INTRODUCTION

Cell malignization results from the accumulation of numerous genetic and epigenetic anomalies, which break the control of cell division, differentiation, and apoptosis [1, 2]. Signaling networks in the cell include many redundant components; therefore, suppression of a single cellular factor involved in signal transduction can be compensated for by an alternative pathway [2]. Therefore, the top-priority task in the development of targeted therapeutical agents is the search for key cellular targets at intersections of regulatory pathways, whose suppression would result in irreversible and uncompensated therapeutical effect. The main causes of uncontrollable cell proliferation in tumors are overexpression of normal genes or expression of aberrant genes coding for receptors, transcription factors, tyrosine kinases, and other regulatory proteins. However, as expression of a large set of genes is distorted, it is difficult to determine the event succession and, consequently, the prime cause of malignization [2]. Nevertheless, suppression of genes coding for proteins that operate at intersections of regulatory pathways or at the beginning of these pathways can restore cell division control [3].

Presently, RNA interference is broadly used for elucidating the role of particular genes in cell cycle distortion and finding targets for new antitumor drugs. Small interfering RNAs (siRNA), complementary to a fragment of a target gene mRNA, allow efficient suppression of the corresponding gene to bring the level of the mRNA, overexpressed in tumor cells, to its normal value [4].

The \textit{CCNB1}, \textit{HER2}, and \textit{PKC} genes, encoding proteins that belong to different groups of cell cycle regulators, are promising targets for siRNAs [5–7]. Experiments and clinical studies confirm that deregulation of these genes is associated with various types of malignant tumors in humans [8–10]. Amplification and overexpression of these genes are detected in patients with breast, cervical, endometrial, Fallopian tube, and ovarian cancers; gynecological adenocarcinomas; and lung carcinomas in humans [8, 13, 14]. The \textit{HER2} gene has been most comprehensively studied as a target for gene-specific cancer treatment. Antibodies against the transmembrane form of the \textit{HER2} protein have a cytostatic effect. Trastu-
zumab, developed on their base, was the first monoclonal antibody-based pharmaceutical to be licensed for breast cancer treatment [15].

Cyclin B1, encoded by the CCNB1 gene, is a regulatory subunit in the cyclin-dependent kinase 1 complex (CDK1). This complex governs the transition between cell cycle phases G2 and M [16]. In mitosis, this complex phosphorylates amino acid Thr125 of the inhibitory domain of caspase 9, which triggers apoptosis in response to treatment with microtubular toxins [16]. The overexpression of the CCNB1 gene can suppress cell damage-induced apoptosis and lead to the accumulation of genetic abnormalities in the cell. [17]. Elevated CCNB1 expression has been detected in carcinoma cells and human prostate tumors [9]. It often precedes aneuploidy [18]; therefore, it is likely to be among early events in cell transformation.

Protein kinase C (PKC) is encoded by the PKC gene. It is expressed in many tissues and organs and is essential for signal transduction from biologically active substances that activate various cell functions, including proliferation [19]. Diacylglycerol, which is formed in the membrane by signal-induced conversion of inositol-containing phospholipids, transiently activates PKC. Carcinogenic phorbol esters intercalated into the cell membrane mimic diacylglycerol and cause long-term PKC activation [20]. Protein kinase C overexpression is observed in various human carcinomas [10].

It is known that small interference RNAs (siRNAs) efficiently and specifically suppress the expression of target genes [21]. The analysis of the aforementioned data allowed us to choose HER2, CCNB1, and PKC genes as targets. Although the deregulation of genes encoding proteins that mediate cell cycle signaling is considered to be the key factor in the development of various tumors in humans [1–4], inhibition of a certain gene can have different effects in cases with different tumors. For this reason, we compared the antiproliferative effects of target gene inhibitors in various tumor cell lines.

The designed siRNAs complementary to HER2, CCNB1, and PKC mRNAs, suppressed the expression of corresponding genes and retarded the division of tumor cells from various tissues with different efficiencies. The siRNA complementary to CCNB1 exerted a pronounced antiproliferative action in SK-N-MC neuroblastoma cells, and the siRNA complementary to PKC efficiently inhibited the growth of MCF-7 breast carcinoma cells.

**EXPERIMENTAL**

siRNA. We designed the following siRNAs of 21 bp in length each: siHER2, complementary to the 1297–1317 region of the human HER2 mRNA (sense strand 5'-GCmAGUUACCmAGUGCCmAAUAUU-3'; antisense strand 5'-UmAUUmGmGGCmACUmGGUmAACUmGCC-3'); siCycB1, complementary to the 189–209 region of the human CCNB1 mRNA (sense strand 5'-CmACCmAGGAACUCGAAAAU-UUU-3'; antisense strand 5'-AAUUUUCGCAG-UUCCUmGGUmGAC-3'); and siPKC, complementary to the 1079–1099 region of the human PKC mRNA (sense strand 5'-GCmGmCCmAGAGAA-GGAAAAUU-3'; antisense strand 5'-UUUUUUCCUm-GCUCUmGGCGCmGmG-3'). The siRNAs were obtained by annealing of the sense and antisense strands, which were synthesized by the solid-phase phosphoramidite method in an automated ASM-102U synthesizer (Biosset, Russia) at the Laboratory of RNA Chemistry, Institute of Chemical Biology and Fundamental Medicine, Novosibirsk. The siScr RNA (sense strand 5'-CmAAmGUCUmAmUmGm-AGUmGGUmUU-3'; antisense strand 5'-CCmACUm-mACmUmAGAGACUmGUU-3') was used as a negative control. Its sequence shows no notable similarity to murine, rat, or human mRNAs.

**Cell cultures and siRNA transfection.** Cell Lines of human oral carcinoma KB-3-1, neuroblastoma SK-N-MC, breast adenocarcinoma MCF-7, and human myeloblastoma HL-60 were received from the collection of the Institute of Cytology, St. Petersburg. KB-3-1 and SK-N-MC were grown in Dulbecco’s Modified Eagle Medium (DMEM), and HL-60 was grown in Roswell Park Memorial Institute medium RPMI-1640, supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin, in humid atmosphere with 5% CO2 at 37°C. Cells in the log phase were inoculated to 96-well plates 24 h before the measurement: KB-3-1, 1.5 × 103 cells per well (cpw); SK-N-MC, 7.5 × 103 cpw; MCF-7, 3 × 103 cpw; and HL-60, 20 × 103 cpw. Alternatively, cells were inoculated to 24-well plates: KB-3-1, 105 cpw; SK-N-MC, 1.25 × 105 cpw; MCF-7, 1.5 × 105 cpw; and HL-60, 1.5 × 105 cpw. The cells were transfected with siRNA at concentrations 50–200 nM with Lipofectamine 2000 (Invitrogen, United States) or Oligofectamine (Invitrogen) for SK-N-MC according to the manufacturer’s recommendations. Corresponding mRNAs were assayed 1 or 2 days after the transfection. Cells treated with the transfection reagent alone were used as reference.

**Reverse transcription polymerase chain reaction (RT-PCR).** Total RNA was isolated from cells by the SDS-phenol method [22]. Reverse transcription was conducted in 20 μl of the mixture containing 1 μg of total RNA, 5 μM oligo(dT)12 primer, 50 mM Tris HCl pH 8.3, 75 mM KCl, 3 mM MgCl2, 0.5 mM dNTP, 5 mM dithiothreitol, and 10 U of Moloney murine leukemia virus reverse transcriptase (M-MLV RT). The mixture was incubated at 42°C for 1 h. The resulting cDNA was amplified in 20 μl of the mixture containing 20 μl of the cDNA template, 10 mM Tris HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.01% Tween 20, 0.25 mM dNTP, 0.25 mM each primer, and 2 U of thermostable Thermus aquaticus DNA polymerase (Institute of Chemical Biology and Fundamental