Ultrastructural characteristics of myenteric plexus in patients with colorectal cancer

Agata Zauszkiewicz-Pawlak¹, Janusz Godlewski², Przemyslaw Kwiatkowski², Zbigniew Kmiec¹, ²

¹Department of Histology, Medical University of Gdansk, Gdansk, Poland
²Department of Human Histology and Embryology, Faculty of Medical Sciences, University of Warmia and Mazury, Olsztyn, Poland

Abstract

Introduction. It has been found previously that colorectal cancer (CRC) is accompanied by atrophy of myenteric plexuses (MPs) localized close to the tumor. The aim of the study was to compare ultrastructure of MPs localized in the unchanged part of the colon wall distant to CRC tumor with the ultrastructure of MPs in the vicinity of CRC tumor.

Material and methods. The present study was conducted using post-operative material derived from 11 patients with CRC. Samples of colon wall were taken from the margin of cancer invasion and from a macroscopically unchanged segment of the large intestine, immediately fixed and processed according to the standard protocol for transmission electron microscopy studies.

Results. In the MPs localized in the control part of colon wall the presence of numerous unmyelinated axons and cell bodies of neurons, interstitial cells of Cajal and enteroglial cells were observed. As compared to control samples, in the MPs located close to the tumor invasion, expansion of the extracellular matrix and myelin-like structures accompanying some nerve fibers were found. The appearance of mast and plasma cells was observed within MPs in the vicinity of CRC tumor. Sporadically, apoptotic cells were present inside the MPs.

Conclusions. The presence of myelin-like structures and apoptotic cells within MPs located close to tumor invasion suggests that atrophy of MPs may be caused by factors released from CRC tumor.

(Folia Histochemica et Cytobiologica 2017, Vol. 55, No. 1, 6–10)

Key words: colorectal carcinoma; enteric nervous system; myenteric plexus atrophy; apoptosis; Cajal cells; electron microscopy

Introduction

The enteric nervous system (ENS) is basically built up of two ganglionated plexuses, the submucosal plexuses (of Meissner), and the myenteric plexuses (of Auerbach). The submucosal plexuses can be further subdivided into the inner and outer subdivisions, and the intermediate submucosal plexus located between them in some bigger mammals such as pig or human [1–3]. Neurons of the above mentioned plexuses produce axons communicating between submucosal and myenteric plexuses into one functional network. The classification of the enteric neurons according to their shape has been pioneered by Dogiel at the end of 19th century and continued later on independently by Furness [4] and Stach [5]. They distinguished up to eight classes of neurons based on cell shape. Apart from neurons, there are several other types of cells accompanying the ENS plexuses in the GI track, such as enteric glial cells (ECGs), connective tissue cells (fibroblasts/fibrocytes, plasmocytes, mast cells), and various classes of interstitial cells [6]. Among these last mentioned cells, using immunolabeling methods, morphologists have described “fibroblast-like cells”
which are distributed along processes of enteric motor neurons, close to nerve endings and nerve varicosities, and interstitial Cajal cells (ICCs) which accompany nerve fibers [7, 8]. Interstitial cells play important role in the control of the motor activity of the GI tract, as pacemaker cells and mechanoreceptors [6, 8]. The main function of the EGCs is the structural support for the ENS components. Recent studies showed that the role of EGCs may be more prominent because they form vast communication network via a complex Ca2+-dependent signals that enables EGCs to transmit and integrate information between the cells of the gut microenvironment (neurons, glial cells, connective tissue cells, and smooth muscle cells) [9].

The structure and function of the ENS in functional and inflammatory disorders of the gastrointestinal (GI) tract has been described [10, 11]. However, little is known about the structure of myenteric plexuses in patients with colorectal cancer (CRC). Our group showed that CRC is accompanied by atrophy of galanin-expressing MPs localized close to the tumor invasion as compared to the plexuses localized distantly from CRC tumor [12, 13]. Also the age-related neurodegenerative disorders like Alzheimer and Parkinson diseases are accompanied by a loss of neurons in myenteric and submucosal plexuses [14]. To determine possible morphological mechanisms of the atrophy of myenteric plexuses located close to the CRC invasion we performed analysis at the ultrastructural level.

Material and methods

Patients' recruitment. The present study was conducted using post-operative material obtained from 11 patients (5 women and 6 men) with CRC diagnosis. The mean age of the patients was 71 ± 7.54 years (mean ± SEM, range: 53–79 years). Post-operative histopathological analysis confirmed that patients included in this study formed a homogeneous group presenting the same degree of adenocarcinoma invasion within the colon wall, defined as T3 by the TNM staging as defined by the American Joint Committee on Cancer (AJCC), moderate degree of adenocarcinoma differentiation grading (G2) and similar anatomical localization of the tumor in sigmoid colon or upper part of rectum. The average size of the tumor was 4.45 ± 0.5 cm (mean ± SEM). Four patients who did not have regional lymph nodes metastasis were classified as the stage II of a cancer, while the other seven patients presented stage III due to lymph node involvement. The tissue samples were collected during surgery at the Department of Oncological Surgery of the Regional Oncological Centre in Olsztyn, Poland. The protocol of this study was approved by the University of Warmia and Mazury Bioethics Commission (No. 12/2012) and written informed consent was obtained from all patients in the study. All patients had no evidence of obstruction or other disorders of bowel content passage. Moreover, they did not have another serious illness nor were subjected to neo-adjuvant chemo- or/and radiotherapy.

Material collection and transmission electron microscopy. Immediately after surgical resection of the intestine, very thin slices of colon wall sections, less than 1 mm of thickness, were harvested from the removed part of the large colon. Samples were taken from the margin of cancer invasion (cancer-infiltrated intestinal wall) and from a macroscopically unchanged segment of the colon at a distance of 5–8 cm from the tumor, as a control tissue. Tissue samples were immediately fixed in 2.5% glutaraldehyde in 0.1 mM sodium-cacodylate buffer (pH 7.4) followed by post-fixation in 2% osmium tetroxide and dehydration in graded series of ethanol. After infiltration with propylene dioxide/epon mixture and pure epon, sections were embedded to polymerize. Semi-thin and ultra-thin sections (Reichert U3 ultramicrotome, Vienna, Austria) were obtained and finally contrasted using uranyl acetate and lead citrate prior to the examination in JEM 1200EX II transmission electron microscope (Tokyo, Jeol, Japan) at 80 kV.

Results

Ultrastructure of the myenteric plexuses in the unchanged part of colon wall distant to CRC tumor

The ultrastructural studies revealed normal organization of the myenteric plexus in colon wall distant from CRC invasion (control tissue). Bundles of unmyelinated nerve fibers and perikaryons were located between the circular and longitudinal layers of smooth muscle cells of the muscularis externa (Fig. 1). They were surrounded by extracellular matrix (ECM) containing abundant collagen and less numerous elastic fibers, fibroblasts/fibrocytes and their processes. Nerve fiber bundles (NFBs) were ensheathed by long cytoplasmic processes of the interstitial Cajal cells and fibrocytes, which separated the bundles from surrounding ECM and smooth muscle cells (Fig. 1A). Axonal profiles presented lucent axoplasm, which contained neurofilaments and neurotubules, neurotransmitter vesicles (large granular vesicles with dense core) and occasionally mitochondria (Fig. 1B). Some of the observed axons were embedded in the infoldings of enteric glial cells (Fig. 1C).

Ultrastructure of the myenteric plexuses in the vicinity of CRC tumor

Generally, the ultrastructure of MPs located close to the CRC invasion was similar to distantly located MPs. Nerve fibers and enteric glial cells embedded in
ECM rich in collagen fibers between smooth muscle layers (Fig. 2A), being occasionally accompanied by cell bodies and processes of interstitial Cajal cells (Fig. 2B). However, in some of the samples located close to cancer invasion single myelin-like structures within NFBs were present (Fig. 2C). Furthermore, few apoptotic cells were present in the ECM surrounding NFBs (Fig. 2D, 3A). They presented nuclear condensation and shrinkage of the cell body, typical morphological markers of apoptotic cell death. Additionally, we observed increased number of fibroblast-like cells (Fig. 3A) and mast cells (Fig. 3B, C) that were present in the ECM surrounding myenteric plexuses. Moreover, ECM was abundant in collagen fibers and seemed to be more prominent than in tissue samples derived from distant region of the colon wall.

Discussion

Our study presents an attempt to explain the observations by Kwiatkowski et al. [15] and Kozlowska et al. [16] who applied morphometry to measure the area of MPs close to and distant from CRC invasion. They found significant reduction (by approximately 50%) of the area occupied by galanin-immunoreactive neurons in the MPs located in the vicinity of CRC invasion in comparison to distally located plexuses. The authors suggested that neither apoptosis nor necrosis has been involved in the observed phenomena [15, 16]. These finding have been confirmed by the results of our ultrastructural study, which showed neither neurons’ apoptosis nor necrosis to occur in myenteric plexuses located close to the CRC tumor. Since the mentioned histomorphometric studies clearly demonstrated atrophy of myenteric plexuses in this location we suggest that the atrophic changes must have taken place earlier during the CRC progression. Since in the present study the patients represented an advanced phase of the disease (stages II and III) it was not possible to determine at the TEM level (this study) or at the light microscopy level, which type of cell death (apoptosis, autophagy or necrosis) was responsible for the atrophy of MPs close to the CRC invasion [16]. Although, we found occasionally presence of apoptotic cells close to the bundles of nerve fibers, the morphology of these cells does not allow defining their type due to cell condensation and shrinkage. Therefore, it cannot be excluded that apoptosis still may play some role in the MPs atrophy in CRC patients. It is also possible that some, as yet unknown factors, released during progression of the disease from the adjacent CRC tumor may cause involution of the neighboring myenteric plexuses. However, this suggestion assumes...
we observed them mainly close to the CRC invasion, myelin-like bodies were also observed in suprarenal glands and central nervous system [19, 20]. Moreover, the hypertrophied nerve fascicles with prominent perineural sheath which are similar to the extrinsic nerves were demonstrated in Hirschsprung’s disease, genetically-determined loss of ENS neurons, in which aganglionic region cause impaired colon peristalsis [21]. Moreover, the increased presence of glial cells and abnormal distribution of collagen fibers and ECM components within colon wall were also reported in Hirschprung’s disease [21]. Since we found that samples taken from the margin of cancer invasion were more abundant in collagen fibers and fibroblasts/fibrocytes it may be assumed that these changes may represent the replacement of the atrophied MPs by the connective tissue.

In summary, the presence of myelin-like structures and apoptotic cells within MPs located close to tumor invasion suggests that atrophy of MPs may be caused by factors released from CRC tumor during the progression of the disease.

**References**


