The Regional Variation of Crypt Architecture in the Mouse Colorectal Mucosa with Special Reference to the Process of Proliferation

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Summary: The NaOH cell-maceration method was applied to the mouse colorectal mucosa to demonstrate the three-dimensional architecture of the lamina propria, especially that of the glandular crypts. In the surface of the lamina propria, there existed the longitudinal ridges and furrows. In the ridges, the crypts opened to the lumen individually, and had no internal partitions separating a crypt. The lateral walls of the crypts in the ridges had numerous hemispherical concavities into which the epithelial cells were tightly fitted. In the furrows, on the other hand, the crypts were subdivided into several smaller crypts by the internal partitions, and were termed as the “protocrypts”. Considered from these findings, the crypts in the ridges appear to be matured, while the protocrypts in the furrows to the crypts undergoing process of proliferation. Further, it is suggested that the crypts proliferate in the furrows and, then, populate in the ridges as the mature crypts.

There is still some uncertainty regarding the mucosa in the colorectal region, especially the proliferation of glandular crypts during the postnatal growth, which stands in contrast to many detailed studies on the mucosa in the stomach and the small intestine. In general, it is described that the glands in the colorectal region are simple tubular type in the textbooks on human histology. However, Levine and Haggitt (1992) mentioned that branching of crypts occurred normally. The branching is suggestive of the proliferation of crypts. Further, in rodents there is an evidence suggesting that the proliferation of crypts exists even in the adult (Maskens, 1978; Cheng and Bjerknes, 1985). The mechanism by which the crypts proliferate is a longitudinal partition of pre-existing crypts through a median upheaval of the mucosa at their base; i.e., the epithelial surface topography does not reflect the actual proliferating process (McCarthy and Kaye, 1990). Therefore, direct observation of the bottoms of the crypts by SEM, after removal of the epithelial layer, is useful for the study on the proliferation of crypts.

On the other hand, the crypts is subject to considerable pathologic distortion by colorectal disease such as ulcerative colitis and adenoma. The proliferation of crypts should not be misinterpreted as pathologic distortion of the normal crypts. This paper reports some of the findings of the proliferation of crypts in the colorectal region, and is expected to contribute towards further understanding of morphogenesis and pathologic change in this region.

Material and Method

20 male ddY mice aged from 6 to 8 weeks were killed by chloroform anesthesia and their descending colon and rectum were removed. The specimens were fixed with 2.5% glutaraldehyde in phosphate buffer for 2 hours and rinsed in distilled water. They were processed by the NaOH cell-maceration method by Ohtani (1987): they were immersed in a 10% NaOH solution for 4 days at room temperature (about 25°C) and rinsed in distilled water. They were processed by the NaOH cell-maceration method by Ohtani (1987): they were immersed in a 10% NaOH solution for 4 days at room temperature (about 25°C) and rinsed in distilled water until they became transparent. After immersion in 1% tannic acid for 2–3 hours, they were rinsed in distilled water for several hours, postfixed with 2% osmium tetroxide for 1 hour, dehydrated in a graded ethanol, and dried with t-butyl alcohol freeze-drying method (Inoue and Osatake, 1988). Then, they were coated with gold and observed under the Jeol JSM 6100 scanning electron microscope.

Results

After NaOH treatment, the cellular elements as well as the basement membrane are dissolved to reveal the surface of the lamina propria. In the surface of the lamina propria in the colorectal mucosa,
there are longitudinal ridges and furrows. The ridges and furrows abruptly disappear at the pectinate line, and the surface continues to the annal canal (Fig. 1, 2).

The lamina propria both in the ridges and in the furrows are perforated by numerous openings of glandular crypts (Fig. 2). The openings of the crypts are surrounded by rather smooth sheet of densely interwoven collagen fibrils. A marked regional variation of crypt architecture is seen in between the ridges and furrows (Fig. 2). In the ridges, the crypts are oval or round in shape, and in most cases they opens to the lumen individually (Fig. 2, 3). The internal partitions separating a crypt are only rarely seen (Fig. 3). In some crypts the angle between the crypts and the luminal surface is round, in others it is sharp. When the crypts are cut vertically, the cylindrical structure of the crypts opening to the lumen is revealed. The lateral walls of the crypts have numerous hemispherical concavities about 10 μm in diameter. The concavities are rimmed circularly by thin collagen fibrils (Fig. 4).

In the furrows, on the other hand, the openings of the crypts are polygon is shape of which angles are rounded off. They are much more larger (80–160 μm in diameter) than the crypts in the ridges. The crypts in the furrows are subdivided into several smaller crypts (10–20 μm in diameter) by internal partitions (Fig. 2, 5). For the convenience of description, the crypts with internal partitions are termed as the “protocrypts (McCarthy and Kaye, 1990)”. The lamina propria of the luminal surface is continuous with the lateral walls and the internal partitions of the protocrypts. The internal partitions are much thinner than the interstices between the protocrypts (Fig. 2, 5). The level where the internal partitions terminate is various even within a protocrypt, they are located at deeper level than the luminal margin of the protocrypts (Fig. 5). Cut vertically, the lateral wall as well as the internal partitions have relatively smooth surface, and had no concavities. In cut surface, it is clearly revealed that the internal partitions are more deeply located than the luminal margin of the protocrypts (Fig. 6).

Discussion

The proliferation of crypts in the colorectal mucosa during postnatal growth has been reported (Helander, 1973; Maskens, 1978; Maskens and Dujardin-Loits, 1981). The mechanism by which the number of crypts increases appears to result from a longitudinal partitioning of pre-existing crypts through a median upheaval of the mucosa at their base (Maskens, 1978). This hypothesis was supported by the data that the DNA synthesis activity predominated in the median upheaval of the mucosa (Maskens, 1978). The proliferation of crypts was also demonstrated in the mouse during repair after injection of a carcinogen, 1,2-dimethylhydrazine (Deschner, 1978). An unsolved problem is whether the proliferation of crypts exists in the adult or not. In rodents, there is an evidence suggesting that the proliferation of crypts exists even in the adult (Maskens, 1978; Cheng and Bjerknes, 1985). Maskens (1978) reported in the mouse that the number of crypts increased more than decuple from birth to the 10th week of age, and that by 6th to 10th week the number reached the plateau level. Further, the branching of crypts detected by Levine and Haggitt (1992) in man is reminiscent of the proliferation of crypts. However, these studies based on the light microscopic technique would only be capable of demonstrating the partitioning of crypts in a section and miss the crypts undergoing multiple partitioning. The three-dimensional observation of crypt architecture is indispensable for collection of detailed information.

Recently, various techniques stripping the epithelial layer from the underlying lamina propria have been developed. Magney et al. (1986) treated the various regions of the mouse gastrointestinal tract with EDTA to reveal the basal surface of the epithelium, and observed the lateral enlargements separating from the crypts. Although the enlargements were suggestive of the proliferation of crypts, deformation by vessels could not be ruled out. On the other hand, McCarthy and Kaye (1990) used 2 kinds of the epithelial-stripping techniques (prolonged osmication followed by sonication, and chelation of calcium by EDTA followed by sonication) to the unfixed rat colon. They reported that the EDTA/sonication technique could eliminate the epithelial cells occupying the crypts seen in the specimens prepared by the osmium/sonication technique (McCarthy and Kaye, 1990). However, unfixed specimens are subjected to considerable artifacts. Ohtani and his co-workers introduced the NaOH cell-maceration method to fixed specimens, and reported that the method was able to remove cellular elements much more effectively and consistently than any other methods (Ohtani, 1987; Ohtani et al., 1988). In this study, the NaOH cell-maceration method was applied to the mouse colorectal mucosa to clarify the crypt architecture.

In the surface of the lamina propria of the newborn rat colon, McCarthy and Kaye (1990) observed the crypts subdivided into 2 or more smaller crypts by internal partitions, and termed them the “protocrypts”. This architecture of the protocrypts is unlike the morphology seen in adult rat of which
the crypts exist as simple unbranched tubular glands (Shamshuddin and Trump, 1981). Because the simple tubular glands appear to be revealed as crypts which have no internal partitions and open to the lumen individually. It is suggested that the internal partitions of the protocrypts continue to grow until they reach the same height and thickness of the interstices of the protocrypts and that, when the partitions become inapparent, simple unbranched tubular glands are formed (McCarthy and Kaye, 1990). That is, the protocrypts appear to reveal the crypts undergoing process of proliferation.

McCarthy and Kaye (1990) suggested in the rat colon that the protocrypts are reflection of active proliferation of crypts before weaning. Are the protocrypts a transient structure present only before weaning? Where do the crypts proliferate in the colorectal mucosa? The mouse aged 6–8 weeks was selected in this study, because it is assumed that in this stage both the protocrypts and the usual crypts are present to be suitable for the study on the proliferation of crypts. In the mouse colorectal mucosa treated with the NaOH cell-maceration method, there existed the longitudinal ridges and furrows in the surface of the lamina propria. A marked regional variation in the crypt architecture was observed in between the the ridges and the furrows. In the ridges, the crypts opened to the lumen individually and had no internal partitions. As discussed above, these crypts correspond to the simple tubular glands in light microscopic level. Further, Hummel et al. (1966) stated that the colorectal glands in the mouse were simple tubular type. That is, the crypts without internal partitions in the ridges appear to be matured. For convenience’ sake, the crypts were denominated the “mature crypts” in the following description. In the furrows, on the other hand, there existed the “protocrypts” subdivided into several smaller crypts by the internal partitions which were more deeply located than the luminal margin of the protocrypts. The internal partitions appear to be the median upheaval of the mucosa, through which the number of crypts increases, reported by Maskens (1978). That is, the protocrypts correspond to the crypts undergoing process of proliferation as suggested above. It is reasonable to assume that the furrows characterized by the protocrypts are the site where the crypts are undergoing process of proliferation into several smaller crypts, and that the proliferated crypts populate in the ridges as the mature crypts. Furthermore, the total number of the crypts would be homeostatically controlled by negative feedback, as Cairnie and Millen (1975) suggested in the mouse small intestine during repair after X-ray irradiation.

In human colorectal mucosa in which distinct longitudinal ridges and furrows are absent, the mucosal surface is subdivided by a complex system of innominate grooves (Williams, 1965; Levine and Haggitt, 1992). Levine and Haggitt (1992) mentioned that the sections through the grooves revealed the “cloverleaf-like crypts” in which multiple crypts opened into a single orifice. From their morphological features, the cloverleaf-like crypts in the light microscopic level seem to correspond to the protocrypts in this study. It may be speculated in man that the crypts proliferate in the grooves.

Another interesting finding is the histology of the lateral wall of the crypts. In the mature crypts, the lateral wall had numerous hemispherical concavities surrounded by the collagen fibrils. In the protocrypts, on the other hand, the lateral wall as well as the internal partitions were rather smooth. In the fundic glands of the rat stomach, Sugimoto and Ogata (1989) described that the lateral wall in the glandular pit was smooth, but the neck had numerous concavities. Furthermore, it is well known that the replacement of the epithelial cells in the neck of the fundic glands is much slower than that in the pit (Stevens and Lebrond, 1953; Messier and Leblond, 1960; Kaku, 1966; Hattori, 1974; Hattori and Fujita, 1976). From the preceding references and the present study, it is suggested that in the mature crypts the epithelial cells are fitted in the concavities, and that the tight adhesion between the cells and the underlying lamina propria is thus formed. In the protocrypts undergoing process of proliferation, on the other hand, the newly proliferated epithelial cells are less tightly fixed into the lamina propria. These findings lend further support to the suggestion that the protocrypts are the crypts undergoing process of proliferation.

References


Explanation of Figures

Plate I

Fig. 1. The surface of the lamina propria. There exist the longitudinal ridges (R) and furrows (F). They abruptly disappear at the pectinate line (arrowheads). arrows = anal margin A = anal canal S = dermis of perianal skin. ×50.

Fig. 2. Higher magnification of the longitudinal ridges (R) and furrow (F). In the ridges, the crypts (arrows) open to the lumen individually. In the furrow, the “proto-crypts” (arrowheads) are subdivided into several smaller crypts. ×100.
Plate II

Fig. 3. Higher magnification of the crypts in the ridge. The crypts open individually, and the internal partition is only rarely seen (arrowhead). $\times 500$.

Fig. 4. Vertically cut surface of the crypts in the ridge. The lateral walls have numerous hemispherical concavities (arrows) rimmed by collagen fibrils. arrowheads = openings of crypts. $\times 500$.

Fig. 5. Higher magnification of the protocrypt in the furrow. The protocrypt is subdivided into several smaller crypts by the internal partitions (arrowheads). The internal partitions are located at deeper level than the luminal margin (arrows) of the protocrypt. $\times 500$.

Fig. 6. Vertically cut surface of the protocrypt in the furrow. The internal partitions (arrowheads) are more deeply located than the luminal margin (arrows) of the protocrypt. The lateral wall (W) and the internal partitions (arrowheads) are smooth. $\times 500$. 