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Colorectal Carcinogenesis from Gut-associated Lymphoid Tissue Clinical and Experimental Documentation

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Author's contribution

The sole author reviewed archived sections from previous experiments, compiled the data and wrote the manuscript.

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Review Article

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ABSTRACT

Aims: The conventional (tubular or villous) adenoma-carcinoma pathway and the serrated adenoma-carcinoma pathway evolve in the vast colorectal mucosa built with crypts lined with mucus producing goblet cells and columnar cells. In contrast, few carcinomas developing in the tiny, spotty gut-associated-lymphoid-tissue (GALT) mucosal domain have been reported.
Place and Duration of Study: Department of Pathology, Karolinska Institute and University Hospital, Stockholm, Sweden. The experiments in rats were carried out during four years.
Methodology: All publications on human colorectal GALT-carcinomas were reviewed. Archival sections from previous experiments in carcinogen-treated rats exhibiting colonic GALT follicles, were re-evaluated.
Results: Only 21 GALT-carcinomas found in 18 patients are in record. Four had ulcerative colitis, two were members of a Lynch syndrome family, two of a CRC family, one of a FAP family, two abdominal pain, two rectal bleedings, one diverticular disease, one a submucosal rectal tumor, one protracted constipation, and two had no symptoms or ground diseases. Conversely, 53% of 276 carcinogen-treated rats had developed GALT-carcinomas.

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Conclusions: It is generally recognized that the vast majority of the CRCs in humans evolve via the conventional adenoma-carcinoma pathway or the serrated adenoma-carcinoma pathway in the GALT-free colorectal mucosal domain. Less frequently CRCs in humans develop in the tiny, spotty GALT mucosal domain. Whereas natural exposures to dietary/environmental factors, genome differences, obesity, type 2 diabetes, and the colonic microbiome are important for the development of CRC in the GALT-free colorectal mucosa in humans, no factors have been advanced to explain the development of carcinomas in colorectal GALT domains in humans. On the other hand, more than 50% of the SD rats injected with DMH developed colonic GALT-carcinomas. Although the cause(s) for the difference in frequency of GALT carcinomas in the two species remains mute, the results strongly suggest that the carcinogen DMH was the most important single factor for the induction of colonic GALT carcinoma in SD male rats. More research is necessary to unveil the factor(s) responsible for the development of GALT carcinomas in the human colorectal mucosa.

Keywords: Pathways; carcinogenesis; crypts; dysplasia; adenomas; gut-associated-lymphoid-tissue (GALT) carcinomas.

1. INTODUCTION

Colorectal cancer (CRC) is one of the more intensively studied human malignancies. CRC is 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. Main risk includes advanced age, family history, male sex, lifestyle and environmental factors [1,2].

2. THE HISTOLOGY OF THE COLORECTAL MUCOSA AND CRC

2.1 The Vast Majority of the Colorectal Mucosa is Built with Cypts Lined with Goblet Cells and Columnar Cells

According to Helander and Fändrik, the mean total mucosal surface of the human digestive tract averages ~32 m2, of which about 2 m2 corresponds to that of the large intestine [3]. The colorectal mucosa may be divided into two quantitatively and functionally different domains [4]. One domain, built with crypts lined with mucus producing goblet cells and columnar cells, occupies the vast majority of the organ. The function of this huge mucosal domain is to protect underlying structures, to absorb vitamins and nutrients, to lubricate the faeces by virtue of its mucous production and to absorb fluids. Fluids are absorbed thanks to Aquaporin 8 [5], a specific water-selective channel protein that regulates water absorption in the human colon.

2.2 A Tiny Fraction of the Colorectal Mucosa Contains Gut-associated Lymphoid-Tissue

The other mucosal domain, built with tiny organized lymphoid aggregates (lymphoid follicles), it is being referred to as gut-associated lymphoid tissue (GALT) [6-8]. Present in the anti-mesenteric border of the colon, the lymphoid aggregates are typically found in the mucosa and submucosa. The epithelium covering GALT domain exhibits cuboidal cells, few or no goblet cells, and scattered M cells (so-called because of broad invaginations or microfolds) [9]. The frequency of the GALT aggregates increases from the proximal to the distal colon, being highest in the rectum. Nasciembeni et al. found in colectomies from patients with Crohn's disease a mean of 0.3 lymphoid aggregates/ cm2, and in the mucosa of patients with colorectal cancer a mean of 2.7 lymphoid aggregates/cm2 [10]. The function of this spotty and tiny mucosal domain is to absorb -via M cells-, luminal antigens, macromolecules and microorganisms by clathrin-mediated endocytosis [11]. Luminal antigens, macromolecules and microorganisms are subsequently hauled into antigen-presenting cells (macrophages, B cells and dendritic 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.

2.3 The Colonic GALT Domain is Part of an Organized GALT System

The organized GALT system is widespread, extending from the Waldeyer´s ring (tonsils), the esophagus, the stomach, Peyer´s patches (ileum), and the appendix, down to the colon [8]. 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 "colonic GALT follicles", to differentiate them, among others, from GALT follicles in the distal small intestine (Peyer´s patches), or from newly formed colonic lymphoid aggregates in patients with Crohn´s colitis [12].

3. COLORECTAL CARCINOMAS IN HUMANS

3.1 The Vast Majority of the CRC Evolve in the Colorectal Mucosa Free from Organized Lymphoid Tissue

The majority of the CRC evolve through the conventional (tubular/villous) adenoma-carcinoma pathway [13] or the serrated adenoma-carcinoma pathway [14,15]. It has been estimated that in the general population about 30% of the CRC progress via the serrated pathway [15].

3.2 Fewer CRC Evolve in Colorectal GALT Domains

In 1954 Cuthbert Dukes described in patients with ulcerative colitis a histological lesion in the submucosa characterized by "misplaced" colonic epithelium [16]. 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 [16]. In 1984 we studied the frequency of misplaced (i.e. ectopic) colonic mucosa in 62 colectomy specimens [17]. 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 surrounded by nodular lymphoid tissue invading the submucosa. The presence of this tumour fulfilled the criteria of colonic GALT-carcinoma [17].

3.3 Colonic GALT Carcinomas in the Literature

So far, only 21 GALT carcinomas have been found in 18 patients [4,17-30]. The clinical data, the descriptive and histological characteristics in the 21 GALT carcinomas reported are condensed in Table 1.

3.4 Age

Table 1 shows that the mean age in the 18 patients with GALT carcinomas was 64 years (range 86-44).

3.5 Gender

Ten of the 18 patients with GALT carcinoma were males and the remaining eight females.

3.6 Localization

Of the 21 GALT carcinomas, 12 were located in the right colon (five of these in the cecum), three in the transverse colon, four in the left colon (three of these in the sigmoid colon) and the remaining two in the rectum.

3.7 Ground Disease/Symptoms

Table 1 shows that four patients had ulcerative colitis, two were members of a Lynch syndrome family, one of a FAP family, two of a CRC family, one had diverticular disease, two had abdominal pain, two rectal bleedings, one had a submucosal rectal tumor, one complained of constipation, and in the remaining two, no symptoms or ground diseases were reported.

3.8 Histological Characteristics

Out of 21 GALT carcinomas in Table 1, eight disclosed adenomas on their luminal surface, two had high-grade dysplasia, and one, undifferentiated malignant cells in the ulcerated surface. In the remaining 10 GALT-carcinomas the luminal surface was reported either ulcerated (n=2), with chronic mucosal inflammation (n=1), with normal mucosa (n=4) (Fig. 1b) or it was not given (n=3).

Table 1. Clinical data, descriptive characteristics and clinical data in 21 GALT-carcinomas found in 18 patients reported in the literature

Year/ref.	Senior author	No. GALT ca	Localization	Gross description of the surface	Histology at the surface	Ground disease or symptoms	Age/Gender
1984 [17]	Rubio	One	Left colon	Irregular plaque -like lesion	Chronic inflamation	UC	57 M
1999 [18]	De Petris	Two	Cecum	Dome	Not given	Lynch syndrome	44 M
			Cecum	Ulcerated	Undifferentiated malignant cells		
2000 [19]	Jass	One	Cecum	Dome	Ulcerated	FAP	56 M
2002 [20]	Clouston	Two	Sigmoid	Polypoid lesion	HGD	None	63 F
	Case 1						
	Case 2		Sigmoid	Polypoid lesion	Not given	None	56 M
2002 [21]	Rubio	One	Right colon	Sessile polyp	Adenoma HGD	UC	53 F
2008 [22]	Stewart	Two	Right colon	Polypoid lesion	Dysplasia	UC	70 M
	Case 1						
	Case 2		Transvers. colon	Plaque-like lesion	Not given	Diverticular disease	63 F
2008 [23]	Asmussen	Two	Sigmoid	Flat polypoid lesion	Adenoma HGD	Rectal bleeding	76 F
	Case 1						
	Case 2		Rectum	Plaque-like lesion	Adenoma with invasion	Rectal bleeding	86 F
2010 [24]	Rubio	Three	Right	Dome	Normal mucosa	Lynch syndrome	53 F
			Right colon	Plaque-like	Normal mucosa		
			Right colon	Histological finding	Normal mucosa		
2012 [25]	Coyne	One	Cecum	Irregular sessile polyp	Ulcerated	CRC family	76 M
2012 [26]	Puppa	One	Right colon	Raised plaque	Adenoma HGD	Constipation	56 M
2012 [27]	Yamada	One	Transvers.	Polypoid lesion	Adenoma HGD	Abdominal pain	77 M
2013 [28]	Rubio	One	Transvers.	Polypoid lesion	Adenoma HGD	UC	68 F
2013 [29]	Yamada	One	Rectum	Sessile elevated lesion	Normal mucosa	Rectal submucosal tumor (SMT)	76 F
2015 [30]	Kannuna	One	Cecum	Sessile mass	Adenoma HGD	Abdominal pain/ fever	57 M
2016 [4]	Rubio	One	Right	Irregular polyp	Adenoma HGD	CRC family	65 M

3.9 GALT Carcinoma or Dome Carcinoma?

Based on the presence of a circumscribed elevation in the colorectal mucosa at gross or endoscopic examination, GALT-carcinomas were referred to as dome carcinomas (DC) [18,19,24]. We recently examined the narratives of the gross or endoscopic architecture of the luminal aspect and the pathological reports in the 21 GALT-carcinomas [4]. Only three (14%) fulfilled the gross criteria of dome carcinoma (Table 1); 9 were described as polypoid lesions (Fig. 1a), 5 as plaque-like lesions, 2 as sessile elevated lesions or mass, one as ulcerated and one was a histological finding. Hence, the majority, 86% (n= 18) of the GALT- carcinomas exhibited structures other than dome shapes at gross examination. In light of these results it was concluded that by using the term "dome" in addressing carcinomas in the colorectal mucosa, many cases of GALT carcinomas might be overlooked. Another drawback of using the "dome" nomenclature is that dome-like outlines may be detected in small metastatic tumors in the submucosa or in small colorectal carcinomas arising in non-GALT mucosa. On the other hand, using the histologic diagnosis in addressing these neoplasias, all cases of GALT-carcinoma will be included [4].

3.10 Immunohistochemistry

In one case with Lynch syndrome [24] extra sections were immunohistochemically challenged with MLH1 (BD Biosciences, San Diego, USA), MSH2, MUC1, MUC2, Actin-α SM (Leica Microsystems AB, Bromma, Sweden), Ki-67 (clone MIB1), MNF 116, laminin 5 (Dako Cytomation, Glostrup, Denmark), M30 (Peviva, Bromma, Sweden), p53 (BD Products, Franklin Lakes, USA), p21WAF1 (Oncogene Science, Chicago, USA) and histochemically stained with Alcian blue (pH 2.5), PAS and PAS-D. Actin-α SM showed that the tumor had penetrated through the *Muscularis mucosae* (Fig. 1c), and MIB1, that the tumor was highly proliferating (Fig. 1d). The tumor expressed MUC-1 (Fig. 2a), but no MUC-2 (Fig. 2b) or MUC- 5AC. Study of the mismatch repair proteins revealed that

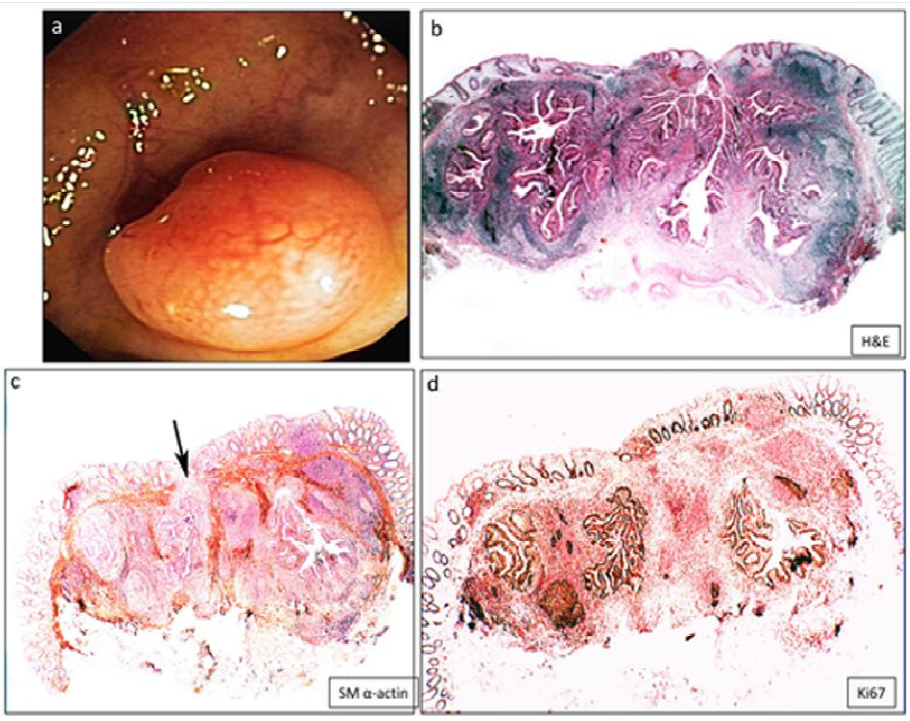

Fig. 1. a: Endoscopic image showing a well-circumscribed polypoid lesion with even surface (right colon) (Courtesy of Dr. Ragnar Befrits, Karolinska University Hospital, Stockholm). b: Histology demonstrated a GALT carcinoma (H&E ×2), c: GALT-carcinoma. Note carcinoma penetrating through the *muscularis mucosae*, at arrow (Actin α SM x2), d: GALT-carcinoma. Low power view to demonstrate high cell proliferation (Ki67, Batch MIB1, x2)

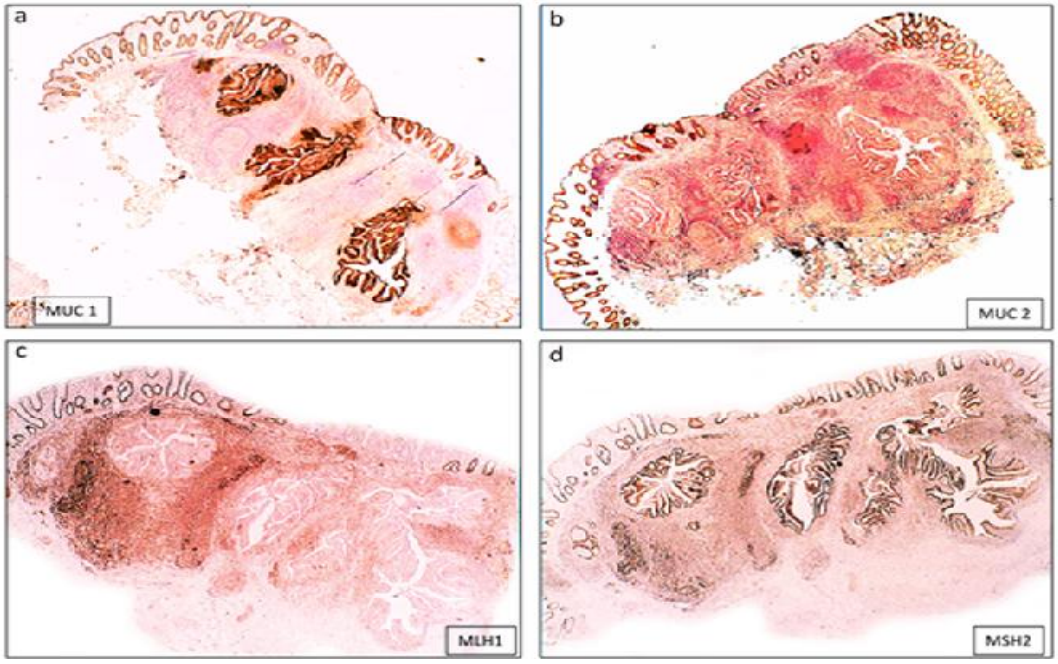

Fig. 2. a: MUC1is intensely expressed, b: MUC2 shows lack of expression, c: MLH1 was not expressed, whereas d: MSH2 was normally expressed (a to d, low power view, x2)

MLH1 was not expressed (Fig. 2c) whereas MSH2 was normally expressed (Fig. 2d). p53 was expressed in 7% of the tumor cells while staining p21WAF1 was negative. M30 revealed only minimal apoptosis. Alcian blue, PAS and PAS- D stains showed absence of sialomucins and glyco-proteins in tumor cells.

4. LESSONS FROM THE ANIMAL WORLD

4.1 A Serendipitous Discovery

While attempting to produce amyotrophic lateral sclerosis by feeding nuts of *Cycas circinalis* (a tropical fern from a family of *Cycadaceae*), Laqueur et al. [31] 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 [32]. This discovery, lead Druckey et al. [33] 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.

4.2 Further Studies in Colonic Carcinogenesis in Rodents

In later years, a vast amount of literature on colorectal neoplasias evoked by different carcinogens by genetic engineering or by spontaneous mutations in mice and rats has been published. A recent search in PUBMED (29/12/2016) using the key words “colon cancer rats” and “colon cancer mice” yielded 21976 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.

Several studies have demonstrated genome variations between different strains [34,35]. Ingested nitrate that triggers endogenous synthesis of N-nitroso compounds (NOCs) are potent carcinogens in the digestive tract [36]. ABCB1/P-glycoprotein a membrane protein encoded by ABCB1/MDR1, transports substrates from the enterocytes to the intestinal lumen, thereby restricting the exposure of the enterocytes to the substrates of the ABCB1 transporter. In animal models the ABCB1 has been implicated in intestinal carcinogenesis. Abcb1/mdr1a knock-out mice develop colitis and later intestinal adenocarcinoma, suggesting that the absence of ABCB1 confers risk of

inflammation-related CRC [36,37,38]. Masuda and Takayama [39,40] found that the oral administration of GLU1 to F-344 rats, elicited colonic tumours.

5. THREE PATHWAYS OF COLONIC CARCINOGENESIS IN RODENTS

5.1 The Conventional Adenoma-Carcinoma Pathway in Rats

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. [41] classified colonic tumours into tubular, tubulovillous and villous adenomas or carcinomas. Perse and Cerar [42] 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. [43] 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. [44] published a Progress Report and Recommendations of Boivin et al. original paper [43]. 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” [44]. In a more recent review, Ward et al. [45] 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. Zalatnai et al. [46] classified colonic tumours in AOM-treated rats into adenomas with severe dysplasia and adenocarcinomas. Finally, Mełeń-Mucha and Niewiandomska [47] 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.

5.2 The Serrated Adenoma-Carcinoma Pathway in Rats

In a recent re-evaluation of archival sections from early experiments [48], we found that out of the 215 colonic neoplasias evolving in Sprague-Dawley (SD) rats injected with 1,2 dimethylhydrazin (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. Thus, the DMH treatment in SD rats prompted not only conventional adenomas and conventional carcinomas, but also serrated adenomas and serrated carcinomas [48].

5.3 The Colonic GALT Carcinoma Pathway in Rats

Years ago, Deasy et al. [49] and Rubio et al. [50] reported that 64% and 62%, respectively of the colonic carcinomas in DMH-treated rats evolved in lymphoid aggregates. In another experiment with DMH-treated rats we found that 37% of the colonic neoplasias had a subjacent lymphoid nodule [51]. Taken together, these findings indicate that adenomas and carcinomas often evolve from the mucosa covering gut associated lymphoid tissue (GALT) in rats (Fig. 3e, f, g, and Fig. 4).

5.4 Colonic GALT Adenomas and Carcinomas in DMH-treated Rats

In a more recent review of archived sections from DMH-treated rats with colonic GALT follicles, dysplastic crypts exhibiting asymmetrical bifurcations in GALT mucosa were found in 49% and colonic GALT carcinomas in 53% of 276 DMH-treated rats [52]. 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 in the remaining 2%. Highly differentiated carcinomas were seen to evolve from dysplastic crypts with asymmetric bifurcations and from adenomas and signet-ring cell carcinomas, and from non-dysplastic crypts having goblet cells with marked anisocytosis. It is apparent that DMH-treatment in SD rats also induce GALT carcinomas [52].

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.

5.5 An Animal Model without Colonic GALT Carcinomas

An important factor in the development of colorectal cancer in humans is the lifestyle, especially dietary habits. Early experiments in rodents demonstrated that extracts of scorched broiled fish and meat contained highly mutagenic heterocyclic amines [39]. Accordingly, pyrrolate from scorched amino acids and proteins were given to rodents to study potential carcinogenesis [40]. Takayama et al. found 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 [40]. 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. GALT carcinomas did not occur in GLU1-treated F-344 rats [53]. But when Shamsuddin and Hogan challenged the same strain of rats (Fisher-344) with AOM, foci of dysplastic crypts and carcinomas were found associated with lymphoid aggregates [54]. Based on these findings it would appear that GALT carcinomas were induced by the chemical composition of the carcinogen administered (DMH/AOM) and not by the strain of rats chosen in those experiments (SD or F-344).

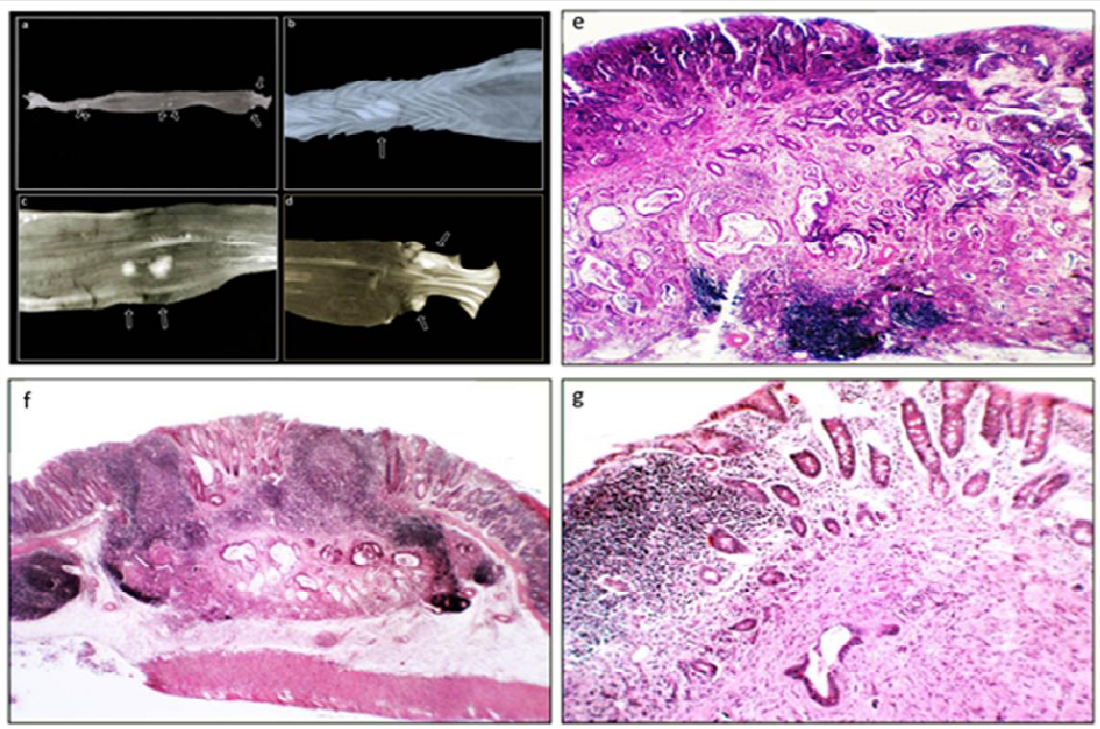

Fig. 3. Upper-left panel, a-d: Colonic GALT domains (at arrows), highlighted by trans-illumination of a colectomy specimen (adult Sprague-Dawley rat, untreated), e: GALT-carcinoma with adenoma on top (H & E, x10, Sprague-Dawley rat), f: Low-power view of a GALT carcinoma (H & E, x2, Sprague-Dawley rat), g: Detail from a GALT carcinoma invading into the submucosa surrounded by a fibrous-reaction (H & E, x10, Sprague-Dawley rat)

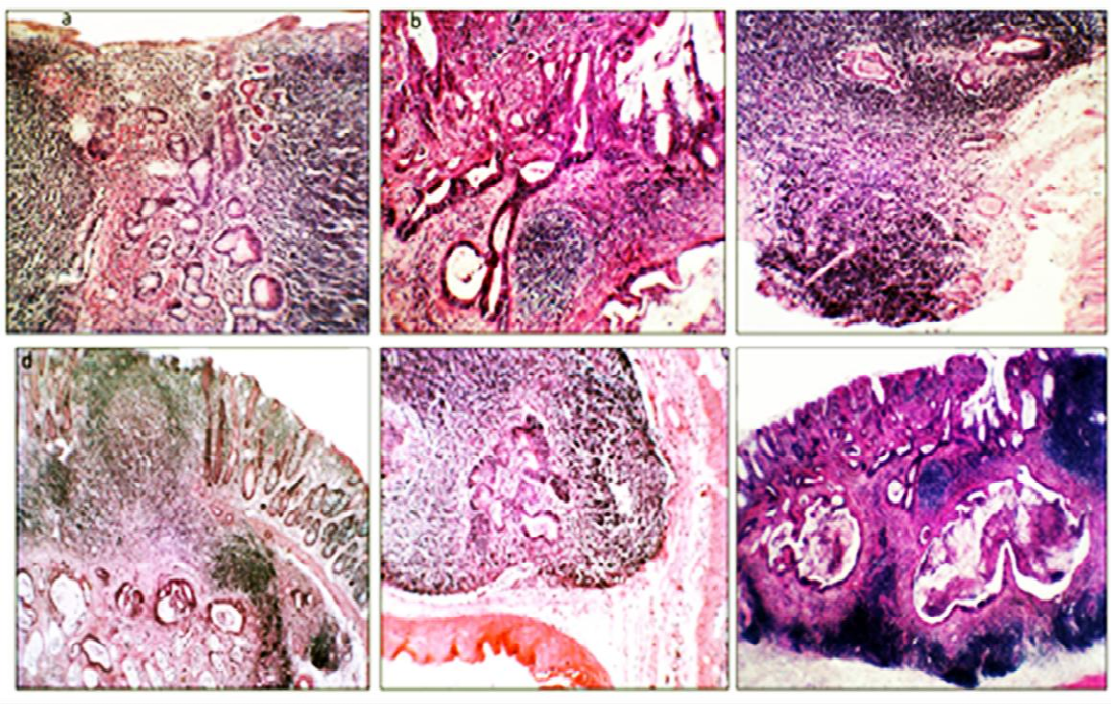

Fig. 4. Different views of GALT carcinomas (a, c, d: x10, b, e: x20, f: x4, Sprague-Dawley rats)

6. DIFFERENCES BETWEEN CRC IN HUMANS AND IN RODENTS

Several factors have been considered to explain the increased risk for cancer in humans evolving in the GALT-free colorectal mucosa. The most important are *i)* the gender (the risk being higher in males), *ii)* genetic differences (the risk being higher in patients with germline mutations in one of the DNA mismatch repair (MMR) genes, *iii)* obesity, *iv)* type 2 diabetes, *v)* differences in the intestinal microbiome and *vi)* differences in intake of dietary environmental carcinogens, such as polycyclic aromatic hydrocarbon-carcinogens produced when meats are heated above 180°C for long periods. Heterocyclic amines represent an important class of carcinogens in foods. Despite that several of the aforementioned factors are important for the development of CRC in the GALT-free colorectal mucosa in humans, no candidates have been proposed to explain the development of GALT carcinomas in the human colorectal GALT-domain. In rodents, on the other hand, the s.c. injection of DMH was the most single important factor for the induction of colonic GALT-carcinomas in male SD rats. Puzzlingly, the oral administration of GLU1 to Fisher-344 rats for 24 months induced colonic adenomas and carcinomas in the GALT-free colonic mucosa, but failed to induce GALT carcinomas.

7. CONCLUSIONS

It is generally recognized that the vast majority of the CRCs in humans evolve in the GALT-free colorectal mucosal domain via the conventional adenoma-carcinoma pathway or the serrated adenoma-carcinoma pathway. Less frequently, CRCs develop in the tiny, spotty GALT mucosal domain in humans. Whereas natural exposures to dietary/environmental factors, genome differences, obesity, type 2 diabetes, and the colonic microbiome are important for the development of CRC in the GALT-free colorectal mucosa in humans, no factors have been advanced to explain the development of carcinomas in colorectal GALT domains in humans. On the other hand, more than 50% of the SD rats injected with DMH developed colonic GALT-carcinomas. Although the cause(s) for the difference in frequency of GALT carcinomas in the two species remains mute, the results strongly suggest that the carcinogen DMH was the most important single factor for the induction of colonic GALT carcinoma in SD male rats. More research is necessary to unveil the factor(s) responsible for the development of

GALT carcinomas in the human colorectal mucosa.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was approved by the The Ethical Committee of the Karolinska Institute, Stockholm, Sweden approved the old experiments (N 48/1989).

COMPETING INTERESTS

Author has declared that no competing interests exist.

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