審査の結果の要旨

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This thesis investigates the DNA damage response in 1-cell stage embryos. Cell cycle checkpoints and DNA repair coordinate with each other to safeguard a proliferating cell from DNA damage arising from endogenous and exogenous stress. Although the first cell cycle in mammalian embryos comprises four phases resembling those of a somatic cell cycle, how DNA damage checkpoint works in each cell cycle phase is unclear. Furthermore, embryos start with a loosened chromatin structure after fertilization, which is gradually tightened during preimplantation development. Chromatin modifications also change throughout this period. Since DNA damage signaling involves changes in chromatin features, the specific chromatin context in 1-cell stage embryos may contribute to an unusual response to DNA damage. However, the distinct DNA damage response in the first cell cycle and its connection with embryonic sensitivity remain to be clarified. Therefore, this study examined the DNA damage response in the first cell cycle and its connection with embryonic sensitivity. This thesis consists of two chapters.

In chapter I, the radiosensitivity and the DNA damage checkpoints were examined in 1-cell stage embryos. The development until the blastocyst stage was monitored in zygotes which had been irradiated at the G1, S, G2 or M phase at the first cell cycle after fertilization. Unlike the high radiosensitivity shown by G2 and M phase somatic cells, zygotes at the G2 phase seemed to be the most resistant to DNA damage, whereas zygotes irradiated during G1, S or M phase barely developed until the blastocyst stage. It is noteworthy that none of the zygotes irradiated at the first mitosis were capable to finish the second round of division. Further examination of the first cell cycle revealed that DNA damage checkpoints were absent in G1, S and M phase of the first cell cycle, which plausibly led to the hyper-radiosensitivity of zygotes in these phases. All zygotes irradiated during the interphase were arrested in the G2 phase and then most of the embryos restarted cell cycle progression later while a small part stopped developing permanently. However, once zygotes entered the mitosis, cell cycle progression was not retarded by DNA damage. Consistently, Chk2 was activated during the G2 phase but not the G1 or M phase, suggesting the role of Chk2 in G2 phase arrest. On the other hand, the checkpoint in the S phase was absent despite the active Chk2, which indicates that the effect of phosphorylated Chk2 on cell cycle arrest is somehow contradicted during the first S phase.

In Chapter II, DNA repair in the first cell cycle and the consequent chromosomal aberrations were examined. Embryos irradiated at the interphase of the first cell cycle

featured micronuclei, which likely resulted from unrepaired double strand breaks induced by irradiation and acentric chromosome fragments. It was noted that micronuclei were more prevalent in embryos irradiated during the G1 and S phase. The lack of checkpoints in the G1 and S phase would contribute to the insufficient DNA repair, and even the temporal arrest in G2 phase did not ensure complete repair of all DNA lesions. Chromatin bridges, which were rarely observed in embryos treated with γ-ray in the first interphase, replaced micronuclei to be the most prevalent chromosomal aberration in embryos irradiated during the first mitosis. It has been known that chromatin bridges form due to telomere fusion and consequently cause the failure in sister chromatid separation when DNA repair system is fully activated during mitosis in somatic cells. Chromatin bridges occur in irradiated somatic cells at a much lower frequency because DNA repair pathways are partially silenced at M phase. Telomere fusion was directly detected in irradiated zygotes using fluorescence in situ hybridization (FISH), suggesting that DNA repair system may be functional during the first mitosis. γH2AX is commonly considered as a marker for DNA damage sites and thus the quantification of γH2AX foci or intensity can be used as an index for the numbers of DSBs. γH2AX signals increase when DSBs form and decrease as DSBs are repaired during the somatic interphase. While DNA damage sites in somatic mitosis are marked by γH2A.X, the downstream pathways are silenced. Artificial restoration of these pathways leads to telomere fusion in mitotic cells exposed to irradiation. Previous studies showed that γH2AX foci formed but persisted in somatic cells damaged during mitosis. However, γH2AX signals in mitotic zygotes rise and then gradually weaken as time passed after irradiation, providing another piece evidence of the functional DNA repair system during the first mitosis.

Taken together, Chk2-dependent DNA damage checkpoint is activated during the G2 phase, but not in the G1, S or M phase, presumably contributing to the hypersensitivity of G1, S and M phase zygotes. The defective cell cycle checkpoints contribute to insufficient DNA repair in the first interphase, which in turn causes micronucleus formation in the subsequent 2-cell stage and low blastocyte developmental rates. Finally, DNA repair system is activated during the zygotic mitosis, leading to telomere fusion, failure in sister chromatid separation and high formation rate of chromatin bridges.

This thesis has thus provided important knowledge about the cell cycle check points and DNA repair system in 1-cell stage embryos and would surely contribute to understanding the mechanism underlying DNA damage response in zygotes.

よって本論文は博士（生命科学）の学位請求論文として合格と認められる。

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