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ALDEHYDE DEHYDROGENASE 1 (ALDH1) EXPRESSION IN SERRATED COLONIC POLYPS

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Abstract

Introduction. Immunohistochemical prognostic markers are promising direction for a search. The **purpose** of the study was analyze ALDH1 immunohistochemical expression in different histological types of serrated polyps of the distal colon. **Materials and methods.** Histopathological and IHC studies of 30 serrated polyps were conducted. IHC study was carried out using antibodies against ALDH1. Results of IHC reactions were estimated by photo digital morphometry and were expressed in immunostained cells relative area (%). **Results.** Hyperplastic polyps are characterized by the median of ALDH1+ stromal cells relative area equal to 15,08 (11,12;21,46) % and the median of ALDH1+ epitheliocytes relative area equal to 18,28 (10,14;26,15) %. The basal-middle pattern of ALDH1+ cells distribution was revealed. Traditional serrated adenomas are characterized by the median of ALDH1+ stromal cells relative area equal to 25,63 (18,26;30,42) % and the median of ALDH1+ epitheliocytes relative area equal to 22,13 (17,22;36,05) %. The upper-middle

pattern of ALDH1+ cells distribution was revealed. Sessile serrated adenomas are characterized by the median of ALDH1+ stromal cells relative area equal to 23,15 (17,21;29,14) % and the median of ALDH1+ epitheliocytes relative area equal to 20,56 (15,25;26,94) % in these polyps. The basal-middle pattern of ALDH1+ cells distribution was revealed. **Conclusions.** Number of ALDH1+ stromal cells is significantly higher in traditional serrated adenomas and sessile serrated adenomas comparing to number of those in hyperplastic polyps.

Key words: Intestinal neoplasms; Polyps; Stem Cells; Aldehyde Dehydrogenase 1.

Introduction. Until recently, colorectal cancer (CRC) was considered the result of adenomas malignization that is well known “adenoma-carcinoma” sequence [1]. Hyperplastic polyps were not regarded as possible CRC precursors. However, nowadays hyperplastic polyps are included in group of neoplasms named as serrated polyps which are CRC precursors. Besides hyperplastic polyps this group includes traditional serrated adenoma and sessile serrated adenoma. All these polyps are characterized by superficial serrated architecture. But these polyps have significant differences as well, including various malignization rates. According to the literature data, hyperplastic polyps undergo malignant transformation in 6-21% cases while serrated adenomas undergo malignant transformation in 19-43 % cases [2-3].

Immunohistochemical prognostic markers are promising direction for a search. These markers would make it possible to assess the risk of cancer growth in polyp’s biopsy samples [4-5]. Among modern and still insufficiently studied markers - aldehyde dehydrogenase 1 (ALDH1). ALDH1 is a type of NAD(P)⁺-dependent enzyme that provides oxidation of intracellular aldehydes. This way ALDH1 protects cells against oxidative damage. Moreover, this enzyme participates biosynthesis of molecules that regulate cellular homeostasis, such as retinoic acid, γ -aminobutyric acid, and betaine [6]. Presence of ALDH1 in stem cells was revealed decades ago. After that active search for practical implementation of ALDH1-positive cells started. It was confirmed that protective function of this enzyme plays key role in stem cells survival rate. Furthermore, presence of ALDH1 was detected in cancer stem cells [7]. It was proved that ALDH1-positive cancer stem cells provide chemoradiotherapy resistance and correlate with a poor prognosis of breast cancer [8]. ALDH1-positive cells have been studied in different solid tumors, including head and neck neoplasms, pancreatic cancer, and colorectal cancer [9-11]. Despite numerous studies of ALDH1-positive cells in malignancies,

the prognostic value of this marker remains controversial. The ALDH1 expression in CRC precursors is poorly characterized [7, 11].

The **purpose** of our study was to analyze ALDH1 immunohistochemical expression in different histological types of serrated polyps of the distal colon.

Materials and methods. Biopsy samples of 120 distal colonic polyps that were removed during diagnostic colonoscopy at the Endoscopy Unit of ZSMU University Clinic were analyzed retrospectively from January 2019 to January 2020. Biopsy samples of 30 serrated polyps were selected for the current histopathological and immunohistochemical (IHC) study.

The features of histological structure of the studied samples were examined in sections stained by hematoxylin and eosin, as well as PAS-staining. Based on histopathological examination studied samples were divided into the following groups: 1 study group – hyperplastic polyps (10 cases), 2 study group – traditional serrated adenomas (10 cases), 3 study group – sessile serrated adenomas (10 cases).

IHC study was conducted according to protocols provided by used antibodies manufacturers. Monoclonal antibodies against ALDH1 (ALDH1A1, Clone 5A11, Thermo Scientific, USA) and visualization system EnVision FLEX with diaminobenzidine (DAKO, USA) were used. The results of IHC reactions were studied using Axioplan-2 microscope (Carl Zeiss, Germany). In each case, the area of ALDH1-positive cells was estimated in 5 standardized microscope fields of view at $\times 200$ magnification. The area of ALDH1+ cells was estimated by the method of photo digital morphometry: calculation of immunopositive pixels number in a digital image with further comparing to total pixels number in the image was carried out. Number of immunopositive pixels was expressed in % and indicated the relative area of ALDH1+ cells. Moreover, distribution of ALDH1+ cells in colonic crypts was examined.

Statistical processing of the results was performed on a personal computer using program “Statistica® for Windows 13.0” (StatSoft Inc., License № JPZ804I382130ARCN10-J). The median (Me), the lower and the upper quartiles (Q1; Q3) were calculated. Comparison was performed using the Mann-Whitney U-test. The correlation analysis was performed using Spearman’s rank correlation coefficient (r). The results were considered as statistically significant when $p < 0,05$.

Results. According to the results of IHC study, 100% cases of hyperplastic polyps are characterized by cytoplasmic ALDH1 expression in stromal cells, and 40% of hyperplastic polyps which are with dysplastic changes are characterized by cytoplasmic ALDH1 expression

in epitheliocytes as well. The median of ALDH1+ stromal cells relative area equal to 15,08 (11,12;21,46) % in these polyps. The median of ALDH1+ epitheliocytes relative area equal to 18,28 (10,14;26,15) % in these polyps. Regarding the features of ALDH1+ cells distribution it was revealed that in 60% studied cases ALDH1+ cells located exclusively within basal part of the colonic crypts and in 40% studied cases ALDH1+ cells located in both basal and middle thirds of the crypts. Direct medium correlation ($r = 0,65$) between presence of dysplasia and lower-middle pattern of immunopositive cells distribution in hyperplastic polyps was revealed.

Traditional serrated adenomas are characterized by cytoplasmic ALDH1 expression in both stromal and epithelial cells in all studied cases. The median of ALDH1+ stromal cells relative area equal to 25,63 (18,26;30,42) % in these polyps. The median of ALDH1+ epitheliocytes relative area equal to 22,13 (17,22;36,05) % in these polyps. Regarding the features of ALDH1+ cells distribution in traditional serrated adenomas it was revealed that immunopositive cells located diffusely in upper and middle thirds of the crypts. Dysplasia was present in all studies cases of traditional serrated adenomas.

Sessile serrated adenomas are characterized by cytoplasmic ALDH1 expression in both stromal and epithelial cells in all studied cases. The median of ALDH1+ stromal cells relative area equal to 23,15 (17,21;29,14) % in these polyps. The median of ALDH1+ epitheliocytes relative area equal to 20,56 (15,25;26,94) % in these polyps. Regarding the features of ALDH1+ cells distribution in sessile serrated adenomas it was revealed that immunopositive cells located exclusively within basal part of the colonic crypts in 80% studied cases, and these cells located diffusely in both basal and middle parts of the colonic crypts in 20% studied cases. Dysplasia was present in all studies cases of sessile serrated adenomas.

Comparative analysis revealed statistically significant difference between relative areas of ALDH1+ cells in hyperplastic polyps and serrated adenomas, and absence of statistically significant difference between relative areas of ALDH1+ cells in traditional serrated adenomas and sessile serrated adenomas. Therefore, number of ALDH1+ stromal cells is significantly higher in traditional serrated adenomas and sessile serrated adenomas comparing to number of those in hyperplastic polyps (25,63 (18,26;30,42) % vs. 15,08 (11,12;21,46) %, $p < 0,05$; 23,15 (17,21;29,14) % vs. 15,08 (11,12;21,46) %, $p < 0,05$). Moreover, number of ALDH1+ epitheliocytes is significantly higher in traditional serrated adenomas and sessile serrated adenomas comparing to number of those in hyperplastic polyps (22,13 (17,22;36,05) % vs. 18,28 (10,14;26,15) %, $p < 0,05$; 20,56 (15,25;26,94) % vs. 18,28 (10,14;26,15) %, $p < 0,05$).

Discussion. According to the obtained results, hyperplastic polyps are characterized by predominant basal distribution of ALDH1+ cells (60% of studied cases) and mixed basal-middle distribution of these cells that appears in dysplastic polyps (40% of studied cases). Histological features of hyperplastic polyps include elongation of the crypts which associates with extension of proliferation zone [2]. According to the literature data, ALDH1 participates biosynthesis of molecules (retinoic acid, γ -aminobutyric acid, betaine, and others) that are involved in mitotic process activation [6]. The revealed patterns of ALDH1+ cells distribution in hyperplastic polyps correspond with typical extension of proliferation zone that confirms aforementioned literature data.

In this study traditional serrated adenomas are characterized by upper-middle distribution of ALDH1+ cells (100% of studied cases). The main histological feature of traditional serrated adenoma is the presence of ectopic crypts located perpendicular to longitudinal axis of the normal crypts [2-3]. Presence of these crypts may explain the revealed pattern of ALDH1+ cells distribution.

Sessile serrated adenomas are characterized by predominant basal distribution of ALDH1+ cells (80% of studied cases) and mixed basal-middle distribution of these cells (20% of studied cases). The main histological features of sessile serrated adenoma are elongation of the crypts, and presence of serrated architecture along the whole surface of the crypts [2-3]. The pattern that was revealed in sessile serrated adenomas is similar to those in hyperplastic polyps and most probably appear due to the extension of proliferation zone.

Based on the obtained numbers of ALDH1+ cells relative areas in the studied polyps it was concluded that the number of ALDH1+ stromal cells is significantly higher in traditional serrated adenomas and sessile serrated adenomas comparing to number of those in hyperplastic polyps. Furthermore, the number of ALDH1+ epitheliocytes is significantly higher in traditional serrated adenomas and sessile serrated adenomas comparing to number of those in hyperplastic polyps. Based on the literature data, ALDH1 is a widely used stem cells marker [11-13]. Over the last decade, investigation of ALDH expression by stem cells has been focused on cancer stem cells [10]. ALDH+ cells with tumor-initiating ability have been identified in many types of tissues including colon [14]. However, the literature data regarding ALDH+ cells in CRC precursors is still incomplete. Our findings prove the presence in ALDH+ cells in polyps-precursors as well as correspondence between pattern of these cells distribution and proliferation zones.

Conclusions:

1. Hyperplastic polyps are characterized by basal-middle pattern of ALDH1+ cells distribution and significantly smaller number of these cells comparing to serrated adenomas.
2. Traditional serrated adenomas are characterized by middle-upper pattern of ALDH1+ cells distribution and significantly higher number of these cells comparing to hyperplastic polyps.
3. Sessile serrated adenomas are characterized by basal-middle pattern of ALDH1+ cells distribution and significantly higher number of these cells comparing to hyperplastic polyps.

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