Science and Technology Group
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1 Introduction

Cellular wounding and repair of local plasma membranes occurs constantly in our bodies. Plasma membrane damage can be induced by various triggers ranging from physical disruption and pathogen invasion to physiological cellular activities, such as muscle contraction, cell division, and the secretion of vesicles. Accumulating evidence suggests the involvement of cellular wound healing in various diseases. However, the detailed molecular mechanisms and physiological consequences of plasma membrane repair are poorly understood. We recently discovered that plasma membrane damage activates a cell cycle checkpoint, resulting in transient or permanent arrest of the cell cycle during plasma membrane repair (Kono *et al*., Proc. Natl. Acad. Sci. U. S. A., 2016). Furthermore, the damaged site memories the membrane damage as a small tubular bud on the outer surface of plasma membrane, and it affects transient or prominent cell cycle arrest depending on the plasma membrane damage quantities. Permanent cell cycle arrest is characterized by its specific metabolic activity and dramatic changes in cell morphology. Originally, it was proposed to be due to the shortening of telomeres after the repeated proliferation. Now that it is known that the cell cycle arrest is also induced by DNA damage response (DDR), oncogene expressions and several stresses.

In my study, I found that the plasma membrane damage induces acute and prominent cell cycle arrest in human cultured cells without showing DDR. The key regulator of this membrane damage repair is considered to be extracellular calcium influx to the cytosol, and I found that this calcium influx induced many drastic changes in many organelles such as ER, lysosomes (not shown here) and mitochondria. To reveal how the membrane damage repair affect the cell cycle progression, I am now focusing on most important changes observed in a mitochondria.

2 Activities and Findings

2.1. Calcium is necessary for membrane repair

I have examined the importance of calcium for the membrane repair. By depleting or reducing the amount of extracellular calcium, human cell cells showed acute rupture after membrane damage introducing treatment
(Fig.1).

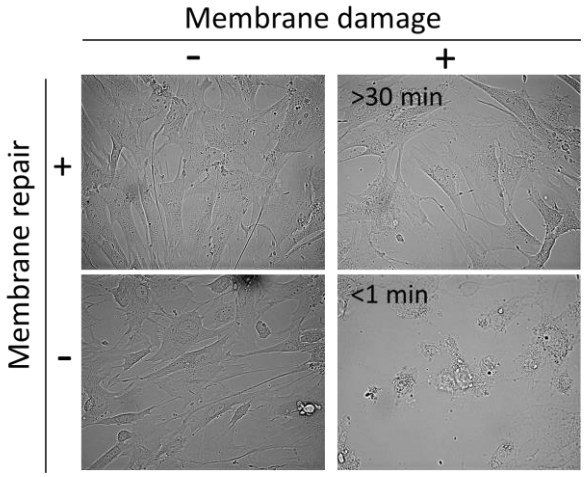

Figure1 Membrane repair ability is necessary for cell survival. When the culture condition lacks extracellular calcium, acute rupture of plasma membrane was observed.

2.2. membrane damage induces morphological changes of mitochondria.

I tried to find key phenomena for the cell cycle arrest after the plasma membrane damage repair, and found that mitochondria showed rapid fragmentation (fig.2). It was a quite rapid process (<20min after the plasma membrane damage induction). Calcium is known to be a main regulator of mitochondrial fission, and this morphological change is due to the calcium influx to the cytosol. Mitochondrial shape is known to correlated with its ATP production capacity, and this morphological change can be

considered as the key regulator of cell cycle arrest after the plasma membrane damage repair.

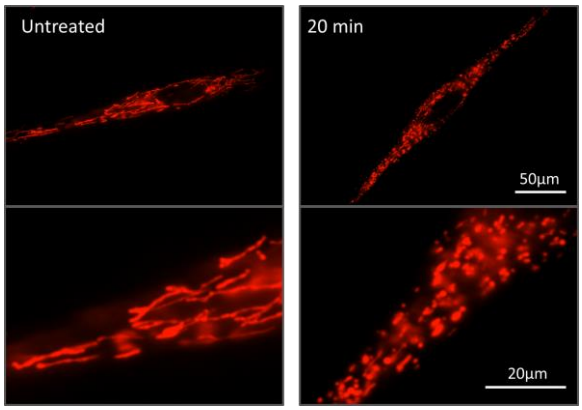

Figure2 Rapid mitochondrial fragmentation was observed shortly after the membrane damage introducing treatment.

2.3. Other membrane damage treatment also invites permanent cell cycle arrest.

To see the effect of calcium influx to the cytosol, I tried to treat cells with other membrane damage inducing treatments, like pore forming toxins, Ethanol, and KCl addition as a stimulator of voltage dependent calcium channel. As a result, any treatment that induce calcium influx showed acute and permanent cell cycle arrest. I will continue to analyze this cell cycle arrest in the perspective of calcium influx and changes in mitochondrial function.

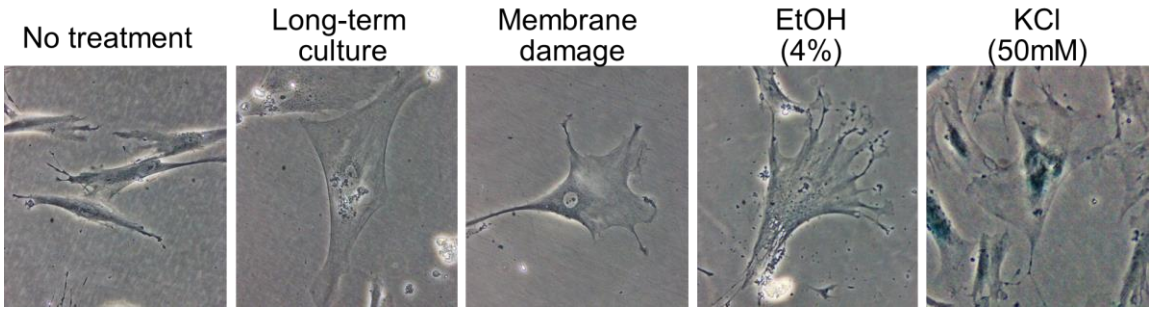

Figure4 Other membrane damage treatment invited permanent cell cycle arrest.

3 Collaborations

Kono Unit, OIST

4 Publications and other output

<presentation>

Keiko Kono and Yohsuke Moriyama, *Fate determination by the cellular surface wound in the budding yeast,* Japanese Society for Biotechnology annual meeting (2019.09.07)

Yohsuke Moriyama, *Membrane Damage Repair and Cellular Senescence*, OIST internal seminar (2019.11.29)

<Lecturer>

OIST Children's School of Science 2019 lecturer: Yohsuke Moriyama, Try to make future from the silk, OIST Children's School of Science 2019 Onna elementary school, Onna (2019.08.20-21)

After school lecturer: Yohsuke Moriyama, Try to see tiny things by the microscope. Science club of Okita elementary school, Nago (2019.12.09)