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Short Report

Attenuated expression of SECIS binding protein 2 causes loss of telomeric reserve without affecting telomerase

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ABSTRACT

The family of selenoproteins have a broad range of functions, including protection against oxidative damage. Previous studies have shown that elevated levels of oxidative damage can induce accelerated loss of telomeric DNA during proliferation of mammalian cells. The incorporation of selenocysteine (Sec) into proteins in mammalian cells requires the Sec insertion sequence (SECIS) binding protein 2 (SBP2). Thus in the present study we have assessed the effect of knocking down the expression of SBP2 on telomere length. Following knock-down of SBP2 expression in two different human cell lines, the MSTO mesothelioma cell line (~5 Kb average telomere length) and SY5Y neuroblastoma cell line (~4.2 Kb average telomere length), we observed a significant reduction (−0.6 to −1.1 Kb; $P \leqslant 0.01$) in telomere length as compared to control cells. This reduction in telomere length was independent of affects on telomerase, since both telomerase activity levels and Tert mRNA expression levels were not altered by knock-down of SBP2 expression. Furthermore, telomeres were particularly sensitive to S1 nuclease digestion following SBP2 knock-down, indicating an increased frequency of oxidative damage-induced lesions in the telomeric DNA in these cells. Together, these observations imply that selenoproteins may help protect telomeric reserve in mammalian cells.

1. Introduction

Mammalian cell senescence, defined as an irreversible state of growth arrest (Hayflick and Moorhead, 1961), can be induced by a number of events, including excessive oxidative stress, DNA damage, dysregulated mitogenic stimuli, and telomere shortening (Campisi and d'Adda di Fagagna, 2007). Even in an optimal growth environment devoid of external stressors, normal human somatic cells still ultimately succumb to cell senescence due to the incremental loss of telomeric DNA at each round of cell division and eventual shortening of the terminal TTAGGG tract below a critical threshold required for telomere function (Harley et al., 1990; Hemann et al., 2001). This proliferation-dependent loss of telomeric DNA is attributed to the absence of sufficient levels of the enzyme telomerase, a ribonucleoprotein complex which functions to complete the replication of telomeres during DNA replication [S phase]—over-expression of telomerase reverse transcriptase (Tert), the limiting component of the telomerase complex, in normal human cell strains is sufficient to restore telomerase activity and prevent both telomere shortening and cell senescence (Bodnar et al., 1998; Blackburn, 2005).

The rate of telomere shortening observed for mammalian cells in a stress-free environment either in vivo or in vitro is typically in the range of 50–100 bp per cell division. A number of factors can lead to acceleration of the rate of telomere shortening, including inhibition or suppression of the levels of telomerase activity (Mitchell et al., 1999; Allsopp et al., 2003), defects in non-telomerase components of the telomeric replication machinery, such as the WRN helicase (Schulz et al., 1996), and increased oxidative damage at the telomere (von Zglinicki et al., 1995). In regards to this latter mechanism, telomeric DNA is particularly sensitive to oxidative damage in some types of human cells, wherein elevated levels of reactive oxygen species react with the terminal TTAGGG tract and create unrepaired single strand breaks (Petersen et al., 1998). It has been shown that accumulated oxidative damage at telomeres can lead to a >5-fold increase in the rate of telomere shortening, accompanied by a dramatic decrease in replicative lifespan (von Zglinicki et al., 1995). Elevated oxidative stress is a well established feature of inflammatory responses of the immune system as well as a number of age-related diseases, including Alzheimer's, cancer and cardiovascular disease, and therefore accelerated telomere shortening induced by increased oxidative damage may have significant physiological relevance.

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In higher eukaryotes, the family of selenoproteins, including thioredoxin reductases, glutathione peroxidases, methionine sulfoxide reductase and selenoprotein P, have a number of functions, including playing an important role in responding to oxidative stress (Papp et al., 2007). Recent studies have shown that Sec insertion sequence (SECIS) binding protein 2 (SBP2) differentially regulates the incorporation of Sec into selenoproteins, and that suppression of SBP2 expression causes dysregulation of the expression levels of selenoproteins (Low et al., 2000; Squires et al., 2007). These observations support a role for SBP2 in establishing the hierarchy in selenoprotein synthesis. Therefore, in the present study, we have examined the effects of knocking down SBP2 expression levels on telomere length homeostasis in human cells in vitro.

2. Materials and methods

2.1. Cell culture and transfection

The MSTO-211H human mesothelioma cell line and SY5Y neuroblastoma cell line were cultured in RPMI 1640 medium (Gibco-Invitrogen) supplemented with 10% fetal bovine serum and incubated with 5% CO2 at 37 °C. Cells were transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Stable shRNA transfectants derived from single cells were selected and maintained in puromycin-supplemented medium (2 μg/ml). Transient siRNA transfectants were harvested 96 h post-transfection. For hydrogen peroxide (H2O2) treatment, MSTO cells were cultured in serum free media containing 250 μM H2O2 for 2 h, followed by 48 h continued growth in the absence of H2O2 in regular growth media.

2.2. siRNA and shRNA expression constructs

SBP2 small interfering RNAs (siRNAs) and non-targeting control siRNAs were purchased from Dharmacon. The retroviral expression vectors (pSM2) for expression of SBP2 or control, scrambled, short hairpin (sh) RNAs were purchased from Open Biosystems. The SBP2 shRNA is designed to target the sequence, AAGTATTTATCTTCTGAGATAA, near the 5′ end of the SBP2 mRNA transcript. To generate an SBP2 shRNA expression vector that co-expressed GFP, the mir30-shRNA cassette of the SBP2-pSM2 vector was PCR amplified and subcloned into the GIPZ lentiviral expression vector (Open Biosystems) upstream of an IRES GFP sequence.

2.3. RNA isolation, cDNA synthesis, and real-time qPCR analysis

RNA was isolated from the transfected cells using RNeasy spin columns and treated with RNase-free DNase I (QIAGEN). Concentration and purity of the extracted RNA were determined using the A260/A280 value measured on an ND1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). One microgram of the RNA was used for cDNA synthesis. All cDNA was synthesized using the Applied Biosystems high-capacity cDNA synthesis kit. Real-time PCR was performed using Platinum SYBR Green quantitative PCR (qPCR) SuperMix (Invitrogen) in a Light Cycler 2.0 (Roche). Cycling conditions were used as suggested in the SYBR Green kit instructions, and results were analyzed using relative quantification software (Roche). Hypoxanthinephosphoribosyltransferase (HPRT) was used as an internal standard.

2.4. TRAP assay

Telomerase extracts were prepared using CHAPS lysis buffer, for equal numbers of cells (200,000) per sample. The TRAP assay was performed using the TRAPeze telomerase detection kit (Chemicon) according to manufacturers' protocol.

2.5. Analysis of telomere length

Telomere length was measured by Southern analysis of terminal restriction fragment (TRF) length, as previously described (Allsopp et al., 2007; Vaziri et al., 1993).

2.6. Analysis of sensitivity to S1 nuclease

Following transient knock-down of SBP2, DNA was digested with restriction enzymes HinfI and RsaI to generate TRFs (Allsopp et al., 2007; Vaziri et al., 1993), and then treated with S1 nuclease (Promega) at 1 U/μg of DNA for 45 min at 37C in the 1× S1 nuclease buffer provided. Reactions were stopped by adding 25 mM EDTA, followed by phenol/chloroform extraction of the DNA. Southern analysis of TRF length was then performed as described (Allsopp et al., 2007; Vaziri et al., 1993).

3. Results and discussion

We have previously shown that SBP2 is expressed at relatively high levels in the mesothelioma cell line MSTO-211H (Squires et al., 2007). Furthermore, SBP2 expression levels were successfully knocked down in this cell line using siRNA technology. Therefore, MSTO-211H cells were also used in the present study to examine the effect of siRNA-mediated knock-down of SBP2 expression levels on telomere length. Sub-confluent MSTO-211H cells were either transiently or stably transfected with siRNA or an shRNA expression construct, respectively, that specifically targeted SBP2 mRNA. A typical southern blot showing analysis of terminal restriction fragment (TRF) length for two independent stable transfection experiments is shown in Fig. 1A. Both of the MSTO cultures stably transfected with the SBP2 targeting shRNA construct showed substantial reduction in telomere length (∼−1.1 Kb, ∼48% of the total initial telomere length) as compared to untreated MSTO cells, or MSTO cells stably transfected with a non-specific shRNA expression construct. Quantitative analysis of mean TRF length for all transiently or stably transfected samples confirmed that telomere length is significantly reduced upon knock-down of SBP2 (Fig. 1B; $P \leqslant 0.01$). Furthermore, slot blot analysis of telomeric signal intensity for these same samples revealed a ∼45% reduction in total telomeric DNA in MSTO cells stably selected for SBP2 knock-down ($P = 0.002$; data not shown).

To verify that this finding could be extended beyond a specific cell line, we repeated the analysis of the affect of knocking down SBP2 expression on telomere length in the SY5Y neuroblastoma cell line. Previous experiments had confirmed SBP2 expression in these cells (data not published). Southern analysis of mean TRF length following transient knock-down of SBP2 or transfection with a scrambled siRNA (control) in SY5Y revealed a reduction in telomere length following SBP2 knock-down (∼−0.6 Kb; Fig. 1C and D; $P = 0.005$). Importantly, this observation supports the potential broad applicability of the role of SBP2 in telomere length regulation.

To examine whether accelerated cell turnover might contribute to the loss of telomeric DNA in MSTO cells transfected with the shRNA expression construct targeting SBP2, we compared the growth rate of MSTO cells transfected with either the SBP2 shRNA expression construct or the control shRNA expression construct. The growth rate of stable transformants over a 60 day period was similar for MSTO cells transfected with the SBP2 targeting construct or the control construct (Fig. 2A). We also assessed affect of SBP2 knock-down on the proportion of apoptotic cells, and did