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　６ 年　 ６ 月 １３ 日現在

機関番号：１７７０１
研究種目：若手研究
研究期間：2021～2023
課題番号：２１Ｋ１５４０１

研究課題名（和文）EPS8L3 mediates YAP nuclear translocation and promotes liver tumorigenesis

研究課題名（英文）EPS8L3 mediates YAP nuclear translocation and promotes liver tumorigenesis

研究代表者
ＮＧＵＹＥＮ　ＴＨＡＯ（NGUYEN, THAO）

鹿児島大学・医歯学域歯学系・助教

研究者番号：４０７３３８３７

交付決定額（研究期間全体）：（直接経費）　　3,600,000円

研究成果の概要（和文）：本研究では、EPS8L3がHippo経路とは独立してYAPの安定性と活性を制御する新規因子として同定された。EPS8L3はYAPと物理的に相互作用し、YAPをユビキチン化とプロテアソーム分解から保護することが示された。また、EPS8L3の発現はヒトのがんにおけるYAPレベルと予後と相関していた。
これらの知見は、EPS8L3-YAP軸を標的とすることでYAP依存的ながんの治療戦略となる可能性を示唆しており、YAP制御の新たな洞察を提供するものである。

研究成果の学術的意義や社会的意義
The Hippo signaling pathway and its key effector, Yes-associated protein (YAP), play crucial roles in regulating organ size, tissue homeostasis, and cell proliferation. Dysregulation of this pathway can lead to the development and progression of various types of cancer.

研究成果の概要（英文）：In this study, EPS8L3 was identified as a novel regulator of YAP that controls its stability and activity independently of the Hippo pathway. EPS8L3 was shown to physically interact with YAP and protect it from ubiquitination and proteasomal degradation. Moreover, EPS8L3 expression correlated with YAP levels and prognosis in human cancers.
These findings suggest that targeting the EPS8L3-YAP axis could potentially serve as a therapeutic strategy for YAP-dependent cancers and provide new insights into YAP regulation.

研究分野：Cancer research

キーワード：cancer　cholangiocarcinoma　EPS8L3　YAP

1. 研究開始当初の背景

Liver cancer, primarily hepatocellular carcinoma (HCC), is one of the most common cancers worldwide, with poor overall survival for advanced diseases. The epidermal growth factor receptor kinase substrate 8 (EPS8) related proteins family, including EPS8L3, has been implicated in cancer development and progression. The Hippo pathway, which regulates YAP and TAZ transcription factors, is frequently dysregulated in various cancer types, including HCC. In this research, we investigated the interaction between EPS8L3 and YAP, their biological activity in cell lines and mouse-TAA induced HCC, and their co-expression in HCC patients. Our data suggest that YAP promotes the tumorigenesis and progression of HCC via EPS8L3 modulation.

2. 研究の目的

In this research, we investigated the interaction between EPS8L3 and YAP, their biological activity in cell lines and mouse-TAA induced HCC, and their co-expression in HCC patients. Our data suggest that YAP promotes the tumorigenesis and progression of HCC via EPS8L3 modulation.

3. 研究の方法

a. Examine the carcinogenesis roles of EPS8L3 and effects of EPS8L3 on malignant behaviors of liver cancers: We overexpressed *EPS8L3* in normal hepatocytes (Hc3716). In addition, to confirm the role of EPS8L3 in regulating malignant behaviors of HCC cells, we used *EPS8L3 siRNA* to knock down *EPS8L3* in HCC cell lines; then examine the cell growth, mammosphere formation, apoptosis, cell cycle, migration and invasion ability, and the epithelial-mesenchymal transition (EMT) using a three-dimentional culture model. Besides, we will clarify the molecular mechanisms using real time PCR, Western blotting, immunohistochemistry and immunofluorescent staining.

b. Investigate the correlation of EPS8L3 and YAP expression during liver cancer progression: Positivity, intensity and localization of the EPS8L3 and YAP immunosignals was compared in tumor tissues and in normal tissues and investigated whether they are associated with clinical parameters. The expressions and alterations of EPS8L3 and YAP were also analyzed using data extracted from The Cancer Genomic Atlas (TCGA) database and the cBioPortal online analyzing tool.

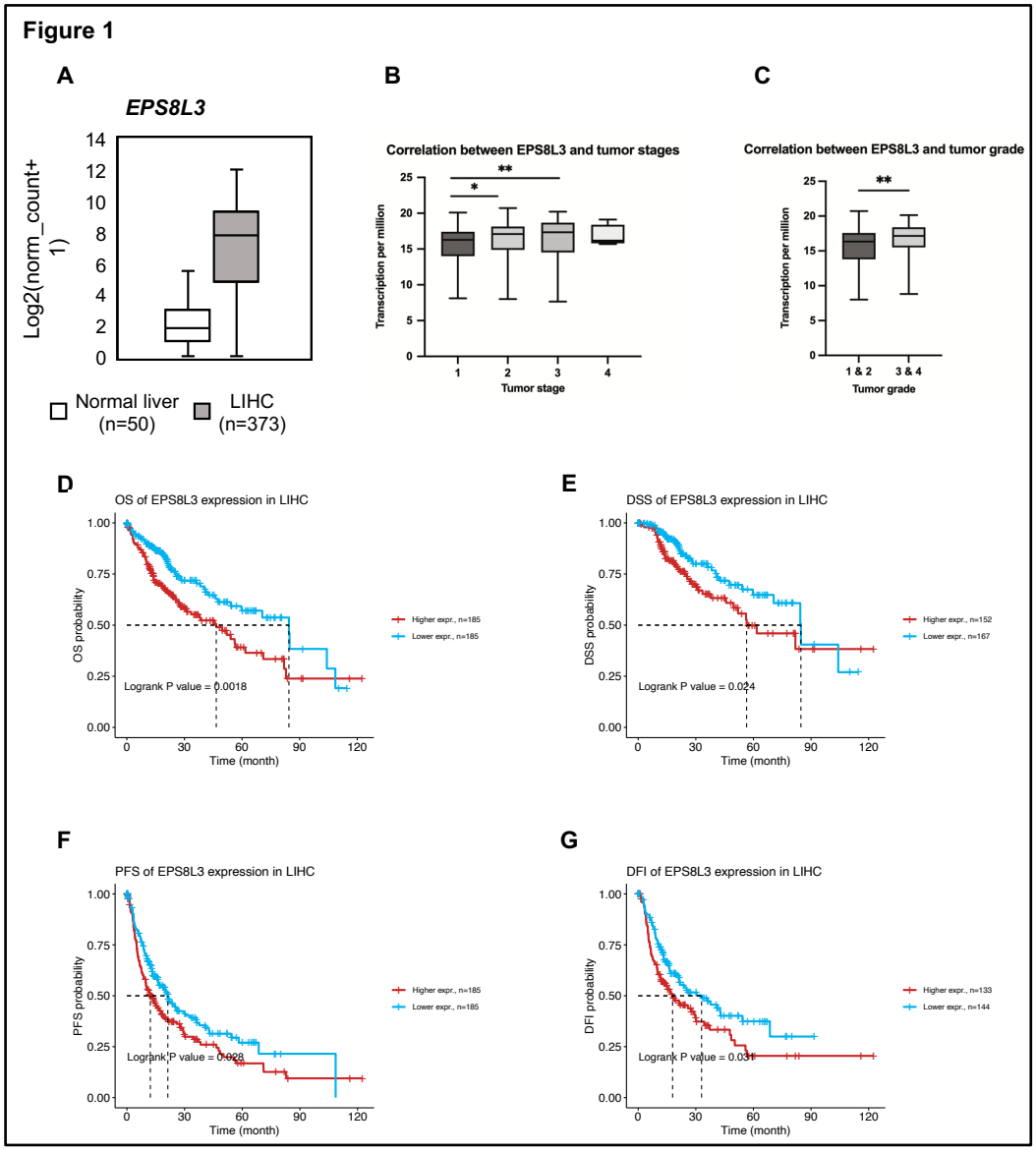
c. Clarify the overexpression of EPS8L3 promotes YAP-driven biological effects: We examined the changes in the expression of YAP target genes in *EPS8L3*-overexpressing cells and *EPS8L3*-knockout cells using real time PCR, Western blotting, immunofluorescent staining. Nuclear localization of YAP, the degradation and phosphorylation of YAP was also examined in *EPS8L3*-overexpressing cells and *EPS8L3*-knockout cells.

d. Validate whether YAP is required for EPS8L3-driven tumorigenesis effects: We performed knock down *YAP* and overexpress EPS8L3 in HCC cells, then examine the proliferation rate, migration and invasion ability of these cells.

4. 研究結果

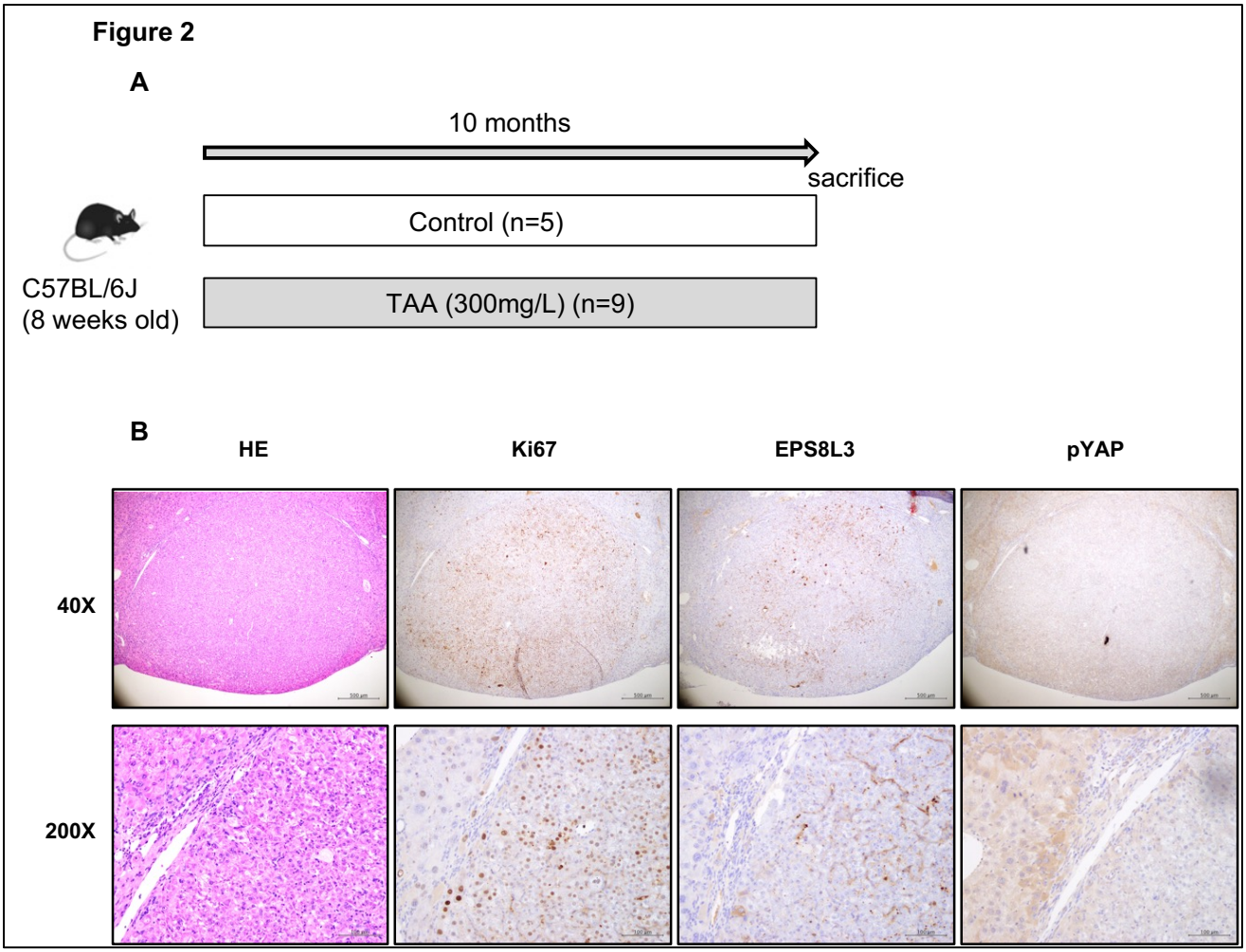
a. Overexpression of EPS8L3 was associated with poor outcome in HCC patients:

We first analyzed the expression of EPS8L3 using RNA-seq data from TCGA database and found that its expression was significantly higher in tumor compared to normal tissue (Fig. 1A). Protein expression analysis using data from CPTAC also showed higher EPS8L3 levels in various cancer types, including liver, pancreatic, and colon cancer (Fig. S1A). EPS8L3 expression was associated with tumor stages and grades (Fig. 1B-C) and significantly correlated with overall survival (OS), disease-specific survival (DSS), progression-free survival (PFS), and disease-free interval (DFI) in HCC patients (Fig. 1D-G).



b. EPS8L3 was overexpressed and related to pYAP expression in mouse TAA-induced HCC model:

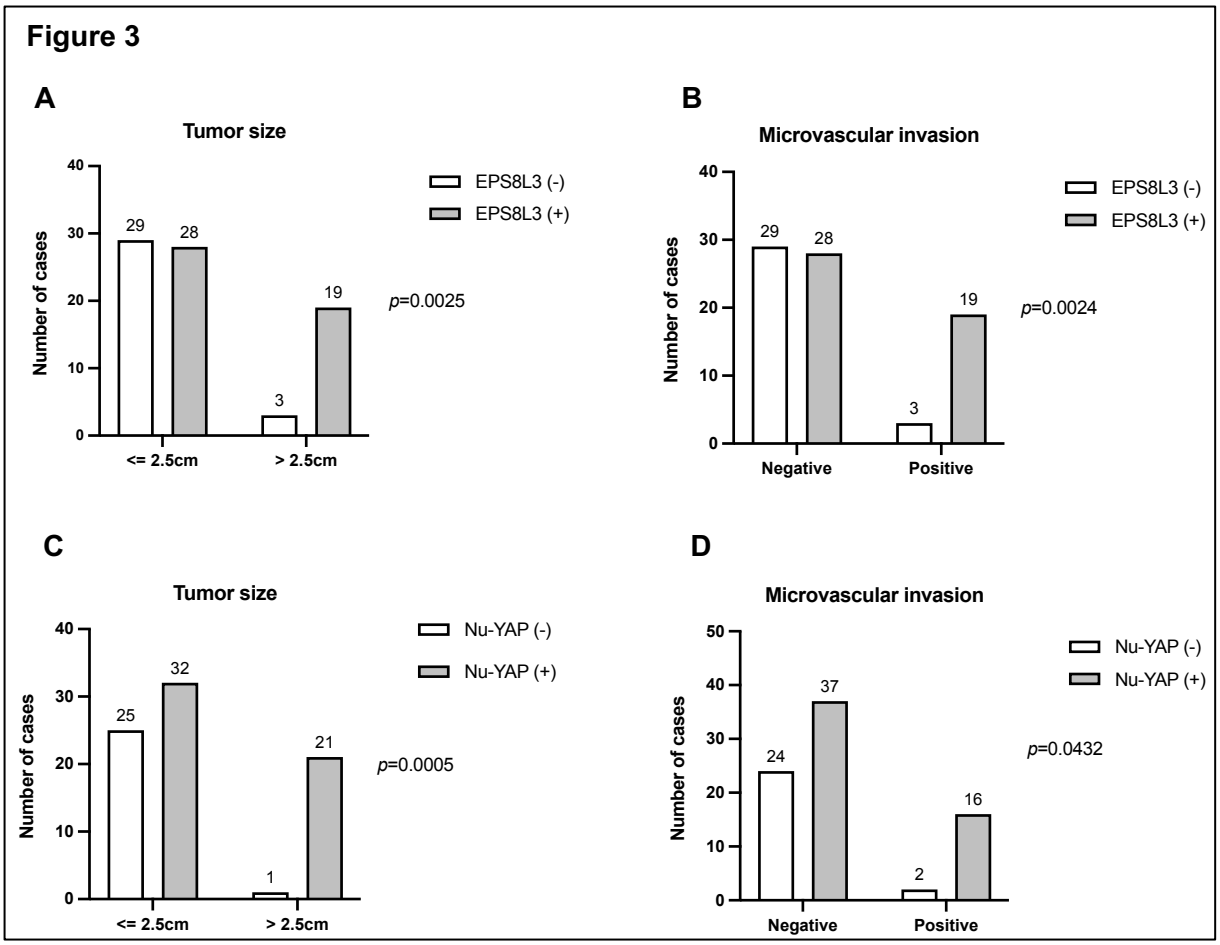
In a mouse model of TAA-induced HCC (Fig. 2A), we observed a progressive increase in EPS8L3 expression during HCC development. Immunohistochemical analysis revealed a strong positive correlation between EPS8L3 and pYAP expression ($r=0.78$, $p<0.001$) (Fig. 2B), suggesting a potential link between EPS8L3 and YAP activation in HCC.



c. EPS8L3 promotes YAP/TAZ activity and stability:

In vitro experiments demonstrated that overexpression of EPS8L3 in HCC cell lines significantly increased YAP/TAZ transcriptional activity, as measured by luciferase reporter assays ($p<0.01$). Cycloheximide chase assays showed that EPS8L3 overexpression enhanced YAP/TAZ protein stability, while EPS8L3 knockdown led to accelerated YAP/TAZ degradation. Mechanistic studies revealed that EPS8L3 promoted YAP/TAZ nuclear localization and inhibited their ubiquitin-mediated degradation.

d. Overexpression of EPS8L3 in HCC is associated with tumor size and microvascular invasion (Fig. 3A-D)



e. EPS8L3 directly binds to YAP and promotes tumor cells proliferation:

Co-immunoprecipitation experiments confirmed a direct interaction between EPS8L3 and YAP in HCC cells. Overexpression of EPS8L3 significantly increased HCC cell proliferation, as evidenced by MTT assays ($p<0.01$) and EdU incorporation ($p<0.01$). Mechanistically, EPS8L3-YAP interaction promoted the expression of proliferation-related genes, such as CTGF, TEAD2 and TEAD4.

5. 結論

Our study reveals a novel role for EPS8L3 in HCC progression and highlights its potential as a prognostic biomarker and therapeutic target. We demonstrate that EPS8L3 promotes HCC growth and metastasis through activation of YAP/TAZ signaling and direct interaction with YAP. These findings provide new insights into the molecular mechanisms underlying HCC progression and suggest that targeting the EPS8L3-YAP axis may be a promising strategy for HCC treatment.

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 Hung Trong Hoang , Phuong Thao Nguyen , Nam Cong-Nhat Huynh , Tam Thi-Thanh Nguyen, Trang Thi Huyen Tu, Michael George Botelho, Lan Van Nguyen, Kaori Shima, Tomonori Sasahira	４．巻 18
２．論文標題 Reliability of online dental final exams in the pre and post COVID-19 era: A comparative study	５．発行年 2023年
３．雑誌名 PLOS ONE	６．最初と最後の頁 e0286148
掲載論文のDOI（デジタルオブジェクト識別子） 10.1371/journal.pone.0286148	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6件（うち招待講演　0件／うち国際学会　6件）

１．発表者名 Phuong Thao Nguyen, Kaori Shima, Tomonori Shima
２．発表標題 New insights into YAP nuclear translocation to promote liver tumorigenesis
３．学会等名 81st annual meeting of Japanese Cancer Association（国際学会）
４．発表年 2022年

１．発表者名 Phuong Thao Nguyen, Kaori Shima, Yudai Shimojukkoku, Tomonori Sasahira
２．発表標題 Impacts of KPNA7 amplification and overexpression on tumor infiltrating immune cells of HNSCCs
３．学会等名 112th annual meeting of The Japanese Society of Pathology（国際学会）
４．発表年 2023年

１．発表者名 Ayami Tomishima, Phuong Thao Nguyen, Kaori Shima, Yudai Shimojukkoku, Tomonori Sasahira.
２．発表標題 The roles of MCTP2 in oral squamous cell carcinoma
３．学会等名 112th annual meeting of The Japanese Society of Pathology（国際学会）
４．発表年 2023年

１．発表者名
Yasunobu Oku, Phuong Thao Nguyen, Kaori Shima, Yudai Shimojukkoku, Tomonori Sasahira

２．発表標題
Cytoplasmic TCF19 translocation impairs DNA damage repair in HNSCC

３．学会等名
112th annual meeting of The Japanese Society of Pathology（国際学会）

４．発表年
2023年

１．発表者名
Phuong Thao Nguyen, Kaori Shima, Yudai Shimojukkoku, Tomonori Sasahira

２．発表標題
EPS8L3 mediates YAP nuclear translocation and promotes liver tumorigenesis

３．学会等名
112th annual meeting of The Japanese Society of Pathology（国際学会）

４．発表年
2023年

１．発表者名
Kaori Shima, Phuong Thao Nguyen, Yudai Shimojukkoku, Tomonori Sasahira

２．発表標題
Analysis of METTL5 expression and function in oral squamous cell carcinoma

３．学会等名
112th annual meeting of The Japanese Society of Pathology（国際学会）

４．発表年
2023年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

―

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