

## Histological Changes Pertinent to Local Tumor Progression in Hereditary Nonpolyposis Colorectal Cancer (HNPCC). A Preliminary Report

C.A. RUBIO and A. LINDBLOM

*Departments of Pathology and Clinical Genetics,  
Karolinska Institute and Hospital, Stockholm, Sweden*

**Abstract.** *While the genetic profiles of hereditary colorectal tumors are being unravelled, the mechanisms implicated in their local progression remain to be deciphered. In this work histological features occurring at the invading tumor front were investigated in ten hereditary non-polyposis colorectal cancers (HNPCC). Of eight moderately-differentiated (i.e. gland-forming) adenocarcinomas, six had dilated glands with a thin layer of tumor cells and all eight had dilated glands in which a group of cells was lacking, i.e. with a glandular pore. It was apparent that the thin glandular epithelium was a stage preceding pore formation. In glands with pores, the contents of the neoplastic glands –rich in proteolytic enzymes– were extruded directly into the extracellular matrix (ECM), leading to the local destruction of the juxtaposed matrix. It was assumed that to restore the continuity of the glands new cancer cells would grow from the tip of the free borders of the pore into the damaged ECM, thus guaranteeing a stepwise, but everlasting, tumor progression in untreated patients.*

Much research has been done to unveil the mechanisms connected with the invasion of sporadic colorectal carcinomas. In that endeavour, several microscopic and molecular parameters have been investigated such as the rate of cell proliferation (1), the expression of p53, *c-myc* and *c-erb B-2* (2), survival (3), degree of tumor differentiation (4), racial and genetic dissimilarities (5), cell motility (6) and the pattern of tumor growth (7) (*i.e.* expansive *vs.* infiltrating, with or without lymphocytic infiltration). In 1993, Hase *et al.* (8) reported the presence of small clusters of tumors cells (*i.e.* foci of up to 5 cancer cells, denominated “tumor

budding”) as well as of single tumor cells at the growing edge of colorectal tumors. The authors found a correlation between the occurrence of tumor budding and prognosis. Tumor cell budding at the invasive margin (8) was proposed as a marker of poor 5-year survival. The studies of Hase *et al.* were subsequently corroborated by Ueno *et al.* (9) and, more recently, by Tanaka *et al.* (10).

Notwithstanding, despite many morphological and molecular investigations, the mechanism whereby cancer cells (including tumor cell budding) invade the juxtaposed normal matrix of the host remains unclear.

In a preliminary publication, we reported the occurrence of histological parameters at the growing edge of ten sporadic colorectal adenocarcinomas that were at variance with those previously reported in the literature (11). We found that the neoplastic glands at the invading front (usually containing retained mucin, inflammatory cells and/or necrotic material) exhibited a thin layer of tumor cells or lacked a group of tumor cells. The latter glandular defect was referred to as a glandular pore (11). Through glandular pores the retained contents were extruded directly into the juxtaposed extracellular matrix (ECM). It was entertained that, once in the ECM, those secretions, rich in proteolytic enzymes (12,13), would lead to the breakdown of the ECM. The malignant cells proliferating from the tip of the glandular pores would eventually invade that damaged matrix, thereby guaranteeing a stepwise, but everlasting, tumor progression in untreated patients. Those preliminary results were later confirmed in a larger series of 112 sporadic colorectal adenocarcinomas (14). As the aforementioned histological changes were equally frequent in colonic and rectal tumors, despite the fact that preoperative irradiation was administered only to patients with rectal tumors, it was inferred that those parameters were neither evoked nor abrogated by irradiation. Studies of tumor stage indicated that glandular pore formation was not related to the ability of colorectal tumors to metastasize to regional lymph nodes (14). Subsequent studies at the growing tumor edge in

*Correspondence to:* C. A. Rubio, M.D., Ph. D., Gastrointestinal and Liver Pathology, Research Laboratory, Department of Pathology, Karolinska Institute and Hospital, 171 76 Stockholm, Sweden. Fax: 46 8 51774524, e-mail: Carlos.Rubio@onkpat.ki.se

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Table I. Mutations in 10 patients with HNPCC with colonic carcinomas.

File number	Family code number	Pathogenic mutation	Parameters
1213/01	294	Msh2, 1447G>T	14,21,51,34,26,44+45, 65
733/02	347	Msh2, del ex 1-7	14, 21,35,45,53,56,62
769/02	401	Msh2, del ex 1-8	49, 53, 54,62
1079/00	198	Mlh1, R265C	31,37,24,17,44+46,29,35,50,54,65
496/01	292	Msh2, 2228del(4)	49,52,54,65
1273/00	269	Mlh1, 665delA	23,44,39,43,15,37,29,50,52,54,63
990/98	187	Msh2, 942+3A>T	49, 59, 62
1371/99	149	Msh2, del ex 8	13, 22,27,37,44,56,51,62
EGR 923/01	246	Msh2, 1772del(4)	35, 39+40, 38, 65
40811/01	400	Msh2, del ex 7-8	17,23,43,44,30,36,50+52,62

sporadic colorectal adenocarcinomas from patients with inflammatory bowel disease (15), as well as in chemically-induced colonic adenocarcinomas in rats (16), showed similar histological features. The same features were also recorded in sporadic esophageal adenocarcinomas (17).

The purpose of this study was to investigate the histological characteristics at the growing tumor edge of colorectal adenocarcinomas in patients with hereditary non-polyposis colorectal cancers (HNPCC) (18).

### Materials and Methods

Ten individuals with HNPCC were chosen from among participants in the surveillance program at the Cancer Family Clinic at the Karolinska Hospital, Stockholm, Sweden. HNPCC families were defined by germline mutations in hMHL1 or hMSH2 and MSI-positive tumors (19) (Table I). Sections were stained with hematoxylin and eosin (H&E).

The occurrence of a thin layer of tumor cells and of cellular defects (pores) was evaluated in ten consecutive, unselected glands at the invading tumor front and in ten consecutive, unselected glands within the tumor bulk in the ten tumors.

### Results

**Histological classification.** In eight of the ten patients with colonic adenocarcinomas, the tumor was classified as moderately-differentiated (*i.e.* gland-forming) adenocarcinoma and the remaining two as undifferentiated carcinoma.

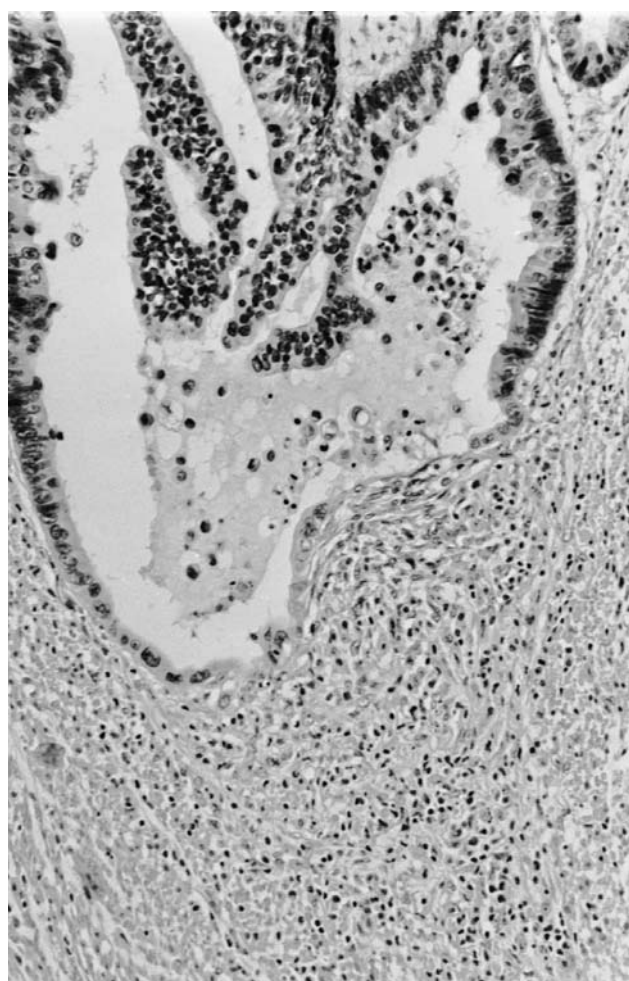


Figure 1. Dilated neoplastic gland with retained mucin. Note a thin layer of tumor cells facing the juxtapsed matrix of the host (invading front, HNPCC colonic adenocarcinoma, H&E 20x).

**Tumor glands with a thinner layer of tumor cells.** Tumor glands with a thinner layer of tumor cells at the invading tumor edge were found in six of the eight moderately-differentiated adenocarcinomas (Figure 1). In one of the eight cases  $\geq 50\%$  of the glands at the invading tumor edge had a thinner layer of tumor cells.

Tumor glands with a thinner layer of tumor cells in the tumor bulk were present in four cases, but none had that phenomenon in  $\geq 50\%$  of the glands.

**Tumor glands with glandular pores.** At the advancing tumor edge juxtaposing the ECM tumor glands with pores were recorded in all eight moderately-differentiated adenocarcinomas. In four of the eight cases  $\geq 50\%$  of the glands had glandular pores. In seven of the eight cases of moderately-differentiated adenocarcinoma the glands with



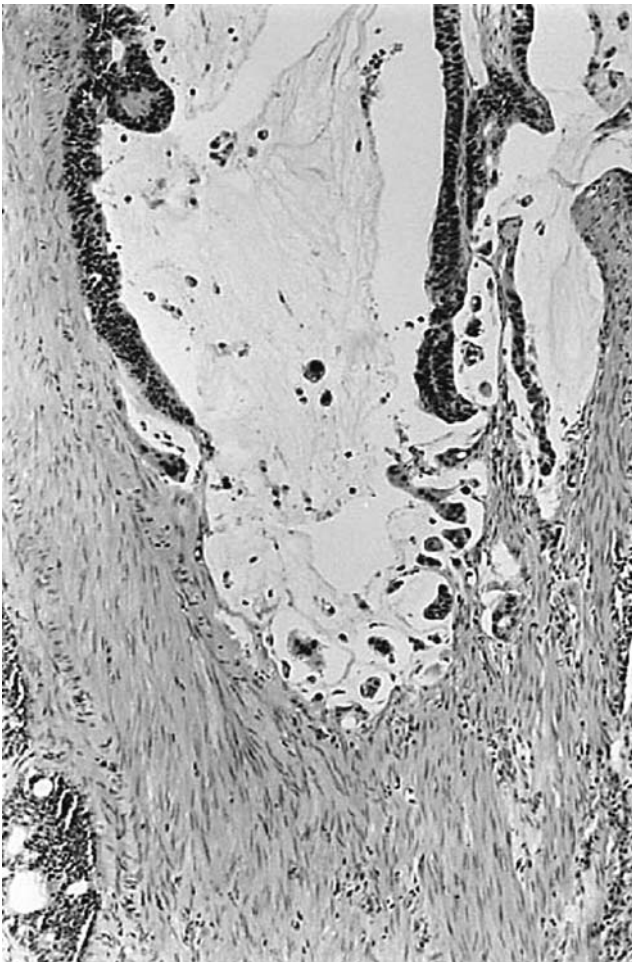


Figure 2. Dilated neoplastic gland with retained mucin being released through a pore into the juxtaposed matrix (invading front, HNPCC colonic adenocarcinoma, H&E 20x).

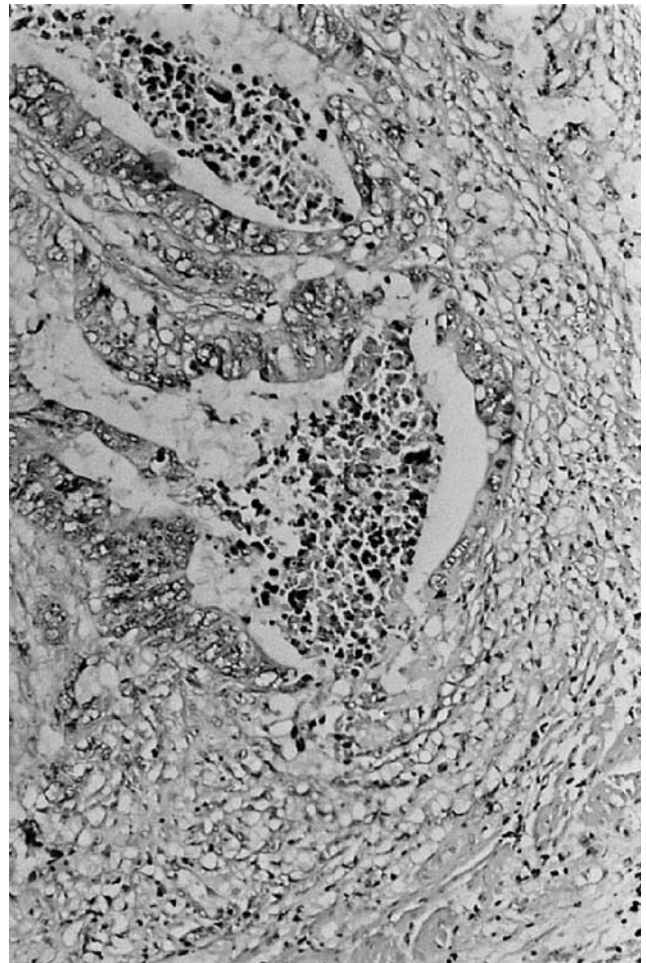


Figure 3. Dilated neoplastic gland with retained inflammatory cells and necrotic material being released through a pore into the juxtaposed matrix (invading front, HNPCC colonic adenocarcinoma, H&E 20x).

pores contained mucin (Figure 2), and the remaining one, neutrophils and necrotic material (Figure 3). Those cellular products were found in direct contact with the ECM.

Tumor glands with pores within the tumor bulk were found in four cases, but only one had glandular pores in  $\geq 50\%$  of the glands.

### Discussion

In this preliminary study, we analysed sections from colonic carcinomas in ten patients with HNPCC. The *avant garde* dilated neoplastic glands facing the immediate vicinity of the host tissues (ECM) often displayed a thin layer of tumor cells and/or glandular pores. As those glands contained mucins, inflammatory cells and/or necrotic material it was inferred that the accumulation of those products had increased the

pressure of the gland against the juxtaposed ECM. The increased intraglandular pressure resulted in the thinning of the outmost layer of the tumor cells and finally in the formation of glandular pores. Through those newly created glandular pores, the contents of the glands poured out into the juxtaposed ECM. Those discharges (rich in proteolytic enzymes (12,13)) resulted in the breakdown of the juxtaposed ECM. Six of the eight gland-forming HNPCC carcinomas (*i.e.* 75%) had, at the invading edge, glands with a thin layer of tumor cells and all eight (100%) had glandular pores.

Friedl *et al.* (12) proposed that the migration of tumor cells is conveyed by proteolytic enzymes native to the ECM, namely matrix metalloproteinases (MMPs), cathepsins and serine proteases. The enzymatic digestion of the ECM leads to pericellular proteolysis. The pericellular breakdown of ECM components generates localized matrix defects and

remodelling along migration tracts (12). The significance of MMPs in extracellular matrix degradation has been much investigated in later years (20).

In contrast to the pure locomotion theory proposed for tumor invasion (12), we found in colonic HNPCC carcinomas that the glands at the tumor front contained mucins (from cancer cells) or neutrophils (from the host) that had leaked through glandular pores. The contact of those extruded materials (rich in proteolytic enzymes (12,13)) led to the breakdown of the ECM (15). It is conceivable that, following the above process, glandular continuity (and subsequent tumor progression) is resumed by the growth of new cancer cells from the tip of the free borders of the pores, into the damaged ECM. Such an enzymatic-tumor-cell locomotion sequence would guarantee a stepwise but everlasting tumor progression in untreated patients.

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