論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	WHOLE GENOME SEQUENCING-BASED MUTATION ANALYSIS OF HUMAN CELLS IN CULTURE
著者(和文)	MILAIEnkhbaatar
Author(English)	Enkhbaatar Milai
出典(和文)	学位:博士(理学), 学位授与機関:東京科学大学, 報告番号:甲第252号, 授与年月日:2025年3月26日, 学位の種別:課程博士, 審査員:松本 義久,塚原 剛彦,片渕 竜也,鷹尾 康一朗,山村 雅幸
Citation(English)	Degree:Doctor (Science), Conferring organization: Institute of Science Tokyo, Report number:甲第252号, Conferred date:2025/3/26, Degree Type:Course doctor, Examiner:,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Transdisciplinary Science and Engineering, Nuclear Engineering 系 コース
Department of, Graduate major in

申請学位（専攻分野）： 博士 (Science)
Academic Degree Requested Doctor of

学生氏名： Milai Enkhbaatar
Student's Name

審査員主査： Yoshihisa Matsumoto
Chief Examiner

要旨（英文800語程度）
Thesis Summary（approx.800 English Words ）

Thesis is titled ″Whole genome sequencing-based mutation analysis of human cells in culture″ and consists of 6 chapters.

Chapter 1: General Introduction

This chapter provides the background and rationale for the study, focusing on the biological effects of radiation, DNA damage, and repair mechanisms. In particular, cancer and hereditary effects, which are considered to be important among the effects of low-dose and low-dose-rate radiation, are assumed to have no threshold and can occur even at low doses, and are thought to be caused by mutations in somatic cells and germ cells, respectively.

Quantitative mutation analysis has relied on gene-targeted approaches, such as the specific locus targeting (SLT) method, which examines mutations in genes associated with readily observable phenotypic changes. However, in recent years, next-generation sequencing (NGS) technologies has revolutionized mutation research by enabling comprehensive whole-genome sequencing (WGS). Despite these advances, it is pointed out that there has not been enough research using the characteristics of WGS in somatic cells, and it is stated that this could lead to significant progress in elucidating the effects of low-dose and low-dose-rate radiation. The purpose of this study is to analyze radiation-induced somatic mutations by combining the creation of single-cell-derived clones with WGS, and the objectives of each chapter are described in Chapters 3-5.

Chapter 2: General Materials and Methodology

This chapter outlines the materials and methodologies employed in this study, detailing the experimental design and procedures. It includes a description of the cell lines used, their culture conditions, and the parameters governing their maintenance and growth. The radiation exposure described the exposure conditions, dose rates, and irradiation setup. Additionally, the genomic DNA extraction and preparation procedures are described, ensuring high-quality samples for downstream analyses. Finally, an overview of the whole-genome sequencing (WGS) analysis.

Chapter 3: Mutation analysis of human fibroblast NB1RGB cells exposed to low dose rate gamma irradiation by whole genome sequencing.

In this chapter were investigate the mutational effects of low-dose-rate gamma irradiation on human neonatal skin fibroblasts (NB1RGB) using whole-genome sequencing (WGS). NB1RGB cells were seeded into 96-well plates as single cells and cultured for 21 days under three experimental conditions: non-irradiation, 1mGy per day, and 20mGy per day. After the incubation period, individual clonal populations were isolated from each condition. Genomic DNA was extracted from these clones and a non-clonal NB1RGB cell population, followed by WGS analysis.

Across six clones (two from each condition), an average of 888 single-base substitutions (SBSs) was detected. Notably, no significant differences were observed in the number or pattern of SBSs between irradiated and non-irradiated cells.

Chapter 4: Mutation analysis of human lymphoblastoid TK6 cells exposed to X-Ray and nuclear reactor radiation by whole genome sequencing

This chapter examines the mutational effects of X-ray and nuclear reactor radiation on human lymphoblastoid TK6 cells through whole-genome sequencing (WGS). Initially, TK6 cells were seeded into 96-well plates to generate single-cell-derived primary clones. Following this, the primary clones were subjected to irradiation with either 1 Gy of X-rays or a combination of 0.5 Gy of neutrons and 0.5 Gy of X-rays in a nuclear reactor. After irradiation, the cells were re-seeded into

96-well plates to establish secondary clones, which were subsequently analyzed by WGS.

The results demonstrated that the number of single base substitutions (SBSs) was higher in X-ray-irradiated cells compared to non-irradiated controls. However, in the nuclear reactor-irradiated cells, no significant increase in SBSs was observed relative to the non-irradiated group. This outcome suggests that the mutagenic effect of nuclear reactor radiation may be influenced by a cell-killing effect of neutrons, or recovery during irradiation

Chapter 5: Mutation analysis of human lymphoblastoid TK6 cells exposed to low dose rate gamma radiation by whole genome sequencing

This chapter investigates the mutational effects of low-dose-rate gamma radiation on human lymphoblastoid TK6 cells using whole-genome sequencing (WGS). TK6 cells were seeded into 96-well plates to establish single-cell-derived primary clones, which were cultured for 10 days under the same three conditions as described in Chapter 3: non-irradiation, 1mGy per day, and 20mGy per day. Following the culture period, primary clones were isolated, and genomic DNA was extracted from these clones, as well as from a non-clonal TK6 cell population.

WGS analysis was conducted to determine whether dose rate influences both the qualitative and quantitative characteristics of mutations. By comparing the mutation spectra and frequencies among the different exposure conditions, this study aimed to elucidate the potential dose-rate dependency of radiation-induced mutagenesis in TK6 cells.

Chapter 6: Conclusion and perspectives

This chapter summarizes the key findings of the study and discusses future research directions.

In summary, this research establishes a novel approach for analyzing radiation-induced somatic mutations by integrating single-cell-derived clonal analysis with whole-genome sequencing (WGS). By applying this methodology, successfully characterized mutation patterns under various radiation exposure conditions, providing new insights into the mutagenic effects of both low-dose-rate and acute radiation exposure.