Discovery of the Mechanism by which Mitochondria are Eliminated from Red Blood Cells and the Involvement of Alternative Autophagy

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Fig. 1: Terminal stage of red blood cell maturation. During erythrocyte maturation, erythroblasts lose their nuclei to become reticulocytes, which are transformed into erythrocytes by the elimination of organelles, including the mitochondria (Fig. 1). It has been considered that autophagy might be involved in this process, but the detailed mechanisms have not been elucidated yet.

Autophagy is a catabolic process where cellular contents, including proteins, lipids, and even entire organelles, are digested within lysosomes. The process of autophagy begins with the formation and elongation of isolation membranes. These membranes invaginate enclosing various intracellular components inside, resulting in the formation of double-membrane vesicles, called autophagosomes. Subsequently, autophagosomes fuse with lysosomes to generate autolysosomes, allowing the degradation of the autophagosome contents. Autophagy occurs constitutively at low levels but is accelerated by various cellular stressors. Autophagy is driven by more than 30 autophagy-related proteins (Atgs) that are well conserved from yeasts to mammals. Among them, Atg5 and LC3 are crucial, because these molecules are required for the expansion and closure of isolation membranes. Furthermore, the translocation of LC3 from cytosol to autophagosomes is recognized as a reliable marker of autophagy.

Despite the crucial role of Atg5 in autophagy, we discovered Atg5-independent autophagy (named alternative autophagy) from ultrastructural analysis (Nishida et al., Nature 1999). The morphology of alternative autophagy was indistinguishable from that of Atg5-dependent autophagy. Alternative autophagy also digests proteins and organelles. Therefore, mammalian cells possess at least two different autophagic pathways: the Atg5-dependent pathway and an alternative pathway. We recently discovered that this alternative autophagy, not Atg5-dependent autophagy, is essential for mitochondrial clearance from reticulocytes during terminal differentiation (Honda et al., Nature Comm. 2014). This conclusion was drawn from following evidence: (1) we have observed that mitophagy is enwrapped and digested by autophagic vacuoles in wild-type reticulocytes and Atg5-deficient reticulocytes; (2) the number of persisting mitochondria in Atg5-deficient reticulocytes and erythrocytes was the same as in wild-type cells of each type; (3) mitochondrial clearance in reticulocytes did not occur in mice lacking ULK1, an essential molecule for alternative autophagy; (4) abnormality in mitochondrial clearance in Ulk1/Atg5-double deficient mice was approximately the same as that in Ulk1-deficient mice, indicating limited involvement of Atg5. Thus, it is likely that the Ulk1-dependent Atg5-independent alternative autophagy is the dominant force for mitochondrial elimination from reticulocytes. This study identified one of the physiological roles of alternative autophagy, and also identified the mechanism of final differentiation in erythrocytes.

References

Fig. 2A: Electron micrographs of wild-type, Atg5-deficient, and Ulk1-deficient erythrocytes. Mitophagy can be observed in the Atg5-deficient, but not Ulk1-deficient erythrocytes. Arrowhead indicates the isolation membrane-autophagosomal structure. Arrow indicates the autophagosome containing mitochondria (*).

Fig. 2B: Schematic model. Mitochondrial elimination in erythrocytes is largely dependent on alternative autophagy regulated by ULK1.

DNA Demethylation-Dependent Regulation of Hepatic Lipid Metabolism

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Fig. 1: Impact of the Nutritional Status in Early Life on Susceptibility to NCDs in Later Life

Fig. 2: Hepatic Fatty Acid β-oxidation Genes During the Fetal and Postnatal Liver

Epidemiological and animal studies have suggested that a number of chronic disorders, especially non-communicable diseases (NCDs) in later life may be acquired during the fetal and postnatal periods (Fig. 1). However, how the nutritional status in early life affects the susceptibility to NCDs in later life has been underexplored.

The methylation of cytosine residues in DNA is a major epigenetic modification, and its role is well studied in organism development and cell differentiation. In most cases, DNA methylation of the promoter region suppresses gene expression. DNA methylation may be affected by environmental factors, thereby regulating a diverse range of biological processes. Although the fetal and postnatal periods, which are highly plastic to environmental changes, should be under epigenetic control, the role of DNA methylation in early life has been ill-defined.

The metabolic function of the liver changes dramatically during early life in mammals so that they can adapt to sequential changes in nutritional environment. For instance, during the suckling period, when fat intake is high, the rate of hepatic de novo lipogenesis is very low, but it increases with the amount of weaning and decreased intake of milk. We have reported the role of DNA demethylation in the induction of glyceraldehyde 3-phosphate dehydrogenase 1, a rate-limiting enzyme of triglyceride biosynthesis, in the postnatal mouse liver (Diabetes 64:2442-2450, 2015).

Before birth, when glucose, a major source of energy during the fetal period, is provided via placenta and breast milk, expression of all the fatty acid β-oxidation genes may be suppressed in a DNA methylation-dependent manner, which is partly because PPARα ligands are unavailable. After birth, activation of hepatic PPARα by milk lipids may lead to the induction of the fatty acid β-oxidation pathway via a DNA demethylation mechanism. It is conceivable that during the postnatal period, milk lipids may serve as a nutrient signal as well as nutrients, so that they can be oxidized efficiently as an energy source (Fig. 2). Given that the DNA methylation status is determined in early life is relatively stable throughout life, our data support the concept that the nutritional status in early life affects the metabolic phenotypes in later life, thus providing clues to “preemptive medicine” for adult-onset metabolic diseases in early life in the form of formula milk and functional food for both babies and mothers.