様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）Unveiling Novel STAT1 Activation Expands its Anti-viral Role into Host Defense Against Bacterial Pathogens

研究課題名（英文）Unveiling Novel STAT1 Activation Expands its Anti-viral Role into Host Defense Against Bacterial Pathogens

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）：Toll様受容体4（TLR4）によるエンドサイトーシスの誘導は、インターフェロン-β（IFN-β）の産生に必須である。そしてIFN-βはSTAT1のTyr701リン酸化を誘導することにより、抗ウイルス応答遺伝子の転写を活性化させる。この研究では，STAT1の新規リン酸化部位として、Thr749を同定し、STAT1がインターフェロン遺伝子の活性化以外のみならず、炎症や敗血症に関与することを明らかにした。マクロファージにおいて、TLR4のエンドサイトーシスはSTAT1のThr749でのリン酸化を誘導し、異なる機構を介してインターロイキン-6（IL-6）およびIL-12p40の産生を促進する。

研究成果の学術的意義や社会的意義
We found that STAT1 phosphorylation status affects its DNA-binding specificity and transcriptional outcome. We hope that our future studies will assist in the development of more effective therapeutic or diagnostic strategies targeting STAT1 in infection and inflammation contexts.

研究成果の概要（英文）：Signal transducer and activator of transcription 1 (STAT1) regulates a wide-range of biological processes. Janus kinase (JAK)-mediated Tyr701 phosphorylation of STAT1 plays a key role in mediating interferons (IFN) signaling and antiviral response. Following binding to its ligand, the cell surface toll-like receptor (TLR) 4 is endocytosed and drives the production of IFN. Our work unveiled an alternative signaling pathway activated by endocytosed TLR4 in macrophages that contributed to the production of the proinflammatory cytokines IL-6 and IL-12p40 independently of IFN. Endocytosis of TLR4 led to noncanonical Thr749 phosphorylation, which confers STAT1 with distinct DNA binding and proinflammatory gene-regulatory properties. Our findings revolutionize the current JAK-STATs paradigm and adds a dynamic dimension to the current working framework of STAT1, which may apply to other STAT family proteins.

研究分野：Immunology

キーワード：STAT1　Threonine　Phosphorylation　Cytokines　Infection　Inflammation

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Signal transducer and activator of transcription 1 (STAT1) regulates a wide range of biological processes, such as immune modulatory activities, cell proliferation and cell death. For more than three decades, the canonical STAT1 working paradigm has comprised Janus kinase (JAK)-mediated Tyr^{701} phosphorylation of STAT1 followed by STAT1 dimerization and its translocation to the nucleus, where it binds to the canonical gamma interferon (IFN) activation site or IFN-stimulated response element DNA sequences and promotes an antiviral response. However, Tyr^{701} phosphorylation cannot explain the full spectrum of STAT1 activities, particularly in the context of bacterial infections and inflammation. Toll-like receptor 4 (TLR4) is the mammalian receptor for bacterial lipopolysaccharide (LPS), which is major component of the outer membrane of Gram-negative bacteria. While endocytosis of LPS-bound TLR4 is essential for IFN-β production, the IFN-β–JAK–STAT1-Tyr^{701} phosphorylation signaling pathway is unlikely to contribute to proinflammatory cytokine production as shown by deficiencies of IFN-β, IFNAR, or TYK2 did not affect the production of proinflammatory cytokines, such as IL-6, in response to bacterial infections.

２．研究の目的

(1) Identify how TLR4 endocytosis promotes proinflammatory cytokines production

(2) Identify the possible role of STAT1 downstream TLR4 endocytosis independent of it Tyr701 phosphorylation

３．研究の方法

(1) Using human primary macrophages from healthy donors and other cell lines to investigate TLR4 signaling.

(2) Using chemical and genetic methodology to alter different molecules downstream of TLR4 signaling

(3) Generating different vectors expressing different mutations of target molecules involved in TLR4 signaling

４．研究成果

(1) **LPS–Induced TLR4 endocytosis promotes ARID5A, IL-6 and IL-12p40 expression:** We found that TLR4 endocytosis promotes the expression of proinflammatory molecules such as ARID5A, IL-6 and IL-12p40. Chemical inhibition of TLR4 endocytosis using MitMAB diminished the expression of these molecules (**Fig. 1**).

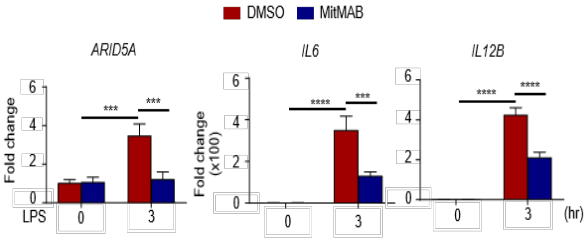

Fig. 1: LPS–Induced TLR4 endocytosis promotes ARID5A, IL-6 and IL-12p40 expression.

(2) **ARID5A stabilizes *IL6* mRNA, but not *IL12B* mRNA, through binding to its 3' UTR:** post-transcriptional regulator (ARID5A) binds to and stabilizes *IL6* mRNA downstream of TLR4 endocytosis. In contrast, TLR4 endocytosis promotes IL-12p40 expression through other mechanism independent of ARID5A.

(3) **Non-canonical phosphorylation of STAT1 at Thr^{749} residue activates *ARID5A* and *IL12B* transcription**: Our findings showed that TLR4 endocytosis–dependent IFN-β–JAK–STAT1-pTyr701 signaling is

dispensable for ARID5A and IL-12p40 expression. Instead, we unraveled that TLR4 endocytosis induces a non-canonical phosphorylation at Thr^{749} residue, which confers STAT1 with distinct gene-regulatory properties such as promoting the transcription of *ARID5A* and *IL12B*.

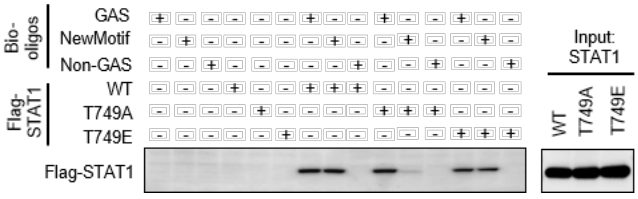

Fig. 2: Thr^{749} phosphorylation of STAT1 facilitates its binding to a non-canonical DNA motif

(4) **Thr^{749} phosphorylation of STAT1 facilitates its binding to a non-canonical DNA motif:** Our work revealed that the phosphorylation of Thr^{749} did not affect the nuclear translocation of STAT1. Instead, it facilitated STAT1 binding to the *ARID5A* and *IL12B* promoters containing the 5'-TTTGAGGC-3' or 5'-TTTGAGTC-3' sequences, respectively (**Fig. 2**).

(5) **A non-canonical TBK1/IKKβ heterodimer mediates pThr^{749} STAT1 downstream of TLR4 endocytosis**: We showed that LPS-induced TLR4 endocytosis promotes the formation of a non-canonical IKKβ-TBK1 kinase complex, where IKKβ kinase domain conducts the phosphorylation of STAT1 at Thr^{749}. Non-phosphomimic mutation of STAT1 (T749A) or kinase defective IKKβ mutation (K44A) abolished STAT1 Thr phosphorylation (**Fig. 3**).

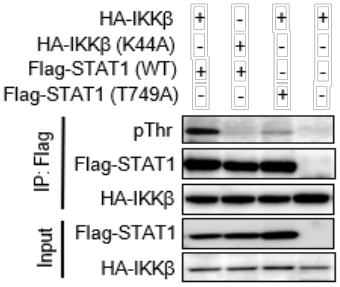

Fig. 3: A non-canonical TBK1/IKKβ heterodimer mediates pThr^{749} STAT1

(6) Conclusion: For more than three decades, the canonical STAT1 working paradigm has comprised JAK-activated pTyr^{701}, followed by STAT1 dimerization and its translocation to the nucleus, where it promotes an antiviral response. Our study provides a plausible answer for how STAT1 function expands beyond that of JAK-pTyr^{701}. Unraveling pThr^{749} and its distinct DNA-binding motif (5′-TTTGANNC-3′) provides a potential mechanistic explanation for the longstanding question of how TLR4 signaling from endosomes promotes proinflammatory cytokines production independent of NF-κB activation and IFN signaling. Collectively, our work revolutionizes the present working paradigm of STAT1 and highlights the importance of differential phosphorylation of STAT1 on its DNA binding specificity and transcriptional outcome.

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(3) **Hozaifa Metwally** and Tadamitsu Kishimoto. The Yin and the Yang of STAT1 Downstream of TLR4 Endocytosis: STAT1 beyond Interferon Signaling. *J Cell Immunol*, 2020.

５．主な発表論文等

〔雑誌論文〕　計4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　0件）

１．著者名 Metwally Hozaifa、Tanaka Toshio、Li Songling、Parajuli Gyanu、Kang Sujin、Hanieh Hamza、Hashimoto Shigeru、Chalise Jaya P.、Gemechu Yohannes、Standley Daron M.、Kishimoto Tadamitsu	４．巻 13
２．論文標題 Noncanonical STAT1 phosphorylation expands its transcriptional activity into promoting LPS-induced IL-6 and IL-12p40 production	５．発行年 2020年
３．雑誌名 Science Signaling	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） 10.1126/scisignal.aay0574	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 Kang Sujin、Narazaki Masashi、Metwally Hozaifa、Kishimoto Tadamitsu	４．巻 217
２．論文標題 Historical overview of the interleukin-6 family cytokine	５．発行年 2020年
３．雑誌名 Journal of Experimental Medicine	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） 10.1084/jem.20190347	査読の有無 無
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 －

１．著者名 Metwally Hozaifa, Kishimoto Tadamitsu	４．巻 1
２．論文標題 Distinct Phosphorylation of STAT1 Confers Distinct DNA Binding and Gene-regulatory Properties	５．発行年 2020年
３．雑誌名 Journal of Cellular Signaling	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） 10.33696/Signaling.1.012	査読の有無 無
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 －

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２．論文標題 The Yin and the Yang of STAT1 Downstream of TLR4 Endocytosis: STAT1 beyond Interferon Signaling	５．発行年 2020年
３．雑誌名 Journal of Cellular 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） 10.33696/immunology.2.044	査読の有無 無
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 －

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4．発表年 2019年

1．発表者名 Hozaifa Metwally and Tadamitsu Kishimoto
2．発表標題 STAT1 displays functionally distinct phosphorylation upon LPS stimulation.
3．学会等名 The 45th IFReC Colloquium（国際学会）
4．発表年 2020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