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Roles of Membrane Contact Sites in Organelle Dynamics and Diseases

ORGANELLA
Tokyo University of Pharmacy and Life Sciences

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I am the representative for the research program entitled “Roles of membrane contact sites in organelle dynamics and diseases” adopted as a MEXT-Supported Program for the Strategic Research Foundation at Private Universities in 2014.

Cell is the structural and functional unit of life. In the cell, there are several kind of organelles surrounded with membrane and they closely communicate with each other. It is necessary for the efficient signal transmission between different organelles to collect information at proximal position without dispersion. Thus, regulation of membrane contact sites in organelle dynamics seems to be important for maintenance of cell function. In particular, biochemical and recent electron microscopy (EM) studies confirmed the presence of specific regions of close apposition between mitochondria and ER, termed mitochondria-associated membranes (MAM). MAM plays a role in many cellular functions, including calcium homeostasis, phospholipid metabolism, formations of autophagosome and inflammasome, apoptosis and cell growth signaling. Furthermore, it has been reported that MAM dysfunction is involved in various diseases such as Alzheimer disease, cardiac failure, cancer, and anti-virus immune response. Thus, it is emerging project to research MAM function for understanding of disease pathology and development of new drug against these diseases.

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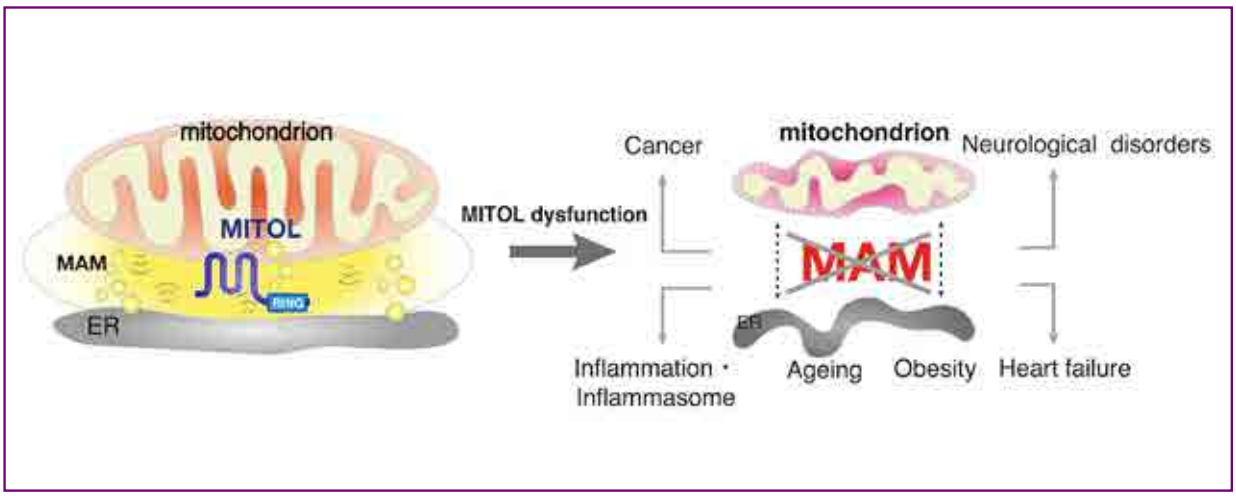
We can challenge for elucidation of MAM function under the circumstances, because excellent scientists with a proven track record for MAM research gather in School of life Sciences of Tokyo university of Pharmacy and life Sciences. In cooperation with many experts for various fields in the inside and outside of university, we try to clarify the roles of MAM in organelle dynamics and diseases. This project may contribute greatly to understanding of pathology and development of new treatments for MAM-associated diseases. In addition, we aim for cultivation of young scientists who are responsible for next generation.



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Elucidation of the roles of MITOL in the formation of mitochondria-associated membrane (MAM) and relationship between MAM disruption and diseases

Roles of
Membrane Contact Sites in
Organelle Dynamics and
Diseases

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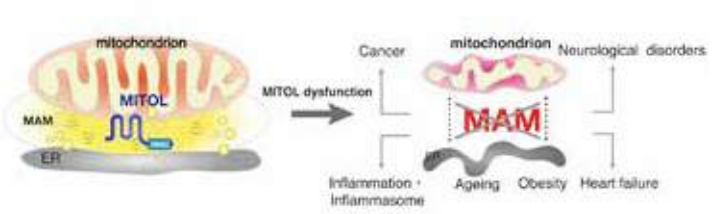
Research summary

The mitochondrion plays an important role in exchanging Ca2+ or metabolizing lipids by positioning at sites situated close to the endoplasmic reticulum (ER), which is called MAM. Mitofusin2 (Mfn2) is a mitochondrial fusion factor which is localized in the endoplasmic reticulum as well as in mitochondria and play a role in the attachment of these organelles to each other. Although a previous study indicates that Mfn2 is required for MAM formation, the mechanisms regulating this process remain unknown. Recently we have demonstrated that MITOL ubiquitinates Mfn2 and induces MAM formation through Mfn2 oligomerization. However, the regulatory mechanism for MITOL-induced MAM formation and the involvement of disrupted MAM in disease pathology are still obscure. In this project, we will explore the roles of MITOL in the MAM formation and MAM-associated diseases such as Alzheimer disease.

Figure

A model of the mechanism by which MITOL regulates MAM formation

MITOL ubiquitinates mitochondrial Mfn2 on its lysine residue in position 192 in a K63-dependent manner, thereby activating Mfn2. In turn, activated Mfn2 binds to Mfn2 localized in the ER, resulting in mitochondria-ER tethering by oligomerization of Mfn2.



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Elucidation of the roles of syntaxin 17 localized in the mitochondria-associated membrane (MAM) and its participation in MAM-associated diseases

Roles of
Membrane Contact Sites in
Organelle Dynamics and
Diseases

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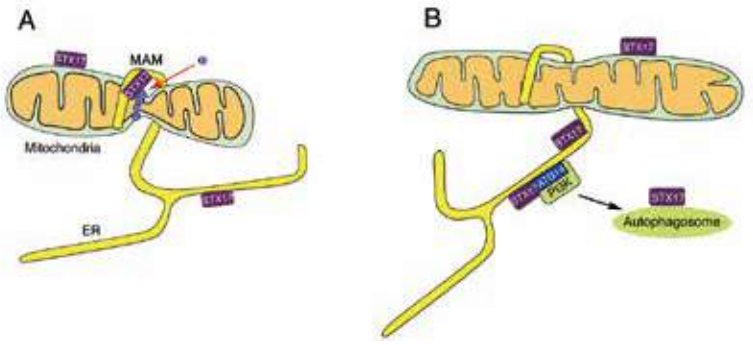
Research summary

The endoplasmic reticulum (ER) contains various subdomains that are in contact with other organelles. The ER subdomain facing mitochondria is called the mitochondria-associated membrane (MAM). The MAM regulates mitochondrial activity through Ca^{2}+ and synthesizes lipids in cooperation with mitochondria. Accumulating data have disclosed that the ER-mitochondria interface is the site for various important cell functions, beyond Ca^{2}+ homeostasis and lipid synthesis. Moreover, the close relationship between this site and neurodegenerative diseases has been pointed out. In this project, we will explore the roles of syntaxin 17 (STX17) in the ER-mitochondria interface and MAM-associated diseases.

Figure

Different roles of STX17 in response to cellular physiology. (A) In fed cells, STX17 promotes mitochondrial fission by regulating Drp1 (represented by D in blue circle) localization/activity. (B) In starved cells, STX17 switches its binding from Drp1 to the PI3K subunit ATG14, leading to mitochondrial elongation and autophagosome formation. This elongation allows mitochondria to escape from autophagic degradation.



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Roles of
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Role of phospholipids and transport proteins in the organization and interplay of ER subdomains

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Research summary

The endoplasmic reticulum (ER) is a multifunctional organelle with various subdomains. Proper localization of lipids and proteins is essential for the formation of these subdomains. In this project, I plan to analyze the localization, movement, and dynamics of phospholipids and proteins in each subdomain and disclose the interplay between the MAM and other ER subdomains.

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Analysis of MITOL-deficient mice

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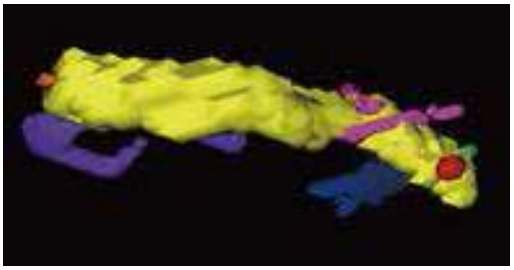
Research summary

Mitochondria are important organelle to produce energy, but mitochondrial dysfunction is closely involved in various diseases. There are several machineries regulating mitochondrial quality to maintain mitochondrial functions. Recently, it has been suggested that the efficient exchanges of lipids and calcium between mitochondria and ER by a direct tethering play an important role in maintaining the function of the mitochondria. We reported that mitochondrial ubiquitin ligase (MITOL) regulates mitochondria-ER contacts via Mfn2. In this project, we try to elucidate the mechanism of mitochondria-ER contact manner through analysis of MITOL-deficient mice.

Figure

Three-dimensional reconstruction of mitochondrion and ER.

Plural ER (red, blue, purple, pink, and green) contact with a mitochondrion (yellow).



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Role of mitochondria-associated membrane (MAM) in *Legionella* infection

Roles of
Membrane Contact Sites in
Organelle Dynamics and
Diseases

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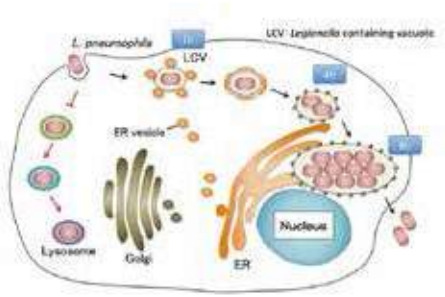
Research summary

Many microbes and viruses create intracellular environments advantageous for their survival and growth by hijacking host physiological machinery. Recent studies have revealed that some microbes and viruses manipulate function of organelle contact sites such as the ER (endoplasmic reticulum)-mitochondria contact site. In this project, I will try to understand the role of organelle contact sites, in particular, the ER-mitochondria contact site in intracellular pathogenesis of *Legionella pneumophila* that is known to cause severe pneumonia.

Figure

Intracellular pathogenesis of *Legionella pneumophila*.

After uptake into the host via phagocytosis, *Legionella pneumophila* prevents its degradation by inhibiting the delivery to lysosome. Simultaneously, Legionella recruits host ER-derived vesicles to the Legionella-containing vacuole to convert it into ER-Golgi intermediate compartment like structures, and then the pathogen-occupied membrane fuse with the ER and *Legionella* start to replicate.



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Roles of
Membrane Contact Sites in
Organelle Dynamics and
Diseases

Possible linkage between peroxisome biogenesis and lipid droplet formation through Sec16B

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Research summary

The endoplasmic reticulum (ER) is the starting site of the secretory pathway. Recent studies revealed that the ER is adjacent to or in contact with other organelles such as mitochondria, peroxisomes, and lipid droplets. We recently reported that Sec16B, which was assumed to participate in the conventional secretory pathway, is involved in protein transport from the ER to peroxisomes. In this project, I will study a system for protein transport from the ER to peroxisomes and compare this with the conventional secretory system. I will also explore the relationship between peroxisome biogenesis and lipid droplet formation.

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Roles of
Membrane Contact Sites in
Organelle Dynamics and
Diseases

Role of Phosphoinositide Turnover in MAM functions

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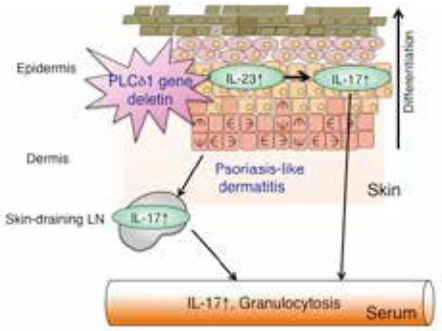
Research summary

Phosphoinositide metabolism is an important intracellular signaling system involved in a variety of cell functions. In this system, phosphatidylinositol 4,5-bisphosphate is hydrolyzed by phospholipase C to generate two second messengers, inositol 1,4,5-trisphosphate (IP3) and diacylglycerol. IP3/calcium mobilization is suggested to be important in maintenance of the MAM function. On the other hand, dysfunction of MAM function often causes various diseases. Therefore we try to focus on the correlation between phosphoinositide metabolism and physiological functions of MAM. We also examine whether dysfunction of phosphoinositide metabolism in MAM links to cancer or skin diseases.

Figure

Importance of PLCδ1 in skin.

Epidermal loss of PLCδ1 causes increased production of IL-23 and IL-17 in the epidermis. This aberrant activation of the local IL-23/IL-17 axis resulted in a phenotype similar to that in human psoriasis. Serum IL-17 levels were also increased, resulting in granulocytosis.
(Kanemaru et al. Nature Commun. 2012)



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Roles of
Membrane Contact Sites in
Organelle Dynamics and
Diseases

The roles of MAM in the activation of intestinal macrophages in inflammation

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Research summary

Tissue-resident macrophages recognize dead cells or tissue damages in various physiological conditions, and initiate inflammation and immune responses. These responses by the macrophages are recently found to play important roles in determining the pathology of various diseases. We have currently identified the CD169-postive macrophages as the cells that orchestrate the immune responses associated with tissue damage. In this study, we are focusing on the roles of MAM in the activation of CD169-postive macrophages in inflammatory conditions, and are investigating the therapeutic strategy with regulating the function of MAM in these macrophages.

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Roles of
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Roles of MAM during Endothelial-to-Mesenchymal Transition (EndMT) in Tumor Microenvironment

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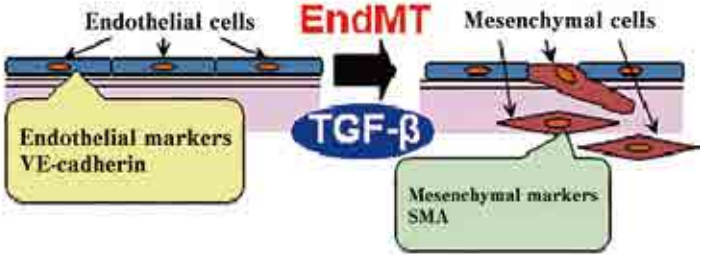
Research summary

Tumor tissues are composed not only of cancer cells but also of tumor vessels, cancer associated fibroblasts (CAFs) that play important roles in cancer progression. This tumor microenvironment is influenced by tumor specific cytokines that alter the structures of various organelle of tumor component cells. However, the roles of such tumor specific cytokines in the formation and maintenance of such organelle structures have not yet been elucidated. We attempt to study how transforming growth factor-β (TGF-β), which is abundant in tumor microenvironment, affect the mitochondria-associated membrane (MAM) of tumor endothelial cells. In tumor microenvironment, endothelial cells undergo endothelial-to-mesenchymal transition (EndMT), which leads to the formation of CAFs. This study will help understand the novel mechanisms how TGF-β-induced alteration of MAM is involved in the progression of cancer and aid developing new therapeutic strategies.

Figure

In tumor microenvironment, TGF-β induces endothelial-to-mesenchymal transition (EndMT) in which endothelial cells lose their characteristics (cell-cell contact and expression of endothelial markers, such as VE-cadherin) and acquire mesenchymal characteristics (high migratory activities and expression of mesenchymal markers such as smooth muscle α-actin).



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Roles of
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In Vitro Reconstitution of the Mitochondria-Associated ER Membranes (MAM) using *Xenopus* egg extracts

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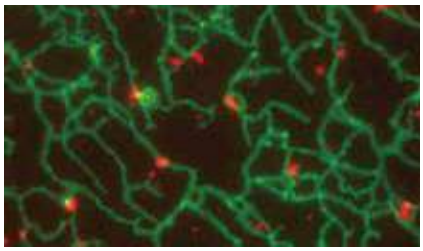
Research summary

The endoplasmic reticulum (ER) is physically connected to mitochondria at a region called the mitochondria-associated membrane (MAM), which plays important roles in regulating not only mitochondrial activity but also various fundamental phenomena such as cell growth, apoptosis and autophagy. In this study, we will develop a novel in vitro system that recapitulate the MAM structure using fractionated *Xenopus* egg extracts for the purpose of analyzing the molecular architecture and biological function of the MAM.

Figure

A microscopic picture showing that mitochondria (red) are deposited along the ER tubule network (green) formed in egg extracts.



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