Cell Proliferation at the Leading Invasive Front of Colonic Carcinomas. Preliminary Observations

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Abstract. Dilated neoplastic glands, some with a layer of flat tumour cells and others lacking a group of consecutive lining tumour cells (i.e., glandular gaps called pores), were previously found at the leading invading tumour edge of colorectal carcinomas. Through the glandular pores, the retained intraglandular material was siphoned off directly into the juxtaposed extracellular matrix (ECM). The tumour cell fabricates, rich in proteolytic enzymes, disrupted the paratumoral anatomy of the ECM and encouraged further tumour penetration. In this work, cell proliferation in the neoplastic glands of the outermost advancing front of seven colonic carcinomas was studied with the proliferation marker Ki67. A total of 105 neoplastic glands were investigated in the seven tumours. In 33 of the 35 neoplastic glands with flat tumour cells, no Ki67 expression could be recorded in the flat cells. In the other two neoplastic glands, only occasional flat tumour cells showed Ki67 expression. The remaining (non-flat) neoplastic cells in the lateral and proximal aspects in the same 35 glands showed Ki67 expression. In 19 out of the 35 neoplastic glands with pores, the tumour cells at the tip of the pores (non-flat) showed Ki67 expression. In the remaining 16 neoplastic glands with pores, the tumour cells at the tip of the pores showed no Ki67 expression. In 15 out of the 35 neoplastic complete glands (i.e., having neither flat tumour cells nor epithelial pores) variable amounts of tumour cells lacking Ki67 expression were seen at the leading invading front. In the remaining 20 complete glands, all tumour cells showed Ki67 expression at the leading invading front. These preliminary results showed, for the first time, that human colonic flat neoplastic cells arrest their proliferation at the invading tumour front. The possibility that these Ki67-negative tumour cells were arrested in G1-phase was entertained. It is not inconceivable that this unexpected paradoxical biological behaviour of flat tumour cells might be connected with the formation of glandular pores at the level of advancing invasion in colonic carcinomas.

Much research has centred on the mechanisms pertinent to the local progression of colorectal carcinomas (CRCs). Some authors studied the histological characteristics of the invading tumour: expansive vs. infiltrative and tumour differentiation (1), foci of ≤5 tumour cells called buds (2) or peritumoral lymphocyte infiltration (3) (to study the reaction of the host). Others (4, 5) assessed the kinetic ability of cancer cells to migrate into the surrounding matrix; they maintained that tumour cell locomotion is the single most important parameter accountable for the local progression of tumours (4, 5). A third group of researchers studied different tumour biomarkers, such as cell proliferation (6), p53 mutations (7), angiogenesis (8), telomerase activation (9) and increased membrane matrix metalloproteinase (MMP (10)), to name some. Nevertheless, despite increased reporting, the mechanism whereby CRCs locally invade the host remains poorly understood.

In a series of studies, we investigated the invading tumour edge of CRCs (11-17) in archival sections stained with haematoxylin and eosin (HE). The sections were also immunostained with cytokeratin MNF 116 to enhance the anatomy of the glands. Those observations revealed the presence of dilated neoplastic glands at the invading front, some with a layer of flat tumour cells and others lacking a group of consecutive lining tumour cells. The latter glandular gaps were called pores (11). The occurrence of neoplastic glands with flat tumour cells and with pores at the invading front was also demonstrated in experimentally-induced colonic adenocarcinomas in rats (15) and in Barrett’s adenocarcinomas of the oesophagus in humans (18).

Through glandular pores, the retained intraglandular material was siphoned off directly into the juxtaposed extracellular matrix (ECM). The tumour cell fabricates, rich in proteolytic enzymes (20-23), disrupted the paratumoral anatomy of the ECM. In this context, it should be mentioned that there is increased awareness that tumour cells produce...
proteolytic enzymes. Kopito and Sitiia (23) claimed that all cells are equipped with a proteolytic apparatus that eliminates misfolded and damaged proteins. In their review, Kroemer and Jäätelä (24) postulated that tumour invasion and metastasis are associated with altered lysosomal trafficking and increased expression of cathepsins, especially the cysteine cathepsins (cystatin B and cathepsin L), as well as the aspartate cathepsin, cathepsin D. In cancer cells, particularly those at the invasive edges of tumours, the localization of lysosomes often shifts from a perinuclear to a peripheral pattern and the lysosomal contents can be secreted into the extracellular space. Together with MMPs and the plasminogen-activator system, secreted cathepsins might participate in the degradation of the ECM (23).

Chen et al. (25) also recently found that 18 out of the 90 up-regulated proteins in pancreatic cancer were enzymes. These authors claim that enzymes generated by tumour cells lead to the proteolytic degradation of the ECM at the tumour cell surface and advancing tumour cell invasion (25).

While investigating neoplastic glands in the outermost advancing front of colonic carcinomas using the proliferation marker Ki67, we recently observed that flat tumour cells lacked Ki67 expression.

Materials and Methods

Histological sections from seven consecutive cases with overt, moderately-differentiated colonic adenocarcinoma were studied. Consecutive sections were histochemically-stained with HE and were immunostained with the proliferation marker Ki67 (Clone MIB-1, Dako Cytomation, Glostrup, Denmark).

Particular attention was paid to the advancing invasive edge at the invading tumour front, all seven invasive carcinomas revealing neoplastic glands with a layer of flat tumour cells. In addition, glands with epithelial pores (i.e., lacking a group of tumour cells) and complete glands (i.e., without thin tumour cells or glandular pores) were seen at the leading invading front.

Ki67 expression at the leading invading front was assessed in five consecutive neoplastic glands displaying a layer of flat tumour cells, in five consecutive neoplastic glands with epithelial pores and in five consecutive complete neoplastic glands (showing neither flat tumour cells nor glandular pores).

Results

Ki67 expression in neoplastic glands at the leading invading front of colonic carcinomas. A total of 105 neoplastic glands were investigated at the leading invading front in the seven colonic carcinomas.

In 33 out of the 35 neoplastic glands investigated, no Ki67 expression could be recorded in flat tumour cells (Figure 1). In the remaining two neoplastic glands, only occasional flat tumour cells showed Ki67 expression. The remaining (non-flat) neoplastic cells in the lateral and proximal aspects of the same 35 glands also showed Ki67 expression.

In 19 out of the 35 neoplastic glands with pores investigated, the non-flat tumour cells at the tip of the pores showed Ki67 expression. In the remaining 16 neoplastic glands with pores, the tumour cells at the tip of the pores showed no Ki67 expression.

In 15 out of the 35 neoplastic complete glands, variable amounts of tumour cells lacking Ki67 expression were seen at the leading invading front. In the remaining 20 complete glands, all tumour cells showed Ki67 expression at the leading invading front.

Discussion

As in previous investigations (10-17), at the leading invading edge of seven human colonic adenocarcinomas, neoplastic glands were found that differed morphologically from other glands in the bulk of the tumour. The morphologically-different neoplastic glands were characterised by flat tumour cells and by the absence of a group of tumour cells (i.e., pore formation). The flat tumour cells and the pores were usually found in the outermost leading edge of the neoplastic glands. It was speculated that the increased intraglandular pressure, conveyed by the accumulated cell secretions, induced cell compression against the juxtaposed ECM, leading to the formation of flat tumour cells. However, this possibility was rejected, since, in the same glands, remnant glandular tumour cells, also subjected to the same pressure, were not flat. Hence, if neither the intraglandular pressure against the surrounding stroma nor the surrounding stroma by itself were responsible for mechanical compression leading to the thinning of tumour cells (and eventually to pore formation by anoxic compression), the possibility that flat cells could be biologically different from those in the rest of the tumour, was considered. We found that the majority of the flat tumour cells was Ki67-negative. The absence of Ki67 expression in those cells strongly suggested that they were not synthesizing DNA. There was no indication that Ki67-negative tumour cells were undergoing apoptosis. Leuchtenberger bodies (26) were absent at the level of the glandular pores. Similarly, there were no histological signs of ongoing cell necrosis at the level of the pores. In contrast, the remnant tumour cells in the same glands showed intense Ki67 expression. The causes for this apparent targeted change in biological behaviour of the leading tumour cells remains unclear, but the change might be part of a molecular strategy aimed at encouraging neoplastic cells to become flat and subsequently disappear, leading to the formation of pores. That strategy would permit tumour-cell derived intraglandular secretions – rich in proteolytic enzymes (19-22) – to inundate and disrupt the surrounding paratumoral anatomy of the ECM, thus encouraging further tumour penetration.
The results of these observations raise a series of questions: a) That neoplastic cells can become flat has not been highlighted previously in the literature. The question here is: Do flat tumour cells mirror some type of "atrophic cell involution"? However, if flattened cells are the result of "atrophic cell involution", why do only the outermost glandular cells become flat and not those present in the proximal and lateral aspects of the same gland? As the glandular pores were often found surrounded by flat tumour cells, the possibility that the flat tumour cells had antedated the formation of pore in those glands was entertained.

b) By definition, tumour cells are cells that have acquired the capacity of autonomous, immortal proliferation. Tumour cells do not respond to normal growth controls (if not halted by anoxic distress, vascular obstruction, radiation, etc.). Hence, why do everlasting flat tumour cells (colonic) refrain from nuclear proliferation when in contact with the outermost stroma?

c) Could the intraglandular pressure alone halt the DNA synthesis of immortal cells? And, if that is the case, why do only the outermost flat tumour cells at the interphase between the advancing cancer and the juxtaposed stroma display cell proliferation arrest and not the cells in the lateral and proximal aspects of the same gland, which are also in direct contact with the peritumoral stroma?

d) Is it possible that the Ki67-negative flat tumour cells were arrested in the G1-phase of the cell cycle? If so, unreported genes might be regulating the proliferation in advancing tumour cells. The prevailing notion that cancer cells are devoted to autonomous, immortal proliferation should perhaps be re-considered (at least for the flat cells at the invading tumour front of colonic carcinomas).

e) If the theory of tumour cell locomotion is the sole mechanism of host invasion (4, 5), why do the outermost tumour cells, responsible for that locomotion, arrest their capacity to replicate?
f) Can a colonic carcinoma launch its invasion and at the same time curb its most powerful weapon to overcome the host by not increasing cell proliferation?

g) To date, it is unknown whether hypothetical crosstalk between the tumour cell-peritumoral ECM matrix, exists. But the possibility that both tumour cells and the stroma co-operate in the mechanism of invasion cannot be disregarded. On the other hand, if there is crosstalk between those two systems, why do only the tumour cells in the proximal and lateral aspects of a neoplastic gland – also in contact with the juxtaposed stroma – proliferate?

In conclusion, the results of this preliminary communication have generated many questions and, unfortunately, few answers. Notwithstanding, we have demonstrated, for the first time, that human colonic flat neoplastic cells at the invading tumour front display arrested proliferation. The possibility that these Ki67-negative tumour cells were arrested in G1-phase was entertained. It is not inconceivable that the unexpected paradoxical biological behaviour of flat tumour cells might be connected with the formation of glandular pores at the level of advancing invasion in colonic carcinomas.

Further research is necessary before one or more of the above questions can be definitively answered.

References


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