacho et al., 2011; Wang et al., 2020). However, the beneficial effects of FBCs are dependent on the dose and route of administration, the rodent species/strain, and the time and stage of administration evaluated, such as initiation stage (I), post-initiation stage (PI), or both stages (EP)

. Over the past few decades, the relationship between gut microbiota and human CRC has been established well-established (Kuno et al 2010; Wong and Yu, 2019; Hanahan, 2022). The drastic change in gut microorganism profile and subsequent disruption of the intestinal barrier, resulting in gut dysbiosis, is considered a risk factor for various diseases, including CRC (Kuno et al 2010; Wong and Yu, 2019); Hanahan, 2022). In human CRC, dysbiosis also involves the development of an invasive polymicrobial bacterial biofilm on RSCC (89% of cases) and LSCRCs (12% of cases) (Dejea et al., 2014). This organization of bacterial communities into biofilms promotes pro-carcinogenic activities that may partially underlie progression along the adenoma-adenocarcinoma axis, associated with increased epithelial permeability and inflammation (Dejea et al., 2014; Li et al., 2017). Of note, CRC patients feature a differential bacterial profile and enhanced blood bacterial DNA load compared to healthy controls, supporting the hypothesis of a higher passage of bacteria from the gastrointestinal tract to the bloodstream in CRC. Bacterial

taxa and operational taxonomic unit abundances were able to predict CRC occurrence and can serve as a basis for evaluating new non-invasive techniques for an early CRC diagnosis (Mutignani et al., 2021). On chemically induced rodent CRC assays, the link between gut microbiota alteration and the development of colon carcinogenesis has been demonstrated (Onoue et al., 1997; Sun et al., 2017). Male ICR mice treated with DMH (20 mg/kg body weight once a week for 6 weeks) were euthanized at 6, 12, 18, and 26 weeks. Fecal Bacteroidaceae and Enterobacteriaceae showed higher relative abundance in the early stages and were gradually replaced with Rikenellaceae, Lachnospiraceae, Ruminococcaceae, and Streptococcaceae at late stage of CRC formation compared to the control group (Sun et al., 2017). In addition, male Wistar rats that received DMH (40 mg/kg body weight, subcutaneous, once a week for 10 weeks) presented a gut microbiota that differed from the vehicle group, showing a higher abundance of *Bacteroides*, *Desulfovibrio*, and *Fusobacterium* and reduced abundance of *Roseburia* and *Eubacterium* in the fecal microbiota of CRC rats (Onoue et al., 1997).

Some previous work has shown that the fecal microbiota of CRC patients may have cancer-promoting properties in germ-free (GF) and conventional rodents (Zhu et al., 2014). Germ-free (GF) and DMH-initiated male F344 Fischer rats were orally inoculated with mixtures of pure culture of *Escherichia coli*, Enterococcus faecium, and several strains of *Bacteroides* and *Clostridium* species (GB) or received feces from conventional rats (Cvd). At 34 weeks, rats receiving GB showed a greater number of total ACFs and ACF with four or more crypts/focus, and mean number of AC/AC (crypt multiplicity) compared to GF group (GF) (Zhu et al., 2014). In male C57BL/6 mice were given AOM, after receiving a course of antibiotics in drinking water, and received gavage twice weekly with stool from 5 patients with CRC or 5 healthy individuals (controls) for 5 weeks. AOM+CRC feces group developed a higher number of intestinal polyps and an increased PCNA and β-catenin levels in colonic mucosa at 32 weeks after stool gavage. In addition, an increase in percentage of Th1 pro-inflammatory lymphocytes (CD4+ and IFN-gamma) and Th17 (CD4+ IL-17+) in colonic mucosa of AOM+CRC feces group was observed compared to AOM-Control feces, which may indicate an important role for dysbiosis in colorectal tumorigenesis (Wong et al., 2017). Male C57BL/6 mice initiated with AOM (10 mg/kg b.w., once a week for 6 weeks), after receiving a course of antibiotics in drinking water, were gavaged either with feces from individuals with normal body mass index (BMI, average 20.9 kg/m^2, range 18.8–22.8 kg/m2) or obese individuals (BMI, average 33.2 kg/m^2, range 31.1–35.4 kg/m^2). The last group showed a significantly higher mean number of colon tumors, showing higher dysplasia and ki-67 labelling indices, compared with those groups gavaged with feces from non-obese

individuals (Kang et al., 2023). These findings suggest that feces from obese individuals promote CRC formation in AOM-treated mice.

As the rodent models of DMH/AOM assays have demonstrated the link between gut microbiota modulation on the promotion of CRC carcinogenesis, the anti-promoting activity of FBCs on gut dysbiosis opens an avenue of opportunities for colon cancer prevention (Fong et al, 2020).

Conclusions and Future Perspectives

In this review, we highlight the translational value of classic chemically induced rodent models of sporadic CRC as a useful tool for; 1) cancer biology, since they recapitulate trending features of adenoma-carcinoma sequence and tumor histopathology, 2) translational screening of preventive and therapeutic strategies for this malignancy (**Fig. 1**). Nonetheless, CRC, as a multifaceted disease, demands a comprehensive understanding that goes beyond singular approaches. Multimodeling, which is the integration of various experimental and computational models, emerges as a pivotal strategy in unravelling the complexities of CRC biology. By synergistically combining other diverse methodologies such as in vitro, in vivo models, and advanced computational simulations - along the backbone of the classical chemically induced models - researchers can gain a more holistic perspective on the dynamic interplay of factors influencing CRC initiation, progression, and response to treatment, also avoiding “model-biased” findings and interpretations.

Legend for figures

Figure 1 - The importance of chemically induced models of colorectal carcinogenesis within the corresponding human disease. While rodent colon is anatomically distinct from humans, the 1,2-dimethylhydrazine- or azoxymethane-induced tumors reflect some of the morphological and molecular features of sporadic colorectal cancer, which are closely related to westernized dietary habits and alcohol drinking. In contrast, there is increasing preclinical evidence on the preventive or therapeutic roles of bioactive food compounds on colon carcinogenesis.

Figure 2 - A) Macroscopic aspect of human right-side (proximal) colon cancer (RSCC, dotted circle) showing exophytic growth polyp-cauliflower-like; B) Macroscopy aspect of human

right-side (distal) colorectal cancer (LSCRCs, dotted circle) showing growth endophytic ring-shaped and showing ulcerating areas. Source: authors' archive

Figure 3 - A-B) Histological sections stained by hematoxylin-eosin showing non-dysplastic ACF and dysplastic ACF (dotted lines) induced by DMH in male Wistar rats, respectively. C) Whole-mount colon stained with methylene blue at 1.0% showing ACF contained 3, 9, or 4 aberrant crypts (dotted circles) induced by DMH in male Wistar rats, respectively (left to right). Bar = 50 or 200 µm.

Figure 4 - Histological sections stained by hematoxylin-eosin showing different phenotypes of well-differentiated colon tumor induced by DMH in male Wistar rats. Exophytic adenoma (A) showing tubular pattern (A1); Flat adenocarcinoma with invasive area (B1); Flat adenocarcinoma (C) with mucinous area (C1) and tubulovillous adenoma (D) with lymphoid tissue-associated mucosa (D1). Bar= 50 or 100 µm.

Figure 5 - A) Macroscopic view of a protruding colon tumor induced by DMH in male Wistar rat; B) Microscopy aspect of the same lesion as shown in A; C) Differential gene expression between exophytic colon tumors and non-tumor mucosal area of the same animals.

Authors' contributions

Guilherme R. Romulado, Luis M. Sarmiento-Machado, Maria A.M Rodrigues, and Luis F Barbisan conceived the review topics and contributed to writing and editing. Guilherme R. Romulado and Maria A.M. Rodrigues were responsible for illustrations, histopathological images and the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflicts of interest

The authors declare no conflicts of interest

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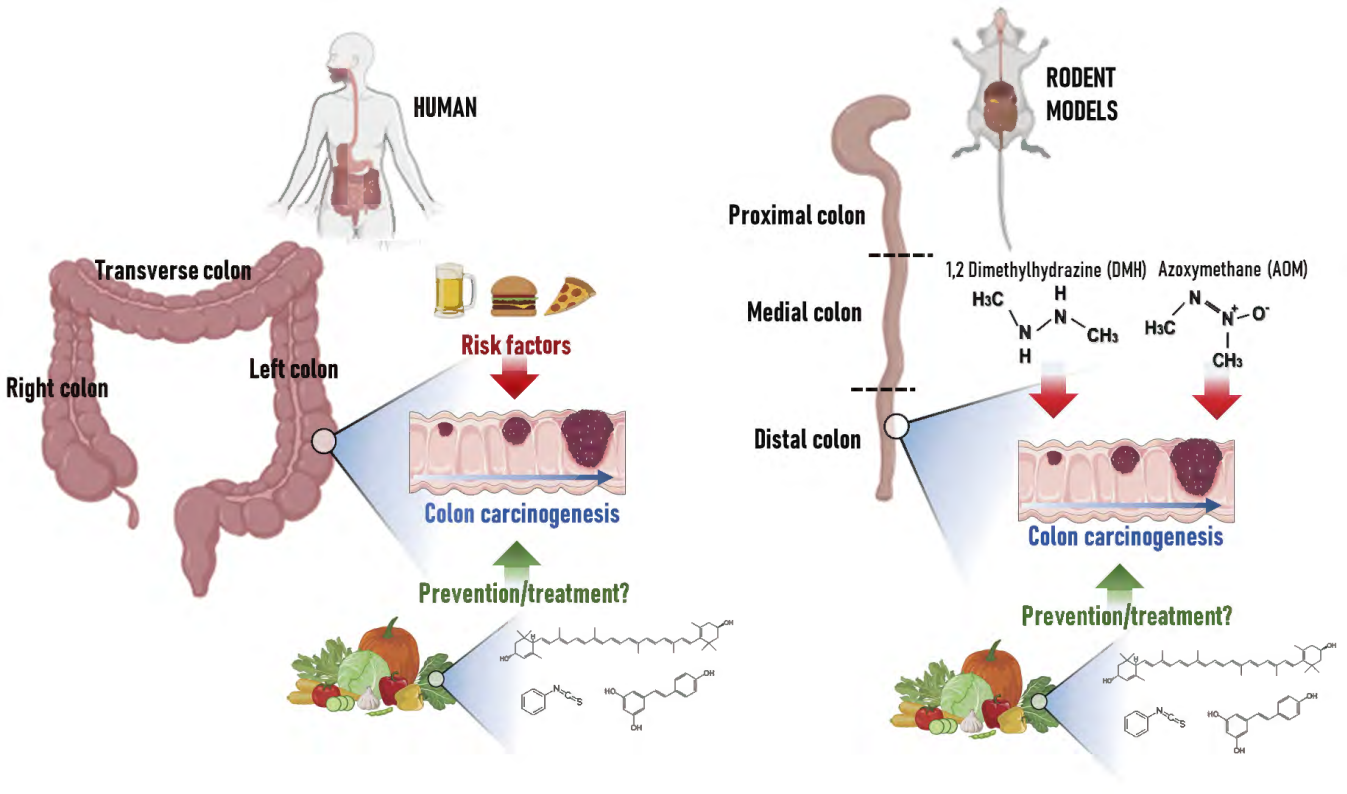
Table 1. Preventive actions of classical food bioactive compounds (FBCs) evaluated in DMH/AOM induced CRC carcinogenesis in rodents.

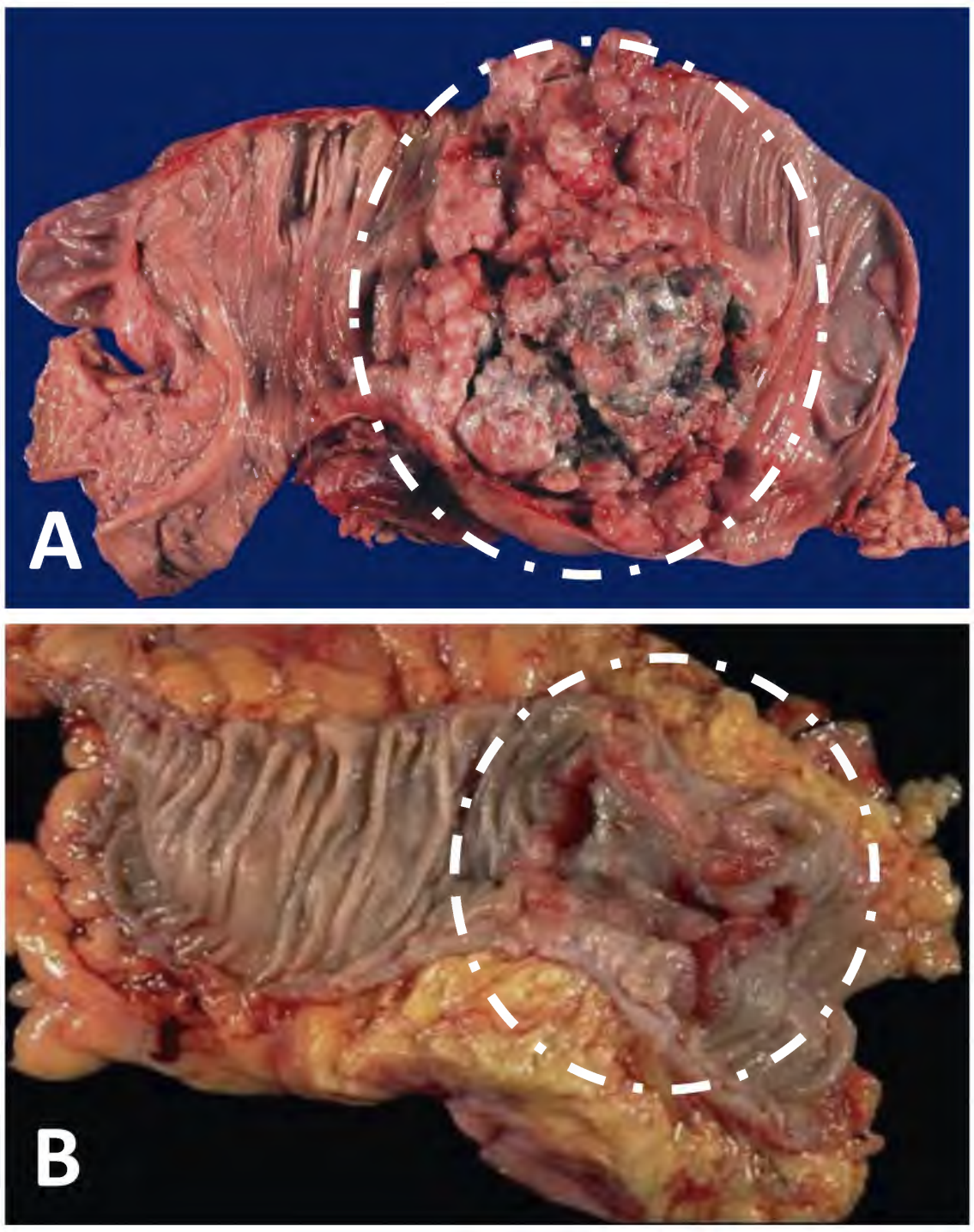
FBC classes	Animal/Age	DMH/AOM model	Treatment (dose/period)	Outcomes
Polyphenols				
Curcumin (38,39)	Male Fischer 344 rats (5 weeks)	AOM (15 mg/kg, s.c), once a week for 2 weeks.	0.2% in chow for 4 weeks (I) or 0.2 and 0.6% in chow from weeks14-52 (PI)	• ↓ Total number of ACF (I) • ↓ Incidence of tumors (%) (PI) • ↑ Apoptosis in tumors (PI)
	Male Wistar rats (6-8 weeks)	DMH (20 mg/kg, s.c), once a week for 18 weeks.	0.2% in chow for 32 weeks (EP)	• ↓ Total number of ACF • ↔ Incidence of tumors (%) • ↓PCNA, ↑TUNEL (apoptosis) in tumors
Resveratrol (40,41)	Male Wistar rats (6-7 weeks)	DMH (20 mg/kg b.w., s.c.) once a week for 15 weeks.	0.008 g/kg bw, i.g.for 15 or 30 weeks (I, PI or EP).	• ↓ ACF, tumor incidence (%), and tumor burden; ↓ β-galactosidase activity;
	Male F344 rats (6-week-old)	AOM (15 mg/kg b.w., i.p)., two weekly doses)	200 µg/kg/day in drinking water for 100 days (EP)	• ↓ ACF, ↑ bax in ACF, ↓ p21 in normal mucosa
Proanthocyanidin (PA) or *Procyanidin B-2 (B-2)* (42)	Male F344 rats (5-week-old)	AOM (15 mg/kg bw, s.c.), three times per week for three weeks.	0.002, 0.01 or 0.05% PA or 0.002, 0.01 or 0.05% B-2 in drinking water for 5 weks (EP).	• ↓Total ACF (0.002% PA and 0.05% B-2) • ↓PCNA (0.002% PA and 0.05% B-2) • ↑ Apoptosis (0.002% PA and 0.05% B-2)
(-)-Epigallocatechin gallate (EGCG) (43)	Male Wistar rats (6-week-old)	DMH (40 mg/kg bw, s.c.), Twice a week for 8 weeks	0.002, 0.01 or 0.05% EGCG in drinking water for 8 weeks (I).	• ↓ ACF (0.002 and 0.01%, 12 weeks) • ↓ Tumor incidence (%) (all doses, at weeks 12 and 20)
Carotenoids				
βcarotene (44,45)	Male F344 rats (7-week-old)	AOM (15 mg/kg, s.c.), once weekly for 2 weeks)	100, 200, 1000 or 2000 ppm in chow for 9 weeks (EP)	• ↓ Total number of ACF (100 and 200 ppm) and ↑ Total number of ACF (2000 ppm)
	Female ICR mice (10-week-old)	DMH (28 mg b.w., s.c.), once a week for 7 weeks)	2 or 22 mg/kg in chow for 21 weeks (EP)	• ↓ Tumor incidence and multiplicity

Lycopene (46,47)	B6C3F1 mice (4-week-old) Male F344 (7-week-old)	DMH (20 mg/kg bw, s.c.), twice a week for 3 weeks. AOM (15 mg/kg, s.c.), once weekly for 2 weeks)	0.005% and 0.0025% in drinking water for 7 weeks (PI) 0.038 or 0.075% in chow for 5 weeks (I or PI)	• ↔Total ACF, • ↔ BrdU proliferation index (%). • ↓ Mean number of ACF (I).
Lutein (46,48)	Male B6C3F1 mice (4 week-old)	DMH (20 mg/kg bw, s.c) twice a week for 3 weeks.	0.05% in chow for 7 weeks (PI)	• ↓ Mean number of ACF and AC; • ↓ BrdU proliferation index (%);
	Male SD rats (4-5-weeks old)	DMH (21 mg/kg b.w., s.c.) once a week for 8 weeks	0.002% in chow for 8 weeks (I or PI)	• ↓ Mean number of tumor (I and PI); • ↓ Tumor K-ras, β-catenin and pPKB protein expression (I and PI)
Probiotics				
Inulin (49)	Male Fisher 344 rats (7-week-old)	AOM (16mg/kg bw, s.c.) twice a week for 2 weeks	10% (w/w) in chow for 5 weeks (I, PI or EP).	• ↓Total ACF and AC/focus (I). • ↓Tumor multiplicity and tumor size (I, PI and EP).
Pectin (50)	Male Fisher 344 rats (5-week-old)	AOM 15 mg/kg bw, s.c.). twice per week for 2 weeks.	10% (w/w) in chow for 9 weeks (EP).	• ↓Total ACF and total AC. • ↑ β-glucuronidase activity. • ↑Short-chain fatty acids (SCFAs).
Resistant starches (R2) (51)	Male Sprague-Dawley rats (age not informed)	DMH (20 mg/kg bw, s.c.), once a week for 20 weeks.	10% (w/w) in chow for 20 weeks (PI)	• ↓ Ki-67-positive crypt cells • ↓ Colonic crypt height • ↑ TUNEL (apoptosis) in colonic crypt cells
Isothiocyanates				
Indole-3-carbinol (I3C), Phenethyl isothiocyanate (PEITC (52)	Male Wistar rats (3-4 weeks-old)	AOM (15mg/kg bw, s.c.) once a week for 2 weeks.	1.36 mmols/Kg or 6.8 mmols/Kg I3C/chow for 8 weeks (EP).; 0.67 mmols/Kg or 3.37 mmols/Kg/chow for 8 weeks (EP)	• ↓ Total ACF (1.36 and 6.8 mmols) and Total MDF (1.36 mmols) (I3C) • ↔ total ACF (0.67 and 3.37 mmols) and ↑MDF (3.37 mmols) (PEITC)
Phenethyl isothiocyanate (PEITC), sulforaphane (SFN) (53)	Male F344 rats (6-week-old)	AOM (15mg/kg bw, s.c.), twice a week for 2 weeks	5 µM by gavage for 3 times weekly for 8 weeks (PI) or 20 µmol by gavage once daily for 3 days for 2	• ↓Total ACF for PEITC and SFN (I and PI)

			weeks (I).	
6-methylsulfinylhexyl isothiocyanate (6-MSITC) (54)	Male F344 rats (4-week-old)	DMH (40mg/kg bw, s.c.), once a week for 4 weeks	200 and 400 ppm in chow for 5 five weeks (I and PI).	• ↓ACF and AC/ACF (400 ppm, I) • ↓BCAC and AC/BCAC (400 ppm , I) • ↓AC/BCAC (400 ppm, PI) • ↓PCNA in BCAC (400 ppm, I)

DMH= 1,2- dimethylhydrazine; AOM= azoxymethane; b.w= Body weght; s.c.= subcutaneous injection; ppm= parts per million; I= Initiation stage; PI= Post-initiation stage; EP= entire experiment (I+PI) stages; AC= aberrant crypt; ACF= Aberrant crypt foci, MDF= mucin-depleted foci, BCAC=β-catenin-accumulated crypts; ↑= increase; ↓ reduce; ↔ not altered; Cell proloieration markers: Ki-67, PCNA= Proliferating cell nuclear antigen, BrdU= Bromodeoxyuridine.


HUMAN
Transverse colon
Right colon
Left colon
Risk factors
Colon carcinogenesis
Prevention/treatment?
RODENT MODELS
Proximal colon
Medial colon
Distal colon
1,2 Dimethylhydrazine (DMH)
Azoxymethane (AOM)
Colon carcinogenesis
Prevention/treatment?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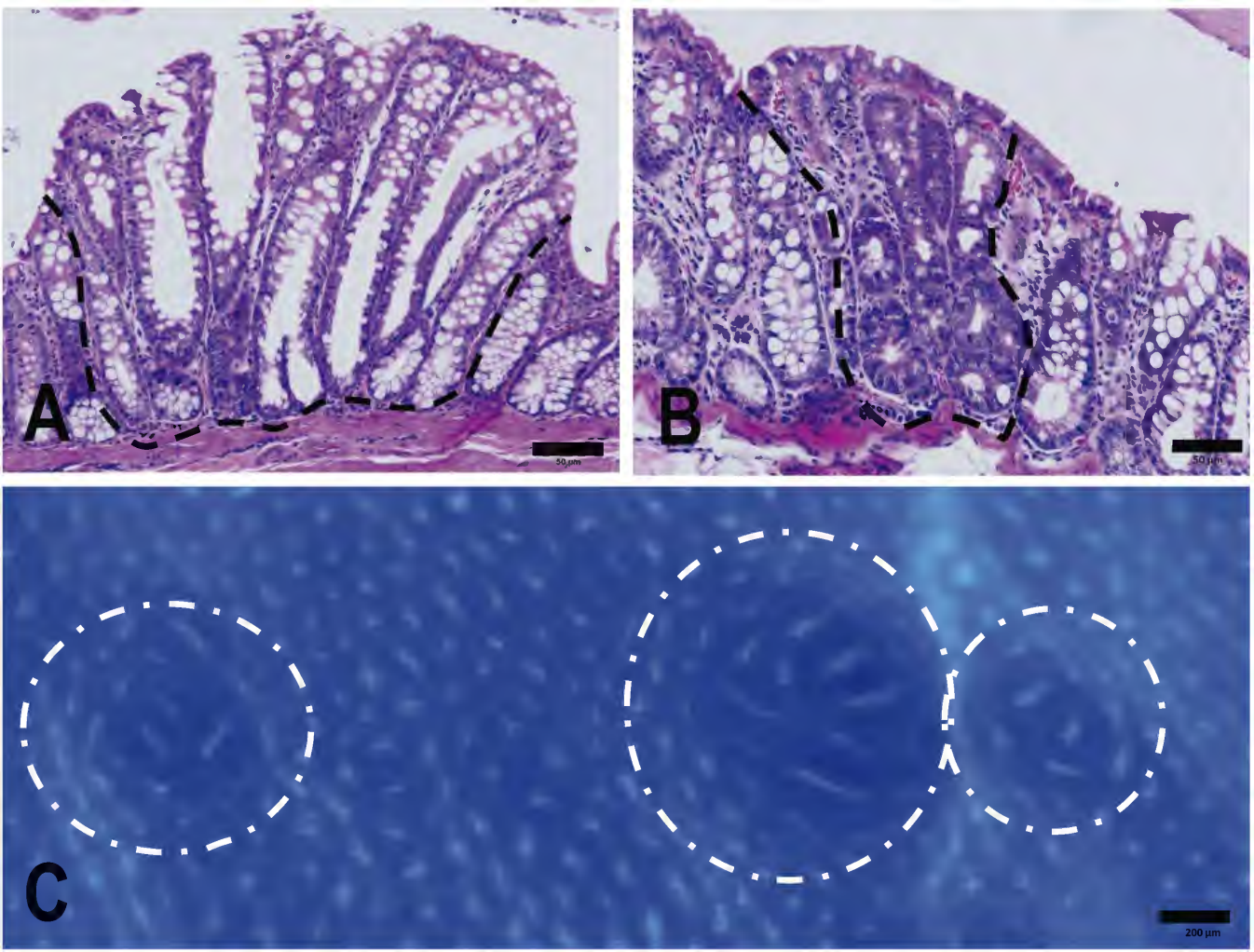
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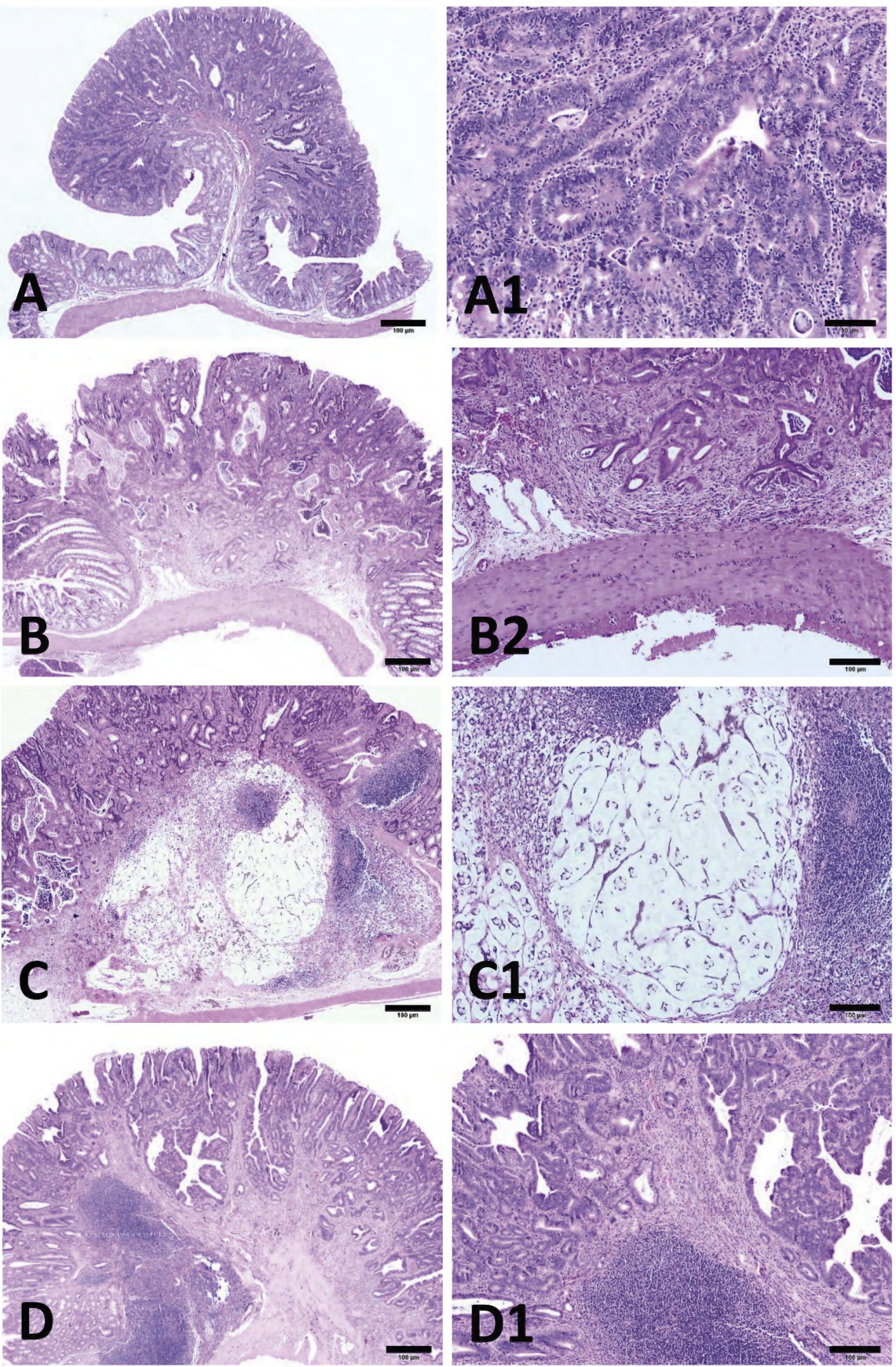
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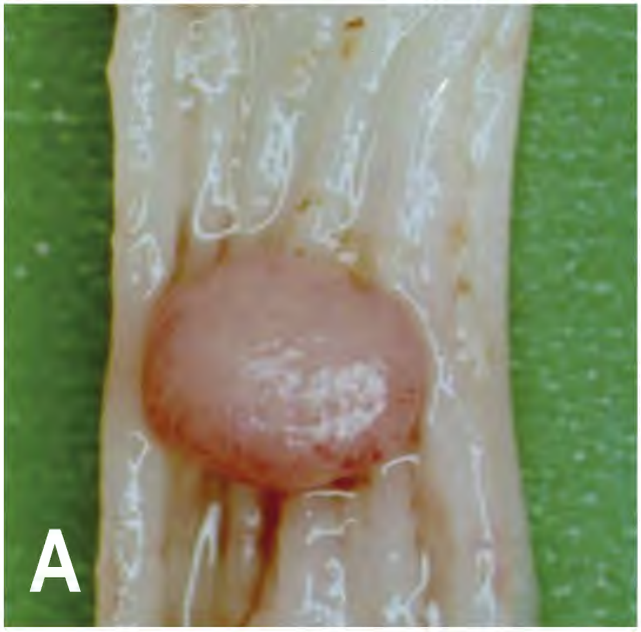

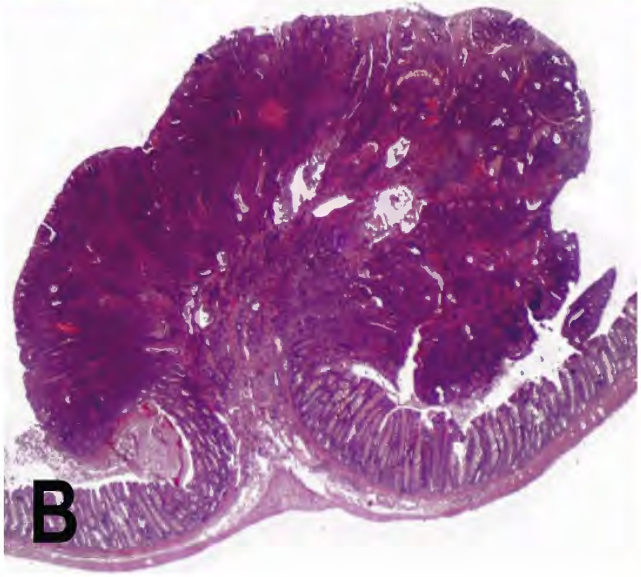

Up-regulated	Down-regulated
Grb2	Ccna1
Nox1	Bax
Cebpd	Gadd45a
Akt2	Jun
Bcl2	Mgmt
Chek1	Trpv1
Cd44	Mapk14
	Gdf15
	Aifm1
	Atm
	Prkcz
	Ccs
	Ogg1
	Tspan8
	Xrcc6
	Jag1