

Original Article

Invasive carcinomas may arise in colorectal adenomas with high-grade dysplasia and with carcinoma *in situ*

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Abstract: Colorectal carcinomas (CRC) usually arise in colorectal adenomas (CRA) displaying high-grade dysplasia (HGD) or carcinoma *in situ* (CIS). The aim was to assess the frequency of adenomas displaying HGD or CIS in a cohort of consecutive CRA with submucosal invasive carcinoma. Ninety-two consecutive adenomas were investigated. Submucosal invasion was present in the 39 adenomas with HGD (42%) and in 58% (53/92) of the adenomas with CIS ($p < 0.05$). Sections from 49 adenomas were stained with the DNA-specific Feulgen reaction and for the proliferation marker Ki-67. Five consecutive high power fields (HPFs) were evaluated using a x40 objective. Marked Feulgen reaction was recorded in 91.8% or in 101 of the 110 HPFs studied in adenomas with HGD, but in none of the 135 HPFs studied in adenomas with CIS ($p < 0.05$). Intense Ki-67 expression ($\geq 75\%$) was present in 98.2% or in 108 of the 110 HPFs studied in adenomas with HGD, but only in 1.4% or in 2 of the 135 HPFs in adenomas with CIS ($p < 0.05$). Hence, HGD cells and CIS cells can be differentiated not only morphologically but also chemically by the semi-quantitative appreciation of their DNA content and immunohistochemically by the apparent difference in cell proliferation. Although submucosal invasion occurred significantly more frequently in adenomas with CIS than in those with HGD, as many as 42% of the adenomas with submucosal invasion displayed HGD at histology. Despite morphological, chemical and immunohistochemical dissimilarities, these 2 non-invasive neoplasias might have similar biological behaviour in terms of progression towards submucosal invasion.

Keywords: Colon adenomas, high-grade dysplasia, carcinoma *in situ*, submucosal invasion

Introduction

Colorectal adenomas (CRA) are foci of atypical cells with aberrant proliferation and the main source of colorectal invasive carcinomas (CRC) [1], the third most commonly diagnosed cancer in Europe and the US [2].

CRA are usually evoked by: 1) environmental factors, including diet, 2) hereditary traits (familial adenomatous polyposis (FAP) or hereditary non-polyposis colorectal cancer (HNPCC)), or 3) inflammatory bowel disease (IBD) such as ulcerative colitis (UC) or Crohn's disease (CD). CRA evoked by environmental factors are called sporadic adenomas, those caused by hereditary traits (FAP-HNPCC), genetically induced adenomas, and those triggered by IBD, IBD-induced adenomas.

The epithelial changes found in CRA are usually classified into low- (LGD) and high-grade dysplasia (HGD) [3-7], or into mild, moderate and severe dysplasia [8]. Most Western pathologists do not use the term carcinoma *in situ* (CIS) for mucosal lesions of the gastrointestinal (GI) tract, despite their regarding CIS in many other organs, such as the skin, breast, pancreas, uterine cervix, vulva, vagina, anus and urinary bladder, as an autonomous lesion [9-16]. In fact, CRA with CIS are usually diagnosed and catalogued as CRA with HGD, even if the term CIS might have appeared in the description of the lesion. This standpoint was adopted during the early 1970s, based on the conviction that: "many pathologists would regard severe dysplasia as synonymous with carcinoma *in situ*. However, this expression should be avoided in the

diagnosis of tumors of the colon and rectum because of its controversial meaning" [17], and because the term could "communicate a sense of anxiety to some surgeons, which can lead to unnecessarily radical operations" [18].

In contrast, Asian pathologists recognize CIS as a separate histological entity among the non-invasive neoplasias of the colorectal mucosa [19, 20]. Because of this difference in diagnostic opinion, a group of Western and Asian pathologists gathered in Vienna [21] years ago to discuss the possibility of unifying the nomenclature of non-invasive intraepithelial neoplasias of the GI tract. The consensus reached in Vienna was that non-invasive neoplasias in the GI tract should be classified into LGD, HGD and CIS. Although non-invasive (and invasive) neoplasias in the GI tract were listed in the Vienna classification [21], the histological description of the criteria necessary to diagnose each of these lesions was not systematically defined, thus postponing the opportunity for its worldwide acceptance.

Recently, the classification proposed in Vienna for non-invasive (and invasive) neoplasias in the GI tract was applied to CRA [22]. In this work, a detailed histological description of the criteria necessary to diagnose the various categories, was presented and illustrated.

Despite many publications on CRA, the question as to whether submucosal invasion arises in areas with HGD or with CIS has not yet being fully elucidated.

In the present work, we reviewed a cohort of consecutive adenomas having submucosal invasive carcinoma, the aim being to explore whether invasion had actually originated in CRA with HGD or with CIS.

Material and methods

Histological sections from 92 consecutive CRA showing submucosal invasion were reviewed. Sections were stained with hematoxylin-eosin (H&E).

Definitions

Following the recommendations of the Vienna classification [21, 22], epithelial neoplasias in CRA adenomas were classified as low-grade

dysplasia, high-grade dysplasia or carcinoma *in situ*.

Low-grade dysplasia (LGD, Category 4:1, Vienna). Includes adenomas showing mild to moderate dysplasia (old nomenclature). The epithelium is lined with spindle shaped, rather uniform hyperchromatic nuclei with regular nuclear membrane. The chromatin particles are uniformly small. The stratified nuclei do not surpass the deeper half of the epithelial thickness.

High-grade dysplasia (HGD, Category 4:2, Vienna). Comprise adenomas with severe dysplasia (old nomenclature). The epithelium is lined with cells having spindle shaped, hyperchromatic, moderately pleomorphic nuclei. The chromatin particles are irregular pleomorphic. The nuclear membrane is regular. The stratified nuclei surpass the superficial half of the epithelium and may reach the luminal epithelial border (**Figure 1**).

Carcinoma in situ (CIS, Category 4:3, Vienna). Include adenomas showing 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 along the nuclear membrane. The nucleolus is conspicuous ($\geq 2.5 \mu\text{m}$ in diameter) and irregular and the nuclear membrane is often notched. The nuclear polarity is disrupted and atypical mitoses are found (**Figure 2**). 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.

Submucosal carcinoma. In the Vienna classification [21, 22] submucosal carcinomas (Category 5:1, Vienna) are those that have penetrated through the muscularis mucosae and unquestionably invaded the submucosal tissues (**Figure 3**).

Sections from 49 adenomas with submucosal invasion were stained with the DNA-specific Feulgen reaction [30] and immunostained with the proliferation marker Ki-67 (clone MIB1, DAKO, Glinstrup, Denmark). Five consecutive HPFs were evaluated in 22 adenomas with HGD and in 27 adenomas with CIS, using a x40 objective. Only HPFs having predominantly ($\geq 95\%$)

Invasive carcinomas may arise in colorectal adenomas with HGD or CIS

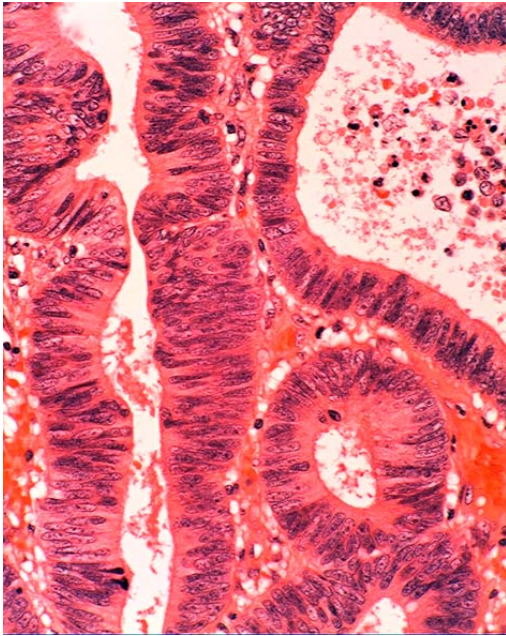


Figure 1. Colonic adenoma with high-grade dysplasia, showing tightly packed hyperchromatic pleomorphic dysplastic cells. (H&E x40).

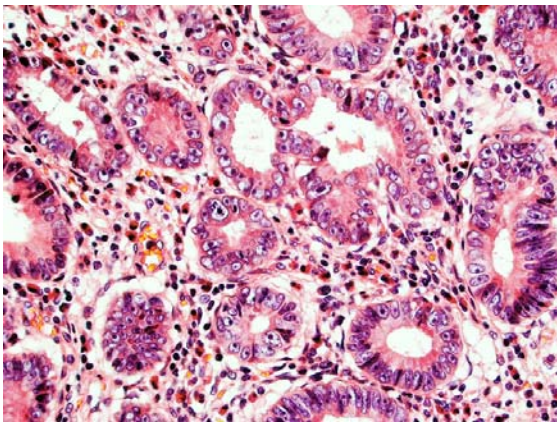


Figure 2. Colonic adenoma with carcinoma *in situ* showing large pleomorphic, pale vesicular nuclei with large, prominent, irregular nucleoli (H&E x40).

HGD or CIS were used for counting. The remaining neoplastic glands often displayed LGD (in adenomas with HGD) or HGD (in adenomas with CIS).

The intensity of Feulgen reaction/HPFs was graded into slight (+), moderate (++) and marked (+++). The number of Ki-67 expressing neoplastic cells/HPFs was graded into $\leq 33\%$, 34%-74% and $\geq 75\%$.

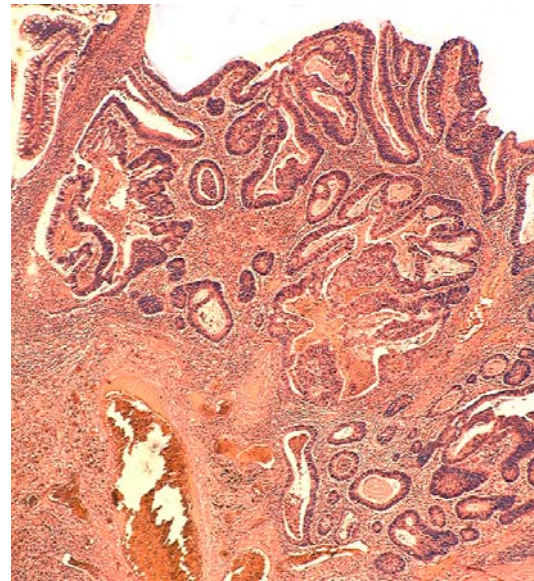


Figure 3. Low power view of an endoscopically resected colonic adenoma with submucosal invasion (H&E, x2).

Statistical analysis: The Kruskal-Wallis test was used to compare differences between a particular degree of Feulgen stain intensity (+, ++ or +++) and of Ki67 expression ($\leq 33\%$, 34%-74% and $\geq 75\%$) both in adenomas with HGD and with CIS.

The Mann-Whitney test was used to compare differences between the number of cases with invasive carcinoma in adenomas with HGD and CIS. Statistical significance was defined as $p < 0.05$.

The Regional Ethical Committee approved the study.

Results

Seventy of the 92 adenomas with submucosal invasion were located in the colon and the remaining 22 in the rectum. Of the 92 adenomas, 39 were histologically classified as with HGD and the remaining 53 as with CIS. They were classified at histological examination as predominantly villous adenomas.

Table 1 shows that in adenomas with HGD, a marked Feulgen reaction (**Figure 4**) was found in 91.8% or in 101 of the 110 HPFs studied. On the other hand, none of the 135 HPFs studied in adenomas with CIS (**Figure 5**) displayed a

Invasive carcinomas may arise in colorectal adenomas with HGD or CIS

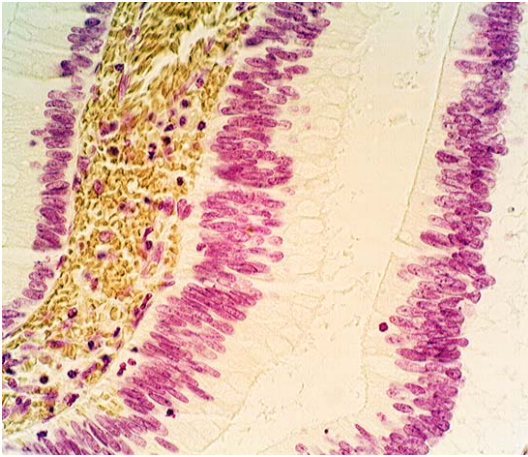


Figure 4. Colonic adenoma with high-grade dysplasia showing markedly Feulgen-stained dysplastic nuclei (Feulgen reaction, x40).

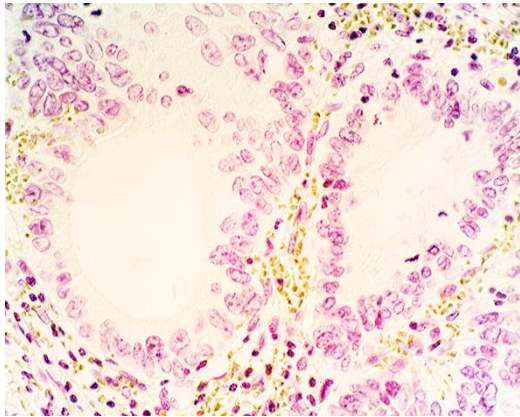


Figure 5. Colonic adenoma with carcinoma *in situ* showing faintly Feulgen-stained nuclei (Feulgen reaction, x40).

marked Feulgen reaction. The difference between adenomas with HGD and with CIS showing marked Feulgen reaction was significant ($p < 0.05$).

Table 2 shows that intense Ki-67 immunostain expression ($\geq 75\%$) was recorded in the majority (98.2%) or in 108 of the 110 HPFs examined in adenomas with HGD (**Figure 6**). In contrast, only 1.4% or 2 of the 135 HPFs studied in adenomas with CIS displayed intense Ki-67 expression. The remaining 98.5% (133 of the 135 HPFs examined) showed a relatively low ($\leq 33\%$) (**Figure 7**) or moderate (34%-74%) Ki-67 expression. The difference between adenomas with HGD and with CIS showing intense Ki-67 immunostain expression was significant ($p < 0.05$).

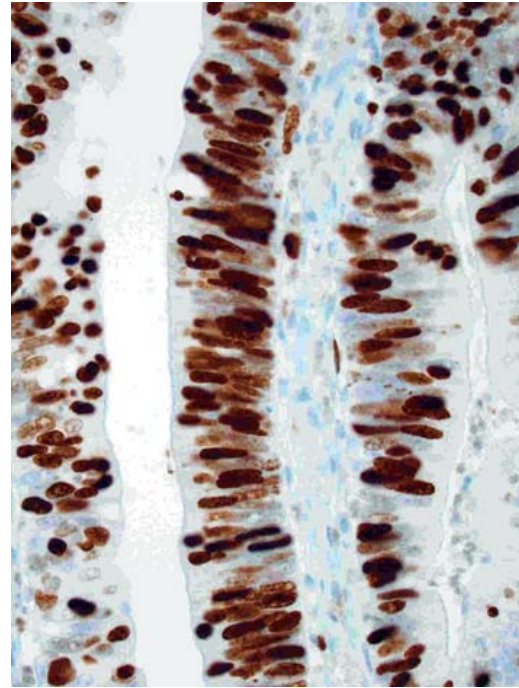


Figure 6. Colonic adenoma with high-grade dysplasia showing marked nuclear Ki-67 expression in the majority of the neoplastic cells (Ki-67 immunostain, x40).

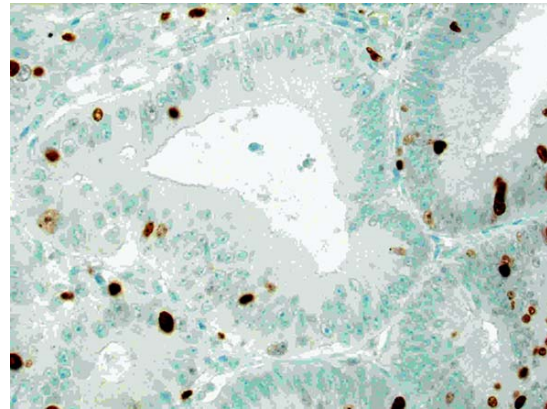


Figure 7. Colonic adenoma with carcinoma *in situ*, showing nuclear Ki-67 expression in only few neoplastic cells (Ki-67 immunostain, x40).

Submucosal invasion was present in the 39 adenomas with HGD (42%) and in the 53 adenomas with CIS (58%). The difference was significant ($p < 0.05$).

Discussion

As early as 30 years ago, HGD and CIS were

Invasive carcinomas may arise in colorectal adenomas with HGD or CIS

Table 1. Number of high power fields (HPFs) with slight (+), moderate (++) or marked (+++) Feulgen reaction in adenomatous cells, in 22 adenomas with high grade dysplasia and in 27 with carcinoma *in situ*.

	Feulgen reaction			Total no. HPFs
	+	++	+++	
Number of HPFs with high grade dysplasia	0	9 (8.2%)	101 (91.8%)	110 (100%)
Number of HPFs with carcinoma <i>in situ</i>	55 (40.7%)	80 (59.3%)	0	135 (100%)
ALL	55 (22.4%)	89 (36.3%)	101 (41.2%)	245 (100%)

Table 2. Number of high power fields (HPFs) with Ki-67 expressing adenomatous cells found in 22 adenomas with high grade dysplasia and in 27 with carcinoma *in situ*.

	Percent of adenomatous cells with Ki-67 expression			Total no. HPFs
	≤ 33%	34%-74%	≥75%	
Number of HPFs with high grade dysplasia	0	2 (1.8%)	108 (98.2%)	110 (100%)
Number of HPFs with carcinoma <i>in situ</i>	64 (47.4%)	69 (51.2%)	2 (1.4%)	135 (100%)
ALL	64 (26.1%)	71 (28.9%)	110 (44.9%)	245 (100%)

considered two separate histological entities in the GI tract [23-25]. In fact, a WHO Expert Committee composed of European, American and Japanese gastrointestinal pathologists met in London in 1978 [23] to discuss terminology of precancerous conditions and epithelial dysplasia of the stomach. The recommendations of the Expert Committee were that "severe dysplasia is used for the description of changes which fall short of the full criteria for the diagnosis of carcinoma *in situ*. The latter must exist at some stage in the progression into invasive carcinoma" [23]. On his return from London, Takeo Nagayo (the Japanese participant) published the concept of dysplasia (a term not used in Japan before 1978) and its relation to the precancerous state [24]. He methodically defined and illustrated the criteria for the differential diagnosis between severe dysplasia and intramucosal cancer of the stomach. Subsequently, a group of Western pathologists headed by C. Fenoglio-Preiser's group [25] reported a similar

classification, but for CRA. They wrote: "The histologic features of colorectal adenomas may be defined as low- and high-grade dysplasia, carcinoma *in situ*, intramucosal carcinoma, and invasive carcinoma". "Intraepithelial carcinoma or carcinoma *in situ* consists of cytologically malignant cells that remain confined to the basement membrane of the original crypts of Lieberhühn", and the "extension of the neoplastic cells through the basement membrane of the crypts into the surrounding lamina propria can be designated as intramucosal carcinoma". Hence, the criteria used by the WHO Expert Committee in England [23], by Nagayo in Japan [24] and by Fenoglio-Preiser in USA [25] should be regarded as the basis for the consensus reached in Vienna regarding the identity of the two different lesions, one being HGD and the other CIS.

Recent molecular studies of colonic neoplasias in humans [26] and in p53-deficient mice [27]

validated the classification of CRA into HGD and CIS. Sugai *et al* [28] found that the frequencies of genetic alterations and DNA aneuploidy increased with an increasing grade as assigned by the Vienna classification. To reach this conclusion Sugai *et al* [28] used flow cytometric analysis of DNA content, polymerase chain reaction microsatellite assays, single-strand conformational polymorphism assays, chromosomal allelic loss, and *Ki-ras* and *p53* gene mutations. The combined genetic and DNA ploidy data supported the conclusion that such analyses may help in the appropriate categorization of colorectal tumors.

The present data substantiate previous preliminary findings [29,30] suggesting that HGD cells and CIS cells can be differentiated not only morphologically [22] but also chemically by the semi-quantitative appreciation of their DNA content in Feulgen stain [29] and immunohistochemically by the state of cell proliferation as shown by Ki-67. The explanation for this paradoxical biological behavior of these two neoplastic cell phenotypes is not fully understood.

Several questions remain to be elucidated: 1) Why do so many CIS cells fail to express Ki-67? 2) How long does the putative arrest of cell proliferation in CIS cells last? [30]. It is assumed that the arrest of cell proliferation might be only temporary, as other CIS cells expressed the Ki-67 proliferation marker (see **Table 2**). 3) If the arrest of cell proliferation is only temporary, when do Ki-67-negative CIS cells resume their cell proliferation? 4) Which molecular signals orchestrate the assumed switch-on, switch-off mechanism of cell proliferation in CIS?

In short, the present investigation strongly suggests that HGD and CIS differ not only morphologically but also chemically, as judged by the semi-quantitative appreciation of their DNA content and immunohistochemically, as ascertained by the difference in cell proliferation. Submucosal invasive carcinomas originated in colorectal adenomas having HGD or CIS morphological phenotypes. Although submucosal invasion occurred significantly more frequently in adenomas with CIS than in those with HGD, as many as 42% of the adenomas with submucosal invasion displayed HGD at histology.

More research is necessary to unveil the mechanism(s) behind the switch-on, switch-off

phenomenon of cell proliferation in CIS cells and the ultimate invasion of the host.

Thanks to recent improvements in medical technology, it is possible to laser-microdissect groups of neoplastic glands [31] with HGD or with CIS for specific molecular analysis. This modern technology [31] will permit missing histological structures such as those in HGD and CIS to be translated into molecular terms.

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Invasive carcinomas may arise in colorectal adenomas with HGD or CIS

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