

STAGE OF DIFFERENTIATION, PROLIFERATIVE INDEX OF TUMOR CELLS AND CYTOTOXIC ACTIVITY OF PERIPHERAL BLOOD LYMPHOCYTES IN COLORECTAL CANCER PATIENTS

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Aim: To evaluate prognostic value of activity of cytotoxic lymphocytes of peripheral blood (CTL) of patients with colorectal cancer (CRC). **Methods:** 2-year survival of 30 patients with colorectal cancer stage II ($T_{2-3}N_{0-1}M_0G_{1-3}$) was investigated in relation with clinical data, cytotoxic activity of peripheral blood lymphocytes (by MTT-test) and immunohistochemical peculiarities of tumor cells using anti-Ki-67-MABs. **Results:** It was revealed that some factors had prognostic significance, namely regional lymph nodes involvement ($r = -0.69$), proliferative activity of tumor cells (Ki-67-positivity) ($r = -0.67$), restoration of CTL function after operation ($r = 0.46$), adjuvant chemotherapy ($r = 0.41$) and differentiation stage of tumor ($r = 0.40$). Negative correlation between CTL activity before operation and tumor proliferative activity also exists ($r = -0.35$). **Conclusion:** the data point to the involvement of natural killer cells in the control of tumor growth.

Key Words: colorectal cancer, cytotoxic activity, Ki-67, survival.

It is well recognised now that efficacy of anticancer therapy strongly depends on application of combined approaches based on surgery, chemotherapy and immunotherapy. That's why the study of immune mechanisms of anticancer defense and the cells involved in its realization are of exceptional importance [1]. The special attention has been paid to the subpopulations of lymphoid cells possessing cytotoxic activity toward tumor cells [2]. Their phenotypes, cytotoxic activity, peculiarities of activation upon action of various cytokines, correlation with prognosis of the diseases have been reported [3–6]. However, the data on prognostic value of the level of activity of cytotoxic lymphocytes in patients with colorectal cancer (CRC) remain controversial [7].

The present study was aimed on evaluation of prognostic value of activity of cytotoxic lymphocytes of peripheral blood of CRC patients. The study of cytotoxic activity (CTA) of natural killer cells (NK) was determined as well as proliferation level and the stage of differentiation of tumor cells.

30 patients (18 females and 12 males, medium age — 63.65 ± 0.81 years) with CRC of stage II ($T_{2-3}N_{0-1}M_0G_{1-3}$), 2nd clinical group, cured in the Department of Abdominal Surgery, Kyiv Municipal Oncological Hospital (Kyiv, Ukraine) were involved in the study. All patients underwent radical surgery, and in 5 cases — chemotherapy (5-fluorouracyl and leucovorin).

CTA of lymphocytes toward cultured K562 cells in relation 1 : 40 [10] was studied by MMT-test before the surgery and 6 months after the surgery [8]. Surgically removed tumor tissue underwent histological ex-

amination and immunohistochemical study for Ki-67 and p53 expression on paraffine slides using monoclonal antibodies (MIB-5 and DO-7, respectively) and immunoperoxidase streptavidine-biotin method (Dacopatts, Denmark). In each calculation, 300 tumor cells have been used. Statistical analysis was performed using computer program SPSS-II and Student's *t*-test. *p* values < 0.05 were considered significant.

During 2-year observation period, in the group of studied CRC patients 7 patients died (2 — in the 1st year, 5 — in the 2nd year), in 8 patients developed recurrence of the primary tumors or distant metastases. So, the 2-year survival rate was 76.66%, and recurrence-free survival rate was 50%, what is in accordance with the data of other authors [9]. On the moment of surgery, no patients have distant metastases, and in 8 patients metastases in regional lymph nodes were revealed. By the stage of tumor cell differentiation, highly differentiated, moderately differentiated and low differentiated adenocarcinoma were diagnosed in 5, 13 and 12 cases, respectively. Proliferative activity of tumor cells varied significantly among studied cases (Ki-67 was expressed in 1 to 60% of tumor cells).

The negative correlation between proliferative activity and differentiation stage of tumor cells was revealed ($r = -0.53$; $p < 0.05$). In the cases of highly differentiated adenocarcinoma the proliferation level was low, whilst in the cases of highly differentiated adenocarcinoma it was significantly higher (Ki-67 positive tumor cells yield 1–7%, ($3.21 \pm 1.54\%$) and 12–48% (34.31 ± 8.43), respectively, Figure). By this marker, the mostly heterogenic group was that with moderately differentiated tumor cells (Ki-67 positive tumor cells represent from 1–2% to 60% of tumor cell population).

One should note that proliferative activity of tumor cells positively correlate with the lymph node metastasis ($r =$

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Abbreviations used: CRC — colorectal cancer; CTA — cytotoxic activity; CTL — cytotoxic lymphocytes; NK — natural killer cells.

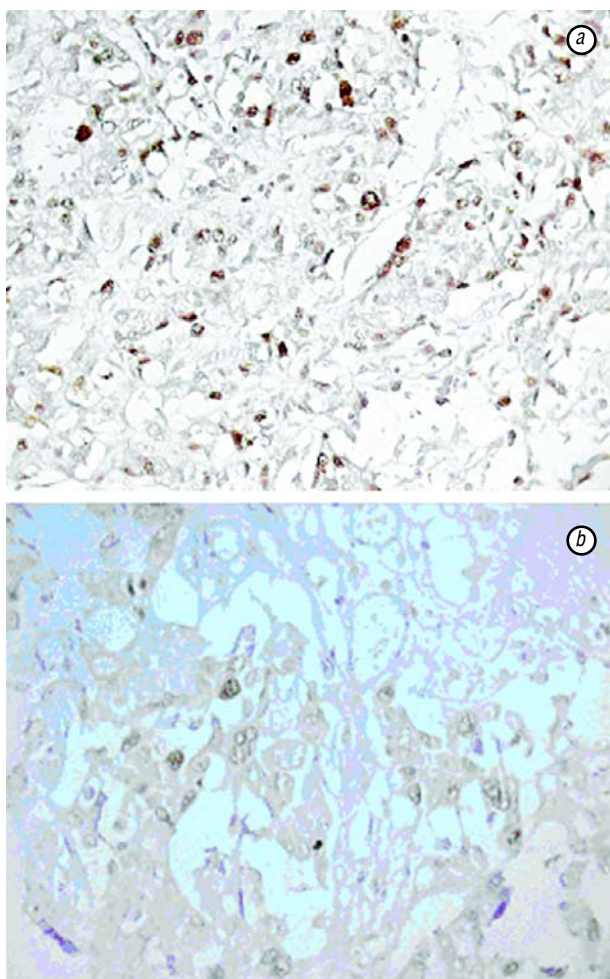


Figure. Immunohistochemical staining of low differentiated adenocarcinoma of stage III (a) and highly differentiated adenocarcinoma of stage I (b) using anti-Ki-67-MABs. Immunoperoxidase ABC-method, x 320

0.76, $p < 0.001$) and negatively correlate with relative content of lymphocytes ($r = -0.45$; $p < 0.05$). At the same time, we have shown that such parameters as the presence of regional lymph node metastasis ($r = -0.69$; $p < 0.001$), proliferative activity ($r = -0.67$; $p < 0.001$) and differentiation stage of tumor cells ($r = -0.40$; $p < 0.05$) may serve as significant prognostic factors for recurrence-free survival (Table 1). Other indexes (age and sex of patients; hemogram's indexes (anaemia syndrome, leukocytosis, thrombocyte's content, relative and absolute lymphocytosis); cell and humoral immunity indexes (subpopulational lymphocyte content, plasma content of immunoglobulins IgG, IgM, IgA or immune complexes)) have no prognostic significance. The level of p53 expression in tumor cells has no significance for prognosis, too (Table 2).

The study of CTA of peripheral blood lymphocytes has revealed the significant decrease of that index in CRC patients before surgery in comparison with healthy donors (46.35 ± 2.43 vs 77.9 ± 2.49 , $p < 0.05$) and its negative correlation with tumor cell proliferation ($r = -0.35$; $p < 0.05$). However, CTA has no prognostic significance in pre-surgical period.

In contrary, the restoration of the level of CTA of EK during post-surgical period positively correlated with recurrence-free survival ($r = 0.46$; $p < 0.01$) (Table 2). For example, in all CRC patients that died during the 2nd year

Table 1. Prognostic factors for CRC patients in correlation with recurrence-free survival

Index	Correlation coefficient, r	$p <$
Presence of regional node metastases	-0.69884	0.001
Proliferation activity of tumor cells	-0.67344	0.001
Restored function of NK after surgery	0.46833	0.01
Performed chemotherapy after surgery	0.406747	0.05
Tumor differentiation stage	-0.40171	0.05

Table 2. Cytotoxic activity of NK cells, stage of differentiation and proliferation rate of tumor cells from CRC patients

Patients	Stage of tumor differentiation			Cytotoxic activity of NK cells, %		p53 and Ki-67 expression in tumor cells (% of positively stained cells)	
	G _I	G _{II}	G _{III}	Before surgery	6 months after surgery	p53	Ki-67
Died (n = 5)	0	3	2	49.36 ± 3.96	46.30 ± 5.16	31.5 ± 12.44	52.14 ± 4.08
Recurrence (n = 8)	0	5	3	50.66 ± 4.35	52.87 ± 3.93	55.67 ± 17.07	15.33 ± 2.07
Recurrence free (n = 15)	5	5	5	42.26 ± 4.85	58.44 ± 2.38	47.15 ± 10.88	10.16 ± 6.22

of observation, CTA level of peripheral blood lymphocytes altered in the range of 1.29 ± 0.57 ($p > 0.05$) in 6 months after the surgery in comparison with that in pre-surgical period; in CRC patients with recurrence — remained unaltered or increased non-significantly (by 13.10 ± 6.25 , $p > 0.05$); in CRC patients with 2-year recurrence-free survival — significantly increased in comparison with the group of patients with recurrence ($p < 0.05$) (by $38.18 \pm 16.91\%$). One should note that post-surgical chemotherapy positively correlated with recurrence-free survival, too ($r = 0.40$; $p < 0.05$).

The data of other authors show that the CTA level of EK may serve as a criterion for application of immunotherapy for CRC patients after surgery [7] and an important factor influencing recurrence-free survival of patients after combined therapy [11]. Presently, the stimulation of activity of EK and other subpopulations of cytotoxic cells either by non-specific immunostimulators or vaccines directed on stimulation of antitumor immunity is considered as a perspective approach in the therapy of CRC patients [11–16].

In conclusion, our data have demonstrated that alteration of CTA of EK cells toward K562 cells may be used for evaluation of prognosis of CRC patients undergoing combined therapy. If CTA indexes remain unaltered and tumor cells possess high proliferation rate, the prognosis of CRC is unfavorable. Restoration of CTA value in post-surgical period and low proliferation rate of tumor cells correlate with good prognosis of the disease.

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СТЕПЕНЬ ДИФФЕРЕНЦИРОВКИ, УРОВЕНЬ ПРОЛИФЕРАЦИИ ОПУХОЛЕВЫХ КЛЕТОК И ЦИТОТОКСИЧЕСКАЯ АКТИВНОСТЬ ЛИМФОЦИТОВ ПЕРИФЕРИЧЕСКОЙ КРОВИ БОЛЬНЫХ КОЛОРЕКТАЛЬНЫМ РАКОМ

Цель: определить прогностическое значение цитотоксической активности лимфоцитов периферической крови больных колоректальным раком (КРР). **Методы:** проанализирована двухлетняя выживаемость 30 больных раком толстого кишечника II стадии ($T_{2-3}N_{0-1}M_0G_{1-3}$) после проведенного комплексного лечения. Цитотоксическая активность естественных киллеров (ЕК-клеток) по отношению к клеткам линии K562 изучалась с помощью МТТ-теста. Пролиферативная активность опухолевых клеток оценена с помощью экспрессии Ki-67 путем иммуногистохимического окрашивания парафиновых срезов опухоли. **Результаты:** установлено, что факторами прогноза заболевания являются поражение регионарных лимфатических узлов ($r = -0,69$), пролиферативная активность опухоли (Ki-67⁺) ($r = -0,67$), восстановление активности ЕК после операции ($r = 0,46$), проведение адьювантной химиотерапии ($r = 0,41$), степень дифференцировки опухолевой ткани ($r = 0,40$). Выявлена негативная зависимость между цитотоксической активностью до операции и пролиферативной активностью опухолевой ткани ($r = -0,35$). **Вывод:** полученные данные свидетельствуют об участии ЕК в контроле опухолевого роста, проведение иммунотерапии больным КРР является перспективным.

Ключевые слова: колоректальный рак, цитотоксическая активность, Ki-67-позитивность, выживаемость.