細則様式第１－２号

学位請求論文の内容の要旨

領　域	生体検査科学	分　野	
氏　名	Valerie Goh Swee Ting		

（論文題目）The study of radiation-induced chromosome aberrations in humans and mice for the improvement of cytogenetic biodosimetry techniques and furthered understanding of low dose-rate ionizing radiation.「細胞遺伝学的線量評価法の改善のためのヒトとマウスにおける放射線誘発染色体異常に関する研究および低線量率電離放射線のさらなる理解」

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This cumulative thesis examines radiation-induced chromosome aberrations used in various applications in both humans and mice. For humans, improvements to various cytogenetic biodosimetry techniques were assessed in protocols, chromosome aberration scoring and interpretation of results, to ultimately improve the reliability of dose estimation. This was discussed in Chapters 1 to 4. In our large-scale mouse study, both short and long-term effects of high and low dose-rate ionizing radiation (HDR-IR, LDR-IR) effects on neonatal mice were explored. Radiation exposure during early immune system development was evaluated to examine possible differences in radiation responses between neonates/children and adults. This was discussed in Chapter 5.

Cytogenetic biodosimetry is the use of a dose-response relationship of accumulating chromosome aberration frequency with increasing radiation dose to estimate exposed dose in a suspected individual after a radiation accident. Chromosome aberrations in PHA-stimulated T lymphocytes are evaluated with whole blood (WB) or isolated peripheral blood mononuclear cell (PBMC) cultures. There are four established cytogenetic endpoints: dicentric chromosomes (Dic), micronuclei (MN), translocations (Tr) and prematurely condensed chromosomes. The type of aberrations used for dose estimation depends on factors such as radiation quality, dose-rate, type of dose exposure and time after initial exposure. For acute irradiation less than 4 Gy for partial and whole-body exposures, both Dic and MN can be used, though the former is the preferred gold standard as Dic is not significantly affected by age and sex. For chronic LDR-IR irradiation and blood collection long after initial radiation exposure, Tr is used instead for retrospective dose estimation due to its long half-life.

In **Chapter 1** [Construction of fluorescence *in situ* hybridization (FISH) translocation dose-response calibration curve with multiple donor data sets using R, based on ISO 20046:2019 recommendations], factors involved in FISH-Tr dose-response calibration curve (DRC) construction for reliable dose estimation were explored. To estimate doses in an individual with cytogenetic biodosimetry, a DRC should be reliably constructed with peripheral blood lymphocytes irradiated *ex vivo* at selected doses. In our study, a linear-quadratic DRC for Tr detected in chromosomes 1, 2 and 4 was constructed with generalized linear modelling, using a modified R-script meant for Dic. This DRC was constructed with most of the guidelines recommended by ISO 20046:2019, and was in good agreement with other DRCs previously published in the absence of ISO regulation. In summary, FISH Tr-DRC should be made with at least 3 donors and at least 5 dose points below 1 Gy, spontaneous Tr should be removed with

Sigurdson's age-adjustment equation, cells scored should be converted to cell-equivalents and simple Tr in cells without unstable chromosome aberrations should be scored. This research was published.

In **Chapter 2** [Improved harvest methodology for cytokinesis-block micronucleus assay (CBMN) with isolated human peripheral blood mononuclear cell culture], an alternative protocol for CBMN assay harvest with isolated PBMC culture was proposed, where PBMCs can be fixed and stored for long-term in a cell suspension with commonly used cell harvest reagents in cytogenetic biodosimetry. The current recommended method of PBMC harvest uses cytocentrifugation, which utilizes specialized equipment not commonly found in cytogenetic laboratories, and does not offer the option to store fixed cells in a cell suspension for long-term. In our protocol (CRG), more than 94 % of 1000 binucleated cells (BNC) were scorable with intact cytoplasm, regardless of donor age, sex and humidity during cell spreading, in contrast to 14 - 89 % in established International Atomic Energy Agency (IAEA) harvest protocols for WB. The CRG protocol was validated in multiple laboratories and with multiple stains (Giemsa, DAPI, acridine orange). The CRG protocol also provided an opportunity to compare CBMN parameters of nuclear division index (NDI) and MN frequency in WB and PBMCs of the same donors. NDI is a cell proliferation indicator and MN is a DNA damage marker. Our results showed higher NDI and lower MN frequency in 2 Gy PBMCs than WB, and a higher age-dependent background MN increase in WB than PBMCs. Thus, parameters of CBMN assay should be separately evaluated for WB and PBMC cultures even though target cells analyzed are PHA-stimulated T cells. This research was published.

In **Chapter 3** [CBMN assay performed in 0 and 2 Gy WB and isolated PBMCs in 6-well transwell co-culture system], a follow-up study was performed as differences in NDI and MN frequency were seen in WB and PBMC cultures in single cultures of 15 ml conical centrifuge tubes in Chapter 2. The presence of cytokines in plasma and other cells in WB but absent in PBMCs could influence cell cycle and DNA induction/repair. In this study, 6-well transwell co-cultures and mono-cultures of 0 and 2 Gy WB and PBMCs were performed in various combinations in four donors, as shown below. Transwell 0.4 μm membrane inserts were used to allow soluble factors to pass through, but not cells.

Co-culture pattern A

	1	2	3	4	5	6	7	8
Upper well	PBMC	PBMC-IR	PBMC	PBMC-IR	-	-	PBMC	PBMC-IR
Lower well	WB	WB	WB-IR	WB-IR	WB	WB-IR	-	-

Co-culture pattern B

	1	2	3	4	5	6	7	8
Upper well	WB	WB	WB-IR	WB-IR	WB	WB-IR	-	-
Lower well	PBMC	PBMC-IR	PBMC	PBMC-IR	-	-	PBMC	PBMC-IR

As donor-specific differences were seen in NDI and MN frequency between upper and lower wells of the same culture condition, wells of the same level were compared. Soluble factors in WB and WB-IR, in the absence of direct cell contact, only affected NDI but not MN frequency in PBMC and PBMC-IR. Furthermore, no radiation-induced bystander effect was seen as non-irradiated cells co-cultured with irradiated cells showed similar NDI and MN frequency. This study also supported the earlier hypothesis that WB and PBMC cultures for CBMN assay should be separately evaluated. Differences in culture vessels (15 ml tubes versus 6-well plates) and materials (polypropylene versus polystyrene) could also be responsible for the differences seen in MN frequency between Chapters 2 and 3.

In **Chapter 4** [Shortened 48 h CBMN assay for triage assessment in a radiological mass-casualty accident], a shortened CBMN assay of 48 h cell culture was developed for triage dose assessment in the event of a mass-casualty radiation accident. Quick data-processing and reliable differentiation between a large population of worried-well from individuals exposed to an equivalent whole-body dose of ≤ 2 Gy are essential for triage assessment. Individuals exposed to ≤ 2 Gy require immediate medical attention as defined by REMM in the U.S. Dept of Health & Human Services. Triage assessment with Dic is recommended by IAEA and ISO, due to the short culture time of 48 h. However, Dic scoring requires scorers with prior training to accurately identify Dic and other aberrations in metaphases of 45 or 46

centromeres, and the time taken to manually score 50 metaphases for triage can take up 150 min per individual per scorer. In contrast, MN scoring can be quickly performed without prior knowledge of chromosome karyotypes. In this study, an alternative to triage assessment using CBMN assay was proposed. The conventional 72 h culture time was reduced to 48 h. Both WB and PBMC cultures were examined. Manual scoring of 200 BNC for triage for one individual can be completed in under 15 min for an experienced scorer for both 2 Gy WB and PBMCs in 48 h CBMN. In our Japanese donor population, similar MN frequency was seen in both 48 and 72 h CBMN, but was not seen in previous experiments conducted in a North American population.

Furthermore, the same type of chromosome aberrations evaluated in human blood can also be used to evaluate radiation-induced damage in mouse splenocytes. As compared to detrimental effects of acute HDR-IR, both biologically damaging and bio-positive effects can be seen after chronic LDR-IR. As defined by UNSCEAR, a dose-rate equal to or lower than 6 mSv/h is considered as LDR-IR. In most LDR-IR mouse studies, researchers focused on long-term effects seen after LDR-IR in adult mice, and developmental effects of the fetus after *in utero* exposure in pregnant mice and trans-generational effects in the offspring after parental exposure. To the best of our knowledge, studies comparing LDR-IR and HDR-IR splenic effects during post-natal immune system development in neonatal mice has yet to be performed.

In **Chapter 5** [The short and long-term splenic effects after LDR-IR and HDR-IR in neonatal B6C3F1 mice], a large-scale mouse experiment was conducted in collaboration with the National Institutes for Quantum and Radiological Science and Technology (QST). Male and female neonatal B6C3F1 at 7 days old were irradiated ^{137}Cs γ-rays of 6 mGy/h or 30 Gy/h, to a total of 4 Gy. Spleens from LDR-IR, HDR-IR and Control mice were collected at 14 21, 28, 35, 42, 75, 100, 200, 300, 400 and 500 d. Physical parameters of body weight, absolute spleen mass and spleen index (spleen mass/body weight * 100 %) were monitored. Spleen histology was performed for 14 d to 35 d mice. Chromosome aberrations of unstable Dic and excess acentric fragments (Ace), and stable Tr marker chromosomes were scored in Giemsa-stained splenocyte metaphases. A total of 420 mice and > 89,000 metaphases were analyzed.

In the early phase (14 ~ 35 d), 7 days after HDR-IR (HDR-IR 14 d) mice showed spleen enlargement with a spleen index of > 1, but decreased to similar levels as LDR-IR and control at 35 d. Spleen index of LDR-IR mice was consistently lower than control mice up to 35 d. From spleen histology, HDR-IR 14 d mice had the highest radiation damage, as seen from reduced lymphocyte population in the white pulp and a surprising decrease in erythrocytes and influx in lymphocytes in the red pulp, but were able to recover within 14 days after HDR-IR. No such splenic damage was seen in LDR-IR mice. For chromosome aberrations, unstable aberrations of Dic and excess Ace preferentially increased in LDR-IR than HDR-IR mice, while marker chromosomes preferentially increased in HDR-IR than LDR-IR mice. In the late phase (42 ~ 500 d), spleen index was comparable among LDR-IR, HDR-IR and control, except for 500 d where a large variance was seen in LDR-IR and HDR-IR mice. One HDR-IR 500 d mouse showed possible spleen myeloid lymphoma as cells were able to proliferate in the absence of mitogen stimulation, and showed a consistent deletion at chromosome 2 with Q-banding. For chromosome aberrations, unstable Dic and excess Ace peaked at LDR-IR 28 and 35 d, and decreased to baseline frequency with increasing time. On the other hand, marker chromosomes also showed some decrease with increasing time, but the frequency was still higher than baseline. No sex-specific differences were seen in all parameters analyzed.

In this experiment, dose-rate was more responsible in inducing radiation damage in the spleen than the overall dose of 4 Gy. Likewise, dose-rate also influenced the type of chromosome aberrations accumulated after HDR-IR or LDR-IR. Moreover, differences in spleen index, histology and chromosome aberrations were seen between our experiment and other experiments of adult exposures during LDR-IR and after HDR-IR. These results could provide a new insight in understanding different radiation responses in the radio-sensitive spleen between neonates/children and adults.

【細則様式第１－２号続き】

学位論文のもととなる研究成果としての筆頭著者原著

論 文 題 目	Construction of fluorescence in situ hybridization (FISH) translocation dose-response calibration curve with multiple donor data sets using R, based on ISO 20046:2019 recommendations
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