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研究課題名（和文）Identification of novel biomarkers unique to the HIV-1 latent reservoir

研究課題名（英文）Identification of novel biomarkers unique to the HIV-1 latent reservoir

研究代表者
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研究成果の概要（和文）：現存の抗ウイルス薬はHIVを根絶できないため、HIV感染を予防するワクチンの開発やHIVを排除可能な新たな完治療法が求められている。このためには、残存するウイルスの特徴を捉えることが重要である。私の母国であるタンザニアではHIV感染症が成人の５％に達することから、母国でコホートを作り、抗ウイルス療法(ART)下でウイルスが抑制された感染者（N=115)をリクルートした。残存するプロウイルス量を測定したところ、これまでに先進国で報告された値の範囲内であった。配列解析をしたところ、薬剤耐性変異はほとんど認められなかった。現在、全長のウイルスゲノムを増幅する条件検討を引き続き実施している。

研究成果の学術的意義や社会的意義
リザーバーウイルスの性状、特に途上国で広がるウイルス変異株での現状を明らかにすることで、エイズが蔓延する地域におけるHIV診療、医療の課題解決に貢献できるとともに、新たな薬剤やワクチン開発に向けた基盤情報を提供できる。

研究成果の概要（英文）：Although the antiretroviral therapy (ART) successfully prolongs lives of HIV-infected patients, the current therapy is insufficient to eradicate infected cells. Understanding the characteristic of the cells harboring provirus is thus crucial. Taking in consideration of the HIV pandemicity in my home country Tanzania, I established a cohort of successfully treated HIV-infected patients (N=115). Firstly, the median copy number of proviral DNA in Tanzania showed within the ranges reported in subtype B-infected patients in the developed contries. Also, sequencing analyses of a part of the viral genome revealed that the treated subjects relatively rarely harbored drug-resistant mutations in proviral genome, suggesting that majority of rebound viruses potentially remained sensitive to ART in Tanzania despite nearly 30% of pretreatment drug resistant mutations were found. Attemts to amplify near full length proviral genome from some of these patients' specimens are still in progress.

研究分野：感染免疫学

キーワード：HIV-1感染症

１．研究開始当初の背景

(Scientific background and the key scientific question)

Latent HIV-1 infection occurs when either activated CD4+ T cells become dormancy or dormant T cells in lymphoid tissues become infected. The proviral DNA harbored in the dormant T cells can hardly be eliminated by antiretroviral (ART) drugs and CTL responses. This is because most of ART drugs and CTL responses target viral proteins that are involved in the viral replication cycle. However, in regards to HIV-1 latency, factors that are associated with induction of HIV-1 latency are still not well understood, and they are very essential for a better design of the HIV therapy.

On the other hand, a plenty of ART-treated patients can control HIV replication down to undetectable level over a long period of time. However, rebound viremia is consistently observed when the ART therapy is interrupted. This rebound viremia suggests the ability of replication-competent viruses to sustain drug pressure over some years and hence serve as reservoir virus in the human host. Therefore, the characterization of both the reservoir virus as well as the cells that harbor these viruses (including both replication-competent and defective ones) is very crucial in order to eliminate the HIV reservoir. However, one major obstacle to this approach is to how we can distinguish the reservoir from uninfected cells in vivo.

２．研究の目的

(The purpose of the research project)

Although recent advance in ART helped in improving health outcome of the HIV-infected individual, but existence of factors like drug toxicities, drug-specific resistance mutations as well as persistence of the residual replication virus in the reservoir hinder the efficacy of these drugs for long-term treatments. As maintaining long-lived latently infected viral reservoir cells, impose a major challenge to the eradication of the virus within the body of individuals.

Also, I would like to emphasize that HIV-1 infection remains pandemic in my home country Tanzania. Poor adherence secondarily to either low ART drug supply or failure for the individual to access the health care facility will always lead to rebound viremia to even a well clinically ART stabilized patients. Therefore, by overcoming various hurdles, I want to contribute to developing new therapeutics and to improving healthy lives in the resources limiting setting such as my home place Sub-Sahara Africa.

３．研究の方法

(1) Identifying the clinically cART stabilized patients in Tanzania cohort

The subjects will be selected among of the participants in an established Tanzania cohort; these individuals should have the undetected plasma viral load for a period of at least two years during their cART at the time of the sample collection. However, the plasma viral loads are always reconfirmed at time of sample collection to avoid including any individual with rebound viremia.

(2) Quantification of proviral DNA copy numbers

Host genomic DNA was prepared from 2-5 million PBMC isolated from Tanzanian HIV-infected patients. Proviral DNA copy numbers were quantified using quantitative PCR method as previously described (Malnati, et. al., Nature Protocol 3, 1240-1248, 2008). The amplification primers span LTR-gag region that is highly conserved among group M viruses including subtype A, C and D dominantly circulating in Tanzania.

(3) Sequencing analysis of proviral DNA

Using host genomic DNA prepared as above, a part of HIV-1 proviral including the genes encoding protease, reverse transcriptase, and integrase was PCR amplified and analyzed for nucleotide sequencing by Sanger method.

４．研究成果

(1) Proviral DNA copies in successfully cART-treated individuals in Tanzania

We first recruited patients who showed suppressed viremia at least 1-2 years with the detailed characteristics shown in the Figure 1. Virally suppressed subjects during ART showed significantly lower proviral DNA load (Figure 2) even in the resource limiting setting in Sub Sahara African countries Tanzania. The extent of proviral copy number seemed to be within the values reported so far in the developed countries.

Figure 1 Study subjects and approach

Virally suppressed subjects (n=115)	
Demographic data	
Gender -female (%)	96(83.5)
Clinical data	
Median Years on treatment (Range)	9 (2-18) 26 missing data
Median recent CD4 count (Range).	554(119-1303) *missing data 57*
Median initial CD4 count (Range)	145 (00-1606) *41 missing data*
Current ART regimen(%)	
NNRTI based first line regimen	94(81.7)
PI based second line regimen	20(17.4)
missing data	1(0.9)

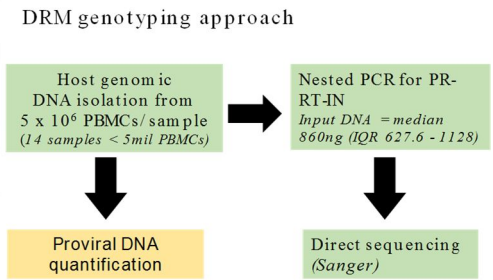


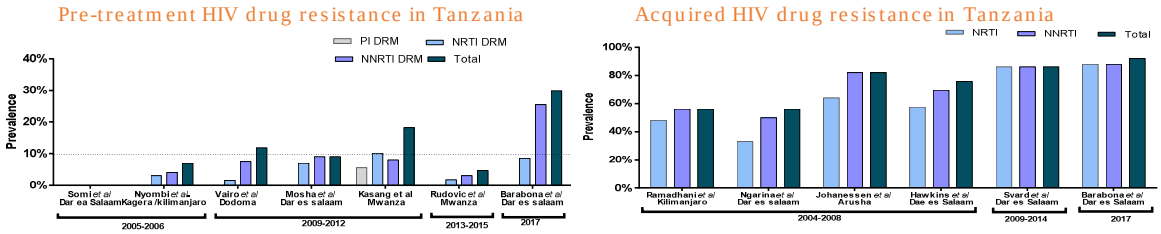
Figure 2 Proviral DNA load among virally suppressed subjects vs ART naïve and treated but viremic subjects

Proviral DNA load is significantly lower in virally suppressed compared to ART naïve or treated but viremic subjects

(2) Drug-resistance mutations in proviral DNA in successfully cART-treated individuals in Tanzania

We initially analyzed the drug-resistant mutations in currently circulating in Tanzania using plasma viral RNA isolated from treatment naïve patients. We found that nearly 30% of treatment-naïve patients harbored at least one mutation causing decreased sensitivity to one of antiretroviral drugs (Figure 3).

Figure 3 DRM in viral RNA



Because of such high prevalence of pre-treatment drug resistant mutations, we next analyzed drug-resistant mutations in proviral DNA from subjects who were ART treated and suppressed viremia over 1 year. The results showed that these successfully treated subjects had relatively rarely harbor drug-resistant mutations in proviral genome, suggesting that majority of rebound viruses potentially could remain sensitive to currently available ART. However, we did not successfully amplify near-full length proviral genome from host genomic DNA of these subjects. This issue remains further attempts.

Figure 4 DRM were relatively rare among virally suppressed subjects

- Amplification = **70/114 (61 %)** DNA samples from PBMCs
- Sequencing = 65/70
- **Stop codon**, **APOBEC mutation**, **frame shift mutations** = **11/65 (17%)**

#	ID	Proviral Load (copies/10^6 PBMCs)	RX	Years on Treament	PI DRM	NRTI DRM	NNRTI DRM
1	SS09	324.6	1ST- LINE	14	_		E138A
2	SS15	341.1	1ST- LINE	2	_		E138A
3	SS18	468.4	2ND LINE	14	_	M184MV	K103KN, Y181YC
4	SS30	491.6	1ST- LINE	10	_		E138A
5	SS 63	418.9	1ST- LINE	_	_	A62AV	
6	SS 68	446.4	1ST- LINE	7	_		E138EA

- **At least one DRM were present in 6/54 (11.1%) intact sequences**
- **NNRTI DRM was dominated by HLA associated polymorphism E138A**
- **Only one subject harboured DRM relevant to ART regimen in Tanzania**

５．主な発表論文等

〔雑誌論文〕　計4件（うち査読付論文　4件／うち国際共著　4件／うちオープンアクセス　0件）

１．著者名 1.Mwimanzi F, Toyoda M, Mahiti M, Mann J, Martin J, Bangsberg D, Brockman M, Goulder P, Kirchhoff F, Brumme Z, Ndung ' u T, Ueno T	４．巻 91
２．論文標題 Resistance of major histocompatibility complex class B (MHC-B) to Nef-mediated downregulation relative to that of MHC-A is conserved among primate lentiviruses and influences antiviral T cell responses in HIV-infected individuals.	５．発行年 2018年
３．雑誌名 J Virol	６．最初と最後の頁 e00725-17
掲載論文のDOI（デジタルオブジェクト識別子） 10.1128/JVI.01409-17	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Barabona Godfrey、Mahiti Macdonald、Masoud Salim、Mbelele Peter、Mgunya Amina Shaban、Minja Lilian、Sunguya Bruno、Shigemi Urara、Matsuda Masakazu、Hachiya Atsuko、Iwatani Yasumasa、Lyamuya Eligius、Ueno Takamasa	４．巻 74
２．論文標題 Pre-treatment and acquired HIV drug resistance in Dar es Salaam, Tanzania in the era of tenofovir and routine viral load monitoring	５．発行年 2019年
３．雑誌名 Journal of Antimicrobial Chemotherapy	６．最初と最後の頁 3016～3020
掲載論文のDOI（デジタルオブジェクト識別子） 10.1093/jac/dkz272	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Barton John P、Rajkoomar Erasha、Mann Jaclyn K、Murakowski Dariusz K、Toyoda Mako、Mahiti Macdonald、Mwimanzi Phillip、Ueno Takamasa、Chakraborty Arup K、Ndung ' u Thumbi	４．巻 5
２．論文標題 Modelling and in vitro testing of the HIV-1 Nef fitness landscape	５．発行年 2019年
３．雑誌名 Virus Evolution	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.1093/ve/vez029	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Masoud Salim、Kamori Doreen、Barabona Godfrey、Mahiti Macdonald、Sunguya Bruno、Lyamuya Eligius、Ueno Takamasa	４．巻 -
２．論文標題 Circulating HIV-1 Integrase Genotypes in Tanzania: Implication on the Introduction of Integrase Inhibitors-Based Antiretroviral Therapy Regimen	５．発行年 2020年
３．雑誌名 AIDS Research and Human Retroviruses	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.1089/AID.2020.0021	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計2件（うち招待講演　1件／うち国際学会　1件）

1．発表者名 Godfrey Barabona, Macdonald Mahiti, Salim Masoud, Peter Mbelele, Amina Shaban Mgunya, Lilian Minja, Bruno Sunguya, Eligius Lyamuya, Takamasa Ueno
2．発表標題 CIRCULATING HIV-1 DRUG RESISTANCE MUTATIONS IN DAR ES SALAAM TANZANIA; IMPLICATION TO PRE EXPOSURE PROPHYLAXIS
3．学会等名 HIVR4P（国際学会）
4．発表年 2018年

1．発表者名 Godfrey Barabona, Macdonald Mahiti, Salim Masoud, Doreen Kamori, George Judicate, Kyoko Yamada, Amina Shaban Mgunya, Lilian
2．発表標題 HIV drug resistance mutations in virally suppressed Tanzanians on long term ART
3．学会等名 第33回日本エイズ学会学術集会（招待講演）
4．発表年 2019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