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

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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. 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 role 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).

Cancers 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-activating 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 non-parametric Pearson and Spearman \( \chi^2 \)-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, $n=40$ |
|----------------|----------------|-----------------|
| SPR≤50 %       | 5 (25.0 %)     | 8 (40.0 %)      |
| SPR>50 %       | 15 (75.0 %)*   | 12 (60.0 %)     |
| Mature type    | 13 (65.0 %)     | 7 (35.0 %)      |
| Intermediate type | 5 (25.0 %)     | 8 (40.0 %)      |
| Immature with mixomatosis | 2 (10.0 %)     | 6 (30.0 %)**    |
| Limited TAF level | 13 (65.0 %)     | 9 (45.0 %)      |
| Expressed level of TAF | 7 (35.0 %)     | 11 (55.0 %)     |

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 epithelium 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.

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**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

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