Biochemical and Biophysical Research Communications 469 (2016) 87–93

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Triptolide inhibits transcription of hTERT through down-regulation of transcription factor specificity protein 1 in primary effusion lymphoma cells

Cong Long [a], Jingchao Wang [a], Wei Guo [b], Huan Wang [a], Chao Wang [a], Yu Liu [a], Xiaoping Sun [a, c, *]

[a] Department of Pathogen Biology, School of Basic Medical Sciences, Wuhan University, Wuhan, 430071, PR China
[b] Department of Pathology and Physiology, School of Basic Medical Sciences, Wuhan University, Wuhan, 430071, PR China
[c] State Key Laboratory of Virology, Wuhan University, Wuhan, 430072, PR China

ARTICLE INFO

Article history:
Received 10 November 2015
Accepted 17 November 2015
Available online 26 November 2015

Keywords:
Triptolide
hTERT
Sp1
PEL cells

ABSTRACT

Primary effusion lymphoma (PEL) is a rare and aggressive non-Hodgkin's lymphoma. Human telomerase reverse transcriptase (hTERT), a key component responsible for the regulation of telomerase activity, plays important roles in cellular immortalization and cancer development. Triptolide purified from *Tripterygium* extracts displays a broad-spectrum bioactivity profile, including immunosuppressive, anti-inflammatory, and anti-tumor. In this study, it is investigated whether triptolide reduces hTERT expression and suppresses its activity in PEL cells. The mRNA and protein levels of hTERT were examined by real time-PCR and Western blotting, respectively. The activity of hTERT promoter was determined by Dual luciferase reporter assay. Our results demonstrated that triptolide decreased expression of hTERT at both mRNA and protein levels. Further gene sequence analysis indicated that the activity of hTERT promoter was suppressed by triptolide. Triptolide also reduced the half-time of hTERT. Additionally, triptolide inhibited the expression of transcription factor specificity protein 1(Sp1) in PEL cells. Furthermore, knock-down of Sp1 by using specific shRNAs resulted in down-regulation of hTERT transcription and protein expression levels. Inhibition of Sp1 by specific shRNAs enhanced triptolide-induced cell growth inhibition and apoptosis. Collectively, our results demonstrate that the inhibitory effect of triptolide on hTERT transcription is possibly mediated by inhibition of transcription factor Sp1 in PEL cells.

1. Introduction

Primary effusion lymphoma (PEL) is a variant of non-Hodgkin's B lymphoma. PEL is often associated with Kaposi's sarcoma-associated herpesvirus (KSHV) infection, and co-infection with Epstein-Barr virus (EBV) has been reported in the majority of PEL cases [1]. PEL usually occurs in immunodeficient patients, such as human immunodeficiency virus (HIV)-infected individuals or those who have undergone organ transplantation [2]. Although treated with traditional chemotherapy or combined with antiretroviral therapy, prognosis of PEL patients is extremely poor upon diagnosis [3]. Therefore, more effective therapies against PEL remain urgently needed.

Telomeres are tandem nucleotide sequences with an average length of 5–15 kilobases at the ends of eukaryotic chromosomes. Telomeres function to maintain genome stability and prevent chromosome degradation, fusions, and loss [4]. The telomeres of normal human somatic cells shorten with each cell division, because lagging strand cannot be completely replicated by DNA polymerase. If telomeres are critically shortened, cells would be induced into senescence. However, malignant cells develop a mechanism to escape from shortening of telomeres by over-expression of telomerase [5].

Telomerase, a ribonucleoprotein enzyme complex, contributes to stabilization of telomere length through the addition of new repeat sequence [6]. Telomerase contains three subunits, including an RNAcomponent, an integral protein component and human

* Corresponding author. Department of Pathogen Biology, School of Basic Medical Sciences, Wuhan University, 185 Eastlake Road, Wuhan, 430071, PR China.
E-mail address: xsun6@whu.edu.cn (X. Sun).

http://dx.doi.org/10.1016/j.bbrc.2015.11.076
0006-291X/

telomerase reverse transcriptase (hTERT). Expression of hTERT is responsible for the telomerase activity [7]. In most normal human cells, hTERT is down-regulated or inactivated during cell differentiation. However, in most types of maglinant cells, hTERT is usualy actived, contributing to cell immortalization. Although expression of hTERT can be regulated at multiple levels, transcriptional regulation is likely to be the major mechanism [8]. Since previous reports have indicated the importance of hTERT overexpression in the development and maintenance of malignancy, the biological insights into hTERT make it a promising target for therapeutic strategies [9,10].

Triptolide is firstly purified from the roots of Chinese herb *Tripterygium wilfordii* [11]. Triptolide displays a broad-spectrum bioactivity profile, including anti-tumor, anti-inflammatory and immunosuppressive. Currently, triptolide has been reported to inhibit proliferation and induce apoptosis against a variety of tumors *in vitro and in vivo*, including cholangiocarcinoma, breast cancer, melanoma and gastric cancer [12]. In addition, triptolide can induce pancreatic cancer cell death via inhibition of Hsp70 and O-GlcNAc modification of transcription factor specificity protein 1 (Sp1) [13]. Minnelide, a water-soluble prodrug of triptolide, has entered clinical trials for treatments of pancreatic cancer with a well toleration [14].

So far, little is known about effect of triptolide on herpesvirus-associated malignancies. Our previous works have demonstrated that triptolide inhibits proliferation of EBV-positive B lymphocytes via inhibition of latent membrane protein 1 (LMP1) [15]. Recently, our lab found that triptolide showed antitumor activity against PEL *in vitro* and *in vivo* through the Latency-associated nuclear antigen 1 (LANA1)-dependent mechanism [Submitted].

Here we show that, in addition to the inhibition of hTERT transcription and protein expression, triptolide reduces the stability of hTERT in BCBL-1 and BC-3 cells. Triptolide decreases hTERT transcription by inhibiting activation of hTERT promoter. Additionally, triptolide down-regulates expression of Sp1 in BCBL-1 and BC-3 cells. Knock-down of Sp1 by Sp1 shRNA2 reduces the expression of hTERT at mRNA and protein levels. Furthermore, Sp1 shRNA2 enhances triptolide-induced cell apoptosis and viability inhibition in BCBL-1 and BC-3 cells.

2. Materials and methods

2.1. Cell lines and reagents

PEL cells (BCBL-1 and BC-3) were obtained from Prof. K. Lan (Institute Pasteur of Shanghai, Chinese Academy of Sciences, Shanghai, China). Human renal embryonic 293T cells were purchased from the American Type Culture Collection (Manassas, VA, USA). PEL cells were maintained in RPMI 1640 medium containing 10% FBS (Gibco, Gaithersburg, MD, USA). Human renal embryonic 293T cells were cultured in DMEM supplemented with 10% FBS. All cells were cultured at 37 °C with 5% CO_2 and 100% humidity. Triptolide (Sigma, USA) and cycloheximide (CHX; Sigma, USA) were dissolved in DMSO.

2.2. Plasmids

The pGL 3.0 luciferase reporter gene vector was purchased from Promega (Promega, Wisconsin, USA). Plasmid *Renilla* Luciferase-Thymidine Kinase (pRL-TK) was kindly provided by Prof. D. Guo (Wuhan University, Wuhan, China). The hTERT promoter regions that span −1126, −792, −461, or −277 to −47 were amplified from BCBL-1 genome by Polymerase Chain Reaction (PCR). The PCR products were digested with enzymes *Kpn I* and *Hind III* (NEB, USA), followed by inserting into the *Kpn I*/*Hind III* sites of the pGL3.0 vector plasmid. All the plasmids were purified through columns (Axygen, San Francisco, USA) as described by the manufacturer. The primers used in this study were listed in Table 1.

2.3. Cell viability assay

The viability of cells was determined by the Cell Counting Kit-8 (CCK-8; Dojindo, Japan) assay. Cells transfected with or without Sp1 shRNA2 were seeded in 96-well plates at a density of 1×10^4 per 100 μl of culture medium. The cells were treated with vehicle control (0.01% DMSO) or triptolide (100 nM) for 24 h, followed by adding 10 μl of tetrazolium substrate to each well of the plate. After incubation at 37 °C for 1 h, the optical density (OD) was detected at a wavelength of 450 nm using a microplate reader (BioTek, EXL800, Vermont, USA).

2.4. Apoptosis assay

The cells transfected with or without Sp1 shRNA2 were placed in 6-well plates and treated with triptolide (100 nM) or vehicle control (0.01% DMSO) for 24 h, respectively. Annexin V-FITC/propidium iodide (PI) Apoptosis Detection kit (Multisciences, ShangHai, China) was used to detected apoptosis. In brief, cells were washed with PBS and resuspended in 500 μl of 1 × binding buffer, followed by staining with 5 μl of Annexin V-FIFC and 10 μl of PI. The samples were analyzed immediately by a Beckman Coulter system (EPICS ALTRA II, Fullerton, USA).

2.5. Cell transfection

Cells were transiently transfected with Sp1 shRNA2 or control shRNA (Genepharma, Shanghai, China) using X-tremeGENE HP DNA Transfection Reagent (ROCHE, Basel, Switzerland) according to the manufacturer's protocol.

2.6. Dual luciferase reporter assay

Human renal embryonic 293T cells (1×10^5/well) were placed in 24-well plates and cultured overnight. Each luciferase reporter gene construct or pGL 3.0 vector control (0.5 μg) and pRL-TK plasmid (0.05 μg) were co-transfected into the cells for 24 h, followed by treatment with triptolide (50 nM) or vehicle control (0.01% DMSO) for further 24 h. The whole protein was prepared and luciferase activity was measured using Dual-Luciferase Assay Kit (Promega, Madison, Wisconsin, USA) according to manufacturer's instructions.

2.7. Real-time PCR

The total RNA was extracted using Trizol Reagent (Invitrogen, Grand Island, New York, USA). All isolated RNA samples were reversely transcribed using Reverse Transcription Kit (TaKaRa, Tokyo, Japan) according to the manufacturer's instructions. The hTERT mRNAs were quantified by a CFX 96 Real-Time PCR Detection System (BIO RAD, USA) using a SYBR green PCR kit (TaKaRa, Tokyo, Japan). The primers used for RT-PCR were listed in Table 1. All data were normalized to the housekeeping gene GAPDH.

2.8. Western blot analysis

Cell lysates were prepared in RIPA lysis buffer (Beyotime, ShangHai, China) supplemented with 0.5% cocktail protease inhibitor (ROCHE, Basel, Switzerland). After centrifugation at 12, 000 × g for 10 min at 4 °C, the supernatants mixed with 5 × loading buffer (250 nM Tris–HCl (PH 6.8), 10% SDS, 0.5% BPB, 50% glycerol,