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Intracellular proton pumps as targets in chemotherapy: V-ATPases and cancer

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Abstract

Cancer cells show a metabolic shift that makes them overproduce protons; this has the potential to disturb the cellular acid-base homeostasis. However, these cells show cytoplasmic alkalinisation, increased acid extrusion and endosome-dependent drug resistance. Vacuolar type ATPases (V-ATPases), toghether with other transporters, are responsible to a great extent for these symptoms. These multisubunit proton pumps are involved in the control of cytosolic pH and the generation of proton gradients (positive inside) across endocellular membrane systems like Golgi, endosomes or lysosomes. In addition, in tumours, they have been determined to play an important role in the acidification of the intercellular medium. This importance makes them an attractive target for control of tumour cells. In the present review we portray the major characteristics of this kind of proton pumps, we provide some recent insights on their *in vivo* regulation, an overview of the consequences that V-ATPase inhibition carries for the tumour cell, such as cell cycle arrest or cell death, and a brief summary of the studies related to cancer made recently with commercially available inhibitors for this kind of proton pump. Some new approaches to affect V-ATPase function are also suggested in the light of recent knowledge on the regulation of this proton pump.

In tumor cells, NADH is recycled to its oxidised form by reducing pyruvate to lactic acid, instead of being transported to and being processed at the mitochondria. As a consequence, two H^+ per glucose molecule consumed are released into the cytosol and need to be disposed of in order to maintain cytosolic pH homeostasis. Strikingly, tumour cells not only maintain a near-neutral pH, but they often display a slightly alkaline cytosol in comparison to normal cells, probably as an acquired advantage against apoptosis induction [1]. The most obvious way to dispose of H^+ is to extrude them to the intercellular space. This has some added advantages for the tumour cell: lactate and H^+ are immunosuppresors that impair cytotoxic T lymphocyte metabolism [2]. Moreover, an acid external medium promotes invasion and cell proliferation by providing the optimal conditions for proteases like cathepsins or matrix metalloproteases that can degrade the intercellular matrix and promote angiogenesis [3] and even help the scape of highly proliferating or metastatic cells from the tumour into adjacent tissues or the blood stream [4]. All this, together with the fact that solid tumours typically display deficient perfusion, especially at the inner core, complicates cytosolic homeostasis because steep H^+ gradients need to be maintained across the plasma membrane of neoplastic cells in order to keep H^+ from flooding back into the cytoplasm. V-ATPases represent the sole primary H^+ transporters in endosomes, Golgi cisternae and lysosomes in mammalian cells. In other organisms, such as parasitic trypanosomatids –responsible for diseases like malaria, Kala azar, sleeping sickness or chagas disease- H^+-pumping pyrophosphatases comprise a second set of pumps and may be an important pharmacological target to fight these ailments [5, 6]. V-ATPases are usually absent from the cell surface, but it is naturally located at the plasma membrane of some cell types such as those at the *vas deferens* and in osteocytes [7, 8]. In tumour cells, its presence at the plasma membrane has been reported [9, 10]. How this location is

achieved by a usually intracellular protein is still obscure, but examples of other proteins of similar origin being found in the extracellular environment are known in neoplasic cells, e.g. the cathepsins [11]. It is thought that plasmalemmal V-ATPases play a significant role in proton extrusion and regulation of cytosolic pH in mammalian cells [12]. At any rate, plasma membrane V-ATPase abundance have been shown to correlate with metastatic and proliferative potential [9, 12].

ACIDIFICATION OF INTERNAL CELL COMPARTMENTS

An overproduction of acid equivalents also has implications in internal organelles since many of them maintain pH values in their lumina that need to differ substantially from that of the cytoplasm. Under physiological conditions, maintenance of these proton gradients has been found to be necessary for vacuole fusion [13], glycosylation of proteins in the Golgi apparatus [14, 15], ligand-receptor dissociation and recycling in endosomes [16], endosomal proteolysis of ligands [17], and protein transport from endosomes to lysosomes [18]. In addition, drug sequestration is also dependent on the maintenance of lysosomal acidification [19]. The endo and exocytic pathways transport cargo and vesicles between different organelles (Fig. **1**). As a gross simplification, it could be considered that the endoplasmic reticulum (ER) lies at one end of these pathways, while the lysosome and the plasma membrane would represent the other end. The ER maintains no pH difference compared with that of the cytosol. However, the closer we move towards the lysosome and the plasma membrane, H^+ gradients (acid inside) maintained in the lumina of the different organelles become steeper (Fig. **1**).

It must be noted that, although V-ATPases are the primary pumps driving the accumulation of protons inside single-membrane organelles, the final proton gradient formed is the result of the interaction of several other factors playing in favour or against the build up of such a gradient. For example, biological membranes are, to some extent, permeable to protons; also, secondary transporters consume accumulated protons to drive the translocation of substrates such as Na^+ or amino acids. Conversely, the action of channels that dissipate the electrical gradient associated to the accumulation of positive charges (e.g. Cl^- channels) allow a greater accumulation of H^+. In addition, resident proteins and solutes with (de)protonable residues provide buffering capacity to organelle lumina. For a detailed view on the determinants acting on these organelles, the reader may refer to more specialised reviews [20, 21]. Although an intervention on any of these factors should produce an alteration of luminal pH, many of these are difficult to target and the extent of the individual importance of some others is still unknown. Hence V-ATPase reveals itself as the best candidate for pharmacological intervention to affect luminal acidification.

STRUCTURE OF V-ATPASES

These H^+-pumps are complex multisubunit enzymes capable of coupling the hydrolysis of ATP or, with a much lower affinity, GTP to the translocation of H^+ across a membrane [22]. Its name derives from being first identified at the plant vacuolar membrane as a H^+-pump different to those found in mitochondria and plasma membrane (F_0F_1 and P-type ATPases, respectively). All in all, the fully assembled mammalian holoenzyme displays a molecular mass nearing 900 kDa.

The V-ATPase was early observed to share some structural homology with the F_0F_1 ATPase of mitochondria and bacteria [23] and thus, much of its domain nomenclature is influenced by knowledge on the mitochondrial pump. Indeed it is considered likely that these two types of ATPases share a common ancestor that behaved as an ATP synthase, probably akin to that found in present archaea [24]. Similarly to bacterial and mitochondrial transporters, V-ATPases are composed of two different multisubunit domains: one composed of lipophylic polypeptides and the other by hydrophylic proteins. The hydrophylic domain, named V_1 by similarity to the F_1 domain in F_0F_1 ATPases, is composed by eight different polypeptides (termed always using capital letters, A to H) (Fig. **2)**. The stoichiometry of these polypeptides is three for A, B, E and G and one for C, D, H, and F. Polypeptides A and B form an hexamer complex alternating A's with B's; single components attach to this substructure and to each other mostly on the V_0-proximal side. On their turn, polypeptides E and G extend their physical interaction further up to provide a bridge between the V1 domain and RAVE complexes involved in regulation of V_0/V_1 assembly [25]. On its turn, the V_0 domain consists of a ring composed of five c subunits and a single c'' one. In close contact with this, there are single copies of a, d and e subunits and, in the case of mammals, an accessory subunit termed Ac45. This last subunit may be specific of plasma membrane localised pumps [26].

Subunits A in the V_1 domain have ATPase hydrolytic activity and, since subunits B immobilise the V_1 domain through their interaction with the actin cytoskeleton [27, 28], the free energy released in these reactions is transmitted, with the help of subunitis Dfd, to the cc" subunit ring providing torque for the rotation of the latter [29]. Noteworthy, subunits C, E, G, H, in the V_1 domain, and subunit a in the V_0 domain remain static

through its physical interaction with the A_3B_3 hexamer. This is important because subunit a contains two hydrophylic hemichannels. The first one feeds H^+ from the external side of the membrane to the c and c'' subunits. Protons are bound by glutamic acid residues in the core of these polypeptides and kept for a full turn with the help of the hydrophobic environment of the membrane until the loaded subunit c or c'' reaches back subunit a and comes in contact with the second hemichannel. This second hemichannel permits the exit of the H^+ from the luminal side of the membrane. Typically, V-ATPase rotors turn clockwise, as seen from the normal to the cytosolic plane of the membrane, and, in a single 360^o rotation, up to two H^+ are translocated per ATP consumed [29]. However, actual H^+ stoichiometry depends on the isoform of subunit a that integrates the pump. Thus, in budding yeast, Stv1p isoform generates pumps that translocate a single H^+ per ATP consumed, while Vph1p harbouring V-ATPases conform to the theoretical 2 H^+/ATP [30]. The implications of this in V-ATPase regulation will be dealt with farther on.

REGULATION OF V-ATPASE ACTIVITY

Knowledge on regulation of V-ATPase activity has received a boost recently. The best characterised mechanism of its H^+-pumping activity regulation is by assembly/dissasembly of the holoenzyme. Under physiological conditions, domains V_0 and V_1 are devoid of H^+ transport or ATP hydrolytic activity, respectively, when not as part of the holoenzyme [31, 32]. This makes possible to use assembly of the pump as a comparatively simple mechanism to regulate dynamically the total H^+ transport capacity on the envelope of an organelle. Domain V_0 is synthesized and assembled in the ER and transported to the Golgi system via COP II coated vesicles where it will receive a V_1 domain already assembled in

the cytosol [33]. However, this holoenzyme can disassemble and reassemble V_1 and V_0 domains in response to cell energy status. In yeast, it was early shown that vacuolar V-ATPases rapidly disassembled upon glucose deprivation but that it could reassemble when the sugar was added back to the growth medium [34]. This same behaviour has been observed in renal epithelial cells [35, 36] and, probably, it plays a role in other tissues, as it is suggested by the results reported on ovine rumen [37]. The first indication of a regulatory link between carbohydrate metabolism and reversible assembly of this H^+-pump came from osteoclasts and kidney cells, where aldolase was identified as a an interacting partner with subunit E influencing the assembly of the holoenzyme [38]. Later it was determined that the enzymatic activity of aldolase was not required for its regulatory function [39]. Anyhow, this behaviour has been best studied in budding yeast and much of the following information comes from this model organism. Noteworthy, the mechanisms for assembly and disassembly are different: while disassembly requires an intact tubulin microtubular network, reassembly of the holoenzyme depends on RAVE (regulator of the H^+-ATPase of vacuolar and endosomal membranes), a protein complex that interact with subunits E, G and C and maintains them in an assembly-competent state [40]. In yeast, reversible (re)assembly of the holoenzyme has been proposed to derive from ras/cAMP/PKA pathway activation [41]. According to this model, intracellular glucose would activate the ras pathway leading to PKA activation through an increase in cAMP [41]. How PKA could be exerting its influence on assembly is still not clear; work by Voss *et al*. has shown a direct phosphorylation of subunit C by PKA that could influence V_1/V_0 assembly [42, 43]. In line with this, PKA has already been reported to phosphorylate the catalytic subunit A and regulate V-ATPase activity in human kidney cells [44], although no data on dissociation of the complex was reported in this case. Related to this, AMPK

(AMP-activated protein kinase) could be exerting an inhibitory role, opposed to activation by PKA, in kidney epidydimal cells [45, 46]; however, in this report AMPK would be affecting the H^+-pump distribution rather than assembly. Alternatively, at least in yeast, glucose metabolism, or to be more exact, a high glycolytic flux, would translate into an increased cytosolic pH that could be sensed by V-ATPases through yet obscure mechanisms and this would drive their reassembly; activation of PKA would result as a downstream effect of the reactivation of H^+ transport [47]. This is in agreement with subunit a being proposed as a pH sensor for the holoenzyme, both in yeast and in kidney epithelial cells [48-50]. Further work is needed to clarify the involvement of PKA in reassembly. It would be interesting to ascertain if this kinase shows any effect on RAVE. Nevertheless, extracellular pH has been shown to affect the ability of yeast V-ATPases to reversibly dissociate their V_1 and V_0 domains [51], although in this case cytosolic pH was shown not to be altered. In addition to this, other signalling pathways may also be contributing to the regulation of V-ATPases. In proximal tubule and kidney cells, PI3-kinase has been reported to activate V-ATPase activity and assembly [35, 36, 52], and in the former type of cells, angiotensin II could be exerting an activation of the enzyme through both PI3K and $p38^{MAPK}$ [53]. Other protein kinases, such as analogs of the yeast stress and cell-cycle related Dbf2p [54], may prove important for V-ATPase activity and assembly in mammalian cells in the future.

Regulation of gene expression is a commonly encountered mechanism for enzyme activity regulation. However, as it is easily understandable for a multisubunit complex that is composed of at least 13 different polypeptides, the concerted gene regulation of all of them is cumbersome. In agreement with this, early studies supported the notion that V-ATPases

were housekeeping proteins. Promoters corresponding to isoforms of proteins A, B and c from *Neurospora crassa* showed no recognisable TATA boxes and a high G+C content, characteristics associated to other known housekeeping genes [55]. However, as it was noted already by then, some subunit B isoforms in human macrophages and in the tobacco hornworm *Manduca sexta* showed indications in their promoters of being inducible. Advances in this field have been sparing and, for the most part, there is little detailed knowledge on inducibility of other isoforms and subunits. However, overexpression of subunits C and c have been found in the context of cancer: it has been shown that ATP6V1C1 human gene (encoding subunit C) is overexpressed in oral squamous carcinoma cells and that this may promote a greater degree of V_1V_0 assembly than in normal tissue [56]. Similarly, different subunit genes have been found to be overexpressed in drug-resistant cell lines, including ATP6L (ATP6V0C, subunit c) in the case of cisplatin resistance [57]. Remarkably, it has been reported recently that active mTORC1 induces the expression of genes encoding several V-ATPase subunits, including isoforms for subunits A, B, C, G, c and c", through TEFB transcription factor in both human cells and mice [58]. Differential expression of subunit isoforms is another way of accomplishing regulation. A thorough recollection can be found in an excellent review published recently [40]. Thus, we will be only give a few hints here. In particular, subunit a isoforms dictate to a large extent the location of the full complex. In yeast, all subunits are encoded by single genes with the exception of subunit a. Assembled complex containing the isoform Vph1p are located exclusively to the vacuole, while those that include isoform Stv1p are found predominantly at the Golgi system and endosomes [59]. In humans, there exist four different isoforms for subunit a (a1 to a4). Isoform a1 is found predominantly in synaptic vesicles and nerve plasma membrane and is thought to be an important element to facilitate

membrane fusion between these two membrane systems [40]. Isoform a2 has been found in renal intracellular organelles while isoform a4 is found predominantly in plasma membrane-localized V-ATPases in these same cell types [40]. Subunit a3 is found also predominantly at the plasma membrane of osteoclasts, although it lies in intracellular compartments in non-resorbing cell precursors [60]. To date, the identity of the subunit a isoform that drives V-ATPases to acquire a plasma membrane location in tumour cells is largely unknown. However, an indication may come from breast cancer MB231 cells. In this system, subunits a3 and a4 have been found overexpressed but only the latter isoform seems to be responsible for these cells displaying V-ATPases at the plasma membrane [61].

As mentioned earlier, the stoichiometry of proton translocation is also affected by the subunit a isoform included in the holoenzyme. In yeast, Vph1p and Stv1p isoforms provide stoichiometries close to 2 and 1 H^+/ATP, respectively, in line with the notion that smaller ratios of H^+/ATP are thought to be helpful for a correct pH homeostasis in non-vacuolar yeast compartments [62]. Analogous to this, lemon-fruit vacuolar V-ATPase displays an apparently variable stoichiometry with an initial $2H^+$/ATP ratio that lowers to 1 H^+/ATP when ΔpH increases across the vacuolar membrane, allowing it to translocate H^+ effectively even in the presence of steep pH gradients [63]. However, the molecular mechanism for these catalytic changes is still unknown. Interestingly, in this same study, it was proposed that organic acids could serve as regulators of the V-ATPase improving H^+/ATP coupling. So far, no studies have dealt with the influence of monocarboxylic acids on tumour cell V-ATPase H^+ translocation.

Other regulatory mechanisms may also contribute to fine tune V-ATPase function in a cell. For example, it has been described that reduction of disulfide bonds at subunit A are

needed for full ATPase activity [64] and that this process may well be at work *in vivo* [65]. Regulation of the number of pumps present in an organelle can also contribute to the control of acidification capacity. This has been observed for epididymal plasma membrane ATPases [66], where V-ATPases have been observed to fluctuate between plasma membrane and endosomal compartments in a concerted manner depending on lumenal pH, cAMP and PKA [67]. Vtc chaperones have also been found to influence vacuolar H^+-pumping through V-ATPases [68], but in this case a physiological role of Vtc complexes in regulating V-ATPase function as a response to environmental or physiological cues is less likely. Human securin (hPTTG1 gene) is a protein involved in the timely separation of chromatids at anaphase and in promoting proliferation in tumour cells [69]. Strikingly, it was also found to be associated to Golgi system and exocytic vesicles [70]. Very recently human securin has been proposed as a novel regulator of endosomal acidification and membrane traffic [71] apparently through a combination of regulation of V-ATPase V_0/V_1 reversible assembly and gene expression.

Being V-ATPases membrane-embedded proteins, it is easy to understand that they may be affected by the lipid composition of the membrane. However, studies dealing with lipid-protein interactions for this H^+-pump have been few and apart. In any case, a clear influence of the lipid environment on V-ATPase functions is starting to emerge. Phospholipids and fatty acids have been shown to influence V-ATPase. In particular, inclusion of phosphatidylserine in the reconstitution liposomes results in greater ATPase activity *in vitro* [72]. More significantly, rats fed an oleic acid-enriched diet showed an accumulation of this fatty acid and a concomitant dramatic increase in both ATPase activity and in V_1/V_0 assembly ratio [73]. Despite lysosomes being sterol-poor organelles and endosomes displaying intermediate contents between ER and plasma membrane [74],

early studies showed that reconstitution of V-ATPase H^+-pumping activity required the presence of cholesterol in the liposomes [75]. Related to this, the presence of abnormal sterols in the membrane have been shown to have deleterious effects for V-ATPases; for example, cellular accumulation of 14α-methylated sterol precursors inhibit fungal V-ATPase activity [76, 77] and Δ^8-unsaturated sterols alter V-ATPase stability (Hernandez, A., Lopez-Lluch, G., Serrano-Bueno, G., Perez-Catiñeira, J.R., Navas, P., Serrano, A., unpublished data). Sphingolipids are a class of lipids that are often associated to sterols in membranes and, along with them, represent the major components of lipid rafts and the related detergent resistant membranes (DRMs). Despite V_1 being the cytoplasmic domain, C26-acyl group containing sphingolipids are necessary to generate ATPase-competent V_1 domains [78]. Other studies have corroborated genetically the importance of sphingolipids for V-ATPase function [79]. In mammals, luminal acidification of melanocyte trans-Golgi system and endo/lysosomes have been suggested to depend on glycosphingolipids, since a cell line devoid of glucosylceramide synthase presents defective luminal acidification [80]. Indeed, a regulatory function for lipids *in vivo* can be envisaged: membrane-bound V-ATPase subunits were associated with DRMs isolated from late endosomes. Also, the V_1/V_0 assembly ratio varies along the endocytic pathway, the relative abundance of membrane-bound V_1 being higher on late endosomes than on early endosomes. This situation is mimicked by the lipid-raft abundance and the luminal pH in these organelles [81].

CELL BIOLOGY OF V-ATPASE INHIBITION

As it is easy to foresee, cellular events or processes that require strict or specific pH conditions, such as membrane fusion events, may be affected by a fault in V-ATPase H^+-

translocation function. Indeed, inhibition, or otherwise impairment, of H^+-translocation by V-ATPases provokes a plethora of effects in the cell that, if severe and sustained enough, can lead to cell death. A brief overview is depicted on Fig. **3**.

In the context of carbohydrate metabolism, the enzyme fructose-1,6-bisphosphatase is a key regulator of gluconeogenesis that is receiving increasing attention in diabetes studies [82] . It was observed in yeast that this enzyme is degraded upon shift to glucose fermentation metabolism and that this process is V-ATPase dependent, at least after long oxidative phosphorylation conditions, since this degradation is done at the vacuole [83]. Also in a yeast model system, it was substantiated that vacuolar V-ATPases regulated cytosolic pH in concert with the plasma membrane P-type pump [84]. In a cellular breast cancer model, silencing of the major V-ATPase subunit a (a3) was also shown to result in impaired cytosolic pH homeostasis and a severe drop in invasiveness [61]. It must be noted, however, that a concomitant alkalinisation of lumina was also observed in this case, making it difficult to ascribe unambiguously the loss of invasive potential to a single effect. Examples of the influence of defective V-ATPase-mediated acidification of lumina are more abundant in the literature. For instance, impairment of membrane traffic: it was shown that yeast cells ablated for any V-ATPase activity missorted Pma1p, the plasma membrane P-type proton pump, to the vacuole and, as a consequence, could not alkalinise their cytosol in response to glucose [85, 86]. In mammalian cells, a change in 0.4 pH units at the Golgi system provokes the mislocalization of glycosyltransferases [14, 15]. A similar situation has been observed for secreted proteins like chromogranin [87].

Another consequence of defective H^+-translocation by V-ATPases is the impairement of autophagy. This process is intimately associated to cancer, although its exact role, either preventing cell death or as a mechanism for it, may differ with cancer type and conditions

[88]. It has recently been proposed that the Warburg effect and autophagy may be connected in solid tumours, where the core of the neoplasic tissue would be feeding the respiration-competent outer tumour cell layers with L-lactate and other nutrients generated through anaerobic glycolysis and autophagic degradation of core components [89]. In a very simplified view (Fig. **4**), this process is characterised by the engulfment of cytosolic components and/or other organelles by a double membrane organelle, the phagophore. After full closure, the phagophore becomes a cargo loaded double-membrane vesicle, the phagosome, this later fuses with endosomes and finally with the lysosome and, after degradation of the inner autophagosomal membrane, releases its contents into the lysosomal lumen. The engulfed cargo is then degraded by lysosomal proteases and other hydrolases [90] . The involvement of the V-ATPase in this process was early observed [91] and, nowadays, inhibition of V-ATPase using bafilomycin A1 or concanamycin A is a standard assay to probe the involvement of autophagy in mammalian cells. The importance of V-ATPases in autophagy is not devoid of controversy, though. In agreement with the need of a low luminal pH for vacuole homotypic fusion events, mitophagy (a particular form of autophagy dealing with the degradation of whole mitochondria) was seen to be affected at the membrane fusion stages. Conversely, those same stages of *Piecemeal Microautophagy of the Nucleus* seems to be independent on V-ATPases [92]. Be that as it may, acidification of lysosomal lumen is agreed to be necessary for proteolytic degradation of autophagosomal cargo at the lysosome and, together with any effects on membrane fusion events, impairment of V-ATPase H^+-transport is agreed to block autophagic flow at its late steps [93]. At any rate, autophagy is known to be a process that precedes, and thus prevents, apoptosis in many instances [94]. Consequently, reports abound on the apoptosis-inducing effects of the inhibition of autophagy by any means (for example: [95-100]),

including the use of bafilomycin A1 (e.g. [101]). However, in this last case a point of caution must be exerted since bafilomycin A1 and other plecomacrolides have been seen to exert conflicting effects on autophagy and apoptosis induction in neurons depending on the concentration used [102]. No data are available on on other cell types or other V-ATPase inhibitors, but still the utilisation of plecomacrolides as potentiators of stress or drug-induced apoptosis through inhibition of autophagy reveals itself as one of the most attractive potential uses for V-ATPase inhibiting compounds.

Many of the chemotherapeutic drugs in present use are cationic molecules. As a consequence, they tend to get excluded from alkaline cytosols and accumulate into acidic compartments like the lysosome [103]. Not surprisingly then, cells that are able to maintain greater pH gradients in these compartments show a greater drug accumulation than normal cells [104] and cells showing a greater luminal acidification prowess are more chemoresistant than those that do not [105, 106]. Conversely, inhibition of V-ATPase activity leads to chemosensitivity [107, 108].

Other important effects are those related to iron uptake and Wnt/β-catenin signalling. Using global gene expression data Straud *et al.* found that the increased sensitivity to V-ATPase inhibitors observed in cancer cells was correlated to their greater dependence on iron uptake, presumably to compensate their ROS production [109] . Wnt/ β-catenin pathway is an important tumourigenic signal in many cancer cell types, e.g. colorectal [110], and ovarian cancers [111]. It has recently been assessed that the Wnt receptor complex component LRP6 binds Prorenin receptor as an adaptor to bind V-ATPase. Upon activation, LRP6 gets endocytosed and phosphorylated in an acidic environment to become active. Furthermore, inhibiton of V-ATPase activity using bafilomycin A1 or apicularen prevented Wnt signalling [112].

Cell cycle arrest is a common outcome of V-ATPase inhibition and often preceds cell death. S-phase arrest has been reported as a consequence of iejimalide A and B action [113], but in general, G1 arrest has been observed. Increased expression of the G1/M transition inhibitor p21 has been found in these cases [114-116]. However, the cellular mechanisms by which this occurs are not known in detail. In HT-29 colon cancer cells, p53 was stabilised following intracellular compartment alkalinization but p21 induction was partially p53 independent [114]. On the other hand, G1 arrest was observed to depend on inhibition of the degradation of hypoxia-inducible factor 1α (HIF-1α) [116]. However, the actual mechanism integrating HIF-1α degradation and V-ATPase inhibition is still unknown. A hint for this may come from the fact that HIF-1α is a transcription factor that induces p21 expression under conditions of hypoxia but that it is kept at low levels under normal aeration conditions [117].

In Hela cells, antisense experiments targeting c proteolipids induced necrosis [118]. However, cell death mechanisms related to V-ATPase inhibition in other reports is exclusively apoptosis so far [113, 119-123]. Most of these studies have been performed using plecomacrolides and, therefore, since there are no data yet for archazolids and indoles, and very few in the case of benzolactone enamides, differences may be found in future studies. In any case, apoptosis seems to follow the intrinsic pathway of through mitochondrial depolarization and liberation of cytochrome c to the cytosol in most cases[107, 119, 121] albeit, in EGFR overexpressing cancer cell lines, the extrinsic pathway of apoptosis has also been reported to play a decisive role through Fas/FasL in concanamycin B-induced cell death [124]. Anyhow, V-ATPase inhibition induced apoptosis is probably caspase-dependent in all cases [107, 119, 121].

PHARMACOLOGICAL INHIBITORS OF V-ATPASES

There is a vivid interest in the pharmacological intervention of V-ATPases, since this may prove helpful to understand a wide range of diseases, among which is cancer, but that also includes osteopetrosis and alzheimer's disease [125, 126]. As a consequence, the list of inhibitory compounds identified along the last 30 years is long and heterogeneous. We will only review here the most important types, with preference for those commercially available, and some strategies that may lead to new approaches towards V-ATPase activity regulation. Due to their particular nature, benzimidazole proton pump inhibitors deserve a detailed review and thus they will be dealt with in another review article in this issue [Ref de Milito]. A more comprehensive list and further details on these and other compounds are available in some excellent reviews [127-131].

Plecomacrolides and Derivatives

The first specific inhibitor described for V-ATPases was bafilomycin A1 [132], but concanamycin A was first reported that same year [133]. Both compounds are collectively known as plecomacrolides and consist of a large macrocyclic lactone ring comprising 16-18 carbons. Their general structures are shown in Fig. **5**. Originally isolated from several species of *Streptomyces* bacteria [129], the total synthesis of bafilomycin A1 was achieved in 1997 [134] and that of concanamycin (concanamycin F) in 2001 [135]. Although it is now well established that the binding site of both compounds lies on subunit c and that causes the inhibition of proton transport through this proteolipid [136], there were some

indications that bafilomycin A1 may also bind to subunit a [137] and these have been confirmed recently [138]. All of them are very potent inhibitors of V-ATPases with IC_{50} in the low nanomolar range. This makes them useful in research to distinguish between different types of ATPases in a cell, since P-type and F_0F_1-type ATPases are not inhibited at concentrations achieving full inhibition of V-ATPases. However, being proteolipid c one of the most conserved subunits across species and there being no isoforms in humans, the downside is that both bafilomycins and concanamycins lack specificity towards different forms of V-ATPases.

Recently, an unexpected activity of bafilomycin A1 was uncovered: using mitochondria isolated from rat liver, bafilomycin A1, in a concentration range between 50 and 250 nM, was shown to transport potassium across the inner mitochondrial membrane in a manner resembling that of valinomycin, causing swelling and depolarisation independently of any inhibition of the F_0F_1 ATPase [139]. These concentrations are above those needed for inhibition of V-ATPases *in vitro*, but may be meaningful to understand the effects observed in *in vivo* assays. For example, this might help to understand some unresolved effects of plecomacrolides, such as the induction of the expression of hypoxia-inducible factor 1α (HSF-1α) and p21 [115, 116]. Similarly, both concanamycin A and bafilomycin A1 have been shown to induce nitric oxide synthase, c-Jun N-terminal kinase and NF-κB concomitant to mitochondrial swelling and depolarisation in RAW 264.7 leukemia cells [140].

Using the information obtained in structural studies of bafilomycins, a series of simpler compounds that were still able to inhibit V-ATPases were described in the late 1990's [141, 142]. These compounds consist of an indole core, hence its name “indoles” (Fig. **5**). Similarly to plecomacrolides, these compounds also bind subunit c [143, 144]. However,

as in the case of the plecomacrolides, it was thought for some time that they may bind to subunit a, a situation that could help explaining their preferential inhibition of osteoclast V-ATPases, as opposed to other mammalian V-ATPases [141, 142, 145], but this hypothesis is now abandoned [146].

Benzolactone Enamides

Compounds sharing a benzolactone enamide core and a cytotoxic profile similar to that of plecomacrolides in NCI's 60-Cell screens [147] were identified by two independent groups in the late 1990's [148-150]. The first of this, salicylihalamide, was originally isolated from *Haliclona sp* sponges, lobatimides were from *Aplidium lobatum* tunicates, while apicularens were extracted from *Chondromyces sp* mixobacteria. This wide range of sources is probably misleading and chances are that they are all produced by symbiotic micro-organisms, most likely mixobacteria [147]. Chemical structures representative of this class of compounds are shown on Fig. **6**. Total synthesis of these compounds were successfully reported a few years later [151-153] and several compounds showing similarities have been described, such as cruentaren, the oximidines or the closely related palmerolides. An excellent review on these latter compounds was published recently [129]. Also similar to plecomacrolides, benzolactone enamides are potent inhibitors of ATP-dependent H^+-translocation showing IC_{50}s in the nanomolar range [129, 154], and bind to the V_0 domain [136, 154]. However, the binding sites of salicylihalamide and lobatimide on the V-ATPase complex are probably different to that of plecomacrolides since the former cannot compete binding of the latter type of compounds [136, 154, 155]. Interestingly, benzolactone enamides have shown selectivity towards animal V-ATPases,

as opposed to their homologous fungal proton pumps [147, 155]. Nevertheless, no information is yet available on the exact subunit benzolactone enamides bind.

Alternative Strategies

The major drawback of traditional V-ATPase inhibitors is their lack of cell-type or tissue specificity. This comes from a common mechanism of action based at binding V_0 domain subunits and, in most instances, specifically on the highly conserved c subunit [136, 146, 154]. Thus, their potential use in cancer chemotherapy is hindered. However, there are alternative approaches and molecules that may give some hope. The development of systemic delivery methods for gene silencing *in vivo* may pave the way to effective cancer treatment, and this strategy is already being tested for other targets in several types of cancer [156]. Gene silencing constructs against tumour-characteristic V-ATPase subunits, either delivered systemically or locally, could be effective at circumventing specificity issues. At any rate, silencing of single subunits in cell culture models have demonstrated its use to reduce tumour cell invasiveness in breast cancer [61]. Moreover, silencing of subunit c gene ATP6V0C inhibited dramatically the proliferation and metastatic potential of HCCLM3 xenografts in mice liver, albeit gene silencing only reduced expression to 60% of untreated controls [157].

Lipid modulation of V-ATPase activity may provide another alternative way for chemotherapeutic intervention. Cancer cells display dramatically increased rates of fatty acid synthesis [158], cholesterol uptake and synthesis [159] and dysregulated sphingolipid metabolism [160]. This makes them susceptible to pharmacological intervention and its the basis of many efforts and proposals (e.g. [161, 162]). Inhibition of any lipid synthesis is

inherently pleiotropic; however, from what we have seen above, both sphingolipids and sterols seem to be paramount for V-ATPase function [76-79] and own unpublished results]. Moreover, abnormal sterol-mediated inhibition of lumenal acidification may well lie at the basis of azole fungicide efficacy [77]. Therefore, administration of sphingolipid or cholesterol analogues may lead to effective inhibition of V-ATPases. Sphingolipid analogues have been tested in cancer models with cytotoxic results in brain, prostate and breast cancer. However, these studies did not evaluate the possibility of an induced V-ATPase dysfunction [160]. LDL receptor-mediated cholesterol uptake mechanisms are used to deliver sterol-conjugated compounds into the cell successfully [163]. In addition, tumour cells display a dramatic increase in LDL receptors and cholesterol uptake that can reach even 100-fold of that found in normal cells [164]. This could be used to increase the specificity of V-ATPase inhibition in cancer cells. Indeed several sterol analogues have been reported to inhibit V-ATPases *in vitro*, for example the well known antibiotic fusidic acid [165] and the marine sponge metabolites adociasulfates-1, -7 and -8 [166]. Nevertheless, no *in vivo* data are available yet.

CONCLUDING REMARKS

The control of cell proliferation through targeting lumenal, cytosolic and/or extracellular pH homeostasis is a strategy that is receiving increasing attention by the scientific community and may well prove helpful in the control of cancer progression if sufficient effort is put in developing appropriate small molecules. Compounds active against V-ATPases raise a great interest, as judged by the number of preclinical tests published and the fair amount of patents issued [167]. However, V-ATPase inhibitors still face a

tremendous challenge to be succesful: their high toxicity. In this sense, inhibition of V-ATPases in pancreas has been proposed to be responsible of increased risk of suffering induced glucose intolerance [168]. Therefore, studies on new derivatives are paramount, specially from those compounds that already show some kind of specificity. Alternatively, the search for new ways to inhibit V-ATPases, making use of different approaches (e.g. by targeting subunit a or by gene silencing) or properties of these proteins that have received little attention, like their sensitivity to the lipid environment, may be instrumental in regulating V-ATPases, and hence cell proliferation, in the future.

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Figure Legends

Fig. (1). Intracellular compartments and their internal characteristic pH values. Vesicle flux between single membrane organelles is shown by arrows.

Fig. (2). Subunit structure of V-ATPases. Subunits in capital letters constitute the hydrophilic V_1 domain; subunits in small letters comprise the V_0 membrane-embedded domain. Asterisks denote the two hemi-channels involved in proton transport.

Fig. (3). Cellular consequences of V-ATPase activity inhibition. Arrow heads indicate increase in response while blunt ends indicate diminution in the response.

Fig. (4). Schematic representation of the (macro)autophagic process and autophagosome maturation.

Fig. (5). Chemical structure of plecomacrolides and derivatives. A, bafilomycin A; B, concanamycin A; C, SB242784 (an indole).

Fig. (6). Chemical structure of benzolactone enamides. A, salicylihalimide A; B, lobatamide A; C, apicularen A.

References

[1] Lagadic-Gossmann, D, Huc, L, Lecureur, V. Alterations of intracellular pH homeostasis in apoptosis: origins and roles. Cell Death Differ 2004; 11(9): 953-961.

[2] Ferreira, LMR. Cancer metabolism: the Warburg effect today. Exp Mol Pathol 2010; 89(3): 372-380.

[3] Joyce, JA, Hanahan, D. Multiple roles for cysteine cathepsins in cancer. Cell Cycle 2004; 3(12): 1516-1619.

[4] Mason, SD, Joyce, JA. Proteolytic networks in cancer. Trends Cell Biol 2011; 21(4): 228-37.

[5] Docampo, R, Moreno, SNJ. The acidocalcisome as a target for chemotherapeutic agents in protozoan parasites. Curr Pharm Des 2008; 14(9): 882-888.

[6] McIntosh, MT, Drozdowicz, YM, Laroiya, K, Rea, PA, Vaidya, AB. Two classes of plant-like vacuolar-type H(+)-pyrophosphatases in malaria parasites. Mol Biochem Parasitol 2001; 114(2): 183-195.

[7] Brown, D, Breton, S. H(+)V-ATPase-dependent luminal acidification in the kidney collecting duct and the epididymis/vas deferens: vesicle recycling and transcytotic pathways. J Exp Biol 2000; 203(Pt 1): 137-145.

[8] Kaunitz, JD, Yamaguchi, DT. TNAP, TrAP, ecto-purinergic signaling, and bone remodeling. J Cell Biochem 2008; 105(3): 655-662.

[9] Nishisho, T, Hata, K, Nakanishi, M, Morita, Y, Sun-Wada, G-H, Wada, Y, Yasui, N, Yoneda, T. The a3 Isoform Vacuolar Type H^+-ATPase Promotes Distant Metastasis in the Mouse B16 Melanoma Cells. Mol Cancer Res 2011; 9(7): 845-855.

[10] Sennoune, SR, Luo, D, Martinez-Zaguilan, R. Plasmalemmal vacuolar-type H^+-ATPase in cancer biology. Cell Biochem Biophys 2004; 40(2): 185-206.

[11] Mohamed, MM, Sloane, BF. Cysteine cathepsins: multifunctional enzymes in cancer. Nat Rev Cancer 2006; 6(10): 764-775.

[12] Sennoune, SR, Martinez-Zaguilan, R. Plasmalemmal vacuolar H^+-ATPases in angiogenesis, diabetes and cancer. J Bioenerg Biomembr 2007; 39(5-6): 427-433.

[13] Ungermann, C, Wickner, W, Xu, Z. Vacuole acidification is required for trans-SNARE pairing, LMA1 release, and homotypic fusion. Proc Natl Acad Sci U S A 1999; 96(20): 11194-11199.

[14] Axelsson, MA, Karlsson, NG, Steel, DM, Ouwendijk, J, Nilsson, T, Hansson, GC. Neutralization of pH in the Golgi apparatus causes redistribution of glycosyltransferases and changes in the O-glycosylation of mucins. Glycobiology 2001; 11(8): 633-644.

[15] Rivinoja, A, Hassinen, A, Kokkonen, N, Kauppila, A, Kellokumpu, S. Elevated Golgi pH impairs terminal N-glycosylation by inducing mislocalization of Golgi glycosyltransferases. J Cell Physiol 2009; 220(1): 144-154.

[16] Contreres, JO, Faure, R, Baquiran, G, Bergeron, JJ, Posner, BI. ATP-dependent desensitization of insulin binding and tyrosine kinase activity of the insulin receptor kinase. The role of endosomal acidification. J Biol Chem 1998; 273(34): 22007-22013.

[17] Authier, F, Metioui, M, Fabrega, S, Kouach, M, Briand, G. Endosomal proteolysis of internalized insulin at the C-terminal region of the B chain by cathepsin D. J Biol Chem 2002; 277(11): 9437-9446.

[18] van Weert, AW, Dunn, KW, Gueze, HJ, Maxfield, FR, Stoorvogel, W. Transport from late endosomes to lysosomes, but not sorting of integral membrane proteins in endosomes, depends on the vacuolar proton pump. J Cell Biol 1995; 130(4): 821-834.

[19] Duvvuri, M, Konkar, S, Hong, KH, Blagg, BSJ, Krise, JP. A new approach for enhancing differential selectivity of drugs to cancer cells. ACS Chem Biol 2006; 1(5): 309-315.

[20] Paroutis, P, Touret, N, Grinstein, S. The pH of the secretory pathway: measurement, determinants, and regulation. Physiology 2004; 19: 207-215.

[21] Weisz, OA. Acidification and protein traffic. Int Rev Cytol 2003; 226: 259-319.

[22] Kakinuma, Y, Ohsumi, Y, Anraku, Y. Properties of H^+-translocating adenosine triphosphatase in vacuolar membranes of SAccharomyces cerevisiae. J Biol Chem 1981; 256(21): 10859-63.

[23] Beyenbach, KW, Wieczorek, H. The V-type H^+ ATPase: molecular structure and function, physiological roles and regulation. J Exp Biol 2006; 209(Pt 4): 577-89.

[24] Grüber, G, Marshansky, V. New insights into structure-function relationships between archeal ATP synthase (A1A0) and vacuolar type ATPase (V1V0). BioEssays 2008; 30(11-12): 1096-1109.

[25] Smardon, AM, Tarsio, M, Kane, PM. The RAVE complex is essential for stable assembly of the yeast V-ATPase. J Biol Chem 2002; 277(16): 13831-13839.

[26] Muench, SP, Trinick, J, Harrison, MA. Structural divergence of the rotary ATPases. Q Rev Biophys 2011; 44(3): 311-356.

[27] Holliday, LS, Bubb, MR, Jiang, J, Hurst, IR, Zuo, J. Interactions between vacuolar H^+-ATPases and microfilaments in osteoclasts. J Bioenerg Biomembr 2005; 37(6): 419-423.

[28] Holliday, LS, Lu, M, Lee, BS, Nelson, RD, Solivan, S, Zhang, L, Gluck, SL. The amino-terminal domain of the B subunit of vacuolar H^+-ATPase contains a filamentous actin binding site. J Biol Chem 2000; 275(41): 32331-32337.

[29] Nakanishi-Matsui, M, Sekiya, M, Nakamoto, RK, Futai, M. The mechanism of rotating proton pumping ATPases. Biochim Biophys Acta 2010; 1797(8): 1343-1352.

[30] Perzov, N, Padler-Karavani, V, Nelson, H, Nelson, N. Characterization of yeast V-ATPase mutants lacking Vph1p or Stv1p and the effect on endocytosis. J Exp Biol 2002; 205(Pt 9): 1209-1219.

[31] Puopolo, K, Forgac, M. Functional reassembly of the coated vesicle proton pump. J Biol Chem 1990; 265(25): 14836-14841.

[32] Zhang, J, Myers, M, Forgac, M. Characterization of the V0 domain of the coated vesicle (H^+)-ATPase. J Biol Chem 1992; 267(14): 9773-9778.

[33] Forgac, M. Vacuolar ATPases: rotary proton pumps in physiology and pathophysiology. Nat Rev Mol Cell Biol 2007; 8(11): 917-29.

[34] Kane, PM, Smardon, AM. Assembly and regulation of the yeast vacuolar H+-ATPase. J Bioenerg Biomembr 2003; 35(4): 313-321.

[35] Nakamura, S. Glucose activates H(+)-ATPase in kidney epithelial cells. Am J Physiol Cell Physiol 2004; 287(1): C97-105-C97-105.

[36] 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(2): 575-89.

[37] Kuzinski, J, Zitnan, R, Warnke-Gurgel, C, Schweigel, M. The vacuolar-type H-ATPase in ovine rumen epithelium is regulated by metabolic signals. J Biomed Biotechnol 2010; 2010: 525034-525034.

[38] Lu, M, Holliday, LS, Zhang, L, Dunn, WA, Jr., Gluck, SL. Interaction between aldolase and vacuolar H^+-ATPase: evidence for direct coupling of glycolysis to the ATP-hydrolyzing proton pump. J Biol Chem 2001; 276(32): 30407-30413.

[39] Lu, M, Ammar, D, Ives, H, Albrecht, F, Gluck, SL. Physical interaction between aldolase and vacuolar H^+-ATPase is essential for the assembly and activity of the proton pump. J Biol Chem 2007; 282(34): 24495-24503.

[40] Toei, M, Saum, R, Forgac, M. Regulation and isoform function of the V-ATPases. Biochemistry 2010; 49(23): 4715-4723.

[41] Bond, S, Forgac, M. The Ras/cAMP/protein kinase A pathway regulates glucose-dependent assembly of the vacuolar (H^+)-ATPase in yeast. J Biol Chem 2008; 283(52): 36513-36521.

[42] Voss, M, Fechner, L, Walz, B, Baumann, O. Calcineurin activity augments cAMP/PKA-dependent activation of V-ATPase in blowfly salivary glands. Am J Physiol Cell Physiol 2010; 298(5): C1047-1056-C1047-1056.

[43] Voss, M, Vitavska, O, Walz, B, Wieczorek, H, Baumann, O. Stimulus-induced phosphorylation of vacuolar H($^+$)-ATPase by protein kinase A. J Biol Chem 2007; 282(46): 33735-42.

[44] Alzamora, R, Thali, RF, Gong, F, Smolak, C, Li, H, Baty, CJ, Bertrand, CA, Auchli, Y, Brunisholz, RA, Neumann, D, Hallows, KR, Pastor-Soler, NM. PKA

regulates vacuolar H^+-ATPase localization and activity via direct phosphorylation of the a subunit in kidney cells. J Biol Chem 2010; 285(32): 24676-24685.

[45] Gong, F, Alzamora, R, Smolak, C, Li, H, Naveed, S, Neumann, D, Hallows, KR, Pastor-Soler, NM. Vacuolar H^+-ATPase apical accumulation in kidney intercalated cells is regulated by PKA and AMP-activated protein kinase. Am J Physiol Renal Physiol 2010.

[46] Hallows, KR, Alzamora, R, Li, H, Gong, F, Smolak, C, Neumann, D, Pastor-Soler, NM. AMP-activated protein kinase inhibits alkaline pH- and PKA-induced apical vacuolar H^+-ATPase accumulation in epididymal clear cells. Am J Physiol Cell Physiol 2009; 296(4): C672-681.

[47] Dechant, R, Binda, M, Lee, SS, Pelet, S, Winderickx, J, Peter, M. Cytosolic pH is a second messenger for glucose and regulates the PKA pathway through V-ATPase. EMBO J 2010; 29(15): 2515-2526.

[48] Brown, D, Paunescu, TG, Breton, S, Marshansky, V. Regulation of the V-ATPase in kidney epithelial cells: dual role in acid-base homeostasis and vesicle trafficking. J Exp Biol 2009; 212(Pt 11): 1762-1772.

[49] Dechant, R, Peter, M. The N-terminal domain of the V-ATPase subunit 'a' is regulated by pH in vitro and in vivo. Channels 2011; 5(1): 4-8.

[50] Marshansky, V. The V-ATPase a2-subunit as a putative endosomal pH-sensor. Biochem Soc Trans 2007; 35(Pt 5): 1092-9.

[51] Diakov, TT, Kane, PM. Regulation of vacuolar proton-translocating ATPase activity and assembly by extracellular pH. j Biol Chem 2010; 285(31): 23771-23778.

[52] Marjuki, H, Gornitzky, A, Marathe, BM, Ilyushina, NA, Aldridge, JR, Desai, G, Webby, RJ, Webster, RG. Influenza A virus-induced early activation of ERK and PI3K mediates V-ATPase-dependent intracellular pH change required for fusion. Cell Microbiol 2011; 13(4): 587-601.

[53] Carraro-Lacroix, LR, Girardi, ACC, Malnic, G. Long-term regulation of vacuolar $H(^{+})$-ATPase by angiotensin II in proximal tubule cells. Eur J Physiol 2009; 458(5): 969-979.

[54] Makrantoni, V, Dennison, P, Stark, MJR, Coote, PJ. A novel role for the yeast protein kinase Dbf2p in vacuolar H+-ATPase function and sorbic acid stress tolerance. Microbiology 2007; 153(Pt 12): 4016-4026.

[55] Merzendorfer, H, Gräf, R, Huss, M, Harvey, WR, Wieczorek, H. Regulation of proton-translocating V-ATPases. J Exp Biol 1997; 200(Pt 2): 225-235.

[56] Otero-Rey, EM, Somoza-Martin, M, Barros-Angueira, F, Garcia-Garcia, A. Intracellular pH regulation in oral squamous cell carcinoma is mediated by increased V-ATPase activity via over-expression of the ATP6V1C1 gene. Oral Oncol 2008; 44(2): 193-199.

[57] Torigoe, T, Izumi, H, Ishiguchi, H, Uramoto, H, Murakami, T, Ise, T, Yoshida, Y, Tanabe, M, Nomoto, M, Itoh, H, Kohno, K. Enhanced expression of the human vacuolar H^{+}-ATPase c subunit gene (ATP6L) in response to anticancer agents. J Biol Chem 2002; 277(39): 36534-36543.

[58] Peña-Llopis, S, Vega-Rubin-de-Celis, S, Schwartz, JC, Wolff, NC, Tran, TAT, Zou, L, Xie, X-J, Corey, DR, Brugarolas, J. Regulation of TFEB and V-ATPases by mTORC1. EMBO J 2011; 30(16): 3242-3258.

[59] Kawasaki-Nishi, S, Bowers, K, Nishi, T, Forgac, M, Stevens, TH. The amino-terminal domain of the vacuolar proton-translocating ATPase a subunit controls targeting and in vivo dissociation, and the carboxyl-terminal domain affects coupling of proton transport and ATP hydrolysis. J Biol Chem 2001; 276(50): 47411-20.

[60] Nyman, JK, Vaananen, HK. A rationale for osteoclast selectivity of inhibiting the lysosomal V-ATPase a3 isoform. Calcif Tissue Int 2010; 87(3): 273-83.

[61] Hinton, A, Sennoune, SR, Bond, S, Fang, M, Reuveni, M, Sahagian, GG, Jay, D, Martinez-Zaguilan, R, Forgac, M. 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(24): 16400-16408.

[62] Kawasaki-Nishi, S, Nishi, T, Forgac, M. Yeast V-ATPase complexes containing different isoforms of the 100-kDa a-subunit differ in coupling efficiency and in vivo dissociation. J Biol Chem 2001; 276(21): 17941-8.

[63] Muller, ML, Taiz, L. Regulation of the lemon-fruit V-ATPase by variable stoichiometry and organic acids. J Membr Biol 2002; 185(3): 209-20.

[64] Feng, Y, Forgac, M. A novel mechanism for regulation of vacuolar acidification. J Biol Chem 1992; 267(28): 19769-19772.

[65] Oluwatosin, YE, Kane, PM. Mutations in the CYS4 gene provide evidence for regulation of the yeast vacuolar H^+-ATPase by oxidation and reduction in vivo. J Biol Chem 1997; 272(44): 28149-57.

[66] Breton, S, Brown, D. New insights into the regulation of V-ATPase-dependent proton secretion. Am J Physiol Renal Physiol 2007; 292(1): F1-10.

[67] Pastor-Soler, NM, Hallows, KR, Smolak, C, Gong, F, Brown, D, Breton, S. Alkaline pH- and cAMP-induced V-ATPase membrane accumulation is mediated by protein kinase A in epididymal clear cells. Am J Physiol Cell Physiol 2008; 294(2): C488-94.

[68] Muller, O, Neumann, H, Bayer, MJ, Mayer, A. Role of the Vtc proteins in V-ATPase stability and membrane trafficking. J Cell Sci 2003; 116(Pt 6): 1107-1115.

[69] Vlotides, G, Eigler, T, Melmed, S. Pituitary tumor-transforming gene: physiology and implications for tumorigenesis. Endocr Rev 2007; 28(2): 165-86.

[70] Minematsu, T, Egashira, N, Kajiya, H, Takei, M, Takekoshi, S, Itoh, Y, Tsukamoto, H, Itoh, J, Sanno, N, Teramoto, A, Osamura, RY. PTTG is a secretory protein in human pituitary adenomas and in mouse pituitary tumor cell lines. Endocr Pathol 2007; 18(1): 8-15.

[71] Bacac, M, Fusco, C, Planche, A, Santodomingo, J, Demaurex, N, Leemann-Zakaryan, R, Provero, P, Stamenkovic, I. Securin and separase modulate membrane traffic by affecting endosomal acidification. Traffic 2011; 12(5): 615-626.

[72] Crider, BP, Xie, X-S. Characterization of the functional coupling of bovine brain vacuolar-type H($^{+}$)-translocating ATPase. Effect of divalent cations, phospholipids, and subunit H (SFD). j Biol Chem 2003; 278(45): 44281-44288.

[73] Grasso, EJ, Scalambro, MB, Calderon, RO. Differential Response of the Urothelial V-ATPase Activity to the Lipid Environment. Cell Biochem Biophys 2011; 61(1): 157-68.

[74] van Meer, G, Voelker, DR, Feigenson, GW. Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol 2008; 9(2): 112-24.

[75] Perez-Castiñeira, JR, Apps, DK. Vacuolar H(+)-ATPase of adrenal secretory granules. Rapid partial purification and reconstitution into proteoliposomes. Biochem J 1990; 271(1): 127-31.

[76] Zhang, YQ, Rao, R. Beyond ergosterol: linking pH to antifungal mechanisms. Virulence 2010; 1(6): 551-4.

[77] Zhang, YQ, Gamarra, S, Garcia-Effron, G, Park, S, Perlin, DS, Rao, R. Requirement for ergosterol in V-ATPase function underlies antifungal activity of azole drugs. PLoS Pathog 2010; 6(6): e1000939.

[78] Chung, J-H, Lester, RL, Dickson, RC. Sphingolipid requirement for generation of a functional v1 component of the vacuolar ATPase. J Biol Chem 2003; 278(31): 28872-81.

[79] Finnigan, GC, Ryan, M, Stevens, TH. A genome-wide enhancer screen implicates sphingolipid composition in vacuolar ATPase function in Saccharomyces cerevisiae. Genetics 2011; 187(3): 771-783.

[80] van der Poel, S, Wolthoorn, J, van den Heuvel, D, Egmond, M, Groux-Degroote, S, Neumann, S, Gerritsen, H, van Meer, G, Sprong, H. Hyper-acidification of trans Golgi network and endo/lysosomes in melanocytes by glucosylceramide-dependent V-ATPase activity. Traffic 2011; In Press (doi: 10.1111/j.1600-0854.2011.01263.x).

[81] Lafourcade, C, Sobo, K, Kieffer-Jaquinod, S, Garin, J, van der Goot, FG. Regulation of the V-ATPase along the endocytic pathway occurs through reversible subunit association and membrane localization. PLoS ONE 2008; 3(7): e2758.

[82] van Poelje, PD, Potter, SC, Erion, MD. In *Diabetes-Perspectives in Drug Therapy. Handb Exp Pharmacol*, Mathias Schwanstecher, ed.; Springer: Heidelberg, 2011, pp. 279-301.

[83] Liu, J, Brown, CR, Chiang, H-L. Degradation of the gluconeogenic enzyme fructose-1, 6-bisphosphatase is dependent on the vacuolar ATPase. Autophagy 2005; 1(3): 146-156.

[84] Martinez-Muñoz, GA, Kane, P. Vacuolar and plasma membrane proton pumps collaborate to achieve cytosolic pH homeostasis in yeast. J Biol Chem 2008; 283(29): 20309-20319.

[85] Perzov, N, Nelson, H, Nelson, N. Altered distribution of the yeast plasma membrane H^+-ATPase as a feature of vacuolar H+-ATPase null mutants. J Biol Chem 2000; 275(51): 40088-40095.

[86] Tarsio, M, Zheng, H, Smardon, AM, Martinez-Muñoz, GA, Kane, PM. Consequences of Loss of Vph1 Protein-containing Vacuolar ATPases (V-ATPases) for Overall Cellular pH Homeostasis. J Biol Chem 2011; 286(32): 28089-28096.

[87] Taupenot, L, Harper, KL, O'Connor, DT. Role of H^+-ATPase-mediated acidification in sorting and release of the regulated secretory protein chromogranin A: evidence for a vesiculogenic function. J Biol Chem 2005; 280(5): 3885-97.

[88] Eng, CH, Abraham, RT. The autophagy conundrum in cancer: influence of tumorigenic metabolic reprogramming. Oncogene 2011; In Press (doi:10.1038/onc.2011.220).

[89] Pavlides, S, Vera, I, Gandara, R, Sneddon, S, Pestell, R, Mercier, I, Martinez-Outschoorn, UE, Whitaker-Menezes, D, Howell, A, Sotgia, F, Lisanti, M. Warburg Meets Autophagy: Cancer Associated Fibroblasts Accelerate Tumor Growth and

Metastasis Via Oxidative Stress, Mitophagy and Aerobic Glycolysis. Antioxid Redox Signal 2011; In Press (doi:10.1089/ars.2011.4243).

[90] Eskelinen, E-L. The dual role of autophagy in cancer. Current Opinion in Pharmacology; In Press(doi:10.1016/j.coph.2011.03.009).

[91] Nakamura, N, Matsuura, A, Wada, Y, Ohsumi, Y. Acidification of vacuoles is required for autophagic degradation in the yeast, Saccharomyces cerevisiae. J Biochem 1997; 121(2): 338-44.

[92] Mijaljica, D, Prescott, M, Devenish, RJ. V-ATPase engagement in autophagic processes. Autophagy 2011; 7(6): 666-8.

[93] Mehrpour, M, Esclatine, A, Beau, I, Codogno, P. Overview of macroautophagy regulation in mammalian cells. Cell Res 2010; 20(7): 748-62.

[94] Platini, F, Perez-Tomas, R, Ambrosio, S, Tessitore, L. Understanding autophagy in cell death control. Curr Pharm Des 2010; 16(1): 101-13.

[95] Mei, S, Ni, H-M, Manley, S, Bockus, A, Kassel, KM, Luyendyk, JP, Copple, BL, Ding, W-X. Differential Roles of Unsaturated and Saturated Fatty Acids on Autophagy and Apoptosis in Hepatocytes. J Pharmacol Exp Ther 2011; In Press (doi: 10.1124/jpet.111.184341).

[96] Mohan, N, Banik, NL, Ray, SK. Combination of N-(4-hydroxyphenyl) retinamide and apigenin suppressed starvation-induced autophagy and promoted apoptosis in malignant neuroblastoma cells. Neurosci Lett 2011; 502(1): 24-29.

[97] Nakamura, O, Hitora, T, Akisue, T, Kawamoto, T, Yamagami, Y, Yamamoto, T. Inhibition of induced autophagy increases apoptosis of Nara-H cells. Intl J Oncol 2011.

[98] Ng, S, Wu, Y-T, Chen, B, Zhou, J, Shen, H-M. Impaired autophagy due to constitutive mTOR activation sensitizes TSC2-null cells to cell death under stress. Autophagy 2011; 7(10): 1173 - 1186.

[99] Noman, MZ, Janji, B, Kaminska, B, Van Moer, K, Pierson, S, Przanowski, P, Buart, S, Berchem, G, Romero, P, Mami-Chouaib, F, Chouaib, S. Blocking hypoxia-induced autophagy in tumors restores cytotoxic T cell activity and promotes regression. Cancer Res 2011; 71(18): 5976-86.

[100] Shimizu, S, Takehara, T, Hikita, H, Kodama, T, Tsunematsu, H, Miyagi, T, Hosui, A, Ishida, H, Tatsumi, T, Kanto, T, Hiramatsu, N, Fujita, N, Yoshimori, T, Hayashi, N. Inhibition of autophagy potentiates the antitumor effect of the multikinase inhibitor sorafenib in hepatocellular carcinoma. Intl J Cancer 2011; In Press (doi: 10.1002/ijc.26374).

[101] Wu, YC, Wu, WKK, Li, Y, Yu, L, Li, ZJ, Wong, CCM, Li, HT, Sung, JJY, Cho, CH. Inhibition of macroautophagy by bafilomycin A1 lowers proliferation and induces apoptosis in colon cancer cells. Biochem Biophys Res Commun 2009; 382(2): 451-456.

[102] Shacka, JJ, Klocke, BJ, Roth, KA. Autophagy, bafilomycin and cell death: the "a-B-cs" of plecomacrolide-induced neuroprotection. Autophagy 2006; 2(3): 228-230.

[103] Chen, VY, Rosania, GR. The Great Multidrug-Resistance Paradox. ACS Chem Biol 2006; 1(5): 271-273.

[104] Millot, C, Millot, J-M, Morjani, H, Desplaces, A, Manfait, M. Characterization of Acidic Vesicles in Multidrug-resistant and Sensitive Cancer Cells by Acridine Orange Staining and Confocal Microspectrofluorometry. J Histochem Cytochem 1997; 45(9): 1255-1264.

[105] Altan, N, Chen, Y, Schindler, M, Simon, SM. Defective acidification in human breast tumor cells and implications for chemotherapy. J Exp Med 1998; 187(10): 1583-1598.

[106] Murakami, T, Shibuya, I, Ise, T, Chen, ZS, Akiyama, S, Nakagawa, M, Izumi, H, Nakamura, T, Matsuo, K, Yamada, Y, Kohno, K. Elevated expression of vacuolar proton pump genes and cellular PH in cisplatin resistance. Int J Cancer 2001; 93(6): 869-874.

[107] Sasazawa, Y, Futamura, Y, Tashiro, E, Imoto, M. Vacuolar H^+-ATPase inhibitors overcome Bcl-xL-mediated chemoresistance through restoration of a caspase-independent apoptotic pathway. Cancer Sci 2009; 100(8): 1460-1467.

[108] Weylandt, KH, Nebrig, M, Jansen-Rosseck, N, Amey, JS, Carmena, D, Wiedenmann, B, Higgins, CF, Sardini, A. ClC-3 expression enhances etoposide resistance by increasing acidification of the late endocytic compartment. Mol Cancer Ther 2007; 6(3): 979-986.

[109] Straud, S, Zubovych, I, De Brabander, JK, Roth, MG. Inhibition of iron uptake is responsible for differential sensitivity to V-ATPase inhibitors in several cancer cell lines. PLoS ONE 2010; 5(7): e11629-e11629.

[110] Myant, K, Sansom, OJ. Wnt/Myc interactions in intestinal cancer: Partners in crime. Exp Cell Res 2011; In Press (doi:10.1016/j.yexcr.2011.08.001).

[111] Barbolina, MV, Burkhalter, RJ, Stack, MS. Diverse mechanisms for activation of Wnt signalling in the ovarian tumour microenvironment. Biochem J 2011; 437(1): 1-12.

[112] Cruciat, C-M, Ohkawara, B, Acebron, SP, Karaulanov, E, Reinhard, C, Ingelfinger, D, Boutros, M, Niehrs, C. Requirement of prorenin receptor and vacuolar H+-

ATPase-mediated acidification for Wnt signaling. Science 2010; 327(5964): 459-463.

[113] McHenry, P, Wang, W-LW, Devitt, E, Kluesner, N, Davisson, VJ, McKee, E, Schweitzer, D, Helquist, P, Tenniswood, M. Iejimalides A and B inhibit lysosomal vacuolar H^+-ATPase (V-ATPase) activity and induce S-phase arrest and apoptosis in MCF-7 cells. J Cell Biochem 2010; 109(4): 634-642.

[114] Castillo-Avila, W, Abal, M, Robine, S, Pérez-Tomás, R. Non-apoptotic concentrations of prodigiosin (H^+/Cl^- symporter) inhibit the acidification of lysosomes and induce cell cycle blockage in colon cancer cells. Life Sci 2005; 78(2): 121-7.

[115] Kawada, M, Usami, I, Ohba, S, Someno, T, Kim, J, Hayakawa, Y, Nose, K, Ishizuka, M. Hygrolidin induces p21 expression and abrogates cell cycle progression at G1 and S phases. Biochem Biophys Res Commun 2002; 298(1): 178-183.

[116] Lim, J-H, Park, J-W, Kim, M-S, Park, S-K, Johnson, RS, Chun, Y-S. Bafilomycin induces the p21-mediated growth inhibition of cancer cells under hypoxic conditions by expressing hypoxia-inducible factor-1alpha. Mol Pharmacol 2006; 70(6): 1856-1865.

[117] Koshiji, M, Huang, LE. Dynamic balancing of the dual nature of HIF-1alpha for cell survival. Cell Cycle 2004; 3(7): 853-854.

[118] Zhan, H, Yokoyama, K, Otani, H, Tanigaki, K, Shirota, N, Takano, S, Ohkuma, S. Different roles of proteolipids and 70-kDa subunits of V-ATPase in growth and death of cultured human cells. Genes Cells 2003; 8(6): 501-513.

[119] Aiko, K, Tsujisawa, T, Koseki, T, Hashimoto, S, Morimoto, Y, Amagasa, T, Nishihara, T. Involvement of cytochrome c and caspases in apoptotic cell death of human submandibular gland ductal cells induced by concanamycin A. Cell Signal 2002; 14(8): 717-22.

[120] Chevallier, C, Laprévote, O, Bignon, J, Debitus, C, Guénard, D, Sévenet, T. Isolation of cytotoxic chondropsins, macrolide lactams from the New-Caledonian marine sponge Psammoclemma sp. and electrospray ion trap multiple stage MS study of these macrolides. Nat Prod Res 2004; 18(5): 479-484.

[121] Hong, J, Yamaki, K, Ishihara, K, Ahn, JW, Zee, O, Ohuchi, K. Induction of apoptosis of RAW 264.7 cells by the cytostatic macrolide apicularen A. J Pharm Pharmacol 2003; 55(9): 1299-1306.

[122] Morimura, T, Fujita, K, Akita, M, Nagashima, M, Satomi, A. The proton pump inhibitor inhibits cell growth and induces apoptosis in human hepatoblastoma. Pediatr Surg Int 2008; 24(10): 1087-1094.

[123] Xu, J, Feng, HT, Wang, C, Yip, KHM, Pavlos, N, Papadimitriou, JM, Wood, D, Zheng, MH. Effects of Bafilomycin A1: an inhibitor of vacuolar H ($^{+}$)-ATPases on endocytosis and apoptosis in RAW cells and RAW cell-derived osteoclasts. J Cell Biochem 2003; 88(6): 1256-64.

[124] Yoshimoto, Y, Imoto, M. Induction of EGF-dependent apoptosis by vacuolar-type H(+)-ATPase inhibitors in A431 cells overexpressing the EGF receptor. Experimental Cell Research 2002; 279(1): 118-27.

[125] Hinton, A, Bond, S, Forgac, M. V-ATPase functions in normal and disease processes. Eur J Physiol 2009; 457(3): 589-598.

[126] Williamson, WR, Hiesinger, PR. On the role of V-ATPase V0a1-dependent degradation in Alzheimer disease. Commun Integr Biol 2010; 3(6): 604-607.

[127] Beutler, JA, McKee, TC. Novel marine and microbial natural product inhibitors of vacuolar ATPase. Curr Med Chem 2003; 10(9): 787-96.

[128] Fais, S, De Milito, A, You, H, Qin, W. Targeting vacuolar H^+-ATPases as a new strategy against cancer. Cancer Res 2007; 67(22): 10627-30.

[129] Huss, M, Wieczorek, H. Inhibitors of V-ATPases: old and new players. J Exp Biol 2009; 212(3): 341-346.

[130] Perez-Sayans, M, Somoza-Martin, JM, Barros-Angueira, F, Rey, JMG, Garcia-Garcia, A. V-ATPase inhibitors and implication in cancer treatment. Cancer Treat Rev 2009; 35(8): 707-713.

[131] Yet, L. Chemistry and biology of salicylihalamide a and related compounds. Chem Rev 2003; 103(11): 4283-4306.

[132] Werner, G, Hagenmaier, H, Drautz, H, Baumgartner, A, Zahner, H. Metabolic products of microorganisms. 224. Bafilomycins, a new group of macrolide antibiotics. Production, isolation, chemical structure and biological activity. J Antibiot (Tokyo) 1984; 37(2): 110-7.

[133] Kinashi, H, Someno, K, Sakaguchi, K. Isolation and characterization of concanamycins A, B and C. J Antibiot (Tokyo) 1984; 37(11): 1333-43.

[134] Toshima, K, Jyojima, T, Yamaguchi, H, Noguchi, Y, Yoshida, T, Murase, H, Nakata, M, Matsumura, S. Total Synthesis of Bafilomycin A(1). J Org Chem 1997; 62(10): 3271-3284.

[135] Toshima, K, Jyojima, T, Miyamoto, N, Katohno, M, Nakata, M, Matsumura, S. The first total synthesis of concanamycin f (concanolide a). J Org Chem 2001; 66(5): 1708-1715.

[136] Huss, M, Ingenhorst, G, Konig, S, Gassel, M, Drose, S, Zeeck, A, Altendorf, K, Wieczorek, H. Concanamycin A, the Specific Inhibitor of V-ATPases, Binds to the Vo Subunit c. J Biol Chem 2002; 277(43): 40544-40548.

[137] Zhang, J, Feng, Y, Forgac, M. Proton conduction and bafilomycin binding by the V0 domain of the coated vesicle V-ATPase. J Biol Chem 1994; 269(38): 23518-23523.

[138] Wang, Y, Inoue, T, Forgac, M. Subunit a of the yeast V-ATPase participates in binding of bafilomycin. J Biol Chem 2005; 280(49): 40481-8.

[139] Teplova, VV, Tonshin, AA, Grigoriev, PA, Saris, N-EL, Salkinoja-Salonen, MS. Bafilomycin A1 is a potassium ionophore that impairs mitochondrial functions. J Bioenerg Biomembr 2007; 39(4): 321-9.

[140] Hong, J, Nakano, Y, Yokomakura, A, Ishihara, K, Kim, S, Kang, Y-S, Ohuchi, K. Nitric oxide production by the vacuolar-type (H+)-ATPase inhibitors bafilomycin A1 and concanamycin A and its possible role in apoptosis in RAW 264.7 cells. J Pharmacol Exp Ther 2006; 319(2): 672-81.

[141] Gagliardi, S, Nadler, G, Consolandi, E, Parini, C, Morvan, M, Legave, MN, Belfiore, P, Zocchetti, A, Clarke, GD, James, I, Nambi, P, Gowen, M, Farina, C. 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(10): 1568-73.

[142] Nadler, G, Morvan, M, Delimoge, I, Belfiore, P, Zocchetti, A, James, I, Zembryki, D, Lee-Rycakzewski, E, Parini, C, Consolandi, E, Gagliardi, S, Farina, C. (2Z,4E)-5-(5,6-dichloro-2-indolyl)-2-methoxy-N-(1,2,2,6,6- pentamethylpiperidin-4-yl)-2,4-pentadienamide, a novel, potent and selective inhibitor of the osteoclast V-ATPase. Bioorg Med Chem Lett 1998; 8(24): 3621-6.

[143] Pali, T, Whyteside, G, Dixon, N, Kee, TP, Ball, S, Harrison, MA, Findlay, JB, Finbow, ME, Marsh, D. Interaction of inhibitors of the vacuolar H(+)-ATPase with the transmembrane V0-sector. Biochemistry 2004; 43(38): 12297-305.

[144] Whyteside, G, Meek, PJ, Ball, SK, Dixon, N, Finbow, ME, Kee, TP, Findlay, JB, Harrison, MA. Concanamycin and indolyl pentadieneamide inhibitors of the vacuolar H^+-ATPase bind with high affinity to the purified proteolipid subunit of the membrane domain. Biochemistry 2005; 44(45): 15024-31.

[145] Farina, C, Gagliardi, S. Selective inhibition of osteoclast vacuolar H(+)-ATPase. Curr Pharm Des 2002; 8(23): 2033-48.

[146] Hesselink, RW, Fedorov, A, Hemminga, MA, Prieto, M. Membrane-bound peptides from V-ATPase subunit a do not interact with an indole-type inhibitor. J Pept Sci 2008; 14(4): 383-388.

[147] Boyd, MR, Farina, C, Belfiore, P, Gagliardi, S, Kim, JW, Hayakawa, Y, Beutler, JA, McKee, TC, Bowman, BJ, Bowman, EJ. Discovery of a novel antitumor benzolactone enamide class that selectively inhibits mammalian vacuolar-type (H^+)-ATPases. J Pharmacol Exp Ther 2001; 297(1): 114-20.

[148] Erickson, K, Beutler, J, Cardellina Ii, J, Boyd, M. Salicylihalamides A and B, Novel Cytotoxic Macrolides from the Marine Sponge Haliclona sp. J Org Chem 1997; 62(23): 8188-8192.

[149] Galinis, DL, McKee, TC, Pannell, LK, Cardellina, JH, Boyd, MR. Lobatamides A and B, Novel Cytotoxic Macrolides from the Tunicate *Aplidium lobatum*. J Org Chem 1997; 62(26): 8968-8969.

[150] Kunze, B, Jansen, R, Sasse, F, Höfle, G, Reichenbach, H. Apicularens A and B, new cytostatic macrolides from Chondromyces species (myxobacteria): production, physico-chemical and biological properties. J Antibiot (Tokyo) 1998; 51(12): 1075-1080.

[151] Lewis, A, Stefanuti, I, Swain, SA, Smith, SA, Taylor, RJ. A formal total synthesis of (+)-apicularen A: base-induced conversion of apicularen-derived intermediates into salicylihalamide-like products. Org Biomol Chem 2003; 1(1): 104-116.

[152] Shen, R, Lin, CT, Porco, JA. Total synthesis and stereochemical assignment of the salicylate antitumor macrolide lobatamide C(1). J Am Chem Soc 2002; 124(20): 5650-5651.

[153] Wu, Y, Liao, X, Wang, R, Xie, X-S, De Brabander, JK. Total synthesis and initial structure-function analysis of the potent V-ATPase inhibitors salicylihalamide A and related compounds. Journal of the American Chemical Society 2002; 124(13): 3245-53.

[154] Xie, X-S, Padron, D, Liao, X, Wang, J, Roth, MG, De Brabander, JK. Salicylihalamide A inhibits the V0 sector of the V-ATPase through a mechanism distinct from bafilomycin A1. J Biol Chem 2004; 279(19): 19755-63.

[155] Huss, M, Sasse, F, Kunze, B, Jansen, R, Steinmetz, H, Ingenhorst, G, Zeeck, A, Wieczorek, H. Archazolid and apicularen: novel specific V-ATPase inhibitors. BMC Biochem 2005; 6: 13-13.

[156] Oh, YK, Park, TG. siRNA delivery systems for cancer treatment. Adv Drug Deliv Rev 2009; 61(10): 850-62.

[157] You, H, Jin, J, Shu, H, Yu, B, De Milito, A, Lozupone, F, Deng, Y, Tang, N, Yao, G, Fais, S, Gu, J, Qin, W. Small interfering RNA targeting the subunit ATP6L of proton pump V-ATPase overcomes chemoresistance of breast cancer cells. Cancer Lett 2009; 280(1): 110-119.

[158] Young, CD, Anderson, SM. Sugar and fat - that's where it's at: metabolic changes in tumors. Breast Cancer Res 2008; 10(1): 202.

[159] Tosi, MR, Tugnoli, V. Cholesteryl esters in malignancy. Clin Chim Acta 2005; 359(1-2): 27-45.

[160] Ryland, LK, Fox, TE, Liu, X, Loughran, TP, Kester, M. Dysregulation of sphingolipid metabolism in cancer. Cancer Biol Ther 2011; 11(2): 138-49.

[161] Buchwald, H. Cholesterol inhibition, cancer, and chemotherapy. Lancet 1992; 339(8802): 1154-1156.

[162] Canals, D, Perry, DM, Jenkins, RW, Hannun, YA. Drug targeting of sphingolipid metabolism: sphingomyelinases and ceramidases. Br J Pharmacol 2011; 163(4): 694-712.

[163] Schroeder, A, Levins, CG, Cortez, C, Langer, R, Anderson, DG. Lipid-based nanotherapeutics for siRNA delivery. J Intern Med 2010; 267(1): 9-21.

[164] Ho, YK, Smith, RG, Brown, MS, Goldstein, JL. Low-density lipoprotein (LDL) receptor activity in human acute myelogenous leukemia cells. Blood 1978; 52(6): 1099-114.

[165] Moriyama, Y, Nelson, N. Inhibition of vacuolar H+-ATPases by fusidic acid and suramin. FEBS Lett 1988; 234(2): 383-6.

[166] Kalaitzis, JA, de Almeida Leone, P, Harris, L, Butler, MS, Ngo, A, Hooper, JN, Quinn, RJ. Adociasulfates 1, 7, and 8: New Bioactive Hexaprenoid Hydroquinones from the Marine Sponge Adocia sp. J Org Chem 1999; 64(15): 5571-5574.

[167] Hernandez, A, Serrano, G, Herrera-Palau, R, Perez-Castiñeira, JR, Serrano, A. Intraorganellar acidification by V-ATPases: a target in cell proliferation and cancer therapy. Recent Pat Anticancer Drug Discov 2010; 5(2): 88-98.

[168] Hettiarachchi, KD, Zimmet, PZ, Myers, MA. The effects of repeated exposure to sub-toxic doses of plecomacrolide antibiotics on the endocrine pancreas. Food Chem Tox 2006; 44(12): 1966-1977.

Figure 1

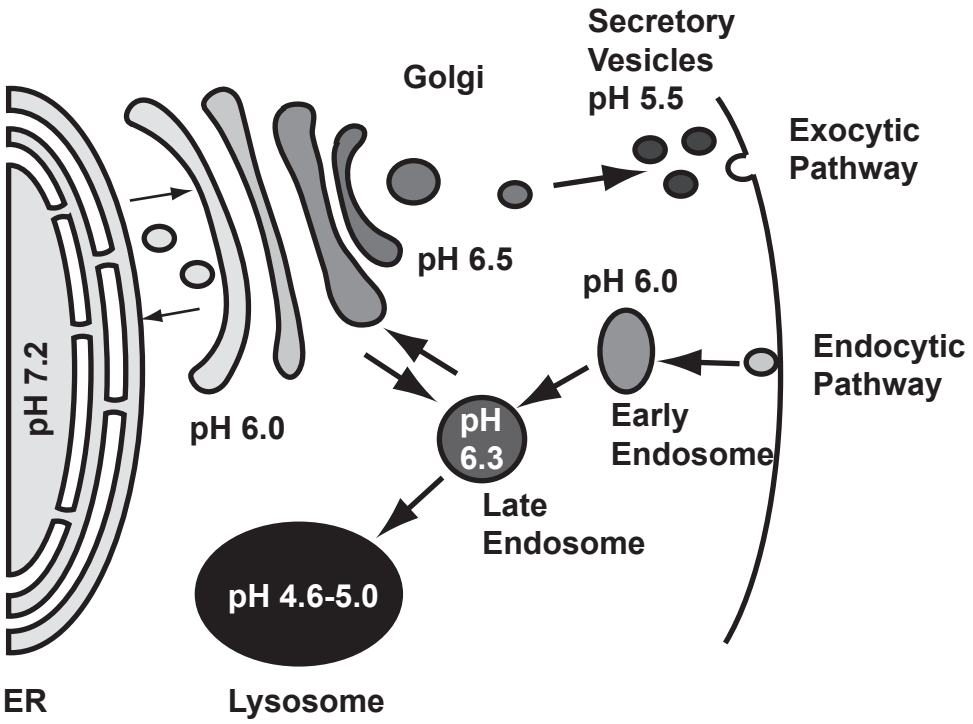

Secretory
Vesicles
pH 5.5
Golgi
Exocytic
Pathway
pH 6.5
pH 6.0
Endocytic
Pathway
pH 7.2
pH 6.0
pH
6.3
Early
Endosome
Late
Endosome
pH 4.6-5.0
ER
Lysosome

Figure 2

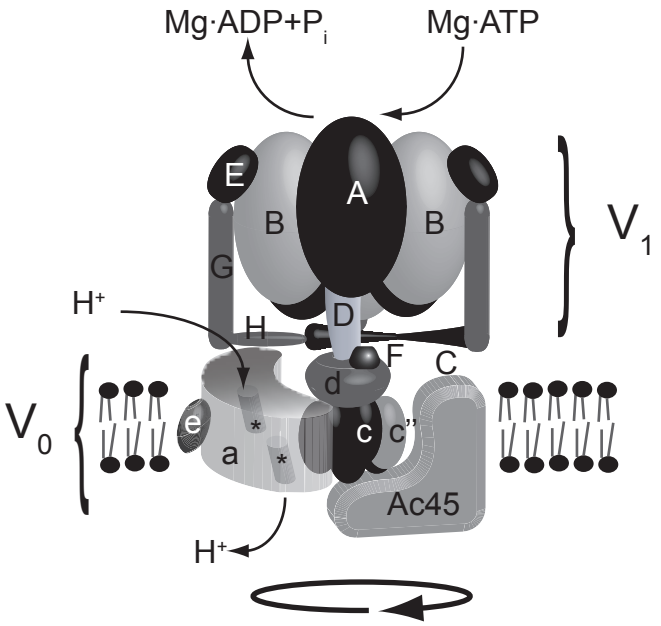


Figure 3

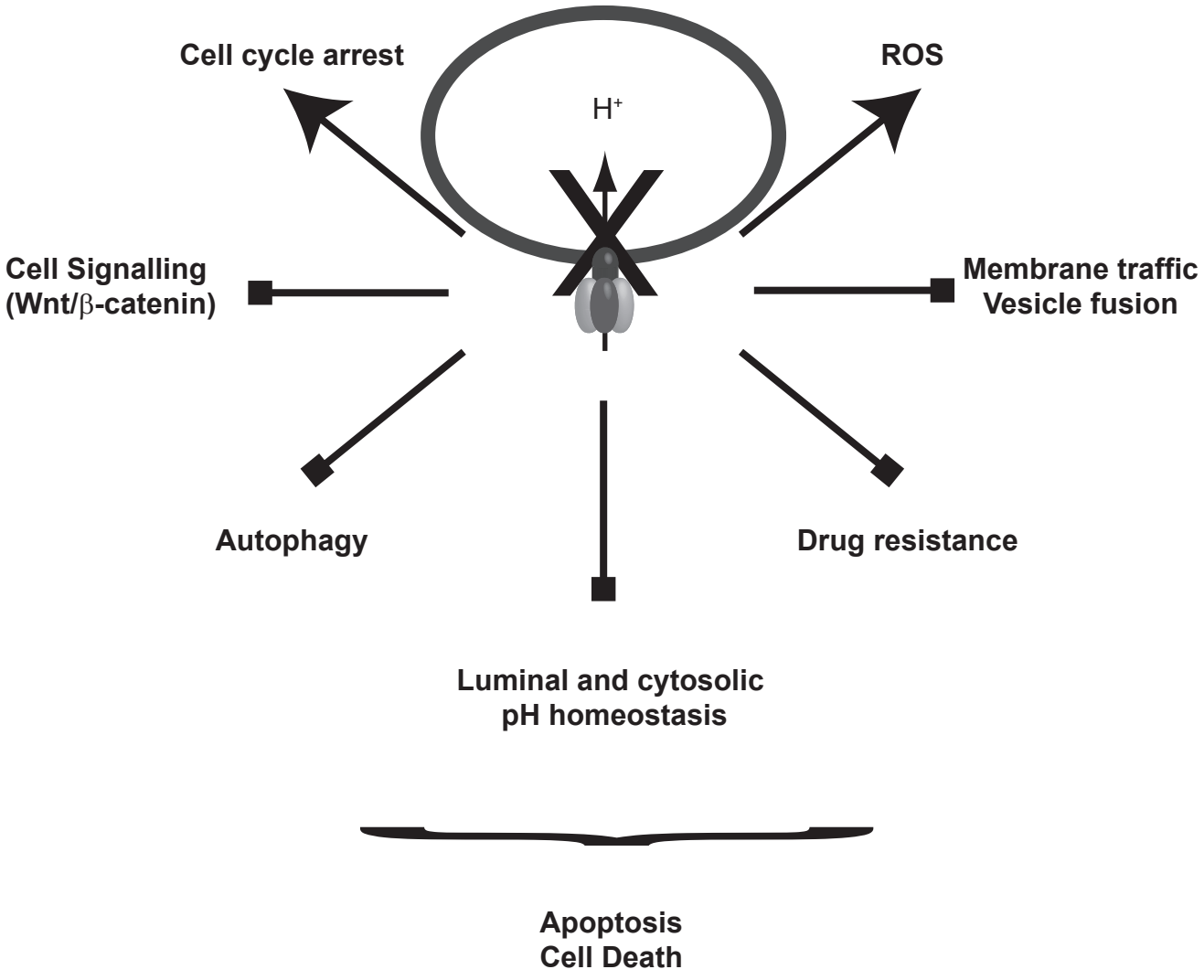

Cell cycle arrest
ROS
H+
Cell Signalling
(Wnt/β-catenin)
Membrane traffic
Vesicle fusion
Autophagy
Drug resistance
Luminal and cytosolic
pH homeostasis
Apoptosis
Cell Death

Figure 4

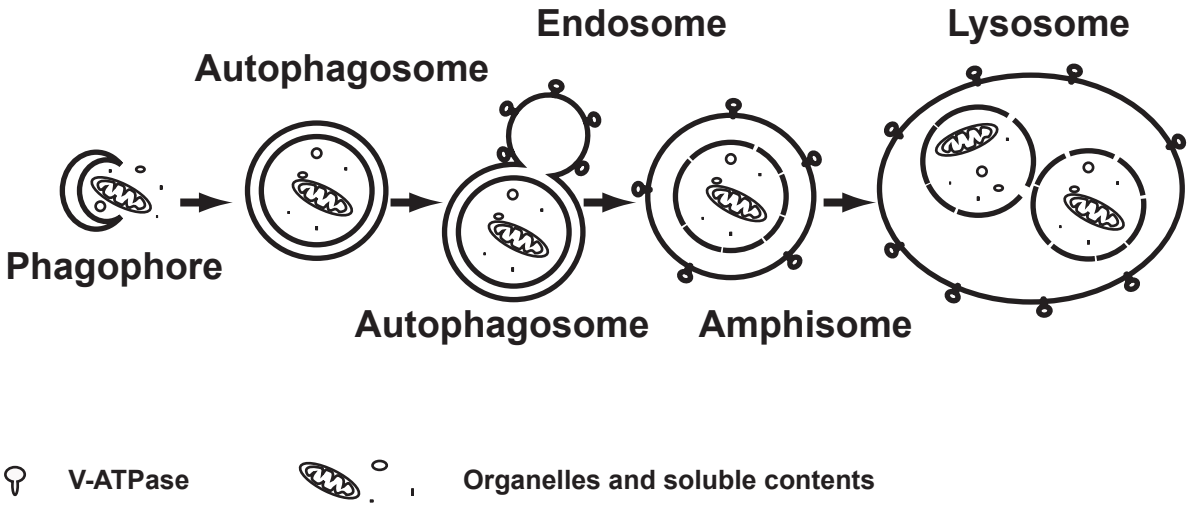
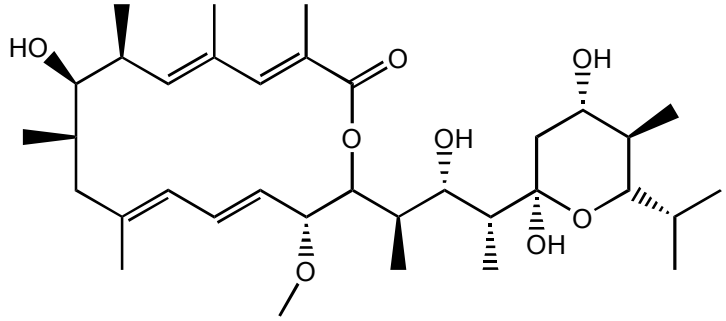

Endosome
Lysosome
Autophagosome
Phagophore
Autophagosome
Amphisome
V-ATPase
Organelles and soluble contents

Figure 5

A



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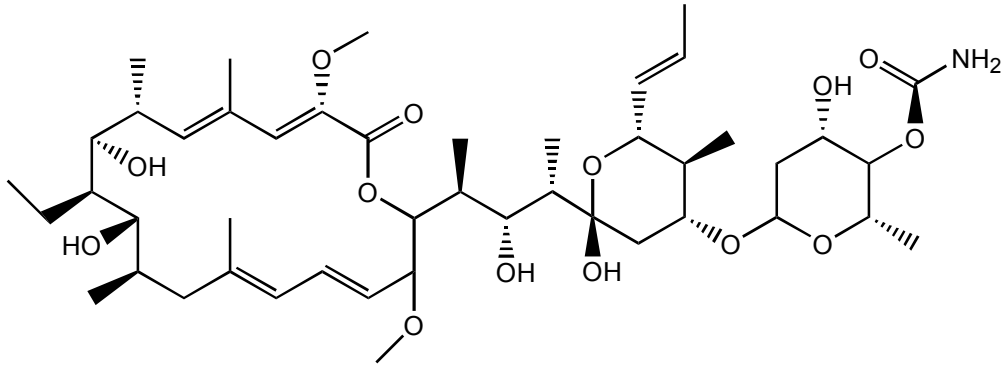

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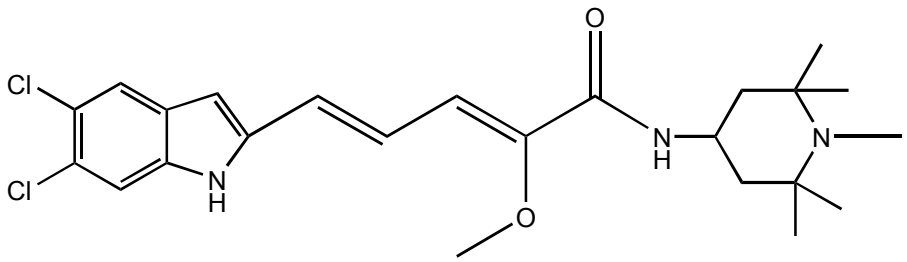
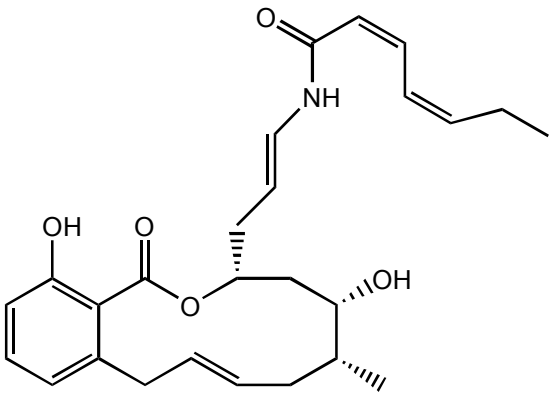


Figure 6

A



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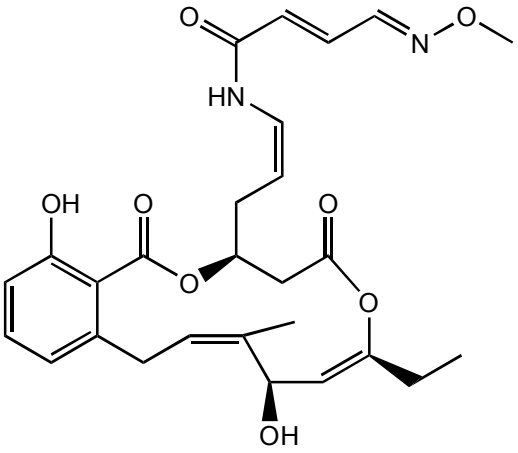

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