Human SUV3 helicase regulates growth rate of the HeLa cells and can localize in the nucleoli

Maciej Szewczyk1, Natalia Fedoryszak-Kuśka3, Katarzyna Tkaczuk1, Jurek Dobrucki4, Agnieszka Waligórska4 and Piotr P. Stępień1,2,3

1Institute of Genetics and Biotechnology, Faculty of Biology, Warsaw University, Warsaw, Poland; 2Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland; 3Centre for New Technologies, University of Warsaw, Warsaw, Poland; 4Department of Cell Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

The human SUV3 helicase (SUV3, hSUV3, SUPV3L1) is a DNA/RNA unwinding enzyme belonging to the class of DExH-box helicases. It localizes predominantly in the mitochondria, where it forms an RNA-degrading complex called mitochondrial degradosome with exonuclease PNP (polynucleotide phosphorylase). Association of this complex with the polyA polymerase can modulate mitochondrial polyA tails. Silencing of the SUV3 gene was shown to inhibit the cell cycle and to induce apoptosis in human cell lines. However, since small amounts of the SUV3 helicase were found in the cell nuclei, it was not clear whether the observed phenotypes of SUV3 depletion were of mitochondrial or nuclear origin. In order to answer this question we have designed gene constructs able to inhibit the SUV3 activity exclusively in the cell nuclei. The results indicate that the observed growth rate impairment upon SUV3 depletion is due to its nuclear function(s). Unexpectedly, overexpression of the nuclear-targeted wild-type copies of the SUV3 gene resulted in a higher growth rate. In addition, we demonstrate that the SUV3 helicase can be found in the HeLa cell nucleoli, but it is not detectable in the DNA-repair foci. Our results indicate that the nucleolar-associated human SUV3 protein is an important factor in regulation of the cell cycle.

Key words: SUV3 helicase, SUPV3L1, dual targeting, nucleolus, mitochondria, cell cycle

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e-mail: stepien@ibb.waw.pl
Abbreviations: mt, mitochondrial; SUV3, Suppressor of Var1

INTRODUCTION

In eukaryotic cells localization of a single gene product to more than one subcellular compartment is a common phenomenon, termed dual targeting, dual localization or dual distribution. Often, the amount of a given protein in the second subcellular compartment is very small or the protein resides in the second compartment only in a specific metabolic status of the cell. Such cases are difficult to detect by standard methods due to a strong signal from the major compartment and are described as an “eclipsed localization” (reviewed by Yogev & Pines, 2011). Dual localization provides a significant challenge in distinguishing which phenotype is related to which subcellular site of activity.

The SUV3 gene encoding an RNA helicase was discovered in the yeast Saccharomyces cerevisiae and the SUV3 protein was found to localize in mitochondria, where together with the DSS1 exoribonuclease, it forms an RNA-degrading complex called mtEXO or mitochondrial degradosome (Stepien et al., 1992; Dziembowski et al., 2003). Inactivation of the SUV3 gene resulted in a massive accumulation of mitochondrial RNA and in the absence of mitochondrial respiration. This prompted a hypothesis that the accumulated mtRNA is toxic for mitochondria (Margossian et al., 1996). Indeed, by lowering the mitochondrial transcription rate, it was possible to rescue the respiratory phenotype of the SUV3 depletion in yeast cells (Rogowska et al., 2006).

The SUV3 gene is present in purple bacteria and all eukaryotes (Dmochowska et al., 1999). The human SUV3 helicase is encoded by a nuclear gene (SUV3, hSUV3, SUPV3L1) and is a DNA/RNA helicase belonging to the family of DExH-box helicases, able to unwind RNA, RNA-DNA or DNA duplexes (Minezuk et al., 2002). The predominant subcellular localization of the SUV3 helicase is the mitochondrion, although small amounts have been detected in the cell nuclei (Szczesny et al., 2007), which is in agreement with the nuclear localization sequence (NLS) present close to the C-terminus of this protein (Shu et al., 2004). In human mitochondria, the SUV3 helicase forms an RNA-degrading complex with PNPase (polynucleotide phosphorylase), localized in the intra-mitochondrial granules (foci) which participate in the mtRNA decay (Szczesny et al., 2010; Borowski et al., 2013). Recently, the SUV3-PNP-polyA polymerase complex has been implicated in modulation of the mitochondrial polyA tail lengths (Wang et al., 2014) in addition, SUV3 haploinsufficiency in mice was found to induce mitochondrial genome instability leading to tumorigenesis and shortened lifespan (Chen et al., 2013). Interestingly, mice with a conditional knock-out of the SUV3 gene developed premature aging, cachexia, sarcopenia and skin disease (Paul et al., 2009).

Silencing of the SUV3 gene in human cell lines results in a significant accumulation of various classes of undegraded mitochondrial RNA (Szczesny et al., 2010); in addition, cell cycle perturbations and apoptosis were observed (Szczesny et al., 2007). This could be analogous to yeast, where a lack of mtRNA degradation resulted in a collapse of mitochondrial functions (Rogowska et al., 2006). On the other hand, since a small fraction of the human SUV3 protein was found in the nucleus, it was not clear which phenotypes could result from the depletion of the nuclear fraction of the SUV3 protein and which from the mi-
tochondrial one. This was due to the fact that experiments reported so far were based on silencing of the whole SUV3 gene.

While mitochondrial functions of the SUV3 protein have been extensively investigated, much less is known about its putative role in the nucleus. The analysis of SUV3 interactors resulted in identification of the WRN and BLM helicases (Pereira et al., 2007), FEN 1 flap endonuclease and replication protein A (Veno et al., 2011). All of these proteins play important roles in chromatin maintenance and DNA repair, but no specific role for SUV3 in these complexes has been proposed.

This paper presents the first experimental approach to dissect the mitochondrially-related phenotypes of the SUV3 helicase depletion from the nuclear ones.

MATERIALS AND METHODS

Cell culture and cell manipulations. T-REx™-HeLa cells (Invitrogen) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) (Gibco) supplemented with 10% fetal bovine serum (Gibco) at 37°C under 5% CO₂. The stable, inducible cell lines obtained in this work were cultured by using TET System Approved FBS (Clontech). For cell growth rate assessment 3.5 × 10⁶ of the appropriate cells were plated, induced (or not) after 24 h and detached after the next 24 h. Every second day the cells were detached, counted and plated at the same density as before.

Development of stable transfected cell lines. We used DNA constructs created earlier by Szczesny et al. (2009): pRS68, pRS97, pRS98 and pRS100. These plasmids were based on the pcDNAs5/FRT/TO vector (Invitrogen) backbone and encoded N-terminally truncated (ΔMTS, non-mitochondrial), full length wild-type (WT), full-length catalytically inactive (G207V) and N-terminally truncated, catalytically inactive (ΔMTS G207V) SUV3, respectively. The host cell-line was co-transfected using TransIT 2020 (Mirus) reagent with 0.4 μg of appropriate construct and 1.6 μg of pOG44 (Invitrogen). Twenty-four hours after transfection, cells were plated and subjected to selection by adding hygromycin B (200 μg/ml) (Invitrogen). Selective medium was replaced every 2–3 days. In all presented experiments, expression of the exogenous genes was induced with tetracycline (Sigma-Aldrich) at the concentration of 100 ng/ml.

Proliferation assay. A real-time cell proliferation assay was performed using the xCELLigence Real-Time Cell Analyzer DualPlate (RTCA DP) (ACEA Biosciences). This system uses measurement of electrical impedance that occurred in the presence and absence of cells in the wells (Xing et al., 2005). The Cell Index was monitored every thirty minutes throughout 4 days of the experiment.

Immunofluorescence. 24 h prior the experiment cells were trypsinized, counted and plated at 50,000 cells per well of a 6-well plate (glass coverslip) in a medium supplemented with 100 ng/ml tetracycline. Then the cells were challenged with 5 μM camptothecin for 0, 3 or 6 h.

Cells were fixed with 4% formaldehyde, permeabilized with 0.5% Triton X-100 (Sigma-Aldrich) in PBS and blocked in 10% FBS in PBS. SUV3 was visualized using primary rabbit polyclonal antibodies described previously by Mińczuk and coworkers (2002), and secondary goat anti-rabbit antibodies coupled with Alexa555 (LifeTechnologies). To visualize phosphorylated histone H2AX we used Abcam mouse monoclonal antibodies (ab22551) and goat anti-mouse antibodies coupled with Alexa488 (LifeTechnologies). Coverslips were mounted with ProLong Gold Antifade Reagent (LifeTechnologies).

Images were acquired by laser scanning confocal microscope Leica TCS SP5 (Leica Microsystems GmbH, Wetzlar, Germany) with 63x plan apochromatic oil immersion objective lens (NA 1.4). The confocal pinhole size was set at 1 Airy unit. Laser line 488nm and 633nm were used for fluorescence excitation. Emission was collected in the range of 496–544nm and 650–735nm. Transmitted light images were also registered.

Western blotting. Wild-type HeLa cells were challenged with camptothecin as depicted above, detached and lysed in a cold RIPA buffer (Sigma-Aldrich). Protein concentration was determined by the Bradford method. 50 micrograms of each total protein extract were separated on 12.5% or 8% SDS-PAGE gels and transferred to PVDF membranes.

SUV3 and γH2AX were immunodetected with the same primary antibodies as used in IF while phosphorylated ATM kinase and β-actin, which was used as a loading control, were detected with CellSignalling mouse polyclonal antibodies (#4526 and #3700, respectively). Primary antibodies were detected with goat anti-rabbit or anti-mouse HRP-conjugated antibodies (Calbiochem) and visualized by enhanced chemiluminescence (BioRad).

RESULTS AND DISCUSSION

Silencing of the human SUV3 gene was previously achieved using three techniques: 1. by employing siRNA (Szczesny et al., 2007), 2. by expression of a missense mutant lacking the helicase activity: complexes harboring such mutant are nonfunctional (Szczesny et al., 2009; Borowski et al., 2012) and 3. by constructing mouse models harboring a deletion or a conditional KO of the SUV3 gene (Pereira et al., 2007; Paul et al., 2009).

In all of the three approaches both, mitochondrial and nuclear functions of the SUV3 helicase were affected, so the resulting cellular or organismal phenotypes could be not be attributed to the SUV3 deficiency in a particular subcellular compartment.

In order to address the question whether the growth rate inhibition depends on the nuclear or mitochondrial SUV3 functions, we constructed HeLa cell lines with inducible gene constructs: two of them were missense mutants in the SUV3 helicase domain with the substitution of glycine for valine at position 207 of the Walker A helicase motif within the SUV3 open reading frame. This mutation completely abolishes the helicase activity (Mińczuk et al., 2002). In addition, we deleted a 46 amino acid N-terminal mitochondrial targeting sequence (MTS) (ΔMTS, non-mitochondrial), thus impairing the transport of the protein into mitochondria. In addition, we also constructed cell lines containing a wild-type helicase, with MTS (wtSUV3) and...
without MTS (Delta46aa; SUV3:G207V). All constructs are schematically shown in Fig. 1. The constructs were stably incorporated into nuclear genomes of the human HeLa cell line using the Flp-In technique. Addition of tetracycline to the medium resulted in induction of the introduced genes. A similar approach has been previously developed in our lab (Szczesny et al., 2010) in human 293 cells, but no growth studies were performed. The growth of the cell lines was measured in the Real-Time Cell Analyzer Dual Plate (RTCA DP, xCELLigence device), where cellular growth was continuously monitored and calculated during 65 hours after gene induction (Fig. 2).

The results presented here indicate that both strains carrying mutations in the helicase domain, either with or without the mitochondrial localization signal (MTS), show a strong inhibition of growth rate. Our previous studies demonstrated that the mutant protein with no MTS does not localize to mitochondria and exclusively localizes to the nucleus (Szczesny et al., 2007); therefore the growth inhibition observed here is not due to the disturbances in mitochondrial RNA metabolism, but is the consequence of the perturbation of a yet unknown nuclear function of the SUV3 protein. Our result is also in line with the previous observations that the down regulation of the SUV3 gene induces caspase- and AIF-dependent apoptotic pathways (Szczesny et al., 2007).

The growth curves presented in Fig. 2 show the unexpected second phenotype associated with the nuclear function of the SUV3 protein: overexpression of the nuclear-localized wild-type helicase results in a higher growth rate. This effect is stronger when the wild-type allele is driven to the cell nuclei by the absence of a mitochondrial targeting sequence. Both constructs, with or without the MTS, drive the growth rate above the control level. Interestingly, similar effects were observed in plants, where overexpression of the full-length wild-type plant SUV3 gene resulted in improved stress resistance in rice, delayed leaf senescence-associated events and resulted in 3.5-fold increase in the...
Human SUV3 protein was found to interact with several nuclear proteins involved in chromatin stability. Therefore we decided to assess if the SUV3 protein co-localizes with the DNA repair foci which form upon DNA damage. Such foci can be visualized by immunofluorescence of 53BP1 and γH2AX proteins. We challenged the cells using camptothecin in order to induce double strand breaks and checked whether signal from the SUV3 antibodies co-localizes with the repair foci. While we could detect the induction of both, the 53BP1 and γH2AX proteins (Fig. 3, panel A) on a Western blot, and the DNA repair foci visualized by immunofluorescence, the SUV3 helicase signal did not co-localize with the foci (Fig. 3 panel B). Thus we suggest that the SUV3 helicase does not participate in the repair of the DNA double strand breaks.

Finally we investigated the sub-nuclear localization of the SUV3 protein. Our previous data indicate that the nuclear level of the SUV3 protein is rather low and it depends on the cell line: signal in the HeLa cells is much stronger than in the 293 cells. Therefore, we induced our SUV3:DELTA46 construct in the HeLa cells, reasoning that the lack of mitochondrial localization signal would drive the overexpressed protein to the cell nuclei and thus increase the signal to noise ratio. Indeed, by using anti-SUV3 antibodies we were able to detect the intranuclear foci of the SUV3 protein by immunofluorescence, which co-localize with nuclei appearing in the visible light (Fig. 4). Recent data suggest that nucleoli exert control over cell cycle progression in both, forward and reverse ways (reviewed by Tsai and Pederson, 2016). Thus, it is tempting to speculate that the SUV3 helicase is a part of this regulatory circuit, but clearly more research is needed.

In summary, we demonstrate here the importance of nuclear localization of the SUV3 protein for regulation of the cell cycle in HeLa cells. To the best of our knowledge, this is the first report dissecting the mitochondrial phenotypes of SUV3 from the nuclear ones. The results presented here are in agreement with previously detected interactions of the human SUV3 protein with nuclear chromatin maintenance factors, yet it seems that SUV3 does not participate in the DNA repair. Our study was conducted on cell lines, but it calls for re-visiting of the data obtained with conditional KO of the SUV3 gene in mice: SUV3-associated phenotypes including cancer (Chen et al., 2013) and premature aging (Paul et al., 2009) could possibly be due to the involvement of the SUV3 helicase in nuclear functions.

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