論文の内容の要旨

論 文 題 目　PTEN, a phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase, differentially regulates endocytosis, migration, and proliferation in the enteric protozoan parasite *Entamoeba histolytica* (腸管原虫赤痢アメーバのホスファチジルイノシトール 3 リン酸脱リン酸化酵素(PTEN)はエンドサイトーシス、細胞運動、増殖を多様に制御する)

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Phosphatidylinositol phosphates (PtdInsPs) are indispensable in eukaryotes and play a pivotal role in a variety of biological processes such as cytoskeletal organization, vesicular trafficking, nuclear function, endocytosis, and motility. There are seven different species of PtdInsPs in mammalian cells tightly controlled via reversible phosphorylation and de-phosphorylation mechanism by PtdInsPs kinases and phosphatases. PTEN (phosphatase and tensin homologue) is a lipid phosphatase that dephosphorylates phosphatidylinositol (3,4,5)-trisphosphate (PtdIns(3,4,5)P_3) to phosphatidylinositol (4,5)-bisphosphate (PtdIns(4,5)P_2) thus depleting cellular signaling processes downstream of PtdIns(3,4,5)P_3. PTEN has been extensively studied in higher eukaryotes where it regulates cell proliferation, cell polarity, and migration via the establishment of a PtdIns(3,4,5)P_3-PtdIns(4,5)P_2 gradient. Many human cancers events are related to PTEN mutations due to inability to catalyze PtdIns(3,4,5)P_3 which activates proto-oncogenic phosphatidylinositol 3-kinase (PI3K)– RAC-alpha serine/threonine-protein kinase (AKT) signaling pathway. Furthermore, PTEN can modulate immune responses by regulating Fcγ receptor-mediated phagocytosis. However, the biological relevance of PTEN on the fundamental cellular process in pathogenic eukaryotes has not been well documented.

Entamoeba histolytica is the etiologic agent of human amebiasis which infect around 50 million people throughout the world and result in 100,000 deaths yearly. Infection by *E. histolytica* usually occurs via ingestion of fecally contaminated food or water. Amoebic trophozoites then invade the intestinal epithelial tissue which causes colitis and amoebic dysentery. Trophozoites can also infect extraintestinal organs and form abscesses in some patients. It is known that major virulence mechanisms of *E. histolytica* are related to actin-mediated processes such as migration, adhesion, trogocytosis (i.e., nibbling or chewing of a part of a live cell), and phagocytosis (i.e., internalization with a single bite) as well as vesicular traffic involved in the secretion of proteases. *E. histolytica* conserves an apparently sufficient set of PI-kinases and -phosphatases to generate 7 species of phosphoinositides. It has been recently identified AGC kinases as PtdIns(3,4,5)P_3-binding proteins and revealed their involvement in trogocytosis and phagocytosis in *E. histolytica*. Thus PtdIns(3,4,5)P_3-mediated signaling is assumed to have a pivotal role in pathogenicity of this parasite. Although a comprehensive analysis of the PTEN functions in higher eukaryotes has been conducted, the role of PTEN in protozoan parasites remained elusive. In the present study, we identified and conducted a functional

analysis of PI 3-phosphatase (PTEN) in the protozoan parasite *E. histolytica*. We also have demonstrated for the first time that PTEN differentially regulates endocytosis (including trogo-/phagocytosis) and motility which play a crucial role in the proliferation and pathophysiology during infection.

In silico analysis using human PTEN as a query for BLAST search showed that *E. histolytica* has 6 proteins that contain PTEN phosphatase domain. The presence of multiple orthologs in amoeba species may reinforce the significance of PTEN for parasitic proliferation and pathogenesis. Our previous transcriptome data verified that one protein (EhPTEN1, EHI_197010) is highly expressed in the trophozoite stage in both *E. histolytica* HM-1: IMSS cl6 and G3 strains, while the 5 other PTENs are expressed at relatively low levels. Additionally, Multiple sequence alignment by Clustal W program showed EhPTEN1 possesses almost all conserved domains in mammalian PTEN including PtdIns(4,5)P_2-binding motif, phosphatase domain, C2 domain, and cytosolic localization signal. These results may propose that this isoform act as a canonical PTEN in *E. histolytica*.

For functional analysis of EhPTEN1, overexpression and gene silenced strains were established and trogocytosis, phagocytosis, endocytosis, and motility were examined. Phosphatase assay using EhPTEN1 recombinant protein was performed to confirm the conservation of PtdIns(3,4,5)P_3 catalytic activity. Live imaging of GFP-EhPTEN1 expressing amebic trophozoites showed localization mainly in the cytosol with higher concentration to pseudopods and to the leading edge of the trogo-phagocytic cup. Effects of augmentation of EhPTEN1 function by overexpression of wild type, or inhibition of EhPTEN1 by gene silencing was investigated on trogo-/phagocytosis, endocytosis, and motility using a confocal quantitative image cytometer. It appears that EhPTEN1 negatively regulates trogo- and phagocytosis where overexpression of EhPTEN1 caused reduction in trogocytosis and phagocytosis while transcriptional gene silencing of *EhPTEN1* gene caused opposite phenotypes. Interestingly, EhPTEN1 was found to act as a positive regulator for fluid-phase and receptor-mediated endocytosis as well as for the motility in *E. histolytica* trophozoites. Moreover, we showed that EhPTEN1 was required for optimal growth of this parasite. This phenotype can be possibly explained by reduced ability in nutrient uptake or dysregulation of cytokinesis when *EhPTEN1* is repressed in *E. histolytica*. However, the phosphatase activity of EhPTEN1 towards PtdIns(3,4,5)P_3 is conserved suggesting that the biological implications of EhPTEN1 are related to its catalytic function. Altogether, these results are consistent with the premise that EhPTEN1 is differentially involved in signaling in different endocytic pathways (including trogo-/phagocytosis) and motility which plays a crucial role in the proliferation and pathophysiology during infection. Our study on the EhPTEN1 functions in *E. histolytica* provides novel implications of phosphatidylinositol signaling which will expand our understanding of the pathogenesis of this parasite as well as other pathogenic eukaryotes.