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Title(English)	Function of XRCC4 extremely C-terminal (XECT) domain in the regulation of DNA double-strand break repair
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論 文 要 旨

THESIS SUMMARY

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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

DNA damage is an important cause of genetic disease. In our everyday life, DNA is continuously damaged by various types of radiation, both endogenous and exogenous radiation. Ionizing radiation, one of the exogenous sources, damages the DNA indirectly by generating free radicals within the cell. These create reactive oxygen species and result in a single-strand and double-strand breaks in the double helix. Ionizing radiation of 1.5-2.0 Gy can cause approximately 1000 single-strand breaks and 40 double-strand breaks. Among these lesions, DNA double-strand breaks (DSBs) are the most lethal to the cells. Defective repair of DSBs can lead to increased radiosensitivity and elevated risk of carcinogenesis. Highly radiosensitive cancer cells are rapidly killed by modest doses of radiation.

Two major pathways mammalian cells used to combat DNA DSBs are homologous recombination (HR) and nonhomologous end joining pathway (NHEJ). HR is an error-free pathway which requires sister chromatids as the template, so HR is active only in the late S and G2 phase, however NHEJ does not require any templates and can join the broken ends directly in a fast kinetic, thus more error-prone. NHEJ is active throughout the cell cycle. NHEJ consisted of seven key players: Ku70, Ku86, DNA-PK catalytic subunits (DNA-PKcs), Artemis, X-ray repair complementing defective repair in Chinese hamster cells 4 (XRCC4), DNA ligase IV and XRCC4-like factor (XLF). NHEJ pathways repaired DNA DSBs in mainly three steps: recognition of the break by DNA-PK complex consisting of Ku70/Ku86 and DNA-PKcs, DNA end processing by Artemis and other end processing enzymes, and finally ligation of DNA ends by the ligation complex (XRCC4/DNA ligase IV/XLF).

One of the key players in NHEJ named XRCC4 exists in tight complex with DNA ligase IV and plays a crucial role in DNA joining step. The N-terminal 200 amino acids of a 336 amino acid XRCC4 protein includes dimerization domain and DNA ligase IV binding domain. This is thought to suffice XRCC4 function in DSB repair. However, the remaining C-terminal region of XRCC4, spanning 130 amino acids are not well elucidated. DNA-PK, the sensor of DNA DSBs, was found to phosphorylate XRCC4 protein during NHEJ pathway. Data analyzed from NCBI database further revealed that XRCC4 extremely C-terminal domain is highly conserved. Thus, this study seeks to elucidate the importance of XRCC4 extremely C-terminal domain in DNA DSB repair.

Throughout this study, two types of cells were used. First, M10 cells harboring mutation in XRCC4 gene, thus are defective in DSB repair and are radiosensitive. Second, human cervical carcinoma cell line HeLa were used in the analysis of nuclear localization function. In this study, series of mutants were constructed by systematically modifying their charge, side chain as well as polarity in the highly conserved amino acids. Constructed plasmids were transfected into M10 cells to establish stable tranformants and their radiosensitivity were assessed. Cobalt-60 was used as the irradiation source.

As a result, among these XRCC4 mutants, R325F and N326L showed elevated radiosensitivity compared to wild type XRCC4 and other mutants. Further study was done to elucidate the mechanism behind these radiosensitive mutants. One of the techniques used in this study was live-cell imaging by monitoring the protein subcellular localization under fluorescent microscope with the use of fluorophores such as green fluorescent protein (GFP) fused with wild type XRCC4 as well as systematically generated XRCC4 mutants. GFP-fused N326L mutants revealed difference in nuclear localization function. This study had unexpectedly found an interesting synthetic nuclear export signal by mutating Asn326 into leucine in XRCC4 protein despite the presence of nuclear localization signal. Fractionation study had revealed that XRCC4 N326L mutant with both NLS and synthetic NES was first imported into the nucleus and then exported out by CRM1 as the export was inhibited with the treatment of CRM1 inhibitor, Leptomycin B. Not all the protein with leucine rich region are targeted for the export to the cytoplasm. Next, this study showed that changing Asn326 therein to other amino acids results in reduced XRCC4 function. N326L mutant, which was created initially, was defective in nuclear localization due to synthetic nuclear export signal. However, Leptomycin B, which restored nuclear localization of XRCC4, could only partially rescue the radiosensitivity of M10-N326L.

By mutating Asn326 to either leucine, alanine, aspartic acid or glutamine compromised XRCC4 function in terms of cell survival after irradiation. It is interesting to note that C-terminal regions of other NHEJ proteins are shown to be essential as well. Considering this, XECT may also act as a module for protein-protein interactions. This present study shed light on the conservation and importance of the XECT region. These interesting results indicated that C-terminal part, as well as N-terminal part, of XRCC4 plays an essential role in DSB repair through nonhomologous end joining pathway. (782 words)