様式 4-1

研究助成 2021－がん領域－

研究成果報告書（最終）＜概要＞

所　　属	国立研究開発法人　理化学研究所
氏　　名	ROBBE Pauline
研 究 テ ー マ	慢性リンパ性白血病を駆動する非コードエレメントの機能解明

- 研究助成報告として広報資料に掲載される点を留意すること。
- 概要の構成は自由とするが、研究目的、研究手法、研究成果などを、1 ページにまとめること。（図表、写真などの貼付を含む）

Despite recent progress in the treatment of Chronic Lymphocytic Leukaemia (CLL), this blood cancer remains largely incurable. This is to a large extent because we do not really fully understand the biology of CLL. We think that one of the underlying reasons for this heterogeneity is the fact that each patient's leukaemia cells carry different genetic changes. Therefore, this research proposal is aiming to identify these genetic changes comprehensively, by using whole genome DNA and transcriptome RNA sequencing. This work especially focus on genetic changes that are noncoding elements, meaning that they are transcribed in RNAs that do not lead to proteins.
Doing so, we aim to improve the ways we predict how patients with CLL respond to treatment using the summary of all the genetic changes that are present in leukaemia cells.

Aim 1: Defining active non-coding regulatory elements and investigating the dysregulation patterns of enhancer RNAs (eRNAs)
We firstly defined enhancers using publicly available data derived from DNA. These regions were defined across the whole genome based on chromatin state data from CLL primary cells. We defined active 56,137 enhancers. Secondly, we performed validation experiments to confirm our findings based on DNA-derived data using RNA-derived data. We obtained clinical samples from the UK Liverpool CLL biobank from 30 patients. We isolated RNA and performed Cap Analysis of Gene Expression (CAGE) is a transcriptomics technique that detects and quantifies transcription starts sites of genes and regulatory elements like enhancers (which transcribes eRNA when they are active). Data is currently being analyzed to precisely measure the dysregulation patterns. Once finalized, the results will be published.

Aim 2: Defining the mutational profile of non-coding elements and identifying non-coding drivers
We investigated the 56,137 enhancers and found 25 enhancers were recurrently mutated more frequently than expected (FDR < 0.1), defined as significantly mutated. We selected some enhancer for further study based on whether they regulated a target gene important in CLL / B-cell biology. Six discrete regions spanning 117 Kb contained 50 variants and were annotated in the previously reported PAX5 super-enhancer. Another region spanning 325kb on chr3q27.2 contained seven significantly mutated enhancers and linked to BCL6. CRISPR knock-out experiments confirmed that this region was necessary to BCL6 expression and cell proliferation. RNA-seq of 8 samples with mutations in this region showed overall increased expression of BCL6, although the effect was heterogenous and suggesting that some variants are more or less pathogenic than others and variants might exert a positional effect. Further validation experiments have been performed and the data is being analyzed. Once finalized, the results will be published.

Aim 3: Integrating all WGS data to accurately predict prognosis in patients with CLL
We combined information of the 25 candidate driver enhancers with other noncoding elements, as well as driver genes, recurrent structural alterations, and many other genomic features. We applied non-negative matrix factorization (NMF) to identify robust subgroups of CLLs sharing subsets of these genomic features. We defined 5 subgroups. Our model predicted individual patients who achieve a plateau after chemo-immunotherapy, and therefore are "functionally cured".

With this research, we wanted to reveal how non-coding regulatory elements can participate to tumorigenesis and influence patients' prognosis.

様式 4-3①

研究助成 2021－がん領域－

研究成果報告書（最終）<発表実績/予定一覧>

所　　属	国立研究開発法人　理化学研究所
氏　　名	ROBBE Pauline

1. 論文発表実績

- 研究助成報告として広報資料に掲載される点を留意すること。
- 掲載年次順（新しいものから）に記入すること。ただし、本研究助成金交付後のものに限る。
- 著者名、論文名、掲載誌名、巻、最初と最後の頁、発表年(西暦)、査読の有無について記入する。
  なお、著者名は省略せず、全てを記入し、自分の名前に下線を引く。
- 国内外雑誌を問わない。
- 印刷中は in press と記入、投稿中の論文はその旨を記載すること。なお学会のアブストラクトは含めない。
- 欄が足りない場合は、増やして記入すること。

1	Pauline Robbe, Kate E. Ridout, Dimitrios V. Vavoulis, Helene Dréau, Ben Kinnersley, Nicholas Denny, Daniel Chubb, Niamh Appleby, Anthony Cutts, Alex J. Cornish, Laura Lopez-Pascua, Ruth Clifford, Adam Burns, Basile Stamatopoulos, Maite Cabes, Reem Alsolami, Pavlos Antoniou, Melanie Oates, Doriane Cavalieri, Genomics England Research Consortium, CLL pilot consortium, Jane Gibson, Anika V. Prabhu, Ron Schwessinger, Daisy Jennings, Terena James, Uma Maheswari, Martí Duran-Ferrer, Piero Carninci, Samantha J. L. Knight, Robert Månsson, Jim Hughes, James Davies, Mark Ross, David Bentley, Jonathan C. Strefford, Stephen Devereux, Andrew R. Pettitt, Peter Hillmen, Mark J. Caulfield, Richard S. Houlston, José I. Martín-Subero & Anna Schuh. Whole-genome sequencing of chronic lymphocytic leukemia identifies subgroups with distinct biological and clinical features. Nat Genet 54, 1675–1689 (2022). https://doi.org/10.1038/s41588-022-01211-y peer-reviewed
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様式 4-3②

2. 学会発表実績

- 発表年順（新しいものから）に記入すること。ただし、本研究助成金交付後のものに限る。
- 発表学会名、発表者名、演題を記入する。
- 国内外を問わない。
- 欄が足りない場合は、増やして記入すること。

	発表時期	発表学会名、発表者名、演題
1	October 2023	International Workshop on Chronic Lymphocytic Leukemia, Boston, USA. Pauline Robbe. Whole-Genome Analysis of CLL and Interplay of Coding, Non-Coding, Transcriptome and Epigenetic Events.
2	July 2023	Japanese society of Hematology International symposium, Tsukuba, Japan. Pauline Robbe. Risk-stratification of chronic lymphocytic leukaemia using whole-genome sequencing
3	December 2022	American society of Hematology congress, New-Orleans, USA. Pauline Robbe. Subclassifying CLL using whole genome sequencing
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3. 投稿、発表予定

	投稿/発表時期	雑誌名、学会名等
1		One publication about enhancer RNAs planned for 2024. Not yet determined
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