Recent Studies on Digestive System Anatomy

Chapter 3

The Histologic Structure of the Large Bowel Mucosa and the Evolution of the Three Pathways of Colonic Carcinogenesis in Humans and in Experimental Animals

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1.Introduction

The Normal Histological Structure of the Mucosa of the Large Bowel in Humans

The mean total mucosal surface of the human digestive tract averages ~32 m^2, of which about 2 m^2 corresponds to that of the large intestine [1]. The latter is built of a single layer of epithelial cells with inward folds called crypts. Crypts replicate by symmetric fission, beginning at their base, and proceeding upwards until two identical, individual crypts are created [2]. Sections cut perpendicular to the surface epithelium show a characteristic appearance of "row of test tubes" due to tightly packed, parallel crypts, "resting" on the *muscularis*mucosae [2]. A slight variation in the configuration of the crypts and in the space between the crypts may occur, but crypt branching is rare. This architecture is retained throughout the colon, except in innominate grooves (cloverleaf-like crypts connecting to a single lumen) [3].

The main segment of the large bowel mucosa is built with crypts lined with mucus producing goblet cells and columnar cells [4]. The function of this huge mucosal domain is to protect underlying structures, to lubricate the faeces by virtue of its mucous production, to absorb vitamins, nutrients and fluids. Fluids are absorbed by the aid of the peptide transporter PEPT1 [5] and Aquaporin 8 [6], a specific water-selective channel protein that regulates water absorption in the colon.

The remnant tiny mucosal domain in the large bowelis spotty and tiny. It is built

with organized lymphoid aggregates (lymphoid follicles) being referred to as gut-associated lymphoid tissue (GALT) [7-9]. It should be understood that the organized GALT is widespread in the gastrointestinal tract, extending from the Waldeyer´s ring (tonsils), the esophagus, the stomach, Peyer´s patches (ileum), and the appendix, down to the colon [9]. In addition, isolated lymphocytes infiltrating the epithelium and lymphocytes and plasma cells infiltrating the *lamina propria* of the stomach, the duodenum, the small and large intestine of the gut are also included in the GALT system. Thus, the term GALT follicle only refers to an organized lymphoid tissue that can be found from the tonsils down to the large intestine. Accordingly, GALT follicles in the colon will be referred to as "large bowel GALT follicles" or "large bowel GALT mucosal domains", to differentiate them from GALT follicles in the distal small intestine (Peyer´s patches) [10], or from newly formed colonic lymphoid aggregates in patients with Crohn´s colitis [11], among others. The large bowel GALT follicles, present in the anti-mesenteric border of the colon, are typically organized into lymphoid aggregates within the mucosa and submucosa. The epithelium covering GALT follicles exhibits cuboidal cells, few or no goblet cells, scattered M cells (so-called because of broad invaginations or microfolds) [7-9] and mucosal crypts 4. The function of this spotty and tiny mucosal domain is to absorb, via M cells, luminal antigens, macromolecules and microorganisms by clathrin-mediated endocytosis [12]. Luminal antigens, macromolecules and microorganisms are subsequently hauled into antigen-presenting cells (macrophages, B cells and dendritics cells) from where they are transferred to gut-indigenous, thymus-independent lymphoid tissue, for immediate immunological processing. The constellation lymphoid tissue-M cells build a lympho-epithelial immunological cross-talk unit, a relay complex for antigen-gut recognition.

2. Colorectal Cancer (CRC)

CRC is one of the more intensively studied human malignancies, being the third most common cancer, the fourth most common cause of cancer death, and the second most common cancer worldwide, in terms of the number of individuals living with cancer five years after diagnosis. An estimated 1,361,000 people are diagnosed with CRC annually; approximately 694,000 people die from CRC annually; and 3,544,000 individuals are living with CRC [13]. Main risk factors for CRC development include advanced age, family history, male sex, lifestyle factors, natural exposures to dietary/environmental factors, genome differences, obesity, type 2 diabetes, and the colonic microbiome [14].

The Majority of the Sporadic CRC Evolves in the Mucosal Domain Lined with Goblet Cells and Columnar Cells

In this vast mucosal domain two pathways of sporadic colorectal carcinogenesis might evolve:

i) The conventionaln (tubular or villous) adenoma-carcinoma pathway

In 1951, Jackman and Mayo launched the concept of adenoma to carcinoma sequence [15]. Adenomas were histologically classified into tubular, villous and a mixed phenotype (tubulovillous). This sequence of events is today being referred to as the conventional (tubular or villous) adenoma-carcinoma pathway. For many years, the general view was that the vast majority of the human colorectal carcinomas developed from conventional adenomas.

ii) The serrated adenoma-carcinoma pathway

In 1990, Longacre and Fenoglio-Preiser first described polyps characterised by serrated architecture and unequivocal dysplasia [16]. More recently, hyperplastic polypyposis [17], serrated colorectal polyps, sessile serrated adenoma/polyps (SSA/P) [18] and traditional serrated adenomas (TSAs) [19] have emerged as an alternative pathway of colorectal carcinogenesis. It has been estimated that in the general population about 30% of the CRC progress via the serrated pathway [20].

iii) The thirtd pathway of colorectal carcinogenesis

Few cases of sporadic GALT-carcinomas have been reported in the literature. So far, only 23 sporadic GALT carcinomas are in record [22]. HGD and conventional adenomas seemingly antedated GALT carcinogenesis in humans [22].

3. Histological Characteristics of the Main Segment of the Colonic Mucosa in Ulcerative Colitis (UC)

The vast majority of the large bowel mucosa in UC also lined with goblet and columnar cells. In addition to high numbers of inflammatory cells, the colorectal crypts show architectural distortions (CAD) [23]. These architectural distortions usually persist despite regress of inflammation due to treatment. Nearly 30 years ago, Allen *et al*. used semi-automatic image analysis to assess the architectural features of colorectal mucosa in UC [24]. Discriminant analysis using the variables mean epithelial height and mean *lamina propria* area per unit length of *muscularis mucosae,* separated normal from UC. Subsequently, Hamilton *et al.* [25] applied morphometry and stereology, to evaluate the architectural characteristics of regenerative and of dysplastic colorectal mucosa in ulcerative colitis. Using neural networks on a mosaic of pixilated images (without any image analysis or image segmentation) the authors concluded that quantitative histological analysis of mucosal abnormalities may be of use in the objective diagnosis of reactive and dysplastic change in patients with ulcerative colitis [25]. More recently, Ficsor *et al*. [26] reported architectonic irregularities in the colonic mucosa by the aid of automated digital microscopy and advanced digital analysis. Shape-related morphological changes helped to distinguish between normal mucosa and UC. Albeit

those studies, the systematic analysis of the spectrum of crypts with architectural distortions (CAD) in UC has remained unattended. We recently classified the histologic repertoire and assessed the frequency of CAD in UC [23]. Five-hundred and sixteen histologic sections from 29 colectomy specimens with UC (24 having adenocarcinoma and five, high-grade dysplasia, HGD) were reviewed. CADs were subdivided into four groups: i) Crypts with fission distortions, ii) Crypts with length distortions, iii) Crypts with outlines distortions and iv) Crypts with axial polarity distortions. The most frequent CAD group had axial polarity distortions (33.4%), and the less frequent CAD group, outline distortions (21.1%) ($P< 0.05$). No apparent differences in frequency between groups were found in colectomies with HGD/carcinoma, or in colectomies preformed for medically-refractory UC without HGD/carcinoma. Nevertheless, most mucosal areas portrayed countless crypts with normal shapes (CNS) lined with normal epithelium, excepting 45 CNS: 28 showed inconclusive-suspected cellular changes (ISCC), and 17, HGD. In contrast, out of the 902 CAD present in the specimens, 343 (38.0%) displayed ISCC, 186 (20.6%) HGD, and the remaining 373 (41.4%) normal epithelium. It may be deduced that out of the 203 crypts exhibiting HGD, 186 (91.6%) were CAD and the remaining 17 (8.4%) CNS ($P< 0.05$) [23]. Based on these findings it was suggested that the microscopic search for HGD in UC colectomy-specimens should preferentially be focused to mucosal areas exhibiting CAD. This view is validated by recent reports showing that p53 over expression (a biomarker of epithelial carcinogenesis) significantly correlated with architectural distortions of the crypts in UC [27].

4. Colorectal Cancer in Ulcerative Colitis

Patients with UC are at increased risk to develop CRC, particularly those with early onset and/or with total-longstanding colitis [32]. Some population-based studies showed that patients with UC have a 2.4-fold increased overall CRC risk [33]. The cumulative probability of UC patients developing CRC is 2% by 10 years, 8% by 20 years, and 18% by 30 years, according to a meta-analysis [34]. CRC in UC might be preceded by different epithelial lesions: *i)* dysplasia in flat mucosa [35], *ii)* UC-related conventional adenomatous lesions [36], *iii)* UC-related serrated adenomatous lesions [37-39], *iv)* dysplasia in UC-related subtle villous changes [40], *v)* conventional adenomas on top of gut-associated lymphoid tissue [41], or *vi)* UC-unrelated, synchronously growing, age-dependent, sporadic adenomas [38]. Importantly, these precursor lesions develop in the vast colorectal mucosa built with mucus producing goblet cells and columnar cells. When no precursor lesions are found in early UC carcinomas, the term *de novo* carcinoma has been applied [38,42].

4.1. UC-Associated GALT-Carcinoma, a Background

In 1954 Cuthbert Dukes described in UC patients a histological lesion in the submucosa characterized by “misplaced” colonic epithelium [43]. Dukes submitted that the misplaced

epithelium was the result of mucosal repair following regeneration of a mucosal ulcer, and that the epithelium detached and buried in the submucosal would encourage cancer development [43]. In 1984, we studied the frequency of misplaced (i.e. ectopic) colonic mucosa in 62 colectomy specimens [44]. One or more foci of misplaced mucosa was found in 72% of the 22 colectomies with ulcerative colitis, in 55% of the 20 colectomies with Crohn´colitis, and in none of the 20 colectomies without IBD. In one patient with ulcerative colitis we found an adenocarcinoma invading the submucosa, surrounded by nodular lymphoid tissue; this tumour fulfilled the criteria of colonic GALT-carcinoma [44]. We recently reviewed the four cases of colonic carcinomas developing in GALT mucosa in UC in the literature, searching for possible precursor-lesions connected with the evolution of these tumours [41]. The luminal surface in three of the four carcinomas revealed conventional (tubular/villous) adenomas or high-grade dysplasia. All four UC-GALT-carcinomas were detected at an early stage (T1N0) [41]. Thus, GALT-carcinomas do occur, albeit infrequently, in patients with UC. The finding that three out of the four GALT-carcinomas in record were covered by conventional adenomas or high-grade dysplasia, strongly suggest that conventional non-invasive neoplasias often precede GALT-carcinomas in UC.The low frequency of UC-GALT-carcinomas could be due to the fact that only minute areas of the colorectal mucosa are occupied by GALT domains.

5. Experimental Colonic Carcinogenesis

5.1. A Serendipitous Discovery

While attempting to produce amyotrophic lateral sclerosis by feeding nuts of Cycascircinalis (a tropical fern from a family of Cycadaceae), Laqueur *et al*. [45] accidentally found that rats had developed colonic cancer. The same author subsequently demonstrated that the active carcinogen in these nuts was cycasin, a water- soluble á-glucoside of methylazoxymethanol [46]. This discovery lead Druckey*et al*. [47] to administer a structurally similar compound, 1,2- dimethylhydrazine (DMH), to rats. DMH and its carcinogenic metabolites (azoxymethane (AOM) and naethylazoxy methanol) are today the most commonly used compounds to induce colonic tumors in rodents and to study morphology, pathogenesis, prevention and treatment of experimentally induced colonic tumors. In later years, a vast amount of literature on colorectal neoplasias evoked by different carcinogens, by genetic engineering or by spontaneous mutations in rodents has been published. A recent search in PUBMED (12/27/2017) using the key words “colon adenomas mice”, “colon cancer mice”. “colon adenomas rats” and “colon cancer rats”, yielded 24086 publications. This vast literature is a testimony of the expectations that experimental models might contribute to grasp the elusive process of colorectal carcinogenesis in humans.

5.2. The colonic mucosal domain in Sprague-Dawley rats

As in the human counterpart, the vast majority of the colonic mucosal domain in

Sprague-Dawley (SD) rats is lined with goblet cells and columnar cells**.** The remnant tiny, spotty colonic mucosal domain is built of gut-associated lymphoid tissue (GALT) [4]. When colectomy specimens removed at autopsy from untreated rats were trans illuminated by the aid of a translucent photography light box, two lumps were found in the proximal colon (ceacum), two in the transverse colon and two in the distal colon, near the rectal border. The histological examination of the six colonic lumps revealed to be organized GALT follicles [4].

The Colonic Mucosa in Carcinogen-Treated Rats displays crypts with architectural distortions

The colonic crypts in rats reproduce themselves by symmetric fission at the base of the crypts, and proceeding upwards, generate two separate identical crypts. We previously reported crypt fission distortions (CFD) in the colon of Sprague-Dawley (SD) rats treated with the colonotropic carcinogens 1,2 dimethyl hydrazine (DMH) having carcinomas [48]. More recently we investigated whether CFD also occurred in the colonic mucosa of DMH-treated SD rats without carcinoma [49]. For this purpose, filed Swiss-roll sections from 35 male rats (25 treated with DMH suspended in EDTA solution, and 10 EDTA-treated) were reviewed [49]. CFD were regarded those with either asymmetric basal fission, asymmetric lateral sprouting/lateral fission, basal dilatations, or spatial aberrations of the normal (vertical) axis. A total of 202 CFD (38%) were recorded amongst 533 crypts with fission in DMH-treated rats. In EDTA-treated rats only one CFD (0.1%) was found amongst the 571 crypts with fission ($p<0.05$).The development of CFD in non-dysplatic crypts in carcinogen-treated rats probably reflected the first histologic event in experimental colonic carcinogenesis [49].

Three pathways of colonic carcinogesesis in DMH-treated Sprague-Dawley rats

i) The conventional (tubular or villous) adenoma-carcinoma pathway

Until recently it was widely recognized that the administration of colonotropic carcinogens to rodents induced conventional (tubular or villous) adenomas that eventually progressed to conventional carcinomas. Several histological classifications have been proposed to address the adenoma-carcinoma pathway in rodents. Working with AOM-treated rats van Kouwen*et al*. [50] classified colonic tumours into tubular, tubulovillous and villous adenomas or carcinomas. Peršeand Cerar [51] also classified colonic tumours in DMH/AOM-treated rats into tubular, villous, or tubulovillous adenomas or carcinomas. Adenocarcinomas were classified into moderately differentiated (tubular, tubulovillous, or villous), poorly differentiated, mucinous, signet-ring cell, and undifferentiated. Summarizing a Consensus Report and Recommendations of the pathology of mouse models of intestinal cancer, Boivin *et al*. [52] classified adenomas into tubular, villous, or tubulovillous, and adenocarcinomas into well differentiated, moderately differentiated, or poorly differentiated. The histologic carcinoma phenotypes were: tubular/tubulovillous/villous carcinoma; mucinous carcinoma, signet-ring

cell carcinoma and undifferentiated carcinoma. Ten years after, Washington *et al.* [53] published a Progress Report and Recommendations of Boivin*et al*. [52] original paper. Based on the new knowledge regarding the serrated pathway of CR carcinogenesis in humans, Washington *et al.* wrote: "The morphologic characteristics of serrated architecture have not been clearly defined in animal models, and the panel agreed that none of the models reviewed developed neoplasms that were morphologically similar to human serrated intestinal neoplasms" [53]. In a more recent review, Ward *et al.* [54] postulated that adenomas in rodents often develop stalks and intestinal adenocarcinomas often develop *de novo* from flat lesions and not from adenomas. Adenomas were not histologically classified. On the other hand, adenocarcinomas were subdivided into scirrhous, tubular, papillary, tubular–papillary, mucinous, signet ring, solid, undifferentiated, and mixed types [54]. Zalatnai *et al.* [55] classified colonic tumours in AOM-treated rats into adenomas with severe dysplasia and adenocarcinomas. Finally, Mełeń-Mucha and Niewiandomska [56] divided adenomas into three groups: adenoma with mild, moderate, and severe dysplasia and adenocarcinomas into well, moderately, poorly differentiated, and signet-ring cell carcinomas. Hence, despite disparate classifications of colonic adenomas and carcinomas in carcinogen-treated rodents, the general view has been that colonic carcinomas evolve via the conventional adenoma (tubular or villous) carcinoma pathway.

ii) The serrated adenoma-carcinoma pathway in SD rats

In a re-evaluation of archival sections from early experiments [57], we found that out of the 215 colonic neoplasias evolving in SD rats injected with DMH for 27 weeks, 11% were conventional (tubular/villous) adenomas, 9% were TSA, 3% serrated carcinomas, 3% microtubular carcinomas, 39% tubular carcinomas, 21% GALT carcinomas, 17% signet-ring cell carcinomas, and 1% villous carcinomas. Obviously, the DMH treatment in SD rats prompt not only conventional adenomas and conventional carcinomas, but also serrated adenomas and serrated carcinomas [58, 59]. Importantly, the conventional (tubular or villous) adenoma-carcinoma pathway and the serrated adenoma-carcinoma pathway in SD rats evolved in the vast majority of the large bowel mucosalined with goblet cells and columnar cells.

iii) The third pathway of colonic carcinogenesis in SD rats

Years ago, Deasy*et al.* [60] and subsequently Martin *et al.* [61] and Rubio *et al.*[62] reported that 64%, 73% and 62%, respectively of the colonic carcinomas in DMH-treated rats, evolved in lymphoid aggregates. In another experiment with DMH-treated SD rats we found that 37% of the colonic neoplasias had a subjacent organized lymphoid nodule [63]. In a more recent review of colonic GALT domains in archived sections from 276 DMH-treated SD rats, we found in the crypts covering those domains, dysplastic crypts in 49%, adenomas in 7%, and GALT-carcinomas in 53% [57]. Thus, crypt dysplasia, adenomas and carcinomas often evolve from the mucosa covering GALT domains in DMH-treated SD rats. Histology of the

146 colonic GALT-carcinomas revealed highly differentiated carcinoma in 75%, signet-ring cell carcinoma in 20%, mucinous carcinomas in 3% and mixed phenotypes in the remaining 2%.

Highly differentiated carcinomas were seen to evolve from dysplastic crypts with asymmetric bifurcations and from adenomas. Signet-ring cell carcinomas apparently evolved from goblet cells with marked anisocytosis. The results obtained were not influenced by the age or the gender of the rats, since at the time of initiating the DMH treatment, all rats were approximately of the same age and only male rats were used in all experiments [58].

Whereas molecular studies of the pathway in GALT-carcinogenesis in humans are restricted by the rarity of these tumours [22,41], GALT-carcinomas frequently develop in DMH treated SD rats [57]. This animal model might permit to further investigate the possible molecular signals required for the development of GALT-carcinomas in rats having synchronously experimentally-induced ulcerative colitis [64,65].

A rat model without colonic GALT carcinomas

Early experiments demonstrated that extracts of scorched broiled fish and meat contained highly mutagenic heterocyclic amines [66]. Accordingly, pyrrolate from scorched amino acids and proteins were given to rodents to study potential carcinogenesis [67]. The results showed that the oral administration of 2-amino-6-methyldipyrido[1,2-a:3’,3’-d] imidazole (GLU-1) isolated from a glutamic acid pyrrolate, induced tumors in the large and small intestine, liver, ear duct and clitoral gland of F344 rats [67]. In a review of sections from 53 colonic neoplasias evolving in 101 Fisher-344 (F-344) rats fed with GLU-1 for 24 months, we found that 85% were conventional adenomas, 23% serrated adenomas, and 15% highly differentiated carcinomas (58, 68). GALT carcinomas did not occur in GLU1-treated F-344 rats. Conversely Shamsuddin and Hogan [69] found dysplastic crypts and carcinomas associated with lymphoid aggregates in Fisher-344 rats when challenged with AOM. Puzzlingly, GALT carcinomas are not induced by GLU1 in F344 rats, but by DMH and AOM, underpinning the significance of the chemical composition of the carcinogen administered in the development of GALT-carcinomas in rats.

6. Conclusions

In humans, the vast majority of the sporadic and ulcerative colitis (UC)-carcinomas evolve in the huge colorectal mucosal domain built with goblet and columnar cells, via the conventional (tubular or villous) adenoma-carcinoma pathway or the serrated adenoma-carcinoma pathway. In the remnant, tiny gut-associated-lymphoid-tissue (GALT)-mucosal domain, carcinoma develop via the dysplasia- conventional adenoma-GALT-carcinoma pathway (referred to as the third pathway of colorectal carcinogenesis). UC-CRC might also be preceded by dysplasia in flat mucosa, dysplasia in subtle villous changes or without precursor lesions

(*de novo*-carcinoma). In UC-colectomies, 92% of the crypts exhibiting HGD had architectural distortions, substantiating recent reports claiming that p53 overexpression (a biomarker of epithelial carcinogenesis) significantly correlated with architectural crypt distortions in UC. In carcinogen-treated SD rats, the three pathways of colonic carcinogenesis were recreated; GALT-carcinomas were very common. In contrast, reported GALT-carcinomas in humans are rare. The main risk factors for human CRC development include advanced age, family history, male sex, lifestyle factors. natural exposures to dietary/environmental factors, genome differences, obesity, type 2 diabetes, and the colonic microbiome. In SD rats, dimethylhydrazine (DMH)-treatment was the single most important risk factor for the development of the three pathways of colonic carcinogenesis.In this survey, we shed some light on the significance of the histological build-up of the large bowel mucosa in the histogenesis of CRC. This knowledge might stimulate further studies focused on the dependency of the histological structure of the colonic mucosa in the evolution of the three pathways colonic carcinogenesis in humans and in laboratory animals.

7. References

1. Helander H F, Fändrik L. Surface area of the digestive tract – revisited. Scand J Gastroenterol 2014; 49: 681-689.

2. Boman BM, Fields JZ. An APC:WNT Counter-Current-Like Mechanism Regulates Cell Division Along the Human Colonic Crypt Axis: A Mechanism That Explains How APC Mutations Induce Proliferative Abnormalities That Drive Colon Cancer Development. Front Oncol 2013; 3: 244-255.

3. Dahl J, Greenson J. Colon. In Histology for Pathologists Ed. Stacey E Mills. Lippincott Williams & Wilkins 2007, 3rd. edition, pp627-648.

4. Rubio CA. Colorectal Carcinogenesis from Gut-Associated Lymphoid Tissue, Clinical and Experimental Documentation. Brit J Med Med Res 2017; 21: 1-12.

5. Wang CY, Liu S, Xie XN, et al. Regulation profile of the intestinal peptide transporter 1 (PepT1).Drug Des Devel Ther. 2017; 11: 3511-3517.

6. Rubio CA, Befrits R, Jaramillo E, et al. Mechanism of diarrhea in collagenous colitis. Gastroenterology 2003; 124: 2000-2001.

7. Liebler EM, Pohlenz JF, Woode GN. Gut-associated lymphoid tissue in the large intestine of calves. I. Distribution and histology.Vet Pathol 1988; 25: 503-508.

8. Elmore S. Enhanced histopathology of mucosa-associated lymphoid tissue. Toxicol Pathol 2006; 34: 687–696.

9. Butler, JE, Sinkora M. The enigma of the lower gut-associated lymphoid tissue (GALT). J LeukocBiol 2013; 94: 259–270.

10. Autenrieth IB, Firsching R. Penetration of M cells and destruction of Peyer's patches by Yersinia enterocolitica: an ultrastructural and histological study. J Med Microbiol 1996; 44: 285-294.

11.Rubio CA, Ásmundsson J, Silva P, et al. Lymphoid aggregates in Crohn's colitis and mucosal immunity.VirchowsArch 2013; 463: 637-642.

12. He K, Marsland Iii R, Upadhyayula S, et al. Dynamics of phosphoinositide conversion in clathrin-mediated endocytic traffic.Nature 2017; Dec 13.

13. Ferlay J, Soerjomataram I, Ervik M, et al.GLOBOCAN 2012 V1.0, Cancer Incidence and Mortality Worldwide: IARC Cancerbase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer, 2013.

14. Rabeneck L, Horton S, Zauber AG, et al. Colorectal Cancer In: Gelband H, Jha P, Sankaranarayanan R, Horton S, editors. Cancer: Disease Control Priorities, Third Edition (Volume 3). Washington (DC): The International Bank for Reconstruction and Development. The World Bank; 2015 Nov 1. Chapter 6, pp 344-366.

15. Jackman RJ, Mayo CW. The adenoma-carcinomasequence in cancer of the colon. Surg Gynecol Obstet. 1951; 93: 327-330.

16. Longacre TA, Fenoglio-Preiser CM. Mixed hyperplastic adenomatous polyps/serrated adenomas. A distinct form of colorectal neoplasia.Am J SurgPathol 1990;14: 524-537.

17. Rubio CA, Stemme S, Jaramillo E, et al. Hyperplastic polyposis coli syndrome and colorectal carcinoma. Endoscopy 2006; 38: 266-270.

18. O'Brien MJ, Gibbons D. The adenoma–carcinoma sequence in colorectal neoplasia. Surg Oncol Clin N Am 1996;5: 513-530.

19. Torlakovic EE, Gomez JD, Driman DK et al. Sessile serrated adenoma (SSA) vs. traditional serrated adenoma (TSA). Am J Surg Pathol. 2008; 32: 21-29.

20. O'Brien MJ, Zhao Q, Yang S. Colorectal serrated pathway cancers and precursors. Histopathology. 2015; 66:49–65.

21. Ball CB: The Erasmus Wilson Lectures on adenoma and adenocarcinoma of the rectum. Br Med J 1. 1903; i: 413-416.

22. Rubio CA, Puppa G, de Petris G, et al.The third pathway of colorectal carcinogenesis. J Clin Pathol. 2018; 71: 7-11.

23. Rubio CA, Kis L, Schmidt PT. Systematic classification of colonic crypts with architectural distortions in ulcerative colitis. ARC J Cancer Science. 2017; 3:13-20.

24. Allen DC, Hamilton PW, Watt PC, et al.Architectural morphometry in ulcerative colitis with dysplasia. Histopathology 1988; 12: 611-621.

25. Hamilton PW, Bartels PH, Thompson D, et al. Automated location of dysplastic field in colorectal histology using image texture analysis. J Pathol 1997; 182:68–75.

26. Ficsor L, Varga VS, Tagscherer A, et al. Automated classification of inflammation in colon histological sections based on digital microscopy and advanced image analysis. Cytometry A. 2008; 73: 230-237.

27. Popp C, Luciana L, Voiosu T, et al. Expression Profile of p53 and p21 in Large Bowel Mucosa as Biomarkers of Inflammatory-Related Carcinogenesis in Ulcerative Colitis. Disease Markers 2016; 2016: 3625279.

28. Rubio CA, May I, Slezak P. Ulcerative colitis in protracted remission. A quantitative scanning electron microscopic study.Dis Colon Rectum. 1988; 31: 939-944.

29. Rubio CA. Putative Stem Cells in Mucosas of the Esophago-Gastrointestinal Tract. In: Stem Cells. Regenerative Medicine and Cancer. Ed. Shree Ram Singh, Nova Science Publishers, Inc, 2010, pp: 279-308

30. Harris MP, Williamson S, Fallon JF, et al. Molecular evidence for an activator-inhibitor mechanism in development of embryonic feather branching Proc Natl Acad Sci U S A. 2005; 102: 11734-11739.

31. Phelep A, Laouari D, Bharti K, et al. MITF - A controls branching morphogenesis and nephron endowment. PLoS Genet. 2017; 13: e1007093.

32. Counsell PB, Dukes CE.The association of chronic ulcerative colitis and carcinoma of the rectum and colon. Br J Surg. 1952; 39: 485-495.

33. Jess T, Rungoe C, Peyrin-Biroulet L. Risk of colorectal cancer in patients with ulcerative colitis: a meta-analysis of population-based cohort studies. Clin Gastroenterol Hepatol. 2012; 10: 639–645.

34. Eaden JA, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. Gut. 2001;48: 526–535.

35. Lennard-Jones JE, Melville DM, Morson BC, et al. Precancer and cancer in extensive ulcerative colitis: findings among 401 patients over 22 years. Gut. 1990; 31: 800-806

36. Torres C, Antonioli D, Odze RD. Polypoid dysplasia and adenomas in inflammatory bowel disease: a clinical, pathologic, and follow-up study of 89 polyps from 59 patients. Am J Surg Pathol. 1998; 22: 275-284.

37. Rubio CA, Befrits R, Jaramillo E, et al.Villous and serrated adenomatous growth bordering carcinomas in inflammatory bowel disease. Anticancer Res. 2000; 20: 4761-4764.

38. Rubio CA. Serrated neoplasias and de novo carcinomas in ulcerative colitis: a histological study in colectomy specimens. J Gastroenterol Hepatol. 2007; 22: 1024-1031.

39. Lee LH, Iacucci M, Fort Gasia M, et al.Prevalence and Anatomic Distribution of Serrated and Adenomatous Lesions in Patients with Inflammatory Bowel Disease. Can J Gastroenterol Hepatol 2017; 2017: 5490803.

40. Hamamoto N, Rubio CA, Befrits R, et al. Magnifying endoscopy in upper and lower digestive tract. Subtle villous changes detected at endoscopy in patients with inflammatory bowel disease. Digestive Endoscopy 2005; 17 (Suppl.): S34–S39.

41. Rubio CA, De Petris G, Puppa G. Gut-associated lymphoid tissue (GALT) carcinoma in ulcerative colitis. Antica Res. 2018; 38: 919-921.

42. Hornick JL, Farraye FA, Odze RD. Clinicopathologic and immunohistochemical study of small apparently “de novo” colorectal adenocarcinomas. Am J SurgPathol 2007; 31: 207-215.

43. Dukes C E.The surgical pathology of ulcerative colitis.Ann R Coll Surg Engl 1954; 14: 389-400.

44. Rubio CA. Ectopic colonic mucosa in ulcerative colitis and in Crohn’s disease of the colon. Dis Colon Rectum 1984; 27: 182-186.

45. Laqueur GL, Mickelsen O, Whiting MG et al. Carcinogenic properties of nuts from CycasCircinalis l. indigenous to Guam J Natl Cancer Inst. 1963; 3: 919-951.

46. Laqueur GL.The induction of intestinal neoplasms in rats with the glycoside cycasin and its aglycone.Virchows Arch Pathol Anat Physiol Klin Med. 1965; 340: 151-163.

47. Druckrey H, Preussmann R, Matzkies F et al. Selective production of intestinal cancer in rats by 1,2-dimethylhydrazine. Naturwissenschaften. 1967; 54: 285-286.

48. Rubio CA. Corrupted colonic crypt fission in carcinogen-treated rats. PLoS One 2017; 12: e0172824.

49. Rubio CA. Are Corrupted Non-dysplastic Colonic Crypts the First Histological Event in Experimental Colonic Carcinogenesis? Anticancer Res. 2017; 37: 2265-2268.

50. van Kouwen MC, Laverman P, Hanm J, et al. Noninvasive monitoring of colonic carcinogenesis: feasibility of [18F] FDG-PET in the azoxymethane model. Nucl Med Biol. 2006; 33: 245–248.

51. Perše M,Cerar A.Morphological and molecular alterations in 1,2 dimethylhydrazine and azoxymethane induced

colon carcinogenesis in rats. J Biomed Biotechnol. 2011; 473964.

52. Boivin GP, Washington K, Yang K. et al. Pathology of mouse models of intestinal cancer: consensus report and recommendations.Gastroenterology. 2003; 124: 762-777.

53. Washington MK, Powell AE, Sullivan R. et al. Pathology of rodent models of intestinal cancer: progress report and recommendations. Gastroenterology. 2013; 144: 705-17;.

54. Ward JM, Treuting PM. Rodent intestinal epithelial carcinogenesis: pathology and preclinical models.ToxicolPathol 2014; 42: 148-161.

55. Zalatnai A, Lapis K, Szende B. et al. Wheatgerm extract inhibits experimental colon carcinogenesis in F-344 rats. Carcinogenesis. 2001; 22: 1649-1652.

56. Mełeń-Mucha G, Niewiadomska H. Frequency of proliferation, apoptosis, and their ratio during rat colon carcinogenesis and their characteristic pattern in the dimethylhydrazine-induced colon adenoma and carcinoma. Cancer Invest. 2002; 20: 700-712.

57. Rubio CA. Three Pathways of Colonic Carcinogenesis in Rats. Anticancer Res. 2017; 37: 15-20.

58. Rubio CA. Traditional serrated adenomas and serrated carcinomas in carcinogen-treated rats. J Clin Pathol 2017; 70:301-307.

59. Deasy JM, Steele G Jr, Ross DS et al. Gut-associated lymphoid tissue and dimethylhydrazine-induced colorectal carcinoma in the Wistar/Furth rat. J Surg Oncol.1983; 124: 36-40.

60. Rubio CA. Updated Histologic Classification of Adenomas and Carcinomas in the Colon of Carcinogen-treated Sprague-Dawley Rats.Anticancer Res 2017; 37: 6667-6670.

61. Martin MS, Hammann A, Martin F. Gut-associated lymphoid tissue and 1,2-dimethylhydrazine intestinal tumors in the rat: an histological and immunoenzymatic study Int J Cancer. 1986; 38: 75-80.

62. Rubio CA. Lymphoid tissue-associated colonic adenocarcinomas in rats. *In Vivo*. 1987; 1: 61-64.

63. Rubio CA, Shetye J, Jaramillo E. Non-polypoid adenomas of the colon are associated with subjacent lymphoid nodules. An experimental study in rats. Scand J Gastroenterol. 1999; 34: 504-508.

64. YangY, ZhuX, QinY, et al. The Anti-inflammatory effect of guchangzhixie-pill by reducing colonic EC cell hyperplasia and serotonin availability in an ulcerative colitis rat model. Evid Based Complement Alternat Med 2017; 2017: 8547257.

65. Suluvoy JK, Sakthivel KM, Guruvayoorappan C et al. Protective effect of AverrhoabilimbiL. fruit extract on ulcerative colitis in Wistar rats via regulation of inflammatory mediators and cytokines. Biomed Pharmacother. 2017; 91: 1113-1121.

66. Masuda M, Takayama S. Intestinal tumors in rats induced by mutagens from glutamic acid pyrolysate. Exp Pathol 1984; 26: 123-129.

67. Takayama S, Masuda M, Mogami Met al. Induction of cancers in the intestine, liver and various other organs of rats by feeding mutagens from glutamic acid pyrolysate. Gan 1984; 75: 207-213.

68. Rubio CA, Takayama S. Difference in histology and size in colonic tumors of rats receiving two different carcinogens. J Environ Pathol Toxicol Oncol 1994; 13:191-197.

69. Shamsuddin AM, Hogan ML.Large intestinal carcinogenesis. II. Histogenesis and unusual features of low-dose azoxymethane-induced carcinomas in F344 rats. J Natl Cancer Inst 1984; 73:1297-305.