Difference in Cell Proliferation between Two Structurally Different Lesions in Colorectal Adenomas: High-grade Dysplasia and Carcinoma *In Situ*

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Abstract. *Background: Despite the fact that most Western pathologists diagnose carcinoma in situ (CIS) in many organs the same pathologists generally diagnose similar histological aberrations in colorectal adenomas, as high-grade dysplasia (HGD). Materials and Methods: Five large colorectal adenomas (measuring ≥20 mm) having areas of both HGD and CIS on staining with hematoxylin and eosin (H&E) were assessed with the proliferation antibody Ki-67 (clone MIB1). Results: HGD is built of tightly packed, spindle shaped, hyperchromatic cells with moderate pleomorphic nuclei having coarse chromatin and CIS of marked pleomorphic, vesicular, hypochromatic nuclei with a prominent nucleolus. Ki-67 was expressed in 96% of the HGD cells but only in 3.5% of the CIS cells ($p<0.05$). Conclusion: The results of this and of previous investigations using the DNA-specific Feulgen stain, suggest that HGD and CIS in colorectal adenomas are two dissimilar morphological entities with dissimilar molecular behaviour. HGD cells in colorectal adenomas seem to be proliferating at any given time, whereas the majority of the CIS cells are not.*

Despite most Western pathologists recognize carcinoma *in situ* (CIS) in many organs such as the breast, uterine cervix, skin, vulva, vagina, anus, testis, pancreas, extrahepatic bile ducts and the urinary bladder (1-11), the same pathologists generally diagnose similar histological aberrations in colorectal adenomas, as high-grade dysplasia (HGD). In the middle of the last century it was proposed that the term carcinoma *in situ* should be banned from the diagnostic terminology in colorectal adenomas as it could lead to misinterpretation by surgeons and to unnecessary surgical interventions (12-13). This maybe the reason why most Western pathologists continue to regard colorectal adenomas with HGD or with CIS, as synonymous.

Nearly 10 years ago, a group of Western and Asian pathologists gathered in Vienna (14) to discuss the nomenclature for gastrointestinal intraepithelial neoplasias (*i.e.* non-invasive intraepithelial neoplasias) and of neoplasias with invasion. The final consensus reached there was that intraepithelial neoplasias in the GI tract should be sub-classified into low-grade dysplasia (LGD), HGD and CIS. Despite a consensus being reached by both schools, the histological criteria necessary to diagnose each of these lesions were not clearly defined, thus postponing the opportunity for its worldwide acceptance.

More recently, the histological criteria for classification of the various categories proposed in Vienna were described for colorectal adenomas (15): of the 1552 adenomas studied, 591 (38.1%) had LGD (51.9%), 806 had HGD and 31 (2.0%) CIS.

More recently, while studying sections from colorectal adenomas using Feulgen stain (16, 17) (used to specifically identify DNA material in cell specimens), we found that the nuclei of HGD cells were intense stained, whereas the nuclei in CIS cells often remained unstained.

In the present work this phenomenon was further investigated by studying the pattern of cell proliferation in areas with HGD and with CIS in colorectal adenomas.

Materials and Methods

A total of five large colorectal adenomas (measuring ≥20 mm) were endoscopically removed from five patients (3 males and 2 females, being 61, 64, 64, 69 and 72 years old, respectively). No colonic or rectal carcinoma was present in the five patients.

Sections stained with hematoxilin and eosin (H&E) showing areas characteristic of both HGD and CIS according to the Vienna classification (14, 15), were selected. Additional sections were immuno histochemically stained with the proliferation antibody Ki-67 (clone MIB1, DAKO, Glinstrup, Denmark) (18)).

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Key Words: Dysplasia, carcinoma *in situ*, DNA, RNA.

Table I. *The number of Ki-67 labelled cells in 5 colorectal adenomas in areas with high grade dysplasia (HGD) and in areas with carcinoma in situ (CIS).*

Case #	HGD	CIS
1	192/200 (96%)	3/200 (1.5%)
2	189/200 (94.5%)	10/200 (5%)
3	193/200 (96.5%)	8/200 (4%)
4	191/200 (95.5%)	6/200 (3%)
5	195/200 (97.5%)	8/200 (4%)
All	960/1000 (96.0%)	35/1000 (3.5%)

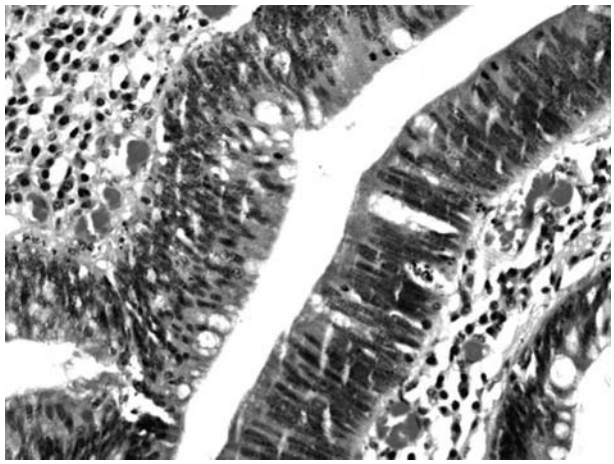

Figure 1. *High-grade dysplasia. Detail from a colonic adenoma, with high-grade dysplasia. Note spindle-shaped dysplastic cells with hyperchromatic nuclei even in the upper half of the epithelium (H&E, original magnification, x40).*

Cell proliferation was assessed at high-power examination by counting the number of Ki-67-labelled cells among 200 consecutive HGD cells and 200 consecutive CIS cells.

Statistical analysis. The non-parametric Wilcoxon test was performed using StatView Version 4.5 software (Abacus Concepts, Berkley, CA, USA). Statistical significance was defined as $p<0.05$.

Results

Table I shows the number of Ki-67-labelled cells in the 5 adenomas. It is seen that 96% of the cells with HGD expressed the Ki-67 immunostain, whereas only 3.5% of the CIS cells were immunostained. The difference between the frequencies of labelled cells was significantly higher in HGD than in CIS ($p<0.05$).

In areas with HGD cells (Figure 1) an intense Ki-67 expression was found (Figure 3). In contrast, the majority of the CIS cells (Figure 2) remained unlabelled with the Ki-67 immunostain (Figure 4).

Discussion

The Vienna classification (14) highlighted the importance of sub-classifying intraepithelial neoplasias to assess the significance of CIS in colorectal carcinogenesis. At present it is unknown whether HGD (category 4.1) progresses to CIS (category 4.2) or whether CIS evolves without any prodromic HGD phase before reaching the lamina propria mucosa (category 5.1 or intramucosal carcinoma), or the submucosal layers (category 5.2). Rationally, HGD should precede CIS.

The results of previous work (15, 17) substantiate the notion that HGD and CIS are not one single entity, as they are morphologically (14) and chemically different (in terms of DNA content (17)). The results of the present work also showed that HGD cells are immuno-histochemically different from CIS cells, as the former cells expressed the proliferation marker Ki-67, whereas the majority of the latter cells failed to do so. Although the explanation for this unexpected paradoxical biological behaviour of neoplastic cells is not fully understood, it is known that only Ki-67 labelled cells are actively involved in the proliferation cycle (G1, S, G2 and M phases (18)) while unlabelled nuclei correspond to cells that have been removed from the cell cycle (G0) (19).

Why only CIS cells are removed from the proliferation cell cycle remains poorly understood. The presence of atypical mitosis and the lack of apoptosis or of cell necrosis in areas with Ki-67-negative CIS in colorectal adenomas indicates that these cells were not undergoing cell death (19).

The nucleolus, the organelle where ribosomal gene expression takes place, was prominent in CIS cells. The organelle is generated around the specific acrocentric chromosomes that contain rDNA repeats (20). The nucleolus is able to modify the cellular requirements for protein synthesis. Gene transcription seems to takes place in the peri-nucleolar compartment (PNC) that surrounds the nucleolus. Recently, Zaidi *et al.* (20) reported that several tumor suppressors and oncoproteins, such as p53, MDM2, p19ARF (encoded by CDKN2A), IRS, B23 (nucleofosmin) and MYC are sequestered in the nucleoli of tumor cells suggesting that a cancer-related role for nucleoli might go beyond protein synthesis.

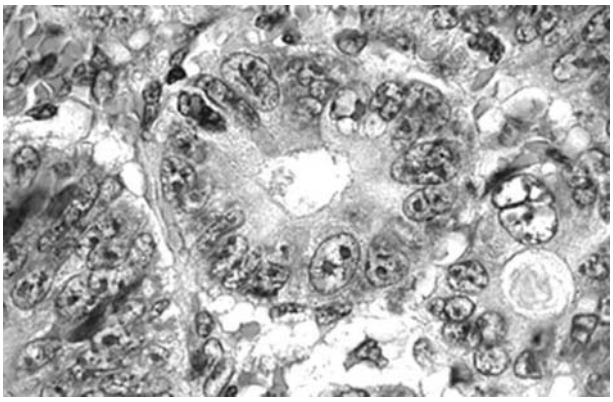

Figure 2. *Carcinoma in situ. Detail from a colonic adenoma with carcinoma in situ. Note pleomorphic, vesicular nuclei and the prominent nucleoli. The nuclei lack the hyperchromasia seen in the nuclei of cells with high-grade dysplasia (H&E, original magnification, x40).*

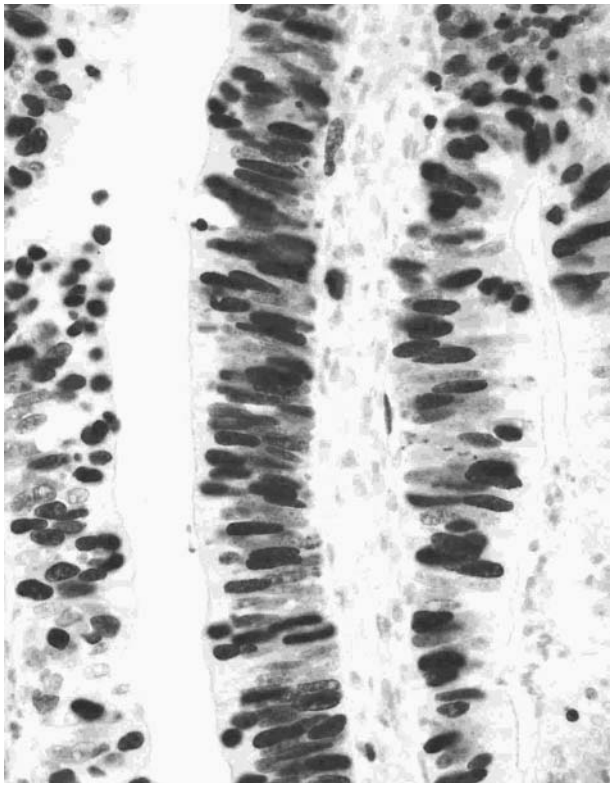

Figure 3. *High-grade dysplasia. Detail from a colonic adenoma with high-grade dysplasia treated with the antibody Ki-67. Note the intense labelling in the nuclei of the dysplastic cells with high-grade dysplasia (Ki-67 (clone MIB1, original magnification, x40).*

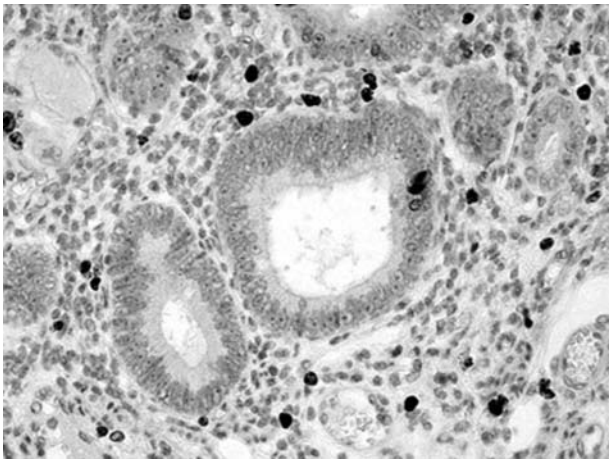

Figure 4. *Carcinoma in situ. Detail from a colonic adenoma, with carcinoma in situ (CIS) treated with the Ki-67. Note that the majority of the CIS nuclei as well as the nucleoli (rich in RNA) are not labelled. Only occasional nuclei in CIS cells and lymphoblasts in the lamina propria are MIB1 positive (clone MIB1, original magnification, x40).*

It would appear that following the S-phase in HGD, the neoplastic cells transcribe their genome from DNA to RNA in CIS cells. The re-arrangement of the pathway of nucleotide synthesis would encourage CIS cells, *via* messenger-RNA signals, to synthesize the mutated cytoplasmic proteins, that are required for the ultimate cellular invasion of the lamina propria mucosa, and beyond.

Tumour cells are by definition those that have acquired the capacity for autonomous, immortal proliferation, unable to respond to normal growth control (21, 22) (when not abrogated by anoxic distress, vascular obstruction, or radiation). The present findings in CIS cells seem, however, not to reconcile with the notion that neoplastic cells have an immortal cell proliferation (21, 22). A not yet envisaged molecular system may exist that orchestrates active proliferation in HGD cells on the one hand, and arrest of cell proliferation in CIS cells on the other. At this stage it should be mentioned that a similar arrest of cell proliferation was recently found in the cancer cells present at the invading edge of colorectal carcinomas (23).

Several years ago Vogelstein and his group (24) launched a molecular paradigm of colorectal carcinogenesis, according to which the loss of the gate-keeper APC (adenomatosis polyposis coli) gene changes the histology of the normal colorectal mucosa into a histological lesion known as adenoma with LGD, and *k-ras* activation and loss of DCC (deleted in colorectal cancer) gene to a lesion known as adenoma with HGD. In adenomas showing invasive carcinoma, mutation of the *p53* gene occurred. The sequence of events described by Vogelstein *et al.* (24) has been axiomatic for the past 19 years; it correlates molecular aberrations with the histology of colorectal adenomas having either LGD or HGD and invasive carcinoma. In that paradigm, however, adenomas with CIS were not included. The question arises: How would CIS fit into that molecular model of colorectal carcinogenesis?

In conclusion, it was demonstrated, for the first time *in vivo*, that HGD cells are frequently proliferating at any given time, whereas the majority of the CIS cells are not. This and previous investigations in colorectal adenomas (15, 16) strongly suggest that HGD and CIS are two dissimilar morphological entities with dissimilar molecular behaviour. It is possible that the Ki-67-negative CIS cells have been removed from the cell cycle (G0).

More research is necessary to disclose the molecules responsible for the switch-on, switch-off mechanism of cell proliferation which takes place in HGD and CIS cells of colorectal adenomas.

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Received July 4, 2007
Revised October 18, 2007
Accepted October 22, 2007