**FULL PAPER** *Pharmacology*

# Impact of Amino Acid Substitutions in Two Functional Domains of Ku80: DNA-Damage-Sensing Ability of Ku80 and Survival after Irradiation

Manabu KOIKE[1)]*, Yasutomo YUTOKU[1, 2)] and Aki KOIKE[1)]

[1)]*DNA Repair Gene Res., National Institute of Radiological Sciences, 4–9–1 Anagawa, Inage-ku, Chiba 263–8555, Japan*
[2)]*Graduate School of Science, Chiba University, Chiba 263–8522, Japan*

(Received 4 June 2013/Accepted 27 August 2013/Published online in J-STAGE 10 September 2013)

ABSTRACT. Various chemotherapeutic drugs, such as etoposide, and ionizing radiation (IR) have been clinically applied for the treatment of many types of animal and human malignancies. IR and chemotheraputic drugs kill tumor cells mainly by inducing DNA double-strand breaks (DSBs). On the other hand, unrepaired or incorrectly repaired DSBs can lead to chromosomal truncations and translocations, which can contribute to the development of cancer in humans and animals. Thus, it is important to clarify the molecular mechanisms underlying the chemosensitivity or radiosensitivity of mammalian cells in order to develop medical treatments and next-generation chemotherapeutic drugs for cancer. Previously, we established and analyzed cell lines stably expressing chimeric constructs of EGFP and the wild-type Ku80 (XRCC5) protein or its mutant protein to which mutations were introduced by the site-directed mutagenesis. We found that the Ku70 (XRCC6)-binding-site mutations (A453H/V454H) of Ku80 and nuclear localization signal (NLS)-dysfunctional mutations (K565A/K566A/K568A) affected the ability to complement etoposide sensitivity. In this study, we examined the radiosensitivity of these cell lines. We found that either or both amino acid substitutions in two functional domains of Ku80, i.e., Ku70-binding-site mutations (A453H/V454H) and NLS-dysfunctional mutations (K565A/K566A/K568A), affect the ability to complement radiosensitivity. Moreover, these mutations in the two domains of Ku80 affect the DSB-sensing ability of Ku80. These information and Ku80 mutant cell lines used might be useful for the study of not only the dynamics and function of Ku80, but also the molecular mechanism underlying the cellular response to IR and chemotherapeutic drugs in mammalian cells.
KEY WORDS: anticancer treatment, cell death, γH2AX, Ku70, Ku80.

doi: 10.1292/jvms.13-0283; *J. Vet. Med. Sci.* 76(1): 51–56, 2014

Chemotherapy and radiotherapy are well-established treatments for cancer. Various chemotherapeutic drugs, such as etoposide and bleomycin, and ionizing radiation (IR) have been clinically applied for the treatment of many types of animal and human malignancies. On the other hand, in clinical settings, cellular resistance to chemotherapy and radiotherapy is a significant component of tumor treatment failure. Thus, it is important to clarify the molecular mechanisms underlying the chemosensitivity or radiosensitivity of mammalian cells in order to develop medical treatments and next-generation chemotherapeutic drugs for cancer.

DNA double-strand breaks (DSBs) are considered the most critical DNA damage [3]. Unrepaired or incorrectly repaired DSBs can lead to chromosomal truncations and translocations, which can contribute to the development of cancer in higher eukaryotic organisms. In addition, one DSB is sufficient to kill a cell, when it is not repaired. DSBs are induced following exposure to ionizing radiation as well as treatment with etoposide or bleomycin [15, 22, 27]. At nascent DSB sites, histone H2AX molecules near these sites are rapidly phosphorylated at serine 139 (γH2AX) [23]. The detection of γH2AX using a specific antibody is the most sensitive method currently available for identifying DSBs.

The heterodimer formation between Ku70 (also named XRCC6) and Ku80 (also named XRCC5) and the DSB-sensing ability of Ku might play a key role in the regulation of Ku-dependent DNA repair activity [1, 6, 17]. The nonhomologous DNA-end-joining (NHEJ) repair process is responsible for repairing a major fraction of DSBs in mammalian cells [2, 3, 17]. The NHEJ repair pathway starts with the binding of Ku70 and Ku80 to a DNA end. Ku70 and Ku80 accumulate at laser-induced DSB sites immediately following irradiation, and these are essential for the accumulation of core-NHEJ factors, such as DNA-PKcs, XLF and XRCC4, but not other core-NHEJ factor, i.e., Artemis, at DSB sites [6, 9, 10, 17, 18].

Previously, we generated and analyzed cell lines stably expressing chimeric constructs of EGFP and the wild-type Ku80 protein or its mutant protein to which mutations were introduced by the site-directed mutagenesis technique [5, 7]. Xrs-6 cells are a DSB repair mutant of the CHO-K1 line and are defective in a core-NHEJ factor Ku80 [3, 21]. Our data revealed that the EGFP-Ku80 transformants showed higher etoposide resistance than xrs-6 cells. Moreover, our data showed that the Ku70-binding-site mutations (A453H/V454H) of Ku80 and nuclear localization signal (NLS)-dysfunctional mutations (K565A/K566A/K568A) affected the ability to complement etoposide sensitivity. However,

* CORRESPONDENCE TO: KOIKE, M., DNA Repair Gene Res., National Institute of Radiological Sciences, 4–9–1 Anagawa, Inage-ku, Chiba 263–8555, Japan.
e-mail: m_koike@nirs.go.jp

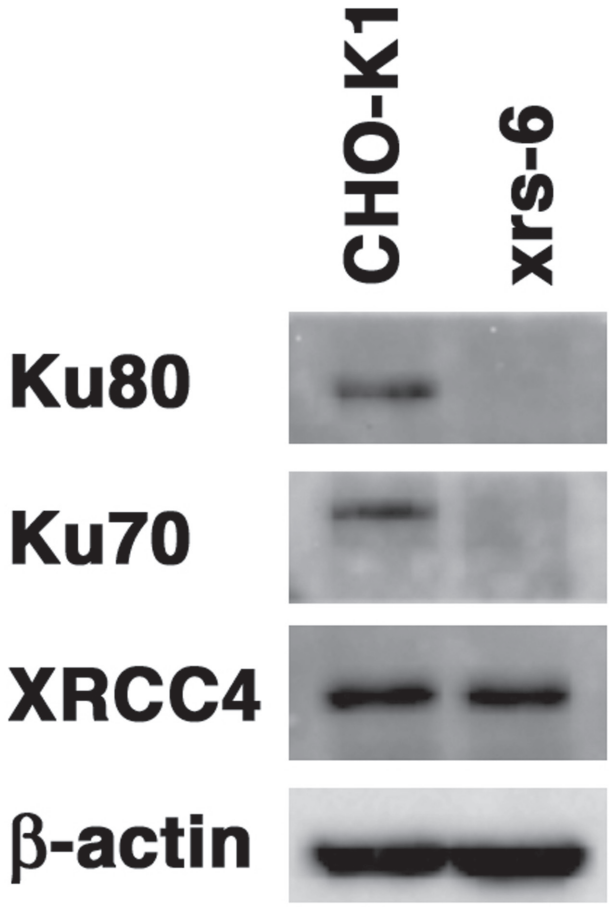


Fig. 1. Comparison of Ku80 protein expression level between xrs-6 and CHO-K1 cells. Total cell lysates from the cell lines were analyzed by Western blotting using an anti-Ku70 antibody, an anti-Ku80 antibody, an anti-XRCC4 antibody or an anti-β-actin antibody.

it remains unclear whether these Ku80 mutations modify the radiosensitivity of cells and the DSB-sensing ability of Ku80.

In this study, to clarify whether the Ku70-binding-site mutations (A453H/V454H) of Ku80 and NLS-dysfunctional mutations (K565A/K566A/K568A) affect the ability to complement radiosensitivity, we first analyzed the radiosensitivity of CHO-K1, xrs-6 and three transformants. Next, we examined whether these mutations in the two domains of Ku80 affect Ku80 accumulation at DSBs.

## MATERIALS AND METHODS

*Cell lines and culture*: A Chinese hamster ovary cell line (CHO-K1) (Riken Cell Bank, Tsukuba, Japan) and Ku80-deficient CHO-K1 mutant cell lines (xrs-6) were cultured as described in a previous study [8]. The xrs-6 cell line stably expressing chimeric proteins of EGFP and the human Ku80 (EGFP-Ku80) or EGFP alone was described previously [5]. The xrs-6 cell line stably expressing EGFP-Ku80 (A453H/V454H), EGFP-Ku80 (K565A/K566A/K568A) or EGFP-Ku80 (A453H/V454H/K565A/K566A/K568A) was described previously [7].

*X-irradiation*: Cells were exposed to X-rays at room temperature, as described previously [5]. X-rays were generated at 200 kVp/20 mA and filtered through 0.5 mm Cu and Al filters.

*Western blot analysis*: Western blot analysis was performed as described previously [10, 12]. The following antibodies were used: a rabbit anti-GFP polyclonal antibody (FL) (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.), a mouse anti-Ku70 monoclonal antibody (N3H10) (NeoMarkers, Fremont, CA, U.S.A.), a goat anti-Ku80 polyclonal antibody (M-20) (Santa Cruz Biotechnology), a goat anti-XRCC4 polyclonal antibody (C-20) (Santa Cruz Biotechnology) and a mouse anti-β-actin monoclonal antibody (Sigma, St. Louis, MO, U.S.A.). The binding to corresponding proteins was visualized using an ECL Western blotting detection system (GE Healthcare Bio-Sci. Corp., Piscataway, NJ, U.S.A.), in accordance with the manufacturer's instructions.

*Fluorescence microscopy*: The fluorescence in cells was visualized as previously described [6, 16]. Briefly, the cells were fixed in PBS containing 4% paraformaldehyde. The fixed cells were then blocked using a blocking solution and incubated for 30 min at room temperature with a mouse anti-γ-H2AX monoclonal antibody (JBW301) (Upstate Biotechnology Inc., Charlottesville, VA, U.S.A.). After washing with PBS, antibody binding was detected using Alexa fluor 568-conjugated secondary antibodies (Molecular Probes, Eugene, OR, U.S.A.). DNA was stained with 0.025 $\mu$g/m*l* 4,6-diamino-2-phenylindole (DAPI) fluorescent dye (Boehringer Mannheim, Mannheim, Germany). The cells were examined under an Olympus IX70 fluorescence microscope (Olympus, Tokyo, Japan). Images were acquired using an FV300 confocal laser scanning microscope (Olympus).

*Localized DNA damage induction using laser and cell imaging*: Localized DNA damage induction using a laser and confocal imaging were performed as described previously [6, 9, 10]. Briefly, confocal images of living cells expressing EGFP-tagged proteins were obtained using an FV300 confocal scanning laser microscopy system (Olympus). A 1% power scan (for 1 sec) from a 405 nm laser was used to induce localized DSBs.

*Clonogenic survival assay*: Clonogenic survival assay was performed as previously described [11, 13].

## RESULTS

Ku80 is a key contributor to chemoresistance to anticancer agents, such as etoposide, or radioresistance in CHO-K1 cells [3, 8, 25]. First, we confirmed the expression of Ku80 in CHO-K1 and Ku80-deficient xrs-6 cells. Consistent with previous studies [8, 24], Western blot analysis showed that Ku80 was expressed in CHO-K1 cells, but not in xrs-6 cells (Fig. 1). Expectedly, the Ku70 expression level was markedly reduced in the xrs-6 cells. A core-NHEJ factor XRCC4 as well as β-actin was expressed in both cell lines.

DSBs are considered the most critical DNA damage [3].

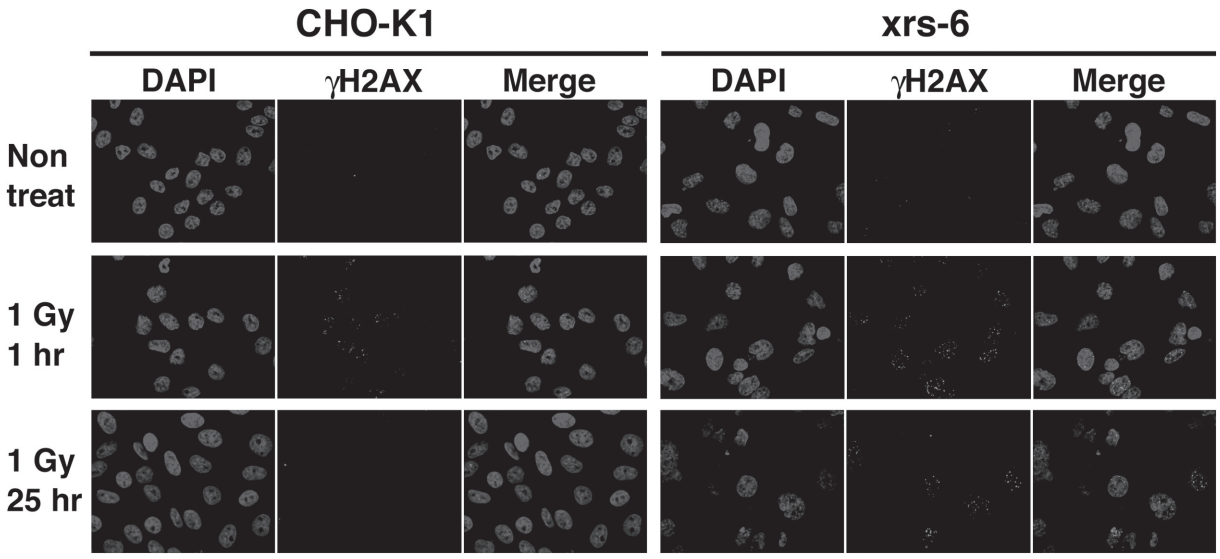


Fig. 2. Immunofluorescences of γH2AX after X-irradiation. The xrs-6 and CHO-K1 cells were irradiated with 1 Gy X-rays and immunofluorescence-labeled for γH2AX at the indicated times. Nuclear DNA was counterstained with DAPI.

One DNA DSB is sufficient to kill a cell, when it is not repaired. γH2AX is a sensitive indicator of DSBs induced by X-irradiation [23]. To confirm the roles of Ku80 in γH2AX elimination after X-irradiation in CHO-K1 cells, we examined X-irradiation-induced H2AX phosphorylation and γH2AX elimination in xrs-6 cells and CHO-K1 cells by confocal laser scanning microscopy using the anti-γH2AX antibody (Fig. 2). Following X-irradiation, γH2AX foci were detected in both cell lines at 1 hr postirradiation. Although γH2AX elimination from 1 to 25 hr after X-irradiation was detected in both cell lines, the elimination from xrs-6 cells was slower than that from the CHO-K1 cells. These findings confirm the persistence of unrepaired DSBs in Ku80-deficient xrs-6 cells.

Previously, we established and characterized stable cell lines expressing EGFP-Ku80 or EGFP in xrs-6 cells [5]. In addition, we established and characterized cell lines stably expressing EGFP-Ku80 mutants with point mutations in either or in both the NLS and heterodimerization domains [EGFP-Ku80 (K565A/K566A/K568A), EGFP-Ku80 (A453H/V454H) or EGFP-Ku80 (A453H/V454H/K565A/K566A/K568A)] using Ku80-deficient xrs-6 cells [7]. In this study, we reconfirmed that the wild-type Ku80 fusion protein or its mutant Ku80 fusion proteins were expressed in these transformants by Western blot analysis (Fig. 3). We also reconfirmed that hamster Ku70 was detected in extracts prepared from the xrs-6 cells transformed with EGFP-Ku80 or EGFP-Ku80 (K565A/K566A/K568A) and from the CHO-K1 cells, but not in those from the other transformants, confirming that mutations of Ku80 at amino acids 453 and 454 affect the interaction of Ku80 with hamster Ku70 (Fig. 3). To determine whether the Ku80 point mutations in the 2 domains in Ku80 affect the sensitivity of xrs-6 cells to X-rays, we examined the radiosensitivity of EGFP-Ku80/xrs-6 cells, xrs-6 cells and the three transformants by colony formation assay. As shown in Fig. 4, the EGFP-Ku80/xrs-6 cells exhibited higher radioresistance than xrs-6 cells, which is consistent with our previous report [5]. The double mutants with mutations in 2 functional domains were unable to complement the radiosensitivity of xrs-6 cells (Fig. 4), suggesting that these domains of Ku80 are important for complementing the radiosensitivity of Ku80-deficient cells. The NLS-dysfunctional Ku80 transformant EGFP-Ku80 (K565A/K566A/K568A)/xrs-6 cells exhibited a slightly higher radiosensitivity than the EGFP-Ku80/xrs-6 cells, suggesting that the NLS of Ku80 is important for Ku-dependent radioresistanse. Similarly, another domain transformant EGFP-Ku80 (A453H/V454H)/xrs-6 cells exhibited a slightly higher radiosensitivity than the EGFP-Ku80 transformants. These findings suggest that both or either of the Ku80 point mutations in the 2 domains of Ku80 affects the sensitivity of xrs-6 cells to X-rays.

Previously, we showed that EGFP-Ku80 accumulation at DSBs began immediately after laser microirradiation in EGFP-Ku80/xrs-6 cells [6]. We examined whether the mutations in both the NLS and heterodimerization domains of Ku80 affect the accumulation of Ku80 at DSBs (Fig. 5A–D). Expectedly, EGFP-Ku80 was localized in the nucleus and accumulated at microirradiated sites in the living cells (Fig. 5B). On the other hand, EGFP-Ku80 (A453H/V454H/K565A/K566A/K568A) was observed to be localized in the cytoplasm, and the mutant did not accumulate at DSBs (Fig. 5C). As shown in Fig. 5D, EGFP alone was localized in the nucleus and cytoplasm and not accumulated at microirradiated sites. These findings demonstrate that the Ku80 point mutations in the 2 domains of Ku80 affect the accumulation of Ku80 at DSBs.

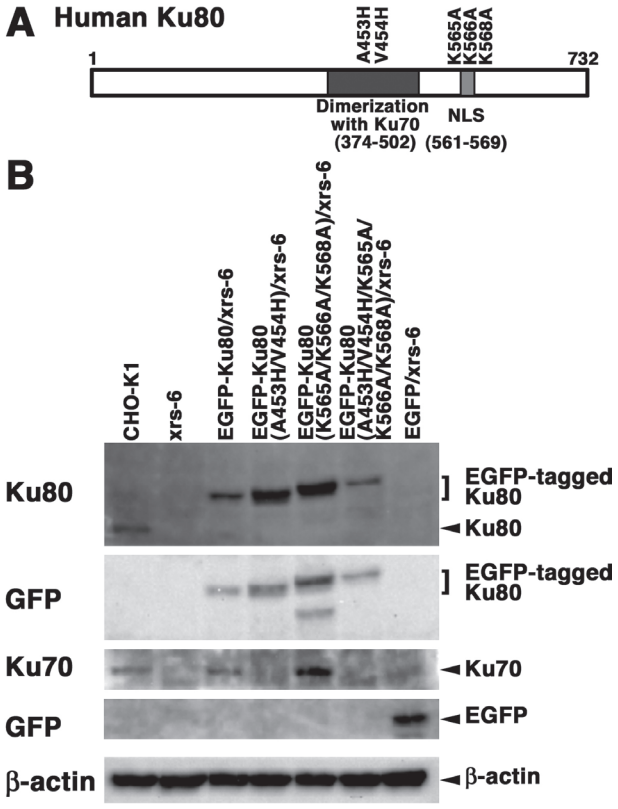


Fig. 3. Ku expression in transformed cells expressing Ku80-site-specific mutants. (A) Schematic diagrams of full-length human Ku80 protein (732 amino acids) showing the location of nuclear localization signal (NLS: amino acids 561–569) and the region involved in dimerization with Ku70 (amino acids 374–502) previously identified. Amino acid changes are designated by listing the wild-type residue, the amino acid positions and then the introduced mutant amino acid. (B) Western blot analysis of untransfected CHO-K1 cells, xrs-6 cells and xrs-6 cells expressing EGFP-Ku80, EGFP-Ku80 (A453H/V454H), EGFP-Ku80 (K565A/K566A/K568A) or EGFP-Ku80 (A453H/V454H/K565A/K566A/K568A). Total cell lysates were separated by 5–20% SDS-PAGE and analyzed by Western blotting using an anti-Ku80 antibody, an anti-Ku70 antibody, an anti-GFP antibody or an anti-β-actin antibody. Lane 1, CHO-K1 cells. Lane 2, xrs-6 cells. Lane 3, EGFP-Ku80/xrs-6 cells. Lane 4, EGFP-Ku80 (A453H/V454H)/xrs-6 cells. Lane 5, EGFP-Ku80 (K565A/K566A/K568A)/xrs-6 cells. Lane 6, EGFP-Ku80 (A453H/V454H/K565A/K566A/K568A)/xrs-6 cells. Lane 7, EGFP/xrs-6 cells.

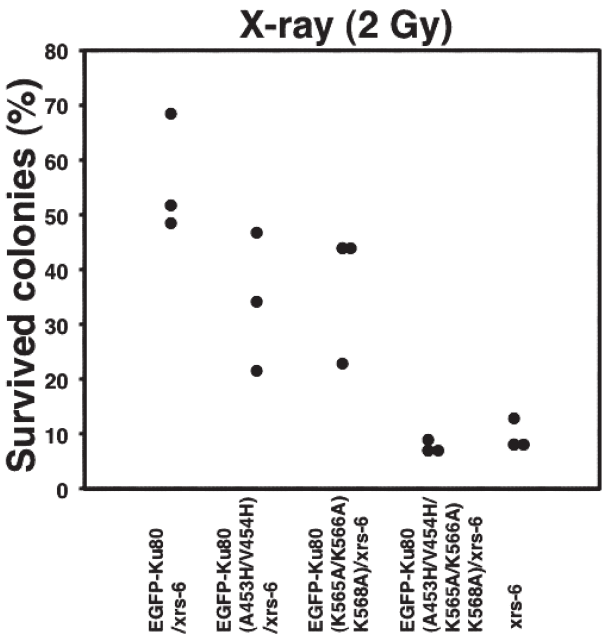


Fig. 4. Percent survival of xrs-6 cells transformed with EGFP-tagged Ku80 mutants following X-irradition. xrs-6 cells or xrs-6 cells transformed with EGFP-Ku80, EGFP-Ku80 (A453H/V454H), EGFP-Ku80 (K565A/K566A/K568A) or EGFP-Ku80 (A453H/V454H/K565A/K566A/K568A) were plated in triplicate and then exposed to X-rays at a dose of 2 Gy and were further incubated for 5 days postirradiation. The cells were subsequently stained, fixed with 2% methylene blue containing 50% methanol and visualized under a microscope. Colonies containing more than 50 cells were scored as survivors. The colonies were counted, and the survival rate was calculated by dividing the number of colonies of treated cells by that of the nontreated control (black circle).

## DISCUSSION

It is important to elucidate the molecular mechanisms underlying the radioresistance, as well as the chemoresistance, of human and animal cells in order to develop next-generation medical treatments for cancer. DNA repair genes, particularly core-NHEJ genes, are key players in radioresistance and chemoresistance. We previously identified and characterized the Ku70-binding-site of Ku80 and the NLS of Ku80 [4, 14]. In this study, our findings showed that either or both of the amino acid substitutions in the 2 functional domains of Ku80, i.e., the Ku70-binding-site mutations (A453H/V454H) and NLS-dysfunctional mutations (K565A/K566A/K568A), affect the ability to complement X-ray sensitivity. Moreover, our findings showed that these mutations affect the ability to repair DNA damage of Ku80 at microirradiation-induced DSBs.

Radiation and etoposide induce DSBs via different mechanisms and with different efficiencies. The rates of rejoining DSBs differ between etoposide and radiation [19]. In previous studies, we demonstrated that EGFP-Ku80 used in this study complemented not only X-ray sensitivity but also etoposide sensitivity in EGFP-Ku80/xrs-6 cells [5]. Others and we demonstrated that the two mutations of Ku80 at amino acids 453 and 454 in the Ku70 binding domain affect the interaction of Ku70 with Ku80 [8, 14, 20]. In addition, we showed that the Ku70-binding-site mutations (A453H/V454H) of Ku80 affected slightly the ability to complement etoposide sensitivity. In this study, Ku70-binding-site mutations (A453H/V454H) of Ku80 only affected slightly the ability to complement radiosensitivity. Another minor DSB repair pathway, such as homologous recombination, may be activated complementarily in EGFP-Ku80 (A453H/V454H)/xrs-6 cells, although further studies are needed to clarify this.

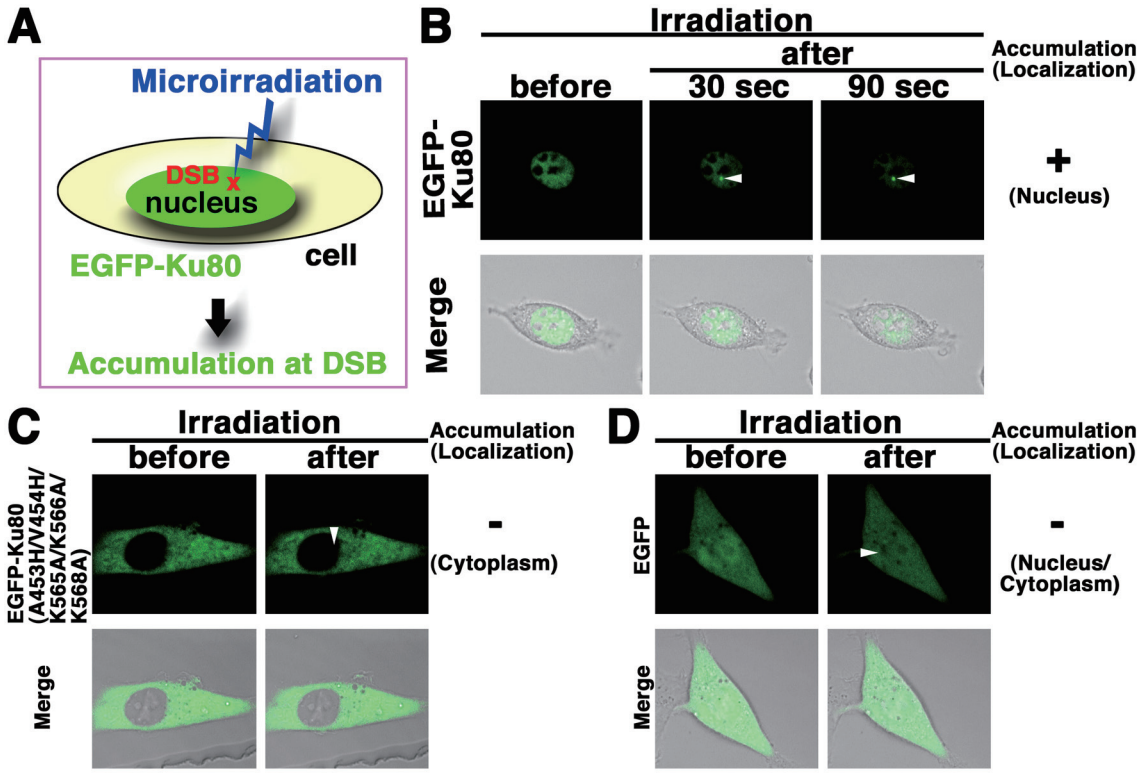


Fig. 5. Mutations in the two functional domains of Ku80 affect the ability to accumulate at microirradiation-induced DSBs. The localization and accumulation of EGFP-tagged proteins at DSBs induced by 405 nm laser irradiation were examined (A). Live-cell images were taken by confocal laser scanning microscopy before and 30 sec and 90 sec (B) or 60 sec (C, D) after microirradiation in the EGFP-Ku80/xrs-6 cells (B), EGFP-Ku80 (A453H/V454H/K565A/K566A/K568A)/xrs-6 cells (C) and EGFP/xrs-6 cells (D). Arrowheads indicate the irradiated sites.

Alternatively, we cannot completely exclude the possibility that the Ku70 binding activity and DNA binding activity may remain slightly below the detection sensitivity *in vivo*.

Previously, we showed that in addition to Ku80 NLS, the heterodimerization between Ku70 and Ku80 is also important for nuclear entry of Ku80 [4, 8, 14]. In this study, NLS-dysfunctional mutations (K565A/K566A/K568A) also only affected slightly the ability to complement radiosensitivity. On the other hand, the double mutants EGFP-Ku80 (A453H/V454H/K565A/K566A/K568A) with mutations in the 2 functional domains were unable to complement the radiosensitivity of xrs-6 cells. In addition, the mutant was not able to accumulate at DSBs, when the DSBs were induced using our simple live-cell imaging technique with a laser, suggesting that the mutations affect the ability to repair DNA damage of Ku80. Altogether, we consider that the double mutants were unable to repair DSBs of xrs-6 cells, since the double mutants were not able to localize and accumulate at DSBs. These findings support the idea that the localization and accumulation mechanism of Ku80 at DSBs might play a key role in regulating NHEJ activity [6, 17].

The DNA repair system protects the genome in humans and animals. It has been reported that genetic variations in NHEJ genes can affect DNA repair capacity and confer predisposition to several types of clinical human cancer [26]. This study and further studies will lead to a better understanding of not only the molecular mechanism underlying the cellular response via the NHEJ pathway to radiation and chemotherapeutic drugs, but also carcinogenesis induced by defects of NHEJ functions in mammalian cells.

ACKNOWLEDGMENTS. We thank Dr. P. Jeggo for providing xrs-6 cells. We also thank Mr. S. Ikenaga for technical assistance with the immunoblotting. This work was supported in part by grants from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

REFERENCES

1. Downs, J. A. and Jackson, S. P. 2004. A means to a DNA end: the many roles of Ku. *Nat. Rev. Mol. Cell Biol.* **5**: 367–378. [Medline] [CrossRef]
2. Hopfner, K. P., Putnam, C. D. and Tainer, J. A. 2002. DNA double-strand break repair from head to tail. *Curr. Opin. Struct. Biol.* **12**: 115–122. [Medline] [CrossRef]
3. Khanna, K. K. and Jackson, S. P. 2001. DNA double-strand breaks: signaling, repair and the cancer connection. *Nat. Genet.* **27**: 247–254. [Medline] [CrossRef]
4. Koike, M. 2002. Dimerization, translocation and localization of

Ku70 and Ku80 proteins. *J. Radiat. Res.* **43**: 223–236. [Medline] [CrossRef]
5. Koike, M. and Koike, A. 2004. Establishment and characterization of stable cell lines expressing human Ku80 tagged with enhanced green fluorescent protein. *J. Radiat. Res.* **45**: 119–125. [Medline] [CrossRef]
6. Koike, M. and Koike, A. 2008. Accumulation of Ku80 proteins at DNA double-strand breaks in living cells. *Exp. Cell Res.* **314**: 1061–1070. [Medline] [CrossRef]
7. Koike, M. and Koike, A. 2005. The Ku70-binding site of Ku80 is required for the stabilization of Ku70 in the cytoplasm, for the nuclear translocation of Ku80, and for Ku80-dependent DNA repair. *Exp. Cell Res.* **305**: 266–276. [Medline] [CrossRef]
8. Koike, M., Shiomi, T. and Koike, A. 2001. Dimerization and nuclear localization of Ku proteins. *J. Biol. Chem.* **276**: 11167–11173. [Medline] [CrossRef]
9. Koike, M., Yutoku, Y. and Koike, A. 2011. Accumulation of p21 proteins at DNA damage sites independent of p53 and core NHEJ factors following irradiation. *Biochem. Biophys. Res. Commun.* **412**: 39–43. [Medline] [CrossRef]
10. Koike, M., Yutoku, Y. and Koike, A. 2011. Accumulation of Ku70 at DNA double-strand breaks in living epithelial cells. *Exp. Cell Res.* **317**: 2429–2437. [Medline] [CrossRef]
11. Koike, M., Yutoku, Y. and Koike, A. 2011. Establishment of Ku70-deficient lung epithelial cell lines and their hypersensitivity to low-dose X-irradiation. *J. Vet. Med. Sci.* **73**: 549–554. [Medline] [CrossRef]
12. Koike, M., Yutoku, Y. and Koike, A. 2013. Ku80 attentuates cytotoxicity induced by green fluorescent protein transduction independently of non-homologous end joining. *FEBS Open Bio.* **3**: 46–50. [Medline] [CrossRef]
13. Koike, M., Yutoku, Y. and Koike, A. 2013. The defect of Ku70 affects sensitivity to X-ray and radiation-induced caspase-dependent apoptosis in lung cells. *J. Vet. Med. Sci.* **75**: 415–420. [Medline] [CrossRef]
14. Koike, M., Ikuta, T., Miyasaka, T. and Shiomi, T. 1999. Ku80 can translocate to the nucleus independent of the translocation of Ku70 using its own nuclear localization signal. *Oncogene* **18**: 7495–7505. [Medline] [CrossRef]
15. Koike, M., Koike, A., Sugasawa, J., Toyooka, T. and Ibuki, Y. 2010. Dynamics of Ku80 in living hamster cells with DNA double-strand breaks induced by chemotherapeutic drugs. *J. Vet. Med. Sci.* **72**: 1405–1412. [Medline] [CrossRef]
16. Koike, M., Awaji, T., Kataoka, M., Tsujimoto, G., Kartasova, T., Koike, A. and Shiomi, T. 1999. Differential subcellular localization of DNA-dependent protein kinase components Ku and DNA-PKcs during mitosis. *J. Cell Sci.* **112**: 4031–4039. [Medline]
17. Mahaney, B. L., Meek, K. and Lees-Miller, S. P. 2009. Repair of ionizing radiation-induced DNA double-strand breaks by non-homologous end-joining. *Biochem. J.* **417**: 639–650. [Medline] [CrossRef]
18. Mari, P. O., Florea, B. I., Persengiev, S. P., Verkaik, N. S., Bruggenwirth, H. T., Modesti, M., Giglia-Mari, G., Bezstarosti, K., Demmers, J. A., Luider, T. M., Houtsmuller, A. B. and Gent, D. C. 2006. Dynamic assembly of end-joining complexes requires interaction between Ku70/80 and XRCC4. *Proc. Natl. Acad. Sci. U.S.A.* **103**: 18597–18602. [Medline] [CrossRef]
19. Olive, P. L. and Johnston, P. J. 1997. DNA damage from oxidants: influence of lesion complexity and chromatin organization. *Oncol. Res.* **9**: 287–294. [Medline]
20. Osipovich, O., Durum, S. K. and Muegge, K. J. 1997. Defining the minimal domain of Ku80 for interaction with Ku70. *J. Biol. Chem.* **272**: 27259–27265. [Medline] [CrossRef]
21. Ouyang, H., Nussenzweig, A., Kurimasa, A., Soares, V. C., Li, X., Cordon-Cardo, C., Li, W., Cheong, N., Nussenzweig, M., Iliakis, G., Chen, D. J. and Li, G. C. 1997. Ku70 is required for DNA repair but not for T cell antigen receptor gene recombination *in vivo*. *J. Exp. Med.* **186**: 921–929. [Medline] [CrossRef]
22. Rogakou, E. P., Pilch, D. R., Orr, A. H., Ivanova, V. S. and Bonner, W. M. 1998. DNA double-stranded breaks induce histone H2AX phosphorylation on serine139. *J. Biol. Chem.* **273**: 5858–5868. [Medline] [CrossRef]
23. Rothkamm, K. and Löbrich, M. 2003. Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. *Proc. Natl. Acad. Sci. U.S.A.* **100**: 5057–5062. [Medline] [CrossRef]
24. Singleton, B. K., Priestley, A., Steingrimsdottir, H., Gell, D., Blunt, T., Jackson, S. P., Lehmann, A. R. and Jeggo, P. A. 1997. Molecular and biochemical characterization of xrs mutants defective in Ku80. *Mol. Cell. Biol.* **17**: 1264–1273. [Medline]
25. Svetlova, M. P., Solovjeva, L. V. and Tomilin, N. V. 2010. Mechanism of elimination of phosphorylated histone H2AX from chromatin after repair of DNA double-strand breaks. *Mutat. Res.* **685**: 54–60. [Medline] [CrossRef]
26. Tseng, R. C., Hsieh, F. J., Shih, C. M., Hsu, H. S., Chen, C. Y. and Wang, Y. C. 2009. Lung cancer susceptibility and prognosis associated with polymorphisms in the nonhomologous end-joining pathway genes: a multiple genotype-phenotype study. *Cancer* **115**: 2939–2948. [Medline] [CrossRef]
27. Watters, G. P., Smart, D. J., Harvey, J. S. and Austin, C. A. 2009. H2AX phosphorylation as a genotoxicity endpoint. *Mutat. Res.* **679**: 50–58. [Medline] [CrossRef]