論　文　内　容　要　旨

Regulation of homologous recombinational repair by lamin B1 in radiation-induced DNA damage.

（放射線誘発 DNA 損傷の相同組換え修復における Lamin B1 による制御）

The FASEB Journal, 2015, pii: fj.14-265546. [Epub ahead of print]

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DNA double-strand breaks (DSBs) are among the most deleterious forms of DNA damage induced by ionizing radiation (IR) and, if not properly repaired, may cause genomic instability, cell death, and cancer. Two distinct pathways, homologous recombination (HR) and non-homologous end joining (NHEJ), are responsible for repair of DSBs. Distinguished from NHEJ, HR is a process that requires the presence of a sister chromatid to serve as a template for DNA repair but with greater accuracy and complexity. RAD51, a recA homolog that forms helical filaments binds on the single-strand DNA, thus promoting recombinational repair. Previous studies indicate that RAD51 is a critical protein in HR repair and cell survival. However, mechanistic insights into the activation of RAD51 in response to DNA damage remain unclear.

To understand the mechanism of RAD51-dependent HR, we searched for interacting partners of RAD51 by a combination of proteomics analysis and immunoprecipitation in human cells, and identified lamin B1.

The B-type lamins are nuclear lamina proteins that constitutively expressed in all cell types and during all stages of development. Numerous studies have implicated the B‑type lamins in multiple aspects of cell physiology, including DNA replication and transcription, mitotic spindle assembly, resistance to oxidative stress, and chromosome distribution. However, the potential involvement of the B-type lamins in the DNA repair pathways has not been well characterized.

In this study, to understand how the interaction with lamin B1 influences the function of RAD51, we examined whether the loss of lamin B1 affected the level of the RAD51 protein. Immunoblotting analyses revealed that siRNA-mediated lamin B1 depletion repressed the DNA damage-dependent increase of RAD51 after IR.

This finding led us to explore how lamin B1 regulates the amount of RAD51:

1) Since A-type lamins regulate HR repair by influencing the transcription of RAD51, we hypothesized that loss of lamin B1 may reflect the effects of A-type lamins. To test this possibility, we examined the effect of lamin B1 depletion on the expression of lamin A. An immunoblotting analysis showed no significant effect of lamin B1 depletion on the expression of lamin A, suggesting that the effect of lamin B1 depletion on the regulation of RAD51 was not via lamin A expression.
2) It has been reported that RAD51 protein levels fluctuate during the cell cycle, and are reduced during G1 and increased at the S and G2/M phases. Therefore, we assessed whether lamin B1 depletion induces the accumulation of cells in the G1 phase, which in turn would decrease the amount of RAD51 protein. The flow cytometry analysis revealed that the lamin B1 depletion had minimal effects on the cell cycle progression of U2OS cells, suggesting that the effect of lamin B1

depletion on the RAD51 protein levels is not linked to cell cycle arrest.

3) We then investigate whether lamin B1 regulates RAD51 protein levels in more direct ways, by repressing RAD51 protein stability and/or its transcriptional activity. A Quantitative Reverse-Transcription PCR (qRT-PCR) analysis revealed that the depletion of lamin B1 had no effect on the RAD51 mRNA levels in U2OS cells treated with or without IR. This finding suggests that lamin B1 is not involved in the transcriptional process of RAD51. On the other hand, the repression of RAD51 by lamin B1 depletion after IR was abolished by the proteasome inhibitor MG132. These results suggest that lamin B1 stabilizes RAD51 by preventing proteasome-mediated degradation in cells with IR-induced DNA damage.

Given the role of lamin B1 in maintaining RAD51 stability after IR shown in this study, we postulated that lamin B1 may also play a role in HR repair by regulating RAD51 function. Indeed, immunoblotting and immunofluorescence staining analysis suggesting that lamin B1 facilitates RAD51 nuclear accumulation and focus formation at DSB sites after DNA damage. Furthermore, Direct Repeat-Green Fluorescent Protein (DR-GFP) assay revealed that the depletion of lamin B1 represses the activity of HR repair. The repression of HR activity by the depeltion of lamin B1 was confirmed by Sister Chromatid Exchange (SCE) assay. Taken together, these findings suggest that lamin B1 is involved in the positive regulation of HR repair by regulating RAD51.

Considering the role of lamin B1 in the regulation of HR repair, we finally examined the effect of lamin B1 depletion on the cellular radiosensitivity by a colony formation assay. As a result, we found that the depletion of lamin B1 increase the radiosensitivity of cells.

Based on these findings, we conclude that lamin B1 positively regulates the HR repair activity, by facilitating the nuclear accumulation and focus formation of RAD51 after the induction of DSBs. This report provides the first evidence that B-type lamin participates in the regulation of the HR repair pathway in response to DNA damage.