News Release

Title

Novel mechanisms for the removal of strong replication-blocking lesions generated by crosslinking reaction between the apurinic/apyrimidinic site and the human HMCES protein

Key Points

• Apurinic/apyrimidinic (AP) site is one of the most frequently generated endogenous DNA damages, which causes cell death and mutagenesis.

• The HMCES protein covalently binds to the AP site and forms the secondary DNA damage, DNA-HMCES protein cross-link, to protect cells from the toxicity of the AP sites, however, the repair mechanism of the cross-link remains to be elucidated.

• This study determines the repair processes of the DNA-HMCES cross-link damage.

Summary

A graduate student of Nagoya University Graduate School of Medicine Yohei Sugimoto, an associate professor Yuji Masuda, and an assistant professor Rie Kanao, members of the research group led by Prof. Chikahide Masutani in Research Institute of Environmental Medicine, Nagoya University and Nagoya University Graduate School of Medicine, and Prof. Shigenori Iwai in Graduate School of Engineering Science, Osaka University and a project researcher Yumi Miyake in Forefront Research Center, Graduate School of Science, Osaka University found novel repair pathways of apurinic/apyrimidinic (AP) sites of DNA.

Endogenous DNA damages induced by intracellular metabolites cause aging and carcinogenesis. AP site is one of the most frequently generated endogenous DNA damages. Although AP sites on double-stranded DNA are repaired by base excision repair, human tissues accumulate 50,000–200,000 AP sites per single cell. The AP site is a damage in which genetic information is lost and is susceptible to DNA strand-breakage through a chemically unstable structure. During DNA replication, the AP site exposed on the single strand of the template DNA not only impedes the progress of DNA polymerases due to the loss of genetic information but also causes serious DNA double-strand break

due to AP site breakage, which would induce cell death.

Recently, it has been discovered that the HMCES protein prevents DNA cleavage by forming the DNA-protein cross-link with the AP site and demonstrated that the DNA-HMCES cross-link protects cells from the toxicity of the AP sites. However, the mechanism by which the DNA-HMCES cross-link as the secondary DNA damage is repaired remains to be elucidated. In this study, the research team determined the repair mechanisms of the DNA-HMCES cross-link damage. This work was published online in Nucleic Acids Research on April 6, 2023.

Background

Endogenous DNA damages caused by intracellular metabolites cause aging and carcinogenesis. Apurinic/apyrimidinic (AP) sites are one of the most frequent DNA damages generated by not only exogenous sources such as ionizing radiation, ultraviolet rays, and various chemical substances but also chemical reactions mediated by endogenous metabolites and oxidative stress, inducing cell death and mutations. In addition, side reactions by an antiviral protein APOBEC, somatic hypermutation in immune cells, and reprogramming of fertilized eggs also cause the generation of AP sites enzymatically. Thus, the repair mechanisms of AP sites are a very important biological function. AP sites on double-stranded DNA are repaired by a series of enzymatic reactions called base excision repair (Fig. 1A). However, 50,000–200,000 AP sites per single human tissue cell are accumulated. AP sites are characterized by loss of genetic information and susceptibility to breakage of DNA strands via a chemically unstable structure. During DNA replication, the AP site exposed on the single strand of the template DNA not only impedes the progression of DNA polymerases due to lack of genetic information but also leads to DNA strand breakage, making it more serious DNA double-strand break, which induces cell death (Fig. 2).

Recently, it has been discovered that the HMCES protein covalently cross-links specifically to the AP site and forms the HMCES-DNA cross-link conjugated with the chemically stable thiazolidine structure (Fig. 1B, Fig. 2), and demonstrated that cells lacking the HMCES protein become sensitive to AP site toxicity, increasing DNA double-strand breaks. However, the effect of DNA-HMCES crosslink as the secondary DNA damage on DNA replication and the mechanism by which the DNA-HMCES crosslink is repaired to the undamaged DNA remain to be elucidated.

Results

To investigate the effects of DNA-HMCES cross-link on DNA replication, the research team first purified human HMCES protein produced in *Escherichia coli* and reacted with synthetic DNA containing an AP site. Consequently, the research team has developed a method for producing a single-stranded DNA containing a DNA-HMCES cross-link *in vitro*. Next, the research team analyzed the DNA replication reaction using the single-stranded HMCES-crosslinked DNA as a template *in vitro* and found that DNA-HMCES crosslink impedes the progression of DNA polymerases more strongly than the AP site itself. The research team suggested that the DNA-HMCES cross-link may be replicated by a mechanism of the homology-mediated reaction using the sister chromosome as a template (Fig. 2).

Next, the research team measured the stability of double-stranded DNA-HMCES crosslink damage, which would be generated by the homology-mediated reaction. The research team found that HMCES dissociated from the double-stranded DNA with a half-life of 3.5 hours, whereas the single-stranded HMCES crosslink was very stable (Fig. 2). This result suggests that when the single-strand region containing DNA-HMCES crosslink is converted to the double-stranded DNA by the homology-mediated reaction, the AP site is restored by the de-cross-linking reaction. The research team suggests that the resultant AP site is repaired by base excision repair (Fig. 2).

A previous study suggested that the HMCES portion of the DNA-HMCES cross-link is digested by the proteasome, exposing another type of DNA damage, DNA-thiazolidine damage (Fig. 1B). The effect of DNA-thiazolidine damage on DNA replication and the mechanism by which the DNA-thiazolidine damage is repaired to intact DNA remained unclear. Therefore, the research team established a method to generate a single-stranded DNA exposing DNA-thiazolidinedione damage by treating single-stranded HMCES-crosslinked DNA with a protease to remove most of the HMCES portion. Next, the research team analyzed the effect of DNA-thiazolidine damage on DNA replication by *in vitro* DNA replication reaction and found that DNA-thiazolidine damage inhibits DNA replication as well as the AP site (Fig. 2). The research team also found that single-stranded DNA-thiazolidine damage and double-stranded DNA-thiazolidine damage are relatively stable, reverting to the AP site with half-lives of 20-24 and 12 hours, respectively (Fig. 2). These results suggested that the double-stranded DNA-thiazolidine damage generated after replication may be repaired by cellular functions.

To determine the repair enzyme of the double-stranded DNA-thiazolidine damage, the research team purified various types of human

DNA repair enzymes produced using *E. coli* and analyzed the repair reaction of the double-stranded DNA-thiazolidine damage. Consequently, the research team identified the APE1 endonuclease, which functions in base excision repair, as the repair enzyme of the double-stranded DNA-thiazolidine damage. Based on these results, the research team believes that the repair mechanisms of DNA-HMCES crosslink damage caused by AP sites are determined (Fig. 2).

Perspective

Base excision repair, which repairs AP sites, is an essential mechanism for cell survival, and its dysfunction causes genome instability disorders, including various cranial nerve diseases. The findings in this study will lead not only to molecular mechanisms on the repair of the AP sites but also to the investigation of the causes of unexplained intractable genomic instability diseases.

Figure 1

A, Generation of AP sites and base excision repair. B, The DNA-HMCES cross-link conjugated with the thiazolidine structure.

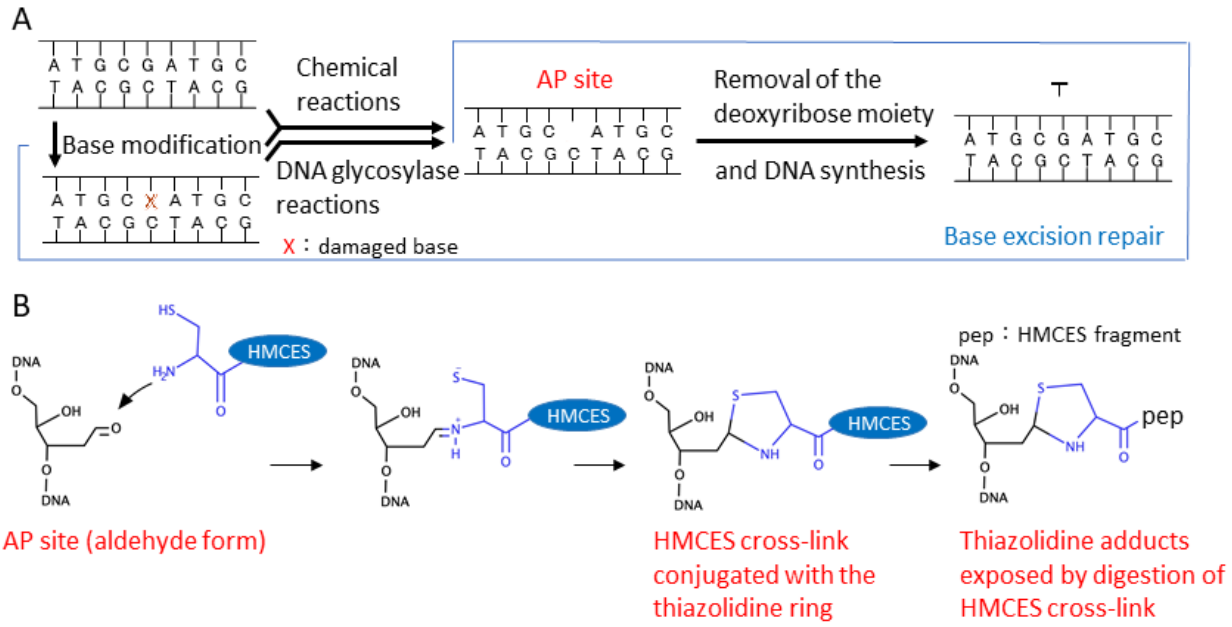
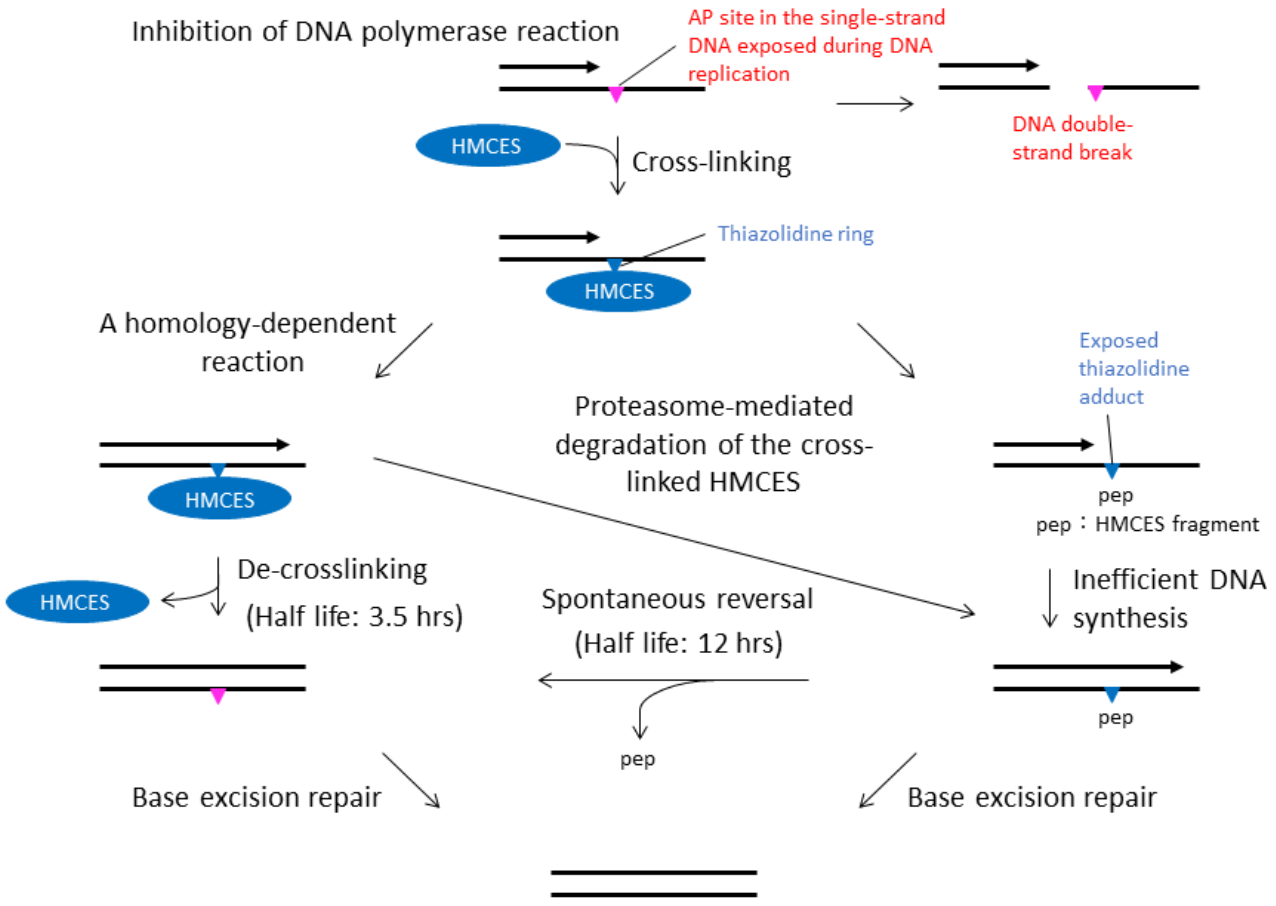


Figure 2
Hypothetical repair pathways of the DNA-HMCES cross-link damage.



Publication
Novel mechanisms for the removal of strong replication-blocking HMCES- and thiazolidine-DNA adducts in humans

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