Arrest of Cell Proliferation in Budding Tumor Cells Ahead of the Invading Edge of Colonic Carcinomas. A Preliminary Report

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Abstract. *Background: Single or small groups of dedifferentiated tumor cells, referred to as tumor budding, may be found ahead of the defined invasive edge of colorectal carcinomas. Tumor budding is claimed to correlate with lymph node metastasis and local recurrence. Materials and Methods: Sections from six surgically removed invasive colonic carcinomas were stained with hematoxylin-eosin (H&E), MNF 116 (an epithelial marker) and for Ki-67 (a proliferation marker) and four specimens also for laminin-5 (a cell-locomotion marker). Results: In comparative fields, a mean of 86.2 tumor buds (range 60-132) were detected with MNF 116 immunostain, 5.8 tumor buds (mean, range 5-9) with H&E stain, 9.7 tumor buds (range 7-18) with Ki-67 immunostain and 9.3 tumor buds (range 8-12) with laminin-5. Conclusion: The majority of the MNF 116-positive budding tumor cells ahead of the invading tumor front were not detected with H&E, or for Ki-67 or laminin-5. Host invasion by budding tumor cells might be activated only after the cell cycle has been switched off (G0).*

Several reports have indicated that single or small groups of dedifferentiated tumor cells, referred to as tumor budding, often occur ahead of the defined invasive edge in different cancer locations such as the lip (1), the tongue (2), the lung (3) and the colorectum (4, 5).

In recent years, much interest has centred on tumor budding in the colorectum due to its reported association with tumor aggressiveness (4-24). It has been postulated that tumor budding is more useful than other accepted parameters (massive submucosal invasion, vascular invasion or poor differentiation) in predicting lymph node metastasis or local recurrence (18).

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We have previously investigated tumor behavior of colorectal carcinomas at the leading invading edge (25-30), not only by conventional hematoxylim-eosin (H&E) stains but also with the broad-spectrum epithelial marker MNF 116 immunostain. Neoplastic glands were found to display either flattened cells or cellular gaps called glandular pores. The flattened glandular tumor cells and those at the tip of the pores were often *p53* mutated but lacked signs of cell proliferation, as deduced by Ki-67 (clone MIB1) immunostain. The possibility that the Ki-67-negative neoplastic cells had been removed from the cell cycle (G0) was proposed. The apparent paradoxical biological behaviour of the neoplastic cells at the tumor edge suggested the possible existence of two independent molecular systems, one transferring the *p53* mutation to daughter cells and the other supervising cell proliferation.

In the present work, the proliferation of tumor budding cells ahead of the invading edge of colorectal carcinomas, was investigated.

Materials and Methods

A total of six consecutive surgically resected colonic carcinomas (from 3 males aged 58, 72 and 82 years, and from 3 females aged 69, 78 and 79 years) were investigated,

A representative area showing the most numerous dedifferentiated tumor cells (single or in small groups of neoplastic cells) ahead of the defined invasive edge was selected in H&E-stained sections. From the same blocks in the six cases, consecutive sections were immunohistochemically stained with cytokeratin MNF 116 (Dako Automation) or with the proliferation antibody Ki-67 (clone MIB1, Dako Automation). In addition, consecutive sections in four of the six specimens were immunohistochemically stained for laminin-5, gamma 2 chain (Dako Automation). Laminin-5 is claimed to be part of the epithelial anchoring system and of cell locomotion (3, 16).

The number of H&E, MNF 116, MIB1 and of laminin-5 labeled cells ahead of the defined invasive edge was calculated in comparative areas (at 10× magnification) previously selected by H&E stain.

Table I. *The number of immunostained budding tumor cells in corresponding fields ahead of the invading tumor edge of colonic adenocarcinomas.*

Case #	MNF 116	H&E	MIB1	Laminin-5
1	60	5	7	9
2	92	6	9	8
3	132	9	18	12
4	82	5	9	8
5	67	4	7	
6	78	6	8	
Total	511	35	58	37
Mean	85.2	5.8	9.7	9.3

MNF 116 (an epithelial marker), hematoxylin-eosin (H&E), MIB1 (a proliferation marker) and laminin-5 (a cell locomotion marker).

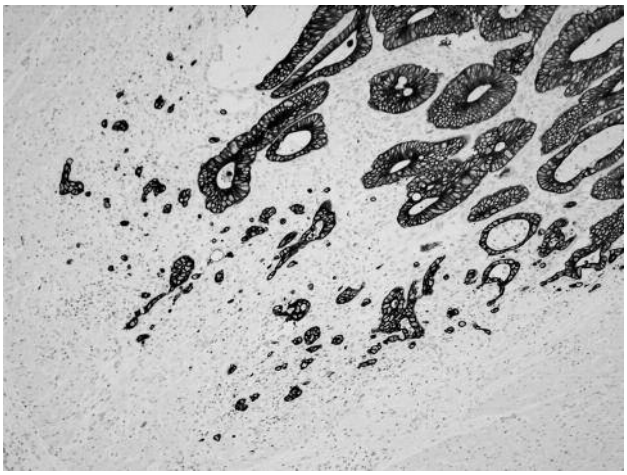

Figure 1. *Numerous budding tumor cells ahead of the invading edge of a colonic carcinoma (MNF 116 immunostain, ×10).*

Results

Table I shows the number of budding tumor cells recorded in selected areas stained with MNF 116 and in comparative fields stained with H&E stain and with MIB1 in all six cases and with laminin-5 in four of the cases.

Whereas a mean of 86.2 tumor buds (range 60-132) was detected with MNF 116 immunostain (Figure 1), only 5.8 tumor buds (mean, range 5-9) were found with H&E stain, 9.7 tumor buds (mean, range 7-18) with MIB1 immunostain (Figure 2) and 9.3 tumor buds (mean, range 8-12) with laminin-5 (Figure 3).

Discussion

Immunostain for Ki-67 is known to label all cells actively participating in the various phases of the cell cycle, namely G1, S, G2 and M (31), unlabeled cells being in a resting phase (G0) (32). Cyclin A was not used since it binds mainly to the S-phase Cdk2.

As the broad-spectrum cytokeratin MNF116 labels all epithelial cells, including budding tumor cells, the observation that the proliferation marker Ki-67 detected only a small fraction of the MNF 116 labeled cells, was unexpected. Similarly, only a few budding tumor cells were recorded in the H&E stained sections and with the laminin-5 immunostain (when compared to those found by the broad-spectrum cytokeratin MNF 116 in comparative areas).

Several authors have assessed the number of budding tumor cells in H&E-stained sections (4, 5, 10-12, 14, 15, 18, 22) and with laminin-5 immunostain (3, 16, 20-24). However, laminin-5 detected only a small fraction of the MNF 116-labeled cells in the present study. Thus, laminin-5 appears to be an unreliable stain in assessing the actual number of budding tumor cells.

Tumor cells are by definition those that have acquired the capacity of autonomous, immortal proliferation; that is, they do not respond to normal growth control (31) when not halted by anoxic distress, vascular obstruction or radiation (25). Notwithstanding, the majority of the budding tumor cells were shown to refrain from cell proliferation in the present study. It remains enigmatic why budding tumor cells with an invasive "instinct" to overcome the host should at the same time curb their most (assumed) powerful weapon, that is, increased cellular proliferation. In the light of these and of previous findings (25), it would appear that cell proliferation at the invading tumor edge in colonic carcinomas might be regulated by a novel molecular system.

As each surgical case was fixed on a different day and at a different time of the day, it might be deduced that the majority of the budding tumor cells were in G0, at any given time. A possible explanation for this conundrum might be that after being sequestered from the cell cycle, G0-budding tumor cells redeploy their molecular attributes, focusing on signals that trigger local invasion, a crucial biological process linked to lymph node metastasis and local recurrence (8, 18-20).

Hence, the arrest of the cell cycle might be an essential prerequisite to encourage budding tumor cells to resume local tumor invasion. It should be mentioned that a similar switch-off phenomenon of the cell cycle has recently been demonstrated in carcinoma *in situ* cells (26, 27) and in neoplastic glandular cells at the invasion edge of colonic carcinomas (25).

In summary, it was demonstrated for the first time that the majority of the budding tumor cells ahead of the invading tumor front are not actively participating in the proliferation cycle. It was speculated that host invasion by budding tumor cells might be activated only after the cell cycle has been switched off (G0).

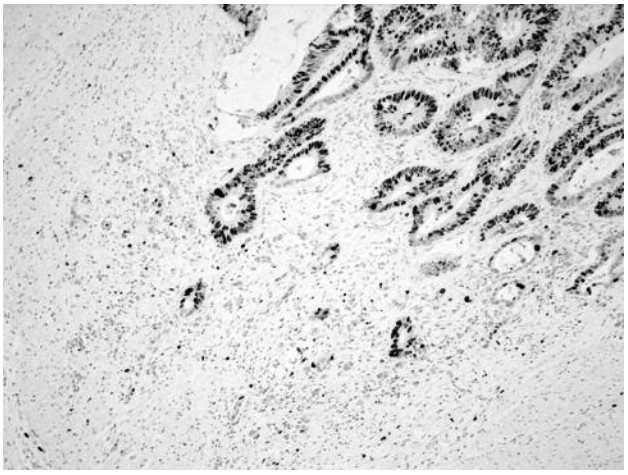

Figure 2. *A comparative field to that shown in Figure 1, stained with the proliferation marker Ki-67, clone MIB1 (×10).*

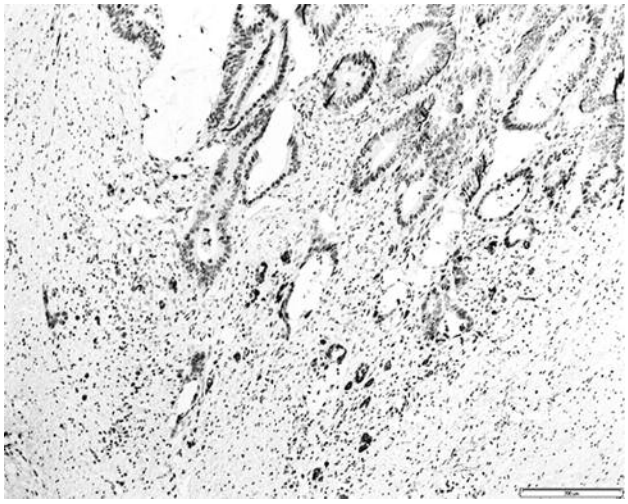

Figure 3. *A comparative field to that shown in Figure 1, stained with laminin-5 (×10).*

A previously non-envisaged regulatory molecular pathway, able to orchestrate the arrest of the proliferation cycle (G0) in budding tumor cells and to promote increased cell locomotion, a *sine qua non* machinery that secures host invasion, might occur.

More research is necessary to disclose the molecular events responsible for the arrest of the proliferation cycle of budding tumour cells and for the assumed increased cell locomotion ahead of the invading edge of colorectal carcinomas.

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