Qualitative DNA Differences between Two Structurally Different Lesions: High-grade Dysplasia and Carcinoma *In Situ* in Colorectal Adenomas

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Abstract. *Background: Despite the fact that the Vienna classification of neoplasias in the gastrointestinal (GI) tract acknowledged low-grade dysplasia (LGD), high-grade dysplasia (HGD) and carcinoma in situ (CIS) and that most Western pathologists recognize CIS in many organs, both CIS and HGD are still used synonymously in colorectal adenomas. Differences between CIS and HGD in colorectal adenomas are reported. Materials and Methods: Five large colorectal adenomas (measuring ≥20 mm) having areas of both HGD and CIS were stained with hematoxylin and eosin (H&E) and with Feulgen stains. Results: The HGD areas showed tightly packed, spindle shaped, hyperchromatic cells with slight to moderate pleomorphic nuclei having coarse chromatin. In contrast, the CIS cells displayed marked pleomorphism, large vesicular nuclei and a prominent nucleolus. In H&E stain the hyperchromasia found in HGD nuclei was much less evident in CIS nuclei. The HGD nuclei were intensively stained (+++) with the DNA-specific Feulgen reaction but the CIS nuclei were not. Conclusion: It would appear that following the completion of chromosomal mutations in the nuclei of HGD-cells, their DNA, carrying the new genetic information, is transcribed into RNA in the nuclei of CIS. Thus, through messenger-RNA, the production of mutated cytoplasmic proteins, required for the ultimate invasion of the lamina propria mucosa (and beyond), would be triggered.*

Based on the degree of cellular aberration most Western pathologists classify colorectal adenomas into those with low-grade dysplasia (LGD) or with high-grade dysplasia (HGD) (1,2), despite the fact that carcinoma *in situ* (CIS) is recognized by most Western pathologists as an intraepithelial carcinoma in other organs such as the skin, breast, pancreas, uterine cervix, vulva, vagina, anus and the urinary bladder (3-10), CIS and HGD in colorectal adenomas are considered as synonymous.

In 1998 a group of Western and Asian pathologists gathered in Vienna (11) to discuss the nomenclature for gastrointestinal epithelial neoplasias showing no submucosal invasion. The final consensus reached in Vienna was that in the GI tract, non-invasive (*i.e.* intraepithelial) neoplasias should be classified into LGD, HGD and CIS.

Despite both schools reaching a consensus on these various categories, the histological criteria necessary to diagnose each one of these lesions were not clearly defined, thus postponing the opportunity for its worldwide acceptance.

Recently, the histological criteria for the various categories proposed in Vienna, applied to colorectal adenomas have been described and illustrated (12).

The purpose of the present work is to report additional findings in colorectal adenomas that substantiate the distinction between HGD and CIS.

Materials and Methods

Five large colorectal adenomas (measuring ≥20 mm) showing at histology areas of both HGD and CIS according to the Vienna classification (12) were investigated. Sections were stained with hematoxilin and eosin (H&E) and by the DNA-specific Feulgen reaction(13).

The sections stained with Feulgen were analyzed on a microspectrophotometer, particular attention being paid to the HGD and CIS areas.

The intensity of the Feulgen reaction was semi-quantitatively assessed in 200 consecutive HGD cells and in 200 consecutive CIS cells, as follows: marked (+++), moderate (++), slight (+) or negative (0) stain.

Results

Four of the five adenomas were localized in the colon and the fifth in the rectum. The H&E stained histological sections showed villous configurations having a group of glands with HGD and a group of glands with CIS in all five adenomas.

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

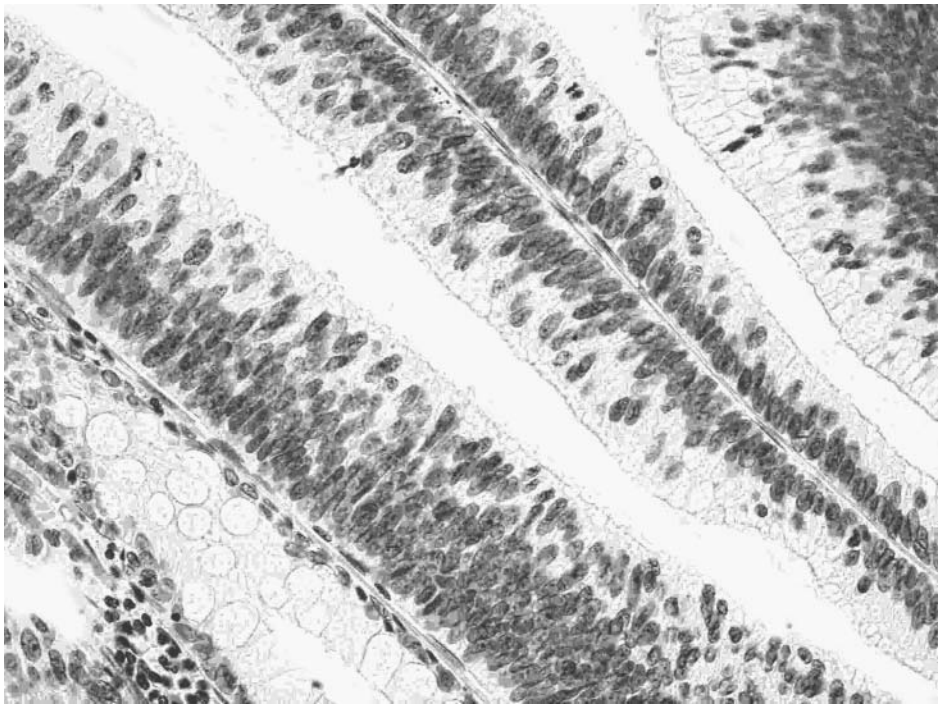

Figure 1. *High-grade dysplasia. Detail from a colonic adenoma with high-grade dysplasia. Note spindle-shaped dysplastic cells with hyperchromatic nuclei even in the upper half of the epithelium (H&E. 40x, original magnification).*

H&E stained sections. The HGD area was lined with tightly packed cells having spindle shaped, hyperchromatic, moderately pleomorfic nuclei (Figure 1) with coarse chromatin particles. The stratified cells surpassed the superficial half of the epithelium often reaching the luminal epithelial border. The nuclear membrane was regular.

The CIS cells (Figure 2) displayed marked pleomorphism and large vesicular (oval or round-shaped) nuclei. The thin chromatin bridges of the nucleolus-associated chromatin reached angular chromatin deposits. The hyperchromasia seen in HGD was much less evident. The nucleolus was prominent (≥2.5 μm in diameter) and irregular. The nuclear membrane was often notched. The nuclear polarity was disrupted and atypical mitoses were usually present.

Feulgen-stained sections analyzed in a microspectrophotometer. In order to analyze the DNA in individual nuclei by microspectrophotometer, the cells in tissue sections should be isolated, without contact with adjacent cells. Unfortunately, attempts to quantify the nuclear DNA content in areas with HGD and with CIS by this method failed, as only few isolated, independent cells without contact with other adjacent cells were present in the sections.

Qualitative observations of the Feulgen reaction. Observation of the DNA-specific Feulgen stain by conventional microscopy, showed an intense reaction (+++) in the 200 nuclei of the HGD cells in all five adenomas (Figure 3) whereas the reaction in the 200 nuclei of the CIS cells was weak (+).

Discussion

Differences in nuclear morphology and degree of Feulgen stain between HGD and CIS in colorectal adenomas, has been demonstrated in this preliminary report.

It should be mentioned that in the past several authors had also considered HGD and CIS in the GI tract as two different entities. In 1978 (15), a WHO Expert Committee composed of European, American and Japanese gastrointestinal pathologists met in London to discuss terminology of precancerous conditions and epithelial dysplasia of the stomach. The recommendations of the Expert Committee (chaired by BC Morson) 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" (15). At his return from London, Takeo Nagayo (the Japanese participant) published, in his country(16), the concept of dysplasia (a term not used in Japan before 1978) and its relation to the precancerous state. He methodically defined the criteria for the differential diagnosis between severe dysplasia and

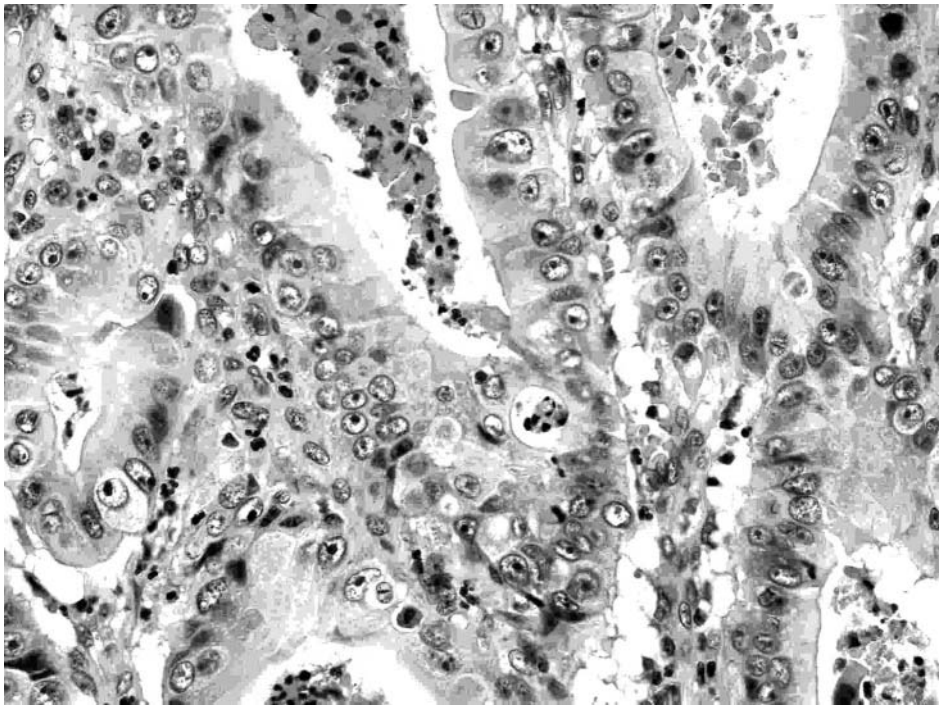

Figure 2. *Carcinoma in situ. Detail from a colonic adenoma with carcinoma in situ. Note pleomorphic, vesicular nuclei having prominent nucleoli. The nuclei lack the hyperchromasia seen in the nuclei of cells with high-grade dysplasia (H&E, 40x, original magnification).*

Figure 3. *DNA-specific Feulgen stain to demonstrate the strong reaction in the nuclei of cells with high-grade dysplasia (lower half of the illustration) and the weak reaction in the nuclei of cells with carcinoma in situ (upper half of the illustration). (Feulgen stain, 20x, original magnification). The nucleoli (rich in RNA) are not stained.*

intramucosal cancer of the stomach. Subsequently, a group of Western pathologists headed by C Fenoglio-Preiser (17) reported a similar classification, but for colorectal adenomas. She 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 remains confined to the basement membrane of the original crypts of Lieberhühn", and the "extension of the neoplastic cells though 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 (15), by Nagayo in Japan (16) and by Fenoglio-Preiser in USA (17) should be regarded as the fundamentals for the consensus reached in Vienna regarding the identity of the two different lesions, one being HGD and the other CIS.

Nevertheless, during the 1960's, the notion emerged among Western pathologists, that carcinoma *in situ* should be banned from the diagnostic terminology as it could lead to misinterpretation by surgeons and to unnecessary surgical intervention. That may be the reason why Western pathologists continue to consider HGD and CIS as synonymous.

It is of interest to mention that recent molecular studies of colonic neoplasias in humans (18) and in p53 deficient mice (19) have validated the Vienna classification. Sugai *et al.* (20) 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.* (20) used flow cytometric analysis of DNA content, polymerase chain reaction microsatellite assays, single-strand conformational polymorphism assays, chromosomal allelic loss, K-*ras* and p53 gene mutations The combined genetic and DNA ploidy data supported the conclusion that those analyses may help in the appropriate categorization of colorectal tumors.

Since it remains unsettled whether HGD progresses to CIS or whether CIS evolves without any prodromic phase (such as HGD) (12) it goes without saying that it is crucial to distinguish between those two morphologically different lesions, separately.

Thanks to recent improvements in medical technology, it is possible to laser-microdisect groups of neoplastic glands with HGD or with CIS for specific molecular analysis. This modern technology will permit translation of the histological structures of HGD or of CIS into molecular terms, in the future.

In conclusion, it would appear that following the completion of chromosomal mutations in the nuclei of HGD-cells, their DNA, carrying the new genetic information, is transcribed into RNA (21) in the nuclei of CIS. The result would be to trigger, through messenger-RNA, the production and amplification of mutated cytoplasmic proteins (22-28), required for the ultimate invasion of the *lamina propria mucosa* (and beyond).

References

1 Morson BC, Whiteway JE, Jones EA, Macrae FA and Williams CB: Histopathology and prognosis of malignant colorectal polyps treated by endoscopic polypectomy. Gut *25*: 437-444, 1984.

2 Loeve F, Boer R, Zauber AG, Van Ballegooijen M, Van Oortmarssen GJ, Winawer SJ and Habbema JD: National Polyp Study data: evidence for regression of adenomas. Int J Cancer *111*: 633-639, 2004.

3 Ackermann S, Gehrsitz C, Mehlhorn G and Beckmann MW: Management and course of histologically verified cervical carcinoma *in situ* during pregnancy. Acta Obstet Gynecol Scand *85*: 1134-1137, 2006.

4 Gonzalez RJ, Buzdar AU, Fraser Symmans W, Yen TW, Broglio KR, Lucci A, Esteva FJ, Yin G and Kuerer HM: Novel clinical trial designs for treatment of ductal carcinoma *in situ* of the breast with trastuzumab (herceptin). Breast J *13*: 72-75, 2007.

5 Ondo AL, Mings SM, Pestak RM and Shanler SD: Topical combination therapy for cutaneous squamous cell carcinoma *in situ* with 5-fluorouracil cream and imiquimod cream in patients who have failed topical monotherapy. J Am Acad Dermatol *55*: 1092-1094, 2006.

6 Raju RR, Goldblum JR and Hart WR: Pagetoid squamous cell carcinoma *in situ* (pagetoid Bowen's disease) of the external genitalia. Int J Gynecol Pathol *22*: 127-135, 2003.

7 Wilkin TJ, Palmer S, Brudney KF, Chiasson MA and Wright TC: Anal intraepithelial neoplasia in heterosexual and homosexual HIV-positive men with access to antiretroviral therapy. J Infect Dis *190*: 1685-1691, 2004.

8 Abe K, Suda K, Arakawa A, Yamasaki S, Sonoue H, Mitani K and Nobukawa B: Different patterns of p16INK4A and p53 protein expressions in intraductal papillary-mucinous neoplasms and pancreatic intraepithelial neoplasia. Pancreas *34*: 85-91, 2007.

9 Yin H, He Q, Li T and Leong AS: Cytokeratin 20 and Ki-67 to distinguish carcinoma *in situ* from flat non-neoplastic urothelium. Appl Immunohistochem Mol Morphol *14*: 260-265, 2006.

10 Snijders PJ, Breuer RH, Sutedja TG, Egging M, Voorhorst FJ, Steenbergen RD, van der Linden HC, Risse EK, Berkhof J, de Vries EG, van der Zee AG, Postmus PE, Meijer CJ and Smit EF: Elevated hTERT mRNA levels: A potential determinant of bronchial squamous cell carcinoma (*in situ*). Int J Cancer *109*: 412-417, 2004.

11 Schlemper R J, Riddell, Kato Y, Borchard F, Cooper HS, Rubio CA, Rugge M, Shimuzu M, Watanabe H and Yamabe H: International concensus on the classification of gastrointestinal epithelial neoplasia. The Vienna classification. Gut *47*: 251-255, 2000.

12 Rubio CA, Nesi G, Messerini L, Mandai K, Itabashi M and Takubo K: The Vienna classification applied to colorectal adenomas. J Hepatology and Gastroenterology *21*: 1697-1703, 2006.

13 Rubio CA, Alm T, Aly A and Poppen B: Intraepithelial bodies in colorectal adenomas: Leuchtenberger bodies revisited. Dis Colon Rectum *34*: 47-50, 1991.

14 Rubio CA and Porwit-McDonald A: A method to quantitate the relative nuclear area of colorectal polyps by image analysis. Anal Quant Cytol Histol *13*: 155-158, 1991.

15 Morson BC, Sobin LH, Grundmann E, Johansen A, Nagayo T and Serck-Hanssen A: Precancerous conditions and epithelial dysplasia in the stomach. J Clin Pathol *33*: 711-721, 1980.
16 Nagayo T: Dysplasia of the gastric mucosa and its elation to the pecanceous state Gann *72*: 813-823, 1981.
17 Fenoglio-Preiser C, Lantz P, Lindstrom M, Davies M and Rilke F: Gastrointestinal Pathology. An Atlas and Text. Raven Press, New York, Second Edition, pp. 909-1068, 1989.
18 Miyaki M, Iijima T, Yamaguchi T, Hishima T, Tamura K, Utsunomiya J and Mori T: Germline mutations of the MYH gene in Japanese patients with multiple colorectal adenomas. Mutat Res *578*: 430-433, 2005.
19 Fujii S, Fujimori T, Kawamata H, Takeda J, Kitajima K, Omotehara F, Kaihara T, Kusaka T, Ichikawa K, Ohkura Y, Ono Y, Imura J, Yamaoka S, Sakamoto C, Ueda Y and Chiba T: Development of colonic neoplasia in p53 deficient mice with experimental colitis induced by dextran sulphate sodium. Gut *53*: 710-716, 2004.
20 Sugai T, Habano W, Uesugi N, Jiao YF, Nakamura S, Sato K, Chiba T and Ishii M: Molecular validation of the modified Vienna classification of colorectal tumors. J Mol Diagn *4*: 191-200, 2002.
21 Galamb O, Sipos F, Dinya E, Spisak S, Tulassay Z and Molnar B: mRNA expression, functional profiling and multivariate classification of colon biopsy specimen by cDNA overall glass microarray. World J Gastroenterol *12*: 6998-7006, 2006.
22 Kim JH, Kang MJ, Park CU, Kwak HJ, Hwang Y and Koh GY: Amplified CDK2 and cdc2 activities in primary colorectal carcinoma. Cancer *85*: 546-553, 1999.
23 Bosari S, Viale G, Bossi P, Maggioni M, Coggi G, Murray JJ and Lee AK: Cytoplasmic accumulation of p53 protein: an independent prognostic indicator in colorectal adenocarcinomas. J Natl Cancer Inst *86*: 681-687, 1994.
24 Lu M, Nakamura RM, Dent ED, Zhang JY, Nielsen FC, Christiansen J, Chan EK and Tan EM: Aberrant expression of fetal RNA-binding protein p62 in liver cancer and liver cirrhosis. Am J Pathol *159*: 945-953, 2001.
25 Zhang JY, Chan EK, Peng XX and Tan EM: A novel cytoplasmic protein with RNA-binding motifs is an autoantigen in human hepatocellular carcinoma. J Exp Med *189*: 1101-1110, 1999.
26 Neufeld KL and White R: Nuclear and cytoplasmic localizations of the adenomatous polyposis coli protein. Proc Natl Acad Sci USA *94*: 3034-3039, 1997.
27 Smith DP, Rayter SI, Niederlander C, Spicer J, Jones CM and Ashworth A: LIP1, a cytoplasmic protein functionally linked to the Peutz-Jeghers syndrome kinase LKB1. Hum Mol Genet *10*: 2869-2877, 2001.
28 Chedin M, Filhol O, Duminy C, Bolla M, Benistant C, Roche S, Chambaz EM and Cochet C: Characterization of two different cytoplasmic protein tyrosine kinases from human breast cancer. Carcinogenesis *18*: 1463-472, 1997.

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