

EDITORIAL

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Colorectal Cancer – a Tumour with Open Borders

Rak okrężnicy i odbytnicy – nowotwór o otwartych granicach

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Abstract

Despite a burgeoning literature regarding the histologic and molecular events taking place at the invasive tumour front of colorectal carcinomas (CRC), the mechanisms whereby this tumour progress from adenomas into the extracellular matrix (ECM) have not been disclosed... until now. In a series of studies we found in colorectal adenomas and at the growing edge of sporadic CRCs dilated neoplastic glands with pores (i.e. default glands due to the lack of a group of consecutive lining tumour cells). Through those glandular pores the retained glandular stuff, namely mucin, inflammatory cells and/or necrotic material is siphoned off directly into the juxtaposed ECM. These produces rich in proteolytic enzymes disrupt the paratumoural anatomy of the ECM. 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. The sealing of the glandular flaws will permit the re-accumulation of new intra-glandular proteolytic material, a mechanism that will replicate a new wave of host invasion at the growing edge, thus ensuring a stepwise but everlasting tumour progression in untreated patients (*Adv Clin Exp Med* 2005, 14, 5, 863–867).

Key words: colorectal tumours, progression, mechanisms.

Colorectal carcinoma (CRC) is the second most common cancer in Europe when both males and females are being considered together.

Several mucosal precursors are known to antedate a CRC. Today one can divide the cause(s) that lead to those precursors into three main categories: a) environmental (including diet), b) hereditary (mainly familial adenomatosis polyposis – FAP and hereditary non-polyposis colorectal cancer – HNPCC) and c) chronic inflammatory. These causes may encourage the formation of dysplastic foci of abnormal cell proliferation known as adenomas and non-adenomatous dysplasias.

The dysplastic foci induced by environmental factors are called sporadic adenomas, those induced by hereditary syndromes, genetically-induced adenomas, and the ones induced by chronic mucosal inflammation such as in IBD – ulcerative colitis (UC) and Crohn's disease of the colon (CC – Crohn's colitis) dysplasia in flat mucosa. Occasionally, extended irregular foci of flat or exophytic dysplastic mucosa – known as dysplasia-associated lesion or mass (DALM) – evolve in IBD patients. IBD patients may also develop sporadic adenomas in areas without inflamma-

tion. It should be born in mind that each one of these dysplastic lesions may proceed to invasive carcinoma.

It has been calculated that CRCs accounts for 11–15% of all cancer cases in the Western world. About 2% of all colon cancer cases are attributed to HNPCC, 1% to FAP and 1% to IBD. The remaining CRCs are called sporadic.

It is to be understood that HNPCC is not a form of cancer, but a syndrome that includes people at high risk for colon cancer. Although HN in HNPCC means nonpolyposis, colonic polyps – even if small – are the ultimate precursors of CRCs in HNPCC patients.

The crucial question is how these dysplastic areas of abnormal cell proliferation acquire the property to become hostile and invade the host? A fair comparison would be with that of a volcano that is dormant for many years but suddenly erupts, without warning. Its ferocious magma invades surrounding fields, devastating near and distant villages, often killing many inhabitants. In this respect it has been calculated that it takes 20 to 40 years for an adenoma to become hostile and erupts (i.e. to become invasive). As regards killing

people, it should be mentioned that the crude survival rate after curative resection for CRC in most large series leaves only 40% to 60% of survivors after 5-years.

A visit to Google shows 221.000 entries for adenomas and colon, 52.000 for adenomas and rectum, 5.240.000 for cancer and colon and 576.000 entries for cancer and rectum. So there appears to be some concern for the study of colorectal neoplasias. In attempts to unveil the histological parameters involved in neoplastic growth that are useful in estimating the potential aggressiveness of CRCs several investigators have concentrated their observations on the growing tumour edge. Some of the parameters studied are the growth pattern (expansive vs. infiltrating, the degree of tumour differentiation, foci of up to 5 cancer cells (called tumour "budding") and the occurrence of peritumoral lymphocytes, the latter to estimate the immunologic reaction of the host. Other authors have focused their studies on the kinetic ability of cancer cells to migrate into the surrounding matrix; they maintain that tumour cell locomotion is the single most important parameter accountable for the local progression of the tumour. Others have investigated the immunohistochemical expression of tumour cells such as cellular proliferation, p53 expression, K-ras mutations, cyclin E and CDK, bcl-2, K(+) ion channels from the HERG1 protein family, and carbonic anhydrase-related protein VIII (CA-RP VIII). To assess the role played by the extracellular matrix (ECM) in tumour penetration, parameters such as angiogenesis, telomerase activation, cathepsin B, CD10 expression, increased membrane type 1 matrix metalloproteinase, TGF- α signaling in fibroblasts, and trimeric laminin 5 expression have been explored.

Proteolytic enzymes have been found necessary for the dissolution of the peritumoral stroma. Proteolytic enzymes native to the ECM (v.gr. matrix metalloproteinases – MMPs), cathepsins and serine proteases are claimed to cause the disintegration of the paratumoural ECM, thus encouraging tumour cell progression. Masaki et al. maintain that the proteolytic degradation of 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, membrane-type MMPs (MT-MMPs) and other MMPs. According to Friedl and Wolf the peritumoral breakdown of the ECM would generate localized matrix defects and remodelling along migration tracts.

However, increased collagen degradation by MMPs can even be evoked in experimentally induced colonic obstruction (i.e. in the absence of

a growing tumour). Another argument against the significance of MMPs in tumour progression is the failure of broad-spectrum MMP inhibitors in clinical trials. Joyce et al. postulated that although ECM degradation has been attributed to MMPs, it is clear that different classes of cancer cell proteases contribute to tumour penetration, with cathepsins being directly involved in the degradation of ECM (including laminin, fibronectin, and collagen). Degradation of the ECM may also come about through modulation of protease-sensitive regulatory networks, involving other proteases and non-proteases such as annexin II (found at the cellular surface of cancer cells). Other recently found enzymes produced by cancer cells are heparanase, serine/threonine kinase AKT and lysozyme.

Notwithstanding, despite that burgeoning literature the series of histologic events telescoping from dysplastic glands in adenomas to invasion into the submucosa or beyond have remained enigmatic... until now! In a preliminary investigation we found at the growing edge of sporadic CRCs dilated neoplastic glands with pores (i.e. default glands due to the lack of a group of consecutive lining tumour cells). In those studies it was observed that through the glandular pores the retained glandular stuff, namely mucin, inflammatory cells and/or necrotic material was being siphoned off directly into the juxtaposed ECM (Fig. 1–3). These produces rich in proteolytic enzymes disrupt the paratumoural anatomy of the ECM. It was inferred that this mechanism would facilitate tumour penetration. We have demonstrated that colorectal tumour cells are able to neo-produce lysozyme. Lysozyme is an innate enzyme with potent nonimmunological antibacterial properties in the upper intestinal tract. Under normal conditions, lysozyme is not secreted in the lower intestinal tract (i.e. colon and rectum). It is therefore surprising that neoplastic cells deriving from lysozyme-nonsecretory

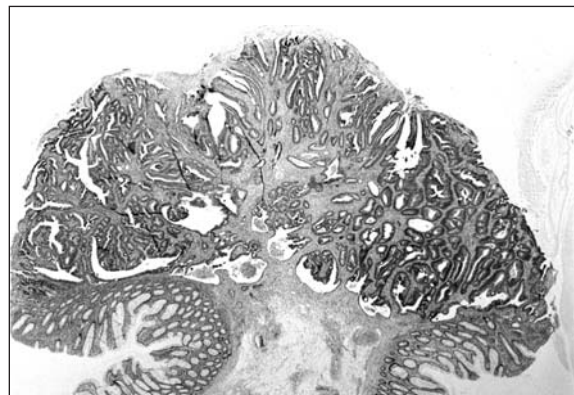


Fig. 1. Overview showing a colonic adenoma with submucosal invasion. Note dilated submucosal neoplastic glands with pore formation at the invading tumour edge (H&E 4 \times)



Fig. 2. Detail from a submucosal neoplastic gland at the invading tumour edge. Note pore formation at arrows and direct contact between the glandular material and the peritumoural ECM (MNF 116 immunostain, 40×)



Fig. 3. Another example to show pore formation in a submucosal neoplastic gland at the invading tumour edge. Note broad pore formation at arrows, the release of the glandular contents and destruction of the periglandular ECM (MNF 116 immunostain, 40×)

normal colorectal cells acquire the capacity to produce that enzyme. The complex biochemical manufacture of lysozyme suggests that its neo-production may not be a haphazard, capricious event in mutated colorectal epithelial cells but part of

a more elaborated molecular task. One of the options for that disparate secretion is that the acquired lysozyme in neoplastic colorectal cells differs from the innate lysozyme found in Paneth cells of the small intestine and from the acquired lysozyme in metaplastic Paneth cells in ulcerative colitis and Crohn's colitis. The aim of acquired lysozyme in neoplastic colorectal cells may be other than antibacterial. It should be pointed out that lysozyme is only a generic name, and that under this word at least 80 different compounds might be listed. Lysozyme is present in materials discharged through tumoral glandular pores into the paratumoral ECM. Neutrophils, sometimes found in neoplastic glands at the tumour edge, also release lysosomal enzymes into the ECM. It is therefore conceivable that acquired lysozyme in colorectal neoplasia mirrors a molecular event which is at variance with the antibacterial task of innate or acquired lysozyme in IBD.

Subsequent studies of large sections from 112 colectomies and rectal amputates revealed that the aforementioned histological parameters were similarly frequent both in colonic and rectal adenocarcinomas. As preoperative irradiation was administered only to patients with rectal tumours, it was inferred that those glandular pores were neither evoked nor abrogated by irradiation. Studies of tumour stage indicated that glandular pore formation was unrelated to the ability of colorectal tumours to metastasise to regional lymph nodes. Further studies of the growing tumour edge in sporadic CRCs in patients with inflammatory bowel disease [19], in carcinomas from patients with HNPCC, and in chemically induced colonic carcinomas in rats, as well as in sporadic esophageal adenocarcinomas in patients with Barrett's esophagus, showed a similar sequence of events, namely dilated glands – pore formation and release of glandular products into the paratumoral ECM. A similar proteolytic mechanism appears to be valid for signet ring cell carcinomas.

In a recent survey we investigated 61 colonic polyps: 47 were adenomas without invasion and 14 were hyperplastic polyps. Glandular pores were recorded above the muscularis mucosa in 25% (3/12) of the tubular adenomas, in 33% (2/6) of the serrated adenomas, in 50% (4/8) of the tubulo-villous adenomas, and in 67% (14/21) of the villous adenomas. None of the 14 hyperplastic polyps had glandular pores. While cell locomotion is considered to be the most important parameter accountable for the local progression of tumours, the results obtained with colonic adenomas offer an alternative view to the cell-migration theory (as the sole pathway of invasion). In concert with our earlier studies we assumed that the release of proteolytic se-

cretions through glandular pores in some colonic adenomas disrupted the surrounding matrix of the lamina propria mucosa, a mechanism that would facilitate the intramucosal penetration by neoplastic cells, setting aflame a committed process of host invasion. It may be argued that pore formation in invading tumour glands (described here and in our previous publications) is a haphazard event. But if that is the case, why that phenomenon mainly occurs at the growing edge of CRC and not within the tumour mass? And why are glandular pores more often found at the growing edge of overt invasive carcinomas than in adenomas without invasion?

More recently we found at the invading front of 56 colonic adenomas having submucosal invasion that 98% or 55 of the 56 areas with invasion had one or more neoplastic dilated glands with pores. In 82% of the tumours submucosal glands with po-

res recorded at the invading tumour front were numerous. In similarity to overt CRCs the accumulated intra-glandular material was discharged through glandular pores into the peritumoral ECM. Even here the proteolytic enzymes released through the pores lead to the breakdown of the peritumoural ECM at the growing tumour edge. It was entertained that to remodel the defective glands, malignant cells proliferating from the tip of the free borders of the pores will invade the enzymatically-disrupted matrix, with the goal to achieve glandular continuity. The sealing of the glandular flaws will permit the re-accumulation of new intra-glandular proteolytic material, a mechanism that would replicate a new wave of host invasion at the growing edge, thus ensuring a stepwise but everlasting tumour progression in untreated patients.

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