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Updated Histologic Classification of Adenomas and Carcinomas in the Colon of Carcinogen-treated Sprague-Dawley Rats

CARLOS A. RUBIO

Department of Pathology, Karolinska Institute and University Hospital, Stockholm, Sweden

Abstract. *Background: Recent studies have disclosed novel histological phenotypes of colon tumours in carcinogen-treated rats. The aim of this study was to update the current histological classification of colonic neoplasias in Sprague-Dawley (SD) rats. Materials and Methods: Archival sections from 398 SD rats having 408 neoplasias in previous experiments were re-evaluated. Results: Of the 408 colonic neoplasias, 11% (44/408) were adenomas without invasive growth and 89% (364/408) invasive carcinomas. Out of the 44 adenomas, 82% were conventional (tubular or villous), 14% traditional serrated (TSA; with unlocked serrations or with closed microtubules) and 5% gut-associated lymphoid tissue (GALT)-associated adenomas. Out of 364 carcinomas, 57% were conventional carcinomas, 26% GALT carcinomas, 8% undifferentiated, 6% signet-ring cell carcinomas, and 4% traditional serrated carcinomas (TSC). Thus, conventional adenomas, conventional carcinomas and GALT-associated carcinomas predominated ($p<0.05$). Conclusion: The updated classification of colonic tumours in SD rats includes conventional adenomas, TSA, GALT-associated adenomas, conventional carcinomas, TSC, GALT-associated carcinomas, signet-ring cell carcinomas and undifferentiated carcinomas. Several of the histological phenotypes reported here are not included in any of the current classifications of colonic tumours in rodents. This updated classification fulfils the requirements for an animal model of human disease, inasmuch as similar histological phenotypes of colon neoplasias have been documented in humans.*

Lorenz and Stewart were the first to induce carcinomas in the colon of rodents (1). Since then, many workers have investigated the morphological, biological and molecular characteristics of colonic adenomas and carcinomas in mice and rats evoked by dimethylhydrazine (DMH) or by its metabolites (azoxymethane and methylazoxymethanol) (2) by spontaneous mutations (3) or genetic manupulations (4).

In more recent years, several histological classifications of experimentally-induced colonic adenomas in rats have been proposed (5-13). Some researchers classify adenomas into tubular, villous and a mixed phenotype and tubulo-villous, others into adenomas with mild, moderate and severe dysplasia, and a third group regard adenomas as only those exhibiting severe dysplasia. Similarly, some authors classify invasive carcinomas into well-, moderately and poorly differentiated, and signet-ring cell carcinomas; others into tubular, mucinous, signet-ring cell and undifferentiated; and by a third group into scirrhous, tubular, papillary, tubular–papillary, mucinous, signet-ring, solid, undifferentiated, and mixed types (5-13). In contrast, Deschner (14), Maskens (15) and, more recently, Ward and Treuting (16) have asserted that adenocarcinomas often develop *de novo*, and not from adenomas, therefore, adenomas were not histologically classified.

Despite the disparate classifications of colonic neoplasias in rodents, the general view has been that colonic carcinomas evolve from conventional (tubular, tubulo-villous or villous) adenomas (5-13). However, recent studies have disclosed additional histological phenotypes of colonic adenomas and carcinomas in DMH-treated Sprague-Dawley (SD) rats (17-19) as follows.

Serrated adenomas and serrated carcinomas. Following activation of a *KrasG12D* mutant allele or inactivated *Apc* alleles, Feng *et al.* (3) found epithelium to be hyperplastic and serrated morphological features in the mouse colon, and Bongers *et al.* (4) found that transgenic expression of the epidermal growth factor receptor in conjunction with ligand heparin-binding epidermal growth factor in mice promoted caecal serrated polyps, but not serrated adenomas. Recently in 215 DMH-treated SD rats, tumours were found to be 1% serrated adenomas, 8% microtubular adenomas, 3% serrated carcinomas and 3% microtubular carcinomas (19). Thus, serrated adenomas and serrated carcinomas can be evoked in

Correspondence to: Carlos A. Rubio, MD, Ph.D., Department of Pathology, Karolinska Institute and University Hospital, 17176, Stockholm, Sweden. Tel: +46 851774527, Fax: +46 851774524, e-mail: Carlos.Rubio@ki.se

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SD rats by DMH treatment, a process referred to as the serrated adenoma–carcinoma pathway (17, 18).

Gut-associated lymphoid tissue (GALT) adenomas and GALT carcinomas. Working with DMH-treated Wistar/Furth(W/Fu) rats, Deasy *et al*. found that 64% of colonic carcinomas had originated in organized lymphoid tissue (20). A similar percentage (62%) was subsequently found in DMH-treated SD rats (21). In another experiment, it was found that 37% of the colonic neoplasias in DMH-treated SD rats had a subjacent lymphoid nodule (22). In a more recent survey of 276 DMH-treated SD rats, it was found that 53% of tumours were GALT-associated carcinomas (19). Adenomas covering GALT-associated carcinomas were found in 7%. These findings implied that GALT-associated carcinomas often evolve in SD rats, a sequence referred to as the third pathway of colonic carcinogenesis in humans (23).

Based on these new alternative pathways of colonic carcinogenesis, the aim of this communication was to update the histological classification of colonic adenomas and carcinomas in a cohort of DMH-treated SD rats.

Materials and Methods

Archival sections from early experiments in 398 Sprague-Dawley (SD), injected with DMH for 27 weeks, were re-evaluated.

Tumour definitions. Adenomas. Circumscribed lesions built with crowded, irregular crypts lined with dysplastic cells with loss of goblet-cell differentiation and a fibro-vascular core.

Traditional serrated adenomas (TSA): Adenomas exhibiting either unlocked serrated dysplastic crypts or closed dysplastic microtubules (17). Colonic dysplastic microtubules are similar to those called ectopic crypt units or ectopic crypt formations in the small intestine of transgenic mice (24).

Adenomas with invasive carcinoma: Adenomas with neoplastic glands penetrating through the *muscularis mucosae* into the submucosal layer or beyond.

Carcinomas. Microtubular carcinomas: Typified by microtubular neoplastic configurations. The descriptive term microtubular carcinoma was preferred to ectopic crypt formation carcinoma since it reflects the ostensibly microtubular neoplastic configurations. GALT-associated carcinomas: GALT mucosal domains exhibiting conventional (tubular) carcinomas or signet-ring cell carcinomas.

Undifferentiated carcinomas: Characterized by large clusters of undifferentiated cancer cells. In some tumours, single tumour cells detached from large clusters exhibit a single cytoplasmic mass containing several neoplastic nuclei, in a syncytium-like arrangement.

Mucinous carcinomas: Carcinomas with rich mucin material (>50%). Large mucin lakes were often seen partially lined by neoplastic tubular or villous neoplastic structures or included signet-ring carcinoma cells. Mucinous carcinomas were herein considered a subgroup of tubular carcinomas or signet-ring cell carcinomas inasmuch as mucin, a secretory attribute, only reflects the degree of secretion in cancer cells.

Overt carcinomas: Carcinomas without remnant adenomatous or GALT-associated tissue. Histologically they were classified into conventional carcinomas (tubular carcinomas or villous carcinomas), traditional serrated (serrated carcinomas or microtubular) carcinomas, GALT-associated carcinomas, signet-ring cell carcinomas (>50% of tumor cells with a prominent mucin vacuole, and a crescent-shaped, eccentrically placed nucleus) and undifferentiated carcinomas.

Tumour with two or more histological phenotypes. It was possible to find two or more histological phenotypes in the same tumour, but only the most conspicuous phenotype appears in the classification here proposed.

Statistical analysis. The non-parametric Mann–Whitney *U*-test was applied to compare difference between groups. Statistical significance was defined as $p<0.05$.

The Ethical Committee of the Karolinska Institute, Stockholm, Sweden approved the experiments (N 48/1989).

Results

A total of 408 neoplastic lesions found in 398 SD rats were investigated. Colonic adenomas without invasion were recorded in 10.8% (44/408) and colonic adenomas with invasive carcinoma in 29.4% (120/408) ($p<0.05$).

From Table I it can be deduced that invasion occurred in 66.4% (71/107) of the conventional (tubular/villous) adenomas, in 83.3% (30/36) of the traditional (serrated/microtubular) adenomas and in 90.5% (19/21) of the GALT-associated adenomas. The difference between invasive growth in GALT-associated/TSA adenomas in the one hand and conventional adenomas in the other was significant ($p<0.05$).

Of all neoplastic lesions, 89.2% (364/408) were carcinomas (including the 120 adenomas with invasive growth in Table I). Out of the 364 carcinomas, 62.6% (228/364) were overt carcinomas, 56.6% (206/364) conventional carcinomas, 3.8% (14/364) traditional serrated carcinomas, 25.5% (93/364) GALT-associated carcinomas, 8.2% (30/364) undifferentiated carcinomas, and 5.8% (21/364) signet-ring cell carcinomas. Of all invasive lesions, 53.6% (195/364) were tubular carcinomas, 17.3% (63/364) GALT-associated signet-ring cell carcinomas, 8.2% (30/364) GALT-associated tubular carcinomas, 8.2% (30/364) undifferentiated carcinomas, 5.8% (21/364) signet ring cell carcinomas, 3.0% (11/364) villous carcinomas, 2.5% (9/364) microtubular carcinomas, and 1.4% (5/364) serrated carcinomas. The difference in frequency between tubular carcinomas on the one hand and the remaining invasive carcinoma phenotypes in the other was significant ($p<0.05$).

Discussion

DMH treatment induced adenomas and carcinomas in the colon of SD rats. Tubular adenomas evolved more frequently. Adenomas without invasion were recorded in 10% and with invasive growth in 29% ($p<0.05$), implying that the DMH

Table I. *Histological phenotypes found in 164 colon adenomas (120 showing invasive growth) in 1,2-dimethylhydrazine-treated Sprague-Dawley rats.*

	Adenomas without invasion, n (%)	Adenomas with invasion, n (%)*
Conventional adenomas		
Tubular adenomas	34 (77.3%)	56 (46.7%)
Villous adenomas	2 (4.5%)	15 (12.5%)
Traditional serrated adenomas		
Serrated adenomas	2 (4.5%)	16 (13.3%)
Microtubular adenomas	4 (9.0%)	14 (11.7%)
GALT-associated adenomas	2 (4.5%)	19 (15.8%)
Total	44 (100%)	120 (100%)

GALT: Gut-associated lymphoid tissue. *Included together with invasive carcinomas.

Table II. *Updated histological classification of colonic adenomas and colonic carcinomas induced by 1,2 dimethylhydrazine in Sprague-Dawley rats.*

Adenomas	Carcinomas
Conventional adenoma	Conventional carcinoma
Tubular phenotype	Tubular phenotype*
Villous phenotype	Villous phenotype*
Traditional serrated adenoma	Traditional serrated carcinoma
Serrated phenotype	Serrated phenotype
Microtubular phenotype	Microtubular phenotype
GALT-associated adenoma	GALT-associated carcinoma
Tubular phenotype	Serrated phenotype
	Microtubular phenotype
	Tubular phenotype
	Signet ring cell phenotype*
	Signet-ring cell carcinoma*
	Undifferentiated carcinoma
	Syncytium-like phenotype

GALT: Gut-associated lymphoid tissue. *Carcinomas with increased mucin production.

treatment induced adenomas prone to evolve into invasive carcinomas. The adenomas with the highest proclivity to progress to invasive carcinoma were GALT-associated adenomas (91%), followed by serrated adenomas (81%), villous adenomas (88%), and microtubular adenomas (78%). When all invasive carcinomas (evolving from adenomas or overt carcinomas were considered, tubular carcinomas were by far the most frequent (52%) ($p<0.05$).

Several histological classifications of carcinogen-induced colonic adenomas and carcinomas in rats have been proposed (5-13). The present study shows that SD rats treated with the colonotropic carcinogen DMH develop supplementary histological neoplastic phenotypes such as traditional (serrated and microtubular) adenomas, GALT-associated adenomas, villous carcinomas, serrated carcinomas, microtubular carcinomas, GALT-associated tubular carcinomas and GALT-associated signet-ring cell carcinomas, in addition to the colonic tumors in rodents reported in the literature (5-13).

Only four to six GALT mucosal domains were found in colectomy specimens of SD rats (18). And yet in this survey, 27% (93/348) were found to be GALT-associated carcinomas. Thus, it is not inconceivable that in SD rats, GALT mucosal domains might have a particular 'appetite' for the colonotropic carcinogen DMH.

Notably, 24% (84/348) of the carcinomas were signet-ring cell carcinomas. Puzzlingly, primary signet-ring cell carcinomas of the colon and rectum in humans account for fewer than 1% of all colorectal carcinomas (25). The cause of the high frequency of signet-ring cell carcinomas in the DMH SD model remains unknown.

Mucinous carcinomas of the colon are included as a separate group in the current literature. However, mucin is a common secretion in adenocarcinoma cells. Large lakes of mucin are often surrounded by expanding tubular neoplastic glands or include signet-ring cells. For some obscure reason, some adenocarcinoma cells release more mucin than others. Based on these deliberations, mucinous carcinomas were regarded here as a subgroup of conventional tubular or villous carcinomas and of signet-ring cell carcinomas. In undifferentiated carcinomas, single tumour cells detaching from large clusters showed syncytial-like arrangement. In this context, Shousha *et al*. found that 10 of 45 colorectal carcinomas contained human chorionic gonadotrophin-positive tumor cells mostly at the periphery of the tumours (26). Some were arranged in syncytial clumps or columns, or singularly, thus resembling trophoblastic tissue, and Chetty *et al*. found that medullary colorectal carcinoma has a syncytial growth pattern admixed with lympho-plasmacytic infiltrate (27). Based on these deliberations, it appears safe to submit that undifferentiated colonic carcinomas in SD rats exhibiting neoplastic cells in syncytial-like arrangement in this survey cannot be classified as medullary carcinomas, inasmuch as they lack the characteristic lymphocytic infiltration.

In conclusion, the DMH SD model permits for detailed monitoring of early and advanced histological stages evolving during colonic carcinogenesis in rats. Several of the histological phenotypes reported here are not included in any of the current classifications of carcinogen-induced colon neoplasias in rodents (5-13). The purpose of studying colonic carcinogenesis is to explore whether the histological changes found in rodents are similar to those evolving during colorectal carcinogenesis in humans. Once the corresponding histological steps in rodents are clearly defined, then each histological phenotype can be tested for specific molecular markers, the final goal being to transfer that knowledge to human pathology. The histological classification proposed herein (Table II) fulfils the requirements for an animal model of human disease,

inasmuch as similar histological phenotypes of colorectal neoplasias have been documented in humans (28-30). The DMH SD model might be useful in analysing different molecular aberrations developing during the conventional adenoma–carcinoma pathway, the serrated carcinoma pathway, and the GALT-associated carcinoma pathway of colonic carcinogenesis under standard laboratory conditions.

The future challenge is to explore whether this updated classification of colonic adenomas and carcinomas in DMH-treated SD rats is applicable to other rat strains treated with different colonotropic carcinogens.

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