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NANOBIOMEDICINE AND NANOPHARMACEUTICALS

Assessment of Inhibition of the Growth of Breast and Colon Tumors when Blocking VEGFR-1 with Monoclonal Antibodies

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Abstract—Vascular endothelial growth factor receptor-1 (VEGFR-1) plays a critical role in tumor-associated angiogenesis. VEGFR-1 is found on the surface of tumor cells and cells in the tumor microenvironment. Blocking this receptor leads to the suppression of proliferation and increased apoptosis of tumor cells, reduction of tumor vascularization, inhibition of the production of immunosuppressive cytokines by tumor-associated macrophages, and the suppression of tumor invasion and metastasis. The creation of monoclonal antibody drugs that block VEGFR-1 is an urgent task in the development of potential antitumor therapeutic drugs. Target molecules created on the basis of antibodies that bind to VEGFR-1 are a promising basis for the creation of theranostic radiopharmaceuticals for the diagnosis and treatment of malignant neoplasms. To study the therapeutic potential of VEGFR-1 inhibition in breast and colon cancers using antibodies, monoclonal antibodies against recombinant human VEGFR-1 protein are developed. The resulting monoclonal antibodies bind to the VEGFR-1 receptor on the cell surface and effectively inhibit the proliferation of breast and colon cancer cells in vitro, reduce the growth rate of the tumor node in vivo, and prolong the survival of tumor-inoculated mice.

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INTRODUCTION

Vascular endothelial growth factor receptor-1 (**VEGFR-1**) is a tyrosine kinase receptor (**TKR**), which binds to members of the VEGF family: VEGF-A, VEGF-B, and placenta growth factor (**PlGF**) [12]. VEGF-A also interacts with VEGFR-2 and TKR, which is responsible for the activation of signal transduction pathways that mediate most of the biological effects of VEGF-A [3, 4].

VEGFR-1 is expressed in endothelial cells, during vascular formation and remodeling, and macrophages and myoepithelial cells, promoting cell migration and survival [5–8]. Moreover, it is involved in the mobilization of bone-marrow myeloid cells generating tumor-associated macrophages [1]. VEGFR-1 is often expressed on the surface of tumor cells in various types of human cancer; its increased expression is a marker of poor prognosis and a high probability of disease recurrence [1, 5]. In tumor cells, VEGFR-1 signaling inhibits apoptosis and induces chemoresistance [1, 9–11]. In addition to the transmembrane form of VEGFR-1, cells produce a soluble form of the receptor (sVEGFR-1), which arises as a result of alternative splicing of the same gene transcript [12, 13] and includes the first six Ig-like domains of membrane VEGFR-1 plus a specific sequence from 31 amino acids in the C-terminal domain. Soluble VEGFR-1 includes the growth factor binding region of membrane VEGFR-1 (residues 1–656) and thus prevents the interaction of VEGF-A and PlGF with their transmembrane TKRs [14, 15].

VEGFR-1 plays a critical role in tumor-associated angiogenesis, but not in physiological angiogenesis, unlike VEGFR-2 [16, 17]. In addition to expression in the tumor endothelium, VEGFR-1 is found on the surface of tumor cells themselves and other cells of the tumor microenvironment. Blocking this receptor leads to the suppression of proliferation and increased

apoptosis of tumor cells, a reduction in vascularization of the tumor node, inhibition of the production of immunosuppressive cytokines by tumor-associated macrophages, and the suppression of tumor invasion and metastasis.

VEGFR-1 blockade exerts antitumor activity through three distinct mechanisms: inhibition of tumor-associated angiogenesis by inhibiting endothelial activation in response to angiogenic factors released by tumor cells (i.e., VEGF-A and PlGF); reducing the mobilization of hematopoietic precursors from the bone marrow and tumor infiltration by myelomonocytic cells secreting cytokines and proangiogenic factors, which in turn can contribute to tumor aggressiveness and resistance to anti-VEGF-A therapy; direct impact on VEGFR-1-positive tumor cells by inhibiting their invasiveness and proliferation [18, 19].

Antiangiogenic therapy, which has so far been used to treat various solid tumors, interferes with VEGF-A signaling mediated by both VEGFR-2 and VEGFR-1 or exclusively by VEGFR-2 [17, 18]. The humanized monoclonal antibody (**mAb**) bevacizumab (trade names: Avastin, Avegra, and B-Mab) targets VEGF-A, thereby preventing the activation of both VEGFRs; small-molecule tyrosine-kinase inhibitors (for example, axitinib, cabozantinib, lenvatinib, pazopanib, regorafenib, sorafenib, sunitinib, vandetanib) interact with the catalytic domain of several TKRs, including VEGFR; and the fully humanized mAb ramucirumab is directed against VEGFR-2 [18, 20–22]. Unfortunately, the therapeutic use of molecules that interfere with VEGF-A/VEGFR-2 signaling results in serious side effects (e.g., bleeding, delayed wound healing, gastrointestinal perforation, hypertension, thromboembolic complications, proteinuria) due to the inhibition of physiological angiogenesis [17, 23, 24]. Molecules selectively targeting VEGFR-1 are expected to cause less toxic effects than molecules targeting VEGFR-2 or VEGF-A, since PlGF is able to transmit its own signals by phosphorylating tyrosine residues different from those phosphorylated by the stimulation of VEGFR-1 by VEGF-A [25], and VEGFR-1 does not play a significant role in physiological angiogenesis in adults [18].

Experimental approaches taken so far to selectively inhibit VEGFR-1 include targeted polymer-drug conjugates, VEGFR-1 antagonist peptides or peptidomimetics, and mAbs that block ligand binding to the receptor [4, 26–28]. There is one known therapeutic antibody against VEGFR-1, which has shown antitumor activity in a number of studies and is currently undergoing clinical trials: icrucumab (IMC-18F1) is a recombinant fully humanized mAb of the IgG1 isotype, which binds specifically and with high affinity to the VEGFR-1 receptor and blocks the binding of ligands that activate it from the family of vascular endothelial growth factors: VEGF-A, VEGF-B, and PlGF [18, 19]. To create an original theranostic radiopharmaceutical for the diagnosis and treatment of malignant neoplasms, this study chose the strategy of developing targeted molecules based on antibodies that bind to VEGFR-1. MAbs (3B12 and 4C1) are obtained against the commercially available recombinant human VEGFR-1 protein. By characterizing the resulting antibodies, in particular, the therapeutic potential of VEGFR-1 blockade in models of breast and colon cancers is studied. The resulting mAbs have an affinity for the VEGFR-1 receptor on the surface of a number of cell lines and effectively inhibit the proliferation of breast- or colon-cancer tumor cells in vitro, reduce the growth rate of the tumor node in vivo and prolong the survival of mice with transplanted tumors.

EXPERIMENTAL

Hybridomas secreting mAbs to VEGFR-1 were obtained using the Milstein–Keller method. Balb/c mice were immunized with a preparation of recombinant human VEGFR1 (RPB818Hu01, Cloud-Clone Corp.), which is a Ser27~Ile328 fragment of the extracellular domain of VEGFR1. After second immunization, the hybridization of lymphocytes of inguinal and abdominal lymph nodes with mouse myeloma SP2/0 was performed. After hybridization, hybrid clones producing mAbs against VEGFR1 were selected and cloned to obtain monoclones. The resulting single clones were screened using enzyme-linked immunosorbent assay (**ELISA**), grown, and cryopreserved.

Production of monoclonal antibodies in ascites was carried out in the body of the BALB/c mice, which were intraperitoneally inoculated with hybridoma cells. Ascitic fluid with a volume of 3–5 mL containing mAb was collected and the antibody titer in it was determined by ELISA. The production of mAbs in the ascitic fluids of mice varied from 1 to 2 mg/mL.

Isolation and purification of monoclonal antibodies. The resulting ascitic fluid was clarified by centrifugation, after which antibodies were isolated from the supernatant by precipitation with ammonium sulfate. Purification of the mAbs was carried out using affinity chromatography using a sorbent with protein A. The resulting mAb solution was concentrated by ultrafiltration and dissolved in Na-phosphate buffer. The antibody concentrations were determined using spectrophotometry. The purity and homogeneity of the resulting product was assessed by polyacrylamide-gel electrophoresis.

Monoclonal antibody testing. The isotype of the heavy chains of the resulting mAbs was studied by indirect solid-phase ELISA using the Mouse Monoclonal Antibody Isotyping Reagents kit (SigmaAldrich, category no. ISO2-1KT). The specificity of the obtained mAbs to the mouse VEGFR1 orthologue was studied by ELISA using recombinant mouse antigen VEGFR1 (Mus musculus, Ser27~Val329, Cloud-

Table 1. Primer and probe sequences

Target	Primers and probes, 5'–3'
VEGF-A (human)	GAGGCAGCTTGAGTTAAACG
	TTCTGTCGATGGTGATGGTG
	FAM-TGCAGATGTGACAAGCCGAGGC **BHQ1**
VEGFR-1 (human)	TCAGCACATTCCCTAGTGAG
	CACAGGTGGTTTGCGTATGT
	FAM-TACTGGCTCCTGGCAGCGGCT-**BHQ1**
VEGFR-1 (mouse)	TAGGAAGGCTTCTAGCCA
	GCTGGATATCTGGATGAGAAA
	FAM-TCAGGTAGGGCTGGCCAAAGAC**-BHQ1**
Actin (human)	ATGCAGAAGGAGATCACTGC
	ATACTCCTGCTTGCTGATCC
	FAM-ATCATTGCTCCTCCTGAGCGCAA-**BHQ1**
Actin (mouse)	ATGTACCCAGGCATTGCTGA
	TCCTGCTTGCTGATCCACAT
	FAM-GGCTCCTAGCACCATGAAGATCA-**BHQ1**

Clone Corp. RPB818Mu01). Analysis of the competition between binding to VEGFR1 of the resulting mAbs and the control recombinant antibody, icrucumab, was carried out using competitive ELISA.

Cell cultures. We used a panel of breast-cancer (**BC**) cell cultures: MCF-7 and MDA-MB-231; and colon-cancer (**CC**) cell cultures: Hutu-80, SW-480, and human LoVo; as well as the mouse BC cell line EMT-6 and CC cell line CT-26. The cell lines were cultured in complete DMEM-F12 medium (Biolot, Russia), containing 10% fetal bovine serum (HiMedia, India) and 0.5% gentamicin at 37°C in an atmosphere of 5% CO_2.

Total RNA extraction and real-time RT-PCR. The cell single layer was filled with LIRA reagent (LRU-100-50, Biolabmix, Russia) and incubated for 10 min. Next, total RNA was isolated according to the manufacturer's instructions. The concentration of total RNA in the samples was changed using a NanoDrop One spectrophotometer (Thermo FS, USA). To degrade genomic DNA, the total RNA was treated with DNase I (Thermo FS, USA): 4 units per 40 μL of reaction mixture at 37°C for 40 min. The expression of *VEGF-A* and *VEGFR-1* genes was assessed by RT-PCR. The Genta Single-tube RT-PCR master mix kit (RT-M-003 GenTerra, Russia) was used for the reaction. The reaction mixture (25 μL) contained a single master mix, 375-nM forward and reverse primer and probe (Table 1), and 0.5 μg of total RNA. The reaction was carried out using a CFX96 Touch™ thermal cycler (BioRad, USA): 30 min at 50°C (reverse transcription), 15 min at 95°C (polymerase activation), and 45 cycles: DNA fragment denaturation (95°C, 15 s), primer and probe annealing (58°C, 30 s), elongation (72°C, 60 s). Relative gene expression was determined by threshold cycle values and normalized to *Actin* gene expression.

Flow cytometry. The human tumor cell lines MCF-7, MDA-MB-231, Hutu-80, SW-480, and LoVo, as well as the mouse cell lines EMT-6 and CT-26 were fixed with 4% formaldehyde. Some cells were additionally treated with a 0.5% Triton solution to permeabilize the membrane. Incubated with mAb 4C1 or 3B12 (0.1 μg/mL) for 16 h, 4°C. They were stained with secondary antibodies anti-mouse-Alexa488 (17c01220, Hansa BioMedLife Science, Estonia, 1 ng/mL) for 1 hour at 4°C and visualized on a CytoFlex flow cytometer (Beckman Coulter, USA) at a laser wavelength of 480 nm, accumulating 20000 events.

Real-time cell-proliferation analysis. All experiments were performed using the xCELLigence DP instrument (ACEA Biosciences, USA). Cells (20000 cells/well) were seeded into E-plates 16 (ACEA Biosciences, USA) and placed in the xCELLigence DP instrument (ACEA Biosciences, USA) for continuous recording of the resistance at the electrodes. After 24 h, the medium was replaced with a new one with antibodies to the VEGFR-1 receptor (clones 4C1, 3B12) or Avastin at concentrations of 1 mg/mL or their combination at a concentration of 0.5 mg/mL each. The electrical resistance was recorded every 15 min for 10 days.

In vivo experiments. To evaluate the growth of the tumor node and survival during the administration of mAbs to the VEGFR-1 receptor (3B12) in animal models, male DBA/BALB mice were used. The study was carried out in accordance with the rules for manipulating laboratory animals and in compliance with bioethics. The corresponding experimental protocols were approved by the Bioethics Committee of

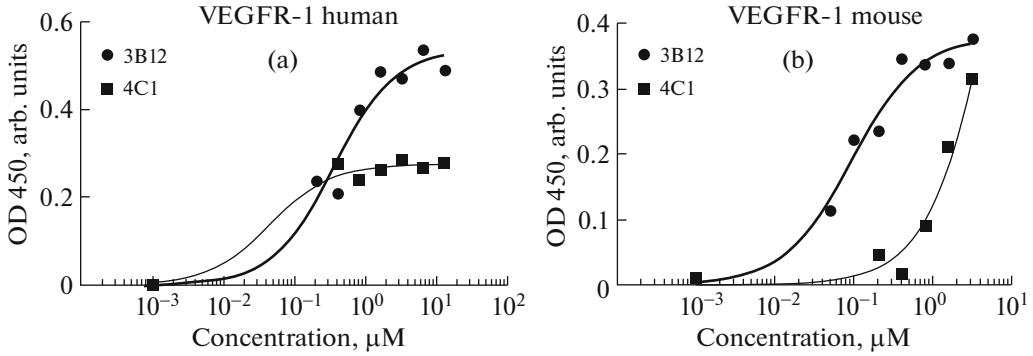

Fig. 1. ELISA assay of monoclonal antibodies 3B12 and 4C1 to the human (a) and mouse (b) VEGFR-1 target antigen.

the Department of Molecular and Radiation Biophysics, National Research Center "Kurchatov Institute", Konstantinov St. Petersburg Institute of Nuclear Physics. Each mouse was injected subcutaneously with 2×10^5 CT-26 cells. Three days after tumor inoculation, intravenous administration was performed: PBS (control), mAb (180 mg/kg), mAb (50 mg/kg), Avastin (180 mg/kg), and Avastin (50 mg/kg). Administrations were carried out 5 times every three days. Each group consisted of five individuals. The growth of the node during the administration of mAb to the VEGFR-1 receptor (3B12) was analyzed using direct measurements of tumor formations. In addition to the size of the node, the life expectancy after inoculation of tumor cells was assessed in all groups. The survival time of the mice from the experimental groups was compared with the corresponding characteristics of control animals that did not receive treatment.

Statistical processing of results. VEGFR-1 expression analysis experiments, as well as cell proliferation were carried out at least in triplicate. Data visualization and analysis were performed using GraphPad Prism software. Flow-cytometry data were processed and visualized using the freely available Floreada.io plugin. The results in the histograms are presented as mean ± SD (standard deviation). The data were compared using one-way ANOVA with Tukey's test to correct for multiple comparisons. The differences were considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

Specificity and binding activity of mAbs against VEGFR-1. Monoclonal antibodies that bind human VEGFR-1 were generated from mice immunized with recombinant human VEGFR-1 protein. The specificity and binding efficiency of mAbs to human VEGFR-1 were tested by ELISA using recombinant human and mouse VEGFR-1 proteins as antigens. Binding analysis showed that both selected mAbs (4C1 and 3B12) against human VEGFR-1 have strong binding activity to human VEGFR-1 (k_m = 0.05 and 0.4 μM, respectively), while 3B12 mAb demonstrated binding activity to mouse VEGFR-1 (k_m = 2.0 μM) (Fig. 1).

Expression of VEGF-A and VEGFR-1 in breast and colon carcinoma cell lines. The expression of VEGF-A and VEGFR-1 was analyzed using RT-PCR in real time on BC cell lines: MCF-7, MDA-MB-231 and CC cell lines: Hutu-80, SW-480, human LoVo, as well as on the mouse BC cell lines EMT-6 and CC cell line CT-26. VEGF-A was expressed at the mRNA level in all the human cell lines tested and was not detected in the mouse cell lines (Fig. 2). VEGFR-1 transcripts were detected in all the cell lines except LoVo cells (Fig. 2), which may be due to the presence of a mutation or deletion in these cells in the selected RT-PCR primer system. Overall, our results indicate that VEGFR-1 and its ligand VEGF-A are widely coexpressed in breast and colon carcinoma cell lines.

All cell lines were positive for cell surface expression of VEGFR-1, which was verified using flow cytometry (Fig. 3). The expression of VEGFR-1 on the surface of Hutu-80 cells shown in Fig. 3c as an example of a representative result. Cytometric visualization of the receptor was carried out using the obtained mAbs to human VEGFR-1 4C1 and 3B12. The results demonstrate more efficient binding of the mAb of the clone 4C1 to the VEGFR-1 epitope on the surface of human cells compared to the mAb of the clone 3B12 (Fig. 3). At the same time, the efficiency of binding of the 3B12 mAb clone to the surface epitope of mouse VEGFR-1 cells is higher compared to the 4C1 mAb clone, which is consistent with ELISA data demonstrating the cross-affinity of the 3B12 mAb to the recombinant mouse VEGFR-1 antigen (Fig. 1b).

mAbs to human VEGFR-1 inhibit the proliferation of breast and colon cancer cells in vitro. To evaluate the growth of breast or cancer cells when VEGFR-1 was blocked with monoclonal antibodies, the proliferation of all cell lines was analyzed using the xCELLigence DP tool, which allows detection of the cellular index in real time. The use of antibodies 4C1 and 3B12 did not significantly affect the proliferation of cells of the MCF-7 and SW-480 lines and significantly sup-

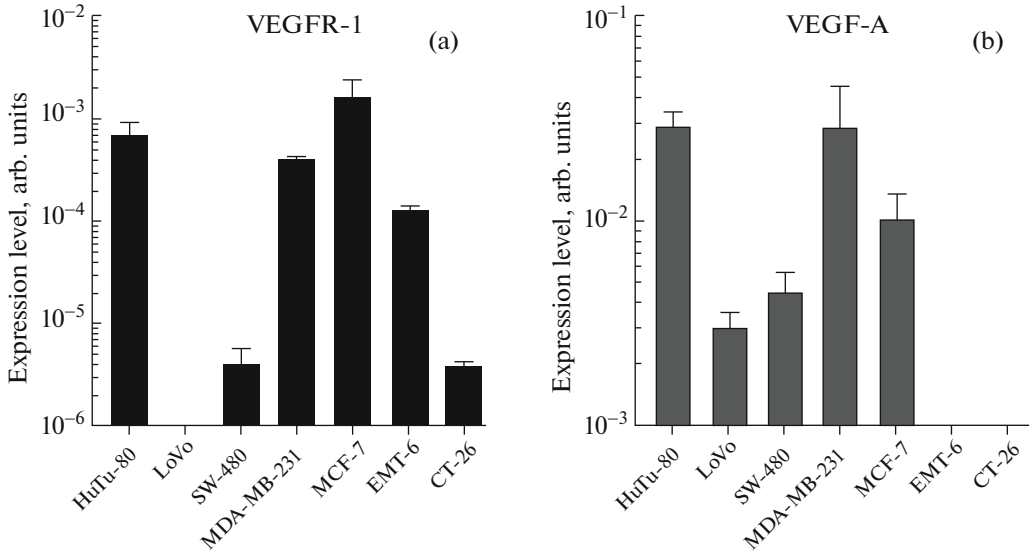

Fig. 2. Relative levels of VEGFR-1 (a) and VEGF-A (b) mRNA in human and mouse breast cancer (BC) and colon cancer (CC) cell cultures. RT-PCR data are presented as the mean ratio of the target gene mRNA/Actin mRNA ± SD.

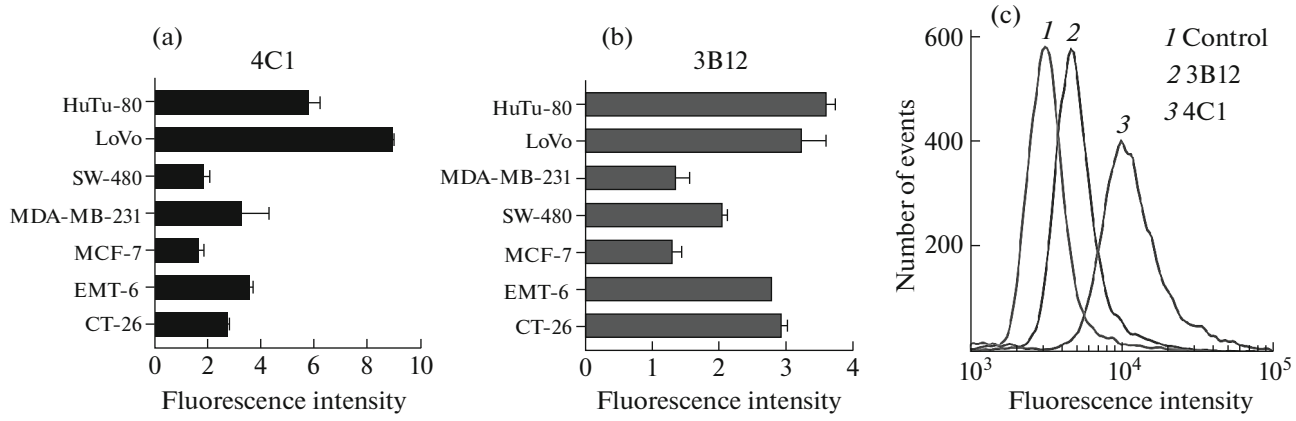

Fig. 3. Immunofluorescence analysis of surface VEGFR-1 expression by the cell lines Hutu-80, LoVo, SW480, MDA-MB-231, MCF-7, EMT6-HER2, and CT-26. Flow-cytometry data for visualizing VEGFR-1 by mAbs 4C1 (a) and 3B12 (b). Example of flow-cytometry results for Hutu-80 cells (c).

pressed the growth of cells of the MDA-MB-231, EMT-6, Hutu-80, LoVo, and CT-26 lines (Fig. 4a), which is obviously associated with the significant presence of the VEGFR-1 antigen on the surface of these cells (Figs. 3a and 3b).

An immunotherapeutic treatment regimen using the drug Avastin, which is a humanized antibody that binds VEGF-A, and a combination of mAb 4C1 to the VEGFR-1 receptor with Avastin was also tested on all cell lines. Avastin did not affect the growth of SW-480 cells, in which the expression level of both VEGFR-1 and VEGF-A was recorded as minimal among all analyzed lines; did not significantly slow down the growth of MCF-7 culture; and significantly suppressed the proliferation of the cell lines Hutu-80, LoVo, MDA-MB-231, and CT-26 (Fig. 4b). When cells were incubated in the presence of a combination of a half dose of both Avastin and mAb 4C1, a synergistic effect was observed compared to the use of Avastin alone for cells of the Hutu-80, LoVo, and CT-26 lines (Figs. 4b and 4c).

Inhibition of VEGFR-1 by the specific mAb 3B12 suppresses in vivo growth of mouse colon carcinoma CT-26. To evaluate in vivo whether VEGFR-1 blockade prevents the growth of colon tumors, cells of the CT-26 line were transplanted into DBA/BALB mice and three days after tumor inoculation the following was administered intravenously: PBS (control), mAb 3B12 (1 mg/mouse), mAb 3B12 (3.5 mg/mouse), Avastin (1 mg/mouse), and Avastin (3.5 mg/mouse). The systemic administration of mAb 3B12 at a dose of

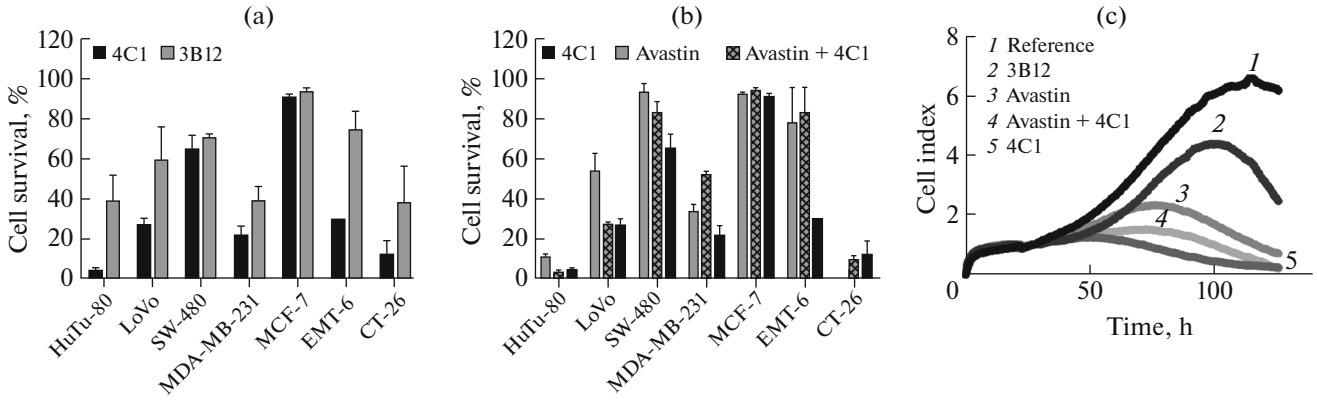

Fig. 4. Analysis of the proliferative activity of breast- and colon-cancer cell lines when incubated in the presence of mAbs to human VEGFR-1 4C1 and 3B12 (a), the drug Avastin, which binds VEGF-A, and their combination (b). An example of the analysis of proliferation of Hutu-80 cells during coincubation with: *1* is PBS (control), *2* is the mAb 3B12 (1 mg/mL), *3* is the drug Avastin (1 mg/mL), *4* is the combinations Avastin (0.5 mg/mL) with mAb 4C1 (0.5 mg/mL), and *5* is mAb 4C1 (1 mg/mL) (c).

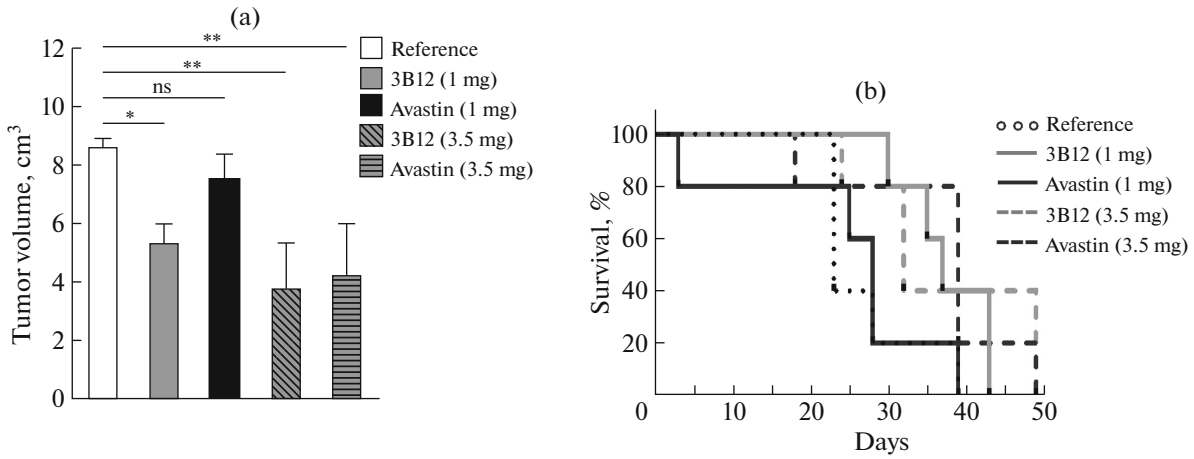

Fig. 5. Inhibition of VEGFR-1 by monoclonal antibodies slows tumor growth in vivo (a) and increases the life expectancy of animals with transplanted CT-26 tumors (b).

1 and 3.5 mg/mouse every 3 days resulted in the statistically significant ($p < 0.05$) suppression of the growth of CT-26 tumor grafts (Fig. 5a).

The average life expectancy after the inoculation of tumor cells was: in the control group that did not receive treatment, it was 23 days; in groups receiving the systemic administration of mAb 3B12 in a volume of 1 and 3.5 mg/mouse, it was 37 and 32 days, respectively; and Avastin in a volume of 1 and 3.5 mg/mouse, it was 28 and 39 days, respectively (Fig. 5b). At the same time, significant ($p < 0.5$), an increase in the life expectancy of animals compared to the control was detected for groups receiving mAb 3B12 (1 mg/mouse) or Avastin (3.5 mg/mouse) (Fig. 5b). Thus, mAb 3B12 to the VEGFR-1 receptor obtained in this study, as well as the mAb included in the drug Avastin and binding VEGF-A, reduce the growth rate of the tumor node in vivo and prolong the survival of mice implanted with CT-26 colon carcinoma cells.

The participation of VEGFR-1 in the induction of angiogenic switching in pathological conditions, the mobilization of stem progenitor cells from the bone marrow, as well as in the growth and migration of tumors confirms the hypothesis of the therapeutic effectiveness of targeting this receptor [4, 8, 16, 18, 29]. In addition to activation in various tumors, VEGFR-1 is expressed in monocytes/macrophages and is involved in their recruitment to tumor sites, where they secrete proangiogenic factors that further stimulate tumor growth and promote resistance to anti-VEGF-A therapy [30]. The selective inhibition of VEGFR-1 by mAbs may enhance the effects of VEGF-A antiangiogenic therapy and counteract the development of resistance to this type of drug [29]. The mechanisms of tumor resistance to bevacizumab include the increased expression of VEGFR-1 (in tumor cells, endothelial cells, and monocytes/macrophages) and signaling and/or activation of

a specific VEGFR-1 ligand, PlGF [1, 6]. Thus, decreased modulation of the PlGF/VEGFR-1 pathway may delay or prevent resistance to anti-VEGF-A agents. The resistance to anti-VEGF-A therapy may also be associated with the formation of blood vessels through mechanisms alternative to angiogenesis, such as intussusception and vasculogenic mimicry [29].

This work describes new mAbs 4C1 and 3B12, which recognize VEGFR-1 and prevent its activation by ligands VEGF-A or PlGF. The resulting mAbs 4C1 and 3B12 are directed against a peptide whose amino-acid sequence is included in the extracellular domain of the receptor, which is confirmed by flow-cytometry data recording the presence of native VEGFR-1 on the surface of a number of tumor lines. The resulting mAbs 4C1 and 3B12 likely inhibit the cellular response that follows the binding of VEGF-A and/or PlGF ligands to the receptor, thereby slowing down the rate of proliferation of a number of tumor cells in vitro. Besides this, mAb 3B12 recognizes both human and mouse VEGFR-1, as demonstrated by ELISA. Thus, it became possible to analyze the effect of treatment with mAb 3B12 on the tumor graft. mAb 3B12 had antitumor activity in vivo. In fact, the effectiveness of five doses of 3B12 at 180 mg/kg was comparable to the effectiveness of five doses of bevacizumab (the drug Avastin) at the same dosage.

Despite its involvement in tumor angiogenesis, VEGFR-1 does not play a significant role in physiological angiogenesis in adults [16, 18]. Therefore, anti-angiogenic therapies that selectively target this receptor may exhibit lower systemic toxicity compared with therapies targeting VEGF-A and/or VEGFR-2 [18, 29]. Indeed, the administration of mAb 3B12 at a high concentration of 180 mg/kg in a mouse model was very well tolerated. Besides, in in vitro experiments, the combined effect of mAb 4C1 to the VEGFR-1 receptor and the drug Avastin, which is a humanized antibody that binds VEGF-A, demonstrated a synergistic effect compared to the use of Avastin alone for certain cell lines. On this basis, the simultaneous targeting of VEGFR-1 with mAbs and VEGF-A blockade is likely to result in increased therapeutic efficacy without causing additive toxicity.

CONCLUSIONS

The new monoclonal antibodies obtained in the study have affinity for the VEGFR-1 receptor and effectively inhibit the growth of breast- or colon-cancer tumor cells in vitro and in vivo.

FUNDING

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was carried out in accordance with the rules for manipulating laboratory animals and in compliance with bioethics. The corresponding experimental protocols were approved by the Bioethics Committee of the Department of Molecular and Radiation Biophysics, National Research Center "Kurchatov Institute," Konstantinov St. Petersburg Institute of Nuclear Physics.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

OPEN ACCESS

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