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Perspective

Organelle remodeling in somatic cell reprogramming

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Works based on the generation of induced pluripotent stem cells (iPSCs) mediated by expressing Yamanaka cocktail factors (Takahashi and Yamanaka, 2006) have been extensively reported. During somatic cell reprogramming, many organelles get remodeled on multilayer such as organelle morphology, metabolic patterns, and meanwhile epigenetic landscapes and gene expression changes. In the process of organelle plasticity control, lipids, ions, and metabolites are ferried to particular targets. Such reorientations need the aid of membrane trafficking. However, most related mechanisms and physiological/pathological functions remain largely unknown.

Organelle membrane trafficking

Membranous organelles are abundant in cytoplasm and carry various functions. Mitochondria are the factories of energy production. Ribosome, endoplasmic reticulum (ER), and Golgi apparatus engage in protein synthesis and transportation. Lysosome is capable of degenerating senescent molecules or exogenous germs. Organelle remodeling is a globally occurred event in somatic cell reprogramming. Autophagy signaling and metabolic reprogramming involved in mitochondria remodeling have been studied extensively. In addition, landscape reconstruction of some other organelles such as midbody and ER (Kuo et al., 2011; Simic et al., 2019) have also been reported in the reprogramming process. These intracellular membrane structures compartmentalize the whole intracellular space to maintain regular biological processes. Concomitantly, in order to regulate material exchanges and signal transductions within such segregation, eukaryotes have evolved many structural and functional connections—vesicle trafficking and membrane contact sites (MCSs).

The ubiquitous vesicle trafficking includes cargo-laden vesicular trafficking and autophagy. In Sox2/Klf4/Oct4 (SKO) reprogramming, we observed BCL2 interacting protein 3 like (BNIP3L)-dependent mitophagy and found that the endosome-related protein Ras analog in brain protein 5 (RAB5) was involved in mitophagosome formation (Xiang et al., 2017; Figure 1). This study has demonstrated the high dynamics and interactions of multiple organelles as well as the spatial and temporal remodeling rules under the precise regulation of protein machines. In Sox2/Klf4/Oct4/c-Myc (SKOM) reprogramming, autophagy has been observed and shown to be involved in the early phase of iPSC generation (Wang et al., 2013; Wu et al., 2015; Liu et al., 2016). On the other hand, vesicle-mediated endocytosis has also been implicated in cell fate decision. Feng Liu's team has revealed that Rab5c regulates endocytic trafficking to promote hematopoietic stem and progenitor cell development (Heng et al., 2020). Wei et al. (2018) have demonstrated a previously unrealized lipid tracking pathway showing that mitochondria are involved in endocytic pathway. Whether vesicle-mediated endocytosis is involved in transition of pluripotency is an interesting question.

In addition to vesicle trafficking pathways, the recalibration of membrane component during organelle remodeling process happens via vesicular-independent material delivery pathways. One way in which organelles communicate is by forming MCSs, regions of close contact between two organelles. It is acknowledged that MCSs are hubs for organelle communication, governing membrane remodeling and rewiring organelle destiny. With a distance of ~10–30 nm, these two organelles are in close proximity but not fused. How could MCSs maintain such a distance but membranes here would not fuse? Does it depend on the interaction of the tether proteins or some unknown self-inhibiting regulatory mechanism? Further studies are necessary to elucidate these mechanisms.

Despite that the structures that establish and maintain MCSs are largely unknown, the functions of MCSs such

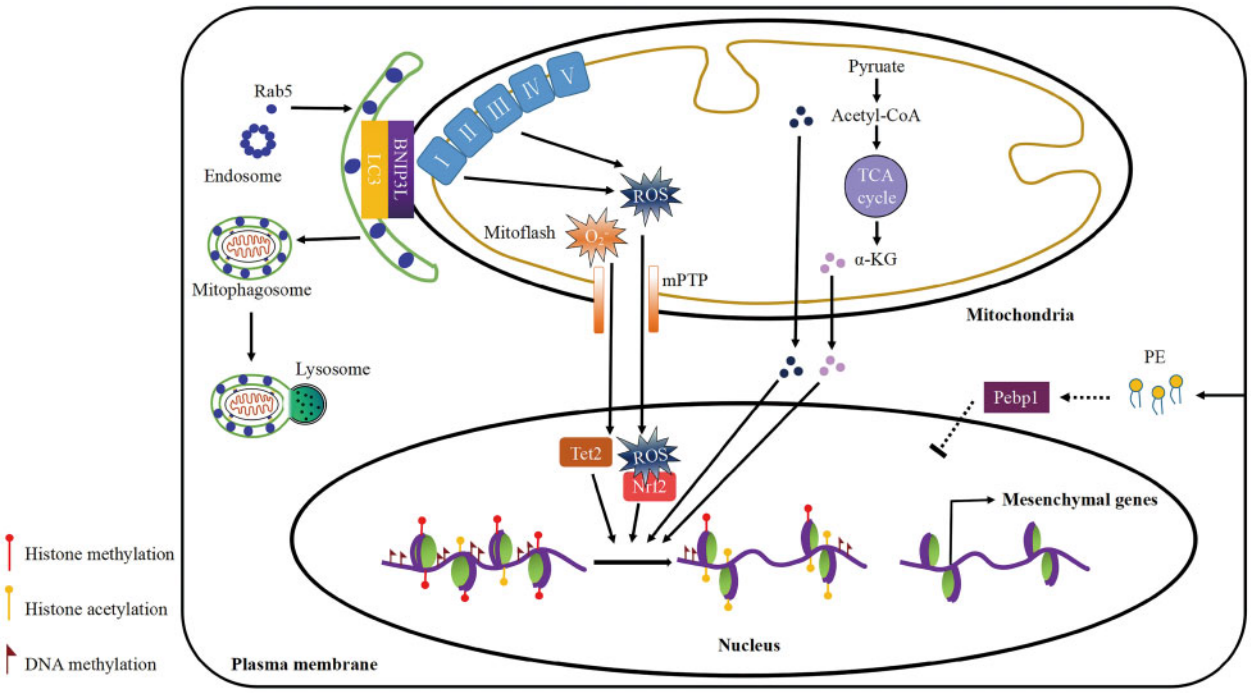

Figure 1 Organelle remodeling is a universal phenomenon in the induction of PSCs. The interaction and communication of membranous organelles, especially the mitochondria, link metabolic rewiring to pluripotency acquisition and maintenance. Concomitantly, organelle ion signaling and metabolites both regulate nuclear epigenetics for pluripotency acquirement. α-KG, α-ketoglutarate; LC3, microtubule-associated protein 1A/B-light chain 3; Pebp1, phosphatidylethanolamine binding protein 1.

as controlling Ca^{2+} signaling, lipid exchanges, and autophagosome formation have been extensively described. MCSs have also been suggested to be involved in organelle division. ER tubules seem to play a crucial role in organelle division. Hoyer et al. (2018) have found that ER tubules wrap around the endosome, shortly after the sequestration of cargo and formation of endosome bud, for regulating endosome fission. Furthermore, MCSs are also involved significantly in many pathological processes that will be exhibited later.

Visualization of structures and dynamics of MCSs has always been considered technically challenging. Encouragingly, Liangyi Chen's group has developed a super-resolution fluorescence-assisted diffraction computational tomography to reveal the 3D landscape of the cellular organelle interactome (Dong et al., 2020), and Dong Li's group has developed a grazing incidence structured illumination microscopy to visualize organelle at nanoscale resolution on millisecond timescales (Guo et al., 2018). These advanced living-cell imaging techniques may help to unmask the MCS dynamics during cell reprogramming and also in some pathological processes.

Organelle oxygen ion signaling

As for organelle remodeling regulation, various stresses trigger the cytoplasmic spatial transfer of ions and metabolites that are involved in pluripotency acquisition and maintenance. The early radical theory has stated that mitochondrial reactive oxygen species (mtROS) evoke senescence because of the toxicity to mitochondrial DNA (mtDNA), mitochondrial proteins, and membrane lipids. ROS overproduction can disrupt redox balance and further induce ferroptosis or apoptosis, while intracellular antioxidant systems like nuclear factor erythroid 2 related factor 2 (Nrf2), sirtuins, and unfolded protein response of mitochondria (UPR^{mt}) can supervise and detoxify excessive ROS. Mitoflash is a phenomenon involving sudden depolarization of $\Delta\Psi m$, bursting production of superoxide, and accelerated extrusion of matrix protons (Wang et al., 2008). We demonstrated that, in early somatic cell reprogramming, mitoflash is transiently activated to regulate Nanog expression via lowering promoter region methylation by ten-eleven translocation methylcytosine dioxygenase 2 (Tet2) occupancy, so as to improve reprogramming efficiency (Ying et al., 2016; Figure 1). Consistent with our study, others have reported that ROS was essential and enhanced gradually during early reprogramming (Hawkins et al., 2016), and reprogramming efficiency was reduced under antioxidant treatments (Zhou et al., 2016). Therefore, oxygen ion signaling activation at early phase facilitates reprogramming.

Long-term and irreversible mitochondrial permeability transition pore (mPTP) induced apoptosis whereas the short-term opening of mPTP may release mitochondrial matrix ions and metabolites

(Elrod et al., 2010). Our lab has further demonstrated that the transient opening of mPTP and ROS release alter histone H3K9me2 and H3K27me3 levels in global genome, ultimately facilitating reprogramming (Ying et al., 2018). In conclusion, the mitochondrial oxygen ion signaling modulates epigenetic events, enhancing reprogramming efficiency. Further studies are needed to investigate the underlying mechanisms of oxygen ion signaling in controlling epigenetics.

Organelle metabolites in nuclear epigenetics

In recent years, many significant progresses in iPSC technology have brought novel insights for making it more amenable to applications. However, the limited efficiency of reprogramming is still the roadblock for application, which is highly related to its epigenetic barriers. It has been well-studied that metabolites are capable of affecting the accessibility of chromosome and regulating gene expression (Figure 1). Citrate lyase that generates acetyl-CoA as acetyl group donor is indispensable for histone acetylation, while sirtuin family members support histone deacetylation accompanied by nicotinamide adenine dinucleotide (NAD) hydrolysis. S-adenosyl methionine (SAM) is the methyl donor for DNA and histone methylation. α-ketoglutarate serves as a co-factor involved in histone demethylation and DNA demethylation, whereas fumarate and succinate could repress α-ketoglutarate-dependent demethylation. We, therefore, would discuss the intimate relationship between metabolites and nuclear epigenetics .

During reprogramming, tubular and cristae-rich mitochondria become rounded and cristae-less immature mitochondria. With respect to metabolism, anaerobic metabolism becomes the main source of energy supply and oxidative phosphorylation (OXPHOS) is gradually degraded. It is presumed that the rapid flux of glycolysis supports the growth of PSCs and reduces ROS production to maintain the genome stability. Temporal sampling indicates the glycolytic gene activation preceding pluripotent marker gene. More importantly, blocking glycolysis impaired reprogramming efficiency, suggesting that anaerobic metabolism fuels the induction of pluripotency (Folmes et al., 2011).

Numerous researches have corroborated that metabolic states of stem cells couple epigenetics and pluripotency. Glucose metabolism aims at providing ATP for various activities. Surprisingly, ATP is suggested to be engaged in histone modification. Nuclear reprogramming has been considered as a stochastic process. iPSCs with different levels of mitochondrial complex I expression and ATP production showed different degrees of pluripotency. Zhang et al. (2018) have found that ATP promotes the expression of Actl6a, a subunit of chromosome remodeling and histone acetylation complex, affecting iPSC pluripotency. Furthermore, OXPHOS seems to be a double-edged sword in reprogramming. Another group has reported that iPSCs derived from aged tissues showed an overactivation of OXPHOS compared to normal ones, while the former showed lower degree of pluripotency than the latter (Zhang et al., 2017).

Not only do lipids serve as main components of cell membrane, but also they are involved in physiological and pathological signal transductions. A previous work in our lab has linked lipid metabolism to pluripotency (Wu et al., 2016). Sterol regulatory element-binding protein (Srebp) is a family of basic helix−loop−helix leucine zipper transcription factors, regulating lipogenesis. We demonstrated that Srebp-1 promotes Yamanaka factors SKO binding to their downstream pluripotent targets via interaction with c-Myc. Recently, by taking advantage of high-coverage lipidomics, we detected temporal variation of phospholipids during somatic cell reprogramming. The level of phosphatidylethanolamine (PE) increased at the early phase and then decreased during the latter days. We found that the cytidine diphosphate ethanolamine (CDP-Etn) pathway, required for PE synthesis, could inhibit mesenchymal-related gene expression and thus promote reprogramming efficiency. Follow-up investigation has unmasked that CDP–Etn pathway inhibits NF-κB signaling by enhancing the interaction of PE-binding protein 1 with IKKα and IKKβ, thus accelerating mesenchymal-to-epithelial transition and pluripotency acquisition (Wu et al., 2019). As such, the interactions between numerous organelle metabolites and various nuclear epigenetics during somatic cell reprogramming are interesting questions that require further studies.

Potential biomedical importance of organelle remodeling

The iPSC technology has not only provided excellent systems for studying the roles of organelle in epigenetic regulation and cell fate determination, but also expanded promising potential applications of organelle remodeling in regenerative medicine.iPSCs derived from human cells could be used as in-a-dish models to explore pathological mechanisms, particularly for subcellular organelle pathological processes. For instance, through iPSC models derived from fibroblasts of two Alers–Huttenlocher syndrome patients with the symptom of valproic acid-induced hepatotoxicity, we found that such hepatotoxicity is associated with targeting mPTP opening-dependent apoptotic sensitivity (Li et al., 2015). Based on iPSC-based disease modeling, we have also linked remodeling of ER–mitochondria interactions to neuritic degeneration in human iPSC-derived neurons. We have observed $\Delta\Psi$m-dependent ER fragmentation and intimate association between ER and mitochondria, which results in inducing IP3R-dependent mitochondrial Ca^{2+} elevation and dysfunction (Bao et al., 2016, 2018). iPSC models contribute to uncovering detailed pathological mechanisms and indicating intensive correlation between organelle interactions and diseases. Furthermore, organoid models based on iPSC

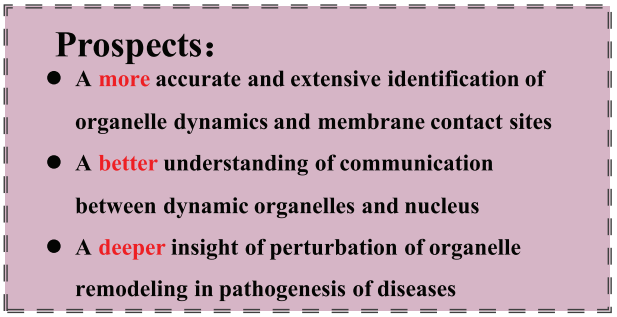

Figure 2 Major challenges in the field of organelle remodeling.

technique may probably provide new models and standpoints of intercellular communications.

In recent years, organelle remodeling has also been implicated in other pathogenesis. Gao et al. (2018) have previously observed organelles abnormal distributions in zygotes and oocytes with the zinc finger BED-type containing 3 (Zbed3) function loss that contributes to aberrant development of some mutant embryos. Well-balanced organelle life circle is significant and its perturbations can be lethal in some cases. As one typical organelle interaction, more and more MCSs have been associated with diseases. Mitochondria-associated ER membrane (MAM) is one of the most well-studied MCSs. Altered mitochondria–ER communication and enhanced Ca^{2+} transfer were reported in either aging or cancer. It has been proposed to be a lifelong switch against aging. In hepatic cells, MAMs have been reported as sensors regulating glucose concentration, suggesting that impaired MAMs may underlie type II diabetes and obesity (Theurey et al., 2016). How abnormal MAMs lead to metabolic imbalance is an interesting question that deserves further investigation.

What's more, mutant mtDNA accumulation is involved in numerous diseases—autosomal recessive chronic progressive external ophthalmoplegia, mitochondrial neurogastrointestinal encephalomyopathy, etc. Mitochondria in different tissues are heterogeneous due to the genetic variation of mtDNA. mtDNA mutations now have been used as barcode to widely evaluate the ability of hematopoietic cloning dynamics in human disease (Ludwig et al., 2019). Moreover, combining with single-cell sequencing, we may better understand cellular dynamics in tissue development and disease and distinguish more unknown cell subtypes. In addition, one of the main challenges within mitochondrial diseases is mysterious relationship between genotype and phenotype. Despite with similar mutant level of mtDNA, patients may show different phenotypes. In this regard, advanced methods to separate tissue-specific mitochondria may be vital. Encouragingly, with new developed cell-specific mitochondrial affinity purification technique, Ahier et al. (2018) have unravelled tissue-specific mtDNA heteroplasmy. With following researches on interaction networks of its proteomics, metabolomics, and lipid omics, the veil of disease mechanisms would gradually be lifted.

In conclusion, organelle remodeling hosts a neoteric but prominent status in somatic cell reprogramming (Figure 1), and there are several challenges in this field (Figure 2). Membrane trafficking performs as a basal material transport infrastructure to facilitate the delivery of various reprogramming modulators and thus to function in organelle remodeling. Seeking out key steps or stimulators of organelle remodeling could help to better understand certain intractable diseases. As mentioned above, mitochondrial remodeling has been explored broadly and may still hide some undetected truths in reprogramming. Besides mitochondria, other organelles also deserve attention, as MCSs exist almost everywhere in cells. Further studies are required for investigating the significance of organelle remodeling in cell fate determination and pathological processes.

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