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Review

Role of autophagy in acute myeloid leukemia therapy

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Abstract

Despite its dual role in determining cell fate in a wide array of solid cancer cell lines, autophagy has been robustly shown to suppress or kill acute myeloid leukemia cells via degradation of the oncogenic fusion protein that drives leukemogenesis. However, autophagy also induces the demise of acute leukemia cells that do not express the known fusion protein, though the molecular mechanism remains elusive. Nevertheless, since it can induce cooperation with apoptosis and differentiation in response to autophagic signals, autophagy can be manipulated for a better therapy on acute myeloid leukemia.

Key words Autophagy, fusion oncoprotein, acute myeloid leukemia

Autophagy is a ubiquitous process in which "bad" cytosolic molecules, damaged organelles, or invaded pathogens are sequestered within double-membrane vesicles called autophagosomes that deliver their contents to lysosomes for degradation and/or recycling of the resulting macromolecules. Indeed, autophagosomes were found to specifically target damaged mitochondria[1], peroxisomes[2], and protein aggregates that lead to DNA damage and genomic instability[3,4], thereby maintaining intracellular organelle and protein homeostasis[5]. Autophagy may also provide nutrients through lysosomal degradation of intracellular components of mitochondrial oxidation[3,6-8]. Autophagy is more frequently compromised in solid cancer cells compared with their normal counterparts and acts as a protective mechanism in response to both extracellular and intracellular stress. However, stimulation of autophagy in cancer cells was observed in response to anticancer treatments when enhanced autophagy destroys large proportions of the cytosol and organelles, which causes irreversible cellular atrophy and consequent collapse of vital cellular functions[9-13]. Heterozygosity for the autophagy-essential gene Beclin1 led to greatly increased rates of tumorigenesis[13-15], possibly because of genomic instability when autophagy is reduced[3]. Conversely, autophagy has also been shown to promote the survival of solid cancer cells following stress or nutrient deprivation[6,16-18]. Therefore, autophagy can protect cells against death or mediate cellular demise, depending on the autophagic stimuli and cellular context.

Acute myeloid leukemia (AML) is characterized by an accumulation of abnormal hematopoietic progenitor cells, which exhibit the morphology of a certain stage of myeloid differentiation, in the bone marrow and the peripheral blood. The blocked differentiation restrains progenitor cells from maturing and subsequently undergoing apoptosis[19], and it may also enhance their self-renewal capabilities[20,21]. Unlike normal hematopoietic cells that require cell extrinsic signals to maintain metabolism and survival, leukemia cells often express constitutively active oncogenic kinases that promote these processes independent of extrinsic growth factors. When cells receive insufficient growth signals or when oncogenic kinases are inhibited, glucose metabolism decreases and the self-digestive process of autophagy is triggered to degrade bulk cytoplasm and organelles. Given its potential function in metabolism and cell survival, manipulation of autophagy may provide a critical means to eliminate certain type of leukemia cells.

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Autophagy in Response to Therapy for PML-RARa[0] t(15,17) Leukemia

Acute promyelocytic leukemia (APL) is a subtype of AML that can be distinguished from other subtypes by

distinct cytogenetic and clinical features. More specifically, more than 95% of patients with APL bear the t(15;17) translocation that encodes the PML/RAR fusion protein [21]. This fusion oncoprotein suppresses granulocyte development at the promyelocytic stage of differentiation and causes malignant transformation of hematopoietic progenitor cells by acting as a transcriptional repressor of gene expression. All-trans retinoid acid induced disease remission in APL patients by interacting with its ligand, causing proteolytic degradation of the oncoprotein and consequent granulocyte differentiation of APL cells [22,23]. In addition, low-dose arsenic trioxide has been reported to be remarkably effective in treating APL by inducing differentiation, whereas high dose has been reported to trigger apoptosis by causing proteolytic degradation of PML/RARa [24-26]. The molecular mechanism by which arsenic trioxide degrades PML/RARa involves PML SUMOylation and subsequent polyubiquitination and proteolytic degradation of both PML and PML/RARa by a protein molecule called RNF4 [27-29]. All-trans retinoic acid and arsenic trioxide can act synergistically to cause leukemia regression through distinct mechanisms that lead to PML/RARa degradation, ultimately resulting in clinical remission of APL [30,31].

The ubiquitin-proteasome system is largely responsible for degradation of short-lived soluble proteins and misfolded proteins following their labeling with polyubiquitin chains. Previous studies have implicated that the ubiquitin-proteasome system plays an important role in therapy-induced degradation of PML/RARa. SUMO-conjugated PML, RNF4 and components of this system, are corecruited to nuclear compartments called PML nuclear bodies in the presence of arsenic trioxide. Both PML and RARa are targeted primarily to the nucleus, and the PML/RARa chimera is detected mainly within the nucleus of interphase cells. Thus, therapy-induced degradation of PML/RARa is largely thought to occur within the nuclear compartment because PML/RARa, together with ubiquitin and components of the ubiquitin-proteasome system, are recruited to PML nuclear bodies during the first few hours of arsenic trioxide treatment[27].

The PML/RARa oncoprotein is prone to aggregation, which makes it a good substrate for autophagic degradation. Its ability to form protein aggregates is possibly attributable to the PML moiety of the fusion protein, which contains a tripartite motif domain. Together with SUMO-conjugated residues and a SUMO-interacting domain, this tripartite motif in PML facilitates the formation of protein-protein interaction networks[32,33]. Further, the PML/RARa chimera may adapt a different conformation that renders it more prone to aggregation than PML or RARa expressed from non-rearranged genes. Because of its highly insoluble conformation, the PML/RARa oncoprotein may be subjected to clearance by the same or similar mechanisms that degrade other types of misfolded or aggregation-prone proteins in the cell. Consistent with this, cytoplasmic PML/RARa-containing particles formed by ectopic expression of PML/RARa and cytoplasmic PML-positive structures in NB4 cells were found to colocalize with ubiquitin, p62, and LC3, which are involved in autophagy-dependent degradation of protein aggregates. p62 contains a ubiquitin-binding domain and an LC3-interacting region and is therefore able to link ubiquitinated cargo to the core autophagic machinery[5,34]. Because autophagy is a cytoplasmic degradation mechanism, proteolytic clearance of this oncoprotein also occurs in the cytoplasm [35], in addition to its aforementioned degradation via the nuclear compartment. It supports the notion that ubiquitin is a signal for autophagic degradation of PML/RARa. Interestingly, a cytoplasmic compartment referred to as cytoplasmic assemblies of PML and nucleoporins, which appears after mitotic cell division and contains PML or PML/RARa, was cleared from NB4 cells in the presence of all-trans retinoic acid, suggesting a possible link between the stability of these cytoplasmic structures and autophagic activity.

Arsenic trioxide is a potent inducer of autophagy in acute leukemia cells, and such induction is dependent on activation of the MEK/ERK pathway but uncoupled from JNK. Experiments using chloroquine, a pharmacologic autophagy inhibitor, revealed that the inhibitory effects of arsenic trioxide on primary leukemic progenitors are mediated in part via induction of autophagic cell death. The critical role of autophagy in the anti-leukemic effects of arsenic trioxide was further established by experiments in which key elements of the autophagic pathway, such as Beclin1 and Atg7, were knocked down by using RNA interference[36]. Previous evidence shows that in addition to apoptosis, arsenic trioxide induces autophagy in the human tlymphocytic leukemia cell line Molt-4 [37]. Indeed, the catabolism of the PML/RARa oncoprotein is largely affected by autophagy, and its degradation in cells treated with arsenic trioxide or all-trans retinoic acid depends upon and relates with increased autophagic activity. Autophagy also has a stimulatory role on the differentiation of NB4 APL cells, suggesting that this degradation pathway potentiates therapy-induced differentiation of APL cells [35]. This observation supports our previous finding that autophagy is essential for vitamin D3-induced differentiation of leukemia cells[13].

Autophagy also plays a critical role in the anti-leukemic effects of arsenic trioxide in primary hematopoietic progenitors from AML patients. Emerging evidence suggests that treating malignant cells with arsenic trioxide results in negative feedback activation of

cellular pathways that counteract apoptosis, implicating autophagy as a mechanism for the suppressive effects of arsenic trioxide on primitive leukemic progenitors. The findings of this study raise the potential that agents promoting autophagy can sensitize leukemia cells to the suppressive effects of arsenic trioxide [38]. Autophagic death of acute lymphoblastic lymphoma cells treated with dexamethasone was recently shown to be dependent on the PML protein, which is known to inhibit mammalian target of rapamycin (mTOR) activity. Thus, mTOR-regulated autophagy may play a general role in the development and treatment of leukemia. Previous studies show that the ubiquitin-proteasome system facilitates clearance of PML/RARa induced by all-trans retinoic acid and arsenic trioxide. Autophagy and proteasome-dependent degradation may thus cooperate in therapy-induced clearance of the APL-associated oncoprotein [6]. In line with this finding, proteolytic cross-talk has been reported to clearly exist between the autophagy and ubiquitin-proteasome systems[39].

Furthermore, proteolytic degradation of PML/RARa by caspases and lysosomal proteases has also been reported, suggesting the existence of multiple proteolytic pathways with a potential to target PML/RARa for degradation. However, because autophagy appears to become markedly induced in the presence of all-trans retinoic acid and arsenic trioxide, this degradation pathway may have a determinant role in therapy-induced PML/RARa clearance. Thus, both all-trans retinoic acid and arsenic trioxide induce clinical remission in APL patients by stimulating mTOR-dependent autophagy and causing concomitant autophagic degradation of the APL-associated oncoprotein PML/RARa[35].

Consistent with the above observations, a recent report indicated that autophagy regulates leukemic myeloid cell differentiation via p62/SQSTM1-mediated degradation of PML/RARa oncoprotein[40]. PML/RARa has been shown to enhance constitutive autophagic activity through inhibition of the Akt/mTOR signaling pathway[41].

Autophagy in Response to Therapy for t(11;17)-Positive APL

Another APL-specific fusion protein, the t(11;17)-associated PLZF/RARa, causes a different subtype of human APL. Patients with APL positive for t(11;17) do not respond to treatment with either all-trans retinoic acid or arsenic trioxide [42-46]. However, whether activation of autophagy is essential to degrade the PLZF/RARa fusion protein is unknown. APL patients who undergo all-trans retinoic acid monotherapy may eventually suffer relapse, as all-trans retinoic acid is apparently unable to eradicate the leukemic population. In normal hematopoiesis, all-trans retinoic acid not only induces differentiation of committed myeloid cells but may also enhance the self-renewal capabilities of stem cells and progenitors [47,48]. Therefore, all-trans retinoic acid has dual effects on the APL leukemic cell population: induction of differentiation in the mature blast population, resulting in a complete remission, and conversely, induction of proliferation and/or self-renewal of leukemic stem cells. These dual effects probably explain the failure to achieve complete molecular remission and the tendency for relapse after all-trans retinoic acid monotherapy. In contrast, arsenic trioxide can induce complete molecular remission and does not seem to exert any toxic effects on hematopoietic stem cells.

Paradoxically, all-trans retinoic acid-induced up-regulation of Beclin1 has been reported to prolong the life span of differentiated acute promyelocytic leukemia cells[49], whereas down-regulation of autophagy by siRNA-mediated knockdown of S100A8 enhanced arsenic trioxide-induced leukemia cell death [50]. These two observations suggest opposing roles of autophagy in arsenic trioxide treatment on leukemia.

Autophagy in Response to Therapy for Leukemia That Does Not Express Known Oncoproteins

In AML cell lines lacking known oncoprotein expression, activation of autophagy can also cause leukemia cell demise. 1,25-Dihydroxyvitamin D3, the hormonally active form of vitamin D3, was also tried in leukemia therapy. Vitamin D3 inhibits cancer cell proliferation by inducing differentiation. These effects are dependent on Akt down-regulation, vitamin D3 disassociation with Raf1, and subsequent activation of Raf/MEK/ERK2 MAPK signaling. In turn, cyclin-dependent kinase inhibitors are up-regulated, and retinoblastoma protein undergoes dephosphorylation and binding to transcription factors E2F1 and C/EBPa [51-53]. The vitamin D3 analog EB1089 was initially reported to be able to induce apoptosis via a p53-independent mechanism involving p38 MAPK activation and ERK MAPK inactivation in B-cell chronic lymphocytic leukemia cells [54]. More recent studies showed that EB1089 triggered autophagy in breast cancer cells [12], again suggesting that the anticancer effects of the same compound may vary depending on cell types. Although vitamin D3 shows promise in counteracting tumors, significant supraphysiologic concentration of this compound is required for antineoplastic effects. Such concentrations are not achievable in patients due to predictable hypercalcemia and hypercalcuria resulting from the increased intestinal absorption of calcium and the calcium-mobilizing properties of vitamin D3. To reduce the risk of vitamin D3-induced hypercalcemia and

the possible immunosuppressive effects of its analogs, a combination of low-dose vitamin D3 or its analogs with another non-immunosuppressive agent that enhances differentiation but not the levels of circulating calcium has been pursued. For example, combinations of vitamin D3 with docetaxel, dexamethasone, paclitaxel, carboplatin, and carnosic acid were tried in several types of cancers and cancer model cell lines [55-57]. However, the current combinatorial therapies with vitamin D3 in the preclinical stage are restricted to differentiation effects, thus limiting the efficacy on cancer cell suppression. A better understanding of the mechanisms by which vitamin D3 exerts suppressive effects on tumor cells is needed to develop vitamin D3 analogs and combinatorial therapies with better antineoplastic effects. We showed that vitamin D3 triggers autophagic death by up-regulating Beclin1 and triggering differentiation but inhibiting apoptosis in myeloid leukemia cells. Knockdown of Beclin1 not only crippled vitamin D3-induced autophagy but also inhibited vitamin D3-induced differentiation and activated apoptotic signaling. These data suggest that autophagy is linked to differentiation and apoptosis. We further demonstrated that additional up-regulation of autophagy dramatically improved the antineoplastic effect of vitamin D3 on human myeloid leukemia cells, suggesting a better strategy for combating leukemia[13].

A number of chemical compounds including arsenic trioxide[21,35,38,40,50], vitamin D3[13], vitamin K2[58], eupalinin A (sesquiterpene lactone) [59], BAY11-7082 (NF-κB inhibitor) [60], morphinone (a morphin derivative) [61], APO866 (NAD biosynthesis inhibitor)[62], and platonin[63] have been reported to be able to induce leukemia cell death via activation of autophagy (Table 1).

Table 1. Autophagy-inducing agents in acute myeloid leukemia therapy

Compound	Cell line	Mechanism	Cell fate	Reference(s)
Arsenic trioxide	NB4	Autophagic degradation of oncogenic fusion protein	Autophagy mediates cell death	[21,35,38,40,50]
Vitamin D3	HL-60	Up-regulation of mTOR	Autophagy mediates cell death	[13]
Vitamin K2	HL-60	Unknown	Autophagy mediates cell death	[58]
Eupalinin A	HL-60	ROS generation and mitochondrial dysfunction	Autophagy mediates cell death	[59]
BAY11-7082 (NF-κB inhibitor)	U937 cell line, primary cells	NF-κB signaling	Autophagic stress proceeds apoptotic cell death	[60]
Morphinone	HL-60 cell line	Unknown	3-MA blocks morphinone-induced cell death	[61]
APO866 (NAD biosynthesis inhibitor)	NB4, HL-60	Unknown	Autophagy mediates cell death	[62]
Platonin	NB4, HL–60, U937	BNIP3 overexpression	3-MA blocks platonin-induced cell death	[63]

Concluding Remarks

Studies on autophagy in leukemia have been limited to *in vitro* leukemia cell models derived from leukemia patients, and there are still few publications on autophagy in early leukemogenesis. Autophagy is a highly context-dependent process that serves different functions in leukemia and solid cancers. In solid tumors, maintaining nutrient supply is difficult, and autophagy serves as an adaptive survival mechanism for the adverse nutrient supply circumstances. However, in leukemia cells in the blood that do not face a nutrient supply challenge, enhanced activation of autophagy degrades oncogenic fusion proteins to promote terminal differentiation or "over-eats" subcellular organelles to cause autophagic cell death. Despite its observed role in the development and maintenance of cancer in animal models and its opposing effects in response to a wide array of cancer therapies, autophagy in human AML cells predominantly drives a cytodestructive cascade that induces clearance of oncogenic fusion proteins and leads to cell death. Thus, autophagy may be a new strategy for targeted perturbation of oncogenic proteins in certain type of acute myeloid leukemia. A recent study also showed that autophagy is essential to maintain normal hematopoietic stem cell function in quiescence, self-renewal, and multilineage differentiation [64]. The autophagy pathway in stem cells may thus restrain transformation and prevent cancer by removing free radicals and limiting damage within stem cells, thereby

protecting normal stem cells from germination of leukemia. Future strategies for tackling leukemogenesis may focus more on the early prevention of transformation at hematopoietic stem cell level.

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