35 Contribution of mutant p53 protein to hyper recombination leading to enhancement of spontaneous mutation.

Sachiko GOTO[1], Masako IZUMI[1], Masamitu HONMA[3], Keiji SUZUKI[2], Msami WATANABE[2], Fumio YATAGAI[1], [1]RIKEN RI-tech [2]Radiation life science pharm. Nagasaki-univ. [3]Genetic and mutagenesis NIHS

Function of p53 contributes to preservation of accuracy of genomic information. Human lymphoblastoid cell, WTK1, carrying mutant p53 gene, exhibits constitutive overexpression of mutant p53. Moreover, spontaneous mutation-frequency in WTK1 is about 100 times higher than that in p53 wild-type TK6. LOH analysis has suggested the possibility that such high frequency of spontaneous-mutation is due to DNA recombination activity. So, WTK1 mutant p53 is suspected not to bind Rad51 for regulation of recombination process. Therefore, both Protein-protein association and Rad51 filament formation were examined by immunoprecipitation and immunofluoresence after 3Gy X-ray irradiation. However, WTK1 did not show any different characters compared to TK6. Rad51 filament was still formed at high level in p53 knockout NH32 even 12 hrs after X-ray exposure. These results suggested that p53 protein might be involved in regulation of recombination process. At present NH32 cell is transformed with wild type or mutant type p53 gene as an inducible form to examine the relation between p53 protein and recombination.

36 Identification of 5-Formyluracil DNA Glycosylase/AP Lyase Activity of *S. cerevisiae* Ntg1 and Ntg2 Proteins

Qiu-Mei ZHANG[1], Hiroshi SUGIYAMA[2], Shuji YONEI[1], [1]Radiat. Biol. Grad. Sch. Sci. Kyoto Univ. [2]Inst. Biomat. Bioeng. Tokyo Med. Dent. Univ.

Reactive oxygen species cause a wide variety of oxidative modifications of the base and sugar moieties in DNA. Thymine glycols, 7,8-dihydro-8-oxoguanine and 5-hydroxypyrimidines are formed in DNA upon exposure to reactive oxygen species and ionizing radiation. 5-Formyluracil is an oxidatively modified thymine in DNA, which is formed in a yield comparable with that of thymine glycols and 8-hydroxyguanine by ionizing radiation. Recently we found that *E. coli* Nth and human hNTH1 (human Nth homolog) proteins possess the activity that releases 5-formyluracil from DNA as free base. In this study, we found that the Ntg1 and Ntg2 proteins, *S. cerevisiae* endonuclease III homologue, form a Schiff base intermediate with oligonucleotides containing 5-formyluracil and cleave the duplex oligonucleotides at the 5-formyluracil site.

37 Identification of 5-Hydroxymethyluracil-DNA Glycosylase Activities in Extracts from *Escherichia coli*

Masaki HORI[1], Qiu-Mei ZHANG[1], Hiroshi SUGIYAMA[2], Shuji YONEI[1], [1]Radiat. Biol. Grad. Sch. Sci. Kyoto Univ. [2]Inst.Biomat. Bioeng. Tokyo Med. Dent. Univ.

5-hydroxymethyluracil (5-hmU) in DNA is produced via oxidation of the methyl group of thymine and deamination of 5-methylcytosine. In higher eukaryotes, 5-hmU-DNA glycosylase activities had already been characterized. On the other hand, such enzymatic activities have not yet been detected in prokaryote and yeast cells. Recently we found that *E. coli* MutM, Nei, and Nth proteins had a 5-formyluracil-DNA glycosylase/AP lyase activity. 5-hmU is also a derivative of thymine as 5-formyluracil. Hence, in this study, we examined whether or not MutM, Nth and Nei proteins recognize 5-hmU in DNA. 5-hmU-containing duplex oligonucleotides were trapped to form DNA-protein complex when reacted with the purified enzymes in the presence of $NaBH_4$. Furthermore, these three enzymes cleaved the oligonucleotides at the site of 5-hmU. These results suggested that *E.coli* MutM, Nei, and Nth proteins have a 5-hmU-DNA glycosylase/AP lyase activity.