論　文　内　容　の　要　旨
Synopsis of Thesis

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論文題名 Title	A GPI processing phospholipase A2, PGAP6, modulates Nodal signaling in embryos by shedding CRIPTO (GPI特異的ホスホリパーゼA2であるPGAP6は、CRIPTOを遊離させることにより胚におけるNodalシグナルを調節する)

論文内容の要旨

〔目　的(Purpose)〕 - 90 words

The glycolsylphosphatidylinositol (GPI)-anchor is conserved post-translational modification in eukaryotes. To data, more than 150 different proteins in mammalian cells have been identified as GPI-anchored proteins (GPI-APs). One of the characteristic features of GPI-APs is that these proteins are releasable from the cell membrane by cleaving GPI moieties. Shedding of GPI-APs from the cell surface by GPI cleaving enzymes has two potential biological effects: removal of inhibitory GPI-APs and function of released proteins at remote sites. The latter example has not been demonstrated. We aimed to identify a new GPI cleaving enzyme.

〔方法ならびに成績(Methods/Results)〕

(Methods)　- 111 words

As a candidate GPI cleaving enzyme, we chose PGAP6, a member of a putative transmembrane hydrolase superfamily termed CREST (alkaline ceramidase, PAQR receptor, Per1, SID-1 and TMEM8), to which PGAP3 belongs. The released GPI-APs were analyzed by TritonX-114 partitioning, mass spectrometry and hydrophobic chromatography. The activity of released CRIPTO as a Nodal co-receptor was determined using HEK293T cells expressing a Nodal-responsive luciferase reporter, ALK4 and FAST2. To determine roles of Pgap6 in vivo, we analyzed expression of Pgap6 by in situ hybridization in mouse embryos when Cripto functions. We then generated Pgap6 knockout mice and analyzed embryogenesis.

(Results) – 195 words

Overexpression of PGAP6 decreased the surface expression of some GPI-APs. Especially, CRIPTO was highly sensitive whereas its close homologue CRYPTIC was resistant. In human embryonic carcinoma NTERA2 cells, PGAP6 and CRIPTO were expressed endogenously, and CRIPTO was secreted continuously, which was almost completely inhibited by knockdown of PGAP6. The CRIPTO released by PGAP6 was active as a Nodal co-receptor. Cell-autonomous CRIPTO activity was reduced when PGAP6 was expressed. A model GPI-AP secreted from PGAP6-expressing cells was a product of phospholipase D cleavage whereas one still associated with the cells had lyso-GPI. From PGAP6-expressing cells, CRIPTO bearing lyso-GPI was secreted and then cleaved by phospholipase D. GPI structure was critical for PGAP6-dependent shedding because CRIPTO expressed on *PIGN*- or *PGAP1*-defective cells, which express GPI-APs with altered glycan and lipid moieties, respectively, was not released by PGAP6. Consistent with these in vitro data, others reported that *Pign*- and *Pgap1*-mutant mice had developmental forebrain abnormality, a phenotype common with Cripto-hypomorpic mice. *Pgap6* homozygotes were embryonic lethal. In E6.2 embyos, *Pgap6* and *Cripto* transcripts were expressed in epiblasts. In some homozygous *Pgap6* knockout embryos observed at E6.7, formation of anterior-posterior axis was incomplete, which was a common feature with *Cripto* knockout embryos.

(総　括(Conclusion) – 54 words

We conclude that PGAP6 is a GPI-specific phospholipase A2 that selectively secretes CRIPTO. PGAP6 likely recognizes both GPI moiety and protein structure of CRIPTO. CRIPTO released by PGAP6 may act non-cell-autonomously in Nodal signaling during embryogenesis. The regulation of PGAP6 activity needs to be addressed to better understand the spatio-temporal regulation of CRIPTO functions.

[Format-H07]

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

細胞表面膜タンパク質の中に、糖脂質であるグリコシルホスファチジルイノシトール (GPI)によって膜にアンカーされている一群があり、GPIアンカー型タンパク質と呼ばれる。GPIアンカー型タンパク質は、GPI部分が酵素によって切断され、細胞表面から遊離し得ることが特徴の一つであるが、生理活性が明らかになっているGPI切断酵素の例は極めて限られている。本研究では、新規のGPI特異的ホスホリパーゼA2を発見してPGAP6と名付け、それがCRIPTOのGPIアンカーを効率よく切断し、細胞から遊離させることを示した。さらに、PGAP6ノックアウトマウスが、CRIPTO欠損マウスと同様、初期発生における前後軸形成不全を示すことを示し、PGAP6によるCRIPTOの切断遊離の生理的重要性を証明した。本論文は、新規のGPI切断酵素と基質であるGPIアンカー型タンパク質を同定し、その遊離の生理的意義を証明したものであり、学位に値するものと認める。