

論文 / 著書情報
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論文要旨

THESIS SUMMARY

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申請学位 (専攻分野)：	博士
Academic Degree Requested	Doctor of (Philosophy)
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要旨 (英文 800 語程度)
Thesis Summary (approx.800 English Words)

DNA double strand break (DSB) is the most detrimental damage that can happen to DNA. DNA DSBs occur at random upon genotoxic stresses which include ionizing radiation or as intermediates during a process called V(D)J recombination in the immune system. Eukaryotic cells have two major pathways to repair DNA DSBs: homologous recombination (HR) which precisely repair DNA DSBs using sister chromatid as a template, and non-homologous end-joining (NHEJ) which repairs DSBs by ligating two broken ends. NHEJ, which is predominant in mammalian cells and is active throughout the cell cycle, involves several key players such as DNA dependent protein kinase catalytic subunit (DNA-PKcs), heterodimeric Ku protein (Ku86 and Ku70), and the DNA Ligase IV (LIG4)-XRCC4-XLF complex. X-ray Complementing 4 (XRCC4) is one of the most essential proteins for DNA DSB repair through NHEJ pathway. XRCC4 was initially found as the gene which could restore normal V(D)J recombination and DNA double-strand break repair of Chinese hamster ovary-derived XR-1 cells. In NHEJ, XRCC4 has an active role of bridging damaged DNA to LIG4 for ligation of DNA ends. In studies in mice, deficiency in XRCC4 or LIG4 causes embryonic lethality and is associated with massive neuronal cell death, severe growth retardation and failure in neurogenesis. In recent studies, several human patients exhibiting developmental diseases such as microcephaly and growth defect, were found to harbor mutations in XRCC4. There are also reports showing that a polymorphism in XRCC4, which changes its subcellular localization, is associated with increased cancer risk. These XRCC4 mutations were found at or nearby different locations in the XRCC4 gene structure corresponding to different functionalities in its DNA repair capability. These disease-associated mutations have caused varied effect in DNA repair from less or unstable XRCC4 expression to total loss of DNA repair function. Unexpectedly, lymphocytes indicate normal immunological functions. This study aims to analyze the functional characteristics of XRCC4 mutations associated to development and cancer risk to delve deeper to the significance of the structure integrity of XRCC4 in the DNA repair, and in non-development and protection of diseases such as cancer.

Human XRCC4 cDNA was obtained from polymerase chain reaction (PCR) of the cDNA pool of human T-cell leukemia MOLT-4 cells and integrated into p3xFLAG-CMV-10 plasmid to express XRCC4 protein with triple tandem FLAG epitope, or into pEGFP-C1 plasmid to express it as a fusion protein with green fluorescent protein (GFP). Mutations were introduced by PCR primers of different sequences depending upon the mutation. The entire XRCC4 open reading frame of the constructs was then verified to be the correct sequence by sequencing for all the constructs. These cDNAs of XRCC4 mutants were transfected to XRCC4-deficient L5178Y mouse leukemia (M10) cell line, obtained from RIKEN cell bank, and human osteosarcoma (U2OS) cell line. For M10 cells, plasmids were transfected by electroporation and visible colonies formed were expanded to obtain the stably transfected clones of the different mutated XRCC4 constructs. For U2OS cells, plasmids were transfected by lipofection. Cells were irradiated using ⁶⁰Co γ -ray source. The analysis of the expression level of XRCC4 was performed by Western blotting. The radiosensitivity of M10 cells are assessed in terms of the ability of the cells to proliferate by forming colonies in soft agarose 10-14 days after radiation exposure. Nuclear localization of U2OS cells was examined by observation under inverted fluorescence microscope. Repair of DNA double-strand breaks after radiation exposure was analyzed using the phosphorylation status (γ -H2AX) of serine 139 of histone H2AX as an index.

Results showed that these disease-associated mutants showed varying degrees of dysfunction, depending upon the positions of its mutations, the length of deletions, or its proximity to significant positions associated to other NHEJ proteins. For disease-associated mutations, V83-S105del transfectant showed the highest radiosensitivity, which was close to control vector transfectant. D254Mfs*68 transfectant showed the second highest radiosensitivity with diminished protein expression. W43R, R161Q, R225X and R275X transfectants, expressing the protein at equal or higher level than wild-type XRCC4 transfectant, showed slight but statistically significant increase in radiosensitivity compared to wild-type XRCC4 transfectants. When expressed in human osteosarcoma (U2OS) cells, R225X, R275X and D254Mfs*68 localized to the cytoplasm, whereas other mutants localized to the nucleus. As for A247S polymorphism associated to cancer risk, the mutation A247S has shown to be causing a significant change in subcellular localization of XRCC4 in U2OS cells which also reiterates the impact of conserved nuclear localization signal and phosphorylation sites of XRCC4 to its function. The significant increase in gamma H2Ax foci as compared to wild-type XRCC4 which increases after radiation exposure, indicates that A247S polymorphism and the change in location from A247S polymorphism causes deficiencies in DNA DSB repair through NHEJ which may lead to improper gene function leading to tumorigenesis and cancer development. These results highlighted that defects of XRCC4 in disease patients such as insufficiency in protein quantity and impaired functionality affects DNA repair, stressing the significance of XRCC4 in DNA repair, normal development, protection against cancer and in maintaining genomic integrity and stability.

備考：論文要旨は、和文 2000 字と英文 300 語を 1 部ずつ提出するか、もしくは英文 800 語を 1 部提出してください。

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