Biomedicine & Pharmacotherapy 67 (2013) 363–366

Available online at
SciVerse ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

The role of inflammatory chemokines in lymphoid neoorganogenesis in breast cancer

S.K. Gantsev [a,1], K. Umezawa [b,2], D.V. Islamgulov [c,3], E.K. Khusnutdinova [c,3], R.S. Ishmuratova [a,1], V.Y. Frolova [a,1], S.R. Kzyrgalin [a,*]

[a] Scientific Research Institute of Oncology, Bashkortostan State Medical University, Lenina street, 3, 450000 Ufa, Russian Federation
[b] Department of Molecular Target Medicine Screening, School of Medicine, Aichi Medical University, Nagakute, Aichi, 480-1195, Japan
[c] Ufa Institute of Biochemistry and Genetics, Russian Academy of Sciences, Prospect Oktyabrya street, 71, 450054, Ufa, Russian Federation

ARTICLE INFO

Article history:
Received 30 January 2013
Accepted 4 March 2013

Keywords:
Breast cancer
Chemokines
Lymphoid neoorganogenesis

ABSTRACT

The expression profiling analysis of inflammatory chemokines and their receptors in newly formed lymph nodes in breast cancer was carried out. The analysis revealed the increase in expression of the genes CCL16, XCR1, CYFIP2, TNFSF14 and the reduction in expression of chemokine ligands CXCL5 and CXCL12 in tertiary lymphoid organs. The obtained results allow us to suggest that the process of induction of lymph nodes neogenesis is identical (in its key mechanisms) to the process of lymphoid tissue neogenesis in autoimmune diseases and in some infections, but may have different triggers.

1. Introduction

Lymphoid neoorganogenesis is the process of de novo formation of new organized lymphoid tissues in chronic inflammation. The chronic inflammation is associated with the accumulation of mononuclear cells; it can be caused by neoplastic aberration, invasion of pathogens or autoimmune reactions, and it is connected with the formation of tertiary lymphoid organs [1].

These tissues resemble secondary lymphoid organs (Figs. 1–6), the development of which ends after birth, with segregation in the area of T- and B-cells, dendritic cells, follicular dendritic cells, lymphatic vessels and high endothelial venules. In lymphoid organogenesis, stromal cells activation leads to the release of molecules that control the processes of recovery, distribution and survival of leukocytes. One of the main roles in this process is played by interleukin-7 (IL-7R) [2].

Creating a niche for incoming leukocytes happens indirectly through cooperation with extracellular matrix components of adhesion molecules, cytokines and chemokines. Tumor necrosis factor (TNF) and lymphotoxin α (LT-a) are expressed by hematogenic cells and play an important role in the activation of cytokines and chemokines in mesenchymal, stromal and endothelial cells, contributing to the creation of a lymphoid niche [3].

The development of organospecific tertiary lymphoid organs is observed in transgenic mice, which have overexpression of lymphoid chemokines or LT-a. It is known that the overexpression of TNF-α in animals also leads to the formation of tertiary lymphoid organs and to the development of chronic inflammatory reaction [2,4].

Studies of chemokines and their receptors in mice led to the understanding of molecular and cellular interactions that are at the basis of lymphoid organogenesis. When developing lymphoid organs and maintaining micro-architecture of lymphoid tissues, the inflammatory cytokines of TNF family and the LT-a closely interact with the homeostatic chemokines, CXCL13, CCL21, CCL19 and CXCL12. [5].

The fact that the chemokines participate in the specific metastasis of cancer cells (CC) presents a special interest. It was found out that those breast CCs of a human express chemokine receptor CXCR4, whereas its complementary ligand CXCL12 is produced by tissue cells (bone marrow, lung and lymph nodes) in which most often CCs metastasize [6]. Herewith in system in vitro, breast CCs are able to migrate in the direction of the gradient CXCL12, and antibodies neutralizing CXCR4 inhibit the formation of metastasis in lymph nodes in experimental animals. Lymphatic endothelial cells produce other specific chemokines (CCL21) attracting to them CCs from the primary tumor, which correlates with the presence of metastases in the "sentinel" lymph node [7].

In order to study the role of chemokines and their receptors in the process of lymph nodes neoorganogenesis, the expression profiling of these genes in the tertiary lymphoid organs in patients with breast cancer was analyzed.

* Corresponding author. Tel./fax: +7 347 237 43 58.
E-mail addresses: ufa.shamil@gmail.com, kafonco@mail.ru (S.R. Kzyrgalin).
[1] kafonco@mail.ru. Tel./fax: +7 347 248 40 58.
[2] umezawa@aichi-med-u.ac.jp. Tel./fax: +81 561 62 33 11.
[3] prfg@mail.ru. Tel./fax: +7 347 237 43 58.

0753-3322/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.biopha.2013.03.017

Fig. 1. A newly formed lymph node in breast cancer.

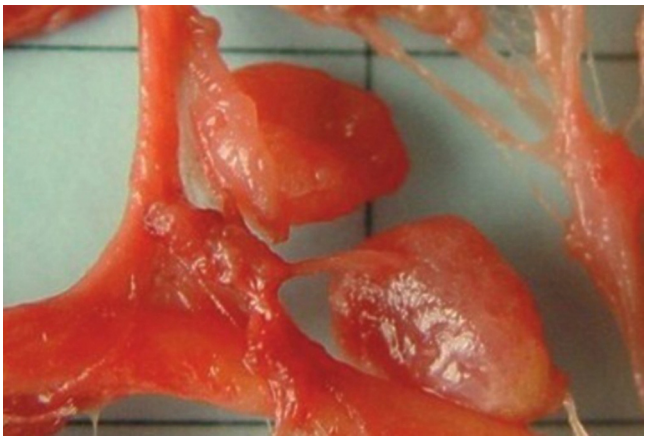

Fig. 2. Newly formed lymph nodes separated using the method of sonolipodestruction.

2. Materials and methods

The material for the study is based on RNA samples isolated from the lymph nodes tissue of 15 patients, who were clinically and cytologically diagnosed with breast cancer of T_2 N_{1-3} M_0 stage and were undergoing testing and treatment at the Republican Oncology Center in 2010. The age of breast cancer patients is ranged from 36 to 68 years (the median is 48 years). All the patients underwent surgical treatment. Total RNA was isolated from 200–300 mg of histologically normal lymph nodes tissue and from newly formed lymph nodes (Fig. 7), isolated using the method of sonolipodestruction [8,9] with Trizol® reagent (Invitrogen, USA), according to a standard protocol. The quality of the isolated RNA was determined on the NanoDrop 1000 spectrophotometer (Thermo FS) by the ratio of absorbencies of A260/A280 (only samples having 1.9–2.1 absorption factor were used). The isolated RNA was treated with DNase. Expression profiling of chemokines and their receptors was determined by fluorescent real-time PCR using Human Chemokines & Receptors PCR Array kit (Qiagen) in two replications. The analysis of the expression of the following genes was carried out:

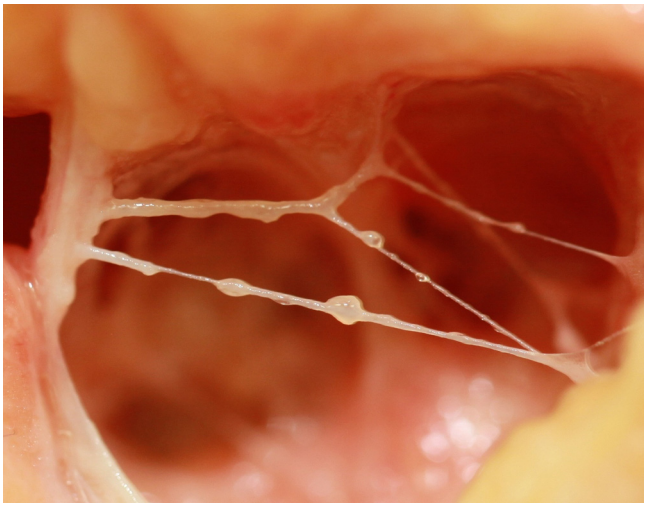

Fig. 3. A newly formed lymph node and lymphatic vessels.

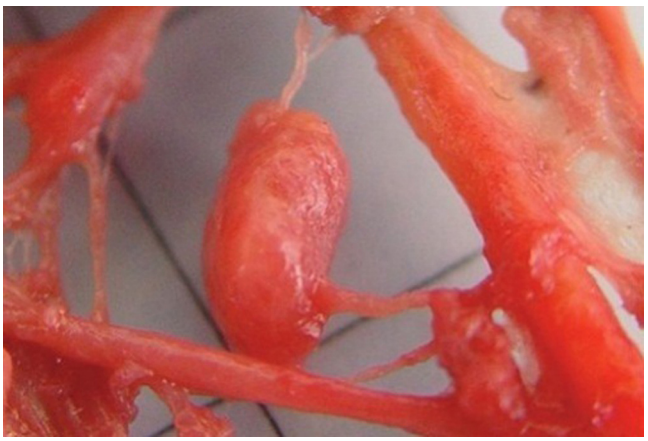

Fig. 4. Newly formed functioning lymph nodes. One afferent and one efferent lymphatic vessel is determined.

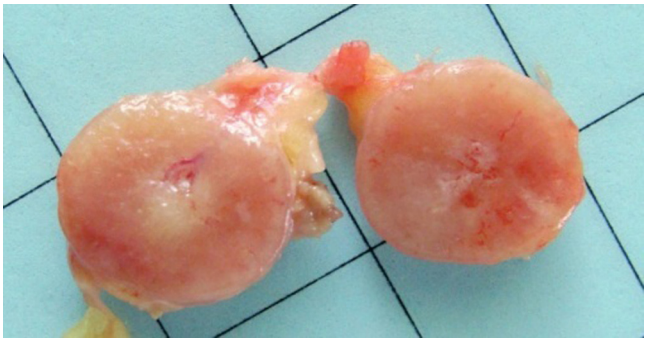

Fig. 5. A newly formed lymph node. Transection.

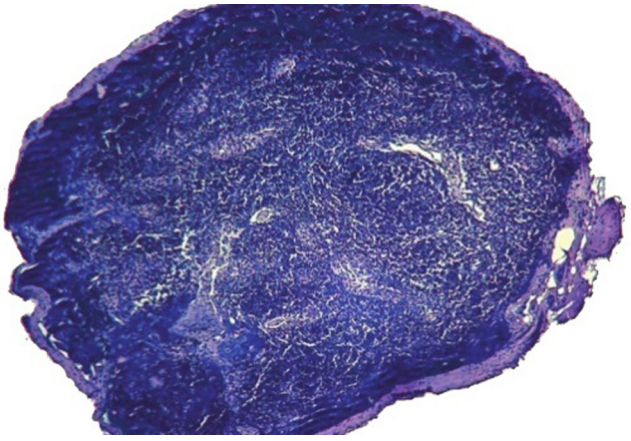

Fig. 6. A newly formed lymph node with the formed stroma. Stained with hematoxylin–eosin, ×60.