Repair roles of hSMUG1 assessed by damage specificity and cellular activity

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ABSTRACT
Single-strand-selective monofunctional uracil-DNA glycosylase (SMUG1) was previously identified as a putative backup enzyme of major mammalian uracil-DNA glycosylase (UDG). However, the subsequent studies have shown conflicting results about the substrate specificity of SMUG1. In the present study, to clarify the repair role of SMUG1, we determined the damage specificity of purified human SMUG1 (hSMUG1) and its contribution to repair of oxidized bases in HeLa cell extracts.

INTRODUCTION
The hydrolytic deamination of cytosine is a major pathway that generates uracil in DNA and results in GC→AT transition mutations. To prevent such mutations, uracil-DNA glycosylase (UDG) efficiently removes uracil from DNA. In fact, Escherichia coli ung mutants deficient in UDG exhibit several fold increases in GC→AT transition mutations. In contrast, UDG knockout mice show only marginal increases in mutation frequencies and no overt phenotypes. These results suggest another activity for removal of uracil in mammalian cells. SMUG1 that was originally found as a single-strand-selective monofunctional uracil-DNA glycosylase is a likely backup enzyme for UDG among the three candidate glycosylases (SMUG1, TDG and MBD4) for uracil.

It has been originally reported that SMUG1 prefers uracil in single-stranded DNA (ssDNA) to that in double-stranded DNA (dsDNA). However, the subsequent study has shown that SMUG1 can efficiently process uracil in dsDNA. In addition, one report shows that the repair enzyme for 5-hydroxymethyluracil (hmU), a methyl oxidation product of thymine, and SMUG1 are the same enzyme, whereas another one shows that repair activity for hmU in calf thymus is distinct from known DNA glycosylases such as UDG, TDG, and SMUG1.

In view of the importance of SMUG1 as a backup enzyme of UDG in mammalian cells and the apparently conflicting results on the activity for hmU, we examined the repair activities of human SMUG1 (hSMUG1) and HeLa cell extracts for uracil and oxidative damage.

RESULTS AND DISCUSSION
hSMUG1 was over-expressed from the cloned cDNA in Escherichia coli, and the substrate specificity of the purified hSMUG1 protein was determined using ssDNA and dsDNA substrates containing various base lesions. The substrates were incubated with hSMUG1 at different salt concentrations, and the resulting abasic sites were cleaved by
NaOH treatment. Products were analyzed by denaturing PAGE. hSMUG1 excised not only uracil (U) but also 5-hydroxyuracil (hoU), hmU and 5-formyluracil (fU) in ssDNA and dsDNA (Fig. 1). Conversely, hSMUG1 did not excise analogous cytosine derivatives (5-hydroxycytosine and 5-formylcytosine) and other oxidative lesions (thymine glycol and 7,8-dihydro-8-oxoguanine). Thus, the lesions recognized are all uracil derivatives bearing an oxidized group at the C5 position.\(^7\)\(^8\) The optimal salt concentration of hSMUG1 varied significantly depending on the strandedness of the substrate (ssDNA vs. dsDNA) and the base opposite the lesion (A or G). The repair activity in HeLa cell extracts was assessed in the presence of a UDG-inhibitor (UGI) to eliminate the UDG activity. The HeLa cell extract and hSMUG1 exhibited a similar damage preference (U:G, hoU:G > hmU:A, fU:A).\(^7\)\(^8\) Furthermore, the activities of the HeLa cell extract and hSMUG1 varied in parallel depending on the salt concentration. Consistent with the damage specificity above, hSMUG1 removed damaged bases from Fenton-oxidized calf thymus DNA, generating abasic sites (Fig. 2).

The present data indicate a dual role of hSMUG1 in base excision repair of damaged bases: a backup enzyme for UDG and a primary repair enzyme for a subset of oxidized pyrimidines such as fU, hmU, and hoU, that are poorly recognized by other DNA glycosylases.

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REFERENCES

Fig. 1 Base lesions recognized by hSMUG1.

Fig. 2 Abasic sites generated by DNA glycosylases in Fenton-oxidized DNA.