博士論文（要約）

DNA damage response in 1-cell stage mouse embryos
(マウス１細胞期胚の DNA 損傷応答に関する研究)

王　媛

DNA damage response in 1-cell stage mouse embryos

December 2020

Yuan Wang

Department of Integrated Biosciences
Graduate School of Frontier Sciences
The University of Tokyo

Contents

Acknowledgements

I would first like to express my earnest appreciation to my supervisor Prof. Aoki for offering me such an interesting research theme, and continuous support and encouragement. Discussions with him have always been enlightening and inspiring. I will bear in mind the words he said to me, “you are a scientist and have to consider things in a scientific way”. What Prof. Aoki taught me will no doubt influence my attitude toward future research and life.

I am grateful to Prof. Mitani, who recommended me to Prof. Aoki three years ago and also guided me through my work involving the use of ionizing radiation.

Special thanks go to Prof. Oda. As required by laws and regulations regarding radioactive materials, I was only allowed to get access to radiation source under supervision. Prof. Oda never rejected my requests to use the machine, no matter what time it was. I will not forget all those early mornings and late evenings when Prof. Oda helped me enter the radiation laboratory and encouraged me with warm words instead of showing any impatience or complaining about the unreasonable requirements.

I want to thank Prof. Suzuki for his advice on studies and also admire his passion and commitment to research, which helped me learn to enjoy the exploration in the science world.

I thank every member in Aoki laboratory and especially appreciate the instructions and assistance from Tsukioka san and Sugie san.

I have to thank my former supervisor Prof. Hua and colleagues Prof. Xu and Dr.

Lu in Zhejiang University for their invaluable help throughout my academic life.

My family has been my unconditional support system. It was my dearest sister who accompanied me through the highest and lowest moments of my life and gave me the courage to chase my dream. It was my beloved Dr. Gong who took care of me and helped me concentrate on studies in the past three years. Much love and gratitude.

Summary

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, whether and how DNA damage checkpoint works in each cell cycle phase is unclear. Besides, it remains elusive how 1-cell stage embryos with the specific chromatin structure respond to DNA damage. Therefore, this study examined the DNA damage response in the first cell cycle and its connection with embryonic sensitivity.

In chapter I, I investigated the radiosensitivity of 1-cell stage embryos and the DNA damage checkpoints in the first cell cycle. The development until the blastocyst stage was monitored in zygotes irradiated at the G1, S, G2 or M phase. 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 the G1, S or M phase barely developed to the blastocyst stage. Further examination of the first cell cycle revealed that DNA damage checkpoints were absent in the G1 and S phases of the first cell cycle, which presumably led to the hyper-radiosensitivity of zygotes in these phases.

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.

Taken together, DNA damage checkpoint is activated during the G2 phase, but not in the G1, S or M phase. 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.

General introduction

The genetic integrity of a cell is frequently threatened by genotoxic stress. Cells are equipped with a set of response to deal with DNA damage and to ensure accurate transmission of genetic information to daughter cells. Several checkpoints stop or slow down the cell cycle progression when DNA is damaged, allowing time for DNA repair system to work on the damaged sites (Murray, 1994). If the lesions are too severe to be restored, the cell enters senescence or apoptosis. Without such proper DNA damage response (DDR), cells with incomplete genetic information may die. Alternatively, dysfunctional DDR will drive accumulation of mutations and even tumorigenesis (Lord et al., 2012).

Preimplantation development refers to the initial 7-9 cell division after fertilization and lasts around 4 days in mice. One-cell embryos, also referred to as zygotes, execute their first cleavage to 2-cell embryos above 10 hours after fertilization. After 3 cleavages without any increase in the whole volume, the embryos reach the morula stage at which blastomeres adhere to each other, i.e. a process called compaction. The embryos reach the blastocyte stage around 90 hours after insemination, consisting of two cell lineages with the outer and inner layers called trophectoderm and inner cell mass, respectively. The blastocyte is characterized by a fluid-filled cavity known as blastocoel. Up to this stage, the embryos have been enveloped by the zona pellucida and prevented from attaching to the oviduct. The preimplantation period ends when the embryos hatch out and adhere to the wall of the uterus (Cockburn et al., 2010) (Fig. 1).

It has long been known that preimplantation embryos are hypersensitive to genotoxic agents. Exposure of preimplantation embryos to ionizing radiation causes a high level of lethality before or shortly after implantation while the same dose on later developmental stages such as organogenesis or fetal period yields less prenatal mortality (Russell et al., 1954). This is attributable to the truncated gap phases and rapid cell cycling during the early development and the consequent weakened DNA damage checkpoints (Palmer et al., 2016). Radiosensitivity was also found to vary dramatically during the preimplantation period: blastocyte formation is more severely impaired by irradiation at the 1-cell and 4-cell stage than at the 2-cell stage, whereas embryos after 8-cell stage become significantly more radioresistant than 4-cell stage embryos (Domon, 1982; Goldstein et al., 1975). The dramatic change in radiosensitivity of preimplantation embryos may be due to the chromatin remodeling during this period. Chromatin is structurally loose after fertilization and becomes gradually tightened during the preimplantation development through histone variant exchange and chromatin modifications (Akiyama et al., 2011; Nashun et al., 2010; Ooga et al., 2016).

The first cell cycle after fertilization strands out in the preimplantation period. It is relatively long and comprises four phases, resembling a somatic cell cycle (Palmer et al., 2016). Yet it remains unclear if DNA damage checkpoints are present in each phase of the first cell cycle or not. Furthermore, the unique chromatin context and transcription pattern at the 1-cell stage may give rise to distinctive DNA damage response. It has been shown that chromatin structure and its composing histone variants are particularly unique at this stage. Fluorescence recovery after photobleaching（FRAP）

analysis revealed that chromatin structure is extremely loosened in 1-cell stage embryos, compared to later preimplantation stage embryos and embryonic stem (ES) cells (Ooga et al., 2016). Some core histone variants are missing and are replaced by other ones specifically expressed in 1-cell stage embryos: H3 variants H3.1 and 3.2 and H2A variants H2AZ and macroH2A are barely deposited in chromatin of zygotes, while H3.3, H2AX and TH2A are abundant (Akiyama et al., 2011; Nashun et al., 2010; Shinagawa et al., 2014). Since the incorporation of H3.1 into DNA damaged sites has been reported (Polo et al., 2006) and H2AX is widely accepted to play an important role in DNA damage response (Lowndes et al., 2005), the response to DNA damage seems to be unique in 1-cell stage embryos. Regarding linker histones at the 1-cell stage, oocyte-specific H1foo is abundant in chromatin whereas somatic-type linker histones except for H1A is not present; yet in later stages H1foo is lost and somatic-type ones become dominant (Funaya et al., 2018). It was reported that somatic-type linker histone H1.2 is involved in DNA damage repair (Li et al., 2018), suggesting again that DNA damage response at the 1-cell stage may differ from the canonical form in somatic cells. Supporting this hypothesis is that studies in my lab and another research group have shown that the signal of γH2AX, a well-established DNA damage marker, is low in irradiated zygotes, compared with that in oocytes and later stage embryos (Adiga et al., 2007; Yukawa et al., 2007), despite the abundance of histone H2AX in zygotes (Nashun et al., 2010). However, whether DNA lesions can be repaired in the first cell cycle and how this affects the sensitivity of zygotes are still under-researched. This study aims to view the DNA damage response in 1-cell stage embryos in a systematic fashion and to

provide an explanation about the embryonic sensitivity.

Chapter I

DNA damage checkpoints in the first cell cycle and radiosensitivity

本章は東京大学が認める「やむを得ない事由」にある「博士論文の全部または一部が、単行本もしくは雑誌掲載等の形で刊行される予定」に該当するため、インターネットでの公表をすることができません。

Chapter II

DNA repair in the first cell cycle and chromosomal aberrations

本章は東京大学が認める「やむを得ない事由」にある「博士論文の全部または一部が、単行本もしくは雑誌掲載等の形で刊行される予定」に該当するため、インターネットでの公表をすることができません。

Figures

Fig. 1

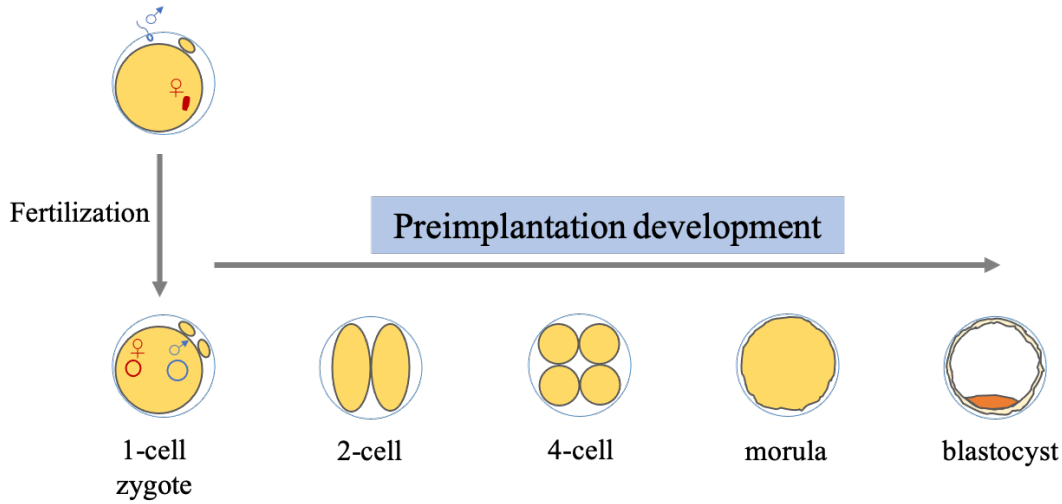

Fig. 1 Outline of the preimplantation development after fertilization

Materials and methods

Animals

Female BDF1 mice at 3 weeks old and male ICR mice retired from breeding were purchased from SLC Japan, Shizuoka. H2AX knockout mice were generated by my colleague Tsukioka san.

All of the procedures using animals were reviewed and approved by the University of Tokyo Institutional Animal Care and Use Committee and were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals.

In vitro fertilization and culture of embryos

Female mice were superovulated with 5 IU of pregnant mare serum gonadotropin (PMSG, ASKA Pharmaceutical Co., Tokyo) for 48 h followed by 5 IU of human chorionic gonadotropin (hCG, ASKA Pharmaceutical). After 16 h. MII oocytes were collected from the ampullae of the mouse oviducts and inseminated in human tubal fluid (HTF) media in a humidified atmosphere of 5% CO_2 / 95% air at 38 °C with the spermatozoa that had been collected from the caudal epididymides of the male mice and capacitated by preincubation for 2 h. The oocytes were washed with KSOM-R media 2 h after the insemination (Lawitts et al., 1993). Successfully fertilized oocytes were selected as two pronuclei became visible around 4.5 HPI.

γ-irradiation

Irradiation was performed at room temperature with γ-rays emitted by ^{137}Cs (Gammacell 3000Elan, MDS Nordion, Ottawa, Canada) at a dose rate of 6.8 Gy/min for certain amount of time (1’28’’ and 5’’) to reach indicated doses (10 Gy and 0.5 Gy). G1, S and G2 phase zygotes were cultured until 3 HPI, 6 HPI and 11 HPI, respectively, when they were exposed to irradiation. Embryos were cultured until 12 HPI when they were transferred to KSOM-R media containing 0.5 μM nocodazole (#140-08531, FUJIFILM) to arrest the development at the metaphase. The arrested M phase zygotes were irradiated at 15 HPI.

Detection of DNA synthesis by BrdU labelling

Bromodeoxyuridine (BrdU, #10280879001, Roche) was preincubated with KSOM-R media at a final concentration of 10 μM for 30 min before use. Embryos were transferred to the KSOM-R media containing BrdU 15 min before the indicated time points and incubated for 30 min. The embryos were then washed with phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA, A3311, Sigma-Aldrich) and fixed with 3.7% paraformaldehyde (PFA) in PBS at 4°C overnight. In the following day, the fixed embryos were washed and treated with 2 N HCl at 37°C for 1 h to denature the DNA, allowing the primary antibody to get access to the incorporated BrdU. The samples were then washed and neutralized in 0.1 M Tris-HCl (pH8.5), at room temperature for 15 min. After several washes, the samples were incubated with the primary antibodies recognizing BrdU (1:100, #11170376001, Roche) and H3K9me3 (1:1000, #07-442, Sigma-Aldrich) at 4°C overnight. The samples were

washed in the following day and incubated with anti-Mouse IgG secondary antibody Alexa Fluor 488 (1:100, #A11001, Invitrogen) and anti-Rabbit IgG secondary antibody Alexa Fluor 647 (1:100, #A31573, Invitrogen) at room temperature for 1 h. All the antibodies were diluted with PBS containing 1% BSA. The embryos were finally washed and mounted on slide glass with VECTASHILD mounting medium containing DAPI (#H-1200, funakoshi). Three independent experiments were conducted, and total more than 75 embryos were examined for each condition. Embryos positive for BrdU signals were counted and representative pictures are shown.

Image shooting and processing

Immunocytochemical images were taken on confocal laser scanning microscope FV3000 (OLYMPUS). Analysis of areas and intensity was performed on ImageJ.

References

Adiga S K, Toyoshima M, Shimura T, et al. Delayed and stage specific phosphorylation of H2AX during preimplantation development of γ-irradiated mouse embryos[J]. Reproduction, 2007, 133(2): 415-422.

Ahn J Y, Schwarz J K, Piwnica-Worms H, et al. Threonine 68 phosphorylation by ataxia telangiectasia mutated is required for efficient activation of Chk2 in response to ionizing radiation[J]. Cancer research, 2000, 60(21): 5934-5936.

Akiyama T, Suzuki O, Matsuda J, et al. Dynamic replacement of histone H3 variants reprograms epigenetic marks in early mouse embryos[J]. PLoS Genet, 2011, 7(10): e1002279.

Aoki F, Schultz R M. DNA replication in the 1-cell mouse embryo: stimulatory effect of histone acetylation[J]. Zygote, 1999, 7(2): 165-172.

Bassing C H, Chua K F, Sekiguchi J A, et al. Increased ionizing radiation sensitivity and genomic instability in the absence of histone H2AX[J]. Proceedings of the National Academy of Sciences, 2002, 99(12): 8173-8178.

Burma S, Chen B P, Murphy M, et al. ATM phosphorylates histone H2AX in response to DNA double-strand breaks[J]. Journal of Biological Chemistry, 2001, 276(45): 42462-42467.

Celeste A, Petersen S, Romanienko P J, et al. Genomic instability in mice lacking histone H2AX[J]. Science, 2002, 296(5569): 922-927.

Chan K S, Koh C G, Li H Y. Mitosis-targeted anti-cancer therapies: where they stand[J]. Cell death & disease, 2012, 3(10): e411-e411.

Chao H X, Poovey C E, Privette A A, et al. Orchestration of DNA damage checkpoint dynamics across the human cell cycle[J]. Cell systems, 2017, 5(5): 445-459. e5.

Chehab N H, Malikzay A, Appel M, et al. Chk2/hCds1 functions as a DNA damage checkpoint in G1 by stabilizing p53[J]. Genes & development, 2000, 14(3): 278-288.

Chen L, Gilkes D M, Pan Y, et al. ATM and Chk2-dependent phosphorylation of MDMX contribute to p53 activation after DNA damage[J]. The EMBO journal, 2005, 24(19): 3411-3422.

Ciccia A, Elledge S J. The DNA damage response: making it safe to play with knives[J]. Molecular cell, 2010, 40(2): 179-204.

Cockburn K, Rossant J. Making the blastocyst: lessons from the mouse[J]. The Journal of clinical investigation, 2010, 120(4): 995-1003.

de Lange T. How telomeres solve the end-protection problem[J]. Science, 2009, 326(5955): 948-952.

Domon M. Radiosensitivity variation during the cell cycle in pronuclear mouse embryos in vitro[J]. Cell Proliferation, 1982, 15(1): 89-98.

Epel D. Protection of DNA during early development: adaptations and evolutionary consequences[J]. Evolution & development, 2003, 5(1): 83-88.

Falck J, Mailand N, Syljuåsen R G, et al. The ATM–Chk2–Cdc25A checkpoint pathway guards against radioresistant DNA synthesis[J]. Nature, 2001, 410(6830): 842-847.

Falck J, Petrini J H J, Williams B R, et al. The DNA damage-dependent intra–S phase checkpoint is regulated by parallel pathways[J]. Nature genetics, 2002, 30(3): 290-294.

Funaya S, Ooga M, Suzuki M G, et al. Linker histone H1 FOO regulates the chromatin structure in mouse zygotes[J]. FEBS letters, 2018, 592(14), 2414-2424.

Giunta S, Belotserkovskaya R, Jackson S P. DNA damage signaling in response to double-strand breaks during mitosis[J]. Journal of Cell Biology, 2010, 190(2): 197-207.

Goldstein L S, Spindle A I, Pedersen R A. X-ray sensitivity of the preimplantation

mouse embryo in vitro[J]. Radiation research, 1975, 62(2): 276-287.

Hayashi M T, Cesare A J, Fitzpatrick J A J, et al. A telomere-dependent DNA damage checkpoint induced by prolonged mitotic arrest[J]. Nature structural & molecular biology, 2012, 19(4): 387.

Hoffmann I. The role of Cdc25 phosphatases in cell cycle checkpoints[J]. Protoplasma, 2000, 211(1-2): 8-11.

Hong Y, Cervantes R B, Tichy E, et al. Protecting genomic integrity in somatic cells and embryonic stem cells[J]. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 2007, 614(1-2): 48-55.

Honjo Y, Ichinohe T. Stage-Specific Effects of Ionizing Radiation during Early Development[J]. International journal of molecular sciences, 2020, 21(11): 3975.

Inoue Y, Kitagawa M, Taya Y. Phosphorylation of pRB at Ser612 by Chk1/2 leads to a complex between pRB and E2F-1 after DNA damage[J]. The EMBO journal, 2007, 26(8): 2083-2093.

Kermi C, Aze A, Maiorano D. Preserving genome integrity during the early embryonic DNA replication cycles[J]. Genes, 2019, 10(5): 398.

Lawitts J A, Biggers J D. Culture of preimplantation embryos[J]. Methods in enzymology, 1993, 225: 153-164.

Li Z, Li Y, Tang M, et al. Destabilization of linker histone H1. 2 is essential for ATM activation and DNA damage repair[J]. Cell research, 2018, 28(7): 756-770.

Lord C J, Ashworth A. The DNA damage response and cancer therapy[J]. Nature, 2012, 481(7381): 287-294.

Lowndes N F, Toh G W L. DNA repair: the importance of phosphorylating histone H2AX[J]. Current biology, 2005, 15(3): R99-R102.

Lu H, Saha J, Beckmann P J, et al. DNA-PKcs promotes chromatin decondensation to facilitate initiation of the DNA damage response[J]. Nucleic acids research, 2019,

47(18): 9467-9479.

Luzhna L, Kathiria P, Kovalchuk O. Micronuclei in genotoxicity assessment: from genetics to epigenetics and beyond[J]. Frontiers in genetics, 2013, 4: 131.

Matsuoka S, Ballif B A, Smogorzewska A, et al. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage[J]. science, 2007, 316(5828): 1160-1166.

Matsuoka S, Huang M, Elledge S J. Linkage of ATM to cell cycle regulation by the Chk2 protein kinase[J]. Science, 1998, 282(5395): 1893-1897.

Murray A. Cell cycle checkpoints[J]. Current opinion in cell biology, 1994, 6(6): 872-876.

Nashun B, Yukawa M, Liu H, et al. Changes in the nuclear deposition of histone H2A variants during pre-implantation development in mice[J]. Development, 2010, 137(22): 3785-3794.

Ooga M, Fulka H, Hashimoto S, et al. Analysis of chromatin structure in mouse preimplantation embryos by fluorescent recovery after photobleaching[J]. Epigenetics, 2016, 11(1): 85-94.

Orthwein A, Fradet-Turcotte A, Noordermeer S M, et al. Mitosis inhibits DNA double-strand break repair to guard against telomere fusions[J]. Science, 2014, 344(6180): 189-193.

Palmer N, Kaldis P. Regulation of the embryonic cell cycle during mammalian preimplantation development[J]. Current topics in developmental biology, 2016, 120: 1-53.

Pawlik T M, Keyomarsi K. Role of cell cycle in mediating sensitivity to radiotherapy[J]. International Journal of Radiation Oncology* Biology* Physics, 2004, 59(4): 928-942.

Polo S E, Roche D, Almouzni G. New histone incorporation marks sites of UV repair in human cells[J]. Cell, 2006, 127(3): 481-493.

Price B D, D'Andrea A D. Chromatin remodeling at DNA double-strand breaks[J]. Cell, 2013, 152(6): 1344-1354.

Rothkamm K, Krüger I, Thompson L H, et al. Pathways of DNA double-strand break repair during the mammalian cell cycle[J]. Molecular and cellular biology, 2003, 23(16): 5706-5715.

Russell L B, Russell W L. An analysis of the changing radiation response of the developing mouse embryo[J]. Journal of Cellular and Comparative Physiology, 1954, 43(S1): 103-149.

Salguero I, Belotserkovskaya R, Coates J, et al. MDC1 PST-repeat region promotes histone H2AX-independent chromatin association and DNA damage tolerance[J]. Nature communications, 2019, 10(1): 1-11.

Santos F, Peters A H, Otte A P, et al. Dynamic chromatin modifications characterise the first cell cycle in mouse embryos[J]. Developmental biology, 2005, 280(1): 225-236.

Scully R, Panday A, Elango R, et al. DNA double-strand break repair-pathway choice in somatic mammalian cells[J]. Nature reviews Molecular cell biology, 2019, 20(11): 698-714.

Sedelnikova O A, Pilch D R, Redon C, et al. Histone H2AX in DNA damage and repair[J]. Cancer Biology and Therapy, 2003, 2(3): 233-235.

Shang Z, Yu L, Lin Y F, et al. DNA-PKcs activates the Chk2–Brca1 pathway during mitosis to ensure chromosomal stability[J]. Oncogenesis, 2014, 3(2): e85-e85.

Shinagawa T, Takagi T, Tsukamoto D, et al. Histone variants enriched in oocytes enhance reprogramming to induced pluripotent stem cells[J]. Cell stem cell, 2014, 14(2): 217-227.

Stolz A, Ertych N, Kienitz A, et al. The CHK2–BRCA1 tumour suppressor pathway ensures chromosomal stability in human somatic cells[J]. Nature cell biology, 2010, 12(5): 492-499.

Stucki M, Clapperton J A, Mohammad D, et al. MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks[J]. Cell, 2005, 123(7): 1213-1226.

Terasawa M, Shinohara A, Shinohara M. Canonical non-homologous end joining in mitosis induces genome instability and is suppressed by M-phase-specific phosphorylation of XRCC4[J]. PLoS Genet, 2014, 10(8): e1004563.

Tsvetkov L, Xu X, Li J, et al. Polo-like kinase 1 and Chk2 interact and co-localize to centrosomes and the midbody[J]. Journal of Biological Chemistry, 2003, 278(10): 8468-8475.

Weissenborn U, Streffer C. The one-cell mouse embryo: cell cycle-dependent radiosensitivity and development of chromosomal anomalies in postradiation cell cycles[J]. International journal of radiation biology, 1988, 54(4): 659-674.

Xu Y, Ayrapetov M K, Xu C, et al. Histone H2A. Z controls a critical chromatin remodeling step required for DNA double-strand break repair[J]. Molecular cell, 2012, 48(5): 723-733.

Yukawa M, Oda S, Mitani H, et al. Deficiency in the response to DNA double-strand breaks in mouse early preimplantation embryos[J]. Biochemical and biophysical research communications, 2007, 358(2): 578-584.

Zhang Y, Qian D, Li Z, et al. Oxidative stress-induced DNA damage of mouse zygotes triggers G2/M checkpoint and phosphorylates Cdc25 and Cdc2[J]. Cell Stress and Chaperones, 2016, 21(4): 687-696.