Cancer Gene Therapy www.nature.com/cgt

REVIEW ARTICLE

The V-ATPases in cancer and cell death

Fangquan Chen[1], Rui Kang[2], Jiao Liu[1]✉ and Daolin Tang[2]✉

Transmembrane ATPases are membrane-bound enzyme complexes and ion transporters that can be divided into F-, V-, and A-ATPases according to their structure. The V-ATPases, also known as H^+-ATPases, are large multi-subunit protein complexes composed of a peripheral domain (V1) responsible for the hydrolysis of ATP and a membrane-integrated domain (V0) that transports protons across plasma membrane or organelle membrane. V-ATPases play a fundamental role in maintaining pH homeostasis through lysosomal acidification and are involved in modulating various physiological and pathological processes, such as macropinocytosis, autophagy, cell invasion, and cell death (e.g., apoptosis, anoikis, alkaliptosis, ferroptosis, and lysosome-dependent cell death). In addition to participating in embryonic development, V-ATPase pathways, when dysfunctional, are implicated in human diseases, such as neurodegenerative diseases, osteopetrosis, distal renal tubular acidosis, and cancer. In this review, we summarize the structure and regulation of isoforms of V-ATPase subunits and discuss their context-dependent roles in cancer biology and cell death. Updated knowledge about V-ATPases may enable us to design new anticancer drugs or strategies.

Cancer Gene Therapy (2022) 29:1529–1541; https://doi.org/10.1038/s41417-022-00477-y

INTRODUCTION

The pH homeostasis of intracellular compartments and organelles involves a complex dynamic process, which is precisely controlled by a variety of ion channels and transporters (e.g., monocarboxylate transporters [MCTs], Na^+/H^+ exchangers [NHEs], anion exchangers [AEs], $Na^+/HCO3^-$ co-transporters [NBCs], carbonic anhydrases [Cas], and vacuolar proton-translocating ATPases [V-ATPases, also known as H^+-ATPases]) [1]. Among them, V-ATPases are evolutionarily conserved protein machinery, containing a V1 domain and a V0 domain. V-ATPases possess multiple isoforms of each domain and exhibit cell- or tissue-specific expression and function. The isoform of the "a" subunit in the V0 domain has key location information for directing V-ATPase to distinct cellular membranes, which explains why cell compartments have differing pH values [2]. V-ATPases use a rotary mechanism to exert biological functions and regulate activity through the reversible dissociation of the V1 and V0 domains [3]. Functionally, V-ATPase-mediated pH acidification is necessary for normal cell activities, such as endocytosis, intracellular membrane traffic, protein degradation, antigen presentation, and sperm maturation. Imbalanced acidification and increased intraluminal pH may inhibit the activity of enzymes in the lysosome and destroy the removal of substrates, including protein aggregates, damaged organelles, and invading pathogens. Thus, it is not surprising that dysfunctional V-ATPases contribute to multiple pathological processes, such as renal tubular acidosis, bone osteopetrosis, tumor cell migration and invasion, and pathogen infection [4].

Malignant tumors remain a big challenge in medicine, and their occurrence and development are affected by internal and external factors, including impaired pH homeostasis [5]. For example, a pH gradient reversal is an early signal for tumorigenesis, which contributes to the development and maintenance of other cancer hallmarks, including increased glycolytic fluxes and resistance to cell death [6]. The acidic tumor microenvironment favors the invasiveness, metastasis, and drug resistance of cancer cells [7]. Destroying the highly acidic pH of cancer cells by inhibiting proton transporters (including V-ATPases) is expected to suppress tumor growth and development [1]. V-ATPases are widely expressed in plasma and organelle membranes and are necessary to maintain lysosome pH gradients of cancer cells. The abnormal expression, mislocalization, and mutation of V-ATPase subunits or isoforms are tightly related to cancer progression, which makes V-ATPase an important therapeutic target in translational medicine [4]. For example, the inhibition of V-ATPase activity by the drug bafilomycin A significantly suppresses tumor cell proliferation and induces cell death [8, 9]. However, due to the complex structure and widespread expression of V-ATPases, there are some challenges in safely and specifically targeting them for tumor therapy.

In this review, we first outline the structure, function, and modulation of V-ATPases, and then focus on their multifaceted roles in tumor biology, including invasion, metastasis, metabolism, and multidrug resistance. Updated knowledge about V-ATPases may provide new insights for developing more efficient targeted therapy using V-ATPase inhibitors.

STRUCTURE OF V-ATPASE

V-ATPases are large multiprotein complexes, initially identified in *Saccharomyces cerevisiae* and plant vacuoles [10]. They are highly conserved in plants, fungi, insects, and mammals, and are structurally related to mitochondrial proton F0F1-ATPases (F-ATPases) responsible for adenosine triphosphate (ATP) synthesis in mitochondria, chloroplasts, and bacteria. V-ATPases and F-ATPases use a similar rotational opening mechanism [11, 12].

---

[1]DAMP Laboratory, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong 510120, China. [2]Department of Surgery, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA. ✉email: 2018683073@gzhmu.edu.cn; daolin.tang@utsouthwestern.edu

Received: 20 December 2021 Revised: 7 April 2022 Accepted: 21 April 2022

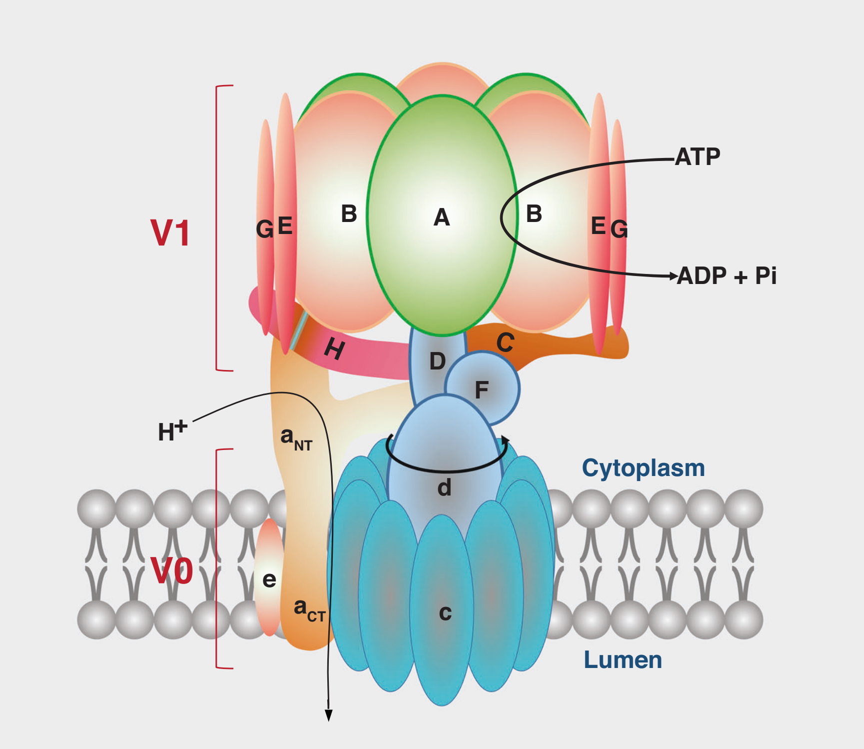

Fig. 1 Structure and mechanism of V-ATPase. V-ATPase consists of the peripheral V1 domain (including subunits A, B, C, D, E, F, G, and H) that hydrolyzes adenosine triphosphate (ATP) and the membrane integration V0 domain (including subunits a, c, d, and e) that transports protons. Subunits A and B form a hexamer that provides the ATP hydrolysis site at the interfaces. The peripheral stalk is composed of E, G, C, and H subunits. Subunits D, F, and d constitute the central stalk. When ATP is hydrolyzed at the AB subunit interface, the central stalk is driven to rotate. The c ring couples the energy of ATP hydrolysis with the proton translocation process from a subunit hemichannel.

F-ATPases not only hydrolyze ATP to form an electrochemical proton gradient, but also participate in ATP synthesis [13]. However, mammalian V-ATPases only participate in the hydrolysis of ATP, although yeast or plant V-ATPases are likely less active in the synthesis of ATP than F-ATPases [14]. In mammals, subunits A, B, C, D, E, F, G, and H form a V1 domain responsible for ATP hydrolysis, whereas subunits a, d, e, c, and c″ make up a V0 domain that is embedded in cell membrane and participates in proton translocation (Fig. 1). Of note, higher eukaryotes lack a c′ subunit, but contain an accessory subunit Ac45 [15]. In mammalian cells, many V-ATPase subunits are present in different isoforms, with their gene names beginning with "ATP6" and then a "V1" or "V0" domain, followed by a subunit or isoform as described above. In yeast, the names of subunits are indicated by "VMA" ("vacuolar membrane ATPase activity") and only one subunit has multiple isoforms [10]. Yeast subunit "a" encodes VPH1 and STV1 isoforms, which direct V-ATPases to vacuoles and Golgi apparatus, respectively [10]. Characterizing these subunit subunits may help us understand the differential expression and localization of V-ATPases in different species and cells.

MECHANISM OF V-ATPASE PROTON TRANSPORT

Analyses of eukaryotic V-ATPase structure using electron microscopy and small-angle X-ray scattering techniques provide a general overview of the V1 and V0 domains and their binding models [16, 17]. The rotary mechanism of V-ATPases is triggered by the hydrolysis of ATP to provide energy, whereas a central stalk is driven to rotate for proton translocation. Using cryo-electron microscopy, three different rotation states have been further identified in humans and yeast, and the switch between each state is accompanied by conformational changes in multiple subunits [18–20]. A and B subunits form a hexamer, serving as a catalytic (mainly through A residues) or noncatalytic (mainly through B residues) site. The B subunit generally plays a role in regulating the activity of V-ATPases [21–24]. Evidence of crystallization in the *Thermus thermophilus* A3B3 complex demonstrate that a hydrophobic cluster region promotes the transition between open and closed catalytic sites, which is essential for ATP hydrolysis [25]. Low-resolution structural analysis of the eukaryote *Saccharomyces cerevisiae* reveals that the A3B3 hydrolysis head is connected to the V0 domain through a central stalk [26]. When cytoplasmic ATP is hydrolyzed, a central stalk composed of D, F, d, and a ring of proteolipid subunits (c or c″ in mammals) in the V0 domain is responsible for the rotating [27]. Rotation of the central stem driven by ATP further facilitates the transport of protons from the cytoplasm to organelles or to the outside of the cell. These findings establish the dynamic and ATP-dependent assembly and regulation of V-ATPase protein machinery.

A dissociation analysis of yeast V0 domain structure using cryo-electron microscopy establishes a molecular model for the proton transport mechanism [28, 29]. First, the proton passes through the cytoplasmic hemi-channel located in subunit a to protonate the glutamate residue on subunit c. When the proteolipid ring rotates, the glutamate residue interacts with the arginine in subunit a. The residues interact and deprotonate, and finally release protons to the lumen side through the second hemi-channel of subunit a [30, 31]. The remaining subunits (C, D, E, F, G, and H) of the V1 domain may act as peripheral/central stalks and perform distinct functions depending on the region they are attached to [26, 32]. A central stalk composed of subunits D/F serves as a rotor that couples ATP hydrolysis to drive the rotation of proteolipid. The peripheral stalk, composed of subunits C, E, G, and H, prevents the

A3B3 subunit head from rotating during ATP hydrolysis, thus acting as a stator [15, 20]. However, the structure and function of the e subunit and Ac45 accessory proteins in the mammalian V0 domain are poorly understood. Future research is needed to determine the mechanochemical differences of V-ATPase proton transport under different pathological conditions.

REGULATION OF V-ATPASE ACTIVITY

The diversity of function of V-ATPase depends on different signals and pathways. Below, we highlight the factors and mechanisms involved in the regulation of V-ATPase activity, which can be shut down and turned on like a switch.

Spatial regulation of V-ATPase activity

An important mechanism for regulating the activity of V-ATPase in vitro is the reversible dissociation/recombination and regulated transport of the V1V0 complex. This process has been characterized in mammalian cells, insects, and yeast [33]. The regulation of V-ATPase activity involves both spatial and temporal aspects. Spatial regulation is manifested in different pH values of cell compartments, which decrease sequentially from early endosomes to lysosomes [34]. Spatial regulation is required for an endocytic pathway to execute many critical steps [35]. For example, when ligands are detached from receptors in the early endosomes, cell surface receptors are recycled from early endocytic compartments, and endocytosed macromolecules are degraded in lysosomes. During intracellular transport, the pH of the lysosome is more acidic than that of the Golgi, which is conducive to combining lysosomal enzymes with mannose 6-phosphate in the trans-Golgi network (TGN) and subsequent dissociation in late endosomes, leading to the recycling of receptors to the TGN [34] (Fig. 2A). These findings highlight that spatial regulation of V-ATPase activity is tightly controlled by the endosomal-lysosomal system. The consequences of a dysfunctional endosomal-lysosomal system is diverse and cell-specific.

Temporal regulation of V-ATPase activity

In mammalian cells, multiple intracellular signaling pathways, especially the PI3K-AKT-mTOR pathway, are involved in regulating the assembly of V-ATPase in response to various stresses [36]. One example of V-ATPase assembly in mammalian cells occurs during the immune response induced by dendritic cells as they transduce signals to T cells. T cells recognize foreign antigens and produce immune responses are an important “fortress” for human health. The recognition leads to foreign antigens being absorbed by dendritic cells and degraded in lysosomes, and then peptide fragments are presented to the surface of dendritic cells. In this process, lysosomes are mediated by assembled V-ATPases to become more acidic, and antigen processing increases. The assembly of V-ATPases creates a favorable environment for the degradation of lysosomal acid-dependent proteases [37], which is dependent on PI3K kinase and mTOR [38].

The assembly of V-ATPase is increased to eliminate excess acid produced in response to glycolysis caused by high glucose concentrations in HeLa cells, renal epithelium cells, Madin-Darby canine kidney cells, and A549 cells [39, 40]. The assembly of V-ATPases stimulated by high glucose concentrations is blocked by inhibiting PI3K kinase activity [39]. In contrast, when cells are exposed to epidermal growth factor (EGF), the assembly of V-ATPases is increased to produce adequate amino acid in lysosomes through the activation of the PI3K-mTOR1 pathway [41]. These findings reveal a lysosomal-related V-ATPase pathway that controls glucose metabolism.

Amino acid starvation has effects that are similar to EGF exposure, and the increased assembly of V-ATPase generates amino acid levels to activate mTOR. However, PI3K and mTORC1 activity are not involved in regulating the assembly of V-ATPases in mammalian cells [42]. Alternatively, rabconnectin family proteins have been implicated in the assembly of V-ATPases in mammalian cells by being regulators of the ATPase of vacuolar and endosomal membrane (RAVE) homologous proteins that regulate the assembly of V-ATPases in yeast [43].

Another key example of increased assembly of V-ATPases is associated with the activation of the endocytosis pathway during viral infection [44]. During this process, V-ATPase-mediated endosomal acidification is conducive to conformational change of the viral coat protein hemagglutinin and promotes the fusion of the viral membrane and the endosomal membrane, thereby releasing the viral genetic material into the cytoplasm of host cells [45]. V-ATPase-mediated pH acidification is also involved in infections of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [46]. When the virus infects the host cells and then accumulates in the lysosome, SARS-CoV-2 is different from other enveloped RNA viruses in that it excretes itself from the cell via an Arf-like small GTPase Arl8b-mediated lysosomal pathway. SARS-CoV-2 further uses this unconventional release pathway to destroy the lysosomal environment and protease activity, leading to virus proliferation [47]. SARS-CoV-2 can also escape autophagy surveillance by encoding the accessory protein ORF3a to disturb autolysosome formation [48]. Thus, endosome/lysosomal acidification mediated by the reversible assembly of V-ATPase plays a significant role in SARS-CoV-2 infection to transmission. A deeper understand of V-ATPase assembly may provide new insights into its physiological implications as well as pathological therapeutic modalities (Fig. 2B).

Regulation of V-ATPase trafficking

V-ATPase trafficking is another mechanism for controlling the activity of V-ATPase in renal alpha intercalated cells, epididymal clear cells, and osteoclasts [49, 50]. Several intrinsic factors, such as adhesion G protein-coupled receptor F5 (ADGRF5, also known as Gpr116), actin, and the microtubule cytoskeleton, are involved in regulating vesicle fusion and endocytosis of V-ATPase–containing vesicles in response to protein kinase A (PKA)-mediated phosphorylation. In cell biology, activated PKA is regulated by bicarbonate-sensitive adenylate cyclase [51–54], and this pathway regulates a plethora of biological processes (Fig. 2C). V-ATPase containing an a3 isoform translocates to the plasma membrane, acidifying the extracellular space to promote bone resorption. Mutations in the T-cell immune regulator 1, ATPase H^+ transporting V0 subunit A3 (TCIRG1) gene encoding the a3 isoform are highly associated with osteoporosis [55]. These findings indicate that abnormal V-ATPase trafficking leads to inflammation-related diseases, such as osteoporosis. The inhibition of PKA-mediated V-ATPase trafficking may help prevent chronic inflammation.

V-ATPASES INHIBITORS

The pH gradient of the cell can be adjusted by different types of V-ATPase inhibitors, including plecomacrolide antibiotics (e.g., concanamycin and bafilomycin), benzolactone enamides (e.g., salicylihamide, apicularens lobatamides, oximidines, and oruentaren), the miscellaneous compound archazolid A, and the indolyls (e.g., omeprazole, lansoprazole, esomeprazole, pantoprazole, and NiK12192). These V-ATPase inhibitors act on soluble domains or membrane sites, resulting in a loss of pH gradient across the plasma membrane, and ultimately slowing cell proliferation or increasing cell death.

Plecomacrolide antibiotics are the most frequently used inhibitor of V-ATPases, are isolated from *Streptomyces* species, and combine with subunit c to disrupt the rotation of V-ATPases [56, 57]. The difference between benzolactone enamides and plecomacrolide antibiotics is that the latter does not distinguish between mammalian and nonmammalian V-ATPases, while the former selectively inhibits mammalian cell V-ATPases [58]. The structures of salicylihalamide A and its derivatives are simple, and

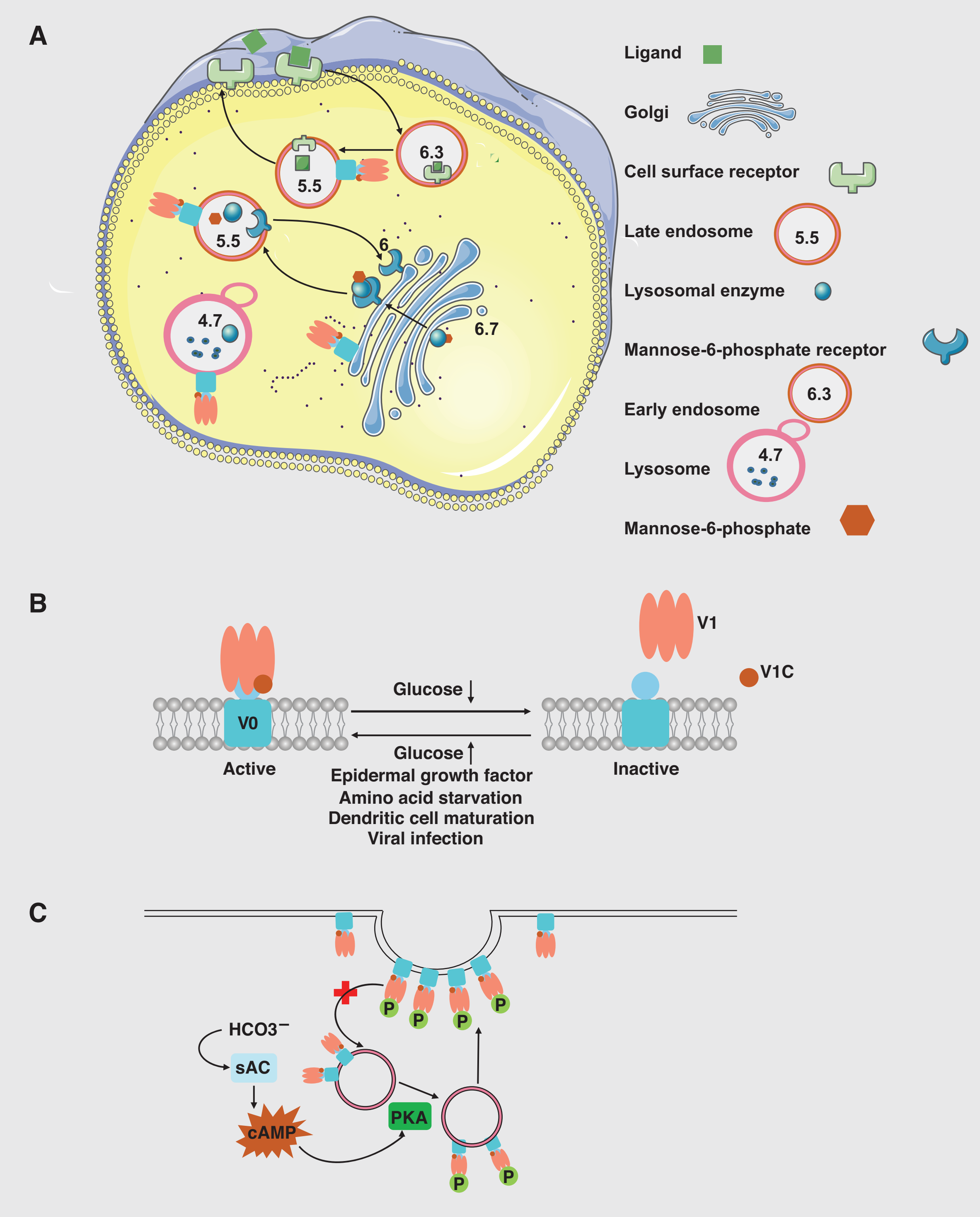

Fig. 2 Regulation of V-ATPase activity. A The pH value of various organelles in the cell is different, which is maintained by V-ATPase. When the endosome is acidified, the ligand is released, and the receptor is recycled back to the cell surface. During intracellular transport, the pH of the Golgi apparatus decreases from cis- to trans-Golgi network (TGN). The pH of the late endosome is lower than that of TGN, which facilitates the binding of lysosomal enzymes to the mannose 6-phosphate receptor (M6PR) of TGN and dissociation in the late endosome. **B** In mammalian cells, the reversible assembly of V-ATPase occurs in response to glucose changes, amino acid starvation, exposure to growth factors, dendritic cell maturation, and viral infection. **C** V-ATPase activity is affected by its trafficking, which is further controlled by the soluble adenylyl cyclase (sAC)-adenosine 3′,5′-monophosphate (cAMP)-PKA pathway.

they have promising anticancer activity on mammalian cells [59]. Archazolid, a compound produced by the mucilaginous bacteria *Archangium gephyra* and *Cystobacter violaceus*, also displays great anticancer activity [60, 61]. Based on the structures of bafilomycin and concanavalin, researchers have designed a simpler class of small molecules, namely indole [62]. A V-ATPase inhibitor from this class, namely omeprazole, is used to treat peptic ulcers and cancer. NiK12192 is a small molecule indole derivative that causes

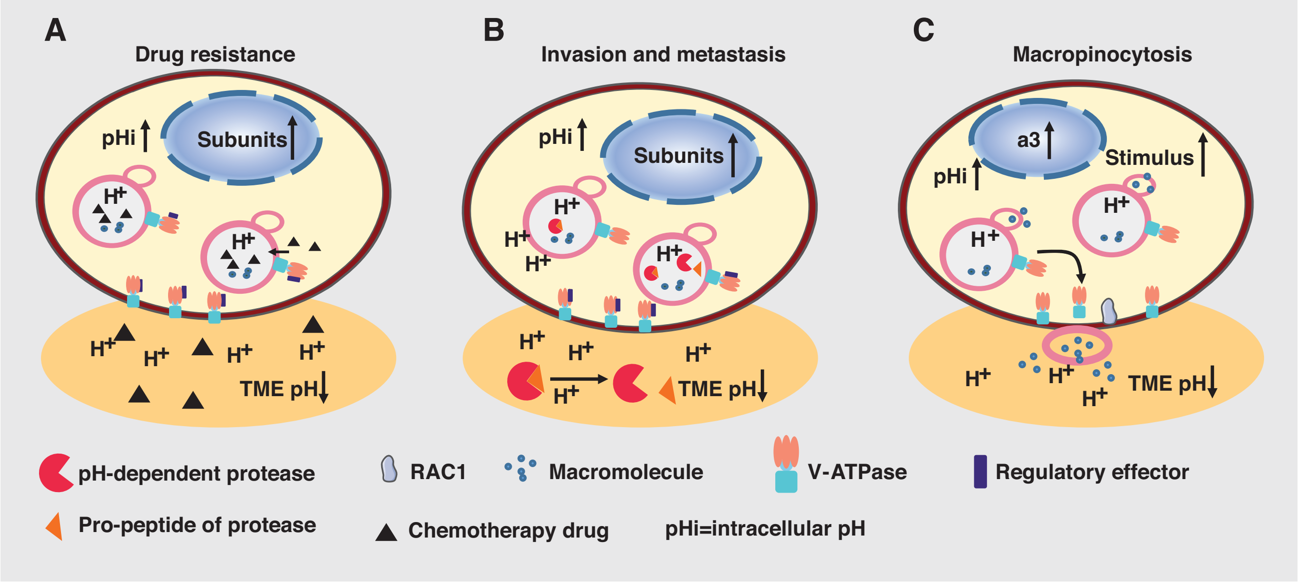

Fig. 3 V-ATPases in cancer. **A** Drug resistance. Some weak base drugs are protonated in the tumor microenvironment (TME). Lysosomal uptake of anticancer drugs in cancer cells increases drug excretion and eventually leads to drug resistance. **B** Invasion and metastasis. The overexpression of a V-ATPase subunit and its regulators are closely related to tumor invasion and metastasis. Plasma membrane V-ATPases regulate pH gradient reversal and promote pH-dependent protease activation, leading to an acidic TME. **C** Macropinocytosis. The plasma membrane localization of RAC1 requires the accumulation of V-ATPase containing an a3 subunit, which is a prerequisite for macropinocytosis.

a reduction in the volume and acidity of lysosomes in cancer and noncancer cells, which may have unpredictable side effects [63]. Although significant progress has been made, there is still a need to screen new V-ATPase inhibitors with high selectivity and low side effects for different cell or tissue types. The specific functions of V-ATPase inhibitors also need to be further studied in various species and disease models.

V-ATPASES IN CANCER

Generally, V-ATPase is overexpressed in cancers and mainly exerts carcinogenic effects at various stages, although some isoforms suppress tumor growth (Fig. 3) [64–69]. The inhibition of V-ATPase significantly limits the growth, migration, and invasion of cancer cells [70]. Specifically targeting subunits/isoforms of V-ATPases is a feasible therapeutic intervention. Below, we focus on the effects of V-ATPases on drug resistance, tumor invasion/metastasis, micropinocytosis, and autophagy, which are well-documented.

Drug resistance

Multidrug resistance (MDR) is a huge challenge for cancer treatment, and its formation involves multiple mechanisms. For example, cancer cells directly remove lipophilic drugs from the cytoplasm by upregulating membrane-associated MDR1 protein, which is encoded by the ATP binding cassette subfamily B member 1 (ABCB1) gene [71]. In addition to their effect on MDR1, cancer cells establish a reversed pH gradient for blocking drugs from reaching the tumor microenvironment, which can be mediated by V-ATPase in the plasma membrane. Compared with nondrug-resistant cancer cells, drug-resistant cancer cells have higher lysosomal amplification and V-ATPase expression on the plasma membrane. The changes in drug-resistant cells promote the release of drug-coated vesicles into the extracellular environment to a certain extent [72]. The acidic extracellular environment of tumors weakens the tumor-killing effect of weak bases or anthracycline anticancer drugs [73, 74]. Given that an alkaline cytoplasm and acidic extracellular environment are hallmarks of cancer cells, it is not surprising that weak base drugs are protonated and lose their anticancer effects [73]. In contrast, the use of the V-ATPase inhibitor bafilomycin reduces drug efflux from HeLa cancer cells that are resistant to vincristine or doxorubicin [75]. Pretreatment with the V-ATPase inhibitor pantoprazole improves the distribution of doxorubicin in the blood vessels of solid tumors [76]. The combination of chemotherapy drugs and V-ATPase inhibitors (e.g., bafilomycin A1, concanamycin A, omeoprazol, and archazolid A) produces a synergistic anticancer effect in drug-resistant cancer cells with stronger V-ATPase activity [77].

The complex structure and ubiquitous distribution of mammalian V-ATPases affect the reversal of pH gradients in cancer cells. Verucopeptin derived from natural products directly interacts with G subunits and inhibits the growth of MDR SGC7901/VCR cells by inhibiting the mTOR signaling pathway and V-ATPase activity [78]. The subunits of d and c are required for the development of MDR in many cases [79, 80]. For example, the positive regulation of yes-associated protein (YAP) expression by the d subunit of V-ATPase is associated with paclitaxel resistance in epithelial ovarian cancer. Treatment with paclitaxel combined with a sub-lethal dose of the proton pump inhibitor esomeprazole significantly improves drug sensitivity in paclitaxel-resistant epithelial ovarian cancer cells by inhibiting YAP expression and limiting the acidic tumor microenvironment [80]. The a2 subunit of V-ATPase is overexpressed in acquired cisplatin-resistant ovarian cancer cells. The inhibition of a2 expression by shRNA blocks DNA repair pathways, induces cell apoptosis, and restores the sensitivity of ovarian cancer cells to cisplatin [81]. A high expression of the V-ATPase C1 subtype is associated with MDR in patients with oral squamous cell carcinoma [82]. The expression of V-ATPase genes in cancer stem cells also favors drug resistance [83], highlighting the wide impact of V-ATPase in mediating MDR in different tumor microenvironment cells.

Serval regulators of V-ATPase are also involved in the production of MDR. For instance, ceramide synthase 2 (CERS2, also known as TMSG1 or LASS2) is a direct negative regulator of V-ATPase and its low expression promotes metastasis and drug resistance in cancer cells [84]. Consistent with this, patients with low CERS2 expression have a poor prognosis [85]. The transmembrane protein transmembrane 9 superfamily member 4 (TM9SF4) is a positive regulator of V-ATPase activity by binding to the V1H subunit in human colorectal cancer cells (e.g., HCT116 and SW480 cells). The inhibition of TM9SF4 enhances the anticancer activity of 5-fluorouracil in colorectal cancer cells [86]. Overall, these findings provide a potential strategy for overcoming MDR by targeting V-ATPase and its regulators.

Invasion and metastasis

A key feature of cancer cells is their ability to spread throughout the body through two related mechanisms: invasion and metastasis. V-ATPases participate in the invasion and metastasis of cancer cells due to its inappropriately high activity and changes in subcellular location. For example, invasive human breast cancer cells express highly active V-ATPases on the plasma membrane, while non-invasive breast cancer cells do not [87]. The V0a subunit of V-ATPases specifically participates in the invasion of breast cancer cells through subunit a3 and a4-mediated translocation to the plasma membrane [2, 88]. The expression of the V1A subunit in gastric cancer tissue is related to lymph node metastasis and vascular invasion, and this change is an indicator of poor prognosis [89]. Subunit V1E or a3 is overexpressed in pancreatic ductal adenocarcinoma and promotes invasion by regulating matrix metalloproteinase 9 (MMP9) activity [90–92]. Similarly, the overexpression of the a2 isoform in ovarian tissues or cell lines (A2780, SKOV-3, and TOV-112D) mediates invasion by enhancing the activity of MMP2 and MMP9 [93]. In invasive breast cancer cells, the V-ATPase a2 subunit is overexpressed on the plasma membrane and secretes a2 peptide, which increases the survival rate of tumor-associated neutrophils and promotes cancer cell invasion and disease progression [94]. The expression of the E1 isoform is related with poor prognosis in esophageal squamous cell carcinoma [95], whereas the overexpression of C1 isoforms in oral squamous cell carcinoma is correlated with higher tumor stage [82]. Together, these findings indicate that different subunits of V-ATPases play a context-dependent role in tumor invasion and metastasis.

The molecular mechanism of tumor invasion and metastasis mediated by V-ATPases have recently been explored. V-ATPases on the plasma membrane not only maintain the intracellular alkaline cytoplasm required for cancer cell proliferation, but also create optimally low pH for cathepsins and matrix metalloproteases to degrade the extracellular matrix [90, 96]. Lysosomal protein cathepsin is activated under acidic conditions and participates in protein degradation. Although MMPs are not directly dependent on pH, they are activated by cathepsin-dependent cleavage at low pH [42]. Both cathepsins (e.g., cathepsin B) and matrix metalloproteinases (e.g., MMP2 and MMP9) are secreted by tumor cells to promote invasion and metastasis by evoking epithelial-to-mesenchymal transition [97, 98]. Alternatively, increased interaction between V-ATPases and the actin cytoskeleton has emerged as an important system that promotes invasion and metastasis. Under normal conditions, subunits B and C of V-ATPases directly interact with actin microfilaments [54, 99]. Conversely, in migrating cancer cells, the interaction between V-ATPases and F-actin increases [100]. Moreover, alkaline pH favors the activation of the actin-binding protein cofilin, which severs agonist filaments to induce cancer cell migration [101]. Consequently, genetic or pharmacological inhibition of V-ATPases blocks cathepsin and MMP activation as well as cytoskeleton dynamics, thereby limiting tumor migration and metastasis [93, 102, 103]. ATP6L, the C subunit of the V-ATPase V0 domain, promotes metastasis of human colorectal cancer cells by inducing epithelial-to-mesenchymal transition, a major event in metastasis [104].

Another possibility is that V-ATPase binding protein provides a fine-tuning mechanism to control tumor migration and metastasis. As expected, the increased binding between V-ATPase and its negative regulator CERS2/TMSG1 inhibits cell invasion and metastasis in various types of cancers, including breast and prostate cancer [84, 105, 106]. The V-ATPase binding protein solute carrier family 48 member 1 (SLC48A1, also known as HRG1) selectively locates in the plasma membrane of aggressive human breast cancer cell line MB231, thereby promoting cancer invasion [107]. In addition, the transmembrane protein TM9SF4 and Rac family small GTPase 1 (RAC1) promote the activity of V-ATPases in human colon cancer cells and breast cancer cells, respectively, which increases invasion and metastasis [86, 103]. Collectively, these studies provide a strategy for inhibiting tumor migration and metastasis by targeting V-ATPase or its partners.

Macropinocytosis

Macropinocytosis is a nonselective endocytosis of solute molecules, nutrients, and antigens in the cytoplasm of mammalian cells [108]. It can be induced by the activation of oncogenic RAS mutations and other stimulating factors, such as EGF, platelet-derived growth factor (PDGF), and macrophage colony stimulating factor [109]. Macropinocytosis is also upregulated in cancer cells in response to various stresses, including autophagy blockade, nutritional deficiencies, oncogene deletion, chemotherapy, and membrane damage [110, 111]. In addition to supporting tumor growth, excessive macropinocytosis can induce non-apoptotic cell death, a type of necrotic rupture characterized by the rapid accumulation of fluid-filled vacuoles [112]. The molecular basis for the formation and maturation of macropinocytosis involves multiple signals and pathways. V-ATPases play a crucial role in macropinocytosis by keeping lysosomes acidified [113]. V-ATPases containing a3 localize to plasma membrane, which is necessary for the distribution of regulatory proteins of macropinocytosis, such as RAC1 [114]. Therefore, inhibiting V-ATPases destroys the process and function of macropinocytosis. Nevertheless, the V-ATPase coupling membrane dynamic may play a dual role in regulating cancer cell survival or death through a dysregulated macropinocytosis pathway.

Autophagy

Autophagy is a highly conserved degradation process that plays a dual role in human diseases, including cancer [115, 116]. The inhibition of autophagy is accompanied by an accumulation of misfolded proteins and damaged organelles, thereby inducing reactive oxygen species (ROS) production and DNA damage, and ultimately promoting tumorigenesis [117–120]. In contrast, in established cancers, autophagy-mediated metabolic homeostasis provides energy for tumor growth. Although autophagy usually promotes tumor survival, the unrestricted activation of autophagy may lead to cell death [121, 122]. The autophagy process involves the formation of multiple membrane structures (phagophores, autophagosomes, and autolysosomes), which is mainly regulated by the autophagy-related (ATG) protein family [123]. Of note, V-ATPase–mediated lysosomal acidification affects the fusing of autophagosomes to lysosomes [124], despite the existence of V-ATPase–independent pathways [125].

Functionally, the modulation of V-ATPase–mediated autophagy affects the effectiveness of tumor treatment. For example, ATP6V1C1 inhibits autophagy in human esophageal squamous cell carcinoma, leading to radiotherapy resistance [126]. As an adaptive pathogenesis, mutations in ATP6V1B2 increase the autophagy flux associated with human follicular lymphoma cells [127]. The marine natural product manzamine A targets vacuolar ATPases and inhibits the autophagy and growth of pancreatic cancer cells [128], although autophagy has a tumor suppressor effect in the early stages of pancreatic cancer [129]. Some subunits of V-ATPase play a significant role in regulating selective autophagy, including xenophagy, mitophagy, aggrephagy, and endoplasmic reticulum-phagy [130, 131], although their direct roles in tumorigenesis and tumor therapy remain largely unknown (Table 1**)**. We also believe that different subunits of V-ATPases may form different complexes with autophagy receptors, thereby establishing the plasticity of selective autophagy.

Table 1. V-ATPase in autophagy and cancer.

Type	Subunit of V-ATPase	Expression and function	Model	References
Macroautophagy	a2	Upregulation; controls the NOTCH pathway	MDA-MB231 and MDA-MB-468 cancer cell lines	[188]
Macroautophagy	C1	Upregulation; confers radiotherapy resistance	TE13 and ECA109 cancer cell lines; BALB/c nude mice	[126]
Macroautophagy	c	Upregulation; maintains lysosomal acidification	HCT116 cancer cell line; BALB/c nude mice	[139]
Macroautophagy	a2	Upregulation; promotes autophagy and platinum resistance	A2780, cis-A2780, and TOV-112D cancer cell lines	[189]
Macroautophagy	A	EN6 target; inhibits mTOR and induces autophagy	HeLa cancer cell line; C57BL/6 mice	[190]
Macroautophagy	c″	Upregulation; inhibits autophagy	*Drosophila* model of gliomagenesis	[191]
Macroautophagy	B2	Mutation; activates autophagic flux	OCI-LY1, SUDHL4, and SUDHL16 cancer cell lines; primary follicular lymphoma B cells	[127]
Xenophagy	c	Upregulation; mediates the interaction between V-ATPase and ATG16L1 and induces xenophagy	HeLa cancer cell line; C57BL/6 mice	[130]

V-ATPASE IN CELL DEATH

Cell death can be classified as accidental cell death or regulated cell death (RCD) [132]. RCD is an active process and has many forms, which can be further divided into apoptotic cell death and non-apoptotic cell death that use different stimulation signals and molecular machinery [133]. Since autophagy and lysosome dysfunction are closely related to the process and outcome of cell death, the role of V-ATPases in cell death is expected to be contextual (Fig. 4).

Apoptosis

Apoptosis is a multi-step and energy-dependent process that usually involves the activation of a group of cysteine proteases, called caspases. The induction of apoptotic cell death involves extrinsic signaling through death receptors and intrinsic signaling mainly through the release of mitochondrial proteins. BCL2 prevents BAX and BAK1 oligomerization, which plays a major role in mediating the release of pro-apoptotic proteins from mitochondria. The development of therapies to eliminate cancer cells by caspase-dependent apoptosis remains mainstream in clinic [134]. The overexpression of V-ATPases in cancer mediates apoptosis resistance during chemotherapy [135–137] (Table 2). In contrast, the inhibition of V-ATPase by inhibitors has been found to increase ROS production, which leads to apoptosis of cancer cells and improves the therapeutic effect [135, 138]. Thus, the selective inhibition of hyperactive subunits of V-ATPases is a potential antitumor strategy. V0 subunit c of vacuolar ATPase (ATP6V0C) is a direct binding site for bafilomycin A1. In human colorectal cancer cells, the suppression of ATP6V0C expression by bafilomycin A1, combined with a novel clinical candidate, IDF-11774, exhibits a synergistic growth inhibitory effect by inhibiting autophagy and inducing caspase 3 (CASP3)-dependent apoptosis [139]. However, the side effects caused by long-term use of V-ATPases inhibitors are also a problem that cannot be ignored [140]. In addition to regulating lysosomal pH, the effect of V-ATPases on inhibiting apoptosis can be achieved by secreting proteins to affect the tumor microenvironment. For example, the human cancer-associated a2 isoform specifically secretes a peptide that reprograms neutrophils by inhibiting the mitochondrial apoptotic pathway, which in turn promotes tumor angiogenesis and invasion [67, 81, 141]. Treatment with anti-a2 antibodies overcomes drug resistance and enhances apoptosis during cancer therapy. V-ATPases expressed in the endoplasmic reticulum also mediate apoptosis resistance by activating the unfolded protein response. In this process, transmembrane BAX inhibitor motif containing 6 (TMBIM6, also known as BI-1) is a conservative endoplasmic reticulum stress protection protein that increases cell survival by activating lysosomal activity to prevent the production of misfolded proteins. Inhibiting the activity of V-ATPases counteracts this compensation and induces apoptosis in human HT1080 fibrosarcoma cells [142]. However, it remains a challenge to distinguish the role of V-ATPases in apoptosis in different organelles.

Although the anti-apoptotic effect of V-ATPases has been widely recognized, they also play a pro-apoptotic effect under certain conditions. In particular, the induction of apoptosis by death receptor ligand TNF superfamily member 10 (TNFSF10, best known as TRAIL) signaling requires V-ATPase-dependent endosomal acidification [143]. Thus, the combination of TNFSF10 and chemotherapy may be specifically sensitive to cancers with a high expression of V-ATPase.

Anoikis

Anoikis is a specific type of apoptosis-like cell death, induced by detached contact with the extracellular matrix [144]. It is a critical mechanism in preventing metastatic colonization during tumor invasion and metastasis. Inhibiting V-ATPase to activate anoikis has become a target for anti-metastatic cancer [145, 146]. For example, treatment of human metastatic cancer cells (T24, MDA-MB-231, 4T1, and 5637 cells) with the V-ATPase inhibitor archazolid induces anoikis at the early stage, as evidenced by its abolishing CASP8 and FADD-like apoptosis regulator (CFLAR, best known as c-FLIP) expression, increasing CASP8 activation, and increasing the pro-apoptotic protein BCL2-like 11 (BCL2L11, also known as BIM). In the later stage, archazolid induces extracellular signal-regulated kinase (ERK), AKT and SRC proto-oncogenes, and nonreceptor tyrosine kinase-mediated degradation of BCL2L11, leading to ROS production and the limiting of anoikis [146], highlighting a dual role of V-ATPase in anoikis. However, the inhibition of signal transducer and activator of transcription 3 (STAT3)-dependent V-ATPase increases ROS production and the sensitivity of aggressive human cervical cancer, breast cancer, and murine melanoma cells to anoikis [145]. These results indicate that V-ATPase helps cancer cells maintain pro-survival signals after detachment and resist anoikis, and thereby constitute a promising target for metastatic cancer.

Alkaliptosis

Alkaliptosis is a form of pH-dependent and caspase-independent cell death induced by JTC801 (an opioid analgesic drug), which was first described in pancreatic cancer cells [147]. The induction of alkaliptosis can eliminate venetoke-resistant acute myeloid leukemia cells [148], providing a new strategy to overcome drug resistance [149]. At the molecular level, JTC801-induced alkaliptosis requires activation of the inhibitor of nuclear factor kappa B

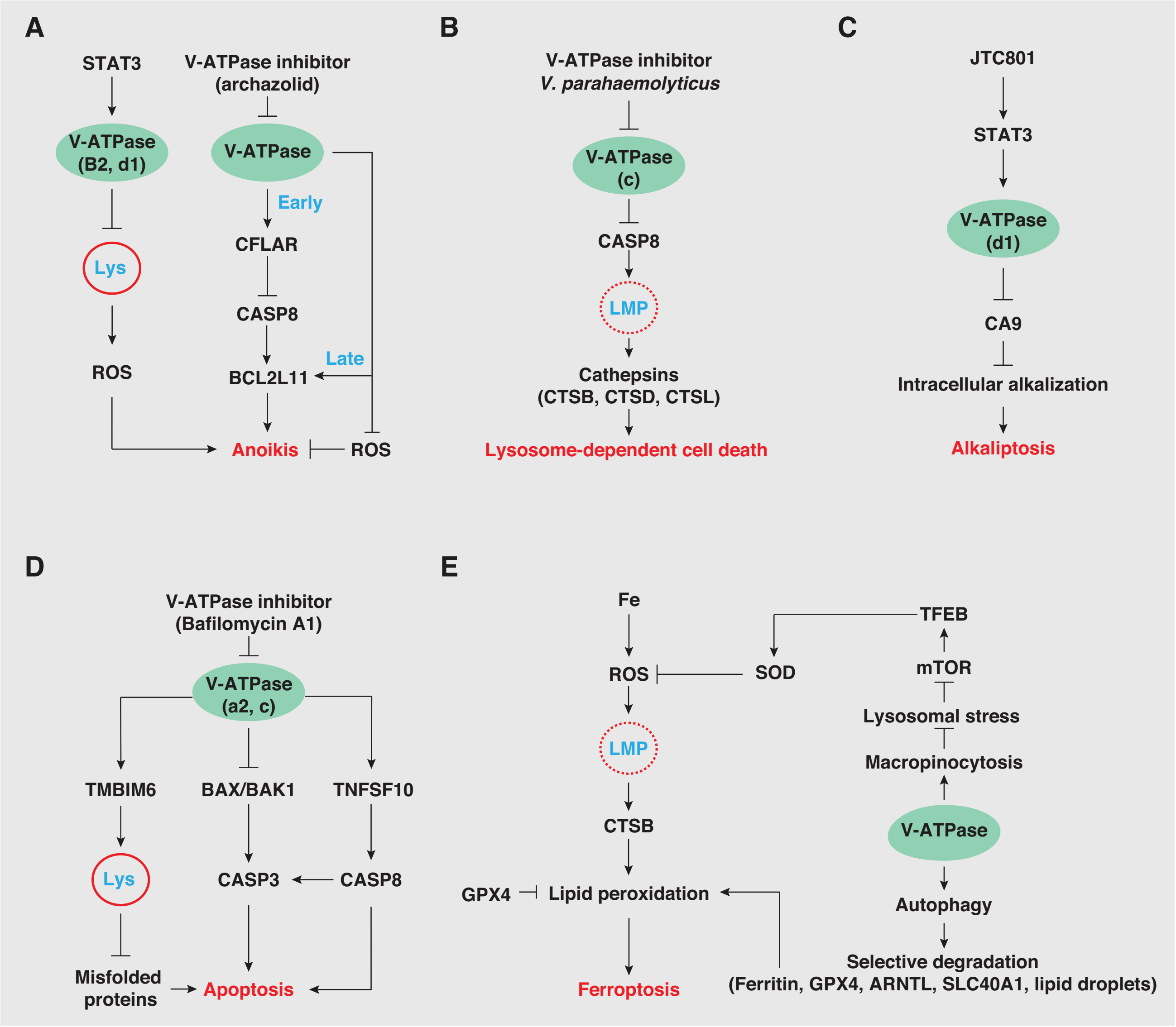

Fig. 4 V-ATPase in cell death and cancer. **A** Anoikis is a mechanism for the elimination of detached cells that can be induced by inhibiting V-ATPase or STAT3 in cancer cells. ROS plays the opposite role in anoikis according to the stage. **B** Lysosome-dependent cell death is driven by lysosomal membrane permeabilization (LMP), which can be inhibited by V-ATPase. **C** Alkaliptosis is caused by CA9 downregulation and subsequent intracellular pH upregulation in cancer cells. Increased interaction between V-ATPase subunit d1 and STAT3 promotes JTC801-induced alkaliptosis. **D** V-ATPase plays a dual role in apoptosis. On the one hand, V-ATPase inhibits the mitochondrial apoptotic pathway by inhibiting the BAX/BAK1 complex or increasing TMBIM6-mediated degradation of misfolded proteins via lysosome (Lys). On the other hand, V-ATPase-mediated TNFSF10 production triggers CASP8-dependent apoptosis. **E** Ferroptosis is a type of iron-dependent cell death caused by lipid peroxidation and oxidative damage of lysosomal membranes to release CTSB. V-ATPase inhibits ferroptosis by activating TFEB-dependent SOD production. V-ATPase-mediated autophagy may promote ferroptosis by selectively degrading anti-ferroptotic regulators. ARNTL aryl hydrocarbon receptor nuclear translocator-like, BAK1 BCL2 antagonist/killer 1, BAX BCL2-associated X, apoptosis regulator, BCL2L11 BCL2-like 11, CA9 carbonic anhydrase IX, CASP8 caspase 8, CFLAR CASP8 and FADD-like apoptosis regulator, CTSB cathepsin B, CTSD cathepsin D, CTSL cathepsin L, GPX4 glutathione peroxidase 4, mTOR mechanistic target of rapamycin kinase, ROS reactive oxygen species, SLC40A1 solute carrier family 40 member 1, SOD superoxide dismutase, STAT3 signal transducer and activator of transcription 3, TFEB transcription factor EB, TMBIM6 transmembrane BAX inhibitor motif containing 6, TNFSF10 TNF superfamily member 10.

kinase subunit beta (IKBKB, also known as IKKβ)-dependent NF-κB pathway, followed by the downregulation of carbonic anhydrase 9 (CA9) [147]. The high-mobility group box 1 (HMGB1, a multi-functional alarm protein that drives innate immunity and autophagy) and antioxidant nuclear factor, erythroid 2-like 2 (NFE2L2, best known as NRF2) pathway regulates alkaliptosis sensitivity and subsequently the sterile inflammatory response [150–153]. This alkaliptotic death-initiated immune response is further related to the activation of a DNA sensor pathway mediated by advanced glycosylation end-product specific receptor (AGER) and stimulator of interferon response CGAMP interactor 1 (STING1) [150]. Early studies demonstrated that STAT3 combined with ATPase H^+ transporting V1 subunit A (ATP6V1A) participates in the cytoplasm and lysosomal pH homeostasis of cancer cells [154]. The use of super resolution structured illumination microscopy also confirms the proximity of STAT3 and ATPase H^+ transporting V0 subunit D1 (ATP6V0D1) in intact HeLa cells. Our data indicate that the upregulation of ATP6V0D1 promotes JTC801-induced alkaliptosis by combining with STAT3 in human pancreatic cancer cells. These findings establish the pro-death effect of ATP6V0D1 in alkaliptosis and may help researchers develop non-apoptotic cell death strategies for tumor treatment.

Table 2. V-ATPase in cell death and cancer.

Type	Subunit of V-ATPase	Expression and function	Model	References
Apoptosis	a2	Upregulation; inhibits apoptosis and promotes angiogenesis, cancer cell invasion, and neutrophil recruitment	Neutrophils; human lung, endometrium, bladder, and kidney cancer tissues	[67]
Apoptosis	a2	Upregulation; inhibits apoptosis and promotes tumor cell survival	A2780 and SKOV-3 cancer cell lines; female athymic nude mice	[141]
Apoptosis	a2	Upregulation; inhibits apoptosis and promotes tumor invasion and drug resistance	A2780, TOV112D, cis-A2780, and cis-TOV112D cancer cell lines	[81]
Apoptosis	c	Upregulation; inhibits apoptosis and promotes drug resistance	Drug-resistant MCF-7 and ADR cancer cell lines	[79]
Apoptosis	c	Upregulation; inhibits apoptosis and promotes drug resistance	KB, PC3, and MCF7 cancer cell lines	[192]
Lysosome-dependent cell death	c	Upregulation; participates in proton transport and maintains lysosomal acidification	HeLa and MCF-7 cancer cell lines	[68]
Lysosome-dependent cell death	c	Upregulation; forms a channel to mediate proton transport	HeLa cancer cell line; RAW264.7 macrophage cell lines	[180]
Ferroptosis	N/A	Upregulation; maintains lysosomal acidification and promotes the fusion of macropinosomes and lysosomes	HK2 cancer cell line; RAW264.7 macrophage cell line	[171]
Alkaliptosis	d1	Upregulation; promotes cytoplasmic pH alkalization and increases alkaliptosis	SW1990 and MIAPaCa2 cancer cell lines	
Anoikis	B2, d1	Upregulation; inhibits anoikis	HeLa, MCF-7, and B16F10 cancer cell lines; BALB/c nude mice	[145]

Ferroptosis

Ferroptosis is a form of regulated cell death characterized by iron-dependent lethal accumulation of lipid hydroperoxides and has been implicated in human disease [155–157]. It is mainly executed by lipid peroxidation on membrane phospholipids containing polyunsaturated fatty acid (PUFA) chains and plays a dual role in tumor biology [158, 159]. PUFAs are converted into phospholipid hydroperoxides through a series of ROS-initiated reactions, which can be neutralized through the enzyme glutathione peroxidase 4 (GPX4) [160]. Although the pharmacological inhibition of GPX4 is one of the main methods of inducing ferroptosis, the phenotype of different GPX4 conditional knockout mice or cells is related to both ferroptotic and nonferroptotic death [161–165], indicating that unknown factors affect the death mode of GPX4-deficient cells. In addition to the plasma membrane, lipid peroxidation on the organelle membrane (including that of lysosomes) also favors ferroptosis [166]. The lysosome is one of the main storage sites of iron in the cell. The damage in the lysosome can cause the release of lysosomal iron and subsequent production of ROS [167]. In turn, increased ROS increases lysosomal membrane permeabilization, which leads to more cathepsin leakage for ferroptosis [168]. As a defense mechanism, lysosomal stress leads to the inactivation of mTOR, leading to nuclear translocation of transcription factor EB (TFEB) [169, 170], enhancing the expression of lysosomal protein and antioxidant superoxide dismutase (SOD), and ultimately reducing ROS and inhibiting ferroptosis [171]. V-ATPase acts as a repressor in the process of lysosomal stress. For example, in carboxyl-modified polystyrene nanoparticle (CPS)-mediated ferroptosis inhibition, CPS-containing macropinosomes fuse with the lysosome in a V-ATPase–dependent manner to form a vacuole, which is a marker of lysosomal stress [171]. However, whether specific subunits of V-ATPase can directly regulate iron death has not been determined. Another possibility is that V-ATPase indirectly participates in iron death by affecting autophagy activity because the selective activation of autophagy mediates the degradation of anti-ferroptosis regulators, including ferritin [172], aryl hydrocarbon receptor nuclear translocator-like (ARNTL, best known as BMAL1) [173], GPX4 [174, 175], solute carrier family 40 member 1 (SLC40A1, also known as ferroportin-1) [176], and lipid droplets [177].

Lysosome-dependent cell death

Lysosome-dependent cell death (LCD) depends on lysosomal membrane permeabilization and is induced by the release of lysosomal hydrolases and contents, especially cathepsins (such as CTSB, CTSD, and CTSL) [178]. Lysosomal leakage in response to different stresses (lysosomotropic detergents, lipid metabolites, and ROS) can amplify or initiate different types of RCD (e.g., apoptosis, necroptosis, and ferroptosis) [179]. The inhibition of V-ATPases leads to lysosomal deacidification and lysosomal membrane permeabilization, thereby inducing LCD and interaction with other types of RCDs [179]. For example, the inhibition of V-ATPase c subunit induces CASP8-dependent LCD, rather than apoptosis, in HeLa and MCF-7 cells [68]. Similarly, lysosomal rupture and LCD is implicated in host cell damage caused by *Vibrio parahaemolyticus* infection [180]. The effector protein VepA secreted by its type III secretion system (T3SS1) interacts with the c subunit to induce HeLa cell lysosome rupture and releases CTSD for LCD [180]. These results suggest that targeting specific subunits of V-ATPase may eliminate apoptosis-resistant cancer cells by inducing LCD. In contrast, V-ATPase inhibitors, such as bafilomycin A1 and concanamycin A, block the cyclin-dependent kinase (CDK) inhibitor abemaciclib from inducing LCD in human non-small cell lung cancer cells [181]. Different CDK inhibitors may trigger different forms of immunological cell death to enhance antitumor immunity [182, 183].

CONCLUSION AND PERSPECTIVES

V-ATPases are highly conserved ATP-driven proton pumps with very diverse functions [15, 36]. In addition to the fundamental physiological functions of V-ATPase in sustaining a pH gradient, the abnormal expression and subcellular locations of V-ATPase are tightly related to the occurrence and development of tumors. A high expression of V-ATPase has a poor prognosis in many types of cancer, whereas plasma membrane V-ATPase promotes tumor cell invasion, metastasis, and drug resistance. Although the functions of the subunits or isoforms of V-ATPase may be not the same, the regulatory assembly of V-ATPase is a mechanism for controlling cellular activity under stresses, especially glucose changes and amino acid starvation [4, 70]. Structurally, conformational changes affect its self-assembly and activation. Activated V-ATPases play a role in mediating lysosomal acidification and autophagy, not only by increasing the recycling of macromolecules, but also through enhancing the defense ability of cells to resist cell death. In contrast, excessive activation of lysosome-dependent autophagy may cause cell death, especially ferroptotic cell death [184, 185].

While V-ATPases are a promising druggable target in cancer therapy, some common problems need to be solved [36]. First, the assembly of V-ATPase involves multiple proteins. We need to define the difference in V-ATPase assembly machinery and modulation signals under different conditions. Identifying the specific components of V-ATPase assembly and subcellular localization signals for cancer cells is essential for subsequent targeted therapy. Second, although V-ATPase inhibitors have shown effective anticancer effects in preclinical studies, their actual clinical application is still controversial. Further research needs to focus on the development of less toxic and more targeted inhibitors of subunit isoforms of V-ATPase, but not other families of transmembrane ATPases. This requires the joint efforts of multiple disciplines to study ATPase structure, drug design, and function. Third, it is necessary to deeply explore the mechanisms and immune consequences of V-ATPase subunits regulating the death of different components of the tumor microenvironment (in immune and non-immune cells) to develop effective tumor immunotherapy [186, 187].

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author on reasonable request.

REFERENCES

1. Neri D, Supuran CT. Interfering with pH regulation in tumours as a therapeutic strategy. Nat Rev Drug Discov. 2011;10:767–77.
2. Hinton A, Sennoune SR, Bond S, Fang M, Reuveni M, Sahagian GG, et al. Function of a subunit isoforms of the V-ATPase in pH homeostasis and in vitro invasion of MDA-MB231 human breast cancer cells. J Biol Chem. 2009;284:16400–08.
3. Kane PM. Disassembly and reassembly of the yeast vacuolar H(+)-ATPase in vivo. J Biol Chem. 1995;270:17025–32.
4. Eaton AF, Merkulova M, Brown D. The H(+)-ATPase (V-ATPase): from proton pump to signaling complex in health and disease. Am J Physiol Cell Physiol. 2021;320:C392–C414.
5. Webb BA, Chimenti M, Jacobson MP, Barber DL. Dysregulated pH: a perfect storm for cancer progression. Nat Rev Cancer. 2011;11:671–7.
6. Matsuyama S, Llopis J, Deveraux QL, Tsien RY, Reed JC. Changes in intramitochondrial and cytosolic pH: early events that modulate caspase activation during apoptosis. Nat Cell Biol. 2000;2:318–25.
7. Boedtkjer E, Pedersen SF. The acidic tumor microenvironment as a driver of cancer. Annu Rev Physiol. 2020;82:103–26.
8. Ohkuma S, Shimizu S, Noto M, Sai Y, Kinoshita K, Tamura H. Inhibition of cell growth by bafilomycin A1, a selective inhibitor of vacuolar H(+)-ATPase. Vitr Cell developmental Biol Anim. 1993;29a:862–6.
9. Yoshimori T, Yamamoto A, Moriyama Y, Futai M, Tashiro Y. Bafilomycin A1, a specific inhibitor of vacuolar-type H(+)-ATPase, inhibits acidification and protein degradation in lysosomes of cultured cells. J Biol Chem. 1991;266:17707–12.
10. Futai M, Sun-Wada GH, Wada Y, Matsumoto N, Nakanishi-Matsui M. Vacuolar-type ATPase: A proton pump to lysosomal trafficking. Proc Jpn Acad Ser B Phys Biol Sci. 2019;95:261–77.
11. Abbas YM, Wu D, Bueler SA, Robinson CV, Rubinstein JL. Structure of V-ATPase from the mammalian brain. Science. 2020;367:1240–6.
12. Kühlbrandt W. Structure and mechanisms of F-Type ATP synthases. Annu Rev Biochem. 2019;88:515–49.
13. Yoshida M, Muneyuki E, Hisabori T. ATP synthase-a marvellous rotary engine of the cell. Nat Rev Mol Cell Biol. 2001;2:669–77.
14. Hirata T, Nakamura N, Omote H, Wada Y, Futai M. Regulation and reversibility of vacuolar H(+)-ATPase. J Biol Chem. 2000;275:386–9.
15. Forgac M. Vacuolar ATPases: rotary proton pumps in physiology and pathophysiology. Nat Rev Mol Cell Biol. 2007;8:917–29.
16. Muench SP, Huss M, Song CF, Phillips C, Wieczorek H, Trinick J, et al. Cryo-electron microscopy of the vacuolar ATPase motor reveals its mechanical and regulatory complexity. J Mol Biol. 2009;386:989–99.
17. Dip PV, Saw WG, Roessle M, Marshansky V, Grüber G. Solution structure of subunit a, $a_{104\text{-}363}$, of the Saccharomyces cerevisiae V-ATPase and the importance of its C-terminus in structure formation. J Bioenerg Biomembranes. 2012;44:341–50.
18. Wang L, Wu D, Robinson CV, Wu H, Fu TM. Structures of a complete human V-ATPase reveal mechanisms of its assembly. Mol cell. 2020;80:501–511.e3.
19. Vasanthakumar T, Bueler SA, Wu D, Beilsten-Edmands V, Robinson CV, Rubinstein JL. Structural comparison of the vacuolar and Golgi V-ATPases from Saccharomyces cerevisiae. Proc Natl Acad Sci USA. 2019;116:7272–77.
20. Zhao J, Benlekbir S, Rubinstein JL. Electron cryomicroscopy observation of rotational states in a eukaryotic V-ATPase. Nature. 2015;521:241–5.
21. MacLeod KJ, Vasilyeva E, Baleja JD, Forgac M. Mutational analysis of the nucleotide binding sites of the yeast vacuolar proton-translocating ATPase. J Biol Chem. 1998;273:150–6.
22. Uchida E, Ohsumi Y, Anraku Y. Characterization and function of catalytic subunit alpha of H+-translocating adenosine triphosphatase from vacuolar membranes of Saccharomyces cerevisiae. A study with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole. J Biol Chem. 1988;263:45–51.
23. Liu Q, Kane PM, Newman PR, Forgac M. Site-directed mutagenesis of the yeast V-ATPase B subunit (Vma2p). J Biol Chem. 1996;271:2018–22.
24. Liu Q, Leng XH, Newman PR, Vasilyeva E, Kane PM, Forgac M. Site-directed mutagenesis of the yeast V-ATPase A subunit. J Biol Chem. 1997;272:11750–6.
25. Maher MJ, Akimoto S, Iwata M, Nagata K, Hori Y, Yoshida M, et al. Crystal structure of A3B3 complex of V-ATPase from Thermus thermophilus. EMBO J. 2009;28:3771–9.
26. Benlekbir S, Bueler SA, Rubinstein JL. Structure of the vacuolar-type ATPase from Saccharomyces cerevisiae at 11-Å resolution. Nat Struct Mol Biol. 2012;19:1356–62.
27. Hirata T, Iwamoto-Kihara A, Sun-Wada GH, Okajima T, Wada Y, Futai M. Subunit rotation of vacuolar-type proton pumping ATPase: relative rotation of the G and C subunits. J Biol Chem. 2003;278:23714–9.
28. Mazhab-Jafari MT, Rohou A, Schmidt C, Bueler SA, Benlekbir S, Robinson CV, et al. Atomic model for the membrane-embedded V(O) motor of a eukaryotic V-ATPase. Nature. 2016;539:118–22.
29. Roh SH, Stam NJ, Hryc CF, Couoh-Cardel S, Pintilie G, Chiu W, et al. The 3.5-Å CryoEM structure of nanodisc-reconstituted yeast vacuolar ATPase V(o) proton channel. Mol Cell. 2018;69:993–1004.e3.
30. Toei M, Toei S, Forgac M. Definition of membrane topology and identification of residues important for transport in subunit a of the vacuolar ATPase. J Biol Chem. 2011;286:35176–86.
31. Kawasaki-Nishi S, Nishi T, Forgac M. Arg-735 of the 100-kDa subunit a of the yeast V-ATPase is essential for proton translocation. Proc Natl Acad Sci USA. 2001;98:12397–402.
32. Zhang Z, Inoue T, Forgac M, Wilkens S. Localization of subunit C (Vma5p) in the yeast vacuolar ATPase by immuno electron microscopy. FEBS Lett. 2006;580:2006–10.
33. McGuire C, Stransky L, Cotter K, Forgac M. Regulation of V-ATPase activity. Front Biosci (Landmark Ed). 2017;22:609–22.
34. Casey JR, Grinstein S, Orlowski J. Sensors and regulators of intracellular pH. Nat Rev Mol cell Biol. 2010;11:50–61.
35. Grant BD, Donaldson JG. Pathways and mechanisms of endocytic recycling. Nat Rev Mol cell Biol. 2009;10:597–608.
36. Stransky L, Cotter K, Forgac M. The function of V-ATPases in cancer. Physiol Rev. 2016;96:1071–91.
37. Trombetta ES, Ebersold M, Garrett W, Pypaert M, Mellman I. Activation of lysosomal function during dendritic cell maturation. Science. 2003;299:1400–3.
38. Liberman R, Bond S, Shainheit MG, Stadecker MJ, Forgac M. Regulated assembly of vacuolar ATPase is increased during cluster disruption-induced maturation of dendritic cells through a phosphatidylinositol 3-kinase/mTOR-dependent pathway. J Biol Chem. 2014;289:1355–63.
39. Sautin YY, Lu M, Gaugler A, Zhang L, Gluck SL. Phosphatidylinositol 3-kinase-mediated effects of glucose on vacuolar H+-ATPase assembly, translocation, and acidification of intracellular compartments in renal epithelial cells. Mol Cell Biol. 2005;25:575–89.
40. Kohio HP, Adamson AL. Glycolytic control of vacuolar-type ATPase activity: a mechanism to regulate influenza viral infection. Virology. 2013;444:301–9.
41. Xu Y, Parmar A, Roux E, Balbis A, Dumas V, Chevalier S, et al. Epidermal growth factor-induced vacuolar (H+)-atpase assembly: a role in signaling via mTORC1 activation. J Biol Chem. 2012;287:26409–22.
42. Stransky LA, Forgac M. Amino acid availability modulates vacuolar H+-ATPase assembly. J Biol Chem. 2015;290:27360–9.

43. Einhorn Z, Trapani JG, Liu Q, Nicolson T. Rabconnectin3α promotes stable activity of the H+ pump on synaptic vesicles in hair cells. J Neurosci: Off J Soc Neurosci. 2012;32:11144–56.
44. Nikitina E, Larionova I, Choinzonov E, Kzhyshkowska J. Monocytes and macrophages as viral targets and reservoirs. Int J Mol Sci. 2018;19:2821.
45. Dou D, Revol R, Östbye H, Wang H, Daniels R. Influenza A virus cell entry, replication, virion assembly and movement. Front Immunol. 2018;9:1581.
46. Yang N, Shen HM. Targeting the endocytic pathway and autophagy process as a novel therapeutic strategy in COVID-19. Int J Biol Sci. 2020;16:1724–31.
47. Ghosh S, Dellibovi-Ragheb TA, Kerviel A, Pak E, Qiu Q, Fisher M, et al. β-coronaviruses use lysosomes for egress instead of the biosynthetic secretory pathway. Cell. 2020;183:1520–1535.e14.
48. Miao G, Zhao H, Li Y, Ji M, Chen Y, Shi Y, et al. ORF3a of the COVID-19 virus SARS-CoV-2 blocks HOPS complex-mediated assembly of the SNARE complex required for autolysosome formation. Developmental Cell. 2021;56:427–442.e5.
49. Breton S, Brown D. Regulation of luminal acidification by the V-ATPase. Physiol. 2013;28:318–29.
50. Duan X, Yang S, Zhang L, Yang T. V-ATPases and osteoclasts: ambiguous future of V-ATPases inhibitors in osteoporosis. Theranostics. 2018;8:5379–99.
51. Alzamora R, Thali RF, Gong F, Smolak C, Li H, Baty CJ, et al. PKA regulates vacuolar H+-ATPase localization and activity via direct phosphorylation of the a subunit in kidney cells. J Biol Chem. 2010;285:24676–85.
52. Pastor-Soler N, Beaulieu V, Litvin TN, Da Silva N, Chen Y, Brown D, et al. Bicarbonate-regulated adenylyl cyclase (sAC) is a sensor that regulates pH-dependent V-ATPase recycling. J Biol Chem. 2003;278:49523–9.
53. Zaidman NA, Tomilin VN, Hassanzadeh Khayyat N, Damarla M, Tidmore J, Capen DE, et al. Adhesion-GPCR Gpr116 (ADGRF5) expression inhibits renal acid secretion. Proc Natl Acad Sci USA. 2020;117:26470–81.
54. Vitavska O, Wieczorek H, Merzendorfer H. A novel role for subunit C in mediating binding of the H+-V-ATPase to the actin cytoskeleton. J Biol Chem. 2003;278:18499–505.
55. Chu A, Zirngibl RA, Manolson MF. The V-ATPase a3 subunit: structure, function and therapeutic potential of an essential biomolecule in osteoclastic bone resorption. Int J Mol Sci. 2021;22:6934.
56. Bowman EJ, Siebers A, Altendorf K. Bafilomycins: a class of inhibitors of membrane ATPases from microorganisms, animal cells, and plant cells. Proc Natl Acad Sci USA. 1988;85:7972–6.
57. Bowman BJ, McCall ME, Baertsch R, Bowman EJ. A model for the proteolipid ring and bafilomycin/concanamycin-binding site in the vacuolar ATPase of Neurospora crassa. J Biol Chem. 2006;281:31885–93.
58. Lebreton S, Jaunbergs J, Roth MG, Ferguson DA, De Brabander JK. Evaluating the potential of vacuolar ATPase inhibitors as anticancer agents and multigram synthesis of the potent salicylihalamide analog saliphenylhalamide. Bioorg medicinal Chem Lett. 2008;18:5879–83.
59. Garcia-Rodriguez J, Mendiratta S, White MA, Xie XS, De Brabander JK. Synthesis and structure-activity studies of the V-ATPase inhibitor saliphenylhalamide (SaliPhe) and simplified analogs. Bioorg medicinal Chem Lett. 2015;25:4393–8.
60. Menche D, Hassfeld J, Sasse F, Huss M, Wieczorek H. Design, synthesis, and biological evaluation of novel analogues of archazolid: a highly potent simplified V-ATPase inhibitor. Bioorg medicinal Chem Lett. 2007;17:1732–5.
61. Luong B, Schwenk R, Bräutigam J, Müller R, Menche D, Bischoff I, et al. The vacuolar-type ATPase inhibitor archazolid increases tumor cell adhesion to endothelial cells by accumulating extracellular collagen. PloS One. 2018;13:e0203053.
62. Gagliardi S, Nadler G, Consolandi E, Parini C, Morvan M, Legave MN, et al. 5-(5,6-Dichloro-2-indolyl)-2-methoxy-2,4-pentadienamides: novel and selective inhibitors of the vacuolar H+-ATPase of osteoclasts with bone antiresorptive activity. J Med Chem. 1998;41:1568–73.
63. Supino R, Petrangolini G, Pratesi G, Tortoreto M, Favini E, Bo LD, et al. Antimetastatic effect of a small-molecule vacuolar H+-ATPase inhibitor in in vitro and in vivo preclinical studies. J Pharmacol Exp Ther. 2008;324:15–22.
64. Zhang F, Shen H, Fu Y, Yu G, Cao F, Chang W, et al. Vacuolar membrane ATPase activity 21 predicts a favorable outcome and acts as a suppressor in colorectal cancer. Front Oncol. 2020;10:605801.
65. Nishie M, Suzuki E, Hattori M, Kawaguch K, Kataoka TR, Hirata M, et al. Downregulated ATP6V1B1 expression acidifies the intracellular environment of cancer cells leading to resistance to antibody-dependent cellular cytotoxicity. Cancer Immunol Immunother. 2021;70:817–30.
66. Whitton B, Okamoto H, Rose-Zerilli M, Packham G, Crabb SJ. V-ATPase inhibition decreases mutant androgen receptor activity in castrate-resistant prostate cancer. Mol Cancer Ther. 2021;20:739–48.
67. Ibrahim SA, Kulshrestha A, Katara GK, Riehl V, Sahoo M, Beaman KD. Cancer-associated V-ATPase induces delayed apoptosis of protumorigenic neutrophils. Mol Oncol. 2020;14:590–610.
68. Zhong B, Liu M, Bai C, Ruan Y, Wang Y, Qiu L, et al. Caspase-8 induces lysosome-associated cell death in cancer cells. Mol Ther. 2020;28:1078–91.
69. Couto-Vieira J, Nicolau-Neto P, Costa EP, Figueira FF, Simao TA, Okorokova-Facanha AL, et al. Multi-cancer V-ATPase molecular signatures: A distinctive balance of subunit C isoforms in esophageal carcinoma. EBioMedicine. 2020;51:102581.
70. Santos-Pereira C, Rodrigues LR, Corte-Real M. Emerging insights on the role of V-ATPase in human diseases: Therapeutic challenges and opportunities. Med Res Rev. 2021;41:1927–64.
71. Robey RW, Pluchino KM, Hall MD, Fojo AT, Bates SE, Gottesman MM. Revisiting the role of ABC transporters in multidrug-resistant cancer. Nat Rev Cancer. 2018;18:452–64.
72. Hraběta J, Belhajová M, Šubrtová H, Merlos Rodrigo MA, Heger Z, Eckschlager T. Drug sequestration in lysosomes as one of the mechanisms of chemoresistance of cancer cells and the possibilities of its inhibition. Int J Mol Sci. 2020;21:4392.
73. Ferguson PJ, Phillips JR, Selner M, Cass CE. Differential activity of vincristine and vinblastine against cultured cells. Cancer Res. 1984;44:3307–12.
74. Vukovic V, Tannock IF. Influence of low pH on cytotoxicity of paclitaxel, mitoxantrone and topotecan. Br J Cancer. 1997;75:1167–72.
75. Marquardt D, Center MS. Involvement of vacuolar H(+)-adenosine triphosphatase activity in multidrug resistance in HL60 cells. J Natl Cancer Inst. 1991;83:1098–102.
76. Patel KJ, Lee C, Tan Q, Tannock IF. Use of the proton pump inhibitor pantoprazole to modify the distribution and activity of doxorubicin: a potential strategy to improve the therapy of solid tumors. Clin Cancer Res. 2013;19:6766–76.
77. Pérez-Sayáns M, Somoza-Martín JM, Barros-Angueira F, Rey JM, García-García A. V-ATPase inhibitors and implication in cancer treatment. Cancer Treat Rev. 2009;35:707–13.
78. Wang Y, Zhang L, Wei Y, Huang W, Li L, Wu AA, et al. Pharmacological targeting of vacuolar H(+)-ATPase via subunit V1G combats multidrug-resistant cancer. Cell Chem Biol. 2020;27:1359–1370.e8.
79. You H, Jin J, Shu H, Yu B, De Milito A, Lozupone F, et al. Small interfering RNA targeting the subunit ATP6L of proton pump V-ATPase overcomes chemoresistance of breast cancer cells. Cancer Lett. 2009;280:110–9.
80. He J, Shi XY, Li ZM, Pan XH, Li ZL, Chen Y, et al. Proton pump inhibitors can reverse the YAP mediated paclitaxel resistance in epithelial ovarian cancer. BMC Mol Cell Biol. 2019;20:49.
81. Kulshrestha A, Katara GK, Ginter J, Pamarthy S, Ibrahim SA, Jaiswal MK, et al. Selective inhibition of tumor cell associated Vacuolar-ATPase 'a2' isoform overcomes cisplatin resistance in ovarian cancer cells. Mol Oncol. 2016;10:789–805.
82. Pérez-Sayáns M, Reboiras-López MD, Somoza-Martín JM, Barros-Angueira F, Diz PG, Rey JM, et al. Measurement of ATP6V1C1 expression in brush cytology samples as a diagnostic and prognostic marker in oral squamous cell carcinoma. Cancer Biol Ther. 2010;9:1057–64.
83. Salerno M, Avnet S, Bonuccelli G, Hosogi S, Granchi D, Baldini N. Impairment of lysosomal activity as a therapeutic modality targeting cancer stem cells of embryonal rhabdomyosarcoma cell line RD. PloS one. 2014;9:e110340.
84. Mei F, You J, Liu B, Zhang M, Liu J, Zhang B, et al. LASS2/TMSG1 inhibits growth and invasion of breast cancer cell in vitro through regulation of vacuolar ATPase activity. Tumour Biol. 2015;36:2831–44.
85. Gu D, Jin H, Jin G, Wang C, Wang N, Hu F, et al. The asialoglycoprotein receptor suppresses the metastasis of hepatocellular carcinoma via LASS2-mediated inhibition of V-ATPase activity. Cancer Lett. 2016;379:107–16.
86. Lozupone F, Borghi M, Marzoli F, Azzarito T, Matarrese P, Iessi E, et al. TM9SF4 is a novel V-ATPase-interacting protein that modulates tumor pH alterations associated with drug resistance and invasiveness of colon cancer cells. Oncogene. 2015;34:5163–74.
87. Sennoune SR, Bakunts K, Martínez GM, Chua-Tuan JL, Kebir Y, Attaya MN, et al. Vacuolar H+-ATPase in human breast cancer cells with distinct metastatic potential: distribution and functional activity. Am J Physiol Cell Physiol. 2004;286:C1443–52.
88. Capecci J, Forgac M. The function of vacuolar ATPase (V-ATPase) a subunit isoforms in invasiveness of MCF10a and MCF10CA1a human breast cancer cells. J Biol Chem. 2013;288:32731–41.
89. Liu P, Chen H, Han L, Zou X, Shen W. Expression and role of V1A subunit of V-ATPases in gastric cancer cells. Int J Clin Oncol. 2015;20:725–35.
90. Chung C, Mader CC, Schmitz JC, Atladottir J, Fitchev P, Cornwell ML, et al. The vacuolar-ATPase modulates matrix metalloproteinase isoforms in human pancreatic cancer. Lab Investig; a J Tech methods Pathol. 2011;91:732–43.
91. Flinck M, Hagelund S, Gorbatenko A, Severin M, Pedraz-Cuesta E, Novak I, et al. The vacuolar H(+) ATPase α3 subunit negatively regulates migration and invasion of human pancreatic ductal adenocarcinoma cells. Cells. 2020;9:465.
92. Ohta T, Numata M, Yagishita H, Futagami F, Tsukioka Y, Kitagawa H, et al. Expression of 16 kDa proteolipid of vacuolar-type H(+)-ATPase in human pancreatic cancer. Br J Cancer. 1996;73:1511–7.
93. Kulshrestha A, Katara GK, Ibrahim S, Pamarthy S, Jaiswal MK, Gilman Sachs A, et al. Vacuolar ATPase 'a2' isoform exhibits distinct cell surface accumulation

and modulates matrix metalloproteinase activity in ovarian cancer. Oncotarget. 2015;6:3797–810.
94. Ibrahim SA, Katara GK, Kulshrestha A, Jaiswal MK, Amin MA, Beaman KD. Breast cancer associated a2 isoform vacuolar ATPase immunomodulates neutrophils: potential role in tumor progression. Oncotarget. 2015;6:33033–45.
95. Son SW, Kim SH, Moon EY, Kim DH, Pyo S, Um SH. Prognostic significance and function of the vacuolar H+-ATPase subunit V1E1 in esophageal squamous cell carcinoma. Oncotarget. 2016;7:49334–48.
96. Gocheva V, Joyce JA. Cysteine cathepsins and the cutting edge of cancer invasion.Cell Cycle.2007;6:60–4.
97. Hendrix A, Sormunen R, Westbroek W, Lambein K, Denys H, Sys G, et al. Vacuolar H+ ATPase expression and activity is required for Rab27B-dependent invasive growth and metastasis of breast cancer. Int J cancer. 2013;133:843–54.
98. Kubisch R, Fröhlich T, Arnold GJ, Schreiner L, von Schwarzenberg K, Roidl A, et al. V-ATPase inhibition by archazolid leads to lysosomal dysfunction resulting in impaired cathepsin B activation in vivo. Int J Cancer. 2014;134:2478–88.
99. Holliday LS, Lu M, Lee BS, Nelson RD, Solivan S, Zhang L, et al. The amino-terminal domain of the B subunit of vacuolar H+-ATPase contains a filamentous actin binding site. J Biol Chem. 2000;275:32331–7.
100. Licon-Munoz Y, Michel V, Fordyce CA, Parra KJ. F-actin reorganization by V-ATPase inhibition in prostate cancer. Biol open. 2017;6:1734–44.
101. Galenkamp KMO, Sosicka P, Jung M, Recouvreux MV, Zhang Y, Moldenhauer MR, et al. Golgi acidification by NHE7 regulates cytosolic pH homeostasis in pancreatic cancer cells. Cancer Disco. 2020;10:822–35.
102. Michel V, Licon-Munoz Y, Trujillo K, Bisoffi M, Parra KJ. Inhibitors of vacuolar ATPase proton pumps inhibit human prostate cancer cell invasion and prostate-specific antigen expression and secretion. Int J Cancer. 2013;132:E1–10.
103. Wiedmann RM, von Schwarzenberg K, Palamidessi A, Schreiner L, Kubisch R, Liebl J, et al. The V-ATPase-inhibitor archazolid abrogates tumor metastasis via inhibition of endocytic activation of the Rho-GTPase Rac1. Cancer Res. 2012;72:5976–87.
104. Wang J, Chen D, Song W, Liu Z, Ma W, Li X, et al. ATP6L promotes metastasis of colorectal cancer by inducing epithelial-mesenchymal transition. Cancer Sci. 2020;111:477–88.
105. Xu X, Liu B, Zou P, Zhang Y, You J, Pei F. Silencing of LASS2/TMSG1 enhances invasion and metastasis capacity of prostate cancer cell. J Cell Biochem. 2014;115:731–43.
106. Zi Y, Zhao W, Zhou J, He H, Xie M. Silencing of TMSG1 enhances metastasis capacity by targeting V-ATPase in breast cancer. Int J Clin Exp Pathol. 2015;8:1312–20.
107. Fogarty FM, O'Keeffe J, Zhadanov A, Papkovsky D, Ayllon V, O'Connor R. HRG-1 enhances cancer cell invasive potential and couples glucose metabolism to cytosolic/extracellular pH gradient regulation by the vacuolar-H(+) ATPase. Oncogene. 2014;33:4653–63.
108. Lim JP, Gleeson PA. Macropinocytosis: an endocytic pathway for internalising large gulps. Immunol Cell Biol. 2011;89:836–43.
109. Hussein NA, Malla S, Pasternak MA, Terrero D, Brown NG, Ashby CR Jr., et al. The role of endolysosomal trafficking in anticancer drug resistance. Drug resistance Updat. 2021;57:100769.
110. Zhang Y, Recouvreux MV, Jung M, Galenkamp KMO, Li Y, Zagnitko O, et al. Macropinocytosis in cancer-associated fibroblasts is dependent on CaMKK2/ARHGEF2 signaling and functions to support tumor and stromal cell fitness. Cancer Disco. 2021;11:1808–25.
111. Kim SM, Nguyen TT, Ravi A, Kubiniok P, Finicle BT, Jayashankar V, et al. PTEN deficiency and AMPK activation promote nutrient scavenging and anabolism in prostate cancer cells. Cancer Disco. 2018;8:866–83.
112. Robinson MW, Overmeyer JH, Young AM, Erhardt PW, Maltese WA. Synthesis and evaluation of indole-based chalcones as inducers of methuosis, a novel type of nonapoptotic cell death. J Med Chem. 2012;55:1940–56.
113. Sønder SL, Häger SC, Heitmann ASB, Frankel LB, Dias C, Simonsen AC, et al. Restructuring of the plasma membrane upon damage by LC3-associated macropinocytosis. Sci Adv. 2021;7:eabg1969.
114. Ramirez C, Hauser AD, Vucic EA, Bar-Sagi D. Plasma membrane V-ATPase controls oncogenic RAS-induced macropinocytosis. Nature. 2019;576:477–81.
115. Klionsky DJ, Abdel-Aziz AK, Abdelfatah S, Abdellatif M, Abdoli A, Abel S, et al. Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition). Autophagy. 2021;17:1–382.
116. Li J, Chen X, Kang R, Zeh H, Klionsky DJ, Tang D. Regulation and function of autophagy in pancreatic cancer. Autophagy. 2021;17:3275–96.
117. Mathew R, Karp CM, Beaudoin B, Vuong N, Chen G, Chen HY, et al. Autophagy suppresses tumorigenesis through elimination of p62. Cell. 2009;137:1062–75.
118. Cai J, Li R, Xu X, Zhang L, Lian R, Fang L, et al. CK1α suppresses lung tumour growth by stabilizing PTEN and inducing autophagy. Nat cell Biol. 2018;20:465–78.
119. Nassour J, Radford R, Correia A, Fusté JM, Schoell B, Jauch A, et al. Autophagic cell death restricts chromosomal instability during replicative crisis. Nature. 2019;565:659–63.
120. Kang R, Xie Y, Zhang Q, Hou W, Jiang Q, Zhu S, et al. Intracellular HMGB1 as a novel tumor suppressor of pancreatic cancer. Cell Res. 2017;27:916–32.
121. Amaravadi RK, Kimmelman AC, Debnath J. Targeting autophagy in cancer: recent advances and future directions. Cancer Disco. 2019;9:1167–81.
122. Liu J, Kuang F, Kroemer G, Klionsky DJ, Kang R, Tang D. Autophagy-dependent ferroptosis: machinery and regulation. Cell Chem Biol. 2020; 27:420–35.
123. Xie Y, Kang R, Sun X, Zhong M, Huang J, Klionsky DJ, et al. Posttranslational modification of autophagy-related proteins in macroautophagy. Autophagy. 2015;11:28–45.
124. Lee JH, Yu WH, Kumar A, Lee S, Mohan PS, Peterhoff CM, et al. Lysosomal proteolysis and autophagy require presenilin 1 and are disrupted by Alzheimer-related PS1 mutations. Cell. 2010;141:1146–58.
125. Mauvezin C, Nagy P, Juhász G, Neufeld TP. Autophagosome-lysosome fusion is independent of V-ATPase-mediated acidification. Nat Commun. 2015;6:7007.
126. Yao X, Chen H, Xu B, Lu J, Gu J, Chen F, et al. The ATPase subunit of ATP6V1C1 inhibits autophagy and enhances radiotherapy resistance in esophageal squamous cell carcinoma. Gene. 2021;768:145261.
127. Wang F, Gatica D, Ying ZX, Peterson LF, Kim P, Bernard D, et al. Follicular lymphoma-associated mutations in vacuolar ATPase ATP6V1B2 activate autophagic flux and mTOR. J Clin Investig. 2019;129:1626–40.
128. Kallifatidis G, Hoepfner D, Jaeg T, Guzman EA, Wright AE. The marine natural product manzamine A targets vacuolar ATPases and inhibits autophagy in pancreatic cancer cells. Mar Drugs. 2013;11:3500–16.
129. Li J, Chen X, Kang R, Zeh H, Klionsky DJ, Tang D. Regulation and function of autophagy in pancreatic cancer. Autophagy. 2021; 17:3275–96.
130. Xu Y, Zhou P, Cheng S, Lu Q, Nowak K, Hopp AK, et al. A bacterial effector reveals the V-ATPase-ATG16L1 axis that initiates xenophagy. Cell. 2019;178:552–566.e20.
131. Li W, He P, Huang Y, Li YF, Lu J, Li M, et al. Selective autophagy of intracellular organelles: recent research advances. Theranostics. 2021;11:222–56.
132. Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P, et al. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death Differ. 2018;25:486–541.
133. Tang D, Kang R, Berghe TV, Vandenabeele P, Kroemer G. The molecular machinery of regulated cell death. Cell Res. 2019;29:347–64.
134. Carneiro BA, El-Deiry WS. Targeting apoptosis in cancer therapy. Nat Rev Clin Oncol. 2020;17:395–417.
135. Graham RM, Thompson JW, Webster KA. Inhibition of the vacuolar ATPase induces Bnip3-dependent death of cancer cells and a reduction in tumor burden and metastasis. Oncotarget. 2014;5:1162–73.
136. Lee YY, Jeon HK, Hong JE, Cho YJ, Ryu JY, Choi JJ, et al. Proton pump inhibitors enhance the effects of cytotoxic agents in chemoresistant epithelial ovarian carcinoma. Oncotarget. 2015;6:35040–50.
137. von Schwarzenberg K, Lajtos T, Simon L, Müller R, Vereb G, Vollmar AM. V-ATPase inhibition overcomes trastuzumab resistance in breast cancer. Mol Oncol. 2014;8:9–19.
138. Hong J, Wuest TR, Min Y, Lin PC. Oxygen tension regulates lysosomal activation and receptor tyrosine kinase degradation. Cancers. 2019; 11:1653.
139. Kim BK, Nam SW, Min BS, Ban HS, Paik S, Lee K, et al. Bcl-2-dependent synthetic lethal interaction of the IDF-11774 with the V0 subunit C of vacuolar ATPase (ATP6V0C) in colorectal cancer. Br J Cancer. 2018;119:1347–1357.
140. Cheung KS, Leung WK. Long-term use of proton-pump inhibitors and risk of gastric cancer: a review of the current evidence. Therapeutic Adv Gastroenterol. 2019;12:1756284819834511.
141. Kulshrestha A, Katara GK, Ibrahim SA, Riehl VE, Schneiderman S, Bilal M, et al. In vivo anti-V-ATPase antibody treatment delays ovarian tumor growth by increasing antitumor immune responses. Mol Oncol. 2020;14:2436–54.
142. Lee GH, Kim DS, Kim HT, Lee JW, Chung CH, Ahn T, et al. Enhanced lysosomal activity is involved in Bax inhibitor-1-induced regulation of the endoplasmic reticulum (ER) stress response and cell death against ER stress: involvement of vacuolar H+-ATPase (V-ATPase). J Biol Chem. 2011;286:24743–53.
143. Horova V, Hradilova N, Jelinkova I, Koc M, Svadlenka J, Brazina J, et al. Inhibition of vacuolar ATPase attenuates the TRAIL-induced activation of caspase-8 and modulates the trafficking of TRAIL receptosomes. FEBS J. 2013;280:3436–50.
144. Gilmore AP. Anoikis. Cell Death Differ. 2005;12 Suppl 2:1473–7.
145. Adeshakin FO, Adeshakin AO, Liu Z, Lu X, Cheng J, Zhang P, et al. Upregulation of V-ATPase by STAT3 activation promotes anoikis resistance and tumor metastasis. J Cancer. 2021;12:4819–29.
146. Schempp CM, von Schwarzenberg K, Schreiner L, Kubisch R, Müller R, Wagner E, et al. V-ATPase inhibition regulates anoikis resistance and metastasis of cancer cells. Mol Cancer Ther. 2014;13:926–37.
147. Song X, Zhu S, Xie Y, Liu J, Sun L, Zeng D, et al. JTC801 induces pH-dependent death specifically in cancer cells and slows growth of tumors in mice. Gastroenterology. 2018;154:1480–93.

148. Zhu S, Liu J, Kang R, Yang M, Tang D. Targeting NF-kappaB-dependent alkaliptosis for the treatment of venetoclax-resistant acute myeloid leukemia cells. Biochem Biophys Res Commun. 2021;562:55–61.
149. Liu J, Kuang F, Kang R, Tang D. Alkaliptosis: a new weapon for cancer therapy. Cancer Gene Ther. 2020;27:267–9.
150. Fang X, Dai E, Bai L, Liu J, Kang R, Zhao Y, et al. The HMGB1-AGER-STING1 pathway mediates the sterile inflammatory response to alkaliptosis. Biochem Biophys Res Commun. 2021;560:165–71.
151. Zhang X, Ding M, Zhu P, Huang H, Zhuang Q, Shen J, et al. New insights into the Nrf-2/HO-1 signaling axis and its application in pediatric respiratory diseases. Oxid Med Cell Longev. 2019;2019:3214196.
152. Kang R, Chen R, Zhang Q, Hou W, Wu S, Cao L, et al. HMGB1 in health and disease. Mol Asp Med. 2014;40:1–116.
153. Tang D, Kang R, Livesey KM, Cheh CW, Farkas A, Loughran P, et al. Endogenous HMGB1 regulates autophagy. J Cell Biol. 2010;190:881–92.
154. Liu B, Palmfeldt J, Lin L, Colaço A, Clemmensen KKB, Huang J, et al. STAT3 associates with vacuolar H(+)-ATPase and regulates cytosolic and lysosomal pH. Cell Res. 2018;28:996–1012.
155. Xie Y, Hou W, Song X, Yu Y, Huang J, Sun X, et al. Ferroptosis: process and function. Cell Death Differ. 2016;23:369–79.
156. Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, et al. Ferroptosis: A Regulated. Cell Death Nexus Link Metab, Redox Biol, Dis Cell. 2017;171:273–85.
157. Tang D, Chen X, Kang R, Kroemer G. Ferroptosis: molecular mechanisms and health implications. Cell Res. 2021;31:107–25.
158. Chen X, Kang R, Kroemer G, Tang D. Broadening horizons: the role of ferroptosis in cancer. Nat Rev Clin Oncol. 2021; 18:280–96.
159. Chen X, Li J, Kang R, Klionsky DJ, Tang D. Ferroptosis: machinery and regulation. Autophagy. 2021;17:2054–81.
160. Lin Z, Liu J, Kang R, Yang M, Tang D. Lipid metabolism in ferroptosis. Adv Biol (Weinh). 2021;5:e2100396.
161. Kang R, Zeng L, Zhu S, Xie Y, Liu J, Wen Q, et al. Lipid peroxidation drives gasdermin D-mediated pyroptosis in lethal polymicrobial sepsis. Cell Host Microbe. 2018;24:97–108 e4.
162. Friedmann Angeli JP, Schneider M, Proneth B, Tyurina YY, Tyurin VA, Hammond VJ, et al. Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. Nat Cell Biol. 2014;16:1180–91.
163. Dai E, Han L, Liu J, Xie Y, Zeh HJ, Kang R, et al. Ferroptotic damage promotes pancreatic tumorigenesis through a TMEM173/STING-dependent DNA sensor pathway. Nat Commun. 2020;11:6339.
164. Canli O, Alankus YB, Grootjans S, Vegi N, Hultner L, Hoppe PS, et al. Glutathione peroxidase 4 prevents necroptosis in mouse erythroid precursors. Blood. 2016;127:139–48.
165. Liu L, Liu B, Guan G, Kang R, Dai Y, Tang D. Cyclophosphamide-induced GPX4 degradation triggers parthanatos by activating AIFM1. Biochem Biophys Res Commun. 2022;606:68–74.
166. Chen X, Kang R, Kroemer G, Tang D. Organelle-specific regulation of ferroptosis. Cell Death Differ. 2021;28:2843–56.
167. Asano T, Komatsu M, Yamaguchi-Iwai Y, Ishikawa F, Mizushima N, Iwai K. Distinct mechanisms of ferritin delivery to lysosomes in iron-depleted and iron-replete cells. Mol Cell Biol. 2011;31:2040–52.
168. Kuang F, Liu J, Li C, Kang R, Tang D. Cathepsin B is a mediator of organelle-specific initiation of ferroptosis. Biochem Biophys Res Commun. 2020;533:1464–9.
169. Martina JA, Chen Y, Gucek M, Puertollano R. MTORC1 functions as a transcriptional regulator of autophagy by preventing nuclear transport of TFEB. Autophagy. 2012;8:903–14.
170. Dai C, Chen X, Li J, Comish P, Kang R, Tang D. Transcription factors in ferroptotic cell death. Cancer Gene Ther. 2020;27:645–56.
171. Li L, Sun S, Tan L, Wang Y, Wang L, Zhang Z, et al. Polystyrene Nanoparticles Reduced ROS and Inhibited Ferroptosis by Triggering Lysosome Stress and TFEB Nucleus Translocation in a Size-Dependent Manner. Nano Lett. 2019;19:7781–92.
172. Hou W, Xie Y, Song X, Sun X, Lotze MT, Zeh HJ,3rd. et al.Autophagy promotes ferroptosis Degrad ferritin.Autophagy.2016;12:1425–8.
173. Yang M, Chen P, Liu J, Zhu S, Kroemer G, Klionsky DJ, et al. Clockophagy is a novel selective autophagy process favoring ferroptosis. Sci Adv. 2019;5:eaaw2238.
174. Wu Z, Geng Y, Lu X, Shi Y, Wu G, Zhang M, et al. Chaperone-mediated autophagy is involved in the execution of ferroptosis. Proc Natl Acad Sci USA. 2019;116:2996–3005.
175. Liu Y, Wang Y, Liu J, Kang R, Tang D. Interplay between MTOR and GPX4 signaling modulates autophagy-dependent ferroptotic cancer cell death. Cancer Gene Ther. 2021;28:55–63.
176. Li J, Liu J, Xu Y, Wu R, Chen X, Song X, et al. Tumor heterogeneity in autophagy-dependent eerroptosis. Autophagy. 2021;17:3361–74.
177. Bai Y, Meng L, Han L, Jia Y, Zhao Y, Gao H, et al. Lipid storage and lipophagy regulates ferroptosis. Biochem Biophys Res Commun. 2019;508:997–1003.
178. Aits S, Jaattela M. Lysosomal cell death at a glance. J Cell Sci. 2013;126:1905–12.
179. Serrano-Puebla A, Boya P. Lysosomal membrane permeabilization as a cell death mechanism in cancer cells. Biochem Soc Trans. 2018;46:207–15.
180. Matsuda S, Okada N, Kodama T, Honda T, Iida T. A cytotoxic type III secretion effector of Vibrio parahaemolyticus targets vacuolar H+-ATPase subunit c and ruptures host cell lysosomes. PLoS Pathog. 2012;8:e1002803.
181. Hino H, Iriyama N, Kokuba H, Kazama H, Moriya S, Takano N, et al. Abemaciclib induces atypical cell death in cancer cells characterized by formation of cytoplasmic vacuoles derived from lysosomes. Cancer Sci. 2020;111:2132–45.
182. Huang J, Chen P, Liu K, Liu J, Zhou B, Wu R, et al. CDK1/2/5 inhibition overcomes IFNG-mediated adaptive immune resistance in pancreatic cancer. Gut. 2021;70:890–9.
183. Liu K, Huang J, Liu J, Li C, Kroemer G, Tang D, et al. HSP90 mediates IFNgamma-induced adaptive resistance to anti-PD-1 immunotherapy. Cancer Res. 2022. https://doi.org/10.1158/0008-5472.CAN-21-3917. Online ahead of print.
184. Zhou B, Liu J, Kang R, Klionsky DJ, Kroemer G, Tang D. Ferroptosis is a type of autophagy-dependent cell death. Semin Cancer Biol. 2020;66:89–100.
185. Li J, Liu J, Xu Y, Wu R, Chen X, Song X, et al. Tumor heterogeneity in autophagy-dependent ferroptosis. Autophagy. 2021;17:3361–74.
186. Legrand AJ, Konstantinou M, Goode EF, Meier P. The diversification of cell death and immunity: memento mori. Mol Cell. 2019;76:232–42.
187. Galluzzi L, Vitale I, Warren S, Adjemian S, Agostinis P, Martinez AB, et al. Consensus guidelines for the definition, detection and interpretation of immunogenic cell death. J Immunother Cancer 2020; 8:e000337.
188. Pamarthy S, Jaiswal MK, Kulshreshtha A, Katara GK, Gilman-Sachs A, Beaman KD. The vacuolar ATPase a2-subunit regulates Notch signaling in triple-negative breast cancer cells. Oncotarget. 2015;6:34206–20.
189. Kulshrestha A, Katara GK, Ibrahim SA, Riehl V, Sahoo M, Dolan J, et al. Targeting V-ATPase isoform restores cisplatin activity in resistant ovarian cancer: inhibition of autophagy, endosome function, and ERK/MEK pathway. J Oncol. 2019;2019:2343876.
190. Chung CY, Shin HR, Berdan CA, Ford B, Ward CC, Olzmann JA, et al. Covalent targeting of the vacuolar H(+)-ATPase activates autophagy via mTORC1 inhibition. Nat Chem Biol. 2019;15:776–85.
191. Formica M, Storaci AM, Bertolini I, Carminati F, Knævelsrud H, Vaira V, et al. V-ATPase controls tumor growth and autophagy in a Drosophila model of gliomagenesis. Autophagy. 2021; 17:4442–52.
192. Torigoe T, Izumi H, Ishiguchi H, Uramoto H, Murakami T, Ise T, et al. Enhanced expression of the human vacuolar H+-ATPase c subunit gene (ATP6L) in response to anticancer agents. J Biol Chem. 2002;277:36534–43.

ACKNOWLEDGEMENTS

We thank Dave Primm (Department of Surgery, University of Texas Southwestern Medical Center) for his critical reading of the manuscript.

AUTHOR CONTRIBUTIONS

FC and DT wrote the manuscript. RK and JL edited the manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Jiao Liu or Daolin Tang.

Reprints and permission information is available at http://www.nature.com/reprints