Abstract. Background: The finding was recently reported of clusters of colonic crypts lined with indigenous normal epithelium displaying irregular shapes underneath the adenomatous glands of conventional (tubular or villous) adenomas. These abnormal crypts were named non-dysplastic corrupted colonic crypts (NDCs). This study explored the characteristics of cell proliferation in NDCs present in a cohort of conventional adenomas. Materials and Methods: Sections from six conventional adenomas were challenged with the proliferation marker Ki-67 (MIB1). MIB+ proliferating clusters were regarded as those exhibiting two or more adjoining MIB+ cells. Results: A total of 46 (range=1-18) NDCs were found underneath the six conventional adenomas. Out of the 46 NDCs, two exhibited only one proliferative cluster/crypt, 14 NDCs two clusters/crypt, 14 three clusters/crypt and the remaining 16 NDCs more than four distinct clusters/crypt. Conclusion: This preliminary study showed, evidently for the first time, that multiple, apparently haphazardly distributed clusters of proliferating cells are present in NDCs. Since the Ki-67 proliferation marker only labels progenitor daughter cells generated by stem cells, each MIB+ cluster in each NDC must have been produced by a single stem cell. Consequently, individual NDCs may harbor several stem cells, a deduction that is in concert with recent studies showing that in the normal human colon, the number of stem cells per crypt is of the order of five to six, or about 5% of the cell population of a single crypt.

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Key Words: Colon, crypts, stem cells, morphology, proliferation.
Materials and Methods

Sections from six conventional adenomas diagnosed at the Department of Pathology, Karolinska University Hospital, were stained with hematoxylin and eosin (H&E) and immunostained with Ki-67 antigen (clone MIB1; DakoCytomation, Glostrup, Denmark). The monoclonal antibody to Ki-67 detects a nuclear antigen that is present only in proliferating cells. Immunostained sections were scrutinized for MIB1 positive (MIB1+) cells present in the NDC underneath conventional adenomas MIB+ proliferating clusters were regarded as those exhibiting two or more adjoining MIB+ cells. The number of MIB1+ cell clusters within each NDC was recorded.

Results

A total of 46 (range=1-18) NDCs were found underneath the six conventional adenomas. Out of the 46 NDCs, two exhibited only one MIB+ cell cluster/crypt, 14 had two MIB+ clusters/crypt, 14 had three and the remaining 16 NDCs had four or more MIB+ clusters/crypt (Figure 1).

Discussion

The normal mucosa of the colon is built with a single layer of epithelial cells with inward invaginations or folds called crypts. Sections cut perpendicularly to the surface epithelium show a characteristic appearance of ‘row of test tubes’ with parallel, tightly packed crypts of about the same size, resting on the muscularis mucosae. This architecture is retained throughout the colon, except in nominate grooves (cloverleaf-like crypts connected to a single lumen) (8). Despite the fact that crypts replicate by symmetric fission, beginning at their base and proceeding upwards until two identical individual crypts are created, crypt branching is rarely observed in fixed preparations (9).

Immunostaining for the nuclear antigen KI-67, a protein marker of DNA-synthesizing epithelial cells, reveals that proliferating cells, which are actively committed to cell division, occupy the slopes of the lower fourth of the crypts. In elderly patients (10) and in patients harboring large adenomas or carcinomas elsewhere in the colon (11, 12), colonic crypts show upper expansion of the normal proliferative zone. In contrast, in this study, a single zone was not found in NDCs but instead there were multiple (isolated) clusters of proliferating cells.

Fission of colonic crypts is orchestrated by the adenomatous polyposis coli (APC) gene. At the base of the crypt within the stem-cell niche, the stem cells generate more stem cells (13), as well as transient amplifying daughter cells, that is, progenitor cells responsible for the bulk of cell proliferation in the crypt (14, 15). Considering that each cluster of proliferating cells is orchestrated by a stem cell (14), the finding of multiple clusters of proliferating cells in NDCs is validated by recent findings indicating that normal colonic crypts are furnished with five to six stem cells (16).

In conclusion, this preliminary study showed, evidently for the first time, that multiple, apparently haphazardly distributed clusters of proliferating cells are present in NDCs. Since the Ki-67 proliferation marker only labels progenitor daughter cells generated by stem cells, each of the multiple MIB+ clusters in each NDC must have been produced by a single stem cell. Consequently, individual NDCs may harbor several stem cells, a deduction that is in concert with recent studies showing that in the normal human colon, the number of stem cells per crypt is of the order of five to six (16), or about 5% of the cell population of a single crypt (17).

Conflicts of Interest

The senior Author (KM) provides clinical and academic consultancy services to Q Medical Technologies.

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