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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 double-strand break (DSB) is the most severe type of DNA damages which can be induced by ionizing irradiation or chemotherapeutic drugs. Eukaryotic cells are able to repair DSB through Homologous-recombination (HR) or Non-homologous end-joining (NHEJ) pathways. HR repair is error-free but it requires sister chromatid as a template for precise repair. So HR pathway is only active in late S and G2 cell cycle. On the contrast, NHEJ directly joins two DNA ends without the need for a template. NHEJ repair is independent of cell cycle, faster than HR, efficient for DSB repair, but usually error-prone. Mutagenesis will be introduced during NHEJ repair, and could eventually lead to carcinogenesis. Nevertheless, NHEJ is still the major pathway for DSB repair, as it is considered as the last resort to rescue the cell from lethal chromatin lesions. In the traditional NHEJ repair model, Ku first recognizes the DSB and helps the recruitment of DNA-PKcs. After the broken DNA ends processed by several DNA polymerases and made compatible to ligation, they are finally joined by Ligase IV, XRCC4 and XLF complex, where Ligase IV is the critical player in NHEJ repair, and XRCC4 acts as a supporting role for Ligase IV. In my thesis work, I found EF1α promotor suitable for Ligase IV gene expression in mammalian cells and successfully expressed Ligase IV in Ligase IV-deficient Nalm-6 cells. Chromatin binding of XRCC4 was missing in Ligase IV deficient cells, but recovered in wild-type Ligase IV transfectant cell, proving that the chromatin binding of XRCC4 requires Ligase IV. XLF however, does not require Ligase IV for chromatin binding, indicating a different recruitment mechanism for Ligase IV, XRCC4 and XLF complex. In this study, I focused on the role of Ligase IV C-terminal region where it has two highly conserved BRCT domains and an XRCC4 interacting region. I created two point mutations W725R and W893R in either of the two BRCT domains. The mutated tryptophan site is not only conserved in Ligase IV proteins across species but also conserved in many BRCT containing proteins involved in DNA repair in human. I analyzed the chromatin binding of Ligase IV and XRCC4 in the two mutants, and found the binding in W725R was much reduced compared to wild-type Ligase IV transfectant, and hardly seen in W893R. I noticed that Ligase IV was expressing at an extremely low level in W893R mutant, which might be due to protein instability. From the colony formation assay, both of the two mutations showed a significant decrease in survival fraction that is similar to the reference mutation R278H, a mutation identified from a patient with Ligase IV syndrome. With the extreme high dose IR, I again showed that BRCT mutants were defective in Ligase IV chromatin binding, especially W893R, indicating a possible involvement of BRCT domains in DSB recognition. To further study the role of C-terminal region, I created a Ligase IV C-terminal fragment named as LigIV-CT, which does not include the DNA binding domain on the N-terminal region that is supposed to facilitate chromatin binding. I found the LigIV-CT was also capable of recruiting itself to chromatin as well as XRCC4, and the binding signal increased with IR dose. It is also suggested that two BRCT domains might be necessary for maintaining a stable conformation of Ligase IV and XRCC4 complex that is needed for Ligase IV kinase activity, as the point mutations W725R and W893R in LigIV-CT reduced the chromatin binding of XRCC4. According to the above discoveries I proposed a model that Ligase IV can bind to chromatin with both N-terminal and C-terminal regions. The N-terminal binding is DSB independent that it dynamically binds to DNA as other DNA ligases do. This binding can be stopped by transcription inhibitors such as Actinomycin D. But the C-terminal region especially recognizes DSB sites after IR and helps the recruitment of Ligase IV. Ligase IV could possibly bridge DNA ends with its two binding domains on the two opposite termini during NHEJ repair. The BRCT mutations disrupt the DSB recognition, and result in high radiosensitivity. This idea was further confirmed by the fact that the introduced Ligase IV C-terminal fragment could effectively sensitize the normal HeLa-Fucci cell after IR induced DSB formation. I also observed the cell cycle progression after IR with fluorescent microscope. The cells treated with DNA-PKcs inhibitor showed a long G2 cell cycle arrest after 20 h, but both wild-type and LigIV-CT transfectant cleared the arrest around 10 h, even though most of the DNA damages were not efficiently repaired in LigIV-CT cells. Increased apoptosis, cell fragmentation and mitotic death could be seen after 60 h, indicating the cell cycle arrest did not ensure a perfect DNA repair by NHEJ molecules. The C-terminal fragment increased the unrepaired DSBs but it did not prolong the G2 cell cycle arrest in HeLa-Fucci cells.