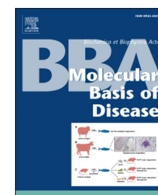
Biochimica et Biophysica Acta 1842 (2014) 2387–2394

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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



Transient overexpression of Werner protein rescues starvation induced autophagy in Werner syndrome cells

Jyotirindra Maity [a], Vilhelm A. Bohr [b], Aparna Laskar [c], Parimal Karmakar [a,*]

[a] Department of Life Science and Biotechnology, Jadavpur University, Kolkata 700032, India
[b] Laboratory of Molecular Gerontology, National Institute on Aging, National Institutes of Health, Baltimore, MD 21224, United States
[c] CSIR-Indian Institute of Chemical Biology, Kolkata 700032, India

ARTICLE INFO

Article history:
Received 12 June 2014
Received in revised form 2 September 2014
Accepted 17 September 2014
Available online 23 September 2014

Keywords:
Autophagy
Aging
Beclin-1
RecQ helicase
Werner protein
Werner syndrome

ABSTRACT

Reduced autophagy may be associated with normal and pathological aging. Here we report a link between autophagy and Werner protein (WRNp), mutated in Werner syndrome, the human premature aging Werner syndrome (WS). WRN mutant fibroblast AG11395 and AG05229 respond weakly to starvation induced autophagy compared to normal cells. While the fusion of phagosomes with lysosome is normal, WS cells contain fewer autophagy vacuoles. Cellular starvation autophagy in WS cells is restored after transfection with full length WRN. Further, siRNA mediated silencing of WRN in the normal fibroblast cell line WI-38 results in decreased autophagy and altered expression of autophagy related proteins. Thus, our observations suggest that WRN may have a role in controlling autophagy and hereby cellular maintenance.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

RecQ helicases are ubiquitous in life and are found in a broad range of prokaryotes, as well as in yeast and human cells [1,2]. Among the five RecQ helicases found in humans, defects in three give rise to clinical disorders associated with cancer predisposition and/or variable symptoms of premature aging. Of these Werner syndrome (WS) and Rothmund Thompson syndrome (RTS) are associated with premature aging and often develop age associated diseases including cancer [3]. Along with the conserved 3′-5′ helicase domain, the WRN gene has other conserved catalytic domains such as 3′-5′ exonuclease, 27 aa direct repeats, a helicase-and-ribonuclease D/C-terminal (HRDC) and a C-terminal NLS [4]. WRN protein (WRNp, 1432 aa) participates in replication [5], transcription [6] and base excision repair, homologous recombination and non-homologous end joining [7–9]. It has been proposed that accumulation of DNA damage in WS cells may be responsible for the accelerated aging and age associated diseases seen among the patients. However, the exact role of WRNp in vivo, in preventing accelerated aging is not yet understood.

Autophagy, specifically macroautophagy is an evolutionarily conserved catabolic process which degrades cellular proteins and damaged or excess organelles [10]. This process is a survival strategy for starving cells involving recycling amino acids [10–12]. Degradation and recycling of building blocks of the organelles or protein are also important for maintenance of cellular homeostasis [13]. Damaged macromolecules or organelles block various cellular functions and their chewed up components are recycled to keep cellular homeostasis. Formation of double membrane autophagic vacuoles, also known as autophagosomes, and transportation of damaged protein to the lysosome for degradation require additional proteins [14,15]. Some of these are highly conserved from flies to mammals. Failure of autophagy accumulates damaged proteins inside the cells, which are responsible for the development of different neurodegenerative disorders [16], autoimmunity [17] and cancer [18]. Similarly, accumulating evidence suggests that autophagy may play an important role in cellular aging [19]. Autophagy decreases with age and this reduced function may underlie the accumulation of damaged non-functional proteins and cause oxidative stress [20]. This perturbs many cellular functions and contributes to the development of many age associated diseases including cancer [13].

Besides the already known impact of WRN on DNA metabolism and cell cycle regulation, defects in transcription have also been observed in WS cells implicating that WRNp may have a role in transcriptional control [21,22]. WRNp participates in transcription of genes induced after stress [23]. Moreover, WRNp involvement in RNA pol I and RNA pol II mediated transcription has been reported [21,24]. WRNp also affects

Abbreviations: ATG, Autophagy-related gene; EBSS, Earle's balanced salt solution; EGFP-LC3, Enhanced green fluorescent protein-microtubule-associated protein-1 light chain 3; MDC, Monodansylcadaverine; mTOR, Mammalian target of rapamycin; WS, Werner syndrome; WRNp, Werner protein

* Corresponding author at: Department of Life Science and Biotechnology, Jadavpur University, West Bengal, India. Tel.: +91 33 24572190; fax: +91 33 2413 7121.
E-mail address: pkarmakar_28@yahoo.co.in (P. Karmakar).

http://dx.doi.org/10.1016/j.bbadis.2014.09.007
0925-4439/© 2014 Elsevier B.V. All rights reserved.

the expression of genes involved in adipogenesis and inflammation [22]. Using array analysis approach, it was shown that both normal old and WS cells lack expression of Beclin-1 by 1.5 fold [25]. Beclin-1 plays a crucial role at the initial stage of autophagic vesicle formation [26–28]. Additionally, proteasomal degradation is also defective in WS cells resulting in the accumulation of damaged proteins [25]. Though autophagy and WS both are hallmarks of aging, these two have not been clearly connected. Thus, we were prompted to investigate autophagy in WS cells. We found that WS cells respond very weakly to starvation induced autophagy and that this was complemented by transfection with full length WRN which restored the expression of genes responsible for the induction of autophagy. Similarly, depletion of WRN from normal cells results in diminished autophagy and down regulation of autophagy related genes. Thus our results suggest a role of WRN in the induction of autophagy.

2. Materials and methods

2.1. Materials

Earle's balanced salt solution (EBSS) and L-glutamine were purchased from Himedia. Fetal bovine serum (FBS), penicillin_streptomycin, MEM NEAA, MEM amino acids, MEM vitamin solution, and Dulbecco's modified eagle medium (DMEM) were obtained from Life Technologies, USA. Bovine serum albumin (BSA) was purchased from SRL (India). 3-MA, monodansylcadaverine (MDC), and osmiumtetraoxide (OsO_4) were obtained from Sigma-Aldrich (USA). Anti-LC3B and anti-Atg5 were purchased from Abcam (Cambridge, England). Anti-beclin-1, anti-β-actin, anti-mTOR, and anti-p-mTOR (Ser2448) antibodies were purchased from Cell Signaling Technologies. Anti-Werner antibody and horse radish peroxidase-conjugated secondary antibodies were obtained from Santa Cruz Biotechnology, USA. The EGFP-LC3B plasmid, which encodes a fusion protein of EGFP and LC3B, was a kind gift from Prof. Tamatsu Yoshimori (Japan). pBABE-puro mCherry-EGFP-LC3B which encodes a fusion protein EGFP, mCherry and LC3B, was a generous gift from Dr. Jayanta Debnath (Department of Pathology, University of California, San Francisco). siRNA against human WRN was purchased from Invitrogen, USA.

2.2. Cell lines and culture conditions

WS cells AG11395 (SV40 transformed fibroblast; age: 60 year, biopsy source: skin; tissue source: skin; gender: male; ethnicity: Caucasia) and WS primary fibroblasts AG05229 (untransformed fibroblast; age: 25 year, biopsy source: thigh; tissue source: skin; gender: male; ethnicity: Caucasian) cells were cultured in minimal essential medium (MEM) supplemented with 10% FBS, 1% penicillin_streptomycin, 1% L-glutamine, 1% MEM NEAA, 1% MEM amino acids, and 1% MEM vitamin solution. WI-38 (SV40 transformed fibroblast; age: 3 months gestation fetus; biopsy source: lung; tissue source: lung; gender: female; ethnicity: Caucasian) cells were cultured in Dulbecco's modified eagle medium (DMEM) with 10% FBS. All the cells were maintained at 37 °C, 5% CO_2 and 95% relative humidity (RH). Starvation was performed by incubating exponential growing cells in Earle's balanced salt solution (EBSS).

2.3. MDC staining

AG11395, AG05229 or WI-38 cells (2×10^4) were seeded in 35 mm plate for overnight. After 16 h cells were starved with EBSS for different time points (2 to 24 h). The cells were then washed with 1X PBS thrice and incubated with 50 mmole/L MDC for 10 min at 37 °C [29,30] After washing with 1X PBS the cells were mounted on glass slides and viewed under a fluorescence microscope (Leica DM 2500).

2.4. Green fluorescent protein-light chain 3 plasmid transfection

AG11395 or WI-38 cells (2×10^4) were seeded in 35 mm plate for overnight. After 16 h cells were transfected 1 μg of EGFP-LC3B plasmid [31] using FuGENE6 as per the manufacturer's instructions (Roche). Next day cells were starved with EBSS for 24 h. The cells were then washed with 1XPBS thrice and mounted on glass slides. Finally cells were observed with a fluorescence microscope (Leica DM 2500).

2.5. Autophagic flux measurement

After 16 h of growth cells were transfected with pBABE-puro mCherry-EGFP-LC3B using Fugene 6 as per manufacturer's instructions (Roche). Next day cells were starved with EBSS for 8 h. Then cells were washed with 1XPBS thrice and mounted on glass slides followed by fluorescence microscopic observation (Leica DM 2500).

2.6. Western bloting

Whole cell lysates were prepared from the cells with lysis buffer containing 1% Triton X-100, 50 mM NaCl, 50 mM NaF, 20 mM Tris (pH 7.4), 1 mM EGTA, 1 mM sodium vanadate, 0.2 mM PMSF, 0.5% NP-40 and protease inhibitors (Bio vision). The supernatant was collected and protein concentration was estimated using Bradford's reagent. Cell lysates containing equal amount of protein (80 μg) were solubilized in Lamellae buffer, boiled for 5 min, and electrophoresed on a 12% SDS-polyacrylamide gel in Tris–glycine buffer (pH 8.8). Proteins were then transferred to polyvinylidine difluoride (PVDF) membrane (Bio-Rad). Nonspecific binding was blocked with 5% non-fat dry milk and 0.05% Tween-20 in 20 mM Tris–Cl, pH 7.6 (TBS-T). After incubation with the appropriate primary antibody, membrane was washed with TBS-T and blot was reincubated with secondary antibodies conjugated with horse radish peroxidase (HRP). Bound antibodies were detected by the ECL detection reagent (Santa Crutz).

2.7. Small interfering RNA (siRNA) mediated silencing of WRN

WI-38 cells were transfected with WRN siRNA (ID: s14907) and scramble (negative control #2 siRNA, Invitrogen, USA) using invitrogen transfection reagent according to the manufacturer's instructions. After 24 h, knockdown efficacy was determined by Western blotting with anti-WRN antibody.

2.8. Transmission electron microscopy (TEM)

AG11395 or WI-38 cells were starved with Earle's balanced salt solution (EBSS) for 24 h. Cells were then collected and prefixed with 2.5% glutaraldehyde. These cells were then post fixed with 1% osmium tetraoxide for 1 h in dark. Cells were then dehydrated by increasing concentrations of acetone. These cells were then embedded with epoxy resin. Polymerization of these cells was done by placing it gradually in oven for 42 °C for 2 h, 52 °C for overnight and then 62 °C for another overnight. Ultrathin sections (50–70 nm) of these blocks were cut using a Leica Ultramicrotome EM UC6. These ultrathin sections were collected form 10% ethanol turf. The sections were contrasted using 1% aqueous uranyl acetate for 5 min and lead citrate in a CO_2-depleted atmosphere for approximate 2 to 4 min. A FEI TECNAI G^2 Spirit BioTWIN (120 kV) electron microscope (Netherland) was used to study the sections in 100 kV.

2.9. Statistical analysis

Non parametric Mann Whitney's U was used to calculate the statistical differences between the groups. $P < 0.05$ was considered as statistically significant. Error bars represent the means ± SD for all plots. Data analysis was performed using the Origin pro v. 8 software (Origin Lab).

Download English Version:

https://daneshyari.com/en/article/8259974

Download Persian Version:

https://daneshyari.com/article/8259974

Daneshyari.com