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Short communication

YNK1, the yeast homolog of human metastasis suppressor NM23, is required for repair of UV radiation- and etoposide-induced DNA damage

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ABSTRACT

In humans, NM23-H1 is a metastasis suppressor whose expression is reduced in metastatic melanoma and breast carcinoma cells, and which possesses the ability to inhibit metastatic growth without significant impact on the transformed phenotype. NM23-H1 exhibits three enzymatic activities *in vitro*, each with potential to maintain genomic stability, a 3′–5′ exonuclease and two kinases, nucleoside diphosphate kinase (NDPK), and protein histidine kinase. Herein we have investigated the potential contributions of NM23 proteins to DNA repair in the yeast, *Saccharomyces cerevisiae*, which contains a single NM23 homolog, *YNK1*. Ablation of *YNK1* delayed repair of UV- and etoposide-induced nuclear DNA damage by 3–6 h. However, *YNK1* had no impact upon the kinetics of MMS-induced DNA repair. Furthermore, *YNK1* was not required for repair of mitochondrial DNA damage. To determine whether the nuclear DNA repair deficit manifested as an increase in mutation frequency, the *CAN1* forward assay was employed. An *YNK1* deletion was associated with increased mutation rates following treatment with either UV (2.6×) or MMS (1.6×). Mutation spectral analysis further revealed significantly increased rates of base substitution and frameshift mutations following UV treatment in the *ynk1*Δ strain. This study indicates a novel role for Y*NK1* in DNA repair in yeast, and suggests an anti-mutator function that may contribute to the metastasis suppressor function of NM23-H1 in humans.

1. Introduction

NM23-H1 was first identified by virtue of its reduced expression in highly metastatic melanoma and breast carcinoma cells, and the ability of forced NM23-H1 expression to inhibit metastatic potential without significant impact on the transformed phenotype [1]. The metastatic process requires the accumulation of mutations and high levels of genomic instability to permit tumor cells to overcome the barriers to metastatic growth [2–4]. Despite the fact that NM23-H1 has been recognized to play a pivotal role in the development of metastasis, the underlying mechanisms by which NM23-H1 exhibits its anti-metastastic effect remains unknown.

Consistent with a role in DNA repair the NM23 molecule possesses at least three distinct enzymatic activities that could participate in genomic maintenance and antimutator activity [5]. NM23-H1 possess significant 3′–5′ exonuclease (3′–5′ EXO) activity [6,7] and these DNA cleaving molecules are predominantly involved with maintaining genomic fidelity during DNA synthesis and repair [8]. Accordingly, deficiencies in 3′–5′ EXO activity have been shown to be associated with the mutator phenotype [8–11]. NM23-H1 also exhibits a nucleoside diphosphate kinase (NDPK) activity that maintains homeostasis of nuclear nucleotide pools which may limit pro-mutagenic mismatches during DNA repair [12,13]. Furthermore, a protein histidine kinase (hisK) activity has been described for NM23-H1, implicated as an inhibitor of signaling pathways underlying cell motility [14], but which could also initiate signaling to DNA repair pathways. Moreover, despite the repair-relevant enzymatic activities of NM23-H1 *in vitro*, its contribution to maintenance of genomic integrity *in vivo* is poorly understood.

Previous studies strongly suggest that NM23 proteins exhibit functions consistent with DNA repair. DNA damage has been reported to induce nuclear localization of NM23-H1 [7]. In addition, co-incubation of NM23-H1 with the base excision repair (BER) enzyme uracil-DNA glycosylase (UDG) results in enhanced 3′–5′ EXO activity against single-stranded oligodeoxynucleotide substrates *in vitro*, suggesting the potential for functional cooperativity between these proteins [7]. A function in genomic stability was also suggested earlier by the marked mutator phenotype of *ndk*-null *Escherichia coli*, which exhibit elevated rates of base substitutions and frameshifts [15]. While a recent study suggested that NDK possesses an intrinsic UDG function, subsequent studies show that *NDK* exhibits little if any intrinsic UDG activity [16–18], but does enhance that of the prototypical UDG, *UNG*, upon physical associa-

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tion between the proteins [17]. Consistent with this function, a very recent study has attributed the mutator phenotype of the *ndk*-null strain of *E. coli* to excess misincorporation of uracil, as well as a defect in the uracil base excision pathway [19].

To explore the potential function of NM23 proteins in maintenance of genomic integrity, we have employed the yeast *Saccharomyces cerevisiae*, which harbors a single NM23 homologue, *YNK1*. Despite of its phylogenetic distance from the eight human NM23 isoforms, ynk1p shares approximately 60% amino acid sequence identity and structural similarities with human NM23-H1 and NM23-H2 [20], including conservation of glutamic acid-5 and lysine-12 residues which are critical for the 3′–5′ EXO function [6], histidine-118, which is essential for NDPK activity, and proline-96, which has been implicated in the histidine kinase function [21]. Our results demonstrate that an *YNK1*-null strain exhibits significantly reduced kinetics of nuclear DNA repair in response to damage induced by UV irradiation, and etoposide, as well as increased rates of UV-induced mutations.

2. Materials and methods

2.1. S. cerevisiae strains and media

S. cerevisiae strains harboring single genetic lesions were obtained commercially (Open Biosystems) and were derived from BY4741 wild-type strains and listed in Table 1 (Supplemental Table 1). Open reading frames for the gene of interest were replaced with a *KanMX* marker by a PCR-based strategy. Yeast strains were grown in standard media consisting of yeast extract/peptone/dextrose (YPD) medium (Fisher Scientific).

2.2. MMS, etoposide and UV treatment

S. cerevisiae strains (1×10^7 cells/ml) were treated with MMS (0.1%; Sigma) or etoposide (1 mM; Sigma) for 1 h at 30 °C shaking at 250 rpm, followed by centrifugation at $5000 \times g$ for 5 min. The pellet was washed in 50 mM potassium phosphate, pH 7.0, aspirated, centrifuged at $5000 \times g$ for 5 min, and YPD added. The repair time course was 0.5, 1, 3 and 6 h for MMS and 0.5, 3, 6, 24 and 48 h for etoposide at 30 °C. For UVB exposure (Model XX-15M, UVP Products), *S. cerevisiae* were grown on YPD plates for 48 h at 30 °C, exposed to UV (192 J/m^2) and maintained at 30 °C and for a repair time course of 0.5, 1, 3 and 6 h.

2.3. Quantitative extended-length PCR (QXL-PCR)

QXL-PCR measures the average lesion frequency and works on the premise that damage on the DNA template will block a thermostable polymerase, resulting in reduced amplification of the DNA fragment. Thus, only DNA templates devoid of polymerase blocking lesions will be amplified. DNA lesion frequencies were calculated as the amplification of damaged (treated) samples (A_d) relative to the amplification of non-damaged fragment controls (A_0) resulting in the ratio (A_d/A_0). To determine average lesion frequency, a random distribution of lesions was assumed, and the following equation was used, $\lambda = -\ln A_d/A_0$ [22,23]. The DNA lesion frequencies were used to determine percentage repair of the initial DNA damage caused by the DNA damaging agent. The PCR conditions and primer sequences used are shown in supplemental information.

2.4. CAN1 forward mutation assay and sequence analysis

The standard CAN1 forward mutation assay was performed and analyzed as previously described [24,25]. Independent *CAN1* colonies were isolated and the *CAN1* gene sequenced at University of Kentucky Genetic Technologies Center.

2.5. Statistical analyses

A two-tailed *t*-test was used for comparison between two treatments and for comparison between three or more experimental groups, one-way ANOVA with the Bonferroni *post hoc* test was used. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. A ynk1Δ strain exhibits attenuated repair of etoposide- and UV radiation-induced nuclear DNA damage

To determine whether *YNK1* has a functional role in DNA repair, we compared the repair rates of *ynk1*Δ vs. wild-type cells following MMS (0.1%), etoposide (1 mM) and UV (192 J/m^2) treatment (Fig. 1). Repair of MMS-induced nDNA damage did not significantly differ throughout the repair period between *ynk1*Δ and wild-type strains. In contrast, *ynk1*Δ mutants demonstrated a significantly reduced capacity to repair etoposide- and UV-induced nuclear DNA damage compared to wild-type up to 6 h and 3 h post-treatment, respectively ($p < 0.05$).

3.2. YNK1 does not have a role in the repair of MMS-, etoposide-, or UV-induced mitochondrial DNA damage

The mitochondrial genome is frequently challenged by DNA damaging agents, and mitochondrial genomic instability is associated with impaired nucleotide metabolism and development of the mutator phenotype [26,27]. Intriguingly, a fraction of the total cellular ynk1p has been localized to the mitochondrion [28]. However, its function within this compartment is not fully understood. Therefore, we aimed to examine whether *YNK1* has a functional role in mitochondrial DNA repair following DNA damage caused by MMS (0.1%), etoposide (1 mM) and UV (192 J/m^2) (Supplemental Fig. 1). The repair of MMS-, etoposide- and UV-induced mtDNA damage did not significantly differ between the *ynk1*Δ and wild-type strains, throughout the respective repair periods. Furthermore, only ~25% of the initial lesions were repaired at 6 h post-treatment for all DNA damaging agents.

3.3. The ynk1Δ strain exhibits a mutator phenotype following MMS and UV exposure

The *ynk1*Δ strain displayed a significantly slower repair of DNA damage induced by UV irradiation. To determine whether this impairment was manifested as an increase in mutation frequency, the *CAN1* forward mutation assay was employed under spontaneous conditions (no treatment), and following exposure to MMS and UV irradiation (Fig. 2). Under spontaneous conditions, no significant difference in mutation rate between *ynk1*Δ and wild-type strains occurred. In contrast, treatment with UV (192 J/m^2) and MMS (0.1%) generated 2.6-fold and 1.6-fold increases in the mutation rate of *ynk1*Δ compared to wild-type, respectively ($p < 0.05$).

Table 1
The percentage of specific mutation types in wild-type and *ynk1*Δ strains.

	−UV exposure				+UV exposure			
	Base substitution	Frameshift	Complex	No mutation	Base substitution	Frameshift	Complex	No mutation
Wild-type	59	0	26	15	53	0	21	26
*ynk1*Δ	70	0	12	18	70	15	10	5

Data is expressed as the percentage of mutation type (e.g. base substitution, frameshift, complex or no mutation) of the total number of mutational events in wild-type and *ynk1*Δ strains.