

Morphological Events Found at the Invading Edge of Colorectal Carcinomas in Baboons

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Abstract. *Background:* Earlier studies at the growing edge of colorectal cancer (CRC) in humans and rats have shown dilated neoplastic glands, some with a thin layer of flattened tumor cells (FTCs), some lacking one or more groups of consecutive lining tumor cells (called glandular pores, GPs). *Materials and Methods:* The characteristics of the neoplastic glands at the invading edge of CRCs were investigated in 39 baboons. A total of 190 neoplastic glands were studied in the 38 cases of glandular-forming adenocarcinomas. *Results:* In the studied neoplastic glands FTCs or GPs were recorded in 44.7% (85 glands). FTCs were found in 9.5% (18 glands) and GPs in 35.3% (67 glands). Only 7.9% or 3 out of the 38 animals showed neoplastic glands with GPs in the bulk of the tumor. *Conclusion:* In similarity to colorectal adenocarcinomas in humans, flattened tumor cells and glandular pores were found at the invading tumor edge of colorectal adenocarcinomas in baboons. A possible mechanism of host invasion is proposed.

Cancer of the colon and rectum (CRC) accounts for 11-15% of all cancer cases in Western countries (1). The disease is common in North America, Europe and Scandinavia and less common in Asia, Africa and South America (2).

Dietary and other possible environmental factors are known to increase the risk for colorectal carcinogenesis (3). Another factor is inheritance, found in patients with FAP (familial adenomatous polyposis) (4) and HNPCC (hereditary non-polyposis colorectal cancer) (5). A third factor is chronic colorectal mucosal inflammation, found in patients with inflammatory bowel diseases (IBD) (6). All these factors encourage the development of precursor lesions, such as sporadic adenomas (dysplastic foci of

abnormal cell proliferation) (7) and, in IBD patients, of dysplasia in flat mucosa (8) or dysplasia-associated lesion or mass (DALM) (9).

In recent years, much research has centred on the mechanisms pertinent to the progression of colorectal carcinomas. Such studies have focused on three main areas, the histological characteristics of the tumor front (expansive vs. infiltrative patterns, degree of differentiation (10), foci of ≤ 5 tumor cells called buds (11) and peritumoral lymphocyte infiltration (12)), the kinetic ability of cancer cells to migrate into the surrounding matrix (13, 14), and the presence of tumor biomarkers related to cell proliferation (15, 16) p53 mutation (16), angiogenesis (17), telomerase activation (18) and increase of membrane matrix metalloproteinase (MMP1 (19)), among others. Notwithstanding, the rapidly increasing literature on colorectal carcinogenesis, the mechanism whereby CRC locally invades the host remains poorly understood.

In a series of studies in humans (20-25) and in rats (26) we investigated the characteristics of the neoplastic glands at the invading front of CRCs. Those studies revealed the presence of dilated neoplastic glands, some with a layer of flattened cells (FTC), i.e. tumor cells having a $>50\%$ reduction in height when compared to other tumor cells in the same gland, and others with a gap in the tumor-lining cells called pores (GP) (20).

The purpose of the present work was to investigate whether such changes occurring at the growing tumor edge of CRC in humans (20-25) and rats (26) also developed in a cohort of baboons with CRC.

Materials and Methods

The baboons were members of colonies at the Southwest National Primate Research Center. The conditions of animal housing have been reported elsewhere (27). Briefly, the animals were housed in metal and concrete indoor-outdoor cages and were fed commercial monkey diets occasionally supplemented with a variety of fruit and vegetables. Water was available *ad libitum*. Baboons were euthanized with a commercial barbiturate euthanasia agent or died naturally, were necropsied and tissue samples were fixed in 10% neutral-buffered formalin, processed conventionally, embedded in paraffin,

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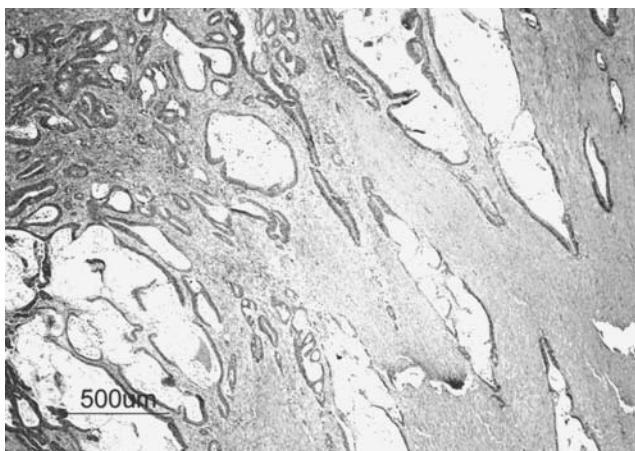


Figure 1. Low power view of an invasive colonic carcinoma (baboon, H&E 2.5x).

cut at 5 μm, stained with hematoxylin and eosin (H&E), and evaluated using light microscopy. All the procedures were conducted in accordance with the Institutional Animal Care and Use.

Between 1984 and 2006 a total of 41 baboons developed a CRC at the Southwest National Primate Research Center. Ten out of the 41 cases having a colonic carcinoma and Crohn's disease have been reported previously (28).

H&E stained sections were retrieved from the files of the Southwest National Primate Research Center. Five consecutive neoplastic glands, found at the invading tumor front, were studied and those glands having FTC and/or GP were particularly noted.

Results

Frequency. Between 1984 and 2006, the mean number of baboons housed per year at the Southwest National Primate Research Center was 3315 animals (range 2578-3931 animals). Hence, during a period of 26 years (between 1984 and 2006), 1.58 CRCs/year developed at this facility.

Localization. Of the 41 CRCs, 40 were found in the colon and the remaining one, in the rectum.

Histological classification. Sections were available for 39 out of the 41 cases.

Of the 39 adenocarcinomas (Figure 1), 38 were glandular-forming adenocarcinomas and the remaining one (2.6%), a signet-ring cell carcinoma. Of the 38 glandular-forming adenocarcinomas, 1 (2.6%) was highly differentiated, 35 (89.7%) moderately differentiated and 2 (5.1%) poorly differentiated.

Neoplastic glands at the invading tumor front. A total of 190 neoplastic glands were investigated in the 38 glandular-forming

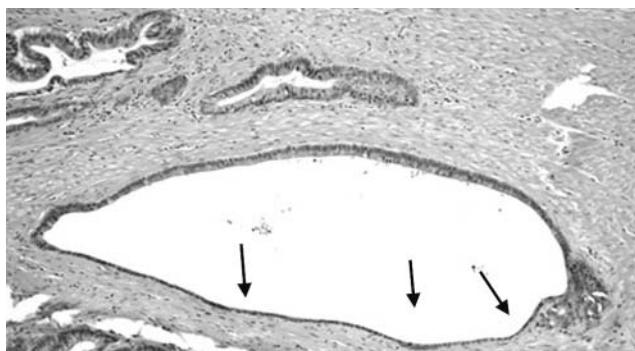


Figure 2. Neoplastic gland at the tumor edge. Note a thin layer of tumour cells (at arrows) facing the juxtaposed extracellular matrix (ECM) of the host (baboon, colonic carcinoma, H&E 10x).

adenocarcinomas. FTC or GP were recorded in 44.7% or in 85 out of the 190 glands. i) With flattened tumor cells. Neoplastic glands with FTC (Figure 2) were found in 9.5% or in 18 out of the 190 neoplastic glands. ii) With pore formation. Neoplastic glands with GP were found in 35.3% or in 67 out of the 190 neoplastic glands studied. Only 7.9% or 3 out of the 38 animals having glandular-forming adenocarcinomas showed neoplastic glands GP in the bulk of the tumor. In neoplastic glands with GP, retained mucus (Figures 3 and 4), occasionally containing neutrophils or necrotic material, was seen released directly into the juxtaposed matrix through the glandular pores. Hence, that material came in direct contact with the surrounding extracellular matrix (ECM).

In the remaining 105 of the 190 neoplastic glands investigated at the invading tumor front, FTC or GP were not found.

ECM changes within the extruded products. The matrix within the extruded products often showed edema, apoptotic granules, fragmented hyaline and cell debris.

Discussion

The CRCs in baboons displayed similar dilated neoplastic glands at the invading tumor edge to those found in CRCs in humans (20-25) and in carcinogen-treated rats (26). Nearly 45% of those glands showed FTC and cellular rifts (GPs). Similar changes were found in both the baboons having colonic carcinoma and Crohn's disease (28) and in the baboons without chronic mucosal inflammation (here called sporadic CRCs).

Those microscopic alterations were mainly found in the outermost aspect of the neoplastic glands juxtaposing the peritumoral ECM. Since occasional tumor cells at the tip of the glandular pores were flat, it would appear that flattened tumor cells might have preceded the formation of pores in those glands. The biological behaviour of the flat tumor

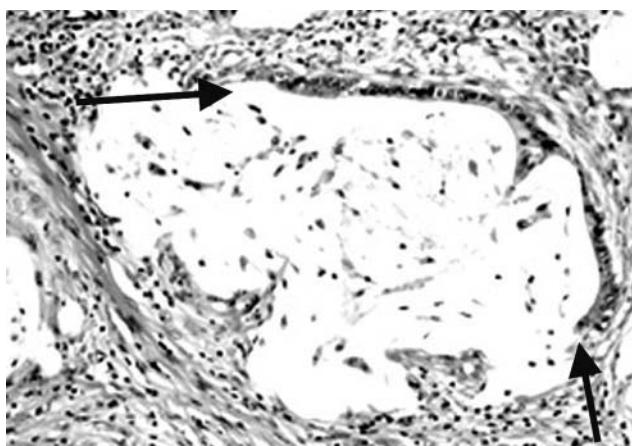


Figure 3. Neoplastic gland at the tumor edge. Glandular pore, (at arrows) through which the intraglandular mucus, is being extruded. The mucus has apparently disrupted the boundaries of the juxtaposed extracellular matrix (baboon, colonic carcinoma, H&E 20x).

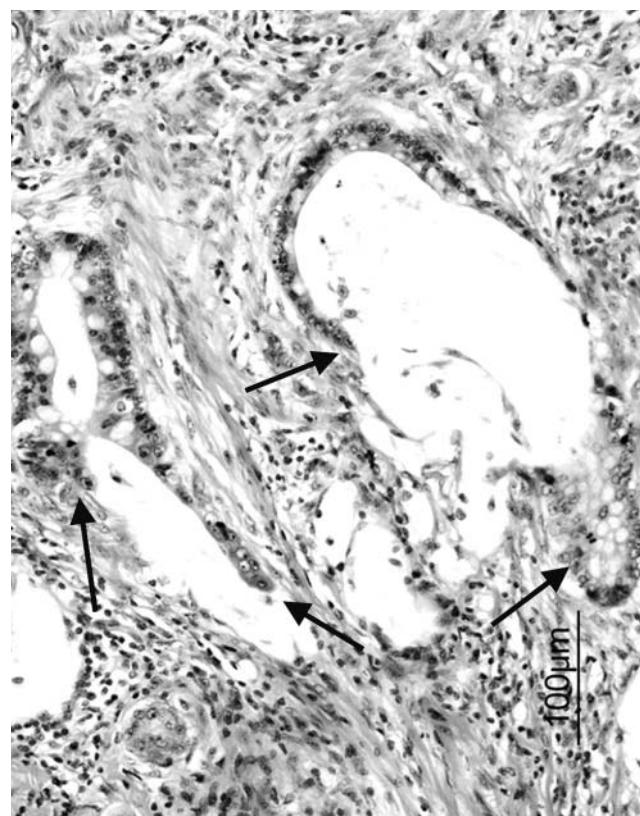


Figure 4. Neoplastic gland at the tumor edge. Another view of glandular pores, (at arrows) through which the intraglandular mucus, is being extruded. The extruded secretion has apparently disrupted the juxtaposed ECM (baboon, colonic carcinoma, H&E 40x).

cells, *i.e.* of the cells preceding the formation of the glandular pores at the invasion tumor front, has recently been demonstrated in CRCs in humans (29).

It may be argued that pore formation in invading tumor glands is a haphazard event. But if that were the case, why would they mainly occur at the growing edge of CRC but only occasionally within the tumor mass?

As in the human and rodent counterparts, the retained glandular material at the invading edge of CRC in baboons was seen to be released directly into the juxtaposed ECM.

In recent years, a series of proteolytic enzymes released by tumor cells have been detected including proteases (30-32), heparanase (33), trypsin (34), serine-arginine protein kinase 1 (35) and Class Pi glutathione transferase (36). More recently a total of 50 differentially expressed proteins in cancer and normal tissues of the colon were identified (37). The intraglandular accumulated secretion, when released through the pores, disrupts the paratumoral composition of the ECM, thus promoting further tumor penetration. Rationally, proteolytic enzymes might also be released by signet-ring tumor cells.

While cell locomotion (13, 14) is considered to be the most important parameter accountable for the local progression of colorectal tumors, the results here reported in baboons offer an alternative view to the tumor cell migration theory (13, 14) as the most important in cancer invasion.

Thus, at the growing tumor edge of sporadic and IBD-associated CRCs in baboons, a similar sequence of events as in humans (21-25) with either sporadic, or IBD-associated CRCs, and inherited CRCs in HNPCC or in rats treated with a colonotropic carcinogen (26). Moreover, a similar sequence of events as in the CRCs in baboons has

been found in adenocarcinomas of the esophagus in humans (38). The release of proteolytic enzymes through glandular pores at the invading front of adenocarcinomas would encourage the subsequent disruption of the surrounding ECM, thus facilitating further tumor penetration. It is assumed that the malignant cells would proliferate from the tip of the free borders of the pores, and invade the enzymatically-disrupted matrix thus restoring glandular continuity. The sealing of the glandular flaws would permit a renewed intra-glandular accumulation of proteolytic material, a mechanism that would initiate a new wave of host invasion at the invading front, thus ensuring a stepwise but everlasting tumor progression in untreated animals.

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