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科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Different roles of C/EBP-alpha in chromosome conformation during lung injury of young and old mice

研究課題名（英文）Different roles of C/EBP-alpha in chromosome conformation during lung injury of young and old mice

研究代表者

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研究者番号：８０８７０５２６

交付決定額（研究期間全体）：（直接経費）　　3,500,000円

研究成果の概要（和文）：がんゲノムアトラスによれば、多くのがんでC/EBP-alphaが増幅され、過剰発現しています。肺腺癌A549細胞のヒドロキシ尿素（HU）処理では、C/EBP-alphaが細胞生存を促進し、化学療法耐性に関与しています。RNAシーケンシング解析では、C/EBP-alpha誘導遺伝子を特定し、C/EBP-alpha過剰発現がんの治療ターゲットを探る予定です。同様に、CXCR5-CXCL13軸ががん細胞の化学療法耐性を引き起こす新たな標的として特定されました。

研究成果の学術的意義や社会的意義
This study explores the role of C/EBP-alpha in cell survival, chemotherapeutic resistance under the DNA-replication stress. The study also explores the mechanism underlying aberrant CXCL13 expression in human cancers. Both findings offer insights into therapeutic avenues for combating human cancers.

研究成果の概要（英文）：As per the cancer genome atlas, the C/EBP-alpha in amplified and over expressed in many cancers. Hydroxyurea (HU) treatment of lung adenocarcinoma A549 cells exhibited C/EBP-alpha's role in promoting the cell viability, suggesting the role of C/EBP-alpha in chemotherapeutic resistance. Ongoing RNA-sequencing of HU-treated 293T cells aims to identify C/EBP-alpha induced genes vital for cell survival. This will allow identifying novel therapeutic targets for treatment of C/EBP-alpha overexpressing cancers. A parallel investigation also uncovered a super enhancer region driving aberrant CXCL13 expression in human cancers. The autocrine signaling in CXCR5-CXCL13 amplified cancers can lead to chemotherapeutic resistance. Several p53 target genes were down regulated by CXCR5-CXCL13 axis in human cancer cells of B-lymphoma origin, Colorectal cancer origin and liver cancer origin. This research identified CXCR5-CXCL13 axis as a new target to overcome the chemotherapeutic resistance in cancer.

研究分野：Epigenetics, Chromosome Conformation

キーワード：　C/EBP-alpha　CXCL13　Super-enhancer　p53　CXCR5

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様　式　Ｃ－１９、Ｆ－１９－１（共通）

１．研究開始当初の背景

Background-

Lung injury causing stress to alveolar type 2 (AT2) cells initiates a homeostasis mechanism in these cells, leading to changes in transcription. The exact mode of altered transcription and its key drivers are not well understood. C/EBP-alpha (CCAAT/enhancer-binding protein alpha) acts as an important regulator for lung homeostasis by maintaining cellular homeostasis.

This study aimed to investigate whether C/EBP-alpha-induced chromosome conformation changes lead to specific gene expression alterations that help maintain cellular homeostasis. C/EBP-alpha is known to play a crucial role in various biological processes. The primary goal of this project was to examine the role of C/EBP-alpha induced gene expression in AT2 cells during lung injury. It was hypothesized that the role of C/EBP-alpha could be due to changes in chromosome conformation of AT2 cells. A *Cebpa* knockout mouse model was planned in collaboration based in Kyoto University Hospital to understand the role of C/EBP-alpha in AT2 cells during lung injury. However, the mouse model was not provided by the collaborators despite the initial promise, necessitating a change in the research approach.

C/EBP-alpha is amplified and overexpressed in several cancers, as per the Cancer Genome Atlas (TCGA). Therefore, an alternative approach was used to investigate the impact of C/EBP-alpha-induced genes on the survival of cancer cells after treatment with hydroxyurea (HU), a chemotherapeutic drug, in lung adenocarcinoma A549 cells. HU prevents DNA replication and induces Poly (ADP-ribose) polymerase (PARP) cleavage.

In parallel, to study the original goal of this study in assessing altered chromosome conformation in altered gene expression, *CXCL13* expression in human cancers was analyzed. This project also aligns with the original proposal to decipher how altered chromosome conformation leads to changes in oncogene expression. The focus was on the chemokine CXCL13, which is aberrantly expressed in certain hematological and solid cancers. While the research atmosphere in the laboratory of Dr. Chiaki Takahashi (CT), was not ideal and my research budget was illegally seized by CT. This caused significant delay in acquiring the necessary consumables in year 2023. Later, few administrative bodies of Kanazawa University partially resolved this situation on my appeal.

２．研究の目的

Purpose of the Study- 1) To investigate the role of C/EBP-alpha in the transcriptional activation of genes affecting cancer cell survival upon chemotherapeutic treatments.
2) To decipher the mechanism of altered chromosome conformation leading to aberrant *CXCL13* expression in hematological and solid cancers.

3. Research Methodology

HEK293T cells were treated with 10 mM hydroxyurea for 24 hours with and without C/EBP-alpha overexpression. The RNA samples were then used for RNA sequencing analysis.
In a second approach, Raji cells were used to investigate the interaction between the super-enhancer and the CXCL13 promoter through a chromosome conformation capture (3C) assay.

３．研究の方法

Research Methodology- HEK293T cells were treated with the 10 mM hydroxyurea for 24 hours with/without C-EBP-alpha overexpression. The RNA-samples were used for the RNA-sequencing analysis.
In second approach, Raji cells were used to investigate the Super-enhancer and CXCL13 promoter interaction by chromosome conformational capture (3C) assay.

４．研究成果

Research Results-

PARP cleavage was rescued by C/EBP-alpha overexpression in A549 cells treated with the Hydroxyurea, implying that C/EBP-alpha plays a role in cell viability under DNA replication stress and chemotherapeutic treatment targeting DNA replication inhibitors.

To determine which genes are induced by C/EBP-alpha to prevent PARP cleavage, RNA sequencing samples were prepared from 293T cells treated with and without HU. Differentially expressed genes are beings analyzed to identify the novel C/EBP-alpha target genes under DNA replication stress. This approach addresses the original goal of identifying C/EBP-alpha-induced genes under cellular stress that play regulatory roles in cell viability and homeostasis.

Additionally, a related project on altered chromosome conformation driving aberrant CXCL13 expression in hematological and solid cancers was conducted. A novel super-enhancer located on the *CCNG2* locus drives *CXCL13* expression in cancer cells. CXCL13 is typically expressed only by certain cells, such as follicular dendritic cells and macrophages, but its exceptional expression in cancers was found to be associated with disrupted chromosomal conformation and loss of epigenetic regulation by DNA CpG methyl-binding protein 1 (MBD1) and Ten-eleven translocases (TET). This work is currently being communicated in peer reviewed journal. In addition, these works were presented in the conference listed below.

1. Gothwal Santosh Kumar, Murakami K, PK Mattila (2024). Functional characterization of CXCR5-CXCL13 axis in human Burkitt ' s lymphoma cells reveals novel therapeutic targets for human cancers. Poster presentation as first author at the 11th Takeda Science Symposium on Pharma Sciences, January 25-26, 2024, Osaka, Japan.
2. Gothwal Santosh Kumar *et al.,* (2022). A mutant p53 driven modulation of CXCR5-CXCL13 axis in the migration of human germinal center derived B-lymphomas. Poster presentation as first author at the 81st Japan Cancer Association meeting, September 29 - October 1, 2022, PACIFICO Yokohama, Japan.
3. Gothwal Santosh Kumar *et al.,* (2022) Mutant p53 R213Q promotes the survival and proliferation of Human Burkitt's lymphoma via CXCL13-CXCR5 axis. Poster presentation as first author at the 18th International p53 Workshop at the Weizmann Institute of Science, Israel, May 18-21, 2022.
4. Gothwal Santosh Kumar (2021). SARS-CoV-2 and the Human Body: Perspectives from Its Genetics, Diagnostics to Therapeutics. Invited talk (30 minutes) at the DAILAB Café Series 57, hosted by Tsukuba University and the Indian Institute of Technology, Delhi.
5. Gothwal Santosh Kumar (2020). SARS-CoV-2 and the Human Body: Perspectives from Its Genetics, Diagnostics to Therapeutics.' Invited talk (30 minutes) at the Indian Scientist Association of Japan Symposium, 2020.
6. Gothwal Santosh Kumar (2023) Regulation of Germinal Center derived B-lymphomas during Humoral Immunity. Invited oral presentation (20 minutes) at the 18th National Research Scholars Meet at the Advanced Center for Training and Research in Cancer (ACTREC), Mumbai, India (December 8, 2023).
7. Gothwal Santosh Kumar (2022). 'Germinal Center Confinement and Suppression of B Lymphomagenesis: New Role of p53.' Presented an invited talk at the Duke-National University of Singapore-Kanazawa University Cancer Research Institute (DUKE-NUS-KUCRI) staff meeting. Venue: World Premium Institute of Nano-Life Science, Kanazawa University, Japan, on October 12, 2022.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計2件（うち招待講演　0件／うち国際学会　2件）

１．発表者名 Santosh Kumar Gothwal
２．発表標題 A mutant p53 driven modulation of CXCR5-CXCL13 axis in human B cell lymphoma
３．学会等名 81st Annual meeting of Japan Cancer Association（国際学会）
４．発表年 2022年

１．発表者名 Santosh Kumar Gothwal
２．発表標題 A mutant p53 P72R driven modulation of CXCR5-CXCL13 axis in human B cell lymphoma-migration
３．学会等名 18th International p53 Workshop（国際学会）
４．発表年 2022年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計1件

国際研究集会	開催年
18th International p53 workshop	2022年～2022年

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関