

Morphological Process of Villi Detachment Assessed by Computer-Assisted 3D Reconstruction of Intestinal Crypt from Serial Ultrathin Sections of Rat Duodenum Mucosa

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Abstract : This work presents an alternative mode of intestine mucosa renewal that may allow to better understand the total loss of villi after irradiation. It was tested a morphological method of 3d reconstruction using micrographs of serial sections of rat duodenum. We used hundreds of sections of each specimen of duodenum placed on glass slides and examined under a light microscope. Those containing the detachment, approximately a dozen, were chosen for observation under a transmission electron microscope (TEM). Each of these sections was glued on a block of epon resin and recut into a hundred of 60 nm-thick sections. Ribbons of these ultrathin sections were distributed on a series of copper grids in the same order of appearance than during the process of microstomia. They were then stained by solutions of uranyl and lead salts and observed under a TEM. The sections were pictured and the electron micrographs showing signs of cells detachment were transferred into two softwares, ImageJ to align the cellular structures and Reconstruct to realize the 3d reconstruction. It has been detected epithelial cells that exhibited all signs of programmed cell death and localized at the villus-crypt junction. Their nucleus was irregular in shape with a condensed chromatin in clumps. Their cytoplasm was darker than that of neighboring cells, containing many swollen mitochondria. In some places of the sections, we could see intercellular spaces enlarged by the presence of shrunk cells which displayed a plasma membrane with an irregular shape in thermowell as if the cell interdigitations would distant from each other. The three-dimensional reconstruction of the crypts has allowed observe gradual loss of intercellular contacts of crypt cells in the longitudinal plan of the duodenal mucosa. In the transverse direction, there was a gradual increase of the intercellular space as if these cells moved away from one another. This observation allows assume that the gradual remoteness of the cells at the villus-crypt junction is the beginning of the mucosa detachment. Thus, the shrinking of cells due to apoptosis is the way that they detach from the mucosa and progressively the villi also. These results are in agreement with our initial hypothesis and thus have demonstrated that the villi become detached from the mucosa at the villus-crypt junction by the programmed cell death process. This type of loss of entire villus helps explain the rapid denudation of the intestinal mucosa in case of irradiation.

Keywords : 3dr, transmission electron microscopy, ionizing radiations, rat small intestine, apoptosis

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