# Advanced Microtubular Colorectal Adenomas: A 10-Year Survey at a Single Hospital

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**Abstract**. *Background: Colorectal carcinoma, the third most commonly diagnosed type of cancer in Europe and the USA, usually originates from colorectal adenoma (CRA). Three main histological phenotypes of CRA are usually recognized: tubular, villous and traditional serrated (TA, VA and TSA, respectively). In 1997, we reported a novel histological phenotype, the microtubular adenoma (MTA), epitomized by dysplastic epithelium arranged in closed rings (microtubules), with sideways-elongated outgrowth. Materials and Methods: The material includes 4,446 CRAs diagnosed at our Department during a 10-year period (2001-2010). Results: Out of 4,446 CRAs, 68 (1.5%) were MTA; of these, 38 (55.9%) exhibited low-grade dysplasia (LGD), 17 (25.0%), high-grade-dysplasia, two (2.9%) intraepithelial carcinoma and three (4.4%), intramucosal carcinoma. Out of the 68 MTA, 22 (32.3%) were advanced MTA. Submucosal carcinoma (SMC) was present in eight (11.8%) MTAs. Ninety-four per cent (64/68) of the MTAs were left-sided adenomas. In previous work, we found that cell proliferation occurred in the dysplastic microtubules in MTA, initially in the luminal dysplastic epithelium in TA and VA, and initially at the bottom of the serrated dysplastic crypts in TSA. Conclusion: Due to these distinctive microscopic and cell proliferative attributes, a predominant left-sided location and the absence of serrated configurations, it is submitted that MTA is a specific CRA phenotype, at variance with TA, VA, and TSA. The high frequency of SMC strongly suggests that MTA is an important alternative pathway in colorectal carcinogenesis.*

Colorectal carcinoma (CRC), the third most commonly diagnosed type of cancer in Europe and the USA (1) usually originates in mucosal foci of mutated cells with proliferative, biochemical and molecular aberrations; they are referred to as colorectal adenomas (CRA) (2). Less frequently, CRC develops from dysplastic crypts in patients with ulcerative colitis (3), from specialized epithelial cells covering gut-associated lymphoid tissue (4), or from mucosa without any recognizable preceding dysplastic alteration (*de novo* carcinoma) (5, 6).

Based on the structural histological configuration, CRAs have been classically classified into tubular (TA), villous (VA) and serrated (SA) subtypes. SA exhibits dysplastic, teeth-like outlines that resemble serrations in a saw; today this lesion is referred to as traditional serrated adenoma (TSA) (7). In 1997, in Japanese patients we found a CRA that was at variance with the aforementioned histological phenotypes, as it displayed closed dysplastic microtubules arranged in a sequential fashion along the slopes of epithelial outgrowths (8). Initially called villo-microglandular adenoma (8) it was later re-named microtubular adenoma (MTA) by the WHO in 2000 (9). Since its description in Japanese patients, MTAs have been also reported in Swedish (10), Italian (11) and English (12) patients. In previous work, we found that cell proliferation in MTA occurred in the dysplastic microtubules (10, 11), in TA and VA initially in the luminal dysplastic epithelium (9, 13), and in TSA initially at the bottom of serrated dysplastic crypts (14).

Based on the degree of cellular dysplasia, CRAs were classified into those exhibiting slight, moderate or severe dysplasia by some (15), and low and high-grade dysplasia (LGD and HGD, respectively), by others (16). More recently, the concept of advanced CRA (17) has received wide acceptance due to its association with invasive carcinoma (18-20). However, the definition of advanced CRA varies. Some authors regard it as those adenomas measuring >1 cm in diameter (21, 22), others >1 cm in diameter with villous histology (23-25); others require the presence of HGD (26-28); others, at least 1 cm or with villous elements at a frequency greater than 20% or with HGD (29); and others as carcinoma *in situ* (intraepithelial carcinoma, IEC) (30-32). Finally, some authors require the presence of intramucosal carcinoma (IMC) (20, 33-35).



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*Key Words:* Advanced adenomas, microtubular configurations, invasive carcinoma, colorectal adenoma.

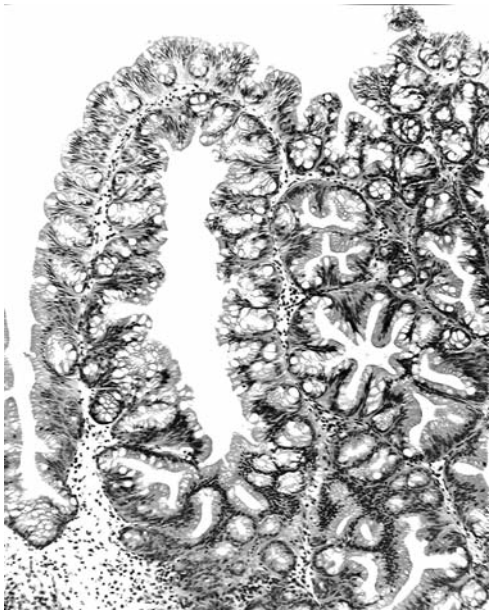

Figure 1. *Microtubular adenoma showing sideways dysplastic microtubules (H&E, ×4).*

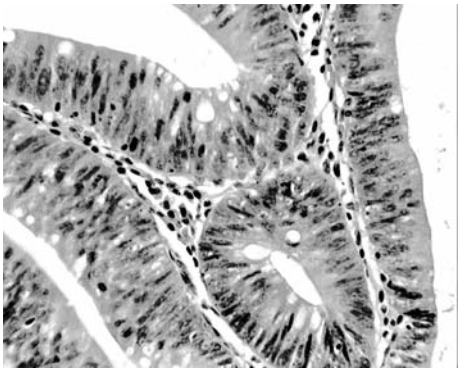

Figure 2. *Microtubular adenoma with high-grade dysplasia. (H&E, ×40).*

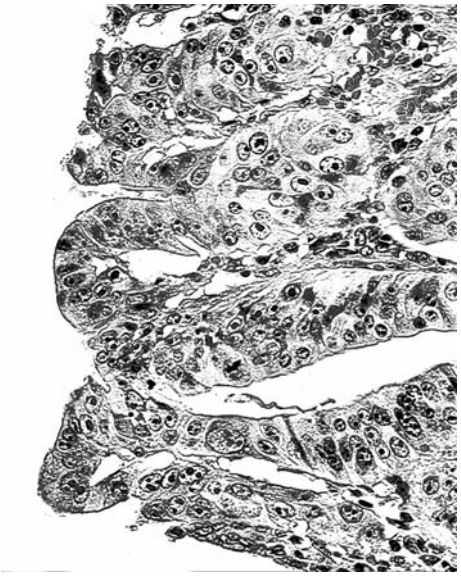

Figure 3. *Microtubular adenoma with intraepithelial carcinoma. Note large pleomorphic, pale vesicular nuclei with large, prominent, irregular nucleoli (H&E, ×40).*

In earlier work of ours, we investigated 92 consecutive CRAs with submucosal carcinoma (SMC) and either HGD or IEC in the remnant adenomatous tissue (36). Although submucosal invasion occurred more frequently in CRA with IEC than in those with HGD, as many as 42% of the SMCs arose in CRA with HGD exclusively. Despite morphological, histochemical and immunohistochemical dissimilarities between the two lesions (37, 38), it was concluded that both HGD and IEC have a propensity to invade host tissue. Against this background, CRA with HGD and with IEC were regarded here as advanced CRA. In addition, and in concordance with other authors (33-35), adenomas with IMC were also regarded as advanced CRA.

The purpose of the present work was to assess, i) the frequency of MTA and ii) the frequency of advanced MTA, in a cohort of CRAs diagnosed between 2001 and 2010 at this Department.

## Materials and Methods

The material comprised of 4,446 polypectomies exhibiting CRA at histology, diagnosed at the Department of Pathology, Karolinska University Hospital during a 10-year period (2001-2010). A total of 3,456 CRAs were diagnosed by the author (CR); and the remaining 990 CRAs by other pathologists at the Department. Sections from these 990 CRAs were retrieved from the files and reviewed; the aim was to detect possible unreported cases of MTA.

TA, VA and TSA were excluded from the study, as sessile serrated polyps.

*Definitions*. MTA: CRA with closed rings (microtubules) of dysplastic epithelium arranged lengthwise along the slopes of elongated outgrowths, in >50% of the adenomatous tissue (Figure 1).

*Classification of MTA according to the degree of neoplastic severity:* LGD: Dysplastic epithelium lined with spindle-shaped, rather uniform hyperchromatic nuclei, with regular nuclear membrane. Chromatin particles are uniformly small and the stratified nuclei do not exceed the deeper half of the epithelial thickness.

*HGD:* Dysplastic epithelium lined with spindle-shaped cells with hyperchromatic, pleomorphic nuclei. Chromatin particles are irregular with angular shapes, and the nuclear membrane is regular. Stratified nuclei exceed the superficial half of the epithelium and may reach the luminal epithelial border. Atypical mitoses are often present (Figure 2).

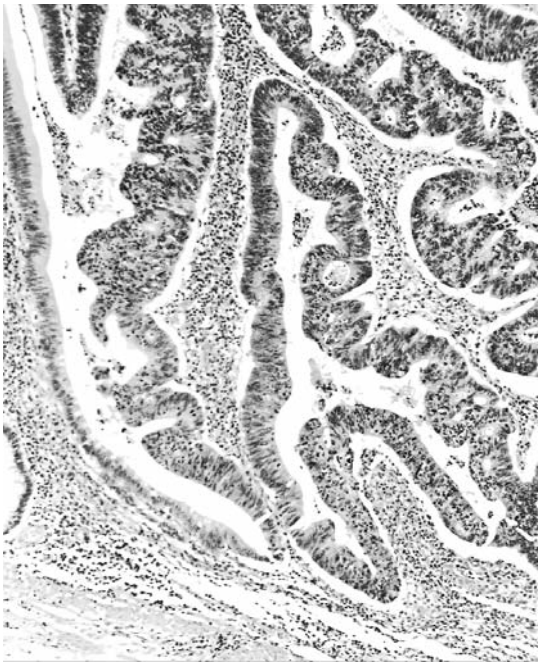

Figure 4. *Microtubular adenoma with intramucosal invasion. Note microtubular pattern on top and lack of penetration of the muscularis mucosa at the bottom of the image (H&E, ×2).*

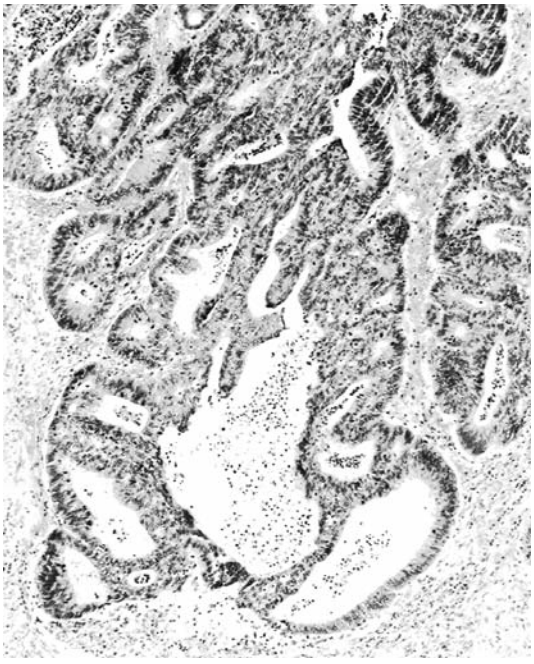

Figure 5. *Microtubular adenoma with invasive carcinoma. Note that the invading tumor retains the microtubular features (H&E, ×10).*

*IEC:* Intraepithelial neoplastic epithelium exhibiting marked pleomorphic cells with swollen large vesicular (oval or round-shaped) nuclei, with bridges of nucleolus-associated chromatin reaching angular chromatin deposits both in the nucleus and on the nuclear membrane. Nucleolus is conspicuous (≥ 2.5 μm in diameter) and irregular and the nuclear membrane is often notched. The nuclear polarity is disrupted and atypical mitoses are found. Structural glandular alterations consist of budding or branching crypts or tubules with epithelial septa, back-to-back glands and cribriform growth. The glands are often arrayed obliquely to the basement membrane (Figure 3).

*IMC:* Adenoma with neoplastic cells with unquestionable invasion into the *lamina propria mucosae*. A desmoplastic reaction in the juxtaposing *lamina propria* and/or a neutrophilic infiltration may accompany that invasion (Figure 4).

*SMC:* Adenoma with neoplastic cells invading across the *muscularis mucosae* and unquestionably reaching the submucosal layer (Figure 5).

*Advanced MTA:* Regarded as those MTA exhibiting HGD, IEC, IMC or SMC.

This study was approved by the Ethics Committee, Department of Pathology, Karolinska University Hospital.

*Statistical analysis*. Comparison between groups was carried out using the Chi-square test; $p<0.05$ was regarded as significant.

## Results

Out of 68 MTAs, 37 (54%) were found in males and the remaining 31 (46%) in females. The mean age of patients with MTA was 59 years (range=25-82 years); 25 (37%) were ≤59 years of age and the remaining 43 (63%), ≥60 years of age.

Out of the 68 MTAs, 64 (94.1%) were located in the left colon-rectum and the remaining four (5.9%) in the right colon ($p<0.05$).

*Histological evaluation*. Out of the 3,456 CRAs reviewed by CR, 58 (1.7%) were MTA. The review of the remaining 990 CRAs diagnosed by other pathologists at the Department, revealed that 10 CRAs initially diagnosed as TA (n=2), VA (n=5), or SA (n=3) were MTA at review. Hence, MTA accounted for 1.5% (n=68) of all CRAs (n=4446) seen at this Department between 2001 and 2010.

Table I shows that out of 68 MTAs, 55.9% had LGD, 25.0% HGD and 2.9% IEC. From Table I, 22 out of the 68 MTAs (32.3%) were advanced MTA.

The same Table also shows that out of 68 MTAs, 4.4% were IMCs and the remaining 11.8% were SMCs.

## Discussion

In the present survey, MTA accounted for 1.5% of 4,446 CRA. Histology showed that 55.9% of the MTAs exhibited LGD, 25.0% HGD, 2.9% IEC, 4.4% IMC and the remaining 11.8% SMC. Advanced MTAs were found in 32.3% of the MTAs and SMC was recorded in 11.8%. These results

Table I. *Degree of neoplastic severity in 68 microtubular adenomas diagnosed during a 10-year period at a single hospital.*

| | Frequency | |
|---|---|---|
| Histology | Cases | % |
| LGD | 38 | (55.9%) |
| HGD | 17 | (25.0%) |
| IEC | 2 | (2.9%) |
| IMC | 3 | (4.4%) |
| SMC | 8 | (11.8%) |
| Total | 68 | (100.0%) |

LGD: Low-grade dysplasia, HGD: high-grade dysplasia, IEC: intraepithelial carcinoma, IMC: intramucosal carcinoma, SMC: submucosal carcinoma.

Table II. *Percentage of microtubular adenomas recorded in 7,724 adenomas examined by the same observer (CR) in Sweden, Italy and Iceland.*

| Country (ref.) | Patients with CRA | Percentage of MTA |
|---|---|---|
| Sweden (31) | 3135 | 1.0% |
| Italy (27) | 1552 | 0.9% |
| Iceland† | 3037 | 5.8% |

CRA: Colorectal adenoma, MTA: Microtubular adenoma. †Rubio and Jónasson, unpublished data.

validate previous results obtained in 1,552 CRA, in Florence, Italy (12, 13) where 35.7% of the MTAs were advanced MTAs, and 7.1% SMC. Thus, previous (12, 13) and present findings strongly suggest that MTAs are aggressive lesions with a propensity to evolve into invasive carcinoma.

Fifty-four percent of the patients with MTA were males, and 63% were ≥60 years of age. These percentages are similar to those recorded in a cohort of Swedish patients with CRA, where all phenotypes of CRA were investigated (10). In that work, 52% of 3,135 CRAs were males and 65% were ≥60 years of age (10). It would appear that the development of MTA is neither influenced by the gender nor by the age of the patients.

According to the molecular paradigm of adenoma progression (39), genetic aberrations accumulate during the various phases of adenomatous growth, from TA with LGD and VA with HGD before invasive carcinoma ensues. It is to be understood that TSA or MTA were not included in the specific paradigm (39). Subsequently Yashiro *et al*. found loss of heterozygosity on 18q in TSA (40) suggesting a different molecular pathway for TSA on the one hand and TA and VA on the other.

In this work, the degree of cellular dysplasia and invasion into the *lamina propria* or in the submucosa were separately analyzed. This was done since progression of cellular neoplastic aberrations requires accumulation of genetic cell mutations (39), whereas penetration of the basement membrane by neoplastic cells requires collagen-degrading proteolytic enzymes, such as collagenase, plasminogen activator (41), heparanase (42), and matrilysin (43).

There is a plethora of literature concerning the cellular mutations that accumulate during carcinogenesis, but the molecular mechanisms that create morphological elements in CRA (TA, VA, SA, MTA) have received little attention. The Vogelstein paradigm (39) does not explain why CRA with disparate histological patterns often exhibit LGD (cf. Table I). The possibility that each histological type has its own molecular pathway of progression towards submucosal invasion cannot be totally excluded (36). On this respect, Harris *et al*. found that morphogenesis is controlled by the interaction between Sonic hedgehog and bone morphogenetic protein-2 (44). Based on this knowledge, it is speculated that the accumulation of cellular mutations leading to invasive carcinoma (39) (that is, carcinogenesis) might act independently of the series of molecular signals that orchestrate dysplastic crypts to adopt different structural configurations (that is, morphogenesis) (44). In this context, it should be mentioned that the administration of two different colonotropic carcinogens to two different strains of rats evoked different histological phenotypes of colonic adenoma with different biological behavior (45). Whether different carcinogens in the microenvironment induce different mutations in colorectal stem cells (46, 47) resulting in disparate architectural configurations such as those in TA, VA, TSA and MTA, remains unknown.

Since the age of the patients was similar in MTA and other adenoma phenotypes (10), there was no indication that TA, VA or TSA had chronologically 're-modelled' into MTA, or that TA, VA, TSA and MTA were transitional patterns capable of converting into a different phenotype with increasing age. Based on these considerations, several questions arise: i) Do MTA evolve, haphazardly in different individuals, or through a stochastic design of cell configuration orchestrated by epigenetic factors affecting susceptible individuals (46)? ii) Do environmental molecular signals program stem cells or more differentiated adenomatous cells to create tubular, villous, serrated or microtubular configurations (47)? iii) What are the molecular signals that instruct dysplastic cells to maintain the characteristic proliferation patterns in MTA (47)? iv) Are molecular signals being transferred from incipient adenomas back to stem cells, so that specific histological configurations can be replicated in subsequent dysplastic cell generations in the same adenoma (46)?

In 2008, Torlakovic *et al*. noted morphological features in TSA, such as filiform projections and ectopic crypt formations (48). The criteria and the illustrations in TSA

with ectopic crypt formations in that publication are identical to those previously reported for MTA in Japanese (8), Swedish (10), Italian (13) and English (14) patients and subsequently endorsed by the WHO in 2000 (9).

TSAs are more commonly found in the left colon, and sessile serrated adenomas, characterized by serrated configurations, in the right colon (48). In the present study 94% of the MTAs were left-sided adenomas.

Previous studies on the histological phenotype in 7,724 colorectal adenomas (Table II) revealed that the frequency of MTA was similar in Sweden (10) (1%) and in Italy (12) (0.9%), but significantly higher in Iceland, namely 5.8% (Rubio and Jónasson, unpublished findings). In the light of this and the present results, we are inclined to speculate that the frequency of MTA might be influenced by genetic and/or epigenetic factors acting in different geographical regions.

In conclusion, due to distinctive microscopic and cell proliferative attributes, a predominant left-sided location, and the absence of serrated configurations, it is submitted that MTA is as a specific CRA phenotype, at variance with TA, VA, and TSA. The high frequency of SMC strongly suggests that MTA is an important alternative pathway in colorectal carcinogenesis. By including MTA amongst TSAs, the particularly aggressive behavior of MTA may be overlooked.

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