THE ROLE OF TUMORAL STROMA IN DETERMINING THE FORECAST OF RECURRENCE AND FATAL CONSEQUENCE OF STAGE IIA-IIIB COLORECTAL CANCER

Alina Yakimenko
Department of Pathological Anatomy
Kharkiv Medical Academy of Postgraduate Education
58 Amosova str., Kharkiv, Ukraine, 61176
alyayakymenko@yahoo.com

Abstract: Colorectal cancer (CRC) ranks 2nd in the structure of mortality from malignant neoplasms (MN). One of the criteria for the prognosis of CRC may be a tumor stroma, which has not been widely used in clinical practice.

The aim was to determine the main morphological features of the stroma CRC T3N0-2M0 and search for prognostic criteria for their recurrence and lethal outcome according to the operating material and autopsy.

Materials and methods. Group I included primary CRC without recurrence. The average recurrence-free period was 5 years (62.5±16.5 months). Group II – primary CRC with recurrence; IIA – with recurrences within 5 years from the date of tumor removal that did not lead to death; IIB – with the appearance of recurrence and lethal consequence of the generalization of the tumor process within 5 years from the moment of removal of the primary tumor. CRC micropreparations made according to the standard method with G+E staining were studied; immunohistochemical study was performed using monoclonal antibodies to smooth muscle actin alpha.

Results. Stromal-parenchymal ratio>50 % is a prognostic criterion for recurrence (p<0.05) and shorter recurrence-free survival (p<0.001) of patients with stage IIA-IIIB CRC. Immature stroma type CRC stage IIA-IIIB is associated with the presence of tumor budding (p<0.001), G3 differentiation (p<0.01), shorter recurrence-free survival (p<0.001); among recurrent CRCs, the immature type of stroma is associated with the lethal outcome of patients (p<0.05). Expressed levels of tumor-activated fibroblasts are one of the criteria for immature CRC stroma (p<0.003), but as an independent prognostic criterion has limited prognostic value.

Conclusions. TSR and immature type of stroma are prognostic criteria for recurrence and recurrence period of CRC pT3N0-2M0, more typical of tumors of patients with recurrence and lethal outcome with the same ratio of tumors by differentiation.

Keywords: stroma, colorectal cancer, recurrence, prognosis.

1. Introduction

In the structure of mortality from malignant neoplasms CRC ranks 2nd after MN of the respiratory system [1]. The recurrence rate of CRC is 30–40 %, which in 40–50 % occurs in the first few years after surgical removal of the tumor [2].

The prognosis of CRC largely depends on the completeness of tumor removal and the stage of the disease. At the same time, the recurrence and lethal outcome of patients among each of the stages according to the TNM system are significantly different. Some histological and molecular biological features may play an important role in determining the clinical behaviour of the tumor and the separate prognosis of the disease [3]. Thus, cancer stroma can play a key role in tumor progression, promote non-angiogenesis, prevent access to immunocompetent cells and serve as a diagnostic criterion for the course of the disease. However, reorganization of the CRC stroma can both promote and limit the aggressive biological behaviour of tumor cells [4].

The aim of the research – determination of the main morphological features of the CRC T3N0-2M0 stroma and search for prognostic criteria for their recurrence and lethal outcome according to the data of surgical material and autopsy.

2. Methods

The material of operated CRCs and sections of sections of patients who died of CRC in the Clinical multidisciplinary hospital No. 17 in Kharkiv for the period from 2010 to 2020 were studied.

The study was approved by the Commission on Bioethics of the Kharkiv Medical Academy of Postgraduate Education on 14.10.21, protocol No. 3.

Selected cases of CRC IIA and IIIB stage, T3N0-1M0 according to the TNM system. Group I included primary CRC without recurrence. The mean follow-up was 5 years (62.5±16.5 months). Group II – primary CRC with recurrence; IIA – with recurrences within 5 years from the date of tumor removal that did not lead to death; IIB – with the appearance of recurrence and lethal consequence of the generalization of the tumor process within 5 years from the moment of removal of the primary tumor. In the study groups, adenocarcinomas were selected with the same ratio of tumors by differentiation: one case of CRC G1, 14 cases of G2, 5 cases of G3 in each group.

The material was paraffin blocks of tumors, from which were made histological, stained with hematoxylin eosin by standard methods. Immunohistochemical study was performed using monoclonal antibodies to DAKO smooth muscle actin alpha.

The ratio of the stromal component to the total area of the CRC in the areas of greatest presence of the tumor stroma was investigated. The field of view was considered to be a magnification of the ×10 microscope lens and in the presence of at least one field of view with a predominance of the stromal component, the tumor was classified as CRC with a high stromal-parenchymal ratio (SPR).

CRCs were divided into three types according to the stroma maturity characteristic. Tumors with immature stroma had a predominance of thin collagen fibers, myxomatosis, with high cellularity due to active forms of fibroblasts. The mature stroma was characterized exclusively by coarse collagen fibers, which could not be determined due to hyalinosis, fibroblasts are absent, the stroma resembles scar tissue. RGZ with an intermediate type of stroma is characterized by intermediate characteristics, absence or weak myxomatosis, possible non-gross hyalinosis.

An IHC study of the CRC stroma for the expression of smooth muscle actin alpha was performed to differentiate fibroblasts from tumor-activated fibroblasts (TAF). The CRC stroma in the reactive change zones was conditionally divided into one that had a limited TAF level and a pronounced TAF level. Stroma with limited levels of TAF was characterized by a few myofibroblasts, which were localized more closely near cancer complexes and fewer or were absent in areas of reactive stroma as they moved away from cancer cells. Stroma with pronounced TAF was characterized by diffuse, while TAF were the dominant cells of the connective tissue stroma.

The relationship between the traits was assessed by the nonparametric Pearson and Spearman χ^2-test. p<0.05 was considered statistically significant. The analysis of the probability of recurrence over a fixed time was assessed by the Kaplan-Meier procedure.

3. Results

CRC with SPR>50 % were in 43.3 % (26/60), respectively in 56.7 % (34/60) – with SPR≤50 %. It was found that the number of cases of CRC with a pronounced stromal component

was higher among recurrent CRC compared with non-recurrent, mainly due to a subgroup of recurrent CRC with a fatal consequence of tumor progression (p<0,05) (**Table 1**). Recurrence-free survival (RFS) was shown to be lower among CRCs with a pronounced stromal component (p<0.001) (**Fig. 1**).

The immature type of stroma with myxomatosis was observed in 15.0 % (9/60) CRC, was more characteristic of recurrent CRC (p>0.05) and was associated with subgroup IIB – recurrent with a lethal outcome (p<0.05), which characterizes this sign as an unfavourable prognostic criterion.

There was a statistically significant relationship between the recurrence period and the type of CRC stroma. As can be seen from **Fig. 1**, the RFS of patients with immature stroma type was lower (p<0.001), the highest value of RFS was among CRC with mature stroma type.

There was a strong correlation between tumor budding (TB) and stroma type (r=0.72, p=0.0002), as well as between CRC differentiation and stroma type (r=0.56, p=0.004). In areas of TB and areas of reduced CRC differentiation with the appearance of tumor cell clusters (which characterize G3), the stroma often lost its signs of mature and myxomatosis or young active fibroblasts appeared. In cases of CRC with TB, the immature type of stroma was observed in 32.1 % (9/28), the mature type – in 25.0 % (7/28), the intermediate type – in 42.9 % (12/28). Among CRC with immature type of stroma, G3 differentiation was observed in 66.6 % (6/9), among mature – in 17.6 % (9/51) (p<0.01).

Table 1

Morphological characteristics of the stroma of CRC study groups

Group Features	I group, n=20	II group, n=40	
		IIA, n=20	IIB, n=20
SPR>50 %	5 (25.0 %)	8 (40.0 %)	13 (65.0 %)
SPR≤50 %	15 (75.0 %)*	12 (60.0 %)	7 (35.0 %)
Mature type	13 (65.0 %)	11 (55.0 %)	7 (35.0 %)
Intermediate type	5 (25.0)	8 (40.0 %)	7 (35.0 %)
Immature with mixomatosis	2 (10.0 %)	1 (5.0 %)	6 (30.0 %)**
Limited TAF level	13(65.0 %)	9 (45.0 %)	7 (35.0 %)
Expressed level of TAF	7 (35.0 %)	11 (55.0 %)	13 (65.0 %)

*Note: * – significant difference from group II, p<0.05; ** – significant difference from subgroup IIA, p<0.05*

It has been shown that the type of CRC stroma is characterized by the peculiarity of the distribution and level of TAF, which were determined by the positive expression of smooth muscle actin alpha. Thus, all CRC cases with immature stroma type had a pronounced level of TAF and, accordingly, these two indicators had an association (p<0.003), the mature stroma type was associated with a limited level of TAF (p<0.03), the intermediate stroma type was characterized by approximately equal relative number of cases with a pronounced and limited level of TAF.

Assessment of TAF levels in invasive areas of the tumor periphery in general complements the characterization of CRC stroma types and when considering the impact on the prognosis of CRC separately, there was also a tendency to a higher number of cases with a pronounced level of TAF among recurrent CRC and recurrent with a fatal outcome (p=0.15) and a tendency to a lower term RFS among CRC with a pronounced level of TAF (p=0.07) (**Fig. 1**).

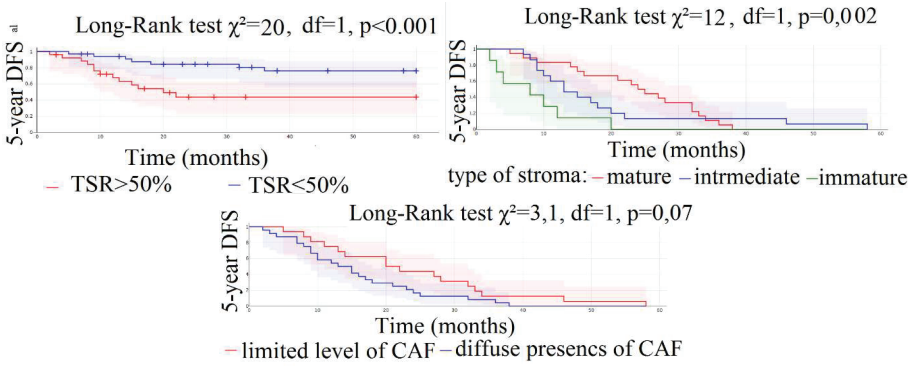

Fig. 1. 5-year recurrence-free survival (5-year DFS) of patients depending on the area of the stromal component (TSR), stroma maturity and TAF level

4. Discussion

Certain CRC factors are important in tumor differentiation, cell migration, tumor progression, and the microenvironment and stromal component itself influence the biological behaviour, differentiation and dissociation of cancer cells. According to some data, type I collagen in the central parts of the tumor is a factor of the microenvironment that inhibits the process of dedifferentiation [5]. Fibrous stroma can initiate tumor progression, promote non-angiogenesis, prevent access to lymphocytes, macrophages and other immunocompetent cells.

In the scientific literature there is a lot of work on the prognostic value of the tumor stroma ratio (tumor cancer ratio) with the definition that the tumor stroma ratio of more than 50 % characterizes the aggressiveness of CRC and is associated with shorter recurrence and overall survival, as well as greater stage T and N [6–8]. However, there are opposite data. Thus, according to Eriksen A. C. and co-authors [9], low TSR CRC stage II, which also did not receive adjuvant PCT, was associated with lower recurrence-free survival, overall survival, microsatellite instability, and increased tumor budding. These data may indicate a protective role of the tumor stroma.

According to our data, evidence and confirmation of the fact that the clinical behaviour and consequence of CRC are due to the nature of the tumor stroma.

It is also confirmed that the reorganization of cancer stroma contributes to the aggressive biological behaviour of tumor cells, which may be due to changes in stromal cell types, their molecular biological characteristics, changes in signalling pathways in and from stromal cells [4].

One of the key components of reactive cancer stroma is myofibroblasts, or TAF. In addition to ECM production, these cells are able to produce lytic enzymes involved in the degradation of the basement membranes of tumor glands and promote the formation of the appropriate cancer phenotype with the formation of individual clusters of cancer cells, increasing their invasive and metastatic activity [10, 11]. The data obtained may indicate that TAF is one of the characteristics of the cancerous stroma and determine its phenotype. TAFs influence the biological behaviour of CRCs, which together with other microenvironmental factors determine the clinical behaviour of cancer and can serve as prognostic criteria for the disease.

Study limitations. To clarify the role of the stroma in the formation of the CRC phenotype and its prognosis, it is necessary to conduct a study using immunohistochemical and

molecular genetic markers characterizing both the stromal and parenchymal components of the tumor.

Prospects for further research. It remains relevant to further study the CRC stroma at the molecular biological level with the determination of its role in the activation of signalling pathways and the formation of aggressive clinical behaviour of the tumor.

5. Conclusions

1. Stromal-parenchymal ratio >50 % is a prognostic criterion for recurrence ($p<0.05$) and shorter recurrence-free survival ($p<0.001$) of patients with stage IIA-IIIB CRC.

2. Immature type of stroma CRC IIA-IIIB stages is associated with the presence of tumor budding ($p<0.001$), G3 differentiation ($p<0.01$), shorter recurrence-free survival ($p<0.001$); among recurrent CRCs, the immature type of stroma is associated with the lethal outcome of patients ($p<0.05$).

3. Expressed level of tumor-activated fibroblasts is one of the criteria for immature CRC stroma ($p<0.003$), but as an independent prognostic criterion has limited prognostic value.

Conflict of interests

The authors declare there is no conflict of interests.

References

1. Fedorenko, Z. P., Hulak, L. O., Mykhaillvych, Yu. Y., Horokh, Ye. L., Ryzhov, A. Yu., Sumkina, O. V., Kutsenko, L. B. (2020). Rak v Ukraini, 2018–2019. Zakhvoriuvanist, smertnist, pokaznyky diialnosti onkolohichnoi sluzhby. Biuleten natsionalnoho kantser-reiestru Ukrainy, 21, 101.
2. Ryuk, J. P., Choi, G.-S., Park, J. S., Kim, H. J., Park, S. Y., Yoon, G. S. et. al. (2014). Predictive factors and the prognosis of recurrence of colorectal cancer within 2 years after curative resection. Annals of Surgical Treatment and Research, 86 (3), 143–151. doi: http://doi.org/10.4174/astr.2014.86.3.143
3. Poornakala, S., Prema, N. (2019). A study of morphological prognostic factors in colorectal cancer and survival analysis. Indian Journal of Pathology and Microbiology, 62 (1), 36. doi: http://doi.org/10.4103/ijpm.ijpm_91_18
4. Conklin, M. W., Keely, P. J. (2012). Why the stroma matters in breast cancer: insights into breast cancer patient outcomes through the examination of stromal biomarkers. Cell Adhesion & Migration, 6 (3), 249–260. doi: http://doi.org/10.4161/cam.20567
5. Ueno, H., Jones, A. M., Wilkinson, K. H., Jass, J. R., Talbot, I. C. (2004). Histological categorisation of fibrotic cancer stroma in advanced rectal cancer. Gut, 53 (4), 581–586. doi: http://doi.org/10.1136/gut.2003.028365
6. Hutchins, G. G. A., Treanor, D., Wright, A., Handley, K., Magill, L. et. al. (2017). Intratumoral stromal morphometry predicts disease recurrence but not response to 5-fluorouracil-results from the QUASAR trial of colorectal cancer. Histopathology, 72 (3), 391–404. doi: http://doi.org/10.1111/his.13326
7. Hynes, S. O., Coleman, H. G., Kelly, P. J., Irwin, S., O'Neill, R. F., Gray, R. T. et. al. (2017). Back to the future: routine morphological assessment of the tumour microenvironment is prognostic in stage II/III colon cancer in a large population-based study. Histopathology, 71 (1), 12–26. doi: http://doi.org/10.1111/his.13181
8. Huijbers, A., Tollenaar, R. A. E. M., v Pelt, G. W., Zeestraten, E. C. M., Dutton, S., McConkey, C. C. et. al. (2013). The proportion of tumor-stroma as a strong prognosticator for stage II and III colon cancer patients: validation in the VICTOR trial. Annals of Oncology, 24 (1), 179–185. doi: http://doi.org/10.1093/annonc/mds246
9. Eriksen, A. C., Sørensen, F. B., Lindebjerg, J., Hager, H., dePont Christensen, R., Kjær-Frifeldt, S., Hansen, T. F. (2018). The prognostic value of tumour stroma ratio and tumour budding in stage II colon cancer. A nationwide population-based study. International Journal of Colorectal Disease, 33 (8), 1115–1124. doi: http://doi.org/10.1007/s00384-018-3076-9
10. Martin, M., Pujuguet, P., Martin, F. (1996). Role of Stromal Myofibroblasts Infiltrating Colon Cancer in Tumor Invasion. Pathology – Research and Practice, 192 (7), 712–717. doi: http://doi.org/10.1016/s0344-0338(96)80093-8
11. Mao, Y., Keller, E. T., Garfield, D. H., Shen, K., Wang, J. (2012). Stromal cells in tumor microenvironment and breast cancer. Cancer and Metastasis Reviews, 32 (1-2), 303–315. doi: http://doi.org/10.1007/s10555-012-9415-3

Received date 14.09.2021
Accepted date 20.10.2021
Published date 28.10.2021

How to cite: *Yakimenko, A. (2021). The role of tumoral stroma in determining the forecast of recurrence and fatal consequence of stage IIA-IIIB colorectal cancer. Technology transfer: innovative solutions in medicine, 13–15. doi: https://doi.org/10.21303/2585-6634.2021.002139*