ONCOLOGY

Subpopulation of Colorectal Adenocarcinoma Cells Co-Expressing CD133 and Cancer Stem Cells Markers of Other Tumors

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 152, No. 12, pp. 684-687, December, 2011
Original article submitted August 17, 2011.

Co-expression of colorectal adenocarcinoma cancer stem cells marker CD133 and a set of surface molecules described in published reports as possible cancer stem cell markers of other solid tumors was analyzed by flow cytometry. Minor cell populations expressing CD29, CD34, CD90, and CD117 against the background of CD133 expression were detected in cancer cells suspensions from the patients with colorectal adenocarcinoma. Our findings suggest that these markers can be used as additional markers of cancer stem cells of human colorectal adenocarcinoma.

Key Words: *cancer stem cells; colorectal adenocarcinoma; surface markers; CD133; flow cytometry*

It was proven that tumor growth in the primary node and metastases is initiated by a special subpopulation of cancer cells, so-called cancer stem cells (CSC). The existence of CSC, or tumor-inducing cells, was demonstrated in studies of leukemias and solid tumors, in particular, in colorectal cancer [2,10].

CSC have high oncogenic potential. Several hundreds CSC can initiate the formation of a new tumor. In comparison with other cancer cells, CSC are characterized by lower proliferation rate and high activity of membrane transporters determining drug resistance, therefore a pool of CSC can survive even after chemo- and radiotherapy, which can lead to relapse. In light of this, the development of methods for detection and elimination of CSC are required for disease control.

There are published data on surface markers expressed on CSC of the most prevalent malignant tumors. We previously analyzed the expression of some potential surface molecular markers of CSC on colorectal adenocarcinoma cells [1]. High relevance of CD133 marker for detection of cell subpopulations associated with CSC was demonstrated. However, CD133 is not a unique marker for colorectal adenocarcinoma CSC. It is also expressed in hemopoietic stem cells, endothelial progenitor cells, neuronal and glial stem cells, and cells of the kidney, breast, trachea, salivary glands, placenta, gastrointestinal tract, and testes [8]. This necessitates the search for other markers expressed together with CD133 on colorectal adenocarcinoma cells and typical of CSC of this tumor.

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The aim of the present study is the search for surface markers of human colorectal adenocarcinoma CSC by analyzing co-expression of CD133 marker and markers of CSC of other solid tumors were analyzed.

MATERIALS AND METHODS

Biopsy specimens obtained from patients with colorectal adenocarcinoma were used without culturing. Tumor samples isolated during surgical removal of colorectal adenocarcinoma were transferred into phosphate buffer (PanEco) containing streptomycin (200 μg/ml), penicillin (200 U/ml), and amphotericin B (0.5 μg/ml). Necrotic foci were removed and the samples were minced to 1-2 mm^3 fragments and transferred to RPMI (Gibco) containing 10% ECS, collagenase (0.1 mg/ml), streptomycin (100 μg/ml), penicillin (100 U/ml), and amphotericin B (0.5 μg/ml), and incubated for 2 h at 37°C with constant stirring. After incubation, the suspension was filtered (Becton Dickinson filter, 70 μ) for removal of cell aggregates. For erythrocyte lysis, the suspension was incubated with lysing buffer (Becton Dickinson) according to manufacturer's instructions.

For immunocytochemical staining we used monoclonal antibodies to human cell surface antigens (CD10, CD34, CD45, CD20, CD24, CD29, CD90, CD44, CD117, CD133) conjugated to one of the following fluorochromes: allophycocyanin (APC), fluorescein isothiocyanate (FITC), or phycoerythrin (PE). Staining was performed routinely. Fluorescence intensity was measured on FACSAria cytofuorometer sorter (Becton Dickinson).

RESULTS

To exclude non-tumor cells from the analysis, the cell suspensions from tumor biopsy specimens were tested for CD45 (total leukocytic antigen) and CD10 (marker of tumor stroma fibroblasts). The expression of each marker was evaluated by fluorescence intensity histogram of a dye conjugated with specific monoclonal antibodies. Stromal fibroblasts were practically absent in the studied samples and their content did not exceed 0.10±0.05% (Fig. 1, *a*). Leukocytes were detected as a separate population (8.6±0.5%, Fig. 1, *b*). Leukocyte population was excluded from further analysis.

For the search for additional surface markers of colorectal adenocarcinoma CSC, co-expression of CD133 and other CSC markers typical of solid tumors (CD20, CD29, CD34, CD90, CD117) was analyzed.

Differentiation marker of B cells (CD20) can serve not only as a marker of B-cell lymphoma and lymphocytic leukemia; it is also expressed on melanoma CSC [4]. However, we did not detect this molecule of colorectal adenocarcinoma cells. Integrin subunit (CD29) was present on colorectal adenocarcinoma cells, including cells expressing CD133. A minor subpopulation of cells simultaneously expressing CD29 and CD133 was identified; these cells constituted 10.7±0.7% total cell population (Fig. 2, *a*). Published data suggest that CD29 is also expressed on breast cancer CSC [6].

Of particular interest is detection of $CD34^+CD133^+$ colorectal adenocarcinoma cells. Normally, cells expressing CD34 are present in the umbilical cord and bone marrow as hemopoietic stem cells; co-expression of CD133 and CD34 was found on endothelial progenitor cells. Moreover, CD34 are also detected in stromal tumors of the gastrointestinal tract, in acute myeloid leukemia, meningioma, schwannoma, neurofibroma, giant-cell fibroblastoma, and other tumors. Published data suggest that CD34 is also expressed on liver stem cells [9]. However, possible presence of endotheliocyte precursors in the test samples made detection of $CD34^+CD133^+$ cells low informative. In light of this,

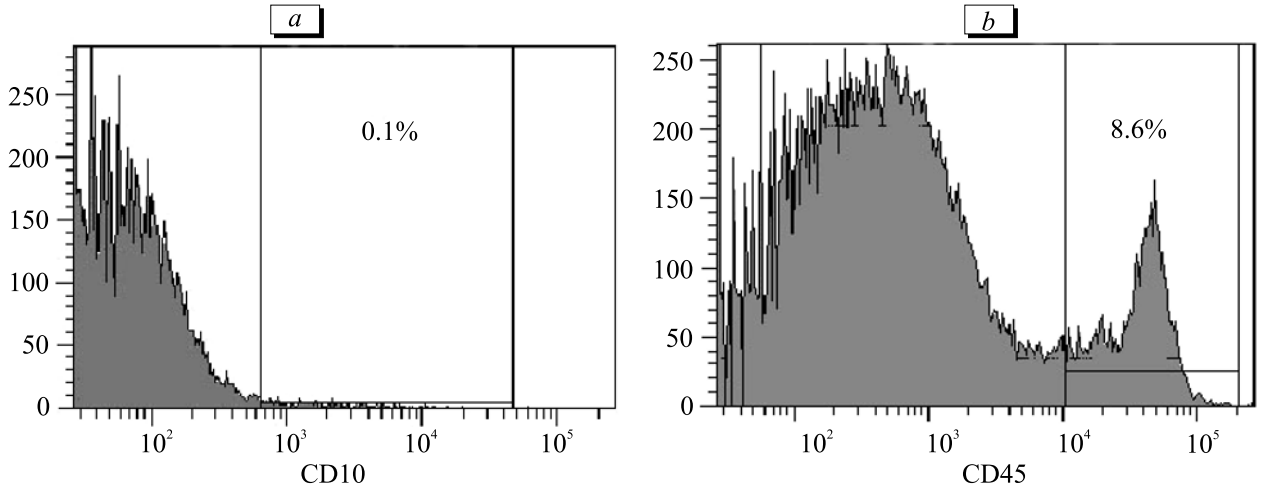

Fig. 1. Expression of markers of tumor stroma (*a*) and hemopoietic cells (*b*) on colorectal adenocarcinoma cells. Abscissa: fluorescence intensity (arb. units); ordinate: number of cells. Here and in Fig. 2: percent of detected subpopulations relative to the total number of tumor cells.

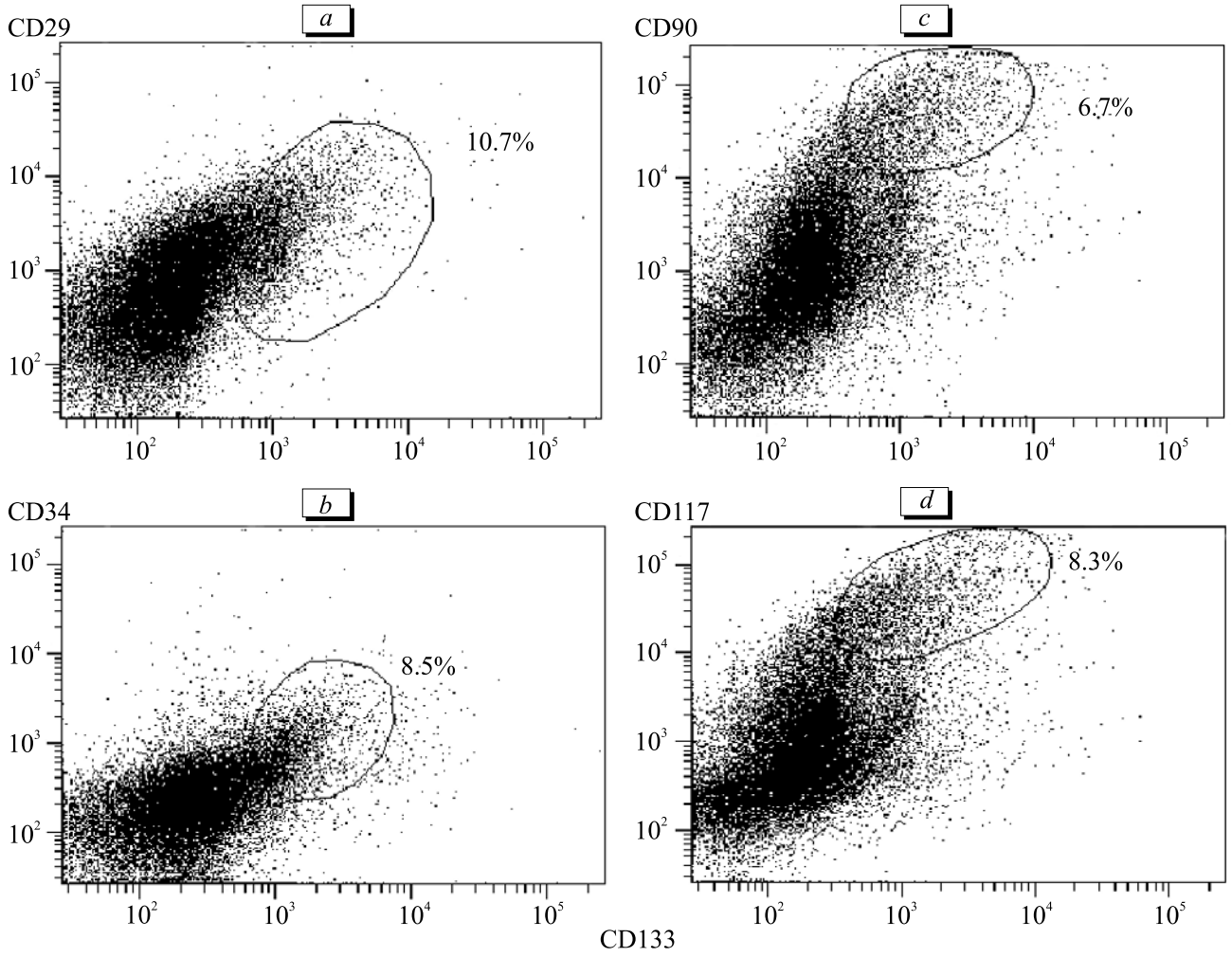

Fig. 2. Co-expression of CD133 and solid tumor CSC markers on colorectal adenocarcinoma cells. Abscissa and ordinate: fluorescence intensity (arb. units).

co-expression of CD34 and CD133 was measured with consideration of CD24 expression (marker of endothelial cells). A minor subpopulation (8.5±0.5%) of $CD34^+CD133^+CD24^+$ tumor cells in human colorectal adenocarcinoma was identified (Fig. 2, *b*).

N-glycosylated surface protein CD90 participating in cell-cell and cell-matrix interactions was described as a marker of lung and liver CSC and circulating metastatic melanoma cells [3,11,13]. CD90 is expressed on hemopoietic and mesenchymal stem cells, hepatic stem cells, some fibroblasts, endothelial cells, and some other cells. Cytometry of tumor cell suspensions from patients with colorectal adenocarcinoma revealed cells simultaneously expressing CD90 and CD133. Subpopulation of $CD90^+CD133^+$ cells constituted 6.7±1.2% (Fig. 2, *c*).

CD117 is a receptor of stem cell factor participating in mobilization of bone marrow cells and stimulation of their release into circulation. Some authors consider it as a marker of liver, lung, and ovarian CSC [3,6,14]. Mutations in CD117 gene are associated with stromal tumors of the gastrointestinal tract, melanoma, and acute myeloid leukemia [5,7,12]. In our study, co-expression of CD117 and CD133 was observed in 8.3±2.2% cells of colorectal adenocarcinoma (Fig. 2, *d*).

Our findings suggest that CD29, CD34, CD90, and CD117 can be considered as additional markers of human colorectal adenocarcinoma CSC. Expression of these markers on CSC of solid tumors can be explained by their important functions. For instance, CD29 and CD90 play an important role in metastasizing of tumor cells.

Our results extend our knowledge on surface phenotype of human colorectal adenocarcinoma CSC and create prerequisites for the development of methods of targeted therapy of this tumor and for further investigation of the mechanisms of its pathogenesis.

The study was performed within the frameworks of State Contract (P811) of Federal Agency of Science and Innovations (17.08.2009).

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