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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）MinIONナノポアシークエンサーを用いたHTLV-1関連疾患新規診断法の確立

研究課題名（英文）Establishment of new diagnostic method for HTLV-1 related diseases using MinION nanopore sequencer

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研究成果の概要（和文）：本研究でマルチオミックスアプローチを用いたHTLV-1感染細胞のクローナリティ解析は主に以下4つの成果を含む①NGSを用いたプロウイルス組込み部位に基づくクローナリティ解析は、ATLの分子診断、HTLV-1感染者のATL発生予測及び予後予測に応用できると示唆した。②MinIonシーケンサを用いた簡便、迅速、安価なクローナリティ解析法を開発した。③NGSエキソーム変異解析に基づきATL患者の腫瘍内不均一性（ITH）を証明した。④RNA-seqによるTCRクローナリティ解析はATL診断の個別化.予後予測および治療の評価に有用であり得ることを示した。

研究成果の学術的意義や社会的意義
我々は当研究の結果を4つの査読有学術雑誌や国内外の学会に発表することができた。
また得られた研究結果はATL・その他HTLV-1関連疾患における診断、予後予測、治療および個別化医療の実現への道を開いた。これらの研究成果を応じて本研究は世界の学術研究の進展または日本だけではなく世界中のHTLV-1感染患者の命と暮らしに貢献し続けることができる。

研究成果の概要（英文）：This study, using multi-omics approaches, in pursuit of determining potential biomarkers for disease development could achieve following findings: ①Clonality analysis based on provirus integration sites using NGS technology suggested possible applications of clonality in the molecular diagnosis of ATL, as well as predicting ATL development among HTLV-1-infected individuals (Blood advances 2017 and Human genomics 2017)②Development of a new methodology for easy-to-do, rapid and cheap enough analysis of clonality using MinIon sequencer③ Showing presence of a high degree of intra-tumor heterogeneity(ITH) based on mutations among ATL patients using Whole Exome Sequencing(Neoplasia 2018).④determining TCR clonality by RNA sequencing might be useful for prognostic purposes and for personalizing ATL diagnosis and assessment of treatments(npj Gen. med.2019).

研究分野：Medical genome sciences

キーワード：HTLV-1　ATL　Personalized Medicine　Molecular Diagnosis　Nanopore Sequencer　Intratumor Heterogeneity　TCR clonality　Clonality analysis

１．研究開始当初の背景

Adult T-cell Leukemia (ATL) is a unique neoplasm, directly caused by infection with Human T-cell Leukemia Virus type-1 (HTLV-1). Following HTLV-1 infection, while majority of the infected individuals remain as asymptomatic carriers (ACs), ～5% of them develop ATL after a long latency period. However, there is no clear determinant to distinguish between the people who develop ATL and those who remain ACs. Moreover, the prevention and treatment of ATL are still-unsolved problems, and the factors that determine ATL development remains to be elucidated. HTLV-1 mainly survives, in vivo, by persistent proliferation of infected cells, and eventually causes ATL after a long latency. The clonal expansion of abnormal cells is the hallmark of ATL. Thus, with revolutionized insights, as a next generation study on ATL risk factors, we have focused on monitoring the clonal composition of infected cells using multi-omics approaches.

２．研究の目的

In the current study, as a potential biomarker for disease development, we have focused on multi-omics approaches that enables us to determine clonality structures using three different criteria including the provirus integration sites, T cell receptor rearrangements as well as somatic mutations. These three criteria are unique and stable genomic fingerprints to monitor the dynamics of clonal growth among HTLV-1 infected cells. We have aimed to examine the possible application of clonality for molecular diagnosis and predicting prognosis of ATL development as well as revealing the mechanism for disease development.

３．研究の方法

Taking advantage of Next Generation Sequencing (NGS) technology, we have previously developed an original methodology that enables precise characterization of HTLV-1 infected clones based on the provirus integration sites (Genome Medicine 2014). In the current study, in addition to employing our NGS-based method, we have developed a new method for analysis of clonality using MinION nanopore sequencer. We also developed a pipeline for analysis of intra clonal heterogeneity based on mutation profiles derived from whole exome sequencing data (Neoplasia 2018), as well as devising a workflow for analysis of clonality based on T cell receptor profiles derived from whole transcriptome sequencing (RNA-seq) data (npj Genomic Medicine 2019).

４．研究成果

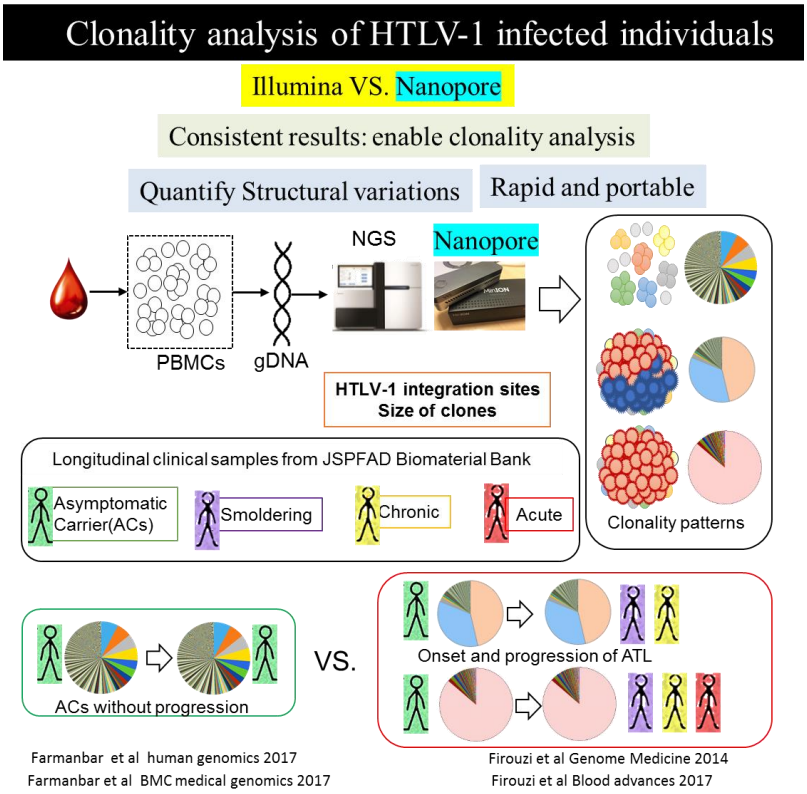
(1) Clonality based on provirus integration sites: Using our NGS-based method (Genome Medicine 2014), we assessed the role of clonality dynamics in ATL onset in 70 single and multiple time-point clinical samples. The results indicated that the clonal expansion of clones occurs several years before onset of aggressive disease, and the patients who progressed to chronic or acute ATL over time, harbored big or very big clones several years in advance. These data proposed potential applications of clonality for molecular diagnosis and predicting prognosis of HTLV-1 infected individuals.

Our study provided the first detailed information regarding the dynamics of HTLV-1clonality and suggested that the clonality could be a useful predictive marker of

ATL onset and progression (Blood advances 2017, Human Genomics 2017, BMC Medical Genomics 2017).

Based on the observed results and using mathematical modeling, we postulated that a quantified clone must be Big(B) or Very Big(B) to dominate over the other rival clones and affect the clinical status of an infected individual thus eventually causes disease progression. Generally, it is known that competition between clones shapes their distribution, but we do not know how a clone wins this competition to undergo clonal expansion. Presumably, a clone needs to become large enough to gain a fitness advantage to out-compete other clones. Moreover, to convert the complex nature of data on clonal expansion into a manageable level of simplicity, we modeled clonal relationships between a set of clones to represent the relationships between clone sizes, their order and individuals' progression status overtime. The proposed mathematical models are fully data-driven, intuitively depicts the clonality patterns of HTLV-1-infected individuals and can assist in early risk assessment of ATL onset by reflecting the prognosis of infected individuals. The proposed models of ATL clonal expansion permits investigation of the impact of clonal expansion and related parameters on the risk of ATL development by offering a new understanding of how clonality patterns contribute to disease progression (Human Genomics 2017, BMC Medical Genomics 2017).

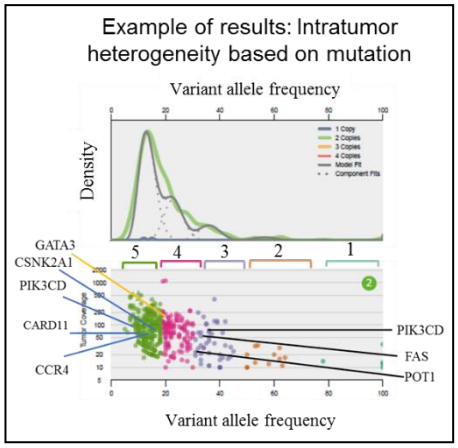
Taken together, we analyzed, modeled and visualized the clonality data by various approaches. we developed an empirical model that illustrates the status of ATL progression and patterns of clonal expansion and enables prediction of the onset and development of ATL. These studies for the first time provided understanding as well as a unique perspective for clarifying the mechanisms of clonal expansion in ATL.

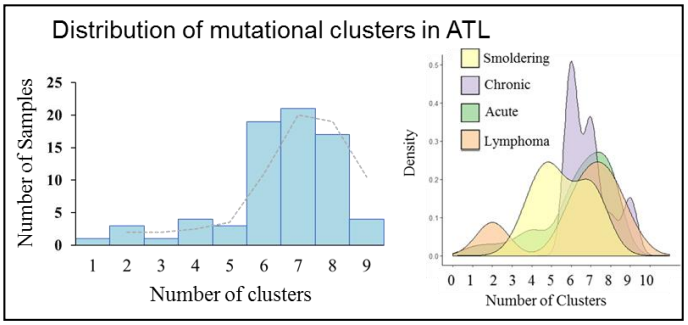


Despite the potential power of my previously developed method based on NGS technology in diagnosis and predicting prognosis, this method is so expensive labor-intensive, slow and needs large sets of laboratory infrastructures and supercomputing systems, also not easily adapted for processing of samples as a routine clinical test. NGS based clonality

analysis requires specialized instrumentation and reagents that are likely to be beyond the limit of diagnostic or regional reference laboratories especially in endemic areas located developing countries. Thus, it is difficult to utilize that method as a first diagnosis test in medical field due to technical and financial drawbacks. Therefore, our main goal for the two years of this project was to devise a method which is easy-to-do, rapid and cheap enough to be performed for personalized medicine and robust genetic tests not only in advanced genomics labs but also in developing countries without any infrastructures. To overcome these limitations, we have used a novel potable USB-size sequencer namely MiniION Nanopore that has been recently developed by Oxford Nonaopore technologies. Taking advantage of this sequencer and a modified library preparation, we could define clonality pattern of HTLV-1 infected individual with minimum handling and equipment. The laboratory infrastructure and computing resources have been used to perform this experiment on the MinION nanopore sequencer would be available not only in most molecular laboratories around the world but also in the field. Realizing the potential advantage of this novel method will have international and local impacts on health of HTLV-1 infected individuals as well as scientific impacts not only in japan but also worldwide.

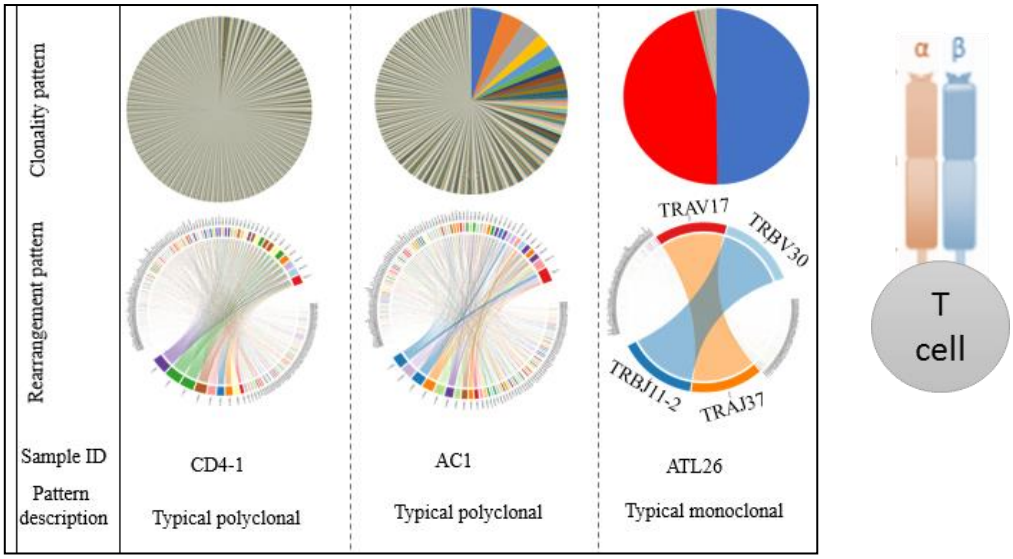
(2) Clonality based on somatic mutation profiles of ATL cells: Intra-tumor heterogeneity (ITH) has been extensively analyzed in a broad range of hematological malignancies and cancers. Recently, different studies have demonstrated the critical role of ITH in development and progression of cancer as well as assessment of therapeutic responses. In this project for the first time, we have conducted a multidisciplinary research on ITH in ATL. I have focused on detecting somatic mutations using 142 tumor/matched-normal samples from 71 patients with different subtypes of ATL by whole exome sequencing (WES) (raw sequencing data from Kataoka et al Nature genetics 2015). We used SciClone to cluster the detected mutations and retrieve clonal architecture from each sample. This study provided invaluable information regarding the mutational clonal architecture of ATL and collectively suggests that ATL displays high degree of ITH and a complex subclonal structure. For the first time, we also defined clonal structure of significantly mutated genes in ATL. The results provide a useful understanding as well as a unique perspective for clarifying the mechanisms of clonal expansion in ATL, and insights into the mechanisms underlying the proliferation of a malignant clone. We published these results in *Neoplasia* 2018





(3) Clonality based on T-cell repertoires:

The clonal distribution of the TCR offers the means to detect and track specific T cells based upon detection of the unique TCR. The TCR repertoire can be altered in the context of infections, malignancies or immunological disorders. Here we examined the diversity of TCR clonality and its association with pathogenesis and prognosis in ATL. Based on these TCR profiles derived from RNA-seq data, CD4-positive cells and ACs showed polyclonal patterns, whereas ATL patients showed oligo- or monoclonal patterns (with 446 average clonotypes across samples). All tumors had some background small TCR clones, but the degree of heterogeneity varied. Expression of TCRa and TCRb genes in the dominant clone differed among the samples. Taken together our results suggested that determining monoclonal architecture and clonal diversity by RNA sequencing might be useful for prognostic purposes and for personalizing ATL diagnosis and assessment of treatments. (*npj Genomic medicine* 2019)



５．主な発表論文等

〔雑誌論文〕（計　5　件）

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npj Genomic Medicine 4, Article number: 10 (2019)　（査読あり）
https://doi.org/10.1038/s41525-019-0084-9

②Farmanbar Amir、**Firouzi Sanaz**、Makalowski Wojciech、Kneller Robert、Iwanaga Masako、Utsunomiya Atae、Nakai Kenta、Watanabe Toshiki
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〔図書〕(計　　0件)
〔産業財産権〕
○出願状況（計　0件)
名称：
発明者：
権利者：
種類：
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〔その他〕
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６．研究組織
(1)研究分担者　なし
研究分担者氏名：
ローマ字氏名：
所属研究機関名：
部局名：
職名：
研究者番号（8桁)：
(2)研究協力者なし
研究協力者氏名：
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