BIOGERONTOLOGY

Neuroendocrine and Proliferative Potential of Human Intestinal Cells during Aging

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 150, No. 12, pp. 682-685, December, 2010
Original article submitted January 19, 2010

A population of neuroendocrine cells secreting chromogranin A was verified in the intestine of subjects aged over 60 years. The count of intestinal cells expressing chromogranin A and Ki-67 proliferation protein increases with aging. More intensive expression of chromogranin A and Ki-67 protein in senile age and in long-livers is presumably a compensatory mechanism aimed at the gastrointestinal function maintenance during its age-associated involution.

Key Words: *intestinal neuroendocrine cells; proliferation; chromogranin A; Ki-67; aging*

The organism realizes contact with the environment through the gastrointestinal organs. Involution of the gastrointestinal tract in subjects over 60 years consists in the development of degenerative changes in the gastrointestinal mucosa [1,3,5]. The maintenance of the appropriate count of neuroendocrine cells and their proliferative activity during aging is an important mechanism supporting the intestinal physiological function [2,4,6,8]. Hence, studies of intestinal cell proliferation and secretory activity in subjects aged over 60 years are a particularly interesting aspect of comprehensive evaluation of body status during aging.

We studied age-related dynamics of chromogranin A and proliferation protein Ki-67 expression in intestinal cells of subjects aged over 60 years.

MATERIALS AND METHODS

The study was carried out on 47 autopsied specimens of the small intestine from subjects of different age. The material was divided into 3 groups in accordance with classification of the World Health Organization: group 1, elderly subjects (60-74 years), group 2, senile subjects (75-89 years), and group 3, long living subjects (90 years and older).

Fragments of the small intestine were fixed in neutral formalin (pH 7.2), processed in ethanols, xylols, and embedded in paraffin by the standard method. Sections (3 μ) were sliced from paraffin blocks on a Leica 54OM microtome.

Intestinal sections for morphological study were stained with hematoxylin and eosin. Immunohistochemical studies were carried out with primary monoclonal antibodies to chromogranin A (1:100, Dako) and Ki-67 (1:50, Dako) and second antibodies (biotin-treated antimouse immunoglobulins from a universal kit). Staining visualization was carried out by avidin complex with biotin-treated horseradish peroxidase (ABC kit) with subsequent development of horseradish peroxidase with diaminobenzidine (all reagents from Dako).

Staining intensity was evaluated morphometrically by two parameters: expression area and optical density (arbitrary units) of immunostained structures

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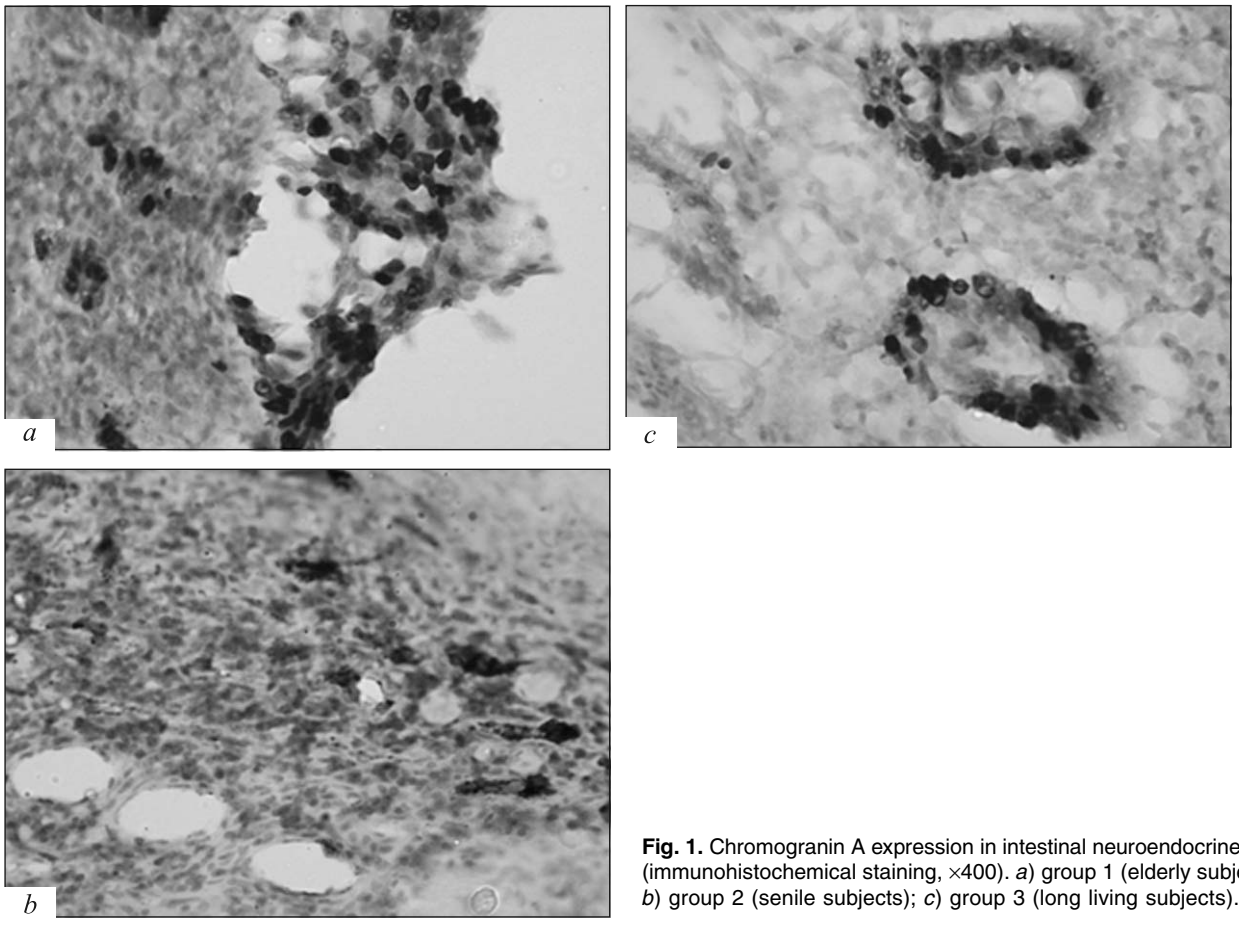

Fig. 1. Chromogranin A expression in intestinal neuroendocrine cells (immunohistochemical staining, ×400). *a*) group 1 (elderly subjects); *b*) group 2 (senile subjects); *c*) group 3 (long living subjects).

by computer-aided analysis of Nikon Eclipse 400 microscopic images using Videotest Morphology 5.0 licensed software. The area of expression was calculated as the ratio of the areas of immunopositive cells to total area of preparation and expressed in percent.

The results were statistically processed using Statistica 7.0 software (ANOVA). The significance of the data was evaluated using nonparametric tests: Kruskal—Wallis test for comparison of two and more samples and Mann—Whitney test for comparison of two samples.

RESULTS

The expression of the main neuroendocrine marker chromogranin A was verified in epithelial and muscle cells of the small intestine in all 3 groups (Fig. 1).

The function of chromogranin A remains unclear. Presumably, this water-soluble protein consisting of 450 amino acid residues is released into the blood together with catecholamines, *e.g.* epinephrine [7]. The synthesis of chromogranin A in intestinal epithelial and muscle cells presumably indicates the involvement of these cells in the regulation of the neuroendocrine processes at the whole body level.

The area of chromogranin A expression in intestinal cells of senile subjects (group 2) was 2.3-fold larger than in elderly subjects (group 1) and 1.5-fold larger than in long-living subjects (group 3; Fig. 2, *a*).

The expression area characterized the count of cells expressing chromogranin A. The increase in the count of chromogranin A immunopositive cells in senile age was presumably due to the development of the compensatory mechanisms, while in long living subjects this reserve was exhausted. Optical density was virtually the same in all 3 groups (Fig. 2, *b*). Optical density reflects the number of signal molecules (in this case, chromogranin A) recorded on the surface of one cell. Hence, the neuroendocrine activity of intestinal cells was not changed in subjects aged over 60 years; however, their counts increased at the age of 60-74, and then decreased.

Immunohistochemical study of human intestine preparations showed expression of Ki-67 proliferative protein in epithelial cells and myofibrils in all studied groups (Fig. 3). The areas of Ki-67 expression was virtually the same in groups 1 and 2 (elderly and senile subjects), while in group 3 (long-living subjects) this parameter was 1.8 times higher than in group 1 and 2.4 times higher than in group 2 (Fig. 2, *c*).

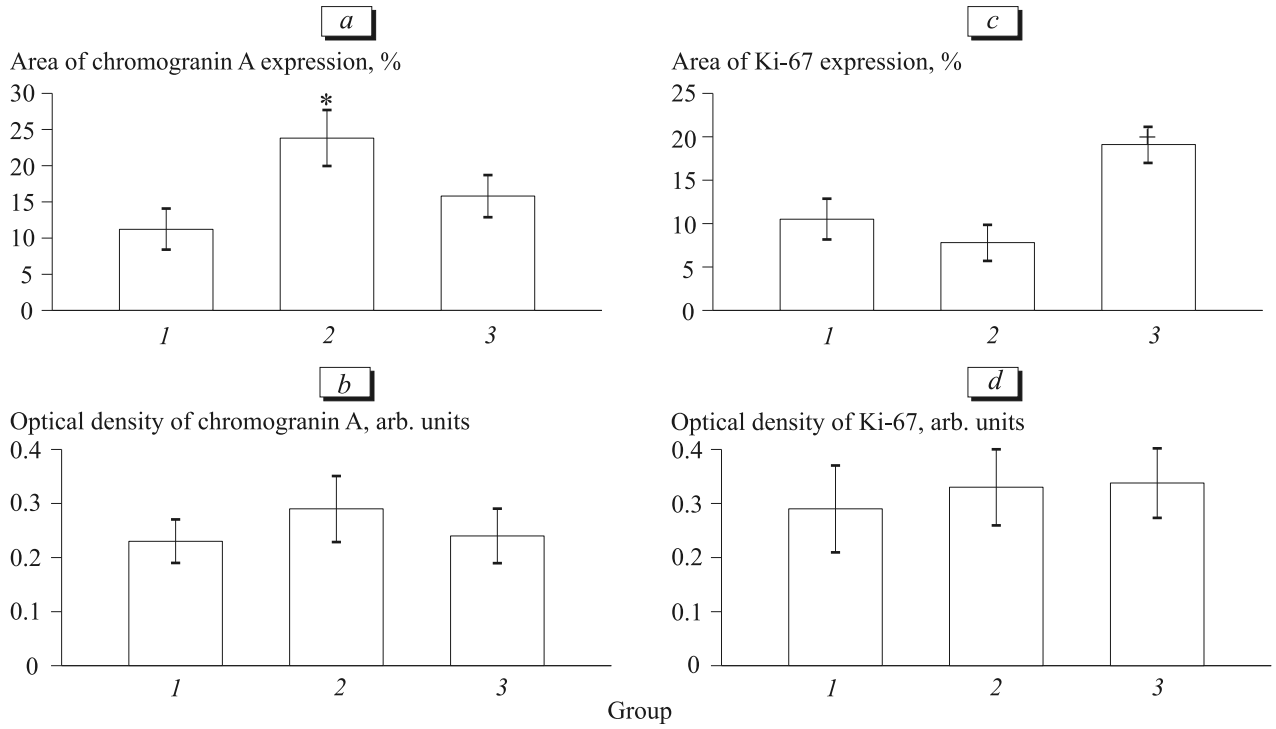

Fig. 2. Chromogranin A and Ki-67 expression in intestinal cells in different age groups. *a*) area of chromogranin A expression; *b*) optical density of chromogranin A expression; *c*) area of Ki-67 expression; *d*) optical density of Ki-67 expression. $p<0.05$ compared to: *groups 1 and 3, +group 1.

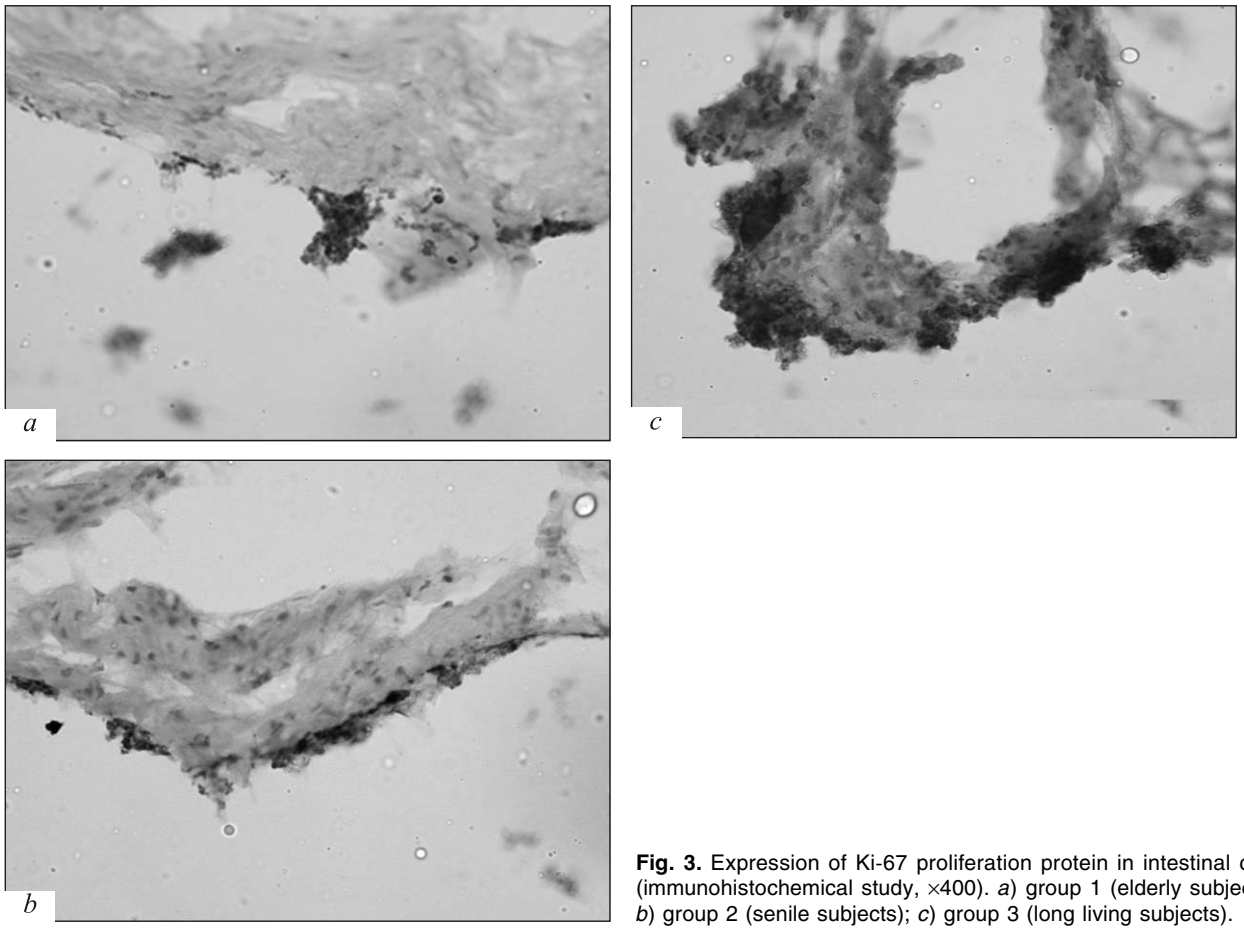

Fig. 3. Expression of Ki-67 proliferation protein in intestinal cells (immunohistochemical study, ×400). *a*) group 1 (elderly subjects); *b*) group 2 (senile subjects); *c*) group 3 (long living subjects).

The increase in the count of intestinal cells capable of proliferation in the oldest age group was presumably one more adaptation mechanism supporting functional activity of the gastrointestinal tract at a level needed for vital activity. Optical density of Ki-67 expression was virtually the same in all groups (Fig. 2, *d*).

The findings indicate the presence of a population of neuroendocrine cells expressing chromogranin A (universal marker of hormonal secretion) in the human intestine. The count of chromogranin A producing cells in the intestine increases at the age of 60-74 years and then decreases indicating the development of compensatory mechanisms aimed at the maintenance of neuroendocrine relationships in the gastrointestinal tract, most manifest in senile age.

High expression of Ki-67 (the key factor of proliferation) by intestinal cells of long living subjects is presumably due to the compensatory mechanisms aimed at preservation of the intestinal cell population under conditions of involution processes unfolding with aging.

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