

**THE ROLE OF CHEMOKINES IN THE RECRUITMENT OF PROGENITOR CELLS INTO THE
TUMOR NICHE IN PATIENTS WITH BREAST CANCER**

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**РОЛЬ ХЕМОКИНОВ В РЕКРУТИРОВАНИИ КЛЕТОК-ПРЕДШЕСТВЕННИКОВ В
ОПУХОЛЕВУЮ НИШУ ПРИ РАКЕ МОЛОЧНОЙ ЖЕЛЕЗЫ**

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***Аннотация.** Развитие первичной опухоли сопровождается формированием опухолевой ниши, которая создает благоприятные условия для выживания и пролиферации раковых клеток. Одним из ключевых элементов эволюции опухолевой ниши является рекрутирование костномозговых клеток-предшественников, включая клетки-предшественники макрофагов, мезенхимальные столовые клетки, эндотелиальные и гемопоэтические клетки-предшественники. Миграция упомянутых клеток в опухоль регулируется рядом хемокинов, в том числе CCL2, CXCL12, MSP (macrophage stimulating protein) и MIF (macrophage inhibitory factor). Целью настоящего исследования являлось изучение параметров опухолевой ниши при раке молочной железы. Исследование включало 24 больных с инвазивной карциномой неспецифического типа молочной железы. В суспензии опухолевых клеток методом проточной цитофлюориметрии определяли содержание клеток-предшественников. Концентрацию хемокинов CCL2, CXCL12, MSP и MIF в венозной крови больных оценивали с помощью твердофазного иммуноферментного анализа. Достоверных различий в содержании исследованных клеточных популяций, а также концентрации изученных хемокинов между пациентами, разделенными на группы в зависимости от наличия или отсутствия лимфогенных метастазов и неoadъювантного лечения, обнаружено не было. В то же время, установлена прямая корреляционная связь между содержанием гемопоэтических клеток-предшественников в опухоли и концентрацией CXCL12 и MIF в крови.*

Introduction. It is becoming more and more recognized that the progression of malignancies is largely determined by the dynamic interaction between tumor cells and cancer niche [1]. Similar to pre-metastatic niche that arises in the site of future metastasis, cancer niche is formed in the location of the primary tumor and provides hospitable environment for tumor growth and progression [2]. Evolution of the cancer niche accompanies the development of the primary tumor and implicates, along with activation of the stromal elements and secretion of chemokines and cytokines, the recruitment of bone marrow-derived progenitor cells including macrophage progenitor cells, mesenchymal stem cells (MSC), endothelial and hematopoietic progenitor cells (EPC, HPC) [1]. The signals that potentially control the recruitment of progenitor cells into the tumor include chemokines CCL2, CXCL12, MSP, and MIF [3–5]. Presumably, the development of the cancer niche not only

potentiates cancer cells survival and proliferation, but also enables some populations of malignant cells to acquire invasive and metastatic capabilities, therefore enhancing the malignant potential of the tumor [6]. In accordance with aforementioned data, the current study was aimed to investigate the parameters of the cancer niche in patients with invasive non-specific breast carcinoma, including the subpopulations of intratumoral progenitor cells and the concentrations of chemokines participating in the cancer niche formation, as well as to determine the connection of investigated parameters with the presence or absence of lymphogenic metastases and neoadjuvant treatment.

Materials and methods. The study included 24 patients with invasive breast carcinoma of no special type (T1-4N0-3M0) aged 36 to 68 years. Eight out of twenty-four patients received neoadjuvant treatment. Eleven patients were diagnosed with lymphogenic metastases, thirteen were free of any metastases. Cell suspensions were prepared from the breast tumor tissue through disaggregation using BD Medimachine System. The percentage of progenitor cells (CD34, CD133, CD90, VEGFR1, CD11b, CD45, CD202) in the acquired cell suspensions was measured with flow cytometry. Cells with CD34+ CD202+CD45–CD133+ phenotype were identified as endothelial progenitor cells, CD34–CD90+CD45– cells – as mesenchymal stem cells, CD34+ CD45+CD11b+ cells – as macrophage progenitor cells, and CD34+CD45+CD133–VEGFR1+ cells – as hematopoietic progenitor cells. The concentrations of CCL2, CXCL12, MSP, and MIF in the venous blood of patients were evaluated by solid-phase enzyme-linked immunosorbent assay (ELISA). The presence of lymphogenic metastases was verified morphologically. The acquired results were statistically processed using Statistica 8.0 (Statsoft, 2007).

Results. Using the flow cytometry, the quantitative data on the subpopulation structure of progenitor cells in tumor tissue of patients with invasive breast carcinoma of no special type was obtained. In order to determine the connection between the investigated subpopulation structure and the presence or absence of lymphogenic metastases and neoadjuvant treatment, all patients were divided into groups accordingly. However, no statistically significant difference in the percentage of precursor cells between given groups was found (Table 1).

Table 1.

The subpopulations of progenitor cells in tumor in patients with invasive non-specific breast carcinoma, %.

	LM-	LM+	NAT-	NAT+
Hematopoietic progenitor cells	1,78 (0,92-4,56)	1,98 (1,66-3,21) $p_1 > 0,05$	2,38 (0,98-4,21)	1,8 (1,33-2,78) $p_2 > 0,05$
Endothelial progenitor cells	2,6 (1,84-4,16)	1,71 (0,29-1,87) $p_1 > 0,05$	2,56 (1,72-3,84)	1,78 (0,86-2,91) $p_2 > 0,05$
Macrophage progenitor cells	1,1 (0,71-2,19)	0,73 (0,73-1,85) $p_1 > 0,05$	0,96 (0,70-2,13)	1,29 (0,73-1,92) $p_2 > 0,05$
Mesenchymal stem cells	6,48 (3,04-17,09)	2,56 (0,01-7,14) $p_1 > 0,05$	6,33 (2,91-14,18)	4,85 (1,28-13,03) $p_2 > 0,05$

“LM-” – absence of lymphogenic metastases; “LM+” – presence of lymphogenic metastases. “NAT-” – absence of neoadjuvant chemotherapy; “NAT+” – presence of neoadjuvant chemotherapy. p_1 – significance of statistical difference in comparison to patients without lymphogenic metastases; p_2 – significance of statistical difference in comparison to patients without neoadjuvant chemotherapy.

The concentrations of chemokines participating in the formation of the cancer niche in patients with invasive breast carcinoma including CCL2, CXCL12, MSP, and MIF were determined. All four chemokines were expressed in the peripheral blood of 100% patients. There was no statistically significant difference in the concentrations of studied cytokines in patients depending on the presence or absence of lymphogenic metastases and neoadjuvant treatment. Nevertheless, a positive correlation between the percentage of hematopoietic progenitor cells in the tumor and the concentrations of chemokines CXCL12 and MIF in the peripheral blood was determined ($R=0,79$ and $0,8$, respectively; $p<0,05$ in both cases).

Conclusion. Taken together, it was shown that the cancer niche is represented by a set of hematopoietic, endothelial, and macrophage progenitor cells as well as mesenchymal stem cells, with varying ratios among patients. Concurrently, the chemokines CCL2, CXCL12, MSP, and MIF that are involved in the recruitment of all the investigated progenitor cells populations into the tumor, were registered in the blood samples of patients in 100% cases. Moreover, the positive correlation between the percentage of hematopoietic progenitor cells in tumor and the concentrations of CXCL12 and MIF in the peripheral blood was determined. Acquired results strongly support the existence of chemokine-mediated recruitment of progenitor cells into the primary tumor site. At the same time, no effect of the neoadjuvant chemotherapy on the process of cancer niche formation was identified.

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