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**Minireview paper**

# Vacuolar $H^+$-ATPase: functional mechanisms and potential as a target for cancer chemotherapy

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**Tumor cells *in vivo* often exist in a hypoxic microenvironment with a lower extracellular pH than that surrounding normal cells. Ability to upregulate proton extrusion may be important for tumor cell survival. Such microenvironmental factors may be involved in the development of resistant subpopulations of tumor cells. In solid tumors, both intracellular and extracellular pH differ between drug-sensitive and -resistant cells, and pH appears critical to the therapeutic effectiveness of anticancer agents. Four major types of pH regulators have been identified in tumor cells: the sodium–proton antiporter, the bicarbonate transporter, the proton–lactate symporter and proton pumps. Understanding mechanisms regulating tumor acidity opens up novel opportunities for cancer chemotherapy. In this minireview, we describe the structure and function of certain proton pumps overexpressed in many tumors—vacuolar $H^+$-ATPases—and consider their potential as targets for cancer chemotherapy. [© 2002 Lippincott Williams & Wilkins.]**

*Key words:* Cancer, chemosensitivity, pH regulation, vacuolar $H^+$-ATPase.

## Introduction

Maintenance of intracellular pH ($pH_i$) is crucial to normal cell function, as many cellular processes have a narrow pH optimum. The vacuolar $H^+$-ATPases (V-ATPases) are universally expressed in eukaryotic cells,[1–5] being situated not only in the membranes of many organelles but also in the plasma membrane.[6–8] The V-ATPases maintain cytoplasmic pH and also play an important role in acidification of intracellular compartments, such as clathrin-coated vesicles, endosomes, lysosomes and Golgi-derived vesicles.[9,10] These enzymes also participate in receptor-mediated endocytosis, renal acidification and bone resorption. The V-ATPases are multisubunit complexes composed of a membrane sector ($V_0$) and a cytosolic catalytic sector ($V_1$).[11] The integral $V_0$ domain functions in proton translocation, while the peripheral $V_1$ hydrolyzes ATP.

The influence of $pH_i$ has been studied with respect to cell growth,[12] cell motility,[13] tumorigenesis,[14] metastasis,[15] apoptosis[16] and drug resistance in cancer cells.[17,18] Recently, acidic pH has been shown to activate transcription factors and increase their target gene expression.[19] V-ATPases are overexpressed in multidrug-resistant cancer cells. Further, we have demonstrated that several genes encoding subunits of V-ATPase are upregulated in cisplatin-resistant cell lines, that $pH_i$ in cisplatin-resistant cells is much higher than in cisplatin-sensitive parental cells and that a V-ATPase inhibitor can synergistically potentiate the cytotoxicity of cisplatin.[20] These results indicate that $pH_i$ is a critical variable for effectiveness of cisplatin. The V-ATPases, then, represent a potential target for cancer chemotherapy.

## Structure and function of V-ATPases

The V-ATPases are 830-kDa multisubunit complexes composed of two functional domains: a membrane-extrinsic $V_1$ domain, and a membrane-embedded $V_0$

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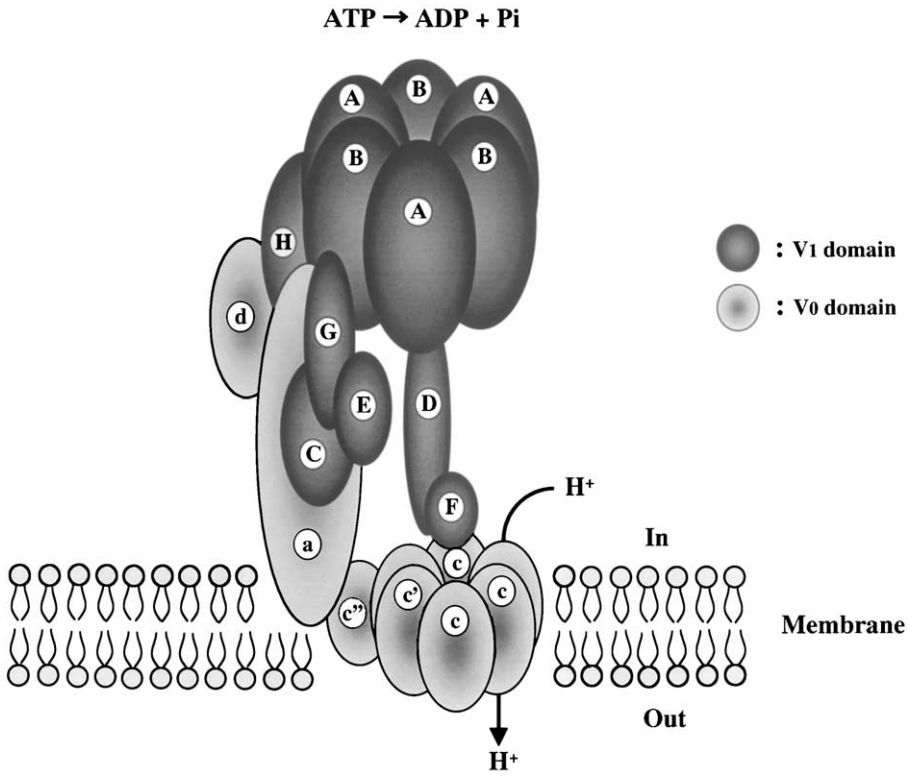



**Figure 1.** Diagram of subunits and domains of vacuolar $H^+$-ATPase. V-ATPases are 830-kDa multisubunit enzymes composed of the cytosolic sector $V_1$ domain (570 kDa) and the membrane sector $V_0$ domain (260 kDa). The $V_1$ domain (subunits A–H) hydrolyzes ATP and the $V_0$ domain (subunits a, c, c′, c″ and d) translocates protons.

domain. The ATP-hydrolyzing $V_1$ domain is a 570-kDa peripheral complex of eight different subunits (subunits A to H). The proton-translocating $V_0$ domain is a 260-kDa integral complex of five different subunits (subunits a, c, c′, c″ and d; Figure 1).[1–5] Information concerning the various subunits is summarized in Table 1.

The V-ATPase family includes several ion-translocating ATPases such as mitochondrial $F_0F_1$-type ATPase (F-ATPase) and gastric ATPase (P-ATPase).[21] While the primary structures of these types of ATPases show an evolutionary relationship, the types differ with respect to function. The V-ATPases pump protons from the cytoplasm to the lumen of the vacuole using the energy released by ATP hydrolysis. As the V-ATPases do not use a counter-ion, their pumping activities are electrogenic, creating an electrical potential difference across the membrane. The V-ATPases are required for maintaining acidification of endosomes, lysosomes, Golgi-derived vesicles, chromaffin granules, the central vacuoles of yeasts and other plants, and some clathrin-coated vesicles.[9,10] Vacuolar acidification is important for a variety of cellular events, such as release of internalized ligand from receptors, viral infection, degradation of macromolecules, transport of small molecules into vesicle lumens and activation of transcription factors.[22]

In addition to their role in intracellular compartments, the V-ATPases are important for plasma membrane functions in various specialized cells. In the kidney, the plasma membrane V-ATPases in several nephron segments make a major contribution to pumping of protons to lumen, an essential homeostatic mechanism.[23] In osteoclasts, cells that are essential for bone remodeling, densely packed V-ATPases reside in a specialized domain of the apical plasma membrane known as the ruffled membrane, where they locally acidify the extracellular compartment at the site of attachment to bone.[24] Furthermore, the V-ATPases are functionally expressed in plasma membranes of human tumor cells and may have specialized functions in cell growth, differentiation, angiogenesis and metastasis.[12–15]

## Regulation of V-ATPase activity

Maintenance of vacuolar pH and $pH_i$ is crucial to normal cell function, so V-ATPase activity is tightly

**Table 1.** Structure and function of human vacuolar $H^+$-ATPase

| Domain | Subunit | Encoding gene | Chromosome | Molecular weight (kDa) | Yeast gene | Function | Interacting protein |
|---|---|---|---|---|---|---|---|
| $V_1$ | A | $ATP6A_1$<br>$ATP6A_2$ | 3p13–q13.2 | 70–73 | VMA1 | ATP hydrolysis<br>NEM/NBD-Cl binding site | |
| | B | $ATP6B_1$<br>$ATP6B_2$ | 2cen–q13<br>8p22–p21 | 56–58 | VMA2 | ATP binding | |
| | C | ATP6C | 8p22–q21.3 | 40–42 | VMA5 | activity, $V_1$–$V_0$ assembly | |
| | D | ATP6M | 14 | 34 | VMA8 | activity, assembly | |
| | E | ATP6E | 22q11.1 | 31–33 | VMA4 | activity, assembly | mSos, aldolase |
| | F | ATP6S14 | 12 | 14 | VMA7 | activity, $V_1$–$V_0$ assembly | |
| | G | ATP6G | 6p21 | 13–15 | VMA10 | activity, assembly | |
| | H | | | 50–54 | VMA13 | activity | HIV-1 Nef |
| $V_0$ | a | ATP6N | 17q21 | 100–116 | VPH1/STV1 | proton translocation<br>bafilomycin-binding site | |
| | c | ATP6L | 16p13.3 | 17 | VMA3 | proton translocation<br>bafilomycin/DCCD-binding site | E5 oncoprotein<br>$\beta_1$ integrin |
| | c′ | | | 17 | VMA11 | proton translocation | |
| | c″ | ATP6F | 1p32.3 | 19 | VMA16 | proton translocation | |
| | d | ATP6N2 | | 38 | VMA6 | activity, assembly | |

regulated at different levels. One possible mechanism controlling V-ATPase activity involves changes in pump density.[25]

Assembly and disassembly of the V-ATPase also can control V-ATPase activity. $V_0$ separated from $V_1$ cannot translocate protons and cytoplasmic pools of $V_1$ appear unable to hydrolyze ATP.[26] The existence of separate pools of $V_1$ and $V_0$ domains in mammalian cells suggests that disassembly may be a widely employed regulatory mechanism.

Feng and Forgac[27] have reported that V-ATPase activity *in vivo* was regulated by reversible disulfide bond formation between cysteine residues at the catalytic site. V-ATPases have been shown to be activated by ADP, salt and phorbol esters. Thus, a number of low-molecular weight activator and inhibitor proteins have been described, but their functions *in vivo* remain to be determined.[28]

## Inhibitors of V-ATPase

Several inhibitors have been found to interact with V-ATPases, interfering with both ATP hydrolysis and proton translocation activities. Inhibitors can be divided into two classes: soluble-domain inhibitors and inhibitors acting at membrane sites. At low micromolar concentrations, soluble-domain inhibitors, such as *N*-ethylmaleimide (NEM) and 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole chloride (NBD-Cl), effectively inhibit ATP hydrolysis.[29] Inhibitors acting at membrane sites such as dicyclohexyl-carbodiimide (DCCD), inhibit the c subunit of V-ATPase.[29,30] Bafilomycin A1 or concanamycin A (macrocyclic lactone class) at nanomolar concentrations selectively inhibit V-ATPase, and also inhibit growth and induce apoptosis in various human cell lines.[31–34] Recently, the described specific inhibitors of mammalian V-ATPase belonging to the benzolactone enamide class, such as salicylihalamide, lobatamides and oximidines, appear promising as anticancer agents (Table 2).[35–37]

## Proteins interacting with V-ATPase

Several V-ATPase-binding proteins have been identified. For example, the c subunit of V-ATPase interacts

with the E5 transmembrane oncoprotein of papillomaviruses and has been proposed to mediate the ability of E5 to cause ligand-independent activation of the platelet-derived growth factor (PDGF)-$\beta$ receptor.[38,39] In addition, $\beta_1$ integrin, which is involved in cell migration, proliferation, differentiation, cytoskeletal organization, signal transduction and cell sensitivity to anticancer agents, binds to the c subunit at the fourth of four transmembrane helices. Overexpression of the c subunit or expression of a subunit c with the fourth helix deleted alters the morphology of myoblasts and fibroblasts.[40] Thus, interactions of the c subunit with $\beta_1$ integrin are important for cell growth control. Recently, the c subunit has been reported to suppress $\beta$1,6 branching *N*-linked oligosaccharides of $\beta_1$ integrin and of epidermal growth factor; this ability to influence glycosylation is located in the second and fourth helices. However, the effect of the c subunit on glycosylation is independent of its binding to $\beta_1$ integrin.[41]

The E subunit of V-ATPase interacts with the Db1 oncoprotein homology domain of mSos-1, which has a dual role in activating Ras and Rac1; this observation suggests that the E subunit may participate in regulation of the mSos-1-dependent Rac1 signaling pathway involved in growth factor-mediated cell growth control.[42] In addition, the E subunit binds to aldolase, an enzyme of the glycolytic pathway. Direct interaction between the V-ATPase and aldolase may underlie the proximal tubule acidification defect in hereditary fructose intolerance.[43]

The accessory protein negative factor (Nef) of human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) decreases expression of CD4 on the surfaces of infected cells. Nef interacts with the H subunit of the V-ATPase, correlating with the ability to internalize CD4.[44,45] This indicates that the H subunit plays an important role in viral infectivity.

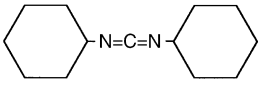
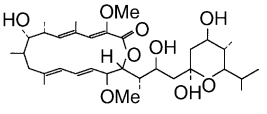
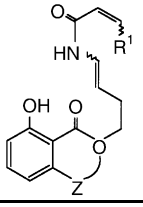
## Regulation of V-ATPase gene expression

V-ATPase genes are considered 'housekeeping genes'. We have isolated and characterized several genomic clones containing the 5′ end of human V-ATPase genes (Torigoe and Kohno, unpublished results). The promoters of five V-ATPase genes encoding A1, C, E, c and c″ subunits were examined regarding structure and transcription factor binding sites. These promoters exhibited a GC-rich region in the area of the first exon and lacked TATA and CCAAT boxes. As shown in Figure 2, a CpG island covers the 5′ end of these genes. We found putative Sp1 and USF sites in their promoter regions, which also are found frequently in mammalian housekeeping genes. Molecular mechanisms of transcription concerning the various subunits encoded by multiple V-ATPase genes remain to be elucidated.

## V-ATPase in human cancer cells

Altered cytosolic pH has been implicated in drug resistance.[46,47] Cells with multidrug resistance contain more acidic organelles than drug-sensitive parental cells, suggesting that acidic organelles are related to resistance. Daunomycin and some anticancer agents such as doxorubicin and vinblastine

**Table 2.** Inhibitors of vacuolar $H^+$-ATPase

| Name | Structure | Molecular weight and description |
|---|---|---|
| DCCD |  | 206.33; peptide-coupling reagent |
| Bafilomycin A1 |  | 622.8; isolated from *Streptomyces griseus* |
| Benzolactone enamide |  | Salicylihalamide; isolated from the marine sponge *Haliclona* sp.<br>Oximidine; isolated from *Pseudomonas* sp. |

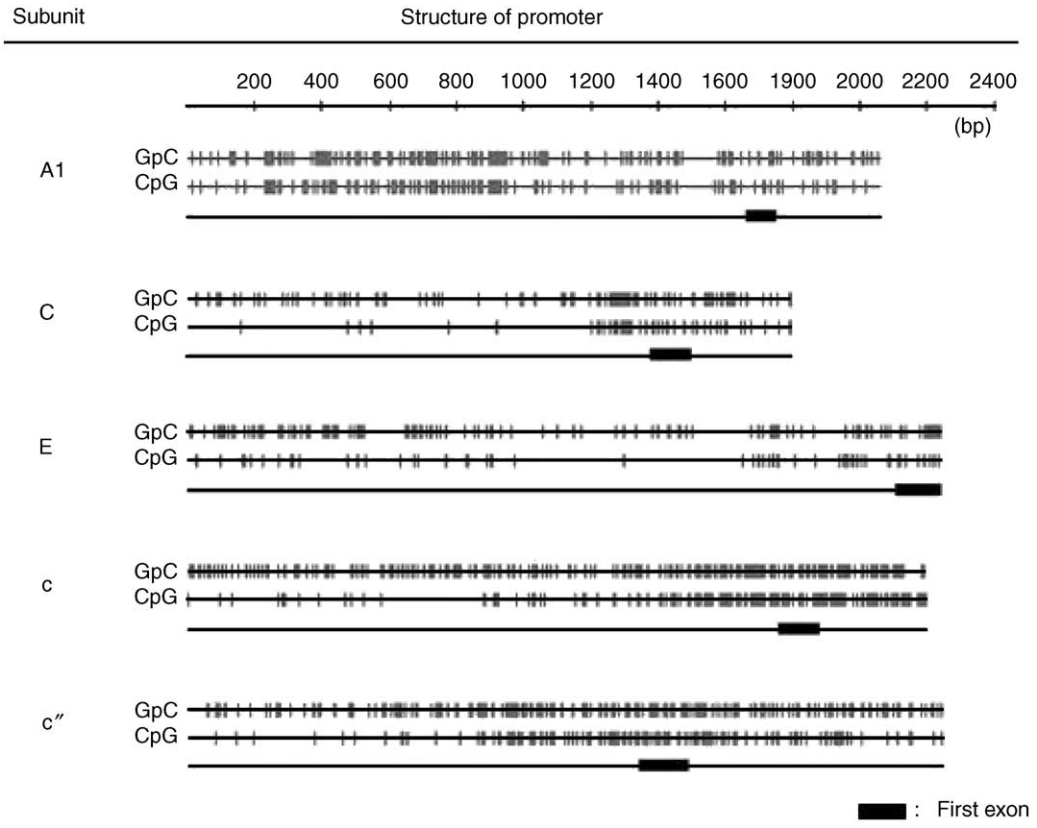



**Figure 2.** Promoters of vacuolar $H^+$-ATPase subunits. Promoter structures of V-ATPase subunits A1, C, E, c and c″ are shown. The diagram shows the distribution of GpC and CpG dinucleotides. Bold line indicates the first exon.

have been found to accumulate in acidic organelles; in cells with multidrug resistance, these agents then are removed from the cell via exocytosis.[48] This accumulation was sensitive to bafilomycin A1, but was insensitive to other transporter inhibitors and P-glycoprotein inhibitors.[49]

We have reported that the genes encoding V-ATPase subunit are inducible by cisplatin treatment and that proton pump gene expression is upregulated in cisplatin-resistant cell lines.[19] Cisplatin forms a charged metabolite in aqueous solutions that binds to DNA or protein in an environment of low pH and low chloride concentration.[50,51] We hypothesize that cisplatin-resistant cells may increase intracellular pH by induction of the V-ATPase subunit genes, thus escaping the cytotoxicity of cisplatin. Intracellular pH in cisplatin-resistant cells is higher than in sensitive parental cells due to upregulation of V-ATPase subunit genes. DNA–cisplatin cross-links form well under acidic conditions, so reduced intracellular pH is associated with increased cisplatin sensitivity.

Recently, ionizing radiation has been reported to induce development of acidic vesicular organelles in neoplastic epithelial cells.[52] Interference with acidification of vesicular organelles by bafilomycin A1, an inhibitor of V-ATPase, results in increased radiosensitivity. Another report suggests that bafilomycin A1-treated cells showed increased accumulation of p53 protein and p53-dependent transactivation of gene expression.[53] These results indicate that the V-ATPases may be a potential target for cancer chemotherapy using inhibitors. Inhibitors of the V-ATPase may produce a supra-additive effect with other anticancer agents or irradiation. Thus, tumor pH is important in the response of cancer cells to irradiation, hyperthermia and chemotherapy.

Intracellular acidification, an early event in apoptosis, increases susceptibility of cells to killing by chemotherapeutic agents and has been found in HL-60 cells undergoing apoptosis in response to etoposide and camptothecin.[54,55] This indicates that intracellular acidification activates endonucleases, inducing cellular DNA fragmentation.[56] These data suggest that the V-ATPase is a promising molecular target for therapy to induce escape from cell apoptosis caused by many stresses including anticancer agents.

## Conclusion

Intracellular and extracellular pH in tumors is important in regulation of cellular functions. A growing body of data from structural and functional analyses of cellular pH regulators has enhanced our understanding of the roles of these molecules in tumor cells. Here, we described the importance of V-ATPases in many cellular processes. Further studies to elucidate tumor-specific pH regulation by the V-ATPases may be important in establishing strategies for molecular target therapy. Development of a useful therapeutic agent for selective inhibition of the V-ATPase in tumors is an important goal.

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