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

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研究課題名（和文）Role of kinesin/dynein adaptor JSAP in reactive oxygen species-induced cell death and lysosome positioning

研究課題名（英文）Role of kinesin/dynein adaptor JSAP in reactive oxygen species-induced cell death and lysosome positioning

研究代表者
Ｉ・ＫＥＴＵＴ　ＧＵＮＡＲＴＡ（I.Ketut, Gunarta）

金沢大学・がん進展制御研究所・助教

研究者番号：９０８３８３９３

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研究成果の概要（和文）：ROSレベルは、細胞内の分子を酸化し、最終的に細胞死を引き起こす可能性があるため、細胞によって厳密に制御されています。 クルクミンなどのROS誘導剤は、癌細胞のリゾソーム局在を変化させることが報告されています。 モータータンパク質アダプターのファミリーであるJSAPが、クルクミンに応答したリソソーム輸送に関与していることを発見しました。 また、JSAPを介したリソソーム輸送とクルクミンによる細胞死の関係を明らかにしました。 私たちの研究は、JSAPファミリーの密接に関連するメンバーであるJSAP1とJSAP2がクルクミン誘発性ストレスにおいて異なる機能を果たしていることを示唆しました。

研究成果の学術的意義や社会的意義
ROSレベルを上昇させ、細胞死を誘発する治療薬は、代替の癌治療を提供します。 ウコン（Curcuma longa）に由来する化合物であるクルクミンは、インビトロおよびインビボで細胞死を誘発する有望な効果があるため、魅力的な薬剤候補です。 この研究の結果は、細胞内輸送が、新しい治療戦略の開発につながる可能性のある急性酸化ストレスの生存にどのように関連していたかについてのより良い理解を与えることが期待されます。

研究成果の概要（英文）：The ROS level is tightly regulated by a cell as it can oxidize molecules inside a cell and ultimately lead to cell death. ROS inducing agent, such as curcumin, has been reported to alter lysosome localization in a cancer cell. We found that JSAP, a family of motor protein adaptors, is involved in lysosome transport in response to curcumin. We also clarified the relationship between JSAP-mediated lysosome transport and curcumin-induced cell death in cancer. Our study suggested that the closely related member of the JSAP family, JSAP1 and JSAP2 (also known as JLP), play a distinct function in curcumin-induced stress.

研究分野：腫瘍生物学関連

キーワード：JSAP　JLP　JSAP1　Curcumin　Autophagy　Lysosome

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

The ROS level is tightly regulated by a cell as it can oxidize molecules inside a cell and ultimately lead to cell death. Cancer cells, which show elevated ROS levels, have developed multiple survival mechanisms to anticipate ROS-induced cell death. In addition to the antioxidant system, autophagy has been proposed as one of the survival mechanisms. Lysosome positioning recently became an interesting topic of research because of its involvement in the various cellular processes, including autophagy. The perinuclear localization of lysosome is required for the autophagosome-lysosome fusion.

The JSAP family proteins, JSAP1 (also known as JIP3) and JSAP2 (also known as JLP, JIP4, and SPAG9), were originally identified as scaffold proteins for JNK and p38 MAP kinase (MAPK) signaling cascades by our group and others. JSAPs are also known as adaptor proteins that link motor proteins (kinesin-1 and dynein) to their cargoes. These proteins are involved in various cellular processes, including endosome transport and axonal transport. These proteins can work redundantly or are mutually dependent on each other. While JSAP1 is expressed mainly in neurons, the expression of JSAP2 is more ubiquitous. JSAP2 is overexpressed in various types of cancer and its variant is linked to familial glioma. It was reported recently that acute oxidative stress induced JSAP2-mediated retrograde trafficking of lysosome. However, the biological consequences and significance of this process remain unknown. In addition, the role of JSAP1 (which has a similar structure with JSAP2) in ROS-induced cancer cell death remains unknown.

２．研究の目的

The purpose of this study is to clarify the relationship between JSAP-mediated lysosome trafficking and ROS induced cell death using curcumin as an inducer. The outcome of this study is expected to give a better understanding of how intracellular trafficking might have related to the survival in acute oxidative stress that may lead to the development of a novel therapeutic strategy.

３．研究の方法

To analyze the role of JSAP, we established several experimental models. We prepared JSAP1 or JSAP2 (JLP) shRNA-mediated knockdown or CRISPR/Cas9-mediated knockout in several cancer cell lines. Then we analyzed the sensitivity of these stable cell lines toward curcumin. The analysis includes cell death assay, immunostaining for lysosome positioning, and autophagy analysis using GFP-LC3. A rescue experiment was performed using wild-type or mutant JSAP protein.

４．研究成果

(1) We confirmed that curcumin can induce cell death in Hela, HCT116, and HepG2 cells in a dose-dependent manner. Knockdown (KD) of JSAP2 (hereafter JLP) or JSAP1 using two different shRNA resulted in increased sensitivity toward curcumin-induced cell death (fig. 1). Furthermore, exogenous expression of JLP in JSAP1 knockout (KO) cell cannot rescue curcumin-induced cell death (fig.1C). These results suggested that although both JSAP1 and JLP played a protective role in curcumin-induced cell death, they might have a distinct mechanism.

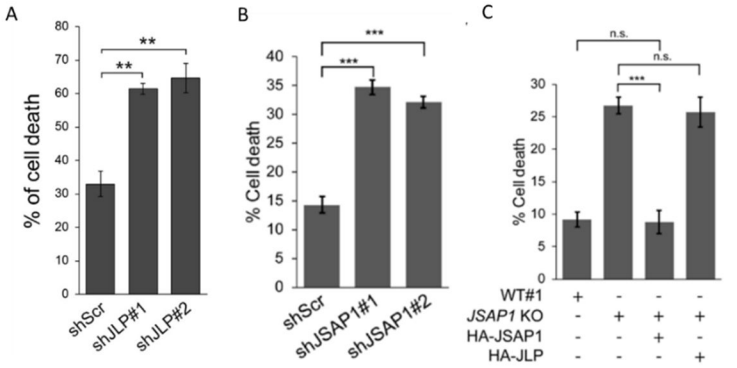

Fig. 1. JSAP1 and JLP play a protective role in curcumin-induced cell death.

(2) Analysis of lysosome position in curcumin-treated Hela cells showed that curcumin induces perinuclear localization of lysosomes. However, in the JLP KD cell this perinuclear localization was impaired (fig.2A). The impairment of lysosome transport in JLP KD cell turn out to be important for the fusion of lysosome and autophagosome to form autolysosome. It is well known that autolysosome formation is critical for the degradation of oxidation products that are toxic to the cell. Additionally, we also observed impairment of p38 phosphorylation in JLP KD cells after curcumin treatment. These results suggest that JLP protects the cell from curcumin-induced cell death by at least two mechanisms. First, JLP mediates perinuclear transport of lysosomes which is required for autolysosome formation. Second, JLP act as a scaffold protein of p38-MAPK which is known to regulate the expression of antioxidant genes.

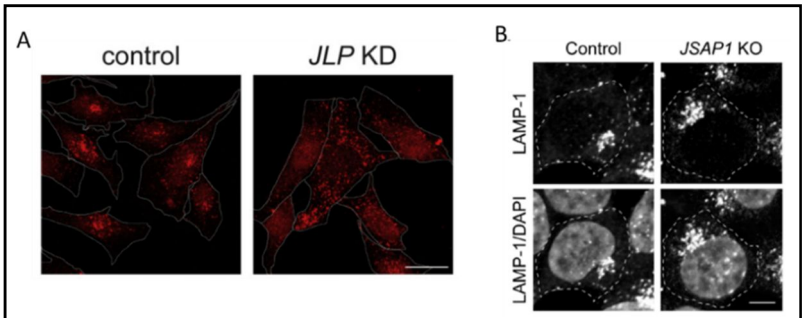

Fig. 2. Effect of JSAP1 or JLP depletion on lysosome localization after curcumin treatment. LAMP1 (lysosome marker), DAPI (nuclei marker). Bar 10 mm.

(3) Unlike JLP, in JSAP1 KO cell we observed a perinuclear localization of lysosome was not impaired following curcumin treatment (fig.2B). Interestingly, we observed an accumulation

of autolysosomes in JSAP1 KO cells. This observation might suggest that although autophagosome-lysosome fusion is not impaired in JSAP1 KO cells, autophagosome degradation might have been attenuated. As a result, the population of free lysosomes, which are required for the newly formed autophagosome, is reduced, and eventually leads to cell death after curcumin treatment. Taken together, our study suggests that JSAP1 and JLP played a protective role in curcumin-induced cell death via a distinct mechanism.

５．主な発表論文等

〔雑誌論文〕　計4件（うち査読付論文　4件／うち国際共著　4件／うちオープンアクセス　4件）

１．著者名 Jambaldorj Boldbaatar, I Ketut Gunarta, Ryusuke Suzuki, Purev Erdenebaatar, Gantulga Davaakhu. Hirohiko Hohjoh, Katsuji Yoshioka	４．巻 522
２．論文標題 Protective role of c-Jun NH2-terminal kinase-associated leucine zipper protein (JLP) in curcumin-induced cancer cell death	５．発行年 2020年
３．雑誌名 Biochemical and Biophysical Research Communication	６．最初と最後の頁 697~703
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.bbrc.2019.11.154.	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

１．著者名 Ryusuke Suzuki, I Ketut Gunarta, Jambaldorj Boldbaatar, Purev Erdenebaatar, Ravdandorj Odongoo, Katsuji Yoshioka	４．巻 14(1)
２．論文標題 Functional role of c-Jun NH2-terminal kinase-associated leucine zipper protein (JLP) in lysosome localization and autophagy	５．発行年 2020年
３．雑誌名 Drug Discoveries & Therapeutics	６．最初と最後の頁 35~41
掲載論文のDOI（デジタルオブジェクト識別子） 10.5582/ddt.2020.01001	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

１．著者名 Norio Hanata, Hirofumi Shoda, Hiroaki Hatano, Yasuo Nagafuchi, Toshihiko Komai, Tomohisa Okamura, Akari Suzuki, I Ketut Gunarta, Katsuji Yoshioka, Kazuhiko Yamamoto, Keishi Fujio	４．巻 11
２．論文標題 Peptidylarginine Deiminase 4 Promotes the Renal Infiltration of Neutrophils and Exacerbates the TLR7 Agonist-Induced Lupus Mice	５．発行年 2020年
３．雑誌名 Frontiers in immunology	６．最初と最後の頁 1095
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fimmu.2020.01095	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

１．著者名 I Ketut Gunarta, Dewi Yuliana, Purev Erdenebaatar, Yuhei Kishi, Jambaldorj Boldbaatar, Ryusuke Suzuki, Ravdandorj Odongoo, Gantulga Davaakhuu, Hirohiko Hohjoh, Katsuji Yoshioka	４．巻 1
２．論文標題 c-Jun NH 2-terminal kinase (JNK)/stress-activated protein kinase-associated protein 1 (JSAP1) attenuates curcumin-induced cell death differently from its family member, JNK-associated leucine zipper protein (JLP)	５．発行年 2021年
３．雑誌名 Drug Discoveries & Therapeutics	６．最初と最後の頁 21
掲載論文のDOI（デジタルオブジェクト識別子） 10.5582/ddt.2021.01021	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5件（うち招待講演　2件／うち国際学会　3件）

1．発表者名
I Ketut Gunarta

2．発表標題
Role of JNK/SAPK-associated protein 1 (JSAP1) in curcumin-induced cell death

3．学会等名
The 43rd annual meeting of the molecular biology association of japan（国際学会）

4．発表年
2020年

1．発表者名
I Ketut Gunarta

2．発表標題
Role of JLP in ROS-induced cell death

3．学会等名
1st Ganken Colloquium For Young Scientist（招待講演）

4．発表年
2019年

1．発表者名
I Ketut Gunarta

2．発表標題
Role of JLP in reactive oxygen species (ROS)-induced cell death and lysosome positioning

3．学会等名
The 42nd Annual Meeting of the Molecular Biology Society of Japan（国際学会）

4．発表年
2019年

1．発表者名
I Ketut Gunarta

2．発表標題
Protective role of JLP-dependent lysosome positioning in reactive oxygen species (ROS)-induced cell death

3．学会等名
International Symposium on Tumor Biology/Joint Symposium of KUCRI and Duke-NUS（招待講演）

4．発表年
2019年

１．発表者名 I Ketut Gunarta
２．発表標題 Role of JSAP2 in cell cycle regulation
３．学会等名 The 44th annual meeting of the molecular biology association of japan（国際学会）
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