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ScienceDirect
Mechanisms of Ageing and Development 128 (2007) 650–661
Mechanisms of Ageing and Development
www.elsevier.com/locate/mechagedev

Expression and localization of Werner syndrome protein is modulated by SIRT1 and PML

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Received 11 May 2007; received in revised form 21 September 2007; accepted 26 September 2007
Available online 2 October 2007

Abstract

Mutations in genes for WRN and BLM RecQ family helicases cause cancer prone syndromes. Werner syndrome, resulting from *WRN* mutation, is a segmental progeria. Endogenous WRN and BLM proteins localize in nucleoli and in nuclear PML bodies defined by isoforms of the PML protein, which is a key regulator of cellular senescence. We further characterized WRN and BLM localization using labeling with monomeric red fluorescence protein (mRFP). When ectopically expressed, mRFP-WRN (or untagged WRN) forms nuclear bodies, which are donut-shaped in some cells. We identified PML isoforms associating with the nuclear bodies. Interestingly, mRFP-WRN relocalizes from nucleoli to the nucleoplasm, frequently showing conspicuous nucleolar exclusion as well as a decrease in frequency of mRFP-WRN nuclear bodies in response to overexpression of wild-type and deacetylase mutant (H363Y) SIRT1 proteins. Similar nucleolar relocalization in response to wild-type SIRT1 was detected for mRFP-labeled BLM. Moreover, increased SIRT1 expression was associated with the downregulation of endogenous WRN and a decreased frequency of cells with BRCA1 foci. Our data indicate for the first time that SIRT1 protein may be functionally associated with WRN and BLM helicases and that some major SIRT1 functions may not require its deacetylase activity.

Keywords: WRN; BLM; PML; SIRT1; Helicase; Senescence; Aggresomes

1. Introduction

Two human RecQ helicases, WRN and BLM, localize in nucleoli and in nuclear structures called PML bodies (Marciniak et al., 1998; Ishov et al., 1999; Yankiwski et al., 2000, 2001; Johnson et al., 2001). Mutant genes coding these proteins cause human cancer prone syndromes (Werner and Bloom syndromes, respectively; Harrigan and Bohr, 2003). The autosomal recessive Werner syndrome is a segmental progeria characterized by the accelerated appearance of many, but not all, aging symptoms (e.g., cataracts, osteoporosis, atherosclerosis, and diabetes). Cells from Werner syndrome patients show genomic instability and accelerated replicative senescence. WRN is a pleiotropic protein involved in the maintenance of genomic stability, especially at telomeric DNA, in the gene transcription, including the transcription of ribosomal RNA genes and in the regulation of apoptosis (Bachrati and Hickson, 2003; Bohr, 2005 and references therein).

The nuclear localization of WRN protein shows a very complex pattern of changes. It was examined using various antibodies as well as the recombinant WRN protein tagged with the green fluorescent protein (GFP). In most of the examined human cell lines, the endogenous WRN is localized in nucleoli (Gray et al., 1998; Marciniak et al., 1998). After inhibition of DNA replication or transcription (e.g., by UV-radiation, hydroxyurea, camptothecin), WRN exits from nucleoli and localizes in the nucleoplasm, frequently showing conspicuous

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doi:10.1016/j.mad.2007.09.004

nucleolar exclusion (Brosh et al., 2001; Shiratori et al., 2002; Karmakar and Bohr, 2005). This change in the localization is accompanied by posttranslational modifications of WRN including phosphorylation (Cheng et al., 2003), SUMOylation (Woods et al., 2004) and acetylation (Blander et al., 2002). The nucleoplasmic staining of WRN is often associated with an increased frequency of WRN foci and bodies (Constantinou et al., 2000; Sakamoto et al., 2001). Some of these WRN-containing structures were shown to contain PML protein, the defining constituent of PML bodies (Johnson et al., 2001; Blander et al., 2002).

Studies of colocalization between WRN and PML proteins were performed on the endogenous proteins (Johnson et al., 2001) or GFP-tagged, ectopically expressed WRN with the detection of the endogenous PML (Blander et al., 2002). These studies showed that endogenous WRN colocalized with endogenous PML protein in nucleoplasmic bodies in some cell lines (Johnson et al., 2001). The ectopically expressed GFP-WRN partially colocalized in large nucleoplasmic foci with the endogenous PML protein in UV-irradiated U-2 OS cells (Blander et al., 2002).

The PML protein positively regulates many anticarcinogenic processes, e.g., cell cycle arrest, apoptosis and cellular senescence (Salomoni and Pandolfi, 2002). It acts by controlling gene transcription. Several hypotheses have been proposed to explain the mechanism of this control (Zhong et al., 2000; Ching et al., 2005 and references therein). According to one of them, PML indirectly post-translationally modifies transcription factors, altering their affinity to the target sequences within gene regulatory elements. This is well-exemplified by the role of PML in activating the p53 tumor suppressor protein. The senescence-inducing signal promotes the formation of the p53, PML and CBP acetyltransferase complex accompanied by p53 acetylation at Lys 382. This acetylation does not take place in PML-null fibroblasts (Pearson et al., 2000; Bischof et al., 2002). The overexpression of the PML isoform IV, but not isoform I or III, also induces p53 acetylation at Lys382, which is accompanied by an increase in the activation of a p53-dependent gene promoter (Bischof et al., 2002).

PML has many isoforms derived from the alternative splicing. For many years, researchers usually used only one isoform to study PML (Jensen et al., 2001 and references therein). Only recently, the isoforms have been studied simultaneously using the same experimental system. They showed significant functional differences. For example, of five isoforms tested (I–V), only isoform IV induced cellular senescence when expressed in primary fibroblasts (Bischof et al., 2002).

One of the proteins recruited to PML nuclear bodies is SIRT1-a NAD-dependent, type III, histone/protein deacetylase (Langley et al., 2002). It is a human orthologue of yeast Sir2 protein. In yeasts, it is required for chromatin modification associated with transcriptional silencing of the mating-type loci and with the suppression of recombination at ribosomal DNA and telomere repeats (reviewed in Guarente, 2000; Anastasiou and Krek, 2006). Human SIRT1 binds p53 protein and promotes its deacetylation in vivo and in vitro. It represses p53-mediated transactivation of gene promoters, antagonizes PML IV-induced cellular senescence and DNA damage-induced apoptosis. Thus, SIRT1 can be viewed as an antisenescence and antiapoptotic protein (Luo et al., 2001; Vaziri et al., 2001; Langley et al., 2002). Apart from p53, SIRT1 has other non-histone substrates, e.g., Ku70 DNA repair protein (reviewed by Anastasiou and Krek, 2006). The SIRT1 homologue extends the lifespan of many organisms, including yeasts and worms (Tissenbaum and Guarente, 2002). It plays a major role in stress signaling, anti-apoptosis, calorie restriction response as well as glucose, fat and insulin biochemistry (reviewed by Anastasiou and Krek, 2006).

In our work, we further characterized the regulation of WRN localization. First, we labeled WRN with monomeric red fluorescent protein (mRFP1) to create a useful tool for protein colocalization studies and we tested in what experimental conditions its localization is consistent with the localization of the endogenous WRN. Second, we identified PML isoforms associating with nuclear bodies formed by mRFP-WRN. Third, we examined the influence of SIRT1 protein on the cellular localization of mRFP-labeled and native WRN. Our study shows, for the first time, the functional relationship between SIRT1 and WRN and may have implications for a better understanding of these two antisenescent proteins.

2. Materials and methods

2.1. Plasmid construction

The mRFP-WRN expression plasmid was constructed based on the pcDNA3.1/HisC-WRN plasmid provided by Vilhelm Bohr (National Institute on Aging, Baltimore, MD, USA) and a plasmid coding for the monomeric red fluorescent protein (mRFP1) provided by Roger Tsien (Campbell et al., 2002). The mRFP coding sequence was amplified using primers with Acc65I (sense) and NotI (antisense) restriction sites, and was ligated into the Acc65I and NotI sites of pcDNA3.1/HisC-WRN plasmid. The resulting expression plasmid codes for 6His/Xpress epitopes, followed by a mRFP sequence joined to WRN with a short amino acid linker (WRPLDISK). The vector employs the CMV promoter to drive the expression of the recombinant protein.

The NotI site was also used, after removing the WRN cDNA sequence, to ligate the PCR-amplified, BLM and RECQ1 cDNA sequences, downstream and in frame with mRFP. The plasmids with BLM and RECQ1 cDNA sequences were obtained from Wang et al. (2001) and from Puranam and Blackshear (1994), respectively. All fusion constructs had identical tags including the short linker. Their open reading frames differed only in the sequence coding the helicase. The linker in the mRFP control vector was followed by the stop codon.

The untagged WRN cDNA sequence with the last 12 residues of the 5′ untranslated region of WRN mRNA was cloned into HinDIII and XhoI sites of pcDNA3.1(+) plasmid (Invitrogen, Carlsbad, CA). The cloning was performed with shuttle plasmids, because WRN cDNA contains two other HinDIII sites, making the direct cloning into HinDIII site impossible. The WRN cDNA was sequenced and no mutations were found.

The EGFP-BLM (in pEGFP-C1 plasmid from Clontech, Palo Alto, CA) was kindly provided by Lisa Spillare (Wang et al., 2001). The PCR amplified EGFP (enhanced green fluorescent protein) sequence (ProofStart polymerase, Qiagen, Hilden, Germany) was ligated into Acc65I–NotI sites of mRFP-WRN plasmid replacing mRFP sequence with EGFP.

The pCI-neo-PML plasmids coding for isoforms I, III, IV, V and VI of FLAG epitope-labeled PML were from Lawrence Banks (Guccione et al., 2004). The numbering of isoforms was based on the system presented by Jensen et al. (2001). The isoform I contained 882 amino acids, isoform III, 641; isoform IV, 633; isoform V, 611; and isoform VI, 560. The PML cDNA sequences were cloned into the pCI-neo expression vector from Promega (Madison, WI). The FLAG-coding sequence is attached to the first PML codon by the SalI sequence.