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REVIEW ARTICLE

# Autophagy reprogramming stem cell pluripotency and multiple-lineage differentiation

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**Abstract:** The cellular process responsible for the degradation of cytosolic proteins and subcellular organelles in lysosomes was termed "autophagy." This process occurs at a basal level in most tissues as part of tissue homeostasis that redounds to the regular turnover of components inside cytoplasm. The breakthrough in the autophagy field is the identification of key players in the autophagy pathway, compounded under the name "autophagy-related genes" (ATG) encoding for autophagy effector proteins. Generally, the function of autophagy can be classified into two divisions: intracellular clearance of defective macromolecules and organelles and generation of degradation products. Therapeutic strategies using stem cell–based approach come as a promising therapy and develop rapidly recently as stem cells have high self-renewability and differentiation capability as known as mesenchymal stem cells (MSCs). They are defined as adherent fibroblast-like population with the abilities to self-renew and multi-lineage differentiate into osteogenic, adipogenic, and chondrogenic lineage cells. To date, they are the most extensively applied adult stem cells in clinical trials. The properties of MSCs, such as immunomodulation, neuroprotection, and tissue repair pertaining to cell differentiation, processes to replace lost, or damaged cells, for aiding cell repair and revival. Autophagy has been viewed as a remarkable mechanism for maintaining homeostasis, ensuring the adequate function and survival of long-lived stem cells. In addition, authophagy also plays a remarkable role in protecting stem cells against cellular stress when the stem cell regenerative capacity is harmed in aging and cellular degeneration. Understanding the under-explored mechanisms of MSC actions and expanding the spectrum of their clinical applications may improve the utility of the MSC-based therapeutic approach in the future.

**Keywords:** Autophagy; Autophagy-related genes; Homeostasis; Mesenchymal stem cells; Regenerative

## 1. INTRODUCTION

In 1963, it was Christian de Duve1 who first discovered the lysosome which earned its trailblazer a Nobel Prize in Physiology, or Medicine 11 years later on. The cellular process responsible for the degradation of cytosolic proteins and subcellular organelles in lysosomes was termed "autophagy" (literally translates to "self-eating" in Greek).[1,2] This process occurs at a basal level in most tissues as part of tissue homeostasis that redounds to the regular turnover of components inside cytoplasm. Basically, starvation and other cellular stress forms can induce autophagy, leading to the degradation inside lysosome, and the degradation products are recycled to produce cellular building blocks and energy for cellular renovation and homeostasis. Besides that, autophagy is increasingly acknowledged as a quality control mechanism for both organelles and proteins.[3] Autophagy's substrates can be soluble factors (such as proteins), higher-order complexes (for example, ribosomes and fluid phase condensates), or membrane-bound organelles (for instance, mitochondria). Besides, a distinct process named endocytosis can help to deliver extracellular material as well as portions of the plasma membrane to lysosomes for degradation.[1,4]

Generally, autophagy was classified into macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). In macroautophagy, the inner autophagosomal membrane is degraded allowing the final degradation of substrates. Meanwhile, the intraluminal vesicle membrane plays this role in microautophagy. In contrast, CMA does not include the degradation of membrane barriers but rather the direct translocation of substrate protein via putative pores.[1] Among them, macroautophagy is the most ubiquitous and substantial characterized form of autophagy.

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The autophagy process includes multiple steps that start from the induction and nucleation of the typical phagophore structure; after autophagosomes are matured, they fuse with the lysosome leading to degrading and recycling of substances.[5]

The breakthrough in the autophagy field is the identification of key players in the autophagy pathway, compounded under the name "autophagy-related genes" (ATG) encoding for autophagy effector proteins. The finding belongs to Yoshinori Ohsumi's laboratory which brought them a Nobel Prize in Physiology or Medicine. Their first identification in the yeast[6] was followed by cloning of their mammalian homologs, which were found to have essentially similar roles as those in yeast.[7] Until now, 42 ATG genes have been identified.[1,2,8]

## 2. INITIATION OF AUTOPHAGY

In the initiation of autophagy, the Unc-51-like kinase 1 (ULK1) serine-threonine kinase complex consisting of ULK1 (the mammalian ortholog of yeast Atg1), the scaffold protein FAK family-interacting protein of 200 kDa (FIP200), the ULK1-mediated phosphorylation of ATG13, and ATG101 (ULK1-ATG13-FIP200-ATG101 complex) plays a major role phosphorylating multiple downstream factors. After reaching the sites of autophagosome initiation, the mentioned ULK1 complex activates a second essential autophagy effector protein complex named the phosphatidylinositol 3-kinase (PI3K) complex.[9,10]

Phosphatidylinositol 3-phosphate (PI3P) was generated via the Beclin 1 (similar to Atg6 in yeast)/class III phosphatidylinositol 3-kinase (PI3KC3) complexes, thus, implicating in the nucleation of autophagosome (PI3KC3-C1 consisting of Beclin 1, VPS15, VPS34, and ATG14) or the maturation of endolysosome and autophagolysosome (PI3KC3-C2 including Beclin 1, VPS15, VPS34, and UVRAG). Among them, the production of PI3P belongs to the responsibility of VPS34.[11] Enrichment in PI(3)P at specialized sites leads to omegasome formation, an initiating autophagosome structure.[12] The structure then presents as a membrane platform in recruiting the subsequent autophagy machinery to elongate autophagosome membrane while keeping in touch with the endoplasmic reticulum (ER) and with other vesicles carrying the ATG9, a transmembrane autophagy protein.[13] Supplying membrane to autophagosomes is played by vesicles bearing ATG9A.[14] Meanwhile, Beclin 1 directly interacts with members of B-cell lymphoma 2 (BCL-2) family[15,16] to negatively regulate autophagy, with the support of BCL-2 adapter protein CISD2/NAF-1 at the ER.[17] Besides, in the early stages of membrane elongation, there are WD repeat domain phosphoinositide-interacting (WIPI) proteins and ATG2A, or ATG2B, also taking place at PI3P generating site.[18,19]

Autophagosome membrane elongation and completion are performed by two ubiquitin-like protein conjugation systems.[20] The first conjugation system consisting of ubiquitin (Ub)-like ATG12 conjugates with ATG5, which is catalyzed by ATG7 and ATG10, and ATL16L1 functions as an E3-like ligase.[21,22]

The second conjugation step is mediated by ATG7 and ATG3, which together with the previous ATG5-ATG12:ATG16L1 complex, is responsible for conjugating membrane-resident phosphatidylethanolamine (PE) to Ub-like light chain 3 (LC3) subfamily microtubule-associated protein 1 LC3 (MAP1LC3)/LC3 (homolog of ATG8 in yeast). LC-I is produced through the LC molecule cleavage via ATG4. After that, through the action of ATG7, ATG3 and the ATG12–ATG5-ATG16L1 complex, LC3-I is covalently bound to PE producing LC-II which is commonly used as an experimental marker to detect and quantify autophagosomes within cells.[23–25] The ubiquitin-like protein conjugation systems determine the efficiency but are not indispensable for autophagosomal membrane completion in mammalian cells that differs from its role in yeast.[22]

Finally, mature autophagosomes are trafficked to lysosomes preparing for the fusion, in which the Rab family of small GTPases, soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins, and membrane tethering proteins take the responsibility.[26–28] The autophagolysosome's contents are then degraded via hydrolases enzyme inside lysosome, recycled, and released back to the cytoplasm.[7,22]

## 3. REGULATION OF AUTOPHAGY

Although autophagy process was renowned as a nonselective, lysosomal degradation mechanism, referred to as general autophagy, there is accreting evidence proving the selective form of autophagy mediating the degradation of specific classes of target molecules.[29–31]

One of the most well-known autophagy pathways was the nutrient-sensing mammalian target of rapamycin (mTOR) pathway.[32] mTOR kinase is a master regulator of cellular growth and metabolism that can negatively regulate autophagy. Under nutrient-rich conditions, mTOR preventing the formation of the autophagy initiation ULK1 Ser/Thr kinase protein complex, therefore, suppresses autophagy. Once autophagy is initiated, ULK1 can negatively regulate mTORC1 and further potentiate the autophagy induction (Jung et al[33]). The phosphorylation of several downstream targets to initiate the autophagic process via ULK1 is following up.[34] Understanding the under-explored mechanisms of MSC actions and expanding the spectrum of their clinical applications may improve the utility of the MSC-based therapeutic approach in the future.

Generally, the function of autophagy can be classified into two divisions: intracellular clearance of defective macromolecules and organelles and generation of degradation products. However, the first-mentioned function can be shown during constitutive autophagy at a basal level (such as a housekeeping function in neurons) or selective targets (such as damaged organelles and invading bacteria-induced autophagy).[1,35] As an intracellular quality control, the function absolutely plays a vital role in long-lived cells and organisms. For instance, the degeneration of the corresponding tissues, with the accumulation of abnormal protein aggregates/condensates and organelles, occurs when deletion of ATG genes in neuronal-cell and hepatocyte-specific deletion.[36,37]

However, the second essential function is especially important for unicellular eukaryotes, such as yeast, and for multicellular organisms in the acute phase of nutrient-shortened conditions, such as during short-term starvation, the postnatal period, and the preimplantation stage.[38] It involves the generation of nutrients to strengthen cell survival in nutrient-limited stage or growth requirements increase. Probably, among different organisms, the ability to adapt to starvation is conserved the best.[39,40]

The dysfunction of autophagy has been ascribed to various pathological conditions, such as cancer,[41] neurodegeneration,[42] muscle[43] and heart disease,[44] infectious disease,[45] as well as aging.[46–48]

## 4. MESENCHYMAL STEM CELLS

Therapeutic strategies using stem cell–based approach come as a promising therapy and develop rapidly recently as stem cells have high self-renewability and differentiation capability. Noticeably, in cell-based therapy, research works have been focused on mesenchymal stem cells (MSCs).[49] MSCs are a class of stem cells that reside in the adult tissues, such as bone marrow,[50] adipose tissue,[51] and dental pulp,[52] as well as in the fetal tissues and fluids, including the umbilical cord–tissue, umbilical cord–blood, and umbilical cord–amniotic fluid.[53] They are defined as adherent fibroblast-like population with the abilities to self-renew and multi-lineage differentiate into osteogenic, adipogenic, and chondrogenic lineage cells.[54] Also, according to the International

Society of Cellular Therapy (ISCT), MSCs express MSC, including ENG/CD105, NT5E/ CD73, and THY1/CD90, and lack hematopoietic markers, such as PTPRC/CD45, CD34, CD14, ITGAM/CD11b, CD79A, CD19, and HLA-DR (major histocompatibility complex, class II, DR).[55] The title "immunoprivileged" cells was conferred on MSCs due to this lack of HLA-DR expression, which is associated with transplant rejections.[56] To date, they are the most extensively applied adult stem cells in clinical trials. Many academic groups and industry performed preclinical and clinical trials to determine the practicability as well as efficacy of MSCs for the treatment of a variety of pathological conditions, for instance, inflammatory and autoimmune disorders, like rheumatoid arthritis,[57–60] diabetes,[61,62] neurodegenerative diseases, such as Alzheimer disease or Parkinson disease,[63,64] ischemia-reperfusion injuries,[65–67] and liver, kidney, and lung fibrosis.[68,69] These studies demonstrated various interesting properties of MSCs, such as immunomodulation, neuroprotection, and tissue repair pertaining to cell differentiation processes to replace lost, or damaged cells, for aiding cell repair and revival.[70] In detail, MSCs could provide trophic effects on cells residing in the injured area, or on recruited immune cells.[71,72] Noticeably, they have the ability to create an important reparative environment via cell-to-cell contact and provide a variety of cytokines, growth factors, as well as extracellular vesicles (carrying mRNA, peptides/proteins, and also micro-RNA)[73] to suppress the inflammatory, prevents apoptosis as well as enhances the survival capacity of dampened tissue cells,[74] reduces oxidative injury, which often involved in tissue damage, and favors angiogenesis/arteriogenesis.[75] The mechanism associated with the immunosuppressive action of MSCs consists of suppressing immune cells' activation and/or proliferation, for example, B cells and T cells[76,77]; suppressing cells' maturation into cells that are able to actively respond to the immunogenic stimulation; promoting regulatory cells' expansion to dampen the immune response ability[78]; reducing proinflammatory cytokine and chemokine secretion, such as (for example, interleukin (IL) 1B, IL2, and tumor necrosis factor [TNF]); and enhancing the anti-inflammatory factor production (for example, IL10, and TGFB).[79,80] Since MSCs have presented felicitous and promising potential, accumulation of more researches to understand the mechanisms underlying the MSC actions is the prerequisite for improving MSC technologies.

Notwithstanding the potential, the use of primary tissue–derived MSCs presents several challenges, including shortages of tissue sources, arduous, and invasive methods to retrieve, cell population heterogeneity, cell senescence, low purity, and long-term expansion–related loss of self-renewal and proliferative capabilities.[81–83] To overcome this deficit, pluripotent stem cell (PSC)–derived MSCs may wherefore be considered auspiciously as a solution.[84]

## 5. THE ROLE OF AUTOPHAGY IN MESENCHYMAL STEM CELL

Autophagy has come into view as a remarkable mechanism for maintaining homeostasis as well as ensuring the adequate function and survival of long-lived stem cells[85] owing to vigorous evidence raised from numerous studies regarding autophagy in hematopoietic stem cells,[86,87] neural stem cells,[88,89] muscle stem cells,[90,91] induced PSCs,[92] and cancer stem cells.[93–95] Autophagy can also exert influence on cell fate decisions through its ability to influence mitochondrial content, energy production, and epigenetic programming.[96,97] Besides, autophagy plays a remarkable role in protecting stem cells against cellular stress when the stem cell regenerative capacity is harmed in the aging and degenerative.[98] Autophagy can be a promising target in regenerative medicine. Recently, it has been proposed that autophagy may be related to MSC activities. On the one hand, the modulation of autophagy in MSCs may affect MSC functions. On the other hand, MSCs may modulate autophagy of the immune and other cells involved in disease pathogenesis.[99,100] These modulations ultimately contribute to the therapeutic action exerted by MSCs.

Several studies have suggested autophagic flux activation through the AMPK/mTOR pathway under hypoxia condition contributes to hypoxia-induced apoptosis in bone marrow–derived mesenchymal stem cells (BMSC).[101] A study has shown that autophagy is involved in hypoxia-induced BMSC proliferation through the activation of the apelin/APJ/autophagy signaling pathway.[102] Compounding evidence suggested autophagy is able to promote BMSC apoptosis and BMSC proliferation.[103] Besides that, there were other studies that claimed the vital role of autophagy in preventing senescence in BMSCs.[104,105] Interestingly, in vice versa, the controversial antisenescence role/prosenescence role of autophagy in MSCs remains to be discussed.[31] Autophagy also promotes BMSC differentiation toward the osteoblastic lineage.[106] A study by Nuschke et al[107] claimed that stimulation of osteogenic differentiation can increase the autophagic turnover in comparison with undifferentiated BMSCs which accumulate nondegraded autophagic vacuoles, with little autophagic turnover. More importantly, they found that through the upregulation of pluripotency genes and autophagy-related genes that activate the PTEN/AKT/mTOR signaling pathway, an AT-rich DNA-binding protein named special AT-rich sequence-binding protein 2 occurs, which can promote osteogenic differentiation as well as bone defect regeneration in BMSCs. A recent study from Cen et al[108] has shown that cell migration and differentiation of $CD4^+$ T cells could be mediated by autophagy of MSCs. The secretion of C-X-C motif chemokine ligand 8 (CXCL8) was promoted leading to the migration of $CD4^+$ T cells. This effect was terminated by exogenous CXCL8 and anti-CXCL8 antibody treatment. The ratio of regulatory T (Treg) cells and the ratio of T helper 1 (Th1) was manipulated in rapamycin-pretreated MSCs and 3-methyladenine–treated MSCs. Noticeably, overexpress and knockdown of TGF-β1 in MSCs fine-tuned these differences.

A study published on Autophagy in 2014 indicated that coculturing amyloid-β (Aβ)–treated neuronal cells and MSCs could promote autophagy, hence, modulating Aβ clearance and providing neuroprotection effect. The author claimed that the cell viability of Aβ-treated neuroblastoma cells was increased owing to MSCs. Interestingly, the fusion of autophagosomes with lysosomes was increased in Aβ-treated cells after coculturing with MSCs.[109]

The therapeutic use of MSCs could obtain a strong improvement through the understanding of underexplored mechanisms in MSC actions and expand the spectrum of their clinical applications.

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