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論文題目	FRET-based detection and quantification of HIV-1 Virion Maturation （FRET を用いた HIV-1 成熟ウイルス粒子の検出と定量）		

（論文内容の要旨）

Several studies have shown that defective proviral HIV-1, which forms the majority of integrated HIV-1 DNA, can lead to the generation of viral proteins and virus-like particles (VLPs). These proteins serve as immunogens for chronic immune system stimulation, contributing to the higher burden of non-infectious chronic diseases seen even in virally suppressed people living with HIV. Furthermore, like HIV virion maturation, VLPs may be capable of Gag processing by the viral protease, increasing their immunogenicity. There is a need, therefore, for tools that can precisely assess maturation rates of both intact and defective HIV-1 particles.

The present thesis describes the development of a fluorescence microscopy tool that can assess virion maturation using Fluorescence Resonance Energy Transfer (FRET). HIV-1 Gag-iFRET (iFRET) is a NL4-3 derivate that contains a FRET fluorescent protein pair (CFP and YFP) inserted between the MA and CA domains of Gag, linked by protease cleavage sequences. In immature virions labeled with iFRET, CFP and YFP are linked and emit an intense FRET signal. In mature virions, they are cleaved apart and the FRET signal decreases. HIV-1 Gag-iFRETΔPro (iFRETΔPro), the protease deficient variant of iFRET, produces solely immature virions and was used as a FRET signal positive control.

Virions were produced using the iFRET or iFRETΔPro constructs together with their NL4-3 or NL4-3ΔPro parent plasmids, respectively, at a 1:10 ratio. Western Blot analysis of both producer cells and virion lysates showed efficient FRET labeling and Gag-iFRET processing, but not Gag-iFRETΔPro processing, as intended. Transmission Electron Microscopy (TEM) of iFRET labeled virions confirmed that they had similar mature and immature morphologies as parental NL4-3, at comparable ratios. iFRETΔPro labeled virions displayed only immature morphologies.

Single virion images were then taken using both FRET (CFP) excitation and control YFP excitation, fluorescence intensity data was extracted from the images and semi-automatically processed to calculate the FRET ratio of every particle. Counts ranged between 17,000 to 77,000 virions. Using the iFRETΔPro labeled virions as reference for FRET signal intensity range of immature virions, the proportion of immature virions in the iFRET samples was quantified to be 22.4% (±2.4%) [mean(±SD)]. TEM images in this study (18%) as well as other studies (10-20%) have shown comparable rates of immature virions.

To determine if the technique was sensitive to changes in the immature virion population, virus was produced under treatment with Darunavir, a protease inhibitor. The technique was capable of detecting dose-dependent shifts in immature virion population and allowed the calculation of a 50% effective concentration (EC_{50}) against virion maturation of 7 nM Darunavir. The 50% inhibitory concentration of infectivity was 2.8 nM, showcasing that infectivity is not a robust surrogate for maturation inhibition.

The technique described in this thesis can perform faster and large-scale determinations of Gag maturation rates and will be useful for the study of VLPs and their role in immune system stimulation. Furthermore, HIV-1 Gag-iFRET labeling and imaging can be used to measure the direct effect on maturation of protease inhibitors and determine their EC_{50}. These applications will deepen the knowledge in the HIV field regarding the long term, non-infectious consequences of living with HIV and regarding the complex pharmacodynamics of protease inhibitors.

（論文審査の結果の要旨）

HIV-1 プロテアーゼによる HIV-1 Gag 分子の切断は、HIV-1 ウイルス粒子が感染性を持つ成熟ウイルス粒子となるために必須の過程であり、そのプロテアーゼ活性の阻害は HIV-1 感染症治療薬の主な治療標的の一つとなっている。その成熟過程は電子顕微鏡を用いて観察できるが、定量的かつ高速にウイルス粒子成熟過程を可視化する方法はなく、新たな方法の開発が期待されている。

本研究では、蛍光共鳴エネルギー移動（FRET）を用いて HIV-1 ウイルス粒子の成熟過程を評価するため実験系を確立した。 HIV-1 Gag 遺伝子内に CFP と YFP 配列を挿入した HIV-1 iFRET を共発現させることで HIV-1 ウイルス粒子をラベルした。蛍光顕微鏡で FRET 励起と YFP 励起による蛍光を単一ウイルス粒子レベルで検出、画像化し、自動処理でウイルス粒子の FRET 比を計算するプログラムを開発した。プロテアーゼ欠損ウイルス粒子の FRET 強度により未成熟ウイルス粒子を定義することで未成熟ウイルス粒子の比率を測定し、電子顕微鏡で観察される未成熟粒子の比率と同程度であることを確認した。プロテアーゼ阻害剤であるダルナビルで処理した際の感染抑制率と本実験系で測定した未成熟ウイルス粒子の比率に相関があることを確認し、本実験系が有効な HIV-1 ウイルス粒子成熟過程を定量的に測定することを実証した。

以上の研究は HIV-1 ウイルス粒子成熟過程の定量化測定技術の開発に貢献し、HIV-1 ウイルスの成熟過程のメカニズム解明に寄与するところが多い。

したがって、本論文は博士（　医学　）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、令和　３年　３月　１日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。

要旨公開可能日：　　年　　月　　日　以降