Journal of Dermatological Science 79 (2015) 57–63

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

RNAi-mediated downregulation of CDKL1 inhibits growth and colony-formation ability, promotes apoptosis of human melanoma cells

Zhiqi Song, Jingrong Lin *, Zhe Sun, Jing Ni, Yang Sha

Department of Dermatology, The 1st Affiliated Hospital of Dalian Medical University, No. 222, Zhongshan Road, Dalian 116011, China

ARTICLE INFO

Article history:
Received 6 October 2014
Received in revised form 26 March 2015
Accepted 27 March 2015

Keywords:
Melanoma
CDKL1
siRNA
Lentivirus

ABSTRACT

Background: Cyclin-dependent kinase-like 1 (CDKL1) is a member of cell division control protein 2 (CDC-2)-related serine threonine protein kinase family, and is reported to be overexpressed in malignant tumors such as breast cancer and gastric cancer.
Objective: This study aimed to evaluate the whether CDKL1 can serve as a potential molecular target for melanoma gene therapy.
Methods: CDKL1 expression in two melanoma cell lines, A375 and MV3 was measured by real-time PCR. To investigate the role of CDKL1 in cell growth of melanoma, we constructed CDKL1-siRNA expressing lentivirus and infected A375 and MV3 cells. The effects of RNAi-mediated CDKL1 downregulation on A375 and MV3 cell proliferation and colony-formation ability were detected by methylthiazolete-trazolium (MTT) assay and colony-formation assay. The effects of CDKL1 downregulation on A375 and MV3 cell cycle and apoptosis were analyzed by FACS analysis.
Results: Human melanoma cell lines A375 and MV3 expressed CDKL1 mRNA. Knockdown of CDKL1 in A375 and MV3 by CDKL1-siRNA lentivirus infection significantly inhibited cell growth and colony formation ability, promoted cell apoptosis, and arrested cells in G1 phase.
Conclusion: CDKL1 is associated with melanoma cell growth, colony formation, apoptosis, and cell cycle regulation. It may be considered as a valuable target for anti-melanoma therapeutic strategies.

1. Introduction

Malignant melanoma is a highly aggressive skin tumor with increasing incidence and poor prognosis [1]. Many attempts have been made to treat the disease. BRAF V600E inhibitors (e.g. vemurafenib, dabrafenib), the first target therapy, have improved both progression-free survival and overall survival, compared with chemotherapy in patients with BRAF V600E-mutated metastatic melanoma [2–7]. However, clinical evidence of tumor resistance developed 5–7 months later in a subset of patients [8,9], due to MAPK reactivation, driven by secondary mutations in NRAS and MEK1 genes [10].

Malignant tumors are characterized by inappropriate cell proliferation, which arises when checkpoint mechanisms that limit proliferation of cells with damaged DNA are degraded, permitting expansion of clones with genetic instability [11]. Disturbed regulation of the cell cycle is a hallmark of cancer [12]. Melanomagenesis is associated with not only defects in nucleotide excision repair of solar radiation-induced DNA damage, but also cell cycle checkpoints that arrest growth after DNA damage [13]. Defective checkpoints may represent potentially selective anti-melanoma therapeutic targets.

The cyclin-dependent protein kinase (CDK) protein family has been demonstrated to be an important regulator of cell division at the G1/S and G2/M checkpoints [14]. The CDK family regulates a wide range of cellular functions such as cell cycle progression, differentiation, and apoptosis [15]. CDKs form active complexes with a specific cyclin, thus controlling the expression of down-stream genes involved in the cell cycle [14]. Cyclin-dependent kinase 1 (CDK1), also known as cell division control protein 2 (CDC2), is a key molecule among them. Cyclin-dependent kinase-like 1 (CDKL1) is a member of CDK1-related serine–threonine protein kinase family [16]. It was cloned on the basis of its similarity to the CDK1 kinase domain [17], and named after the amino acid sequence corresponding to the PSTAIRE motif of CDK1/CDC2 [18,19]. CDKL kinase family is not known to interact with cyclins, but considered as a separate branch of CDK family that is similar to the MAP kinases group of signal transducing enzymes [23]. CDKL1 contains the conserved MAP kinase dual phosphorylation motif Thr-Xaa-Tyr (Thr-Asp-Tyr), and may therefore contribute to signal

* Corresponding author. Tel.: +86 13700089700.
E-mail address: amy4963@163.com (J. Lin).

http://dx.doi.org/10.1016/j.jdermsci.2015.03.020
0923-1811/

transduction [24]. Recent studies reported that CDKL1 was overexpressed in human breast cancer and gastric cancer, and loss of CDKL1 function in these cancer cell lines resulted in inhibition of cell proliferation and increase of apoptosis [20,21]. However, up to now, the expression of CDKL1 in malignant melanoma and its function in melanoma cell regulation are not known.

In this study, we investigated the expression of CDKL1 in human melanoma cell lines A375 and MV3. Furthermore, we constructed CDKL1-siRNA expressing lentivirus, and evaluated the effects of RNAi-mediated CDKL1 downregulation on A375 and MV3 cell proliferation, colony-formation ability, cell cycle and apoptosis.

2. Methods

2.1. Cell culture

Human melanoma cell lines, A375 and MV3 were obtained from Shanghai institutes for biological science (SIBS) cell bank. A375 cells were maintained in DMEM and MV3 cells were maintained in RPMI 1640 medium (Gibco BRL, Grand Island, NY, USA) plus 10% fetal bovine serum (FBS) (Hyclone, Logan, UT, USA) in a 5% CO_2 incubator at 37 °C.

2.2. Lentiviral CDKL-siRNA vector construction and packaging

Small interfering RNA (siRNA) target sequence (CTACTGTGATACCAAGAAA) for CDKL1 gene (NM_004196) was designed and a non-silencing siRNA sequence (TTCTCCGAACGTGTCACGT) was adopted as negative control (NC). siRNA constructs were synthesized and cloned into pGCSIL-GFP plasmid vector with Age I/EcoRI sites (GeneChem, Shanghai, China) which contains the green fluorescent protein (GFP) gene as a reporter with an internal CMV promoter. The CDKL1-siRNA plasmid were then transfected into HEK293T cells based on the instruction of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) when the cell density reached 70–80%. Lentivirus particles expressing CDKL1-siRNA (Lv-CDKL1-siRNA) were harvested from cell culture medium 72 h after transfection. Lentivirus was concentrated using the Centricon-Plus-20 centrifugal ultrafiltration device (Millipore, Billerica, MA, USA), and were kept at −80 °C.

2.3. Infection of melanoma cells by lentivirus

When A375 and MV3 cells were seeded in 6-well plates and grown to 30% confluence in the medium, appropriate amount of lentivirus was added according to the multiplicity of infection (MOI). On the next day, the culture medium was substituted and GFP fluorescence expression was observed under a fluorescence microscopy (Olympus, Tokyo, Japan) three days after infection. The percentages of GFP-positive cells were evaluated.

2.4. Quantitative real-time PCR

A375 and MV3 cells were cultured in 6-well plates and were then infected with lentivirus for 72 h. Total RNA was isolated from cultured cells by Trizol reagent (Invitrogen, Carlsbad, CA, USA), and reversely transcripted to cDNA with random primers by M-MLV reverse transcriptase (Promega Corp.) following the manufacturer's instructions. Two sets of primers were used for PCR. Primers were designed by Beacon Designer 2 software (Premier Biosoft International, Palo Alto, CA, USA) as follows: GAPDH-F, 5′-TGACTTCAACAGCGACACCCA-3′, GAPDH-R, 5′-CACCCTGTTGCTGTAGCCAAA-3′; CDKL1-F, 5′-CGAATGCTCAAGCAACTCAAGC-3′, CDKL1-R, 5′-GCCAAGTTATGCTCTTCACGAG-3′. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was amplified as internal control. The SYBR Green Real-Time PCR assay kit (TAKARA, Otsu, Japan) was used, and quantitative real-time PCR (qRT-PCR) was performed using Thermal Cycle Dice Real Time System TP800 (Takara, Japan). All samples were examined in triplicate. Relative quantitation was done by taking the difference [Delta C (T)] between the C (T) of GAPDH and C (T) of each gene and computing 2 [−Delta Delta C (T)].

2.5. Cell growth assay

A375 cells at the logarithmic phase after infection with CDKL1 siRNA lentivirus and scrambled siRNA lentivirus were digested, resuspended, counted and inoculated in 96-well plates. From the second day, cells with green fluorescence were taken photos and counted by Cellomics ArrayScan VT1 Readers once a day. Cell growth was observed continuously for 5 days, and cell growth curves were drawn.

2.6. BrdU incorporation assay

Cells infected with CDKL1 siRNA lentivirus or control, along with nontreated cells, were cultured in 96-well plates at a density of 2×10^4 cells/well. A 5-bromodeoxyuridine (BrdU) incorporation assay was performed on 1 and 4 days after seeding using the BrdU Cell Proliferation ELISA kit (Roche, USA) according to the manufacturer's information. Briefly, 10 μL of 1/100 diluted BrdU

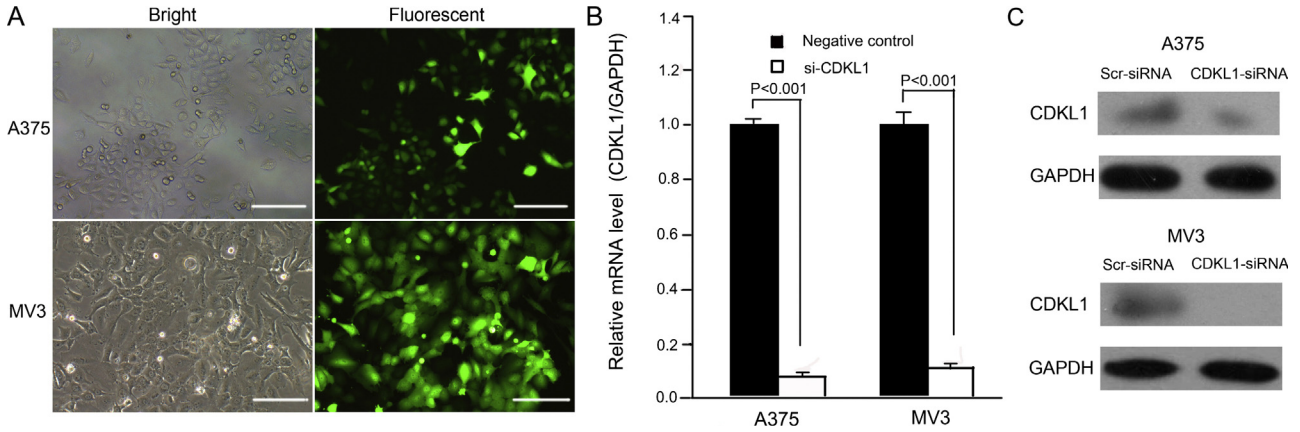

Fig. 1. CDKL1 silencing efficiency by siRNA lentivirus. (A) Lentivirus infection in A375 and MV3 melanoma cell lines. Bright and fluorescent photomicrographs of A375 and MV3 cells were taken 72 h after lentivirus infection at a magnification of ×100 (Scale bar = 250 μm). (B) Identification of CDKL1 knockdown efficiency using siRNA lentivirus by real-time PCR in A375 and MV3 cells. (C) Western blot analysis of CDKL1 protein expression in A375 and MV3 cells infected with scr-siRNA and CDKL1-siRNA lentivirus.