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AMBRA1 and its role as a target for anticancer therapy

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The activating molecule in Beclin1-regulated autophagy protein 1 (AMBRA1) is an intrinsically disordered protein that regulates the survival and death of cancer cells by modulating autophagy. Although the roles of autophagy in cancer are controversial and context-dependent, inhibition of autophagy under some circumstances can be a useful strategy for cancer therapy. As AMBRA1 is a pivotal autophagy-associated protein, targeting AMBRA1 similarly may be an underlying strategy for cancer therapy. Emerging evidence indicates that AMBRA1 can also inhibit cancer formation, maintenance, and progression by regulating c-MYC and cyclins, which are frequently deregulated in human cancer cells. Therefore, AMBRA1 is at the crossroad of autophagy, tumorigenesis, proliferation, and cell cycle. In this review, we focus on discussing the mechanisms of AMBRA1 in autophagy, mitophagy, and apoptosis, and particularly the roles of AMBRA1 in tumorigenesis and targeted therapy.

KEYWORDS

AMBRA1, autophagy, mitophagy, tumorigenesis, targeted therapy

Introduction

The global incidence and mortality of cancers have been dramatically increasing annually (1). In recent decades, cancer has been a leading cause of death and severely impacted life expectancy worldwide (2). At present, the strategies of cancer treatment mainly include surgical excision, chemotherapy, radiotherapy, and immunotherapy. However, the results of these treatments are still unsatisfactory. The molecular and cellular mechanisms of cancer have been explored in the last decades, but metastasis, chemoradiotherapy resistance, and recurrence are still the key obstacles to cancer treatment (3–6). Consequently, there is a dire need of figuring out the underlying mechanisms of cancers and find ways to cure them.

Autophagy is a cellular process that regulates the degradation of its cytoplasmic components *via* lysosomes. There are three major autophagy pathways, including macro-

autophagy, micro-autophagy, and chaperone-mediated autophagy (CMA), which mainly differ in delivery methods and wrapped cargoes. Macro-autophagy wraps and degrades intracellular cargoes through autophagosomes with a bilayer membrane structure by fusing with lysosomes eventually (7, 8), while mitophagy is a selective macro-autophagy for mitochondria decomposition (9). Micro-autophagy, compared with macro-autophagy, directly engulfs the organelles *via* lysosomal deformation (10). CMA degrades the KEFRQ motif-containing proteins with the help of a chaperone heat shock protein of 70 kDa (HSP70) (11). For the macro-autophagy (generally accepted as the term "autophagy") process, the formation of autophagosome is mainly divided into initiation, nucleation, elongation, and maturation. Although autophagy has a controversial effect on tumors in a context-dependent manner (12), autophagy disorder impacts the initiation and progression of cancer. Therefore, autophagy may be a promising target for cancer therapy.

The activating molecule in Beclin1-regulated autophagy protein 1 (AMBRA1), identified as an autophagy-associated protein initially, is a fundamental factor in the process of autophagosome formation (13). Furthermore, AMBRA1 is an intrinsically disordered protein that accounts for its great plasticity, which enables it to be a splendid scaffold protein connecting other intracellular processes related to autophagy (14–16). Through multifarious molecular interaction techniques, such as mass spectrum, genetic engineering technology, co-immunoprecipitation (co-IP), and yeast two-hybrid screening, numerous interaction partners of AMBRA1 have been demonstrated, as summarized in Table 1. Therefore, not surprisingly, AMBRA1 participates in diverse physiological and pathological processes, for instance, embryogenesis, neural development, tumorigenesis and proliferation, differentiation, and epithelial–mesenchymal transition (EMT) (13, 26, 33–36, 42–45).

AMBRA1 plays multi-functional roles in intracellular physiological and pathological processes. The aberrant expression and dysregulation of AMBRA1 positively and negatively control tumor formation and progression through diverse signal pathways, such as c-MYC, cyclin D, mTOR, PI3K, STAT3, and TGFβ (21, 23, 24, 26, 34, 38). Thus, AMBRA1 may be a potential target biomarker for future cancer therapeutics.

AMBRA1-protein structure and subcellular location

AMBRA1 was firstly identified by Francesco Cecconi using a gene-trap expression and mutational analysis to seek genes expressed in the development of the nervous system in 2007 (13, 46). *AMBRA1* gene is located in chromosome 11p11.2 with 24 exons, and it encodes a protein with a linear sequence of 1,298 amino acids. The subcellular location of AMBRA1 is mainly in cytoplasmic structures, such as autophagosome, cytoskeleton, endoplasmic reticulum, and mitochondria, and it is also found to be localized in the nucleus (13, 17, 18, 28, 29). Interestingly, the subcellular localization of AMBRA1 is dynamic, which primarily depends on autophagy induction. In the absence of autophagy induction, AMBRA1 tends to partially locate at mitochondria and cytoskeleton, and AMBRA1 re-localizes to the endoplasmic reticulum to enable autophagosome nucleation upon autophagy induction (17, 18).

AMBRA1 has no obvious domains but the WD40 domain at its N-terminus (13, 26). WD40 domain contains ~40 amino acids and acts as a binding site for the interaction of the protein with protein or DNA, so AMBRA1 can present a scaffold that assembles protein complexes or mediates transient interplay with other proteins (47). Furthermore, AMBRA1 contains 3 motifs-two PxP motifs, two TQT motifs, and one light chain 3 (LC3) interacting region (LIR) motif (Figure 1). The PxP motifs, corresponding to the aa 275-281 and aa 1177-1183 of AMBRA1, resemble the SH3 motif and bind with the catalytic subunit of protein phosphatase 2A (PP2A) to regulate c-MYC (26, 27). The TQT motifs located on the AMBRA1 C-terminal sequence mediate the interaction with the dynein light chain 1 (DLC1), fastening AMBRA1 to the dynein motor complex in the absence of autophagy induction (17). The LIR motif on its C-terminal region is critical for the binding between AMBRA1 and the autophagy-related protein 8 (ATG8) family proteins light chain 3 beta (LC3B) (28). Finally, AMBRA1 is cleaved by caspases at D^{482} during apoptosis (Figure 1). Its C-terminal part generates a BH3-like domain, called $AMBRA1^{CT}$, which acts as a pro-apoptotic factor by directly binding and inhibiting anti-apoptotic factor B-cell lymphoma 2 (BCL2) (20, 48, 49).

The role of AMBRA1 in autophagy initiation and apoptosis

In 1957, autophagy was first noted by Clark in the kidneys of neonatal mice by using an electron microscope (50) and firstly described by Deter and De Duve in the late 1960s without unveiling underlying mechanisms (51). In 1996, Oshumi and co-workers found ~30 autophagy-related genes (ATGs) in yeast (52, 53), which opened a new horizon for surveying this basic cellular process. Autophagy is a self-digestion process that engulfs impaired organelles or proteins to decompose into small molecules for cell reutilization, and this process is fundamental for cell survival.

The paralleled levels of AMBRA1 and autophagy suggest that AMBRA1 is one of the pivotal proteins regulating autophagy. Under normal conditions, AMBRA1 remains in a low or dormant state: 1) AMBRA1 preferentially binds to BCL2 at the outer mitochondrial membrane (18); 2) AMBRA1 is a vital

TABLE 1 The interaction partners of AMBRA1 protein.

The interaction protein of AMBRA1	Binding sites on AMBRA1	Function	Reference
BECLIN1	aa 533–751	Favoring the BECLIN1–Vps34 functional interaction	(13)
DLC1	aa 1075–1077 and 1087–1089	Inhibiting AMBRA1 and BECLIN1–VPS34 complex translocation to ER	(17)
Mito-BCL-2	The N-terminal and C-terminal region of AMBRA1	Harnessing AMBRA1 at mitochondria and inhibiting autophagy	(18)
Parkin	The N-terminal region of Ambra1	Local activation of class III PI3K around depolarized mitochondria	(19)
Caspases	D482 site in AMBRA1	Cleavage at D482	(20)
Calpains	?	Complete decomposition	(20)
TRAF6	aa 618–623 and 681–686	Supporting ULK1 ubiquitylation by LYS-63-linked chains	(21)
ULK1	The N-terminal and C-terminal region of AMBRA1	Activating AMBRA1 by phosphorylation	(21)
DDB1-CULLIN4 complex	The second AMBRA1 WD40 domain	Limiting AMBRA1 protein abundance and promoting AMBRA1 degradation	(22)
ELONGIN B-CULLIN5 complex	The C-terminal region of AMBRA1	Promoting the accumulation of the mTOR inhibitor DEPTOR	(22)
ELONGIN C-CULLIN5 complex	aa 735–1208	Negatively regulating the assembly and ubiquitin E3 ligase activity of CRL5 complexes	(23, 24)
RNF2	?	Ubiquitinating AMBRA1 at lysine 45	(25)
Catalytic subunit of PP2A	aa 275-281 and 1206-1212	Facilitating the dephosphorylation and degradation of the proto-oncogene c-Myc	(26, 27)
LC3	aa 1012-1023	Promoting mitophagy	(28)
FAK/Src	?	Regulating adhesion and invasive migration	(29, 30)
IKKα	Upstream of the LIR motif of AMBRA1	Promoting mitophagy	(31)
HUWE1	?	Promoting PINK1/Parkin-independent mitophagy	(31, 32)
ALDH1B1	?	Inhibiting carcinogenesis	(33)
Cyclin D	?	Regulating cell cycle	(34–36)
ATAD3A	?	Promoting PINK1 stability	(37)
Smad4	?	Facilitating TGFβ-driven metastasis	(38)
Cardiolipin	?	Promoting autophagosome formation	(39)
ERLIN1	aa 533-751 and 767-1269	Driving autophagy initiation	(40)
SUGT1	the C-terminal region of AMBRA1	Inhibiting the activity of CRL7 complexes	(22)
mTORC1	?	Inhibiting the activity of AMBRA1	(21)
CANX (calnexin)/GD3	?	Promoting autophagy	(41)
WIPI1	?	Promoting autophagy formation	(39, 41)
WASH	?	Promoting AMBRA1 degradation by potentiating RNF2	(25)
Akap8/Cdk9	?	Histone modifications and altered chromatin accessibility; transcriptional regulation	(29)

component of the BECLIN1/VPS34 complex, which is harnessed to the cytoskeleton through an interaction between the AMBRA1 and DLC1 (13, 17); 3) mTORC1 phosphorylates and inhibits AMBRA1 and ULK1, a protein kinase responsible for the recruitment of ATG proteins to the pre-autophagosomal structure. Furthermore, the DEP Domain Containing MTOR Interacting Protein (DEPTOR), an inhibitor of mTOR activity, is degraded by SOCS/ELONGIN B (ELO B)/CULLIN 5 (21, 22, 25, 54) (Figure 2). All the above processes prevent the activation phosphorylation of AMBRA1. Upon autophagy induction by glucose starvation, AMP-activated protein kinase (AMPK) inhibits mTORC1 through the phosphorylation of Tuberous Sclerosis 2 (TSC2) and Raptor with the result of reducing phosphorylation of ULK1 on Ser 757 and phosphorylation AMBRA1 on Ser 52 (21, 54). The phosphorylation of ULK1 on Ser 757 is reduced, and subsequently, AMPK directly interacts with and activates the dephosphorylated ULK1 by phosphorylating ULK1 on Ser 317 and Ser 777 (54). Moreover, the dephosphorylated AMBRA1/TRAF6 ubiquitylates ULK1 on Lys 63 to further promote ULK1 self-association, stability, and activity (21). The activated ULK1 kinase phosphorylates AMBRA1 and promotes its release from the dynein motor complex and relocates to mitochondria-associated membranes (MAMs) of the endoplasmic reticulum by interacting with CANX, GD3, WIPI1, ERLIN1, and Cardiolipin to enable autophagosome formation (17, 39–41,

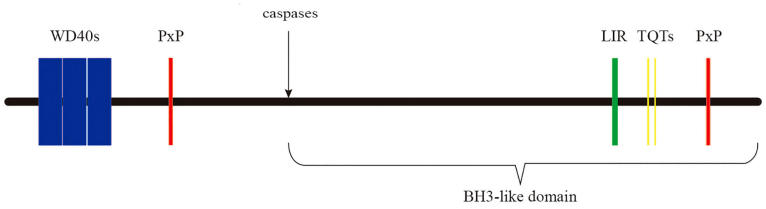

FIGURE 1
The domains and motifs of AMBRA1 protein. AMBRA1 contains WD40 domain (aa 1–175) and three kinds of motifs-two PxP motifs (aa 275–281 and 1206–1212), two TQT motifs (aa 1104–1106 and 1116–1118), and an LIR motif (aa 1043–1052). At the D482 site, AMBRA1 is cleaved by caspases.

55) (Figure 2). The exact phosphorylation site of AMBRA1 by ULK1 is unknown. As for the release of AMBRA1 from mitochondria upon autophagy induction, the underlying mechanism remains elusive, although ULK1 might be involved. In the autophagy induction stage, AMBRA1 not only regulates the activity of ULK1 kinase but also interacts with BECLIN1 and VPS34 and modulates their activity. In 2007, Gian Maria Fimia and colleagues firstly observed that AMBRA1 directly interacts with BECLIN1 and VPS34, and the downregulation of AMBRA1 markedly reduces BECLIN1-associated autophagy because of the reduced interaction between BECLIN1 and VPS34 (13). This corresponds to the characteristics of AMBRA1 as a scaffold protein that offers a platform for BECLIN1 and VPS34 interaction. To further identify the biological functions of AMBRA1, Antonioli et al. performed tandem affinity purification (TAP), sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and mass spectrometry to identify the interacting proteins of AMBRA1, and they found that some Cullin-RING ligase (CRL) components such as Cullin4, Cullin5, DNA damage-

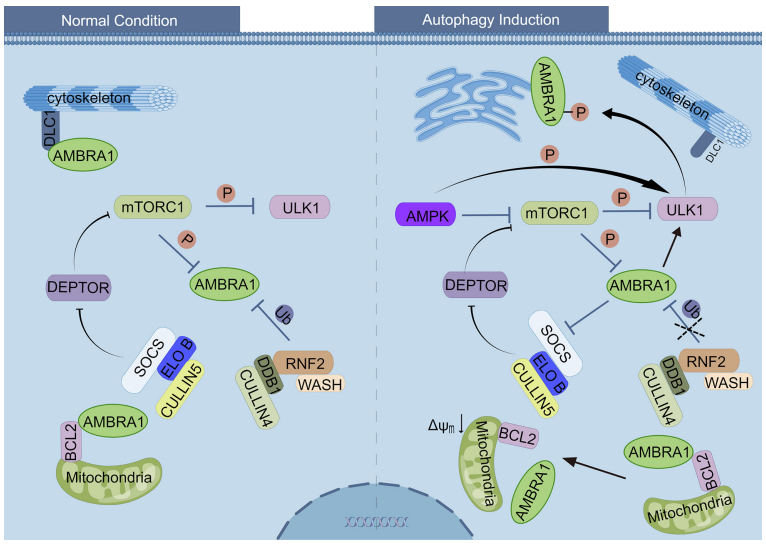

FIGURE 2
Regulation of AMBRA1 under normal conditions and autophagy induction. Left side: under normal conditions, AMBRA1 is relocated to the cytoskeleton through an interaction with DLC1, as well as with BCL-2 at the outer mitochondrial membrane. The mTORC1 inhibits ULK1 and AMBRA1 by phosphorylating ULK1 and AMBRA1, respectively. RNF2/DDB1/CULLIN4, together with WASH protein, ubiquitylates and degrades AMBRA1. SOCS/ELONGIN B/CULLIN5 ubiquitylates and degrades DEPTOR, resulting in activation of mTORC1 complex. Right side: upon autophagy induction of glucose starvation, AMPK inhibits mTORC1 and then reduces the phosphorylation of ULK1 and AMBRA1. Subsequently, AMPK directly interacts and activates the ULK1 by phosphorylation, and the dephosphorylated AMBRA1 ubiquitylates ULK1 to further promote the activity of ULK1. The activated ULK1 kinase phosphorylates AMBRA1, promotes its release from dynein motor complex, and relocates to mitochondria-associated membranes (MAMs). The degradation of AMBRA1 by RNF2/DDB1/CULLIN4 is inhibited, and therefore, AMBRA1 promotes DEPTOR accumulation and inhibits mTORC1 activity. The interaction between AMBRA1 and mito-BCL-2 is disrupted when mitophagy induction.

binding protein 1 (DDB1), Elongin B, and suppressor of G2 allele of SKP1 homolog (SUGT1) interact with AMBRA1, indicating that AMBRA1 is involved in mediating CRL ubiquitination activity (22). The temporal dynamic interaction of AMBRA1 with CULLIN 4 and CULLIN 5 regulates both the initiation and termination stages of autophagy, which keeps the autophagy under control.

Selective engulfment of impaired mitochondria *via* autophagy, namely, mitophagy, is important for the efficient turnover of mitochondria. Experts found that the processes of mitophagy are mainly divided into receptor-mediated mitophagy and ubiquitin-mediated mitophagy in mammals. Emerging evidence indicates that AMBRA1 plays an important role in both processes (28, 56). As mentioned above, AMBRA1 preferentially interacts with mitochondrial BCL-2 (mito-BCL-2) in normal conditions, and the interaction between AMBRA1 and mito-BCL-2 is disrupted when mitophagy is activated (18). Van Humbeeck et al. revealed that AMBRA1 is a non-substrate interactor of E3 ubiquitin ligase Parkin, and the interaction of AMBRA1 and Parkin is enhanced upon mitochondrial depolarization, leading to the clearance of mitochondria in a Parkin-mediated manner (19). By analyzing the protein sequence of AMBRA1 and validating by immunoprecipitation study and point mutation of AMBRA1, Strappazzon and colleagues disclosed that AMBRA1 contains a LIR motif responsible for binding with LC3 in its C-terminus (28) . They also originally constructed a plasmid encoding myc-AMBRA1 fused to Actin assembly-inducing protein (ActA) that can target the AMBRA1–ActA protein to the outer mitochondrial membrane, ultimately proving that AMBRA1 acts as a powerful mitophagy regulator through Parkin-mediated and Parkin-independent mitophagy (28) (Figure 3). For Parkin-mediated mitochondrial clearance, the loss of mitochondrial membrane potential rapidly recruits AMBRA1 to the outer mitochondrial membrane (OMM), where it interacts with ATAD3A–TOMM–PINK1 complex to prevent PINK1 degradation by mitochondrial matrix protease Lon Peptidase 1 (LONP1). Then the increase of PINK1 on the OMM recruits Parkin from the cytosol to damaged mitochondria, leading to mitochondria clearance in a Parkin-mediated manner (37). In terms of PARKIN-independent mitophagy, AMBRA1 acts as a mitochondrial receptor, and E3 ubiquitin ligase HUWE1 promotes the LIR motif of AMBRA1 unfold to interact with LC3 (28, 31). However, for the origin of AMBRA1 in mitophagy, few studies have been conducted. It is speculated that mitophagy regulation by AMBRA1 may be attributed to the dissociation of mito-BCL-2. A study from Strappazzon and colleagues found that GSK-3β phosphorylates MCL-1 to release AMBRA1, while

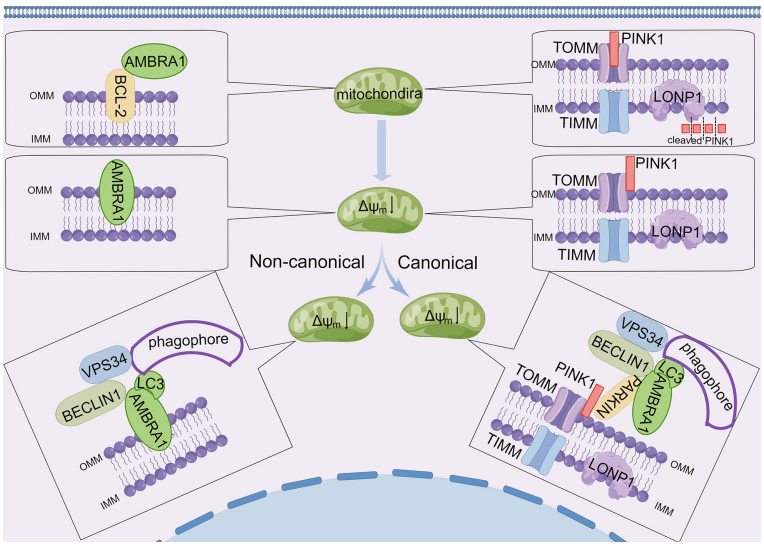

FIGURE 3
AMBRA1 and canonical/non-canonical mitophagy. Under normal conditions, AMBRA1 interacts with BCL-2 at the outer mitochondrial membrane. PINK1 is transported to the inner mitochondrial membrane through the TOM/TIM complex, and then PINK1 is damaged by LONP1. Upon loss of mitochondrial membrane potential (Δψm), AMBRA1 separates with BCL-2 and relocates to OMM. PINK1 accumulates on the outer membrane surface where it associates with the TOM complex. AMBRA1 promotes mitophagy of damaged mitochondria in two major ways: i) in non-canonical mitochondrial clearance, AMBRA1 functions as a mitophagy receptor and accumulates on the OMM, promoting specific binding to LC3 through a conserved LC3-interacting region (LIRs) and regulating the formation of phagophore enclosing mitochondria. ii) In canonical mitochondrial clearance, the accumulation of PINK1 recruits cytosolic PARKIN and AMBRA1, which induces new phagophores through its effect on VPS34 and its LIRs.

HUWE1 promotes MCL-1 degradation in Hela cells and MCF7 breast cancer cells (32), highlighting the need for further studies to elucidate molecular mechanisms of AMBRA1 and mito-BCL-2 in mitophagy. In conclusion, AMBRA1 plays a pivotal role in regulating mitophagy.

The interplay between autophagy and apoptosis is complicated. The stimulus factors for autophagy and apoptosis are similar, but the diverse outcomes may be due to different sensitivity thresholds (57). AMBRA1 is at the intersection of autophagy and apoptosis; namely, AMBRA1 not only participates in autophagy but also plays roles in mitochondrial apoptosis (58). Along with the stress aggravation, AMBRA1-mediated autophagy fails to restore the normal function of the cell, and then the cell will initiate the apoptotic program. The full-length AMBRA1 is cleaved by caspases to remove the N terminus and turn into a pro-apoptotic BH3-like protein. The cleaved form of AMBRA1 binds and inhibits the activity of anti-apoptotic BCL2 family proteins BCL2, MCL1, and BCL2L1 to promote cell death (48). To sum up, AMBRA1 can simultaneously be a regulator in the process of autophagy and apoptosis.

Unfolded protein response (UPR) also regulates the cross-talk between autophagy and apoptosis (59). In general, activation of UPR promotes cell survival by inducing cytoprotective autophagy and inhibiting apoptosis (60). Cancer cells are often subjected to numerous intrinsic and extrinsic insults that result in the destruction of protein homeostasis (proteostasis) and the accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER), which is known as ER stress (61). To counteract ER stress, cells activate a series of adaptive mechanisms of UPR to clear unfolded or misfolded proteins and restore proteostasis (62). UPR is controlled by three ER-transmembrane stress sensors, activating transcription factor 6 (ATF6), inositol-requiring enzyme 1α (IRE1α), and pancreatic endoplasmic reticulum kinase (PERK) (63). Under ER stress conditions, IRE1 phosphorylates BCL2 and BCL-XL by JUN N-terminal kinase (JNK), which promotes the dissociation of BECLIN1 and BCL2 families (59). Although the phosphorylation of BCL2 and BCL-XL by JNK has no effect on the binding between AMBRA1 and BCL2 proteins (18), UPR can also promote the activity of AMBRA1. Specifically, PERK inhibits mTORC1 by enhancing the expression of Tribbles Homolog 3 (TRB3) with the help of C/EBP homologous protein (CHOP) (59), while calcium release from ER also positively regulates the activity of AMPK (64), suggesting that UPR may activate the activity of AMBRA1 through both inhibiting mTORC1 and activating AMPK. However, if the insults are prolonged and severe, pro-survival UPR will transform into pro-apoptotic UPR. UPR can promote apoptosis by activating pro-apoptotic BCL2 proteins, BAX and BAK (65). The cleaved AMBRA1 can enhance the pro-apoptotic role of UPR by inhibiting the activity of anti-apoptotic BCL2 family proteins (48). These indicate that UPR and AMBRA1-mediated autophagy may coordinate with each other in modulating survival and apoptosis.

Role of AMBRA1 in tumorigenesis and tumor progression

c-MYC belongs to the "super transcription factors" family and is deregulated in >50% of cancers, which is an important target for cancer therapy (66). AMBRA1 regulates the activity of c-MYC through different pathways, and the roles of AMBRA1 in regulating c-MYC are controversial. Cianfanelli et al. investigated the cross-talk between two mTOR-dependent cell processes, autophagy induction and proliferation suppression, through four different approaches: gene-trap mutation in *AMBRA1 locus*, siRNA interference, *Ambra1* heterozygous ($Ambra^{+/gt}$) mice, and zebrafish embryo transplantation. The study finally identified that AMBRA1 interacts with the phosphatase PP2A and enhances its phosphatase activity on the proto-oncogene c-MYC, which further prevents tumorigenesis and tumor hyperproliferation (26). However, another study presented a different perspective that AMBRA1 is a tumor stemness-promoting factor in medulloblastoma (MB). Myc-Interacting Zinc Finger Protein 1 (MIZ-1) is a c-MYC cofactor, which is known to regulate AMBRA1 transcription directly (67). In MB subgroups of patients with enhanced levels of the c-MYC oncogene (MB_{Group3}), c-MYC correlating with MIZ-1 promotes the transcription of AMBRA1. Consequently, AMBRA1 promotes the activity of c-MYC through SOCS3/STAT3 pathway, which contributes to MB_{Group3} stem potential, growth, and migration (23). The cancerous inhibitor of protein phosphatase 2A (CIP2A) is an oncoprotein that could inhibit PP2A and stabilize c-MYC in human malignancies (68). The mechanism of AMBRA1 and CIP2A in regulating c-MYC is similar in that they both are under the control of mTORC1 and regulate the activity of PP2A. AMBRA1 inhibits the activity of c-MYC by enhancing the activity of PP2A, which inhibits the proliferation and tumorigenesis of cancer, but CIP2A plays the opposite role on PP2A to AMBRA1 (69). However, whether there is a direct interaction between AMBRA1 and CIP2A is unknown.

In addition to c-MYC, the cell cycle protein cyclin D is another key target in cancer therapy (70). The cyclin D–cyclin-dependent kinase (CDK) 4/6 complex is the fundamental factor for cell cycle progression, which promotes the transition from the G0 or G1 to S phase temporally (71). Thus, the cyclin D–CDK4/6 complex is frequently overexpressed and hyperactivated in various cancers (72). Recently, there is a breakthrough in the mechanism of cyclin D decomposition. Three independent studies unveiled a novel ubiquitylation degradation mechanism of cyclin D. As a

substrate receptor of the CULLIN 4/DDB1 complex, AMBRA1 directly binds and ubiquitinates cyclin D to promote its proteasomal degradation (34–36), while checkpoint kinase 1 (CHK1) is a key kinase in the replication stress response, and its inhibition aggravates DNA damage and leads to cell death in AMBRA1-null cancer cells (35). Furthermore, CDK2 is the catalytic subunit of the CDK complex, whose inhibition recovers the sensibility of AMBRA1-deficient tumors to CDK4/6 inhibitors palbociclib or abemaciclib (34). These findings elucidated therapeutic vulnerabilities in AMBRA1-deficient tumors and shed light on future clinical trials.

AMBRA1 is also associated with cancer development, including EMT, migration, invasion, and metastasis (26, 45, 73) (Figure 4). Interestingly, AMBRA1 plays an oncogenic role in hepatocellular carcinoma, metastatic breast cancer, and medulloblastoma, whereas AMBRA1 seems to be a tumor suppressor in colorectal cancer cell, melanoma, and squamous cell carcinoma (23, 24, 29, 33, 38, 45, 74, 75). The different effects of AMBRA1 in cancers may be due to diverse types and stages of cancer, as AMBRA1 is an autophagy-associated protein and has different roles depending on the gene context (76). Future studies need to focus on ascertaining the underlying mechanisms of how AMBRA1 plays opposite roles in different cancer types and figure out the gene context determining different functions of AMBRA1.

AMBRA1—a potential target for anticancer therapy

One of the major barriers in anticancer therapies is attributed to tumor resistance to apoptosis (30). As mentioned above, AMBRA1, an autophagy-related protein, is the direct substrate of caspases and calpains and acts an important role in apoptosis as well (13, 48). However, AMBRA1 is at the crossroad between autophagy and apoptosis and might be a novel prognostic and therapeutic candidate target for cancer therapy.

The role of autophagy in cancer therapies remains controversial (76). AMBRA1 is an autophagy-related protein and plays an important role in autophagy induction, so it can enhance resistance or sensitivity to chemotherapeutic agents in cancer treatment. In general, AMBRA1-mediated autophagy is pro-tumoral. Specifically, AMBRA1-mediated autophagy reduces the sensitivity to cisplatin in pancreatic cancer cells, ovarian cancer cells, and oropharyngeal squamous cell carcinoma cells (77–79). Sun et al. reported that AMBRA1 inhibited paclitaxel-induced apoptosis and chemosensitivity *via* the AKT–FOXO1–BIM pathway in MCF-7 and MDA-MB-231 breast cancer cells (80, 81). The same group also found that AMBRA1 expression level was negatively correlated with the sensitivity of breast cancer cells to epirubicin previously (82). In contrast, AMBRA1-

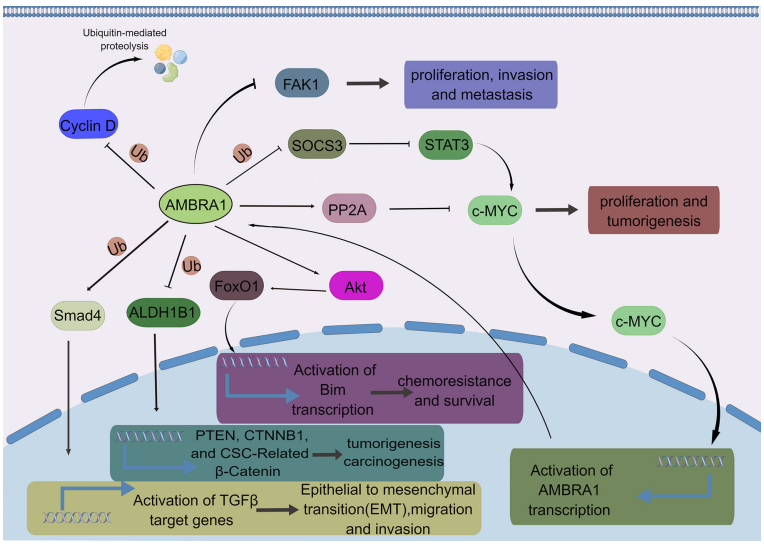

FIGURE 4
The major AMBRA1-related signaling pathways in cancer. AMBRA1 ubiquitinates cyclin D to promote its proteasomal degradation. AMBRA1 inhibits the proliferation, invasion, and metastasis of melanoma by inhibiting the phosphoactivation of FAK1. AMBRA1 inhibits the activity of c-MYC by enhancing the activity of PP2A, thereby inhibiting the proliferation and tumorigenesis of cancer cells, whereas AMBRA1 also promotes the activity of c-MYC through SOCS3/STAT3 pathway, enhancing tumor stem potential, growth, and migration of MBGroup3 stem cells. AMBRA1 promotes chemoresistance and survival in breast cancer cells through the AKT-FOXO1-BIM axis. AMBRA1 inhibits tumorigenesis and carcinogenesis by ubiquitylating ALDH1B1, a cancer stem cell marker. AMBRA1 mediates non-proteolytic polyubiquitylation of SMAD4 to enhance its transcriptional functions. Consequently, AMBRA1 potentiated TGFβ signaling and critically promoted TGFβ-induced epithelial-to-mesenchymal transition, migration, and invasion of breast cancer cells.

associated autophagy may also be anti-tumoral. Shen and colleagues unveiled that AMBRA1 was significantly upregulated in MDA-MB-231 and MDA-MB-453 cells after treating with cisplatin. While treating these cancer cells with a classic autophagy inhibitor 3-methyladenine (3-MA), they found that the cytotoxicity of cisplatin is impaired, which indicates that AMBRA1-mediated autophagy could enhance the cytotoxicity of cisplatin (83). Therefore, in the following studies, the identification of specific cell contexts in which AMBRA1-mediated autophagy exerts chemo-sensitization or resistance will be beneficial to potential AMBRA1 targeting therapies.

Radiation therapy is another classical cancer treatment scheme, and AMBRA1 also regulates tumor sensitivity to radiotherapy. AMBRA1-associated autophagy promotes the transition from hyper-radiosensitivity to induced radio-resistance in A549 and H460 human lung adenocarcinoma cell lines (84). Calcitriol, an active metabolite of vitamin D, enhances the sensitivity to irradiation in SiHa and CaSki cervical cancer cells by promoting AMBRA1 degradation (85). Therefore, the combination of AMBRA1 suppression and chemoradiotherapy may achieve a favorable outcome. Although the effect of AMBRA1-mediated autophagy on chemoradiotherapy is relatively limited at present, many studies indicated that BECLIN1 has a significant impact on chemoradiotherapy (86–89). Given that AMBRA1 is a vital component of the BECLIN1/VPS34 complex and regulates the activity of BECLIN1 (13), it is suggested that AMBRA1-mediated autophagy influences chemotherapy and radiotherapy. However, the difference between AMBRA1- and BECLIN1-associated autophagy needs to be further investigated.

In addition to the two classic treatments mentioned above, cancer immunotherapy as an emerging trajectory has played a more critical role in cancer therapy in the last decade. AMBRA1 is also involved in immune regulation, as AMBRA1 regulates the activities of various subtypes of T cells. Firstly, previous studies have shown that autophagy is associated with the survival, differentiation, and activation of T cells (90). Sato et al. found that AMBRA1 regulates the activity of OVA53 precursor T cells and naive T cells in an autophagy-dependent manner (91). Furthermore, this group also found that AMBRA1 regulates the proliferation of precursor T cells and naive T cells in an autophagy-independent manner (92). This regulation might be attributed to the recent finding that CULLIN4–AMBRA1 E3 ligase regulates the stability of cyclin D to control the cell cycle (35). Becher et al. also presented that AMBRA1 promotes differentiation and maintenance of human regulatory T cells by facilitating FOXP3 transcription *in vitro* and *in vivo* (27). The suppressor of cytokine signaling-3 (SOCS3) is a well-known feedback inhibitor of the JAK/STAT3 pathway, and STAT3 is central in regulating the anti-tumor immune response (93–95), while AMBRA1 activates STAT3 through suppression of SOCS3 in hepatocellular carcinoma and medulloblastoma (23, 24), indicating that the regulation of tumor immunogenicity by AMBRA1 may be in a STAT3-dependent manner.

Finally, a growing number of studies found that microRNAs (miRNAs), ~22-nt non-coding single-stranded RNAs, are directly associated with some important physiology and disease progression of plants and animals in a post-transcription modification manner. Insights into the roles of miRNAs in cancer have made miRNAs attractive targets for novel therapeutic approaches (96). By analyzing four independent databases (97–100) (DIANA-microT v5, TargetScan 8.0, microrna.org, and PicTar), only miR-23a-3p, miR-7-5p, miR-9-5p, and miR-200bc-3p/429 were identified as potential miRNAs targeting *AMBRA1*, suggesting the limited number of conserved miRNA binding sites in 3′-UTR of *AMBRA1* mRNA. These miRNAs can regulate chemosensitivity and cancer proliferation by targeting *AMBRA1* mRNA. MiR-23a-5p and miR-23a-3p derive from the same precursor miRNA-23a but are processed from the 5′ and 3′ arms, respectively. MiR-23a-5p restored the sensitivity of NB4 cells to arsenic trioxide (ATO) by targeting *AMBRA1*, and similar results were obtained in U937 cells. Moreover, clinical samples analysis revealed that miR-23a-5p is correlated with the NF-κB pathway in relapsed acute promyelocytic leukemia patients (101). MIR7–3HG promoted cell proliferation by targeting *AMBRA1* mRNA, which prevented c-MYC degradation to enhance transcription in HeLa cells and A549 cells (102). A recent study found that miRNA-198 targeted *AMBRA1* mRNA and regulated the enzalutamide-resistant prostate cancer growth *in vitro* and *in vivo* (103). Since miRNA is highly tissue-specific and can be used to predict molecular phenotypes of cancers, these specific miRNAs might be used as a basic approach to diagnose and treat cancers of AMBRA1 abnormity.

Conclusions and perspectives

The incidence and mortality of cancer are increasing yearly, and cancer is the major source of the global disease burden. A systematic analysis estimates that the burden of cancer will continue to rise for at least the next 20 years (104). As the pathogenesis of tumors is complicated, tons of studies unveiled many mechanisms of tumor initiation and progression (105–109). AMBRA1 as an emerging haploinsufficient tumor suppressor plays a pivotal role in tumorigenesis and progression (26, 45). Furthermore, AMBRA1-mediated autophagy plays controversial roles in chemoradiotherapy (78, 79, 81–84), and the different roles of AMBRA1-associated autophagy in cancer treatment seem to depend on tumor type, stage, and genetic context (76).

AMBRA1 is an intrinsically disordered protein that was associated with various tumor progressions, including autophagy, tumorigenesis, proliferation, EMT, and apoptosis (26, 34, 45, 48). Studies found that AMBRA1 regulates tumorigenesis by targeting the activity of c-MYC, STAT3, and ALDH1B1 (23, 26, 33). In addition, AMBRA1 also regulated

tumor proliferation, EMT, migration, and invasion by inhibiting cyclin D, FAK1, and Smad4 (34–36, 38, 45). However, these studies were mainly conducted *in vitro*; further work will be focused on validating these findings *in vivo*. In addition, it would be interesting to understand how AMBRA1 itself is regulated, both with relation to the cell cycle and in light of the multiple well-established functions. Moreover, several AMBRA1 isoforms are annotated in the human genome. It also remains to be determined whether these possible protein isoforms exist in cells and, if so, how their functions differ.

Currently, the post-translational modifications (PTMs) of AMBRA1 are only focused on phosphorylation and ubiquitylation (21, 22, 110), which are mainly associated with autophagy. To the best of our knowledge, there is almost no report about other types of PTMs, such as SUMOylation, methylation, and acetylation of AMBRA1, to date. Potential PTM forms of AMBRA1 may be identified through mass spectrometry and investigated in various physiological and pathological conditions.

In sum, targeting AMBRA1 has the potential to inhibit tumorigenesis and tumor progression in some types of malignancies. Furthermore, AMBRA1 tightly correlates with chemoresistance. During chemotherapy, cancer cells could attenuate the cytotoxicity of chemotherapeutic agents through autophagy, thereby promoting cancer survival. Therefore, autophagy inhibition by targeting AMBRA1 might enhance the effect of agents to achieve the therapeutic goal.

Author contributions

XL wrote the manuscript. XW read and approved the final manuscript. YL and JL revised the manuscript, and provided suggestions for the final manuscript. All authors read and approved the manuscript and agreed to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work were appropriately investigated and resolved.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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