Physiological functions of the phospholipid-metabolizing enzyme Phospholipase D2 in anti-tumor immunity : regulation of CD8+ T lymphocyte proliferation

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論文の内容の要旨

Abstract of thesis

In this thesis, Ms. NGO THAI BICH VAN described the role of the phospholipid-metabolizing enzyme Phospholipase D2 in anti-tumor immunity. The abstract is as follows:

【材料と方法　Materials and Methods】

The applicant subcutaneously transplanted cancer cells into wild-type and *Pld2*-knockout mice to form tumors and analyzes tumor-infiltrating $CD4^+$ and $CD8^+$ cells by immunostaining. She also analyzed T cell population in the thymus and spleen by flow cytometry and applied bone marrow transplantation to investigate the effects of PLD2 on immune cells. *In vitro* proliferation of primary $CD8^+$ T cells under co-stimulation of CD3/CD28 is examined by using Carboxyfluorescein succinimidyl ester (CFSE) assay. CD3/CD28-dependent phosphorylation of Erk in primary $CD8^+$ T cells is also analyzed by Western blot analysis. Ras activation was detected by pull-down assay.

【結果　Results】

The applicant found that the growth of the tumor formed by subcutaneously transplanted cancer cells was enhanced in $Pld2^{-/-}$ mice. Unlike PLD1, PLD2 was dispensable for tumor angiogenesis in mice. In line with the increase of tumor size, cell apoptosis was inhibited in tumors formed in $Pld2^{-/-}$ mice. The result of bone marrow transplantation indicated that PLD2 in bone marrow- derived cells showed a suppressive function in tumor growth.

Thus, the applicant examined the function of PLD2 in T lymphocytes as they were the major components of adaptive immunity, being recruited into the tumor microenvironment together with other immune cells to eliminate tumor cells. Interestingly, the number of $CD8^+$ T cells, which are known to induce cancer cell death, was significantly decreased in *Pld2*$^{-/-}$ tumor sections. In addition, CD3/CD28-stimulated proliferation of primary cultured $CD8^+$ T cells isolated from the *Pld2*$^{-/-}$ spleen was significantly suppressed compared to that of WT $CD8^+$ T cells. On the other hand, no significant difference was seen in proliferation between WT and *Pld2*$^{-/-}$ $CD4^+$ T cells. Furthermore, CD3/CD28-dependent phosphorylation of Erk1/2 and Ras activation were inhibited in *Pld2*$^{-/-}$ $CD8^+$ T cells. Production of Interleukin-2 (IL-2), one of the downstream target gene of Erk, which is known to promote $CD8^+$ T cell proliferation, was also decreased in *Pld2*$^{-/-}$ $CD8^+$ T cells.

【考察　Discussion】

Previous reports provided evidences for the central role of $CD8^+$ T cells in anti-tumor immunity and are consistent with the notion that impaired $CD8^+$ T cell infiltration into tumor microenvironment in *Pld2*$^{-/-}$ mice promoted tumor growth. In the present study, the applicant focused on the function of PLD2 in $CD4^+$ and $CD8^+$ T cells. In dissecting the signaling pathway involved in PLD2-mediated T cell proliferation, she found that PLD2 wa required for CD3/CD28-stimulated Ras activation and Erk phosphorylation in primary $CD8^+$ T cells. Other reports showed that PLD2 can activate Ras through the action of RasGEF. Taken together, it was reasonable to speculate that, upon TCR engagement of $CD8^+$ T cells, PLD2 recruits a Ras GEF to the specific compartment such as the plasma membrane, inducing compartmentalized and temporal activation of Ras-Erk signaling to promote cell proliferation and survival.

審査の結果の要旨
Abstract of assessment result

【批評　General Comments】

The applicant provided evidence that PLD2 promotes $CD8^+$ T cell proliferation upon TCR engagement in the spleen through the activation of Ras-Erk pathway and IL-2 production, thereby potentiating the anti-tumor immune response. The findings revealed a novel function of PLD2 in the immune system, which may contribute to eliminate cancer cells.

【最終試験の結果　Assessment】

The final examination committee conducted a meeting as a final examination on June 5, 2017. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結果　Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.