Protruding and Non-protruding Colon Carcinomas Originating in Gut-associated Lymphoid Tissue

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Abstract. *Background: Colon carcinomas arising in gut-associated lymphoid tissue (GALTC) are termed dome carcinomas (DC) because of their protruding phenotype. Only 8 GALTC cases have been reported in the literature. Case Report: A female patient, aged 53, having a familial pedigree of colon cancer, uterine cervix cancer and brain tumour developed a signet-ring carcinoma in the cecum and 10 years later endometrial cancer. While asymptomatic, a plaque-like protrusion in the colon was detected at surveillance colonoscopy. Histology demonstrated a protruding GALTC. The surgical specimen showed four additional carcinomas: 2 GALTC (non-protruding) and 2 carcinomas in lymphoid-free colonic mucosa (LFCMC). Conclusion: Since adenomas could not be demonstrated neither previously nor in the colectomy specimen, it is suggested that the GALTCs in this patient may have followed the GALT-carcinoma pathway.*

About half the lymphocytes of the immune system are found in mucosa-associated lymphoid tissues (MALT) (1). MALT is situated along the surfaces of all tissues, the gut-associated lymphoid tissue (GALT) being its most well known representative.

The colorectal mucosa (CRM) may be divided into two quantitatively, structurally and functionally dissimilar fractions. One fraction comprehends the vast majority of the CRM and is not associated with lymphoid tissues (LT). It will be referred to as the GALT-free fraction. It is built with mucus producing goblet cells and columnar cells with regular microvilli roofed by glycocalix, an extracellular glycoprotein. The function of this huge mucosal fraction is to protect underlying structures and to actively absorb fluids, vitamins and some nutrients. The other fraction,

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microscopically small, is LT associated; it will be referred to as the GALT fraction. This tiny fraction is covered with a single layer of cells having broad invaginations that amplify the cell surface, forming intraepithelial pockets. These microfolds give the cell its name, namely M cell (1). Occasional mucus producing goblet cells are present. The function of the M cells is to absorb luminal antigens, macromolecules and microorganisms *via* clathrin-mediated endocytosis (2) and to haul them into the underlying collection of gut-indigenous, thymus-independent LT for immediate immunological processing. The constellation M cells-LT builds a lympho-epithelial immunological cross-talk unit, a relay complex for antigen–gut recognition.

Nearly all colorectal carcinomas (CRC), the third most frequent cancer worldwide (3), evolve in the GALT-free fraction. Conversely, CRC arising in the GALT fraction are very rare. In 1999, de Petris *et al.* (4) reported the first case of colon carcinoma arising in the GALT fraction. Because of its protruding shape, these authors proposed to call this new phenotype of colon carcinoma, dome carcinoma (DC). Since then, 7 additional cases of DC have been reported in the literature (5-8) (Table I).

Recently, a case of DC was diagnosed at this hospital.

Case Report

Family history: The patient's grandfather died of colon cancer at the age of 50, her mother died of cervical cancer at age 56, as did one aunt at age 50 and a second aunt of a brain tumor when she was young. Of 6 siblings, one brother at age 41 and a sister at age 43 had died of colon cancer.

The patient was a 53-year-old female (2009). Because of her family pedigree, she underwent periodical clinical examinations. In 1992, an X-ray revealed a caecal tumour. An ileo-caecal resection was carried out and histology showed a signet-ring cancer. Ten years later (2002), an hystero-salpingo-oophorectomy was performed because of endometrial cancer.

At the beginning of 2009, being asymptomatic, a colonoscopic check-up revealed a well-circumscribed, plaque-like protrusion with even surface, 2 cm distal to the ileo-colonic
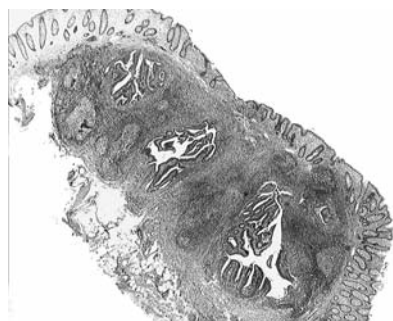
Figure 1. *Low power view of GALTC (protruding phenotype) (H&E ×2).*

Figure 2. *Lack of MLH1 immunoreactivity in GALTC (protruding phenotype, MLH1, ×2).*

Table I. *Cases of dome colorectal cancer published in the literature.*

Author (ref.)	Age (years)/ gender	Site	DC diagnosed at biopsy	DC diagnosed at colectomy	No. synchronous tumors	Family history	Invasion (layer)
De Petris *et al.* (4)	46/M	Ascending colon	No	Yes	2	Probably HNPCC	subm
Jass *et al.* (5)	56/M	Ascending colon	No	Yes, at screening for FH	None	FAP (daughter)	subm
Clouston *et al.* (6)	63/F	Case #1, Sigmoid colon	No	Yes	None	Not-stated	subm
	56/M	Case #2, Sigmoid colon	Yes	No	None	Not-stated	subm
Asmussen *et al.* (7)	76/F	Case #1, Sigmoid colon	Yes	No*	None	Not-specified	subm
	86/F	Case #2, Anorectal	Yes	No**	None	Non-specified	Muscularis propria
Stewart *et al.* (8)	76/M	Case #1, Ascending colon	Yes	No+, surveillance for UC	1	No	subm
	63/F	Case #2, Transverse colon	Yes	No++, routine endoscopy	None	No	subm
Present reported case	53/F	Ascending colon	No	Yes at surveillance for HNPCC	4	HNPCC	subm

*Tubular adenoma (HGD); **carcinoma in adenoma; +carcinoma; ++diverticulitis; subm: submucosa; M: male; F: female; HNPCC: hereditay non-polyposis colorectal carcinoma; FH: familial hypercholesterolemia; FAP: familial adenomatous polyposis.

anastomosis. The rest of the colon and rectum was apparently normal. A mucosectomy of the protruding lesion was performed. The mucosectomy specimen exhibited a centrally located plaque-like protrusion measuring 3 mm × 8 mm. The histological examination revealed a well-circumscribed LT with germinal centres; it was covered with cuboidal to columnar epithelim, partly infiltrated with lymphocytes. No adenomatous structures, either on top or sidewise were discerned. Within the organized LT, a glandular-forming adenocarcinoma was found (Figure 1). High-power microscopic examination revealed pleomorphic tumour cells with inconspicuous nucleolus. Multiple sections proved that the tumour had been locally excised. The lesion was diagnosed as DC (4-8).

Extra sections were immunohistochemically challenged with MLH1 (BD Biosciences, San Diego, USA), MSH2, MUC1, MUC2, Actin 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), $p21^{WAF1}$ (Oncogene Science, Chicago, USA) and histochemically stained with Alcian blue (pH 2.5), PAS and PAS-D.

Study of the mismatch repair proteins revealed that whereas MSH2 was normally expressed (Figure 2), MLH1 was not expressed (Figure 3). The tumour was highly proliferating, expressed MUC-1, but no MUC-2 or MUC-5AC. p53 was expressed in 7% of the tumour cells while staining $p21^{WAF1}$ was negative. M30 revealed only minimal apoptosis. Actin SM showed that the tumor had penetrated through the *muscularis mucosae*. Alcian blue, PAS and PAS-D stains showed absence of sialomucins and glyco-proteins in tumour cells.

A colectomy was subsequently performed. The specimen measured 50 cm in length. About 15 cm from the proximal resection line, a 3 mm × 7 mm non-protruding mucosal
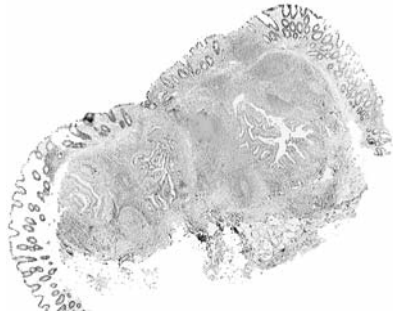
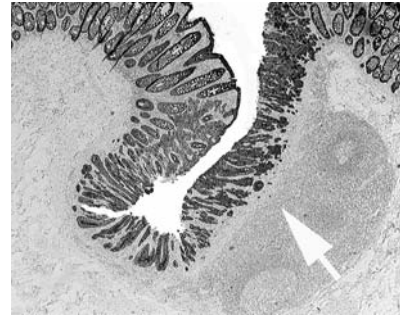Figure 3. *GALTC showing high cell proliferation (protruding phenotype, Ki-67, clone MIB1, ×2).*

Figure 4. *GALTC with invasion into the submucosa (at arrow) found in the colectomy specimen (non-protruding phenotype, MNF116, ×2).*

lesion was found. Histology showed a GALTC (non-protruding phenotype) with early invasion into the submucosa (Figure 4). No adenomatous components were found on top. Multiple sections from the area of previous endoscopic mucosectomy showed no remnant tumour.

From the remnant specimen, 20 new blocks (about 4 cm in length) were randomly sampled from the apparently normal colon mucosa. Histology revealed 3 new synchronously growing adenocarcinomas of non-protruding phenotype; one was GALTC and the remaining two were non-GALTC.

Adenomatous components were not histologically demonstrated in any of the 3 carcinomas or in the remnant specimen.

A total of 14 lymph nodes were harvested from the surgical specimen; none had metastatic deposits. The post-op was uneventful. Eight months after operation the patient is doing well.

Discussion

Despite CRC being the third most frequent cancer worldwide, carcinomas evolving in GALT mucosa are rare. In fact, this is the 9th case of DC ever reported. Of the 9 cases with DC shown in Table I, the family pedigree was known in 5 and notably 2 occurred in patients with Lynch syndrome.

The Lynch syndrome is triggered by germline mutation in one of two DNA mismatch repair (MMR) tumour suppressor genes: *MSH2* or *MLH1*, and much less frequently *MSH6* (which is strongly associated with endometrial cancer). Following inactivation of the wild-type allele, MMR proteins would no longer be expressed resulting in a failure to repair DNA mismatches occurring as spontaneous errors during DNA replication (9). In his early reports, Lynch referred to cancer family syndrome (CFS) (10) and later (11) he introduced the term HPNCC, emphasizing the heritable predisposition to CRC, in the absence of widespread polyposis. Boland and Troncale called this genetic trait,

Lynch syndrome (12).

In the literature, HNPCC and Lynch syndrome have largely been used as synonymous but today many authors favour the term Lynch syndrome, as HNPCC is inexact and potentially misleading, of the disease phenotype (9, 13, 14). In fact, the term HNPCC implies an absence of colorectal polyps, which is not the case, as Lynch patients may harbour similar numbers of polyps as the general population. In addition, the term HNPCC fails to acknowledge the wider spectrum of associated neoplasms such as carcinomas of the endometrium, stomach, small bowel, ovary, pelviureter and skin. More importantly, HNPCC has been variously applied to two overlapping groups of patients, those meeting the Amsterdam Criteria and those evidencing the clinico-pathological and molecular features with demonstrable germline mutation in an MMR gene. This confusion is at least partially attributable to the association of HNPCC with the Amsterdam Criteria, before the demonstration of the molecular genetic basis of the disease (9, 13, 14).

Of the 5 synchronously coexistent carcinomas found in a patient with Lynch syndrome reported here, 3 were GALTC and the remaining 2 GALT-free carcinomas. Of the 3 GALTC, one was of protruding phenotype (DC), whereas the remaining two were of non-protruding phenotype. The remaining 2 GALT-free neoplasias were non-protruding carcinomas. The complete silencing of MLH1 immunoreactivity and the normal expression of MSH2 was consistent with the predominant DNA mismatch deficiency found in patients with Lynch syndome (9, 13, 14).

de Petris *et al*. (4) proposed the term DC when reporting a case of protruding GALTC. Other authors shown in Table I adopted the same term (DC) to define GALTC in general (5-8). We demonstrated, however, that GALTC might be evidenced not only as GALT-DC but also as GALT-non protruding. The occurrence of protruding or non-protruding phenotypes should be included when reporting the architectural profile of the GALTC in histological sections.

Colon GALTC appears to be a rare tumor. Despite the endoscopical removal of thousands of small colonic polypoid and non-polypoid lesions in surveillance programes in Japan, France, Germany, Sweden and U.S.A. (15), we found GALTC only in one patient reported here.

The overwhelming majority of CRC evolve in the GALT-free mucosa through the adenoma–carcinoma sequence (16-18). Since adenomas were not demonstrated, neither previously nor in the colectomy specimen, it is suggested that the GALTCs in this patient might have followed the GALT–carcinoma pathway.

The future challenge is to disclose the sequence of microscopic/molecular changes taking place, most likely in M cells, that antedate the development of epithelial neoplasias in GALT.

This is the first case in the literature harboring multiple synchronously growing GALTC, both protruding (DC) and non-protruding, in a patient with Lynch syndrome.

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