Cancer Letters 290 (2010) 76–86

Contents lists available at ScienceDirect

Cancer Letters

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

Curcumin inhibits nuclear localization of telomerase by dissociating the Hsp90 co-chaperone p23 from hTERT

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ARTICLE INFO

Article history:
Received 14 April 2009
Received in revised form 20 August 2009
Accepted 24 August 2009

Keywords:
Telomerase
hTERT
Curcumin
Hsp90
p23
Ubiquitination

ABSTRACT

The molecular chaperone complex Hsp90–p23 interacts with the rate-limiting catalytic subunit of telomerase, hTERT. Although their interactions are required for proper folding of nascent hTERT as well as the assembly of active telomerase, the precise role of the chaperone proteins in regulation of nuclear localization of hTERT remains unclear. Here we demonstrate that curcumin inhibits telomerase activity in a time- and dose-dependent manner by decreasing the level of hTERT expression. Following curcumin treatment, we observed a clear accumulation of hTERT in the cytoplasmic compartment of the cell. The curcumin-induced cytoplasmic retention of hTERT could be due to failure of nuclear import, and the resulting cytoplasmic hTERT protein was rapidly ubiquitinated and degraded by the proteasome. We also report that curcumin treatment results in a substantial decrease in association of p23 and hTERT but does not affect the Hsp90 binding to hTERT. In contrast, the treatment of the Hsp90 inhibitor geldanamycin promotes dissociation of both Hsp90 and p23 proteins from hTERT. Taken together, these results demonstrate that the interaction of the Hsp90–p23 complex with hTERT is critical for regulation of the nuclear localization of telomerase, and that down-regulation of hTERT by curcumin involves dissociating the binding of hTERT with p23. Thus, inhibition of nuclear translocation of hTERT by curcumin may provide new perspectives for regulation of telomerase activity during tumorigenic progression.

1. Introduction

Eukaryotic telomeres are essential and functional components located at the physical ends of linear chromosomes [1]. In most organisms, telomeres consist of long tandem repeats (TTAGGG in vertebrates) and proteins that associate directly or indirectly with telomeric DNA sequences. Properly capped telomeres protect chromosomal termini from nucleolytic degradation, end-to-end fusion, and other inappropriate processes, thus enabling cells to distinguish chromosome ends from double-strand breaks in the genome [2,3]. Without functional telomere maintenance pathways, dividing cells show a progressive loss of telomeric DNA with each cell division due to a DNA end replication problem [4,5]. Thus, telomere shortening functions as a control mechanism that regulates the proliferative capacity of cells. Continued cell proliferation requires telomerase to compensate for telomere loss and to maintain chromosomal stability [6]. Since telomerase activity is up-regulated in a majority of human cancer cells, telomerase has attracted considerable attention as a potential target for cancer therapy [7–9].

Although the enzymatic activity of telomerase is regulated by hTERT at the transcriptional level [10,11], several lines of evidence have suggested a post-translational

Abbreviations: hTERT, human telomerase reverse transcriptase; Hsp90, heat shock protein 90; GA, geldanamycin; TRAP, telomeric repeat amplification protocol; LMB, leptomycin B; NES, nuclear export signal; ITAS, internal telomerase assay standard; RT-PCR, reverse transcription-polymerase chain reaction; DAPI, 4,6-diamino-2-phenylindole.

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doi:10.1016/j.canlet.2009.08.026

regulation of telomerase activity. A number of telomerase-associated proteins have been identified in vertebrates and appear to regulate telomerase assembly, nuclear translocation, and post-translational modifications of hTERT [6]. The molecular chaperone proteins have been shown to bind specifically to hTERT and promote the assembly of active telomerase both *in vitro* and *in vivo* [12,13]. Ubiquitin ligase MKRN1 has been shown to promote the ubiquitination-mediated degradation of hTERT and subsequently cause a decrease in telomerase activity as well as in telomere length [14]. Phosphorylation of hTERT has been reported to regulate telomerase activity. Up-regulation of telomerase activity is associated with phosphorylation of hTERT by PKC and Akt and its nuclear translocation [15–17]. In contrast, telomerase activity is inhibited by c-Abl kinase-mediated phosphorylation of hTERT [18]. 14–3-3 protein and NF-κB have been shown to directly interact with hTERT and regulate telomerase activity by modulating the nuclear localization of hTERT [19,20]. Other telomerase-associated proteins, KIP and PinX1, positively and negatively regulate telomerase activity and telomere length through direct binding to hTERT, respectively [21,22].

Hsp90 is a highly conserved and abundant molecular chaperone found in all eukaryotes [23]. Hsp90 is required for proper folding and maturation of its substrate proteins and exists in association with other proteins such as p23, Hsp70, and p60. The co-chaperone p23 binds the ATP-bound dimeric form of Hsp90 and serves to stabilize Hsp90–substrate complexes [24]. The molecular chaperone complex Hsp90–p23 interacts with hTERT [12,13], and disruption of the Hsp90 function by geldanamycin (GA) inhibits telomerase activity through a proteolysis of hTERT [14]. Whereas biochemical evidence indicates that the molecular chaperone proteins are important for maintenance of a functionally active telomerase complex, the precise role of Hsp90–associated proteins in regulation of the telomerase function remains unclear. The role of curcumin in the inhibition of telomerase activity has been suggested in various human cancer cell lines [25,26]. Curcumin is a natural compound present in turmeric, the dried rhizome of the plant *Curcuma longa* L., which possess anti-inflammatory, anti-tumor, anti-proliferative, and anti-oxidant activities [27,28]. Recent studies revealed that curcumin inhibits telomerase activity possibly due to suppression of nuclear translocation of hTERT and the inhibition of telomerase activity correlates with induction of apoptosis [29] These findings suggest that inhibition of telomerase followed by induction of apoptosis might be involved in the anti-proliferative effect of curcumin. However, little is known about the molecular mechanisms by which curcumin down-regulates telomerase activity and influences the functions of the molecular chaperones.

In the present study, we show that curcumin inhibits telomerase activity in a time- and dose-dependent manner through the proteasome-mediated degradation of hTERT. We also report that nuclear translocation of hTERT is impaired by curcumin treatment. The resulting cytoplasmic hTERT was rapidly ubiquitinated and degraded by the proteasome. Moreover, curcumin treatment resulted in a substantial decrease in association of p23 and hTERT but did not affect the Hsp90 binding to hTERT. These results demonstrate that the interaction of the Hsp90–p23 complex with hTERT is critical for regulation of the nuclear localization of telomerase, and that down-regulation of hTERT by curcumin involves dissociating the binding of hTERT with p23.

2. Materials and methods

2.1. Telomerase assay

The telomeric repeat amplification protocol (TRAP) was used as previously described [30]. Briefly, cell extracts (200 ng of protein) were added to the telomerase extension reactions and incubated for 20 min at 37 °C. PCR was performed using the HTS primer and HACX primer for 30 cycles (denaturation at 94 °C for 30 s, annealing at 62 °C for 30 s, and extension at 72 °C for 30 s). As an internal telomerase assay standard, NT and TSNT primers were added to the PCR mixture as previously described [31]. Telomerase products were resolved by electrophoresis on a 10% nondenaturing polyacrylamide gel. Bands were then visualized by staining with SYBR Green (Molecular Probes). The signal intensity was quantified with a LAS-4000 Plus Image analyzer (Fuji Photo Film).

2.2. Immunoprecipitation and immunoblot

Immunoprecipitation and immunoblotting were performed as described previously [21]. Briefly, H1299 cells were transiently transfected with the expression vectors as indicated using LipofectAMINE 2000 (Invitrogen). Cell lysates were preincubated with protein A-Sepharose (Amersham Biosciences) and incubated with primary antibodies precoupled with protein A-Sepharose beads for 2 h at 4 °C. The precipitated proteins were washed extensively and subjected to immunoblot analyses. Immunoprecipitation and immunoblotting were performed using anti-FLAG (Sigma), anti-HA (Sigma), anti-Hsp90 (Santa Cruz Biotechnology), anti-p23 (Abcam) antibodies.

2.3. In vivo ubiquitination assay

H1299 cells were transfected with HA-ubiquitin and FLAG-hTERT expression vectors, followed by MG132 treatment to inhibit proteasome function. Cell lysates were subjected to immunoprecipitation with anti-FLAG antibody, followed by immunoblotting analysis with anti-HA antibody to illuminate ubiquitin-modified hTERT.

2.4. Immunofluorescence microscopy

Cells were fixed with 2% paraformaldehyde and permeabilized with 0.5% Triton X-100 in phosphate-buffered saline (PBS). Cells were then blocked in PBS containing 2% bovine serum albumin and incubated with goat anti-hTERT (Santa Cruz Biotechnology) or mouse anti-FLAG (sigma). After washing, cells were incubated with Alexa Fluor 568 goat anti-mouse immunoglobulin and Alexa Fluor 488 goat anti-goat immunoglobulin (Molecular Probes). The cells were examined using an Olympus BX61 fluorescence microscopy.