様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　３ 年　 ４ 月 ２６ 日現在

機関番号：１３３０１
研究種目：研究活動スタート支援
研究期間：2019～2020
課題番号：１９Ｋ２３８４１

研究課題名（和文）Direct visualization of conformational dynamics of hemagglutinin and interaction between hemagglutinin and exosomes using high-speed atomic force microscopy.

研究課題名（英文）Direct visualization of conformational dynamics of hemagglutinin and interaction between hemagglutinin and exosomes using high-speed atomic force microscopy.

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　2,200,000円

研究成果の概要（和文）：本研究では、高速原子間力顕微鏡（HS-AFM）を用い、インフルエンザAのヘマグルチニン（HA）、さらにHAとエキソソームとの相互作用のナノ分子動態を明らかにすることに成功した。pHが中性の溶液中では、HAは楕円形であるが、酸性では融合性遷移を経てY字型になった。この変化は「アンケージング」モデルに適合する可能性が高いことが示唆された。さらに、HAとエクソソームとの相互作用についてHS-AFM解析を行い、酸性条件下でエクソソーム表層にHA融合ペプチドが挿入されることを見出した。この結果は、この後エクソソームの変形や破裂が引き起こされる可能性があることを示唆するものであった。

研究成果の学術的意義や社会的意義
This study could provide important insights that are essential for vaccine and antiviral drug developments to cope annual flu season in Japan. Besides, our study also suggest that HS-AFM is a feasible tool to complement other imaging tools and bioassays to enhance virology research in near future.

研究成果の概要（英文）：The native conformation of human influenza A hemagglutinin (HA) observed using HS-AFM is ellipsoidal, and it undergoes fusogenic transition in acidic condition to become Y-shape. Direct real-time observation of fusogenic transition suggests that the transition mechanism is likely to fit to the Uncaging Model. HA-exosome interaction is weak in neutral condition but firm in acidic condition. The weak interaction could be mediated by HA-receptor (sialic acid) interaction. In contrast, the firm interaction implies that HA fusion peptide could be released after fusogenic transition, and then inserted into exosomal layer and resulted deformation or rupture of exosomes.

研究分野：病理病態学、感染・免疫学およびその関連分野

キーワード：Hemagglutinin　Influenza A　HS-AFM　Fusogenic transition

１．研究開始当初の背景(Background at the beginning of the Research)

Hemagglutinin (HA), one of important virulence factors of Influenza A virus, is synthesized as a trimeric precursor (HA0) and requires host protease cleavage to become functional. HA mediates viral entry by orchestrating fusion between viral membrane and host endosomal membrane, to release viral ribonucleoprotein complex into host cytoplasm for viral replication. Conformational dynamic of HA, especially the acidic-induced fusogenic transition, has been actively studied using various tools such as X-ray crystallography, Cryo-EM, and FRET (Benton et al PNAS 2018; Das et al Cell 2018), to decipher the mechanism behind this process. *However, the real-time visualization of native structure and conformational changes of HA remain elusive due to technical limitation*. The key scientific question in this proposal- "**Can we directly visualize the real-time structure and conformational changes of HA protein at nano-scale range**?". It looks difficult but still possible to answer since the applicant has used HS-AFM to conduct real-time observation on HA0 trimer of H5N1 (Lim et al Biochim Biophys Acta 2019). It has been reported that exosome-like vesicles with sialic acid in human tracheobronchial epithelial (HTBE) cell culture secretions could neutralize influenza A virus (Kesimer et al FASEB J 2009). Furthermore, HA-exosome interaction in acidic environment can be used to mimic the HA-endosome interaction during viral entry because both exosome and endosome are derived from multivesicular bodies. *The other challenge of the project is to investigate the interaction between exosomes and viral factors, which is still undetermined*. HS-AFM is feasible to study interaction of biomolecules and hence the applicant intended to use HS-AFM to record the interaction between exosome and recombinant HA proteins.

２．研究の目的(Purpose of the Research)

The **purpose** of this study is to investigate the native structure of HA of human influenza A viruses (H1N1 and H3N2), fusogenic transition of HA, and interaction between HA and exosome in neutral or acidic condition. The project provides **significant finding that reveal the mechanism of fusogenic transition of HA in real time**. In addition, **HA-exosome interaction in neutral or acidic**

condition could reflect the interaction between HA and its receptor (sialic acid) in neutral condition or the interaction between HA and lipid membrane in acidic condition. The applicant and collaborators are going to be the first team to demonstrate the complex conformational dynamics of HA and its interaction with exosomes using HS-AFM.

３．研究の方法(Research Methods)

Native conformation and fusogenic transition of HA were observed using HS-AFM. Furthermore, western blotting was also performed to support fusogenic transition of HA in acidic condition. High purity exosomes derived from PC-9 cell line were isolated using Tim-4 affinity purification. To observe HA-exosome interaction, HA was added into chamber while scanning exosomes under either neutral or acidic buffer. Imagej was used for image processing of HS-AFM images, spatial dimension analysis, and generation of refined HS-AFM movies.

４．研究成果(Research results)

The native conformation of human influenza A hemagglutinin (HA) observed using HS-AFM is ellipsoidal, and it undergoes fusogenic transition in acidic condition to become Y-shape. Direct real-time observation of fusogenic transition suggests that the transition mechanism is likely to fit to the Uncaging Model. HA-exosome interaction is weak in neutral condition but firm in acidic condition. The weak interaction could be mediated by HA-receptor (sialic acid) interaction. In contrast, the firm interaction implies that HA fusion peptide could be released after fusogenic transition, and then inserted into exosomal layer and resulted deformation or rupture of exosomes.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　1件／うちオープンアクセス　1件）

１．著者名 Lim Keesiang、Kodera Noriyuki、Wang Hanbo、Mohamed Mahmoud Shaaban、Hazawa Masaharu、Kobayashi Akiko、Yoshida Takeshi、Hanayama Rikinari、Yano Seiji、Ando Toshio、Wong Richard W.	４．巻 20
２．論文標題 High-Speed AFM Reveals Molecular Dynamics of Human Influenza A Hemagglutinin and Its Interaction with Exosomes	５．発行年 2020年
３．雑誌名 Nano Letters	６．最初と最後の頁 6320～6328
掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/acs.nanolett.0c01755	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計4件（うち招待講演　0件／うち国際学会　0件）

１．発表者名 Lim Kee Siang
２．発表標題 High-Speed AFM Reveals Molecular Dynamics of Human Influenza A Hemagglutinin and Its Interaction with Exosomes
３．学会等名 MBSJ (The Molecular Biology Society of Japan) 2020
４．発表年 2020年

１．発表者名 Lim Kee Siang
２．発表標題 High-Speed AFM Reveals Molecular Dynamics of Human Influenza A Hemagglutinin and Its Interaction with Exosomes
３．学会等名 WPI NanoLSI Symposium (4th)
４．発表年 2020年

１．発表者名 Lim Kee Siang
２．発表標題 Direct visualization of avian influenza H5N1 hemagglutinin precursor and its conformational changes by high-speed atomic force microscopy.
３．学会等名 1st WPI NanoLSI-iCeMS Joint Symposium on Nanoimaging and Advanced Materials for Life Science
４．発表年 2020年

１．発表者名 Lim Kee Siang
２．発表標題 Direct visualization of avian influenza H5N1 Hemagglutinin precursor and its conformational change by high-speed atomic force microscopy.
３．学会等名 MBSJ 2019
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

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0件

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