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Review Article

Role of autophagy in megakaryocyte differentiation and platelet formation

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Abstract: Autophagy is a conserved biological process for digestion and recycling of cytoplasmic constituents in eukaryotic cells. Autophagy may trigger cell death or promote cell survival following various forms of stress. The emerging roles of autophagy in megakaryopoiesis, thrombopoiesis, and platelet function have been uncovered using not only in vitro and in vivo genetic models, but also in clinical observations of autophagic structure in patients with thrombocytopenic disorders. Inhibition of autophagy in early stage of megakaryocyte differentiation appears to impede megakaryocyte maturation, reduce platelet formation, and affect platelet function, whereas autophagic deficiency in mature megakaryocytes gives rise to abnormal platelet activation and function without changing platelet size and number. On the other hand, induction of autophagy by rapamycin in megakaryocytes exhibited substantial therapeutic benefits in patients with immune thrombocytopenic purpura (ITP). This mini-review is to highlight recent progresses in understanding the regulation of autophagy in megakaryopoiesis, thrombopoiesis and platelet function to bridge the gap between autophagy and megakaryocyte/platelet pathophysiology.

Keywords: Autophagy, megakaryopoiesis, thrombopoiesis, platelets

Introduction

Autophagy is a conserved catabolic process in eukaryotic cells. During autophagy, targeted cytoplasmic components undergo sequestration by double membrane vesicles called autophagosomes, which then deliver their contents to lysosomes for degradation or recycle [1]. Since its discovery in 1960s, autophagy has been flourishingly investigated and comprehensively linked to various biological processes and pathological conditions. According to different transportation and substrates, autophagy can be divided into three distinct forms termed macroautophagy, microautophagy and chaperone-mediated autophagy, of which macroautophagy consists of pexophagy, mitophagy and non-selective autophagy [2].

The crucial roles of autophagy have been implicated in nutrient starvation, infection, cell death and repair [3-5]. Autophagy is considered to play a mainly protective role in cellular stress response by removing aggregated protein and recycling degraded products [6]. On the flip side of the coin, autophagy may trigger programmed cell death under certain conditions [7]. The complete process of autophagy, which includes activation, cargo identification, autophagosome formation, lysosome fusion and degradation, is mediated by a series of autophagy-related genes (ATGs) [8]. Over the past decades, the pleiotropic roles of autophagy in the development of hemocytes have been characterized in erythropoiesis, as well as in blood cancers [9, 10]. Recently, autophagy has been demonstrated to be indispensable for normal megakaryopoiesis and platelet function using animal models with lineage specific deletion of ATG [11, 12]. In addition, accumulating evidence of autophagy has hitherto been noted in ITP, myelodysplastic syndromes, and chronic myelogenous leukemia since the initial discovery of putative autophagic vacuoles from megakaryocytes in 1970s [13-15]. In the present review, we will first discuss the molecular basis

of the autophagic machinery briefly and then move on to more specific regulation of megakaryopoiesis and thrombopoiesis by autophagic modulators, followed by touching upon the clinical relevance of autophagic regulation and thrombocytopenic disorders.

Autophagic signaling

The process of autophagy was initially discovered by Porter et al in 1962 from glucagon treated liver cells, in which lysosomes containing other organelles were observed [16]. Further discoveries in injury response and recycling/degradation of cellular constituents led to the invention of the term "autophagy" by de Duve [17]. Amongst all the three types of autophagy identified, macroautophagy is the canonical pathway that has been most extensively studied [18]. Damaged organelles are subjected to sequestration by double layered autophagosomes that subsequently deliver their contents for acidic hydrolysis and degradation by fusing with lysosomes [2]. Microautophagy, to some extent, resembles macroautophagy in spite of direct engulfment of intracellular constituents by lysosomes [19]. On the other hand, chaperone-mediated autophagy operated in a quite different manner involving the hsc70-containing complex with high selectivity for substrates [20].

Autophagy-related genes (ATGs) are the key regulators of autophagic signaling. Originally cloned from the yeast Saccharomyces cerevisiae, the homologues of ATGs in mammalian cells have been identified, and their functions unveiled [21, 22]. In mammals, nutritional deprivation, growth factors, and oxidative stress can regulate autophagy through AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) [23, 24]. In the setting of starvation, increased ratio of AMP/ATP activates AMPK, which then induces cellular protective autophagy through inhibition of mTORC1 [25]. UNC-51 like kinase 1 (ULK1), focal adhesion kinase family interacting protein of 200 kD (FIP200), ATG101 and ATG13 form a protein complex with mTORC1 that is an inhibitor of autophagy [26]. Increased formation of this complex following dephosphorylation of ATG13 induced by metabolic stress or rapamycin promotes autophagy [27]. Similarly, activated UKL1 phosphorylates Beclin-1, which forms a complex with ATG14L, P150, and phosphatidylinositol 3-kinase VPS34 (Vsp34), to initiate the formation of autophagosomes [28]. Once Vsp34 is activated, the generation of phosphatidylinositol 3-phosphate (PtdInS(3)P) will recruit WD repeat domain phosphoinositide-interacting protein 2 (WIPI2) to the surface of the phagophore through binding with ATG16L [29]. Concurrently, ATG12 and ATG5 bind to ATG16L thus forming an E3-like complex, which then binds to ATG3 and promotes autophagosome nucleation. On the other hand, activated ATG3 covalently binds to LC3, which is lipidated by ATG16L and conjugates with PE on the membrane of autophagosomes [30]. During this step, p62 pinpoints specific organelles as a cargo docking receptor, as well as guiding LC3 into autophagosomes [31]. Eventually, the autophagosomes fuses with the acidic lysosomes to form autolysosome, from which the outside LC3 molecules are conversed to the cleaved form by ATG4, while the inner LC3 and cargos undergo degradation [32].

Recently, the roles of autophagy in hematopoiesis have been recognized. Several studies demonstrate that autophagy is involved in both megakaryopoiesis and erythropoiesis, for instance, in differentiation of reticulocytes to red blood cells, as well as in thrombopoiesis [11, 33, 34]. Induction of autophagy is associated with cell death and differentiation in chronic myeloid leukemia cell line, when the efficacy of induction of autophagy has been tested in thrombocytopenic disorders [35, 36].

Megakaryopoiesis and thrombopoiesis

Megakaryopoiesis is a complicated process mediated by different hematopoietic cells and extracellular factors. Hematopoietic stem cells are committed to megakaryocyte lineage, including mixed progenitor colony (CFU-Mix), or common myeloid progenitor (CMP), colony-forming unit-granulocyte-erythrocyte-monocyte-megakaryocyte (CFU-GEMM), mixed MK/erythroid progenitor cell (MEP), colony-forming unit-megakaryocyte (CFU-MK), burst-forming unit-megakaryocyte (BFU-MK), and eventually differentiated mature megakaryocytes [37]. The maturation of MKs is characterized by endomitosis, cytoplasmic maturation, and assembling of all constituents required for the production of functional platelets. Mature megakaryocytes can be identified by specific cell surface markers including CD41, CD61 (integrin

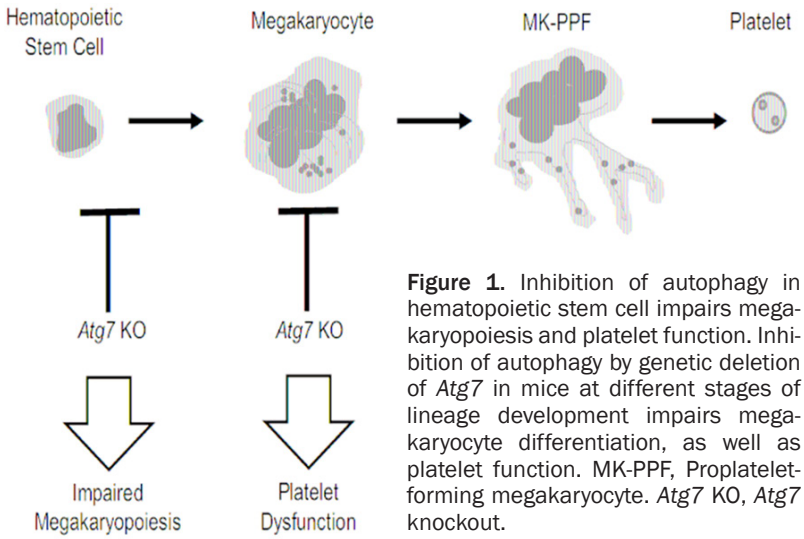

Figure 1. Inhibition of autophagy in hematopoietic stem cell impairs megakaryopoiesis and platelet function. Inhibition of autophagy by genetic deletion of *Atg7* in mice at different stages of lineage development impairs megakaryocyte differentiation, as well as platelet function. MK-PPF, Proplatelet-forming megakaryocyte. *Atg7* KO, *Atg7* knockout.

αIIbβ3), CD42 (glycoprotein Ib) and glycoprotein V [38]. The engagement of transcription factors, cytokines and extracellular stress synergically promotes the maturation of megakaryocytes [39]. Transcription factors, such as SCL, GATA1, GATA2, NF-E2, allows the development of megakaryocyte/erythroid progenitor cells [40]. FOG1 (ZFPM1) regulates the transcriptional activity of GATA-1 and contributes to early megakaryocyte differentiation, when NFE2 and SCL-1 regulate the later differentiation of megakaryocytes and production of platelets [41, 42]. Moreover, PU.1 and Fli-1 also guide the differentiation of megakaryocytes from progenitor cells [43]. In contrast, C-myb (MYB) balances with GATA1 by playing an inhibitory role in megakaryopoiesis [44]. Besides transcription factors, the differentiation of megakaryocytes is also fine-tuned by hormones, especially thrombopoietin. By interaction with its receptor c-MPL, thrombopoietin acts as the canonical potentiator of differentiation of megakaryocytes and thrombopoiesis [45]. Activation of downstream signals consisting of MAPK, PI3K, and STATs, works in concert to promote megakaryopoiesis [46]. Bone marrow niches provide platforms for megakaryocytes and platelet development by supplying gradients of oxygen, chemokines, and infrastructures for megakaryocyte migration [47]. Additionally, megakaryocyte migration is regulated by stromal cell-derived factor-1α (SDF-1), angiopoietin 1 (Ang-1), and TPO, etc. SDF-1α navigates terminal megakaryocytes towards the vascular endothelium, when activation of VEGFR1 promotes SDF-1 mediated migration of megakaryocytes to the vascular niches and increases platelet production [48]. Recently, the gradients of reactive oxygen species are also implicated in megakaryocyte maturation [49].

Upon maturation, megakaryocytes acquire all necessary cellular constituents for thrombopoiesis, which are demarcated into micro anucleated particles prior to shedding [50]. Meanwhile, the cell membrane and cell skeleton including tubulin and actin undergo extensive rearrangement, which allows the protrusion of massive pseudopodial projections termed proplatelets [51, 52]. Eventually, these proplatelets dispatch from megakaryocytes with their constituents, thus giving rise to circulating platelets. The dispatching of platelets from mature megakaryocytes is termed thrombopoiesis. Of note, apoptosis has been shown to be involved in the eventual stage of platelet release [53].

Roles of autophagy in megakaryocyte maturation and platelet function

There is substantial evidence supporting the roles of autophagy in megakaryopoiesis. In 1970s, TEM (Transmission Electron Microscope) studies from patients with carcinoid syndrome identified autophagosome-like structures inside platelets but not megakaryocytes, indicating the possible involvement of increased autophagy [13]. A later study in dogs with burn injury revealed the presence of autophagocytosis in megakaryocytes, which was considered as potential cell recycling process [54]. Evidence of activated autophagy is also noticed in ITP, which exhibits extensive cytoplasmic vacuoles representing programmed cell death. Nevertheless, these vacuoles observed appear to be with single membrane rather than with double membranes resembling autophagosomes [15].

The inhibitor of autophagy, mTORC1, has been reported to regulate both early and late steps of megakaryocyte development [55]. Additionally, inhibition of mTORC1 with rapamycin induces autophagy, decreases the size and

ploidy of megakaryocytes, and impedes the maturation of megakaryocytes through a p21 and cyclin D3 dependent manner [56]. These findings suggest an important role of autophagy in the regulation of megakaryocyte development. Of note, both mTORC1 containing rapamycin-associated TOR protein (Raptor) and mTORC2 containing rapamycin-insensitive companion of Raptor (Rictor) are involved in the regulation of cell cycle [57]. However, it can be difficult to conclude that autophagy directly regulates megakaryopoiesis due to the non-specificity of pharmacological approach such as rapamycin. In that case, genetic approaches using gene knockout mice were developed to underscore the role of autophagy. Consequently, a recent study demonstrated that abrogation of autophagy from stem cell stage by hematopoietic knockout of ATG7 lead to impaired megakaryopoiesis, thrombopoiesis and hemostasis, producing larger but fewer dysfunctional platelets [11]. However, deletion of ATG7 in mature megakaryocytes and platelets using PF4-driven crew method only result in abnormal hemostasis while platelet number and size remain unchanged. Further investigation demonstrated abnormal aggregation and cargo granule packing in these platelets [12]. In light of these findings, autophagy is likely to be indispensable for the early stage of megakaryocyte development, and is required for normal platelet function as well (**Figure 1**).

Being a chronic myelogenous leukemia (CML) cell line, K562 retains the capability of megakaryocytic differentiation, thus providing a favorable tool to study megakaryopoiesis. One group reported that inhibition of autophagy in K562 cells by knockdown of autophagic genes substantially impedes megakaryopoiesis [58]. Consistently, Lapatinib treatment induces autophagic cell death and megakaryocytic differentiation in K562 cells, both of which can be inhibited by knockdown of ATG7 or application of 3-MA [35]. In contrast, another group showed that although autophagy was readily observed during induction of megakaryocytic differentiation by 12-O-tetradecanoyl-phorbol-13-acetate (TPA), it does not seem to be required for cell differentiation [59]. Further studies in MO7e cells showed that cycle progression and nuclear division are regulated by mTORC1, whereas cell size and cell death were controlled by mTORC2 [57]. The relationship between autophagy and megakaryopoiesis should be interpreted cautiously since rapamycin used in this study may also suppress P70S6K and 4E-BP pathways. In addition to megakaryocytes, further studies in platelets confirmed the presence of autophagic proteins and showed that class III PtdIns3K dependent autophagy was required for normal platelet function [60].

Closing remarks

Autophagy, as a conserved biological process, has been well studied and associated with cancer, metabolic disorders, autoimmune disease, and radiation damage. Altered autophagy is implicated in hematopoietic and blood cells indicated by morphological studies. Given that most evidence of autophagy hitherto is from cancer cells, the explicit function of autophagy in megakaryocytes and platelets remains to be elucidated. Fortunately, recent genetic studies uncovered the indispensable role of autophagy in both megakaryopoiesis and platelet function. In addition, results from a clinical trial on ITP suggest that rapamycin is effective for treating immune-induced thrombocytopenia. Thus, targeting autophagy may yield a promising approach for thrombocytopenic disease, for example, in MDS or secondary to chemo-/radiation-therapy.

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Disclosure of conflict of interest

None.

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