Form 3

Abstract of Thesis

Name (NANTAKEERATIPAT TEERACHATE)	
Title	Lysosomal acid lipase-regulating bioenergetic process is important for the cytodifferentiation of human periodontal ligament cells (ライソゾーム酸性リパーゼ誘導性エネルギー代謝制御によるヒト歯根膜細胞の分化制御機構の解明)

Abstract of Thesis

Introduction: Energy-carrying adenosine triphosphate (ATP) is necessary for all cells to maintain their physiological functions and fuel many cellular activities, including cytodifferentiation. Lysosomal acid lipase (LAL), encoded by a lipase A (*LIPA*) gene, plays an important role in energy production by hydrolyzing triglycerides and cholesteryl esters in lipoprotein and lipid droplets (LDs) to produce fatty acids (FAs), which serve as energy substrates for cellular activities via oxidative phosphorylation (OXPHOS). LAL demonstrates some osteogenic functions. Periodontal ligament (PDL) plays an important role as a reservoir of mesenchymal stem cells that can differentiate into osteoblasts and cementoblasts to maintain the homeostasis of periodontal tissue. However, the involvement of energy metabolism mediated via *LIPA*/LAL in the cytodifferentiation of human PDL (HPDL) cells has not been fully elucidated. In this study, we aimed to investigate the mechanism underlying the regulation of cytodifferentiation of HPDL cells mediated by LAL in terms of energy metabolism.

Methods: (1) To analyze the effects of Lalistat-2 (0 – 100 μM) on the LAL activity and cell viability and proliferation, we performed the LAL activity assay, trypan blue exclusion assay, and bromodeoxyuridine incorporation assay, respectively. To analyze the utilization of LD, we induced LD formation using oleic acids and then treated the cells with or without Lalistat-2 in the absence of fetal bovine serum (FBS). (2) To confirm the involvement of *LIPA* in the cytodifferentiation of HPDL cells, we performed osteogenic induction by supplementing the growth medium with 50 μg/ml of L-ascorbic acid and 5 mmol/L of β-glycerophosphate and then analyzed the mRNA expression levels of *LIPA* and calcification-related genes using real-time PCR. To examine the involvement of LAL in the cytodifferentiation of HPDL cells, we performed osteogenic induction with or without Lalistat-2 (10 μM) and then analyzed the mRNA expression of calcification-related genes, alkaline phosphatase (ALPase) activity, and calcified nodule formation. (3) To analyze the involvement of LAL in the energy metabolism pathway during the cytodifferentiation of HPDL cells at the mRNA level, we performed the osteogenic induction with or without Lalistat-2 (10 μM) and then analyzed the mRNA expression of metabolism-related genes. (4) To analyze the involvement of LAL in LD utilization, we stained LDs on day 0, 3, and 7 of the cytodifferentiation of HPDL cells and performed confocal microscopy. To analyze the metabolic profiles of HPDL cells during their cytodifferentiation, we measured the rate of ATP production during OXPHOS and glycolysis along with OXPHOS-related parameters using a real-time ATP rate assay and a Cell Mito Stress assay, respectively.

Results: (1) We found that Lalistat-2 (10 and 100 μM) inhibited at least 90% of LAL activity but did not affect cell viability and proliferation (0 – 100 μM). During starvation in the growth medium without FBS, HPDL cells treated with or without Lalistat-2 (1 μM) showed a significant decrease in LD counts whereas those treated with Lalistat-2 (10 and 100 μM) showed no significant difference in the remaining LDs, suggesting the inhibition of LD utilization by Lalistat-2 (10 and 100 μM). The results indicated that 10 μM was the minimum concentration of Lalistat-2 that was effective in HPDL cells, and hence, was used in subsequent experiments. (2) We found that the mRNA expression of *LIPA* was upregulated in a similar manner as that of alkaline phosphatase (*ALPL*) and collagen type I alpha I chain (*COL1A1*) during cytodifferentiation of HPDL cells, confirming the involvement of *LIPA* in the cytodifferentiation. In the presence of Lalistat-2, the mRNA expression of *ALPL*, *COL1A1,* and integrin-binding sialoprotein (*IBSP*) was significantly downregulated. Lalistat-2 also significantly decreased ALPase activity and calcified nodule formation during the cytodifferentiation of HPDL cells. These results indicated that inhibition of LAL by Lalistat-2 suppressed the cytodifferentiation of HPDL cells. (3) During cytodifferentiation of HPDL cells in the absence of Lalistat-2, the mRNA expression of ATP synthase F1 subunit alpha (*ATP5FA1*), an OXPHOS-related gene, glucose transporter 1 (*GLUT1*), a glucose-uptake-related gene, and carnitine palmitoyltransferase 1A (*CPT1A*), an FA-oxidation-related gene, was significantly upregulated, whereas that of lactate dehydrogenase A (*LDHA*), a glycolysis-related gene, was downregulated, suggesting the importance of FA oxidation and OXPHOS as the major energy production pathways during the cytodifferentiation of HPDL cells. In the presence of Lalistat-2, a significant downregulation of *ATP5FA1* mRNA expression was observed, indicating that LAL was involved in the energy metabolism of HPDL cells during their cytodifferentiation via regulation of the OXPHOS pathway. (4) The LD counts were reduced on day 3 and 7 of the cytodifferentiation of HPDL cells compared to those on day 0, suggesting that FAs were utilized during the cytodifferentiation of HPDL cells. In the presence of Lalistat-2, a higher number of LDs were observed in HPDL cells, suggesting that Lalistat-2 inhibited LD utilization during the cytodifferentiation of HPDL cells. We observed a significantly increased ATP production rate associated with OXPHOS, resulting in an increase in the total ATP production in HPDL cells, confirming that OXPHOS was a major energy production pathway involved in the cytodifferentiation of HPDL cells. In the presence of Lalistat-2, the total ATP production rate was significantly decreased, and the reduction was primarily attributed to the OXPHOS pathway. Furthermore, Lalistat-2 also largely decreased the spare capacity, an ability of the cells to generate more ATP by OXPHOS during the energy demand. The results indicated that LAL enhanced the OXPHOS pathway and supported LD utilization and ATP production during the cytodifferentiation of HPDL cells.

Conclusion: Collectively, we demonstrated that LAL utilized LDs to drive the OXPHOS pathway, resulting in adequate ATP production to support the physiological cytodifferentiation of HPDL cells and maintain the homeostasis of the periodontal tissue. We elucidated a feasible mechanism by which LAL regulates the pathophysiological conditions of periodontal tissue through energy metabolism.

様式 7

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

本研究は、エネルギー代謝に重要な役割を担うライソゾーム酸性リパーゼ（LAL）がヒト歯根膜細胞の分化制御機構に及ぼす影響について検討したものである。

その結果、LAL は、酸化的リン酸化（OXPHOS）経路を正に制御することで、ヒト歯根膜細胞が硬組織形成細胞へと分化する際に必要なアデノシン三リン酸（ATP）の産生を促進し、歯周組織の恒常性維持に寄与することを見出した。

これらの成果は、歯周組織における LAL 誘導性エネルギー代謝の生物学的機能の一端を明らかにし、歯周組織の恒常性維持機構の解明につながる重要な知見を与えるものであり、博士（歯学）の学位論文として価値のあるものと認める。