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Endogenous hSNM1B/Apollo interacts with TRF2 and stimulates ATM in response to ionizing radiation

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ABSTRACT

Human SNM1B/Apollo is involved in the cellular response to DNA-damage, however, its precise role is unknown. Recent reports have implicated hSNM1B in the protection of telomeres. We have found hSNM1B to interact with TRF2, a protein which functions in telomere protection and in an early response to ionizing radiation. Here we show that endogenous hSNM1B forms foci which colocalize at telomeres with TRF1 and TRF2. However, we observed that additional hSNM1B foci could be induced upon exposure to ionizing radiation (IR). In live-cell-imaging experiments, hSNM1B localized to photo-induced double-strand breaks (DSBs) within 10 s post-induction. Further supporting a role for hSNM1B in the early stages of the cellular response to DSBs, we observed that autophosphorylation of ATM, as well as the phosphorylation of ATM target proteins in response to IR, was attenuated in cells depleted of hSNM1B. These observations suggest an important role for hSNM1B in the response to IR damage, a role that may be, in part, upstream of the central player in maintenance of genome integrity, ATM.

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1. Introduction

All living organisms possess mechanisms which respond to DNA-damage and result in the repair of lesions or the elimination of irreparably damaged cells, thus maintaining genomic integrity. We have recently described *hSNM1B* as a new gene involved in this cellular response to DNA damage [1]. The hSNM1B protein belongs to the SNM1-family. The common features of the proteins in this group are two domains, a metallo-β-lactamase domain and a β-CASP region, which are characteristic of members of the β-lactamase superfamily of proteins which interact with nucleic acids [2]. The sequence similarity among the SNM1-family members is restricted to these two regions which are conserved from yeast to mammals.

ARTEMIS is the best investigated member of the SNM1-family with an established function in DNA overhang processing and opening of DNA hairpins generated during non-homologous end joining (NHEJ) and V(D)J recombination [3]. In some cases mutations in the *ARTEMIS* gene have been shown to be the underlying cause of severe combined immunodeficiency in association with radiosensitivity (RS-SCID) [4,5].

Based on its similarity to the *S. cerevisiae SNM1(PSO2)* gene, we originally identified the human *KIAA0086/hSNM1* gene as a potential human DNA-crosslink repair gene with an unusually long 5′UTR [6], a feature which was later shown to play a role in the regulation of hSNM1 translation [7]. Mouse embryonic stem (ES) cells in which *mSNM1* is disrupted display a twofold decrease in their survival upon exposure to Mitomycin C (MMC), but not to other DNA crosslinking agents or ionizing radiation (IR) [8]. However, treatment with either IR or MMC does result in an increased number of nuclear hSNM1 foci [9], suggesting that hSNM1 responds in some way to both DNA double strand breaks (DSBs) and interstrand cross links (ICLs). In addition, mammalian SNM1 has been implicated in an early mitotic stress checkpoint, in tumor suppression, and immunity [10,11].

In contrast to the DNA damage response roles identified for Artemis and hSNM1, several groups have recently suggested that hSNM1B functions primarily in telomere protection. Freibaum and Counter found transiently expressed EGFP-hSNM1B colocalized and Co-immunoprecipitated with TRF2 [12]. Another group identified this interaction by employing a combination of Co-immunoprecipitation and mass spectrometry [13]. Finally, using a fragment of TRF2 as a bait, Lenain et al. found hSNM1B as an interactor in a yeast two hybrid screen [14]. These studies showed that transiently expressed hSNM1B fused with GFP or a myc-tag localizes to telomeres. Following hSNM1B knockdown, the phenotype of TRF2 inhibited cells was exacerbated in terms of growth defects, telomere deprotection and increased fusions [14]. Activation of a DNA-damage signal at telomeres was observed as a consequence of hSNM1B knockdown [13]. Altogether these recent findings strongly suggest that hSNM1B cooperates with TRF2 to protect telomeres from being recognized as damaged DNA.

Our own prior studies of hSNM1B have suggested a more general role for the protein in the cellular response to both DNA double-strand breaks or interstrand crosslinks [1]. In the current study, we extend these findings. Using hSNM1B and TRF2 specific antibodies in Co-immunoprecipitation and indirect immunofluorescence (IF) experiments we confirm the interaction for the native proteins without transfection and expression of exogenous constructs. We further show that hSNM1B, like TRF2, accumulates rapidly following photo-induction of DSB at non-telomeric sites, suggesting the cooperation of these two proteins in the early cellular response to DSBs. Moreover, we show that depletion of hSNM1B by treatment with siRNA, attenuates the autophosphorylation of ATM on Serine 1981 resulting in decreased phosphorylation of its target proteins, SMC1, p53 and H2AX. These findings establish hSNM1B as an early DSB-response protein that stimulates ATM and contributes to the maintenance of genomic integrity.

2. Results

2.1. Subcellular localization of endogenous hSNM1B

Previous reports on the subcellular distribution of hSNM1B were based on experiments employing transiently overexpressed and tagged versions of hSNM1B [12,15]. To validate an hSNM1B-antiserum we have shown before to work specifically in immunoprecipitation experiments [1] for indirect immunofluorescence, we expressed Flag-tagged hSNM1B in GM00637 cells and double stained these cells with antibody against the Flag-tag and with the hSNM1B-antiserum. IF analysis with anti-Flag antibody revealed an almost exclusively nuclear localization of hSNM1B with a subset of the transfected cells displaying nuclear foci, a result which is in agreement with the above mentioned reports on hSNM1B localization. In addition, all foci stained with the anti-Flag also stained positive with anti-hSNM1B indicating that the hSNM1B-antiserum is able to recognize hSNM1B in this experimental setting (Fig. 1A). We then tested the ability of the anti-hSNM1B antiserum to recognize endogenous hSNM1B foci. The antibody detected bright nuclear foci in a considerable subset of cells of all three cell lines tested. The remaining cells showed a diffuse nuclear staining (Fig. 1B). Quantification revealed that ~60% of the GM00637 and HeLa nuclei and ~70% of the U2OS nuclei analyzed stained foci positive (Fig. 1B), however, foci positive HeLa cells appeared to have less foci per nucleus.

2.2. Interaction between TRF2 and hSNM1B

We used a full length *hSNM1B* cDNA as a bait in a yeast two hybrid (Y2H) screen and recovered a single cDNA clone encoding amino acids 40-252 of TRF2 from a HeLa cDNA library. TRF2 is a core component of shelterin, a protein complex involved in chromosome-end regulation and protection [16]. The TRF homology domain of TRF2 mediates homodimerization and interaction with other telomeric proteins and is comprised of amino acids 43-245 of the protein [17]. As shown in Fig. 2A, the cDNA identified in the Y2H screen represented almost exclusively the TRF homology domain (TRFH) amino-terminally fused to the vector encoded B42 domain.

To further explore the interaction between hSNM1B and TRF2 we performed Co-immunoprecipitation (Co-IP) exper-