Mutation Specificity with Incorporation of Oxidized dNTPs by DNA Polymerase η

Hidaka Katsuhiko

Key Words: human DNA polymerase η, oxidative damage, Mutation

1. Introduction

With the development of chemical industry since the beginning of the 20th century, there have been many types and large amounts of chemicals released into the environment. Of these chemicals, there are carcinogens and mutagens, which threaten our health. According to carcinogenesis and mutagenensis studies, most of the carcinogens and mutangens induce DNA damage and mutation by generating reactive oxygen species (ROS) within living cells. Several lines of evidence indicate that the oxidation of DNA precursors (dNTPs) produced by ROS is one of the important sources in the mutation process. Indeed, incorporation of oxidized dNTPs into DNA is considered to induce mutation when incorporated into DNA by DNA polymerases. Y-family DNA polymerases are probably involved in the mutation process because their error rates during DNA synthesis are extremely high and they may incorrectly incorporate dNTPs into a DNA strand.

2. Methods

With the *in vitro* gap-filling assay, I analyzed the mutation specificity with incorporation of 2-OH-dATP and 8-OH-dGTP into DNA by human DNA polymerase η (pol η). These two kinds of oxidized dNTPs were chosen because they may be the most important sources.

3. Results and Discussion

When 200 μM normal dNTPs and an equal amount of 2-OH-dATP were present during DNA synthesis by pol η, the G:C→T:A transition frequency was 8.2-fold higher than that of the control. This result indicates that pol η tends to misincorporate 2-OH-dATP opposite G during DNA synthesis. In mismatch repair (MMR)-defective mouse and human cells, overexpression of human MTH1 which sanitizes 2-OH-dATP and 8-OH-dGTP markedly reduced the G:C→T:A transitions[1)]. In addition, there is no report that other human DNA polymerases misincorporate 2-OH-dATP opposite G during DNA synthesis. Therefore, it is possible that misincorporatoin of 2-OH-dATP by pol η contributes to the induction of G:C→T:A transitions in mice or human cells.

Adding 8-OH-dGTP at equimolar concentrations with the normal dNTPs (200 μM) during the DNA synthesis by pol η, A:T→C:G transversion increased 17-fold. Evaluation in respect to the increasing number, A:T→C:G transversion yielded 0.82 per adenine. This number is much higher than that of the other DNA polymerases. These results indicate that pol η probably has the most tendencies for misincorporation of 8-OH-dGTP opposite A during DNA synthesis. Furthermore, it is notable that increasing frameshifts such as deletion and addition during DNA synthesis with pol η, have not been reported in the case of other DNA polymerases. According to two other reports, it was indicated that frameshifts increased in MTH1-defective mouse[2)], and overexpression of human reduced frameshifts in MMR-defective mouse or human cells. These results suggest that pol η possibly contributes to induce framshifts in mice or human cells.

In the presence of low concentrations 8-OH-dGTP (1/ 1000 vs. normal dNTPs) during DNA synthesis by pol η, the mutation frequency was increased ($p<0.05$). This has biological meanings because the rise in the mutation frequency with closer real concentration of 8-OH-dGTP in real cells was confirmed.

1) M. T. Russo et al./ Mol. Cell. Biol., 24 (2004) 465-474

2) A. Egashira et al./ DNA repair 1 (2002) 881-893