Science and Technology Group
Annual Report FY2018

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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. To reveal how the membrane damage affect the cell cycle progression, I am now focusing on what happens when the cell suffers plasma membrane damage.

2 Activities and Findings

2.1. Transient membrane damage arrest cell cycle permanently.

To establish experimental procedures, I have tested several conditions of membrane damage inducing treatments. Finally, I have adopted one best condition that can induce permanent cell cycle arrest after membrane damage inducing treatment (Fig.1). Once treated with this membrane damage inducing condition, cell cycle was arrested almost same as doxorubicin (DXR) treatment for one day, that induce DNA damage dependent cell cycle arrest.

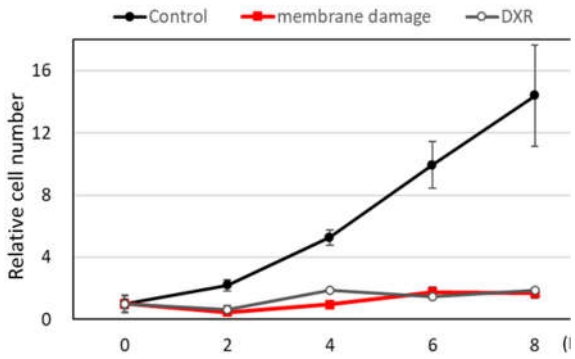

Figure1 Relative cell number of fibroblast cell after 1day treatment of membrane damage induction or Doxorubicin treatment.

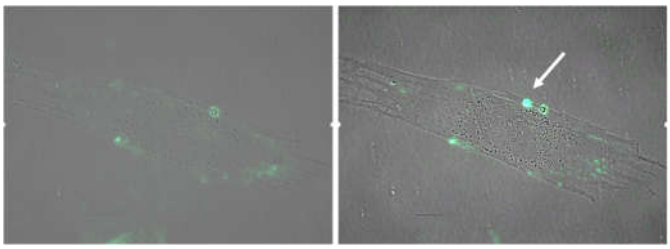

Figure2 Observation of membrane damage sites. The newly made damaged site on the membrane was observed as green fluorescent spots (right image; arrow head).

2.2. Observation of membrane damage site with a fluorescent probe.

To observe damaged site of plasma membrane, I have tested several fluorescent probes, and a kind of lipid specific fluorescent probe could stain damaged membrane site specifically. With this probe, I could observe when and how membrane suffer damages by the live cell imaging (figure2).

2.3. membrane repair factor is necessary for cell survival

I have identified one important factor for membrane repair after induction of plasma membrane damage. By depleting or reducing the amount of this factor, fibroblast cells showed acute rupture after membrane damage induction (Figure3).

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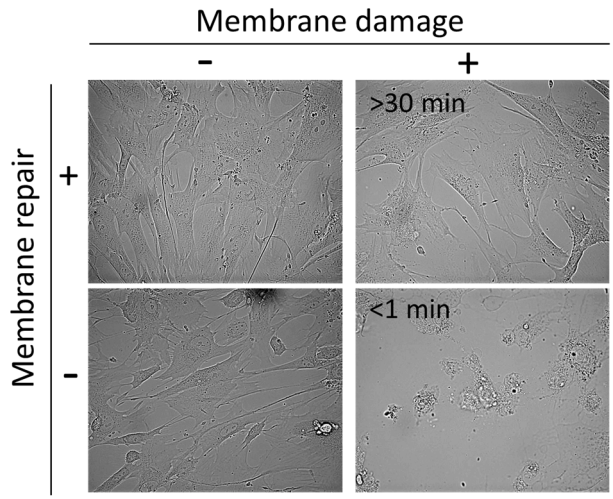

Figure3 Membrane repair ability is necessary for cell survival. When the culture condition lacks membrane repair factor, acute rupture of plasma membrane was observed.

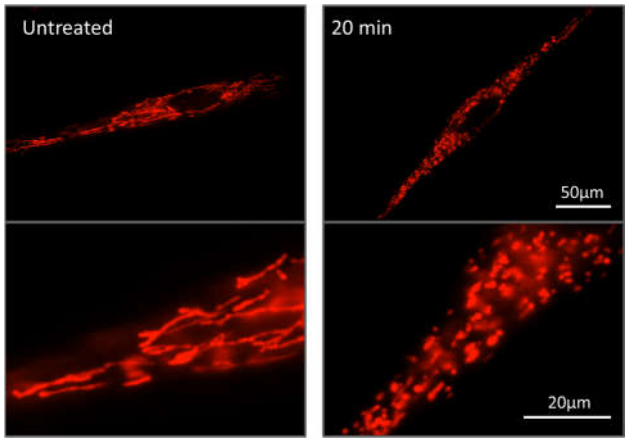

Figure4 Rapid mitochondrial fragmentation was observed shortly after the membrane damage induction.

2.4. membrane damage induces morphological changes of mitochondria.

I had tried to find key phanomena for the cell cycle arrest after the plasma membrane damage induction, and found that mitochondria showed rapid morphological changes. It was a quite rapid process (<20min after the plasma membrane damage induction), and a reversible process after the removal of membrane damage stimulating condition. I am now trying to test whether this mitochondrial fragmentation and accompanying reduction of ATP production and reactive oxygen production can arrest cell cycle.

3 Collaborations

Kono Unit, OIST

4 Publications and other output

Yohsuke Moriyama, Hunter Barbee, Yumiko Masukagami, Yuri Matsui and Keiko Kono, *The plasma membrane ultrastructure after cellular wound healing,* The 16th International Membrane Research Forum (2019.03.19)

Yumiko Masukagami, Yohsuke Moriyama, Hunter Barbee, Yuri Matsui and Keiko Kono, *The plasma membrane ultrastructure of aged cells*, The 16th International Membrane Research Forum (2019.03.19)