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COMPARATIVE STUDY OF KI-67 AND CD44 EXPRESSION IN SERRATED POLYPS OF THE DISTAL COLON

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Abstract

Introduction. Serrated polyps are newly distinguished types of colonic polyps. Nowadays lots of questions about serrated neoplastic pathway are still unclear. The **purpose** of the study was to compare Ki-67 and CD44 immunohistochemical (IHC) expression levels in different histological types of serrated polyps. **Materials and methods.** Histopathological and IHC studies of 30 serrated polyps were conducted. IHC study was carried out using antibodies against Ki-67 and CD44. Results of IHC reactions with antibodies against Ki-67 were estimated by immunostained nuclei counting and were expressed in proliferation index (PI) while results of IHC reactions with antibodies against CD44 were estimated by photo digital morphometry and were expressed in immunostained cells relative area (%). Furthermore, distribution of Ki-67+ and CD44+ cells in colonic crypts was examined.

Results. It was revealed that hyperplastic polyps (HP) were characterized by medium PI and basal-middle pattern of Ki-67+ cells distribution. HP were also characterized by the median of CD44+ cells area equal to 22,36 (13,15;30,41) % and basal-middle pattern of CD44+ cells distribution. Herewith, direct medium strength correlation between Ki-67 and CD44 expression levels in HP was established. Traditional serrated adenomas (TSA) were characterized by medium PI and diffuse pattern of Ki-67+ cells distribution. TSA were also characterized by the median of CD44+ cells area equal to 25,48 (15,19;29,04) % and upper-middle pattern of CD44+ cells distribution. Direct weak strength correlation between Ki-67 and CD44 expression levels in TSA was established. Sessile serrated adenomas (SSA) were characterized by medium PI and basal pattern of Ki-67+ cells distribution. SSA were also characterized by the median of CD44+ cells area equal to 20,54 (11,25;28,15) % and basal-middle pattern of CD44+ cells distribution. Direct medium strength correlation between Ki-67 and CD44 expression levels in SSA was established.

Key words: Intestinal neoplasms; Polyps; Cell Proliferation; CD44 Antigen.

Introduction. Serrated polyps were included in the WHO Histological Classification of Digestive System Tumors ten years ago [1]. The main types of serrated polyps are hyperplastic polyps (HP), traditional serrated adenomas (TSA), and sessile serrated adenomas (SSA) [2].

All these types are distinguished by the typical serrated (so-called “saw-toothed”) appearance of the epithelium. HP are characterized by prominent elongation of the colonic crypts that usually reach lamina muscularis mucosae. TSA are characterized by presence of ectopic crypts which locate perpendicularly relative to the normal cryptal longitudinal axis. These crypts do not reach lamina muscularis mucosae. SSA are characterized by abnormal branching of the colonic crypts, horizontal direction of crypts growth, as well as abnormal dilation of basal parts of the crypts. These crypts may reach lamina muscularis mucosae [2].

Despite the great interest regarding serrated polyps and a large number of publications devoted to studying of their characteristics, lots of questions about serrated neoplastic pathway are still unclear.

Proliferative index (PI) that is counted based on Ki-67 expression is widely used for estimation of neoplastic lesions malignant potential [3]. However, features of Ki-67 expression in different types of colonic polyps were described in single publications. It is well known that HP are characterized by normal PI (PI that does not significantly differ from PI of non-changed colonic mucosa). Herewith, distribution of Ki-67+ cells in HP differs from the

distribution in non-changed mucosa: there is an elongation of Ki-67+ cells zone in HP. There is no consensus regarding PI and zones of Ki-67+ cells distribution in TSA and SSA [4-6].

Results of current studies show that parallel estimation of a few markers that reveal neoplastic lesions malignant potential is more reliable, comparing to estimation of one marker expression [7]. Another one marker that is widely used for estimation of neoplastic lesions malignant potential is CD44. Nowadays this marker is commonly used for detection of cells which acquire stemness. The acquisition of stem cells properties is recently discovered property of malignancies that opens new questions [8-9]. Regarding serrated polyps, it is known that CD44 expression is significantly higher in TSA and SSA comparing to HP, and CD44 expression is significantly higher in HP comparing to non-changed colonic mucosa [10]. However, the data about distribution of CD44+ cells in these polyps were not found in current literature. The question about clinical significance of parallel studying of Ki-67 and CD44 in serrated colonic polyps has not been studied as well.

The **purpose** of our study was to compare Ki-67 and CD44 immunohistochemical expression levels 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 Ki-67 (Clone MIB-1, DAKO, Denmark) and CD44 (CD44 Std. / HCAM Ab-4, Thermo Scientific, USA) were used. In each IHC-stained sample, 5 standardized microscope fields of view at ×200 magnification were studied. Ki-67 expression was estimated using Photoshop CC (2014): using tool <counter> counting of immunopositive nuclei in digital images of the microslides was performed. Ki-67 expression level called as proliferation index (PI) was estimated as low if <25% of nuclei in a field of view were immunopositive, it was estimated as medium if 25-75% of nuclei were immunopositive, and PI was estimated as high if >75% of nuclei were immunopositive. The

area of CD44+ 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 CD44+ cells. Moreover, distribution of Ki-67+ and CD44+ 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 it was revealed that HP were characterized by the medium PI with the median of Ki-67 expression equal to 26,23 (22,19; 48,88) %. Additionally, the following features of Ki-67+ cells distribution were revealed: in 60% studied cases Ki-67+ cells located exclusively within basal part of the colonic crypts whereas in 40% studied cases Ki-67+ cells located in both lower and middle thirds of the crypts.

HP were also characterized by the median of CD44+ cells relative area equal to 22,36 (13,15;30,41) %. Regarding the features of CD44+ cells distribution it was revealed that in 50% studied cases CD44+ cells located exclusively within basal part of the colonic crypts and in 50% studied cases CD44+ cells located in both lower and middle thirds of the crypts. Correlation analysis detected direct medium strength connection (r=0,61) between Ki-67 and CD44 expression levels in lower and middle thirds of the colonic crypts.

It was established that TSA were characterized by the medium PI with the median of Ki-67 expression equal to 38,34 (25,26; 50,02) %. The following features of Ki-67+ cells distribution were revealed: 80% studied cases were characterized by diffuse Ki-67+ cells location (immunopositive cells located in lower, middle, and upper parts of the colonic crypts) whereas in 20% studied cases Ki-67+ cells located within middle and upper parts of the colonic crypts.

TSA were also characterized by the median of CD44+ cells relative area equal to 25,48 (15,19;29,04) %. Regarding the features of CD44+ cells distribution it was revealed that in 100% studied cases CD44+ cells located within middle and upper parts of the colonic crypts. Correlation analysis detected direct weak strength connection (r=0,43) between Ki-67 and CD44 expression levels in the colonic crypts of TSA.

It was revealed that SSA were characterized by the medium PI with the median of Ki-67 expression equal to 28,43 (23,20; 45,68) %. Regarding the features of Ki-67+ cells distribution it was revealed that in 100% studied cases Ki-67+ cells located exclusively within basal part of the colonic crypts.

SSA were also characterized by the median of CD44+ cells relative area equal to 20,54 (11,25;28,15) %. Regarding the features of CD44+ cells distribution it was revealed that in 80% studied cases CD44+ cells located exclusively within basal part of the colonic crypts whereas in 20% studied cases CD44+ cells located in both lower and middle thirds of the crypts. Correlation analysis detected direct medium strength connection (r=0,73) between Ki-67 and CD44 expression levels in lower and middle thirds of the colonic crypts.

Comparative analysis revealed the absence of statistically significant difference between PI in studied polyps. It was also revealed that the medians of CD44+ cells relative area is significantly higher in TSA comparing to HP and SSA. Herewith, there is no significant difference between medians of CD44+ cells relative area in HP and SSA.

Discussion. According to the obtained results, sessile serrated polyps are characterized by medium proliferation index with no significant difference between the medians of Ki-67 expression in HP, TSA, and SSA. According to the literature data, HP are characterized by medium PI [4] that is consistent with the obtained data. The literature data about PI of TSA and SSA are very limited and controversial: there is information about medium PI of these polyps as well as there is information about high PI of these polyps [4-6].

In this study each type of serrated colonic polyps was characterized by specific pattern of Ki-67+ cells distribution: HP differ by location of Ki-67+ cells in basal and middle parts of the crypts, TSA differ by diffuse Ki-67+ cells location, SSA differ by location of Ki-67+ cells in basal part of the crypts. It is well known that Ki-67 expression is revealed exclusively in basal part of the crypts in normal colonic mucosa [11]. Therefore, presence of Ki-67+ cells not only in basal, but also in middle part of the HP crypts indicates elongation of proliferation zone in these polyps that is consistent with the literature data [4]. SSA were characterized by normal pattern of Ki-67+ cells distribution that complements literature data. TSA were characterized by diffuse pattern of Ki-67+ cells distribution that complements literature data as well.

Regarding the medians of CD44+ cells relative area, it was revealed that the median of CD44+ cells relative area is significantly higher in TSA comparing to HP and SSA. Based on the aforementioned data, TSA significantly differ from HP and SSA: 1) TSA are characterized by diffuse pattern of Ki-67+ cells distribution; 2) TSA are characterized by

significantly higher median of CD44+ cells relative area. These observations reflex higher ability to proliferation of TSA cells as well as disorganization of the cellular hierarchy in the crypts. From another side, these observations indicate similarities of HP and SSA: 1) location of Ki-67+ cells in basal or in basal and middle parts of the crypts; 2) the same indexes of CD44+ cells relative area. It may be explained by one of the theories of SSA formation that says that SSA develop based on microvesicular HP. However, some authors say that SSA develop *de novo* [12].

Another one fact that was revealed in this study is that Ki-67 and CD44 expression levels significantly correlate in serrated polyps of the distal colon. Ki-67 expression was characterized by similar distribution pattern in studied polyps. However, CD44 does not participate cell proliferation directly, the revealed correlations may be explained by the functions of this molecule. CD44 participates in signaling transduction through cytoskeleton elements, including signals that activate mitotic activity. Moreover, CD44 participates Wnt/β-catenin signaling pathway, one of the effects of which is activation of cell proliferation [8-9].

Conclusions:

1. Sessile serrated polyps are characterized by medium proliferation index and specific patterns of Ki-67+ cells distribution: HP differ by location of Ki-67+ cells in basal and middle parts of the crypts, TSA differ by diffuse Ki-67+ cells location, SSA differ by location of Ki-67+ cells exclusively in basal part of the crypts.

2. Diffuse pattern of Ki-67+ cells location in TSA and significantly higher median of CD44+ cells relative area in these polyps (comparing to HP and SSA) indicate significant disorganization of the cellular hierarchy in TSA crypts.

3. The indexes of Ki-67 and CD44 expression level correlate in serrated polyps of the distal colon that reflexes indirect involvement of CD44 into cell proliferation activity regulation.

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