90 Role of DNA-dependent protein kinase in initial recognition of DNA double-strand breaks

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DNA-dependent protein kinase (DNA-PK) is composed of Ku70, Ku86 and catalytic subunit (DNA-PKcs) and acts as a sensor of DNA double-strand breaks (DSBs) during non-homologous end-joining (NHEJ). Here we demonstrated that DNA-PKcs formed nuclear foci rapidly after exposure to ionizing radiation. DNA-PKcs foci were observed just after 5 Gy of X-irradiation. Wortmannin inhibited XRCC4 phosphorylation but not DNA-PKcs foci formation. On the other hand, both DNA-PKcs foci formation and XRCC4 phosphorylation were reduced by Ku86 siRNA. These results suggest that DNA-PKcs foci formation requires Ku proteins and precedes its activation. DNA-PKcs did not response to DNA replication arrest, while NBS1 and histone H2AX, which are essential for homologous recombination (HR), were phosphorylated and formed foci. Also, X-ray-induced DNA-PKcs foci did not colocalize with phosphorylated H2AX. These results further suggest that DNA-PK selectively recognize DSBs repaired by NHEJ independent of HR-related proteins.

91 Effect of NP95 Deficiency on Caffeine Action in Mouse Embryonic Stem Cells

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Radiosensitization together with abrogated cell cycle checkpoints are most pronounced actions of caffeine among its many diversified activities. Caffeine has recently been considered to overcome the G2/M and S checkpoint responses through its inhibition of phosphorylation of ATM/ATR kinase substrates including Chlk1, Chk2 and P53. We have found that homozygouly *Np95*-inactivated embryonic stem (ES) cells are more sensitive to X-rays, UV-light, MNNG, and HU than ES wild type cells, mimicking the phenotype of ATRkd human fibroblasts or Chk1–/– cells. To explore the relationship between ATR and NP95 we examined the effect of *Np95* status on the caffeine action in ES cells. Two mM caffeine in the post irradiation medium enhanced the cytotoxicity of UVC in *Np95* +/+ cells, but not in *Np95* –/– cells, while synergism was more pronounced in *Np95* –/– cells than *Np95* +/+ cells following the treatment with X-rays.

92 Phenotypic Assay of *C. elegans* Deficient in Uracil DNA Glycosylase Activity

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Base excision repair (BER) is one of the pathways to avoid mutations in living cells. Damaged bases are excised by DNA glycosylases and then AP lyases nick DNA, followed by repair synthesis and rejoining by DNA polymerases and DNA ligases. Increased frequency of mutations in BER-defective mutants in *E. coli* and *S. cerevisiae* indicates that BER plays an important role in the cells. However, the effects of BER deficiency in multicellular organisms are yet unknown. In human, defect of nucleotide excision repair results in serious hereditary diseases, while no desease has been found to relate to BER deficiency. So we investigated the influence of BER deficiency in multicellular organsim using *C. elegans*. Recently, we identified the uracil DNA glycosylase homolog gene (*Ceung*) in *C. elegans*, and clarified the glysocylase activity of purified recombinant CeUNG. In this study, we examined the phenotypic properties of *C. elegans* defective in UNG activity using RNAi.

93 Is Interaction between p53 and p53-Binding Protein 1 (53BP1) Necessary for the Repair of DNA Double-Strand Breaks?

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After X-irradiation of cells, p53-binding protein 1 (53BP1) binds to chromatin at sites of DNA double-strand breaks in a phosphorylation-dependent manner. The Tudor plus Myb domain, the minimal region of 53BP1 for chromatin binding, binds directly to both double-stranded and single-stranded DNA, and stimulate end-joining by DNA ligase IV/Xrcc4, but not by T4 DNA ligase in vitro, suggesting 53BP1's role in the repair of DNA double-strand breaks. In the last meeting, we showed that a colon cancer cell line SW48 is deficient in 53BP1 expression, and that expression of 53BP1 in SW48 cells significantly reduce the number of cells containing X-ray-induced chromosomal aberrations. To determine whether 53BP1-p53 interaction is necessary for stimulation of repair by 53BP1 for X-ray-induced chromosomal aberrations, we established a SW48 cell line that expressed mutant 53BP1 that did not bind to p53. The importance of interaction between 53BP1 and p53 in the repair of DNA double-strand breaks will be discussed.