Science and Technology Group
Annual Report FY2020

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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 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 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).

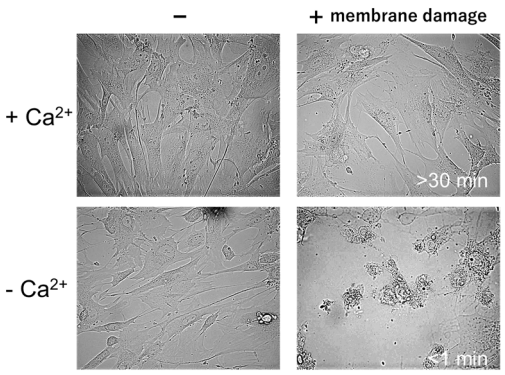

Figure1 Membrane repair ability is necessary for cell survival. When the culture condition lacks extracellular calcium, acute rupture of plasma membrane was observed.

2.2. Transient plasma membrane damage treatment induced permanent cell cycle arrest

I found that transient treatment of normal human fibroblasts with membrane damaging treatment shows cell cycle arrest. As a result, cell proliferation was inhibited after the treatment, and the proportion of SA-β-gal positive cells, a kind of senescence cell marker, was increased.

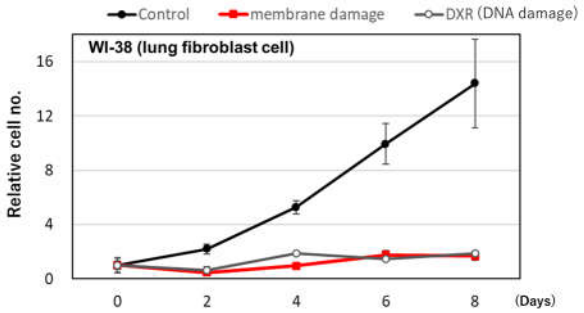

Figure2 Transient plasma membrane damage inducing treatment showed cell cycle arrest.

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2.3. 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.3). 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.

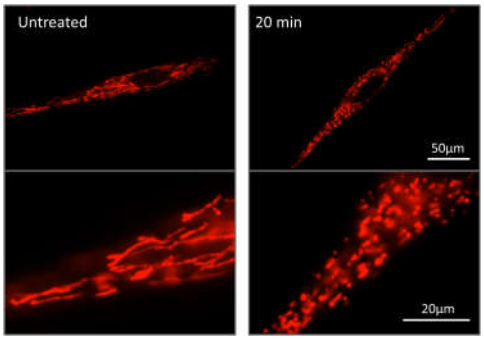

Figure3 Rapid mitochondrial fragmentation was observed shortly after the membrane damage introducing treatment.

2.4. 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 possible membrane damage inducing treatments, like Ethanol, pore forming toxins, 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.

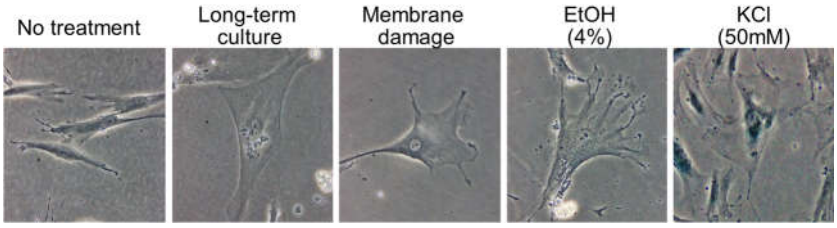

Figure4 Other membrane damage treatment also induced permanent cell cycle arrest.

2.5. Membrane damage inducing treatment left scars.

Serial electron microscopic observation revealed that transient membrane damage inducing treatment left many projections on the plasma membrane even after 8 days.

In addition to the cell cycle arrest in the perspective of calcium influx and changes in mitochondrial function, I will see the significance of these plasma membrane damage dependent "scars" for the establishment of cell cycle arrest and cellular senescence.

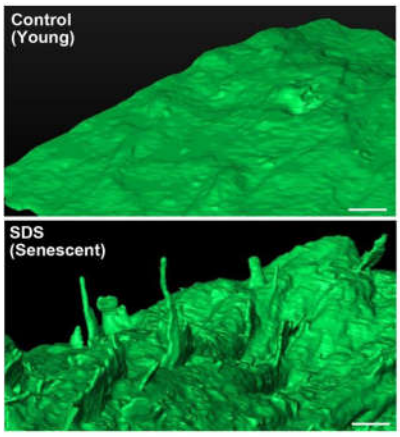

Figure5 membrane damage inducing treatment increased projections on the plasma membrane.

3 Collaborations

Kono Unit, OIST

4 Publications and other output

K Kono, Y Johmura, Y Moriyama, Y Masukagami, K Nishimura, H Barbee, H Takase, S Sugiyama, Y Sato, T Higashiyama and M Nakanishi, **Plasma membrane damage limits replicative lifespan in yeast and human fibroblasts,** BioRxiv, 2021

https://www.biorxiv.org/content/10.1101/2021.03.26.437120v1.full

doi: https://doi.org/10.1101/2021.03.26.437120