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DNA glycosylase activities for thymine residues oxidized in the methyl group are functions of the hNEIL1 and hNTH1 enzymes in human cells

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Abstract

Bacteria and eukaryotes possess redundant activities that recognize and remove oxidatively damaged bases from DNA through base excision repair. DNA glycosylases excise damaged bases to initiate the base excision repair pathway. hOgg1 and hNTH1, homologues of *E. coli* MutM and Nth, respectively, had been identified and characterized in human cells. Recent works revealed that human cells have three orthologues of *E. coli* Nei, hNEIL1, hNEIL2 and hNEIL3. In the present experiments, hNEIL1 protected the *E. coli nth nei* mutant from lethal effect of hydrogen peroxide and high frequency of spontaneous mutations under aerobic conditions. Furthermore, hNEIL1 efficiently cleaved double stranded oligonucleotides containing 5-formyluracil (5-foU) and 5-hydroxymethyluracil (5-hmU) in vitro via β- and δ-elimination reactions. Similar activities were detected with hNTH1. These results indicate that hNEIL1 and hNTH1 are DNA glycosylases that excise 5-foU and 5-hmU as efficiently as Tg in human cells.

Keywords: Oxidative DNA damage; 5-Formyluracil; 5-Hydroxymethyluracil; DNA glycosylase; hNEIL1; hNTH1

1. Introduction

Reactive oxygen species (ROS), including superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^\bullet$OH), is the most important cytotoxic and mutagenic agents, because they are continuously generated in living cells during normal cellular metabolism [1,2]. ROS are also generated by exogenous stimuli such as ionizing radiation. ROS cause a wide variety of oxidative modifications to purines and pyrimidines in DNA [3–7]. Unrepaired oxidative DNA damage may be involved in carcinogenesis, aging and many degenerative pathologies in humans [7–9]. Bacteria and eukaryotes have evolved base excision repair mechanisms for oxidative base damage in DNA [3,5,10–14]. *E. coli* has three types of DNA glycosylase, MutM (formamidopyrimidine DNA glycosylase), Nth (endonuclease III) and Nei (endonuclease VIII), that recognize and remove oxidatively damaged bases from DNA [5,10–13].

Human hOgg1 and hNTH1 enzymes, homologues of *E. coli* MutM and Nth, respectively, had been identified in human cells [15,16]. hOgg1 and hNTH1 show overlapping substrate specificities with *E. coli* MutM and Nth, respectively [12–16]. Recently, Hazra et al. [17], Bandaru et al. [18] and Takao et al. [19] found three orthologues of the *E. coli nei* gene, *hNEIL1*, *hNEIL2* and *hNEIL3*, in the human genome database. It is important to clarify the critical roles of human Nei orthologues in the protection against oxidative stress in vivo. In this study, we first examined whether or not hNEIL1, when expressed in the *E. coli nth nei* mutant, could protect

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the mutant cells from oxidative stress mediated cell death and mutations. This paper shows that hNEIL1 reduces the frequency of spontaneous mutations and prevents the killing by H_2O_2.

hNEIL1 is a bifunctional glycosylase/AP lyase that catalyzes β- and δ-elimination reactions at the site of the damaged base [17–20]. hNEIL1 recognizes similar substrates as *E. coli* Nei [21,22], which excises guanine- and adenine-formamidopyrimidines, oxidized pyrimidines such as dihydrouracil, thymine glycol (Tg), 5-hydroxycytosine and 5-hydroxyuracil from DNA [17–19]. It shares a potential helix-2-turn-helix motif and catalytic residues at the N-terminus with *E. coli* MutM and Nei [17–19].

In this study, the abilities of hNEIL1 to cleave double-stranded oligonucleotides containing Tg, 5-formyluracil (5-foU) and 5-hydroxymethyluracil (5-hmU) were assayed. These lesions are the major products of oxidative damage to thymine in DNA [3–6,10–14]. Tg prevents DNA synthesis and is primarily recognized and removed from DNA by *E. coli* Nth and Nei and human hNTH1 and hNEIL1 [5,10–14,17–19]. 5-foU is potentially mutagenic in *E. coli* and mammalian cells [23–27]. Therefore, much attention should be paid to the cellular repair functions for 5-foU in DNA. Recent works revealed that 5-foU is removed by AlkA [28], MutM, Nth and Nei in *E. coli* [23,25], Ntg1 and Ntg2 in *S. cerevisiae* [29]. Previously we found that cell-free extracts from HeLa cells contained two enzymatic activities that recognize 5-foU in DNA [26]. Furthermore, hNTH1 has been characterized to remove 5-foU from DNA [30]. Another 5-foU DNA glycosylase should be present in human cells. In this report, we show that hNEIL1 has a DNA glycosylase/AP lyase activity for 5-foU and 5-hmU in DNA as efficiently as hNTH1.

2. Materials and methods

2.1. Enzymes and chemicals

Tetracycline hydrochloride, kanamycin, chloramphenicol and phenylmethylsulfonyl fluoride (PMSF) were purchased from Wako Pure Chemicals (Osaka, Japan). Ampicillin was obtained from Meiji Seika (Tokyo, Japan). T4 polynucleotide kinase and isopropyl-1-thiol-β-D-galactopyranoside (IPTG) were obtained from New England Biolabs (Beverly, MA) and Toyobo (Osaka, Japan), respectively. Prepacked columns for fast protein liquid chromatography (FPLC), HiTrap SP Cation Exchange, HiTrap Chelating and Resource S were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). [γ-^{32}P]ATP (>148 TBq/mmol) was obtained from ICN Biomedicals (Costa Mesa, CA).

2.2. Bacterial strains

E. coli KSR4 and KSR7 were *nth*::Cm *nei*::Kan and *nth*::Cm *nei*::Kan *mutM*::Tet derivatives of SY5, respectively [23,31]. BL21-CodonPlus was obtained from Stratagene (La Jolla, CA). LB broth was used to culture *E. coli* cells and, when necessary, ampicillin (100 μg/ml), kanamycin (50 μg/ml), tetracycline (25 μg/ml) or chloramphenicol (30 μg/ml) was added to the medium.

2.3. Cloning of hNEIL1 cDNA

Primers for PCR were designed based on the cDNA sequences in GenBankTM (AK026055 for FLJ22402/hNEIL1). The cDNA for hNEIL1 was amplified from a commercial human testis cDNA library (Invitrogen, Carlsbad, CA) with oligo (dT) primers. The cDNA for FJK31644 was amplified from the cDNA pool reverse-transcribed from mRNA from HeLa cells. The amplified fragment was sub-cloned into *E. coli* expression vector (pFNd) under *taq* promotor derived from pFLAG2 (Sigma, St. Louis). The plasmid containing hNEIL1 cDNA was named phNEIL1.

2.4. Assay for spontaneous mutations

A single colony was inoculated into LB medium and cultured at 37 °C for 18 h with aeration. The mutation to rifampicin resistance was assayed as follows: 0.1 ml aliquot of overnight culture was plated on LB plates containing 100 μg/ml of rifampicin and then incubated at 37 °C for about 30 h. Mutant colonies on the plates were counted. The cell suspensions were also appropriately diluted and plated on LB agar. After incubation for 24 h, the number of viable colonies was counted to estimate the frequency of spontaneous mutation.

2.5. Assay for the killing effect of H_2O_2

Overnight cultures of *E. coli* SY5, KSR4 (*nth nei*)/vector (pFNd), KSR4/phNEIL1 and KSR4/pNTH1 [30] were appropriately diluted and plated (0.1 ml) on LB agar containing H_2O_2 (0–1.5 mM) and appropriate antibiotics. After incubation at 37 °C for about 18 h, the number of colonies was counted to estimate survival.

2.6. Synthesis of 5-foU-, 5-hmU- and Tg-containing oligonucleotides

Oligonucleotides containing 5-foU or 5-hmU at defined sites were synthesized as described previously [32,33]. The oligonucleotides were further purified using HPLC, as previously described [32]. Tg-containing oligonucleotide was prepared according to Dianov et al. [34]. In brief, an oligonucleotide with a single thymine residue (5′-GGACGACATAAGGAACC-3′) was oxidized with 50 mM of osmium tetroxide in the presence of 2% pyridine at room temperature for 15 min. The oligonucleotide containing Tg was purified by gel filtration on Sephadex G-25. Other oligonucleotides were synthesized and purified by Takara