【Grant-in-Aid for Scientific Research (S)】

Biological Sciences (Medicine, Dentistry, and Pharmacy)

Title of Project : Engulfment of Apoptotic Cells and Asymmetry of Plasma Membranes

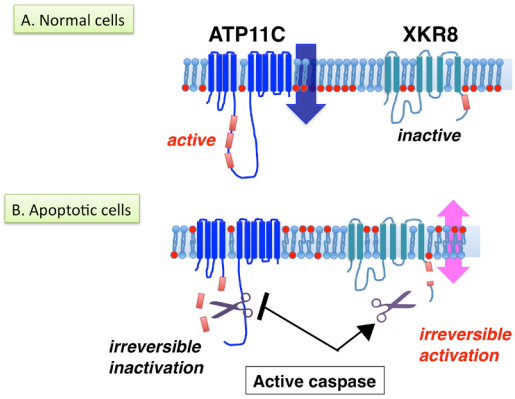
Shigekazu Nagata
(Osaka University, Immunology Frontier Research Center, Professor)

Research Project Number : 15H05785 Researcher Number : 70114428
Research Area : Biochemistry, Molecular biology
Keyword : Apoptosis, Macrophage, Phosphatidylserine, Flippase, Scramblase

【Purpose and Background of the Research】

Macrophages engulf apoptotic cells using Tim-4, MFG-E8 and Mer/Protein S that recognize phosphatidylserine (PtdSer). If this process does not occur smoothly, it will cause autoimmune disease. Plasma membranes consist of two layers. In healthy cells, PtdSer is exclusively localized in the inner leaflets, but exposed in apoptotic cells. ATP11C work as a flippase that translocates PtdSer from outer to inner leaflets, while Xkr8 scrambles PtdSer during apoptosis.

In this project, we will perform the following studies. (1) Interaction of Mer and its homologues (Tyro3 and Axl) with their ligands (Protein S and Gas 6), and their ability to engulf apoptotic cells. (2) Effect of Tim-4 on MerTK kinase activity, and identification of Mer's targets. (3) Physiological functions of Xkr4 and Xkr9 that work as a scramblase. (4) Tissue distribution of other P4-type ATPases and their function as a flippase.



【Research Methods】

(1) By establishing cell lines that express only Mer, Axl, or Tyro3 in the absence or presence of Tim-4, we will study their ability to engulf apoptotic cells. We prepare the extracellular region of Mer, Axl, and Tyro3, and biochemically study their interaction with Protein S or Gas6. (2) Using the immunoprecipitation followed by Western blotting, we will study the association of Tim-4 and Mer. (3) By establishing knock-out mice, we will try to elucidate the physiological function of Xkr4 and Xkr8. (4) By expressing each member of the P4-ATPase family in ATP11C-null cells, we will study whether P4-ATPases other than ATP11C have flippase activity or not.

【Expected Research Achievements and Scientific Significance】

This project is fully based on our own previous results. When this project is accomplished, it will reveal the molecular mechanism and physiological role of the engulfment of apoptotic cells. Our studies on flippases and scramblases in this project will elucidate how the asymmetrical distribution of phospholipids is maintained in healthy cells. It will also reveal why and how the asymmetry of plasma membrane is broken in some physiological settings. Our previous study on the molecules that recognize PtdSer indicated that if apoptotic cells are not properly engulfed by macrophages, it will cause autoimmune diseases. The de-regulation of scramblase and flippase may also cause various diseases, and the outcome of this project would contribute to our understanding of human diseases such as autoimmune diseases.

【Publications Relevant to the Project】

・Segawa, K. et al. (2014) Caspase-mediated cleavage of phospholipid flippase for apoptotic phosphatidylserine exposure. Science 344: 1164-1168.
・Suzuki, J. et al. (2013) Xk-related protein 8 and CED-8 promote phosphatidylserine exposure in apoptotic cells. Science 341: 403-406.

【Term of Project】 FY2015-2019

【Budget Allocation】 118,100 Thousand Yen

【Homepage Address and Other Contact Information】

http://biochemi.ifrec.osaka-u.ac.jp/
snagata@ifrec.osaka-u.ac.jp