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　４ 年　 ４ 月 ２６ 日現在

機関番号：８２６０３
研究種目：基盤研究(C)（一般）
研究期間：2019～2021
課題番号：１９Ｋ０７５８６

研究課題名（和文）Viral regulation of host RNA degradation and its implication on hepatocarcinogenesis.

研究課題名（英文）Viral regulation of host RNA degradation and its implication on hepatocarcinogenesis

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　3,400,000円

研究成果の概要（和文）：我々はHBxタンパク質がRNAエクソソーム因子であるSKIV2Lを誘導、ISGであるOAS2を抑制することで、宿主やウイルスRNAの安定性を制御することを明らかにした。HDVはHBV感染に依存するサテライトウイルスである。我々はHDVにおいてもSKIV2L、OAS2によるHDV複製の上昇を確認し、HBVと同様のHBx-SKIV2L-OAS2を介する複製への関与を考えた。HBx単体の発現ではOAS2に変化はなかったが、HBV WTではSKIVL2-OAS2を介するHDV複製は上昇し、HBx欠損HBVでは変化がなかった。以上のことから、SKIV2LはHBV、HDV複製に重要であることが示唆された

研究成果の学術的意義や社会的意義
We found that the RNA exosome co-factor (SKIV2L) regulate Hepatitis Delta Virus (HDV) replication by the suppression of interferon stimulated gene (OAS2) expression.

研究成果の概要（英文）：We found that HBx protein significantly induced the expression of the RNA exosome co-factor (SKIV2L) suggesting that it may regulate the stability of other host /viral mRNA. Silencing of SKIV2L significantly enhanced the expression of OAS2. Hepatitis Delta Virus (HDV) is a satellite virus that depends on HBV. We found that SKIV2L significantly induced HDV replication. OAS2 played important role in this regulation. We hypothesized that HBx may increase cellular permissiveness to HDV infection by increasing SKIV2L levels and in turn suppressing OAS2 expression. We did not find any significant effect of HBx expression on OAS2 levels. We observed similar results by transfecting WT or a mutant HBV lacking the expression of HBx. From these results we concluded that there are no significant effect of SKIV2L on the HBx-mediated changes in cellular transcriptome. We also found that SKIV2L plays an important role in supporting HDV infection by suppressing host ISGs especially OAS2.

研究分野：Molecular Virolog

キーワード：SKIV2L　HBV　RNA degradation

１．研究開始当初の背景

The abundance and stability of mRNAs can significantly affect the expression of genes encoded by these mRNAs. We found that Hepatitis B x protein significantly induced the expression of the RNA exosome co-factor (SKIV2L). Since SKIV2L is a key player in RNA-exosome mediated RNA decay, our data suggested that through the regulation of HBx expression, HBx can possibly regulate the stability of another host /viral mRNA.

２．研究の目的

We aimed to analyze the HBx-mediated regulation of host and viral transcriptome and the possible contribution of RNA exosome-mediated RNA decay in this regulation.

３．研究の方法

1-We analyzed the changes in cellular transcriptome induced by HBV infection in chronic HBV patients in published microarrays.
2-We also performed in vitro experiment using RNA seq analysis, in which we analyzed the changes in the transcriptome that is induced by HBx overexpression in normal hepatocytes, or when HBx was overexpressed in hepatocytes in which SKIV2L expression was silenced with specific siRNA.

４．研究成果

1- The analysis of the change in the transcriptome of liver cells showed that the expression of MafF and KIF4 was upregulated in chronic HBV patients. These data confirmed the importance of these gens since they were also identified by our siRNA screening as important regulators of HBV infection. We analyzed its mechanisms and found that (MafF) acts as anti-viral HBV host factor that is induced by IL-1β and suppress the transcription of HBV-pgRNA which is required for viral replication **(Ibrahim MK et al. JVI, 2021)**. We also found (KIF4) to play a pro-viral effect by inducing the transport of NTCP from the cytoplasm to the cell surface where it acts as a receptor for HBV and HDV infection **(Gad SA. et al; Plos Pathogens March 2022)**. We analyzed the role of HBx on the regulation of the expression of these genes, and we found no significant effect of HBx on MafF or KIF4 expression (unpublished data).

2- When we performed RNAseq analysis and analyzed the host genes regulated by SKIV2L, we found that silencing of SKIV2L significantly enhanced the expression of several interferon stimulated genes (ISGs), especially OAS2.
We also analyzed the effect of SKIV2L on the RNA of other virus which is known to accompany HBV infection. Hepatitis Delta Virus (HDV) is a satellite RNA virus that depends on HBV. We found that SKIV2L significantly induced HDV replication. Further analysis showed that OAS2 played important role in this regulation. This effect was not observed in cells in which OAS2 expression was silenced.
Based on these data, we initially hypothesized that HBx may increase cellular permissiveness to HDV infection by increasing SKIV2L levels and in turn suppressing OAS2 expression. However, we found no direct effect of HBx on OAS2 levels. These data hypothesized that SKIV2L regulate OAS2 expression in HBx-independent mechanism.
From these results we found that SKIV2L plays an important role in supporting HDV infection by suppressing host ISGs especially OAS2. The mechanism by which SKIV2L affect OAS2 expression and the mechanism by which OAS2 affects HDV replication is under investigation.

Published Papers

1- Marwa K Ibrahim, Tawfeek H Abdelhafez, Junko S Takeuchi, Kosho Wakae, Masaya Sugiyama, Masataka Tsuge, Masahiko Ito, Koichi Watashi, Mohamed El Kassas, Takanobu Kato, Asako Murayama, Tetsuro Suzuki, Kazuaki Chayama, Kunitada

Shimotohno, Masamichi Muramatsu, **Hussein H Aly**, Takaji Wakita. MafF is an antiviral host factor that suppresses transcription from Hepatitis B Virus core promoter. **Journal of Virology**, August 2021 Volume 95 Issue 15 e00767-21. **(Co-Corresponding Author) (Peer Reviewed)**

2- Sameh A Gad, Masaya Sugiyama, Masataka Tsuge, Kosho Wakae, Kento Fukano, Mizuki Oshima, Camille Sureau, Noriyuki Watanabe, Takanobu Kato, Asako Murayama, Yingfang Li, Ikuo Shoji, Kunitada Shimotohno, Kazuaki Chayama, Masamichi Muramatsu, Takaji Wakita, Tomoyoshi Nozaki, **Hussein H Aly**. The kinesin KIF4 mediates HBV/HDV entry through the regulation of surface NTCP localization and can be targeted by RXR agonists in vitro. **Plos Pathogens**, 2022 Mar 21;18(3):e1009983. doi: 10.1371/journal.ppat.1009983. **(Corresponding Author) (Peer Reviewed)**

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1- Sameh A. Gad, Masaya Sugiyama, Masataka Tsuge, Kosho Wakae, Kento Fukano, Noriyuki Watanabe, Takanobu Kato, Asako Murayama, Yingfang Li, Ikuo Shoji, Kunitada Shimotohno, Kazuaki Chayama, Masamichi Muramatsu, Takaji Wakita, Tomoyoshi Nozaki, **Hussein H. Aly**. Kinesin KIF4 is a key regulator for surface localization of NTCP, and HBV/HDV infection. 2021 International Meeting, Biology of the Hepatitis B and D Viruses. Poster presentation, September 26-30 in Toronto, Canada.

2- Sameh A. Gad, Masaya Sugiyama, Masataka Tsuge, Kosho Wakae, Kento Fukano, Noriyuki Watanabe, Takanobu Kato, Asako Murayama, Yingfang Li, Ikuo Shoji, Kunitada Shimotohno, Kazuaki Chayama, Masamichi Muramatsu, Takaji Wakita, Tomoyoshi Nozaki, **Hussein H. Aly**. Kinesin KIF4 is a possible therapeutic host target and key regulator for surface localization of NTCP, and HBV/HDV infection. Oral presentation. 2021, The 68th Annual Meeting of the Japanese Society for Virology. Kobe, November 16 – 18.

3- Sameh A. Gad, Takanobu Kato, Masamichi Muramatsu, Kazuaki Chayama, Takaji Wakita, **Hussein H Aly**. KIF4a, a new host factor required for surface localization of NTCP, and HBV/HDV infection. 17th Hiroshima Liver project research center symposium. Oral presentation, June 2021.

4- Marwa K. Ibrahim, Sameh A. Gad, Koichi Watashi, Li Yingfang, Masaya Sugiyama, Masahiko Ito, Asako Murayama, Tetsuro Suzuki, Takanobu Kato, Kunitada Shimotohno, Masamichi Muramatsu, Takaji Wakita, **Hussein H. Aly**. MafF is a novel host restriction factor for HBV which suppresses transcription from HBV-core promoter. American Society for Virology (ASV) Abstract Accepted as Oral Presentation (meeting canceled due to COVID-19 pandemic). Instead, presented in ASV Virtual Workshop (W-17-6), 19-6-2020.

5- Marwa K. Ibrahim, Sameh A. Gad, Koichi Watashi, Li Yingfang, Masaya Sugiyama, Masahiko Ito, Asako Murayama, Tetsuro Suzuki, Takanobu Kato, Kunitada Shimotohno, Masamichi Muramatsu, Takaji Wakita, **Hussein H. Aly**. The IL-1b/TNF-a-inducible MafF protein is a novel anti-HBV host restriction factor that suppresses transcription from the HBV core promoter. 16th Hiroshima Liver project research center symposium. Oral presentation, June 2020.

6- Marwa Khalil Ibrahim, Sameh A. Gad, Koichi Watashi, Takanobu Kato, Asako Murayama, Kunitada Shimotohno, Takaji Wakita, Masamichi Muramatsu, **Hussein H. Aly**, MAFF IS AN IMPORTANT REGULATOR OF HBV CORE PROMOTER ACTIVITY, AND HBV REPLICATION. The Liver Meeting, AASLD, 2019.

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International Collaboration, and improving scientific abilities of students and postdocs who participated in this work

This project fostered international collaboration between the National Institute of Infectious Diseases, Japan, and the National Research Center, Egypt.
During this project we supervised the following researchers in Japan

1- Post-Doc
   Marwa Khalil Ibrahim, researcher in the National Research Center
2- Post-Doc
   Tawfeek Hussein, researcher in the National Research Center
3- PhD student
   Sameh Ali Gad, The University of Tokyo, Japan

５．主な発表論文等

〔雑誌論文〕　計10件（うち査読付論文　9件／うち国際共著　9件／うちオープンアクセス　1件）

１．著者名 Murayama A、Yamada N、Osaki Y、Shiina M、Aly H H、Iwamoto M、Tsukuda S、Watashi K、Matsuda M、Suzuki R、Tanaka T、Moriishi K、Suzuki T、Nishitsuji H、Sugiyama M、Mizokami M、Shimotohno K、Wakita T、Muramatsu M、Liang T. J、Kato T	４．巻 73
２．論文標題 N‐Terminal PreS1 Sequence Regulates Efficient Infection of Cell‐Culture?Generated Hepatitis B Virus	５．発行年 2020年
３．雑誌名 Hepatology	６．最初と最後の頁 520～532
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/hep.31308	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 Yamada Norie、Murayama Asako、Shiina Masaaki、Aly Hussein Hassan、Iwamoto Masashi、Tsukuda Senko、Watashi Koichi、Tanaka Tomohisa、Moriishi Kohji、Nishitsuji Hironori、Sugiyama Masaya、Mizokami Masashi、Shimotohno Kunitada、Muramatsu Masamichi、Murata Kazumoto、Kato Takanobu	４．巻 50
２．論文標題 Anti‐viral effects of interferon‐λ3 on hepatitis B virus infection in cell culture	５．発行年 2020年
３．雑誌名 Hepatology Research	６．最初と最後の頁 283～291
掲載論文のDOI（デジタルオブジェクト識別子） 10.1111/hepr.13449	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 Ibrahim Marwa K.、Abdelhafez Tawfeek H.、Takeuchi Junko S.、Wakae Kosho、Sugiyama Masaya、Tsuge Masataka、Ito Masahiko、Watashi Koichi、El Kassas Mohamed、Kato Takanobu、Murayama Asako、Suzuki Tetsuro、Chayama Kazuaki、Shimotohno Kunitada、Muramatsu Masamichi、Aly Hussein H.、Wakita Takaji	４．巻 「－」
２．論文標題 MafF is an antiviral host factor that suppresses transcription from Hepatitis B Virus core promoter	５．発行年 2021年
３．雑誌名 Journal of Virology	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） 10.1128/jvi.00767-21	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 Elbahrawy Ashraf、Ibrahim Marwa K.、Eliwa Ahmed、Alboraie Mohamed、Madian Ali、Aly Hussein Hassan	４．巻 「－」
２．論文標題 Current situation of viral hepatitis in Egypt	５．発行年 2021年
３．雑誌名 Microbiology and Immunology	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） 10.1111/1348-0421.12916	査読の有無 有
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2．論文標題 MCPIP1 reduces HBV-RNA by targeting its epsilon structure	5．発行年 2020年
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2．論文標題 Hepatitis B Virus Genotype-Dependent Vulnerability of Infected Cells to Immune Reaction in the Early Phase of Infection	5．発行年 2019年
3．雑誌名 Frontiers in Microbiology	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） 10.3389/fmicb.2019.02427	査読の有無 有
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2．論文標題 Anti‐viral effects of interferon‐λ3 on hepatitis B virus infection in cell culture	5．発行年 2020年
3．雑誌名 Hepatology Research	6．最初と最後の頁 283～291
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2．論文標題 Identification of natural compounds extracted from crude drugs as novel inhibitors of hepatitis C virus	5．発行年 2021年
3．雑誌名 Biochemical and Biophysical Research Communications	6．最初と最後の頁 1～8
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.bbrc.2021.06.022	査読の有無 有
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1．著者名 Gad Sameh A.、Sugiyama Masaya、Tsuge Masataka、Wakae Kosho、Fukano Kento、Oshima Mizuki、Sureau Camille、Watanabe Noriyuki、Kato Takanobu、Murayama Asako、Li Yingfang、Shoji Ikuo、Shimotohno Kunitada、Chayama Kazuaki、Muramatsu Masamichi、Wakita Takaji、Nozaki Tomoyoshi、Aly Hussein H.	4．巻 18
2．論文標題 The kinesin KIF4 mediates HBV/HDV entry through the regulation of surface NTCP localization and can be targeted by RXR agonists in vitro	5．発行年 2022年
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掲載論文のDOI（デジタルオブジェクト識別子） 10.1371/journal.ppat.1009983	査読の有無 無
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〔学会発表〕　計7件（うち招待講演　1件／うち国際学会　4件）

1．発表者名 Marwa K. Ibrahim, Sameh A. Gad, Koichi Watashi, Li Yingfang, Masaya Sugiyama, Masahiko Ito, Asako Murayama, Tetsuro Suzuki, Takanobu Kato, Kunitada Shimotohno, Masamichi Muramatsu, Takaji Wakita, Hussein H. Aly.
2．発表標題 The IL-1 β induced (MafF) is an important regulator of HBV core promoter activity
3．学会等名 The 67th Annual Meeting of the Japanese Society for Virology.
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2．発表標題 MafF is a key player of IL-1β-induced suppression of transcription from HBV-core promoter, and the resulting suppression of viral replication
3．学会等名 2019 International Meeting, molecular Biology of Hepatitis B virus（国際学会）
4．発表年 2019年

１．発表者名
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MafF is a novel host restriction factor for HBV which suppresses transcription from HBV-core promoter.

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MAFF IS AN IMPORTANT REGULATOR OF HBV CORE PROMOTER ACTIVITY, AND HBV REPLICATION

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Sameh A. Gad, Takanobu Kato, Masamichi Muramatsu, Kazuaki Chayama, Takaji Wakita, Hussein H Aly.

２．発表標題
KIF4a, a new host factor required for surface localization of NTCP, and HBV/HDV infection.

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２．発表標題 Kinesin KIF4 is a key regulator for surface localization of NTCP, and HBV/HDV infection
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４．発表年 2021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