論 文 内 容 の 要 旨
Synopsis of Thesis

氏 名 Name	郝飞克
論文題名 Title	The PtdIns3-phosphatase MTMR3 interacts with mTORC1 and suppresses its activity (ホスファチジルイノシトール-3-ホスファターゼMTMR3はmTORC1と結合してその活性を制御する)

論文内容の要旨

〔目 的(Purpose)〕

We previously reported that MTMR3 negatively regulates autophagy, via PI3P metabolism. However, some components of the autophagy-executing machinery, such as ULK1, are believed to function upstream of PI3P dependent step. This suggests that there may remain a missing link between MTMR3 function and autophagy regulation to be further determined. To understand this novel function of MTMR3, we searched for proteins that interact with it.

〔方法ならびに成績(Methods/Results)〕

Method: To search for the binding proteins of MTMR3, we constructed the OSF (One-Strep-FLAG) tagged MTMR3 (wild-type and phosphatese-deficient mutant) and transfected them into HEK293T cells. The MTMR3 binding proteins were purified by using the Strep-Tactin purification system and subsequently identified by LC-MS/MS analysis. Among the binding candidates of MTMR3, mTOR had a high MASCOT search score, which suggested the possibility that mTOR binds to MTMR3. Furthermore, to confirm the interaction of MTMR3 and mTOR, we performed co-immunoprecipitation in the HEK293T cells. The overexpression and knockdown of MTMR3 were performed to investigate the biological significance of MTMR3-mTOR interaction. The dynamic translocation of MTMR3 was observed by the live-cell imaging using a confocal laser scanning microscope.

Results: The interaction between MTMR3 and mTOR was confirmed by the co-immunopricipitation result. And this interaction was independent of nutrient conditions and phosphatase activity of MTMR3. The further investigation showed that MTMR3 interacted with mTOR by its N-terminal half, which includes PH-G domain and PTP (phosphatase) domain but not C-terminal half. The MTMR3-mTOR interaction was unaffected when the cells were treated with rapamycin, a specifically mTOR inhibitor. As mTOR forms mTOR complex1 and mTOR complex2, our co-immunopricipitation result showed that in addition to mTOR, MTMR3 also interacted with another mTORC1 component, Raptor but not the Rictor, which belongs to mTORC2, suggesting that MTMR3 interacts with mTORC1 but not mTORC2. Furthermore, we investigated the biological significance of the interaction between MTMR3 and mTORC1 by overexpression and knockdown of MTMR3 in HEK293T cells. The results showed that overexpression of MTMR3 full-length or its N-terminal half significantly inhibited mTORC1 activity but not the C-terminal, which suggested that MTMR3 inhibits mTORC1 activity in an interaction-dependent manner. In contrast, knockdown of MTMR3 enhanced mTORC1 activity in some extent. The live-cell imaging showed that MTMR3 transiently localized to Golgi. The immunostaining results of truncation mutants of MTMR3 suggested that MTMR3 localizes to Golgi depends on its PTP domain.

〔総 括(Conclusion)〕

In this study, we demonstrated that MTMR3 interacts with mTORC1 and inhibits its activity. Our results suggest that MTMR3 regulates autophagy via its effect on mTORC1 activity, in addition to its phosphatase activity.

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本論文では脂質代謝に関わる酵素ホスファチジルイノシトール３ホスファターゼMTMR3の機能に着目して解析を行った。ホスファチジルイノシトール３リン酸は細胞内の限られた箇所で生成され、オートファジーの時空間的な制御に関わるが、MTMR3はその脱リン酸を介してオートファジーを制御する。HEK293T細胞からMTMR3の共沈降物を質量分析法により解析したところmTORが共沈したことから、MTMR3はｍTORを含む二種の複合体のうち、栄養環境に応答し細胞成長やオートファジーを制御する中心因子であるmTORC1と相互作用することを発見した。結合領域としてMTMR3のアミノ末端側PH-GドメインとPTP ドメインが関わり、さらに全長のMTMR3やその結合領域を発現させることにより、mTORC1のキナーゼ活性が抑制されることを明らかにした。これらの成果は、mTORC1活性が脂質代謝酵素MTMR3の影響下にもあることを示したことから、オートファジーや細胞成長の制御機構理解の新局面を切り開くものであり、学位の授与に値すると考えられる。