

## Review Article

# Morphologic and Molecular Events at the Invading Edge of Colorectal Carcinomas

Carlos A. Rubio

*Gastrointestinal and Liver Pathology Research Laboratory, Department of Pathology, Karolinska Institute and University Hospital, Stockholm, Sweden*

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**Abstract:** The mechanisms whereby colorectal carcinomas invade the extracellular matrix remain elusive. In a series of studies on the growing edge of colorectal carcinomas, we found dilated neoplastic glands, some with a layer of flat tumor cells, and some lacking one or more groups of consecutive lining tumor cells (called glandular pores). Through the glandular pores, the retained glandular material was siphoned off directly into the juxtaposed extracellular matrix. The substances secreted by the tumor cells, rich in proteolytic enzymes, disrupted the anatomy of the extracellular matrix. To remodel the defective glands, the malignant cells, proliferating from the tip of the free borders of the pores, invade the enzymatically disrupted matrix to achieve glandular continuity. Sealing of these glandular flaws permits intraglandular accumulation of new proteolytic material, a mechanism that replicates a new wave of host invasion at the invading edge, thus ensuring stepwise but everlasting tumor progression in untreated patients. More recent findings indicated that the flat tumor cells at the advancing edge failed to express the proliferation marker Ki67 but overexpressed the mutated p53 protein. This paradoxical biologic behavior of tumor cells may be connected with the subsequent formation of glandular pores and strongly suggests that the arrested cell proliferation at the advancing tumor edge occurs independently of p53 mutation. Possibly, two independent molecular systems exist at the advancing edge of colonic carcinomas, one supervising cell proliferation and the other actively transferring the mutated p53 protein to daughter cells.

**Key Words:** Colorectal, adenocarcinomas, growing edge, pore formation, proteolysis

Colorectal carcinoma (CRC) is the third most commonly diagnosed tumor in Europe and the United States [1]. CRCs usually arise in foci of abnormal mucosal cell proliferations known as adenomas and nonadenomatous dysplasias. The triggers for the development of these abnormal mucosal areas can be 1) environmental (including diet [2]), 2) hereditary (as in familial adenomatosis polyposis (FAP) or hereditary nonpolyposis colorectal cancer [HNPCC] [3,4]), and 3) inflammatory (e.g., inflammatory bowel disease [IBD], such as ulcerative colitis and Crohn's colitis [5]). CRCs account for 11%–15% of all cancer cases in the Western world. About 2% of all colon cancer cases are attributed to HNPCC and 1% to IBD. The remaining CRCs are sporadic [6].

The dysplastic foci evoked by environmental factors are called sporadic adenomas, those induced by hereditary traits, genetically

induced adenomas, and the ones induced by chronic mucosal inflammation, dysplasia in flat mucosa. Occasionally, extended irregular areas of flat or exophytic dysplastic mucosa, known as dysplasia-associated lesion or mass (DALM) evolve in patients with IBD. These patients may also develop sporadic adenomas in areas without inflammation. All these dysplastic lesions may advance toward invasive carcinoma.

HNPCC is not a form of cancer per se but is a syndrome that includes people at high risk for colon cancer. Although “nonpolyposis” is a part of the term “hereditary nonpolyposis colorectal cancer,” colonic polyps (usually small) are the ultimate precursors of CRCs in these patients. The crucial question is how these dysplastic foci of abnormal cell proliferation become hostile and invade the host? This conundrum appears difficult to trace because it takes 40 years for an

adenoma to evolve into an invasive carcinoma [7].

An Internet search using Google (4/24/2007) brought up about 22,530,000 entries for colonic and rectal cancer, suggesting the mounting concern about the biology, diagnosis, treatment, and survival of colorectal neoplasias. Attempting to unveil the histologic parameters involved in neoplastic growth that are useful in estimating the potential aggressiveness of CRCs, several investigators concentrated on the growing tumor edge. Some of the parameters they studied were the growth pattern (expansive vs. infiltrating), the degree of tumor differentiation [8], foci of up to five cancer cells (called tumor budding [9]), and the occurrence of peritumoral lymphocytes [10, 11], the last parameter to estimate the immunologic reaction of the host. Other investigators focused on the kinetics of cancer cells to migrate into the surrounding matrix [12-14], claiming that tumor cell locomotion is the single most important factor accountable for the local progression of the tumor. Still others assessed some of the attributes of tumor cells, such as cellular proliferation [15-17], p53 expression [18], *K-ras* mutations [19], cyclin-dependent kinase [16], Bcl-2, potassium ion channels from the HERG1 protein family [20], and carbonic anhydrase-related protein VIII [21].

More recent investigations suggested that alterations in the Wnt (signaling molecules that regulate cell-to-cell interactions) pathway may be implicated in CRCs [22]. To estimate the role played by the host in tumor penetration, the Fas-Fas ligand mechanism [23,24], angiogenesis [25,26], telomerase activation [27], cathepsin B [28], CD10 expression [29], increased membrane type 9 matrix metalloproteinase (MMP) [30], transforming growth factor signaling in fibroblasts [31], Smad4 [32], and trimeric laminin 5 expression [33] were analyzed.

Proteolytic enzymes are necessary for the dissolution of the peritumoral stroma. It has been proposed that proteolytic enzymes native to the extracellular matrix (ECM) (e.g. matrix metalloproteinases (MMPs)), cathepsins, and serine proteases [34-37] cause the disintegration of the peritumoral ECM, thus accelerating tumor cell progression. Masaki *et al* [38] maintained that proteolytic degradation by extracellular MMPs is one of the essential

events in tumor invasion. The members of the human MMP gene family are classified into subgroups of proteolytic enzymes: collagenases, stromelysins, matrilysins, gelatinases, and membrane-type (MT) and other MMPs. According to Friedl and Wolf [13], the peritumoral breakdown of ECM generates localized matrix defects and promotes remodeling along the migration tracts. In addition, increased collagen degradation by MMPs can be evoked even in experimentally-induced obstruction of the colon (i.e., in the absence of a growing tumor) [39].

An argument against the significance of MMPs in tumor progression is the failure of broad-spectrum MMP inhibitors in clinical trials [40]. Joyce *et al* [37] postulated that although ECM degradation has been attributed to MMPs, different classes of cancer cell proteases clearly contribute to tumor penetration, with cathepsins directly involved in the degradation of the ECM. Degradation of ECM may also come about through the modulation of protease-sensitive regulatory networks involving other proteases and nonproteases such as annexin II present on the surface of cancer cells [41]. Other recently found enzymes produced by cancer cells are heparanase [42] and the AKT serine/threonine protein kinase [43].

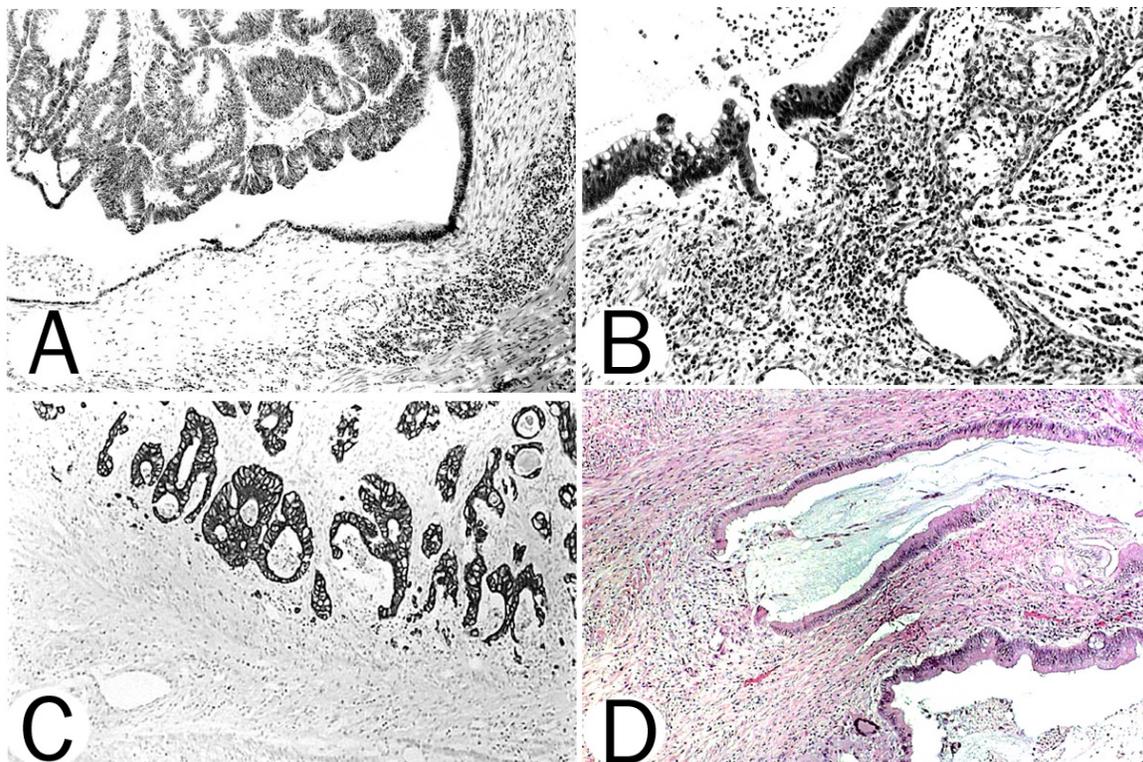
Despite the burgeoning literature on these subjects, however, the series of histologic events that place between the presence of dysplastic glands in adenomas to the submucosal invasion or beyond remains enigmatic. In previous studies of the invading edge of CRCs [44,45], we found dilated neoplastic glands, some with a layer of flat cells (i.e., tumor cells having a >50% reduction in height compared with other tumor cells in the same gland (**Figure 1A**) and some lacking one or more groups of consecutive lining tumor cells. The latter glandular gaps are called glandular pores [44,45] (**Figures 1B-D, Figure 2A**). Further studies of the growing tumor edge in sporadic CRCs in patients with IBD [46], in carcinomas from patients with HNPCC [47], in chemically-induced colonic carcinomas in rats [48], and in patients with Barrett's adenocarcinomas [49] showed a similar sequence of events, namely, dilated neoplastic glands with flat tumor cells and pore formation. We observed in those studies that through the glandular pores, the retained glandular material was being siphoned off

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directly into the juxtaposed ECM. The substances secreted by the tumor cells, rich in proteolytic enzymes, disrupted the juxtaposed peritumoral anatomy of the ECM, a mechanism that facilitates tumor penetration.

It has been proposed that to remodel the defective glands, malignant cells proliferating from the tip of the free borders of the pores

invade the enzymatically disrupted matrix with the goal of achieving glandular continuity. Sealing of the glandular flaws would permit the reaccumulation of new intraglandular proteolytic material, a mechanism that would replicate a new wave of host invasion at the growing edge, thus ensuring a stepwise but everlasting tumor progression in untreated patients [50].



**Figure 1** A. Neoplastic gland at the tumor edge. Note the thin layer of tumor cells facing the juxtaposed tissues of the host (colonic adenocarcinoma; H&E, 100 $\times$ ). B. Neoplastic gland at the tumor edge lacking a group of consecutive tumor cells, called glandular pore formation. Note the release of mucin directly into the juxtaposed extracellular matrix of the host (colonic adenocarcinoma; H&E, 200 $\times$ ). C. Overview of the invading tumor showing neoplastic glands with pore formation (colonic adenocarcinoma; MNF 116 immunization, 25 $\times$ ). D. Neoplastic gland at the invading edge of the tumor with intraglandular mucin being released through the pore into the extracellular matrix (rectal adenocarcinoma; H&E, 100 $\times$ ).

We recently found at the invading edge of CRCs that laminin  $\alpha$ 2 chain, a trimetric matrix protein localized at the basement membrane in many organs, was overexpressed in the neoplastic cells limiting to the glandular pores (Figure 2B) (Lenander and Rubio, unpublished data). The presence of this adhesion-migration macromolecule at the tumor edge suggests its active participation in the events pertinent to the local progression of colonic carcinomas.

Studies of large tissue sections from colo-

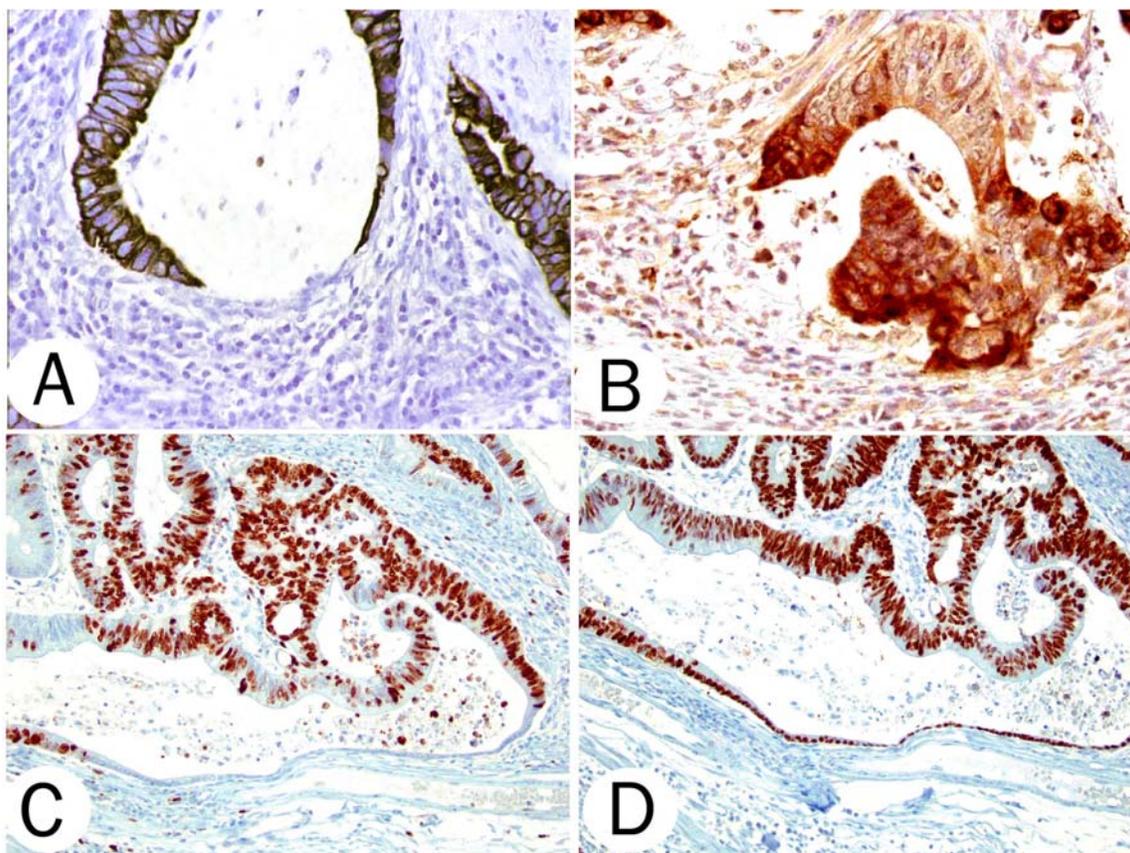
stomies and rectal amputates [51] revealed that the aforementioned histologic parameters (i.e. neoplastic glands with flattened tumor cells and with pore formation) were similarly frequent at the invading edge in both colonic and rectal adenocarcinomas [45]. Because only patients with rectal tumors underwent preoperative radiotherapy, it was inferred that those glandular pores were neither evoked nor abrogated by irradiation [45]. Studies of tumor stage indicated that glandular pore formation at the invading edge was unrelated to the

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ability of colorectal tumors to metastasize to regional lymph nodes [45].

It may be argued that pore formation in

invading tumor glands is a haphazard event. If this is the case, however, why does this phenomenon mainly occur at the growing edge of CRCs but not within the tumor mass?



**Figure 2** **A.** Neoplastic glands with intraglandular mucin. Note the pore formation, putting the mucin produced by the tumor cells in direct contact with the extracellular matrix (rectal adenocarcinoma; MNF 116 immunostain, 100 $\times$ ). **B.** Neoplastic gland with pore formation at the invading edge showing overexpression of laminin 5-2, particularly in the neoplastic cells surrounding the pore (colonic adenocarcinoma; laminin 5-2 immunostain, 200 $\times$ ). **C.** Colonic adenocarcinoma with a dilated neoplastic gland at the invading tumor edge. Note the absence of Ki67 expression in the flat tumor cells (clone MIB 1 immunostain, 200 $\times$ ). **D.** The same gland as in panel C showing p53 expression in all cells, including the flat tumor cells at the invading tumor edge (immunostain for P53, 200 $\times$ ).

In more recent studies, we demonstrated that colorectal tumor cells are able to produce lysozyme [52], an innate enzyme with potent nonimmunologic antibacterial properties that is usually found in the upper intestinal tract. Under normal conditions, lysozyme is not secreted in the lower intestinal tract (i.e., the colon and rectum). It is therefore surprising that neoplastic cells derived from normal colorectal cells that do not secrete lysozyme acquire the capacity to produce it. The complex biochemical manufacture of lysozyme

suggests that the onset of its production may not be a haphazard, capricious event in mutated colorectal neoplastic cells but instead part of a more elaborate molecular task.

One explanation for the disparate secretion in neoplastic colorectal cells is that the acquired lysozyme differs from the innate lysozyme found in Paneth cells of the small intestine and from the acquired lysozyme production in metaplastic Paneth cells in ulcerative colitis and Crohn's colitis [53]. It is conceivable that

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the task of lysozyme in neoplastic colorectal cells may be other than antibacterial. It should be emphasized that lysozyme is only a generic name for at least 80 different compounds. It is interesting that in CRC, lysozyme was found in materials discharged through glandular pores into the peritumoral ECM. It is therefore plausible that acquired lysozyme in colorectal neoplasia mirrors a molecular event that is at variance with the antibacterial task of the innate, natural lysozyme in normal tissues of the upper intestinal tract or of the acquired lysozyme in colorectal tissues with chronic inflammation. A similar proteolytic mechanism appears to be valid for Signet-ring cell carcinomas.

The occurrence of glandular pores was also investigated in colonic adenomas without [54] and with submucosal invasion [55] and in hyperplastic polyps [54]. Glandular pores were found within the confines of the lamina propria in 25% of tubular adenomas, in 33% of serrated adenomas, in 50% of tubulovillous adenomas, and in 67% of villous adenomas [54]. None of the hyperplastic polyps had glandular pores. In colonic adenomas with submucosal invasion [55], we found dilated neoplastic glands with pores in 82% of the areas with invasion. Similar to overt CRCs, we noticed that the accumulated intraglandular material was being discharged through glandular pores into the juxtaposed peritumoral ECM.

Although cell locomotion is considered the most important factor in the local progression of tumors [13, 14], the results obtained in studies of colonic adenomas [54, 55] offer an alternative view to the cell-migration theory as the sole pathway of invasion. As in our earlier studies, we assumed that the release of proteolytic secretions through glandular pores in some colonic adenomas disrupted the surrounding matrix of the lamina propria mucosae, a mechanism that facilitates intramucosal penetration by neoplastic cells and initiates a committed process of host invasion [45, 50].

Preliminary results indicate that the flat tumor cells in the dilated neoplastic glands at the advancing edge of colonic carcinomas fail to express the proliferation marker Ki67 [56] (**Figure 2C**), but overexpress the mutated p53 protein [57] (**Figure 2D**). These results showed, to our knowledge for the first time,

that the proliferation of p53-positive flat neoplastic colonic cells at the invading edge is arrested. It was inferred that a temporary cell-cycle arrest had occurred in these Ki67-negative neoplastic cells. This paradoxical biologic behavior of tumor cells may be connected with the subsequent formation of glandular pores.

In light of these findings, we speculate that the arrest of cell proliferation at the advancing tumor edge in colonic carcinomas occurs independently of p53 mutation [57]. We further propose the possible existence of two independent molecular systems at the advancing edge of colonic carcinomas, one that supervises cell proliferation and the other that actively transfers the mutated p53 protein to daughter cells [56, 57]. Increased knowledge about the enzymatic cellular mechanisms occurring at the invading edge of CRC may lead to alternative therapeutic strategies that are not necessarily targeted at reducing tumor cell proliferation.

Please address all correspondences to Carlos A. Rubio, MD, PhD, Gastrointestinal and Liver Pathology Research Laboratory, Department of Pathology, Karolinska Institute and University Hospital, 17176 Stockholm, Sweden. Fax: 46 8 51774524; Email: [Carlos.Rubio@onkpat.ki.se](mailto:Carlos.Rubio@onkpat.ki.se)

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