

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



V-ATPase Inhibitors in Cancer Treatment and Their Implication in Multidrug Resistance in Oral Squamous Cell Carcinoma

Mario Pérez-Sayáns and Abel García García
*University of Santiago de Compostela
Spain*

1. Introduction

ATPases are enzyme systems that originated in a common ancestor and are distributed universally among all organisms. There are three types of ATPases: those found in archaea (A-ATPases), synthases (F-ATPases), and vacuole or vacuolar ATPases (V-ATPases) (Nelson, Nelson 1989). They are essential for life and have in common the fact that they create an electrochemical ion gradient across the membrane to hydrolyze or synthesize ATP. Structurally, they are enzymatic complexes that work as molecular rotary motors. ATPases are formed by two domains, a hydrophobic domain (A0, V0, and F0) and a hydrophilic domain (A1, V1, and F1) connected by a central axis and either one or two lateral axes. In this chapter, we are going to discuss V-ATPases.

1.1 Biological functions

Unlike F-ATPases, whose primary function in eukaryotic cells is to generate ATP from proton motive force, V-ATPases function exclusively as ATP-dependent proton pumps, performing diverse biological functions within cells (Nelson 1992; Kane 1999; Saroussi & Nelson 2008, Stevens & Forgac 1997).

Regarding to the membrane transport, V-ATPases play an important role in receptor-mediated endocytosis (Forgac 1998), intracellular transport, and the acidification of late endosomes (Kane 1999; Stevens & Forgac 1997; Nishi & Forgac 2002; Kawasaki-Nishi & Forgac 2003; Finbow, Harrison 1997). Vacuolar acidification has also been reported to be involved in the transport of lysosomal enzymes from the Golgi apparatus to the lysosomes (Stevens, Forgac 1997; Moriyama, Nelson 1989). V-ATPases appear to play an important role in the creation of the microenvironment needed for correct protein transport, exchange, and secretion (Schoonderwoert et al. 2000).

Although V-ATPases were initially identified in intracellular compartments, knowledge on the roles they play in the plasma membrane has increased enormously. V-ATPases located at the apical membrane of type A intercalated cells are involved in the secretion of protons in renal fluid (Smith et al. 2005; van Hille et al. 1993). Type B intercalated cells, whose function is to secrete bicarbonate, also contain V-ATPases, but they are located between the apical and basolateral membranes (Nishi & Forgac 2002, van Hille et al. 1993). In macrophages and neutrophils, plasma membrane V-ATPases (pmV-ATPases) are involved

in the homeostasis of cytoplasmic pH (Stevens & Forgac 1997, Nishi & Forgac 2002, Nanda et al. 1996). These ATPases also play an important role in bone reabsorption (Marshansky, Futai, Stevens, Forgac 1997, Nishi, Forgac 2002, Smith et al. 2005, van Hille et al. 1993). Another of their functions is to regulate sperm motility and maturation on the apical membrane of epididymal cells and vas deferens by stabilizing the sperm medium (Nishi, Forgac 2002). The role of V-ATPases in cancer cells will be discussed in a specific place.

Other additional functions of V-ATPases involves the low pH maintained by them in lysosomes and phagosomes, which is necessary for the activity of the degradative enzymes in these compartments (Sun-Wada, Wada & Futai 2003, Sun-Wada, Wada & Futai 2004, Kurashima et al. 1996) and the transport of small molecules and ions (Nishi, Forgac 2002, Kurashima et al. 1996). The driving force necessary for the accumulation of neurotransmitters in synaptic vesicles is proton motive force, which is generated by V-ATPases (Nelson, Harvey 1999). The fusion-fission balance of the vacuolar system of eukaryotic cells is also controlled by V-ATPases, i.e. via the interaction between vacuolar SNARE proteins and GTPase Vps1p (Baars et al. 2007, Muller et al. 2003). Exocytosis in eosinophils and binding to actin cytoskeleton is also regulated by V-ATPases (Kurashima et al. 1996). The association between V-ATPase subunits and other cellular proteins, for example, that which occurs between the C subunit of the V₀ domain and the E5 oncoprotein, or between platelet-derived growth factor (PDGF) and b1 integrin, indicate that these subunits play a role in cell growth and transformation. V-ATPases also allow the entry of certain viruses (e.g. influenza) and toxins (e.g. diphtheria) into the intracellular space via the binding of these pathogens to the endosomal membrane (Stevens, Forgac 1997). In the case of the human immunodeficiency virus (HIV), the association between the V-ATPase H subunit and the HIV-1 Nef protein, which controls the expression of CD4 (the main HIV receptor), facilitates endocytosis of Nef and/or alterations in the acidification of the endosomal pathway by this protein (Nishi, Forgac 2002)(Marshansky, Futai). The most recent function attributed to V-ATPases is their involvement in the regulation of cell-cell fusion to form larger cells, as is the case with osteoclasts and macrophages (Wada et al. 2008).

1.2 V-atpase structure

The V-ATPase proton pump has multiple subunits, each with multiple isoforms, hence the need for a clear, standardized nomenclature system. Initially, the HUGO Gene Nomenclature Committee agreed to use the ATP as the stem, or root, symbol. ATP6, for example, indicated ATPase, H⁺ transport, lysosomal (vacuolar proton pump). In 2003, the nomenclature system for genes encoding V-ATPase subunits was revised and it was decided to maintain the root ATP6 and add the domain to which a particular subunit belonged, followed by the letter of the subunit, and finally the number of the isoform, where relevant, (e.g. *ATP6V1C1*, *ATP6V1E*, etc.) (Smith A.N. et al. 2003).

V-ATPase structure, function, biogenesis, and regulation was widely revised by Stevens and Forgac (Stevens & Forgac 1997). We will use the nomenclature system proposed by these authors to explain the structural subunits of V-ATPase together with relevant modifications based on recent research using transmission electron microscopy (Wilkins, Zhang & Zheng 2005).

V-ATPases have been found to be practically identical in terms of the composition of their subunits in all eukaryotic cells. They have two distinct structures: a peripheral catalytic

sector (V1) and a hydrophobic membrane sector (V0) responsible for driving protons (Gruber 2005). The catalytic sector is composed of five polypeptides known as subunits A, B, C, D, and E, with a molecular weight, in decreasing order, ranging from 72 to 33 kDa. Recent advances in knowledge of the mechanism of action of F-ATPases have clarified the relationship between function and structure for each of the subunits of these enzymes (Qi, Wang & Forgac 2007, Inoue et al. 2005) (Figure 1).

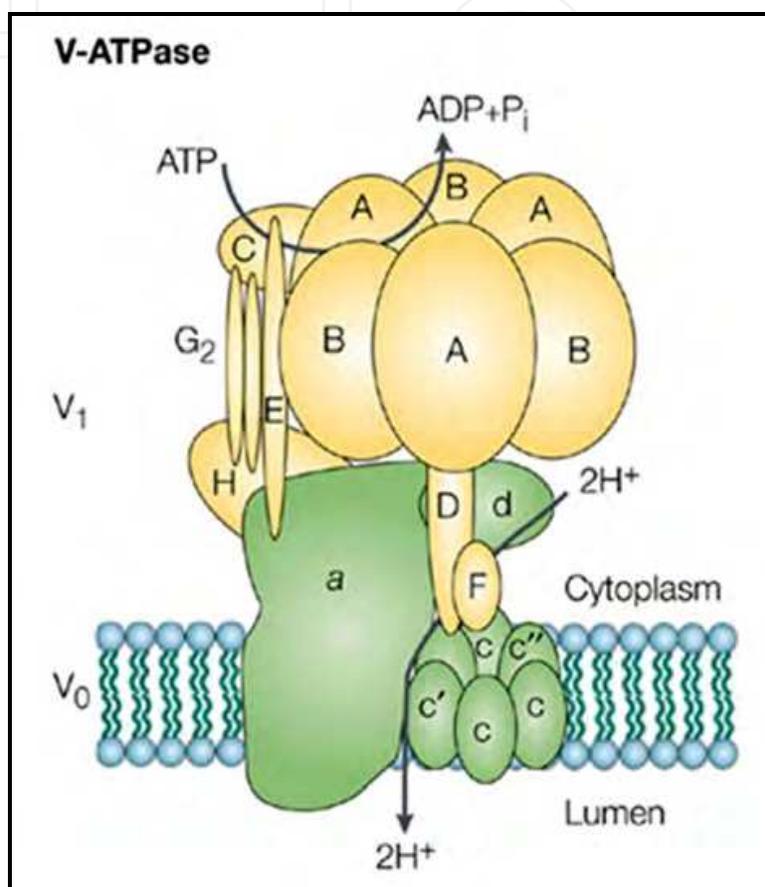


Fig. 1. Diagram of V-ATPase. The cytosolic domain (in yellow) is formed by three A subunits, three B subunits, three G subunits, and one C, D, E, F, and H subunit. The V0 transmembrane domain is formed by five subunits: a, c, c', c'' and d. The V1 domain contains the catalytic unit (Nishi & Forgac 2002).

1.3 V-ATPase regulation

Three major regulatory mechanisms have been described for V-ATPase: 1) the regulation of pump density, which allows different cells to maintain their cytoplasmic and vacuolar pH stable; 2) the regulation of V1 and V0 domain association/dissociation, for example, a decrease in glucose levels can cause a 70% dissociation of the V1 domains of the membrane; and 3) the regulation of secretory activity, via the maintenance of balance in the formation of bisulfite and binding efficiency between H⁺ and the pump. Other mechanisms include the necessary modifications in the membrane potential for the generation of electrogenic force (Forgac 1998; Peng, Stone & Xie 1993) and alterations in the vacuolar transporter chaperone (Vtc) complex, which affect the conformation of the V0 domain and its function in vacuole fusion of the membrane (Muller et al. 2003).

2. V-ATPase inhibitors

Scientific evidence suggests that the acidic tumor microenvironment is key to managing cancer progression and metastasis. In particular, V-ATPases play a major role in metastasis tumor development because many tumor cells secrete lysosomal enzymes that participate in the extracellular matrix degradation necessary for metastatic invasion. These enzymes are most active at low optimal pH; moreover, V-ATPases are responsible for microenvironment acidification (Nishi, Forgac 2002, Martinez-Zaguilan et al. 1993). Among the many mechanisms that regulate the tumor microenvironment, V-ATPases are especially significant because they can be inhibited by proton pump inhibitors. (Fais et al. 2007).

2.1 Classes of V-ATPase inhibitors

Initial attempts to block V-ATPases were made after bafilomycin and concanamycin were discovered in 1988 (Bowman, Siebers & Altendorf 1988). New molecules capable of inhibiting V-ATPase to a greater or lesser extent via different mechanisms of action were later discovered. Such molecules include benzolactone enamides salicylilalamide (Erickson et al. 1997), lobatamide A and B (Galiniis et al. 1997), apicularen (Kunze B., Janse R., Sasse F., Höfle G. and Reichenbach H. 1998), indolyis (Gagliardi et al. 1998, Nadler et al. 1998), oximidine (Kim et al. 1999), macrolactone archazolid (Sasse et al. 2003), lobatamide C (Shen et al. 2003), and cruentaren (Kunze et al. 2006). The latest generation of inhibitors include NiK12192 (Saroussi, Nelson 2008, Petrangolini et al. 2006), FR202126 (Niikura 2007), and PPI SB 242784 (Hesselink et al. 2008). We can see the differences and similarities of V-ATPase inhibitors in Table 1:

The V-ATPase inhibitors studied most thoroughly and used most often are macrolide antibiotics with 18-membered lactone rings, namely, bafilomycins and concanamycins. Bafilomycin and concanamycin are commercially available, and various laboratories have developed in vitro synthesis processes for experimental purposes (Scheidt et al. 2002). The remaining V-ATPase inhibitors are still in experimental phase, due to possible side effects that may occur in humans. However, PPIs are the treatment of choice for peptic diseases such as gastroesophageal reflux (Larsson et al. 1985). While these pumps block the secretion of gastric acid, they also directly inhibit V-ATPase activity. Examples of PPIs include omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole (Horn 2000), all of which accumulate in acidic compartments (De Milito, Fais 2005a). PPI treatment has been associated with V-ATPase activity inhibition and an increase in both extracellular pH and pH in lysosomal organelles. In vivo experiments using mice/human xenografts have shown that pretreatment with PPIs can sensitize solid human tumors to chemotherapy drugs (De Milito, Fais 2005a).

Treatment with PPIs has also been found to sensitize tumor cells to cisplatin, 5-fluoracil, and vinblastine through changes in cellular pH gradients, with retention of the drugs in the cytoplasm, and in the nucleus in the case of doxorubicin (De Milito, Fais 2005a, Luciani et al. 2004, Luciani et al. 2004, Cianfriglia et al. 1990).

It is also known that low pH levels are suitable for the complete activation of PPIs (De Milito et al. 2007), suggesting that tumor alkalinization may be an extremely interesting target for future anticancer treatments (De Milito, Fais 2005a, Luciani et al. 2004, De Milito, Fais 2005b). Specific V-ATPase inhibitors such as concanamycin and bafilomycins are other candidates for investigation, not only to treat cancer but also to reduce MDR in tumors (Perez-Sayans et al. 2009, Sasazawa et al. 2009).

CLASSES OF V-ATPase INHIBITORS					
		Chemistry	Provenience	Binding site	Action
Plecomacrolide	Concanamycin & Bafilomycin	Macrolide antibiotics with 18-membered lactone rings	<i>Streptomyces</i>	Unknown	V-ATPases inhibition Ionophoric properties
	Salicylihamide A	Macrocyclic salicylate	Sponge <i>Haliclona</i> sp.	VO complex	Animal V-ATPases inhibition Cytotoxin
Benzolactone enamides	Apicularens	Lactone ring	<i>Chondromyces</i>	VO complex	Highly toxic for human and animal cell
	Lobatamides	Substitution of enamide NH, salicylate, and phenyl salicylate	<i>Tunicate Aplidium lobatum</i>	VO complex	Animal and mammalian V-ATPases inhibition
	Oximidines	Lactone ring	<i>Pseudomonas</i> sp.	VO complex	Animal and mammalian V-ATPases inhibition
	Cruentaren	Lactone ring	<i>Byssovorax cruenta</i>	VO complex	Cytotoxicity on mammalian and fungal cells at mitochondrial F-ATPases
	Archazolid	Macrocyclic lactone ring with a thiazole side	<i>Archangium gephyra</i> <i>Cystobacter violaceus</i>	VO subunit c	Cytotoxicity on mammalian cell line
Indolylyls	Bafilomycin-based	Synthesis	VO subunit c	V-ATPase inhibitor	
Late-generation V-ATPase inhibitors	NiK12192, SB 242784, FR202126, 3-bromopyruvate (3-Br PA), Tributyltin chloride (TBTCI), FR177995, FR167356				

Table 1. Classes of V-ATPase inhibitors

3. Role of v-ATPases inhibitors in cancer

3.1 Tumor metastasis

The development and maintenance of the proton gradient present in tumors is due directly to the ability of tumor cells to secrete protons (H⁺) (Martinez-Zaguilan et al. 1993, McLean et al. 2000), acidify the extracellular medium (Cardone, Casavola & Reshkin 2005, Perona, Serrano 1988), and keep the cytosolic pH alkaline (Sennoune, Martinez-Zaguilan 2007). This ability also

increases with tumor aggressiveness (Montcourrier et al. 1997, Parkins et al. 1997). In addition, low pH may cause extracellular matrix (ECM) degradation and remodeling through activation of proteolytic enzymes which contribute to invasion and cancer metastasis (Martinez-Zaguilan et al. 1996, Rofstad et al. 2006). Proteases need low extracellular pH to optimize their activation, including metalloproteinases (MMP), morphogenetic bone metalloproteinases (protein type 1), tissue serine proteases, and adamalysin-related proteinases. Among them, MMPs are the proteases basically involved in degradation and remodelling of all extracellular matrix (ECM) structural components (Montcourrier et al. 1994, Rozhin et al. 1994, Johnson et al. 2000, Kato et al. 2005, Gocheva, Joyce 2007).

Sennoune et al. assessed the effect of bafilomycin A1 in breast tumor cells and found that cytoplasmic pH recovery was inhibited in response to acid load, in both highly and lowly metastatic cells, although to a greater extent in highly metastatic cells (Sennoune et al. 2004). This suggests that V-ATPases in the plasma membrane are involved in the acquisition of a more metastatic phenotype and that the use of V-ATPase inhibitors allows distant metastasis to be minimized (Figure 2).

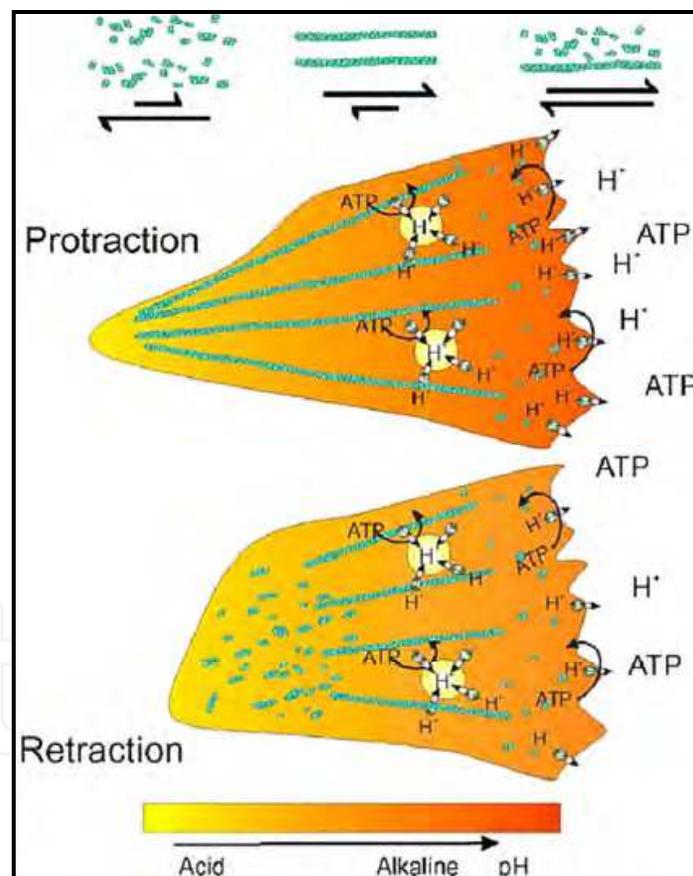


Fig. 2. Proposed mechanism by which overexpression of pmVATPase at the leading edge of the cell modulates cell migration/invasion. The proposed model should be viewed as a framework to explain how pmV-ATPases determine the acquisition of an invasive phenotype needed for angiogenesis and metastasis. Changes in pH_{cyt} are critical for establishing cell polarity needed for cell movement. A critical step in directed motility and migration is the asymmetric actin polymerization at the leading edge (Sennoune, Martinez-Zaguilan 2007).

Using RNA interference techniques, Lu et al. found that distant metastasis could be delayed and suppressed in human hepatocellular carcinoma *in vitro* by reducing proton extrusion and gelatinase activity through the inhibition of V-ATPase subunit c (ATP6L) (Lu et al. 2005). This fact is consistent with subunit c block by bafilomycin and concanamycin, as this is their main binding site to V-ATPase (Bowman et al. 2004). In tyrosinase-positive amelanotic melanoma cells, inactive tyrosinase accumulates in the endoplasmic reticulum because the presence of aberrant V-ATPases blocks trafficking through secretory pathways. The use of V-ATPase inhibitors, such as bafilomycin A1 or concanamycin A, improves transport, demonstrating the involvement of this enzyme and preventing conditions that favor metastatic dissemination (Halaban et al. 2002).

Hence, both *in vitro* or *in vivo*, V-ATPases are a target for anticancer therapeutic agents, either directly by regulating the pH gradient in the tumor environment or indirectly by preventing ECM protease activation (Fais et al. 2007).

3.2 Tumor cell growth and survival

V-ATPases may also play a significant role in tumor cell survival by regulating pH and preventing apoptosis. As previously reported, plasma membrane V-ATPases help regulate cytosolic pH in macrophages and neutrophils (Nanda et al. 1996). This mechanism may also be used by tumor cells, which produce more H⁺ due to high glycolytic activity (Gatenby, Gillies 2004). Treatment with V-ATPase inhibitors lowers H⁺ extrusion, both *in vitro* and *in vivo* (Volk, Albert & Kempinski 1998, McSheehy et al. 2003).

Bafilomycin A1 was assessed as a potential anticancer agent because it inhibits cell proliferation and tumor growth. Although this effect has been attributed to the inhibition of intracellular acidosis by blocking V-ATPases, the precise mechanism remains unknown (Bowman et al. 2004). A study conducted by Lim et al., hypothesized that bafilomycin A1 and its analogue, concanamycin A, stimulate a tumor growth factor, hypoxia-inducible 1 α (HIF-1 α) (Zhong et al. 1999). The interaction of bafilomycin with HIF-1 α increases with hypoxia, causing strong induction of the p21 gene which, in turn, leads to cell cycle detection in cancer cells (Lim et al. 2006).

V-ATPase inhibition has also been shown to trigger apoptosis through caspase-dependent and caspase-independent mechanisms (De Milito et al. 2007, Aiko et al. 2002), and bafilomycin and concanamycin induce apoptosis in other types of cells, including neutrophils (Gottlieb et al. 1995) and osteoclasts (Xu et al. 2003).

Morimura et al. described the growth-inhibiting effect of apoptosis stimulation in human hepatoblastomas using bafilomycin A1. In particular, electron microscopy, morphological observations, and flow cytometry showed higher apoptotic cell ratios and diminished cell reproduction. Cell growth inhibition in normal liver cells was insignificant (Morimura et al. 2008).

In the case of human gastric cancer cells, Nakashima et al. investigated the mechanism of apoptosis induced by bafilomycin A1. Bafilomycin inhibits the growth of MKN-1 cancer cells through apoptosis. Flow cytometry was used to measure alterations in lysosomal pH, which increased in the presence of bafilomycin. Caspase-3 activity was also increased by bafilomycin; such findings suggest that bafilomycin A1 induces apoptosis in MKN-1 cells mediated by proteases released after lysosomal dysfunction, followed by caspase-3 activation of the cytochrome c-independent manner (Nakashima et al. 2003, Hishita et al. 2001).

A study conducted by Wu et al. has shown that bafilomycin A1 suppresses macroautophagy by preventing lysosome acidification (Wu et al. 2009). Macroautophagy is a protein

degradation pathway that allows increased cell survival under stress and in cancer (Meijer, Codogno 2004, Mortimore, Hutson & Surmacz 1983). Macroautophagic inhibition in HT-29, HCT-116, and SW1116 colon cancer cells is accompanied by down-regulation of cyclin D and E and up-regulation of p21^{Cip1} and various caspases, causing an antiproliferative effect (Wu et al. 2009).

Cancer cells are more likely to express V-ATPase than normal cells, causing abnormalities in the acidic microenvironment and affecting cancer cell growth and infiltration significantly (Saroussi, Nelson 2008, Cardone, Casavola & Reshkin 2005, Perona, Serrano 1988). Moreover, neoplastic cells are more sensitive to bafilomycin A1 than normal cells, a fact that may be used in anticancer therapy (Ohta et al. 1996).

3.3 Chemoresistance

Resistance to chemotherapy agents is the main reason for treatment failure in patients with cancer, and multidrug resistance (MDR) occurs in many types of tumors. The main mechanism that gives rise to the MDR phenotype is the overexpression of drug efflux transporters such as the P glycoprotein (Pgp) in the plasma membrane (Nielsen, Skovsgaard 1992) (Figure 3).

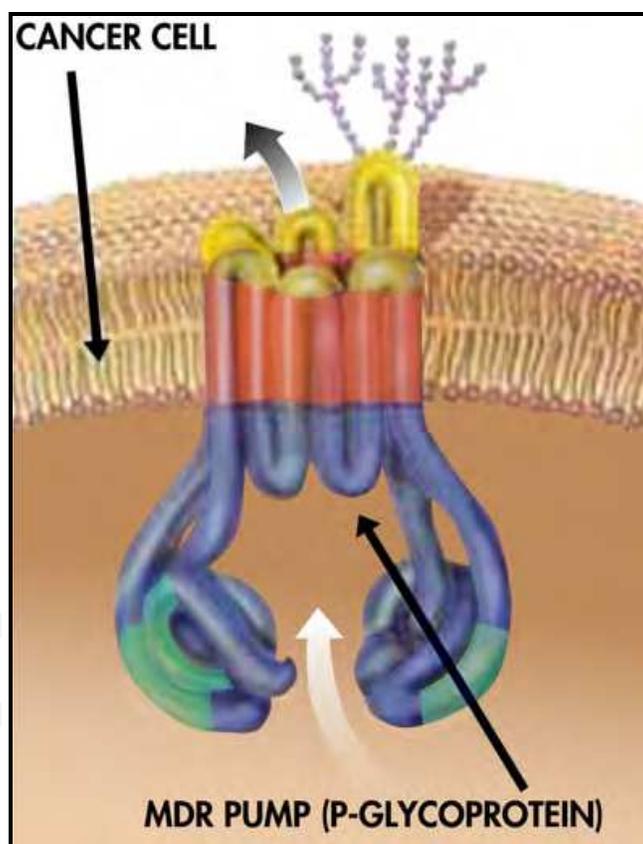


Fig. 3. Pgp resides in the cell membrane, where it may act to pump toxins out of the cell. The painting shows a model of the protein's structure that is based on its known sequence of amino acids. The protein chain is thought to snake back and forth 12 times across the lipid bilayer of the membrane forming a 12-sided pore. The pan of the protein outside the cell bears sugar chains (purple); two large and nearly identical domains protrude into the cell. They include regions (green) that bind the cellular energy-carrying compound ATP, which probably provides the energy that drives the efflux (arrows) (Kartner, Ling 1989).

Extracellular pH is considerably more acidic in oral squamous cell carcinoma (OSCC), a solid tumor, than in normal tissue. This increased acidity interferes with the absorption of standard chemotherapy drugs, reducing their effect on tumors (Griffiths 1991, Negendank 1992). Vacuolar ATPases (V-ATPases) have been reported to be largely responsible for this acidic environment (Newell et al. 1993, Yamagata et al. 1998). While a clear association has been established between MDR and Pgp expression in some tumors, the mechanism by which drug resistance occurs within the multistep process of OSCC has not yet been fully elucidated (Tanigawara 2000). OSCC is highly resistant to a wide range of structurally different drugs with diverse cytotoxic mechanisms of action (McLeod, Evans 1999). This suggests that OSCC may be intrinsically chemoresistant and it is possible that V-ATPases play a key role in this resistance (Perez-Sayans et al. 2009).

3.3.1 Multidrug resistance in OSCC

As already mentioned, while a clear association has been established between MDR and Pgp expression in certain tumors, the mechanism by which drug resistance occurs within the multistep process of OSCC has not yet been fully elucidated (Ling 1997). Several genes have been implicated in MDR, including *MDR1*, *MRP* (multidrug resistance-associated protein), *GST- π* , and *DNA* topoisomerase II.

Pgp is encoded by *MDR1* and flow cytometry studies have shown increased Pgp levels in recurrent OSCCs compared to normal mucosa with oral lesions at different stages of tumorigenesis (Jain et al. 1997). These findings were confirmed by immunohistochemical staining in a study that compared recurrent tumors with untreated primary oral tumors (Chomczynski, Sacchi 2006, Xie et al. 2000). The best known *MDR1* gene product is Pgp/P-170, which has been implicated in resistance to chemotherapy agents such as taxanes, anthracyclines, vinca alkaloids, podophyllotoxins, and camptothecins (Juliano, Ling 1976).

The mechanism by which Pgp-mediated MDR is acquired in head and neck tumors, however, is different. Immunohistochemical studies, for example, have revealed high levels of Pgp in salivary gland adenocarcinoma (SGA) cell lines but insignificant levels in OSCC cell lines (Naramoto et al. 2007). Reverse transcription quantitative polymerase chain reaction analysis of *MDR1* expression has also revealed increased Pgp levels in different cell lines treated with vincristine (alkaloid cancer drug). These results suggest that Pgp-induced MDR in OSCC is essentially an acquired phenotype caused by the genetic induction of Pgp (Uematsu et al. 2001).

MRP has been linked to MDR in multidrug-resistant Pgp-negative cells lines in small cell lung cancer, cancer of the stomach, bladder, cervix, and prostate, and leukemia (Kim et al. 1996, Kim et al. 1995, Endo et al. 1996b, Endo et al. 1996a). In head and neck tumors, overexpression of MRP1 mRNA has been found in human and murine OSCC and SGA cell lines mice treated with vincristine, suggesting the theory of Pgp- and MRP-independent MDR in OSCC.

Overexpression of the isozyme *GST- π* is often associated with malignant transformation and/or MDR (Ruzza et al. 2009). *GST- π* is responsible for detoxifying xenobiotics such as carboplatin (used in chemotherapy) and elevated levels of this enzyme cause treatment resistance (Engel et al. 2005, Koshiyama et al. 2001). Whether or not this is also the case in OSCC, however, is a matter of debate. In a study by Chen et al (Chen, Lin 1997), *GST- π* levels increased with increased severity of oral epithelial dysplasia in line with the development of OSCC. The immunohistochemical expression of placental *GST- π* has been

studied in the oral epithelium of premalignant and malignant oral lesions, and has indeed been proposed as a good marker for premalignant lesions and tumors (Zhang, Xiao & Priddy 1994). Another study, however, that analyzed GST- π levels using enzyme-linked immunoassay failed to find a significant relationship between GST- π and TNM stage (Oude Ophuis et al. 1998). Finally, in a study that analyzed GST- π expression using Northern blot analysis and gene amplification with Southern blot analysis, Wang et al (Wang et al. 1997) concluded that the amplification of the *GST- π* gene was not critically related to the overexpression of GST- π mRNA. Furthermore, they found no relationship between GST- π mRNA overexpression and tumor size, neck nodal status, or patient survival.

The downregulation of topoisomerase II—an enzyme that breaks and rejoins double-stranded DNA in the interconversion of different topological forms of DNA—has also been associated with MDR (Deffie, Batra & Goldenberg 1989, Shi et al. 2008).

The mechanisms underlying MDR response are less clear in OSCC than in other types of tumors (Yajima et al. 2009). MDR holds challenges for both researchers and the pharmaceutical industry. Accordingly, efforts are being made on all sides to find anticancer compounds characterized not only by high tolerability and oral bioavailability but also by the ability to overcome the problem of drug resistance. OSCC is highly resistant to a wide range of structurally different drugs with different cytotoxic mechanisms of action (McLeod, Evans 1999). This suggests that OSCC may be intrinsically chemoresistant, with other molecules, including V-ATPases, playing an important role (Perez-Sayans et al. 2009).

3.3.2 The role of v-ATPases in multidrug resistance

It has been demonstrated that hypoxia and acidity contribute to the transition from benign to malignant growth via the selection of tumor cells capable of surviving in an acidic, oxygen-deprived environment. Acidity, for example, has been associated with chemotherapy resistance (Raghunand et al. 2001), proliferation (Morita et al. 1992), and metastatic behavior (Martinez-Zaguilan et al. 1996). Indeed, alteration of the pH gradient between the extracellular environment and the cell cytoplasm has been suggested as a possible mechanism of resistance to cytotoxic drugs (De Mito, Fais 2005a) (Figure 4).

The alteration of cytosolic pH also plays an important role in drug resistance in chemotherapy. Extracellular pH in solid tumors is significantly more acidic than in normal tissue. This increased acidity interferes with the absorption of basic chemotherapy drugs, reducing their effect on tumors (Griffiths 1991, Negendank 1992). Martínez-Zaguilán et al., found that unlike chemoresistant cells, chemosensitive cells did not recover from acid load (Martínez-Zaguilán et al. 1999). Becelli et al (Becelli et al. 2007) found that reversed pH gradient was directly related to drug resistance.

Anaerobic metabolism is an important determinant of tumor acidity that allows the selection of cells capable of surviving in a hypoxic-anaerobic environment via the synthesis of lactate. A complex system appears to regulate pH homeostasis in mammal cells, and it seems as if malignant tumor cells are capable of exploiting some of these mechanisms to protect themselves from the acidic environment, while maintaining levels of acidity that are poorly tolerated by normal or more differentiated cells (De Mito, Fais 2005b).

Recent studies have suggested that V-ATPases, which secrete protons through the plasma membrane, may play a key role in the acidification of the tumor environment (Perez-Sayans et al. 2009). Several human tumor cells are characterized by increased V-ATPase expression and activity (Perez-Sayans et al. 2010), and pretreatment with proton pump inhibitors (PPIs)

has been found to sensitize tumor cell lines to the effect of different chemotherapy drugs (De Milito, Fais 2005a, Luciani et al. 2004, De Milito, Fais 2005b, Sennoune, Luo & Martinez-Zaguilan 2004). The effectiveness of the drug transport mechanism appears to be comparable to that of drug efflux pumps such as Pgp, although vesicle acid exchange (above all in vesicles that have an active H⁺/cation exchange system) may be an important factor in drug resistance, and particularly in cells that do not overexpress Pgp-type efflux pumps in the plasma membrane (Raghunand et al. 1999).

Murakami et al (Murakami et al. 2001) found cisplatin-resistant tumors to contain elevated levels of all V-ATPase subunits but levels of *ATP6C* were particularly. They also found significantly higher levels of cellular pH in cisplatin-resistant tumor cells than in cells sensitive to vincristine and etoposide. In a later study, however, Zhang et al (Zhang et al. 2006) identified 38 overexpressed genes and 25 underexpressed genes in cisplatin-resistant OSCC. Torigoe et al (Torigoe et al. 2002) showed that treatment with anticancer agents increased *ATP6L* (c subunit). Interfering RNA targeting the c subunit has also been found to improve drug resistance in breast cancer cells (You et al. 2009).

4. Conclusions

The above findings suggest that the induced expression of V-ATPases in MDR is an anti-apoptotic defence and that the combined use of PPIs or V-ATPase inhibitors and low chemotherapy doses might be a possible treatment target (Torigoe et al. 2002). We believe that the future of these molecules in cancer treatment involves measuring the overexpression of specific V-ATPase subunits in tumors to be treated and then using inhibitors specific for the subunits being expressed (Perez-Sayans et al. 2009, Perez-Sayans et al. 2010). This will allow clinicians to provide more specific treatment, while also minimizing adverse effects.

5. References

- Aiko, K., Tsujisawa, T., Koseki, T., Hashimoto, S., Morimoto, Y., Amagasa, T. & Nishihara, T. 2002, "Involvement of cytochrome c and caspases in apoptotic cell death of human submandibular gland ductal cells induced by concanamycin A", *Cellular signalling*, vol. 14, no. 8, pp. 717-722.
- Baars, T.L., Petri, S., Peters, C. & Mayer, A. 2007, "Role of the V-ATPase in Regulation of the Vacuolar Fission Fusion Equilibrium", *Molecular Biology of the Cell*, vol. 18, no. 10, pp. 3873-3882.
- Becelli, R., Renzi, G., Morello, R. & Altieri, F. 2007, "Intracellular and extracellular tumor pH measurement in a series of patients with oral cancer", *The Journal of craniofacial surgery*, vol. 18, no. 5, pp. 1051-1054.
- Bowman, E.J., Graham, L.A., Stevens, T.H. & Bowman, B.J. 2004, "The bafilomycin/concanamycin binding site in subunit c of the V-ATPases from *Neurospora crassa* and *Saccharomyces cerevisiae*", *The Journal of biological chemistry*, vol. 279, no. 32, pp. 33131-33138.
- Bowman, E.J., Siebers, A. & Altendorf, K. 1988, "Bafilomycins: a class of inhibitors of membrane ATPases from microorganisms, animal cells, and plant cells", *Proceedings of the National Academy of Sciences of the United States of America*, vol. 85, no. 21, pp. 7972-7976.

- Cardone, R.A., Casavola, V. & Reshkin, S.J. 2005, "The role of disturbed pH dynamics and the Na⁺/H⁺ exchanger in metastasis", *Nature reviews.Cancer*, vol. 5, no. 10, pp. 786-795.
- Chen, Y.K. & Lin, L.M. 1997, "Evaluation of glutathione S-transferase activity in human buccal epithelial dysplasias and squamous cell carcinomas", *International journal of oral and maxillofacial surgery*, vol. 26, no. 3, pp. 205-209.
- Chomczynski, P. & Sacchi, N. 2006, "The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on", *Nature protocols*, vol. 1, no. 2, pp. 581-585.
- Cianfriglia, M., Cenciarelli, C., Tombesi, M., Barca, S., Mariani, M., Morrone, S., Santoni, A., Samoggia, P., Alessio, M. & Malavasi, F. 1990, "Murine monoclonal antibody recognizing a 90-kDa cell-surface determinant selectively lost by multi-drug-resistant variants of CEM cells", *International journal of cancer.Journal international du cancer*, vol. 45, no. 1, pp. 95-103.
- De Milito, A. & Fais, S. 2005a, "Proton pump inhibitors may reduce tumour resistance", *Expert opinion on pharmacotherapy*, vol. 6, no. 7, pp. 1049-1054.
- De Milito, A. & Fais, S. 2005b, "Tumor acidity, chemoresistance and proton pump inhibitors", *Future oncology (London, England)*, vol. 1, no. 6, pp. 779-786.
- De Milito, A., Iessi, E., Logozzi, M., Lozupone, F., Spada, M., Marino, M.L., Federici, C., Perdicchio, M., Matarrese, P., Lugini, L., Nilsson, A. & Fais, S. 2007, "Proton pump inhibitors induce apoptosis of human B-cell tumors through a caspase-independent mechanism involving reactive oxygen species", *Cancer research*, vol. 67, no. 11, pp. 5408-5417.
- Deffie, A.M., Batra, J.K. & Goldenberg, G.J. 1989, "Direct correlation between DNA topoisomerase II activity and cytotoxicity in adriamycin-sensitive and -resistant P388 leukemia cell lines", *Cancer research*, vol. 49, no. 1, pp. 58-62.
- Endo, K., Maehara, Y., Ichiyoshi, Y., Kusumoto, T., Sakaguchi, Y., Ohno, S. & Sugimachi, K. 1996a, "Multidrug resistance-associated protein expression in clinical gastric carcinoma", *Cancer*, vol. 77, no. 8 Suppl, pp. 1681-1687.
- Endo, K., Maehara, Y., Kusumoto, T., Ichiyoshi, Y., Kuwano, M. & Sugimachi, K. 1996b, "Expression of multidrug-resistance-associated protein (MRP) and chemosensitivity in human gastric cancer", *International journal of cancer.Journal international du cancer*, vol. 68, no. 3, pp. 372-377.
- Engel, J.B., Schally, A.V., Halmos, G., Baker, B., Nagy, A. & Keller, G. 2005, "Targeted therapy with a cytotoxic somatostatin analog, AN-238, inhibits growth of human experimental endometrial carcinomas expressing multidrug resistance protein MDR-1", *Cancer*, vol. 104, no. 6, pp. 1312-1321.
- Erickson, K.L., Beutler, J.A., Cardellina II, J.H. & Boyd, M.R. 1997, "Salicylihalamides A and B, Novel Cytotoxic Macrolides from the Marine Sponge *Haliclona* sp", *The Journal of organic chemistry*, vol. 62, no. 23, pp. 8188-8192.
- Fais, S., De Milito, A., You, H. & Qin, W. 2007, "Targeting vacuolar H⁺-ATPases as a new strategy against cancer", *Cancer research*, vol. 67, no. 22, pp. 10627-10630.
- Finbow, M.E. & Harrison, M.A. 1997, "The vacuolar H⁺-ATPase: a universal proton pump of eukaryotes", *The Biochemical journal*, vol. 324 (Pt 3), no. Pt 3, pp. 697-712.
- Forgac, M. 1998, "Structure, function and regulation of the vacuolar (H⁺)-ATPases", *FEBS Letters*, vol. 440, no. 3, pp. 258-263.

- Gagliardi, S., Nadler, G., Consolandi, E., Parini, C., Morvan, M., Legave, M.N., Belfiore, P., Zocchetti, A., Clarke, G.D., James, I., Nambi, P., Gowen, M. & Farina, C. 1998, "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", *Journal of medicinal chemistry*, vol. 41, no. 10, pp. 1568-1573.
- Galinis, D.L., McKee, T.C., Pannell, L.K., Cardellina, J.H. & Boyd, M.R. 1997, "Lobatamides A and B, Novel Cytotoxic Macrolides from the Tunicate *Aplidium lobatum*†", *The Journal of organic chemistry*, vol. 62, no. 26, pp. 8968-8969.
- Gatenby, R.A. & Gillies, R.J. 2004, "Why do cancers have high aerobic glycolysis?", *Nature reviews.Cancer*, vol. 4, no. 11, pp. 891-899.
- Gocheva, V. & Joyce, J.A. 2007, "Cysteine cathepsins and the cutting edge of cancer invasion", *Cell cycle (Georgetown, Tex.)*, vol. 6, no. 1, pp. 60-64.
- Gottlieb, R.A., Giesing, H.A., Zhu, J.Y., Engler, R.L. & Babior, B.M. 1995, "Cell acidification in apoptosis: granulocyte colony-stimulating factor delays programmed cell death in neutrophils by up-regulating the vacuolar H⁽⁺⁾-ATPase", *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 13, pp. 5965-5968.
- Griffiths, J.R. 1991, "Are cancer cells acidic?", *British journal of cancer*, vol. 64, no. 3, pp. 425-427.
- Gruber, G. 2005, "Structural features and nucleotide-binding capability of the C subunit are integral to the regulation of the eukaryotic V1Vo ATPases", *Biochemical Society transactions*, vol. 33, no. Pt 4, pp. 883-885.
- Halaban, R., Patton, R.S., Cheng, E., Svedine, S., Trombetta, E.S., Wahl, M.L., Ariyan, S. & Hebert, D.N. 2002, "Abnormal acidification of melanoma cells induces tyrosinase retention in the early secretory pathway", *The Journal of biological chemistry*, vol. 277, no. 17, pp. 14821-14828.
- Hesselink, R.W., Fedorov, A., Hemminga, M.A. & Prieto, M. 2008, "Membrane-bound peptides from V-ATPase subunit a do not interact with an indole-type inhibitor", *Journal of peptide science : an official publication of the European Peptide Society*, vol. 14, no. 4, pp. 383-388.
- Hishita, T., Tada-Oikawa, S., Tohyama, K., Miura, Y., Nishihara, T., Tohyama, Y., Yoshida, Y., Uchiyama, T. & Kawanishi, S. 2001, "Caspase-3 activation by lysosomal enzymes in cytochrome c-independent apoptosis in myelodysplastic syndrome-derived cell line P39", *Cancer research*, vol. 61, no. 7, pp. 2878-2884.
- Horn, J. 2000, "The proton-pump inhibitors: similarities and differences", *Clinical therapeutics*, vol. 22, no. 3, pp. 266-80; discussion 265.
- Inoue, T., Wang, Y., Jefferies, K., Qi, J., Hinton, A. & Forgac, M. 2005, "Structure and regulation of the V-ATPases", *Journal of Bioenergetics and Biomembranes*, vol. 37, no. 6, pp. 393-398.
- Jain, V., Das, S.N., Luthra, K., Shukla, N.K. & Ralhan, R. 1997, "Differential expression of multidrug resistance gene product, P-glycoprotein, in normal, dysplastic and malignant oral mucosa in India", *International journal of cancer. Journal international du cancer*, vol. 74, no. 1, pp. 128-133.
- Johnson, L.L., Pavlovsky, A.G., Johnson, A.R., Janowicz, J.A., Man, C.F., Ortwine, D.F., Purchase, C.F., 2nd, White, A.D. & Hupe, D.J. 2000, "A rationalization of the acidic

- pH dependence for stromelysin-1 (Matrix metalloproteinase-3) catalysis and inhibition", *The Journal of biological chemistry*, vol. 275, no. 15, pp. 11026-11033.
- Juliano, R.L. & Ling, V. 1976, "A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants", *Biochimica et biophysica acta*, vol. 455, no. 1, pp. 152-162.
- Kane, P.M. 1999, "Introduction: V-ATPases 1992-1998", *Journal of Bioenergetics and Biomembranes*, vol. 31, no. 1, pp. 3-5.
- Kartner, N. & Ling, V. 1989, "Multidrug resistance in cancer", *Scientific American*, vol. 260, no. 3, pp. 44-51.
- Kato, Y., Lambert, C.A., Colige, A.C., Mineur, P., Noel, A., Frankenne, F., Foidart, J.M., Baba, M., Hata, R., Miyazaki, K. & Tsukuda, M. 2005, "Acidic extracellular pH induces matrix metalloproteinase-9 expression in mouse metastatic melanoma cells through the phospholipase D-mitogen-activated protein kinase signaling", *The Journal of biological chemistry*, vol. 280, no. 12, pp. 10938-10944.
- Kawasaki-Nishi, S., Nishi, T. & Forgacs, M. 2003, "Proton translocation driven by ATP hydrolysis in V-ATPases", *FEBS letters*, vol. 545, no. 1, pp. 76-85.
- Kim, J.W., Shin-Ya, K., Furihata, K., Hayakawa, Y. & Seto, H. 1999, "Oximidines I and II: Novel Antitumor Macrolides from *Pseudomonas* sp", *The Journal of organic chemistry*, vol. 64, no. 1, pp. 153-155.
- Kim, W.J., Kakehi, Y., Hirai, M., Arao, S., Hiai, H., Fukumoto, M. & Yoshida, O. 1995, "Multidrug resistance-associated protein-mediated multidrug resistance modulated by cyclosporin A in a human bladder cancer cell line", *Japanese journal of cancer research : Gann*, vol. 86, no. 10, pp. 969-977.
- Kim, W.J., Kakehi, Y., Kinoshita, H., Arao, S., Fukumoto, M. & Yoshida, O. 1996, "Expression patterns of multidrug-resistance (MDR1), multidrug resistance-associated protein (MRP), glutathione-S-transferase-pi (GST-pi) and DNA topoisomerase II (Topo II) genes in renal cell carcinomas and normal kidney", *The Journal of urology*, vol. 156, no. 2 Pt 1, pp. 506-511.
- Koshiyama, M., Fujii, H., Kinezaki, M., Morita, Y., Nanno, H. & Yoshida, M. 2001, "Immunohistochemical expression of topoisomerase IIalpha (Topo IIalpha) and multidrug resistance-associated protein (MRP), plus chemosensitivity testing, as chemotherapeutic indices of ovarian and endometrial carcinomas", *Anticancer Research*, vol. 21, no. 4B, pp. 2925-2932.
- Kunze B., Janse R., Sasse F., Höfle G. and Reichenbach H. 1998, "Apicularens A and B, New Cytostatic Macrolides from *Chondromyces* Species (Myxobacteria): Production, Physico-chemical and Biological Properties", *J.Antibiot.(Tokyo)*, vol. 51, no. 12, pp. 1075-1080.
- Kunze, B., Steinmetz, H., Hofle, G., Huss, M., Wiczorek, H. & Reichenbach, H. 2006, "Cruentaren, a new antifungal salicylate-type macrolide from *Byssovorax cruenta* (myxobacteria) with inhibitory effect on mitochondrial ATPase activity. Fermentation and biological properties", *The Journal of antibiotics*, vol. 59, no. 10, pp. 664-668.
- Kurashima, K., Numata, M., Yachie, A., Sai, Y., Ishizaka, N., Fujimura, M., Matsuda, T. & Ohkuma, S. 1996, "The role of vacuolar H(+)-ATPase in the control of intragranular pH and exocytosis in eosinophils", *Laboratory investigation; a journal of technical methods and pathology*, vol. 75, no. 5, pp. 689-698.

- Larsson, H., Mattson, H., Sundell, G. & Carlsson, E. 1985, "Animal pharmacodynamics of omeprazole. A survey of its pharmacological properties in vivo", *Scandinavian journal of gastroenterology. Supplement*, vol. 108, pp. 23-35.
- Lim, J.H., Park, J.W., Kim, M.S., Park, S.K., Johnson, R.S. & Chun, Y.S. 2006, "Bafilomycin induces the p21-mediated growth inhibition of cancer cells under hypoxic conditions by expressing hypoxia-inducible factor-1alpha", *Molecular pharmacology*, vol. 70, no. 6, pp. 1856-1865.
- Ling, V. 1997, "Multidrug resistance: molecular mechanisms and clinical relevance", *Cancer chemotherapy and pharmacology*, vol. 40 Suppl, pp. S3-8.
- Lu, X., Qin, W., Li, J., Tan, N., Pan, D., Zhang, H., Xie, L., Yao, G., Shu, H., Yao, M., Wan, D., Gu, J. & Yang, S. 2005, "The Growth and Metastasis of Human Hepatocellular Carcinoma Xenografts Are Inhibited by Small Interfering RNA Targeting to the Subunit ATP6L of Proton Pump", *Cancer Research*, vol. 65, no. 15, pp. 6843-6849.
- Luciani, F., Spada, M., De Milito, A., Molinari, A., Rivoltini, L., Montinaro, A., Marra, M., Lugini, L., Logozzi, M., Lozupone, F., Federici, C., Iessi, E., Parmiani, G., Arancia, G., Belardelli, F. & Fais, S. 2004, "Effect of proton pump inhibitor pretreatment on resistance of solid tumors to cytotoxic drugs", *Journal of the National Cancer Institute*, vol. 96, no. 22, pp. 1702-1713.
- Marshansky, V. & Futai, M. "The V-type H⁺-ATPase in vesicular trafficking: targeting, regulation and function", *Current Opinion in Cell Biology*, vol. In Press, Corrected Proof.
- Martinez-Zaguilan, R., Lynch, R.M., Martinez, G.M. & Gillies, R.J. 1993, "Vacuolar-type H(+)-ATPases are functionally expressed in plasma membranes of human tumor cells", *AJP - Cell Physiology*, vol. 265, no. 4, pp. C1015-1029.
- Martinez-Zaguilan, R., Seftor, E.A., Seftor, R.E., Chu, Y.W., Gillies, R.J. & Hendrix, M.J. 1996, "Acidic pH enhances the invasive behavior of human melanoma cells", *Clinical & experimental metastasis*, vol. 14, no. 2, pp. 176-186.
- Martínez-Zaguilán, R., Raghunand, N., Lynch, R.M., Bellamy, W., Martinez, G.M., Rojas, B., Smith, D., Dalton, W.S. & Gillies, R.J. 1999, "pH and drug resistance. I. functional expression of plasmalemmal V-type H⁺-ATPase in drug-resistant human breast carcinoma cell lines", *Biochemical Pharmacology*, vol. 57, no. 9, pp. 1037-1046.
- McLean, L.A., Roscoe, J., Jorgensen, N.K., Gorin, F.A. & Cala, P.M. 2000, "Malignant gliomas display altered pH regulation by NHE1 compared with nontransformed astrocytes", *American journal of physiology. Cell physiology*, vol. 278, no. 4, pp. C676-88.
- McLeod, H.L. & Evans, W.E. 1999, "Oral cancer chemotherapy: the promise and the pitfalls", *Clinical cancer research : an official journal of the American Association for Cancer Research*, vol. 5, no. 10, pp. 2669-2671.
- McSheehy, P.M., Troy, H., Kelland, L.R., Judson, I.R., Leach, M.O. & Griffiths, J.R. 2003, "Increased tumour extracellular pH induced by Bafilomycin A1 inhibits tumour growth and mitosis in vivo and alters 5-fluorouracil pharmacokinetics", *European journal of cancer (Oxford, England : 1990)*, vol. 39, no. 4, pp. 532-540.
- Meijer, A.J. & Codogno, P. 2004, "Regulation and role of autophagy in mammalian cells", *The international journal of biochemistry & cell biology*, vol. 36, no. 12, pp. 2445-2462.
- Montcourrier, P., Mangeat, P.H., Valembois, C., Salazar, G., Sahuquet, A., Duperray, C. & Rochefort, H. 1994, "Characterization of very acidic phagosomes in breast cancer

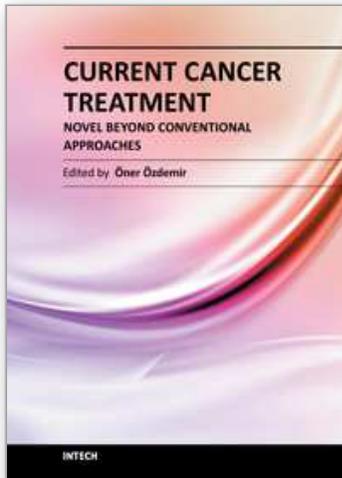
- cells and their association with invasion", *Journal of cell science*, vol. 107 (Pt 9), no. Pt 9, pp. 2381-2391.
- Montcourrier, P., Silver, I., Farnoud, R., Bird, I. & Rochefort, H. 1997, "Breast cancer cells have a high capacity to acidify extracellular milieu by a dual mechanism", *Clinical & experimental metastasis*, vol. 15, no. 4, pp. 382-392.
- Morimura, T., Fujita, K., Akita, M., Nagashima, M. & Satomi, A. 2008, "The proton pump inhibitor inhibits cell growth and induces apoptosis in human hepatoblastoma", *Pediatric surgery international*, vol. 24, no. 10, pp. 1087-1094.
- Morita, T., Nagaki, T., Fukuda, I. & Okumura, K. 1992, "Clastogenicity of low pH to various cultured mammalian cells", *Mutation research*, vol. 268, no. 2, pp. 297-305.
- Moriyama, Y. & Nelson, N. 1989, "H⁺-translocating ATPase in Golgi apparatus. Characterization as vacuolar H⁺-ATPase and its subunit structures", *Journal of Biological Chemistry*, vol. 264, no. 31, pp. 18445-18450.
- Mortimore, G.E., Hutson, N.J. & Surmacz, C.A. 1983, "Quantitative correlation between proteolysis and macro- and microautophagy in mouse hepatocytes during starvation and refeeding", *Proceedings of the National Academy of Sciences of the United States of America*, vol. 80, no. 8, pp. 2179-2183.
- Muller, O., Neumann, H., Bayer, M.J. & Mayer, A. 2003, "Role of the Vtc proteins in V-ATPase stability and membrane trafficking", *Journal of Cell Science*, vol. 116, no. 6, pp. 1107-1115.
- Murakami, T., Shibuya, I., Ise, T., Chen, Z.S., Akiyama, S., Nakagawa, M., Izumi, H., Nakamura, T., Matsuo, K., Yamada, Y. & Kohno, K. 2001, "Elevated expression of vacuolar proton pump genes and cellular PH in cisplatin resistance", *International journal of cancer. Journal international du cancer*, vol. 93, no. 6, pp. 869-874.
- 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. 1998, "(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", *Bioorganic & medicinal chemistry letters*, vol. 8, no. 24, pp. 3621-3626.
- Nakashima, S., Hiraku, Y., Tada-Oikawa, S., Hishita, T., Gabazza, E.C., Tamaki, S., Imoto, I., Adachi, Y. & Kawanishi, S. 2003, "Vacuolar H⁺-ATPase inhibitor induces apoptosis via lysosomal dysfunction in the human gastric cancer cell line MKN-1", *Journal of Biochemistry*, vol. 134, no. 3, pp. 359-364.
- Nanda, A., Brumell, J.H., Nordstrom, T., Kjeldsen, L., Sengelov, H., Borregaard, N., Rotstein, O.D. & Grinstein, S. 1996, "Activation of Proton Pumping in Human Neutrophils Occurs by Exocytosis of Vesicles Bearing Vacuolar-type H⁺-ATPases", *Journal of Biological Chemistry*, vol. 271, no. 27, pp. 15963-15970.
- Naramoto, H., Uematsu, T., Uchihashi, T., Doto, R., Matsuura, T., Usui, Y., Uematsu, S., Li, X., Takahashi, M., Yamaoka, M. & Furusawa, K. 2007, "Multidrug resistance-associated protein 7 expression is involved in cross-resistance to docetaxel in salivary gland adenocarcinoma cell lines", *International journal of oncology*, vol. 30, no. 2, pp. 393-401.
- Negendank, W. 1992, "Studies of human tumors by MRS: a review", *NMR in biomedicine*, vol. 5, no. 5, pp. 303-324.
- Nelson, H. & Nelson, N. 1989, "The progenitor of ATP synthases was closely related to the current vacuolar H⁺-ATPase", *FEBS letters*, vol. 247, no. 1, pp. 147-153.

- Nelson, N. 1992, "Evolution of organellar proton-ATPases", *Biochimica et biophysica acta*, vol. 1100, no. 2, pp. 109-124.
- Nelson, N. & Harvey, W.R. 1999, "Vacuolar and Plasma Membrane Proton-Adenosinetriphosphatases", *Physiological Reviews*, vol. 79, no. 2, pp. 361-385.
- Newell, K., Franchi, A., Pouyssegur, J. & Tannock, I. 1993, "Studies with glycolysis-deficient cells suggest that production of lactic acid is not the only cause of tumor acidity", *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, no. 3, pp. 1127-1131.
- Nielsen, D. & Skovsgaard, T. 1992, "P-glycoprotein as multidrug transporter: a critical review of current multidrug resistant cell lines", *Biochimica et biophysica acta*, vol. 1139, no. 3, pp. 169-183.
- Niikura, K. 2007, "Effect of a V-ATPase inhibitor, FR202126, in syngeneic mouse model of experimental bone metastasis", *Cancer chemotherapy and pharmacology*, vol. 60, no. 4, pp. 555-562.
- Nishi, T. & Forgac, M. 2002, "The vacuolar (H⁺)-ATPases--nature's most versatile proton pumps", *Nature reviews.Molecular cell biology*, vol. 3, no. 2, pp. 94-103.
- Ohta, T., Arakawa, H., Futagami, F., Fushida, S., Kitagawa, H., Kayahara, M., Nagakawa, T., Miyazaki, I., Numata, M. & Ohkuma, S. 1996, "A new strategy for the therapy of pancreatic cancer by proton pump inhibitor", *Gan to kagaku ryoho.Cancer & chemotherapy*, vol. 23, no. 12, pp. 1660-1664.
- Oude Ophuis, M.B., Mulder, T.P., Peters, W.H. & Manni, J.J. 1998, "Plasma glutathione S-transferase P1-1 levels in patients with head and neck squamous cell carcinoma", *Cancer*, vol. 82, no. 12, pp. 2434-2438.
- Parkins, C.S., Stratford, M.R., Dennis, M.F., Stubbs, M. & Chaplin, D.J. 1997, "The relationship between extracellular lactate and tumour pH in a murine tumour model of ischaemia-reperfusion", *British journal of cancer*, vol. 75, no. 3, pp. 319-323.
- Peng, S., Stone, D. & Xie, X. 1993, "Reconstitution of recombinant 40-kDa subunit of the clathrin-coated vesicle H⁽⁺⁾-ATPase", *Journal of Biological Chemistry*, vol. 268, no. 31, pp. 23519-23523.
- Perez-Sayans, M., Garcia-Garcia, A., Reboiras-Lopez, M.D. & Gandara-Vila, P. 2009, "Role of V-ATPases in solid tumors: Importance of the subunit C (Review)", *International journal of oncology*, vol. 34, no. 6, pp. 1513-1520.
- Perez-Sayans, M., Reboiras-Lopez, M.D., Somoza-Martin, J.M., Barros-Angueira, F., Gayoso Diz, P., Gandara Rey, J.M. & Garcia-Garcia, A. 2010, "Measurement of ATP6V1C1 expression in brush cytology samples as a diagnostic and prognostic marker in oral squamous cell carcinoma", *Cancer biology & therapy*, vol. 9, no. 12.
- Perez-Sayans, M., Somoza-Martin, J.M., Barros-Angueira, F., Rey, J.M. & Garcia-Garcia, A. 2009, "V-ATPase inhibitors and implication in cancer treatment", *Cancer treatment reviews*, vol. 35, no. 8, pp. 707-713.
- Perona, R. & Serrano, R. 1988, "Increased pH and tumorigenicity of fibroblasts expressing a yeast proton pump", *Nature*, vol. 334, no. 6181, pp. 438-440.
- Petrangolini, G., Supino, R., Pratesi, G., Bo, L.D., Tortoreto, M., Croce, A.C., Misiano, P., Belfiore, P., Farina, C. & Zunino, F. 2006, "Effect of a Novel Vacuolar-H⁺-ATPase Inhibitor on Cell and Tumor Response to Camptothecins", *Journal of Pharmacology And Experimental Therapeutics*, vol. 318, no. 3, pp. 939-946.

- Qi, J., Wang, Y. & Forgac, M. 2007, "The vacuolar (H⁺)-ATPase: subunit arrangement and in vivo regulation", *Journal of Bioenergetics and Biomembranes*, vol. 39, no. 5-6, pp. 423-426.
- Raghunand, N., Mahoney, B., van Sluis, R., Baggett, B. & Gillies, R.J. 2001, "Acute metabolic alkalosis enhances response of C3H mouse mammary tumors to the weak base mitoxantrone", *Neoplasia (New York, N.Y.)*, vol. 3, no. 3, pp. 227-235.
- Raghunand, N., Martínez-Zaguilán, R., Wright, S.H. & Gillies, R.J. 1999, "pH and drug resistance. II. turnover of acidic vesicles and resistance to weakly basic chemotherapeutic drugs", *Biochemical Pharmacology*, vol. 57, no. 9, pp. 1047-1058.
- Rofstad, E.K., Mathiesen, B., Kindem, K. & Galappathi, K. 2006, "Acidic extracellular pH promotes experimental metastasis of human melanoma cells in athymic nude mice", *Cancer research*, vol. 66, no. 13, pp. 6699-6707.
- Rozhin, J., Sameni, M., Ziegler, G. & Sloane, B.F. 1994, "Pericellular pH affects distribution and secretion of cathepsin B in malignant cells", *Cancer research*, vol. 54, no. 24, pp. 6517-6525.
- Ruzza, P., Rosato, A., Rossi, C.R., Floreani, M. & Quintieri, L. 2009, "Glutathione transferases as targets for cancer therapy", *Anti-cancer agents in medicinal chemistry*, vol. 9, no. 7, pp. 763-777.
- Saroussi, S. & Nelson, N. 2008, "Vacuolar H⁽⁺⁾-ATPase-an enzyme for all seasons", *Pflugers Archiv : European journal of physiology*, .
- Sasazawa, Y., Futamura, Y., Tashiro, E. & Imoto, M. 2009, "Vacuolar H⁽⁺⁾-ATPase inhibitors overcome Bcl-xL-mediated chemoresistance through restoration of a caspase-independent apoptotic pathway", *Cancer science*, .
- Sasse, F., Steinmetz, H., Hofle, G. & Reichenbach, H. 2003, "Archazolids, new cytotoxic macrolactones from *Archangium gephyra* (Myxobacteria). Production, isolation, physico-chemical and biological properties", *The Journal of antibiotics*, vol. 56, no. 6, pp. 520-525.
- Scheidt, K.A., Bannister, T.D., Tasaka, A., Wendt, M.D., Savall, B.M., Fegley, G.J. & Roush, W.R. 2002, "Total Synthesis of (-)-Bafilomycin A1", *Journal of the American Chemical Society*, vol. 124, no. 24, pp. 6981-6990.
- Schoonderwoert, V.T.G., Holthuis, J.C.M., Tanaka, S., Tooze, S.A. & Martens, G.J.M. 2000, "Inhibition of the vacuolar H⁺-ATPase perturbs the transport, sorting, processing and release of regulated secretory proteins", *European Journal of Biochemistry*, vol. 267, no. 17, pp. 5646-5654.
- Senoune, S.R., Luo, D. & Martinez-Zaguilan, R. 2004, "Plasmalemmal vacuolar-type H⁺-ATPase in cancer biology", *Cell biochemistry and biophysics*, vol. 40, no. 2, pp. 185-206.
- Senoune, S.R. & Martinez-Zaguilan, R. 2007, "Plasmalemmal vacuolar H⁺-ATPases in angiogenesis, diabetes and cancer", *Journal of Bioenergetics and Biomembranes*, vol. 39, no. 5-6, pp. 427-433.
- Senoune, S.R., Bakunts, K., Martinez, G.M., Chua-Tuan, J.L., Kebir, Y., Attaya, M.N. & Martinez-Zaguilan, R. 2004, "Vacuolar H⁺-ATPase in human breast cancer cells with distinct metastatic potential: distribution and functional activity", *AJP - Cell Physiology*, vol. 286, no. 6, pp. C1443-1452.
- Shen, R., Lin, C.T., Bowman, E.J., Bowman, B.J. & Porco, J.A., Jr 2003, "Lobatamide C: total synthesis, stereochemical assignment, preparation of simplified analogues, and V-

- ATPase inhibition studies", *Journal of the American Chemical Society*, vol. 125, no. 26, pp. 7889-7901.
- Shi, H., Lu, D., Shu, Y., Shi, W., Lu, S. & Wang, K. 2008, "Expression of multidrug resistance-related proteins p-glycoprotein, glutathione-s-transferases, topoisomerase-II and lung resistance protein in primary gastric cardiac adenocarcinoma", *Hepato-gastroenterology*, vol. 55, no. 86-87, pp. 1530-1536.
- Smith A.N., Lovering R.C., Futai M., Takeda J., Brown D. & Karet F.E. 2003, "Revised Nomenclature for Mammalian Vacuolar-Type H⁺-ATPase Subunit Genes", *Molecular Cell*, vol. 12, pp. 801-803.
- Smith, A.N., Jouret, F., Bord, S., Borthwick, K.J., Al-Lamki, R.S., Wagner, C.A., Ireland, D.C., Cormier-Daire, V., Frattini, A., Villa, A., Kornak, U., Devuyst, O. & Karet, F.E. 2005, "Vacuolar H⁺-ATPase d2 subunit: molecular characterization, developmental regulation, and localization to specialized proton pumps in kidney and bone", *Journal of the American Society of Nephrology : JASN*, vol. 16, no. 5, pp. 1245-1256.
- Stevens, T.H. & Forgac, M. 1997, "Structure, function and regulation of the vacuolar (H⁺)-ATPase", *Annual Review of Cell and Developmental Biology*, vol. 13, pp. 779-808.
- Sun-Wada, G.H., Wada, Y. & Futai, M. 2003, "Vacuolar H⁺ pumping ATPases in luminal acidic organelles and extracellular compartments: common rotational mechanism and diverse physiological roles", *Journal of Bioenergetics and Biomembranes*, vol. 35, no. 4, pp. 347-358.
- Sun-Wada, G., Wada, Y. & Futai, M. 2004, "Diverse and essential roles of mammalian vacuolar-type proton pump ATPase: toward the physiological understanding of inside acidic compartments", *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, vol. 1658, no. 1-2, pp. 106-114.
- Tanigawara, Y. 2000, "Role of P-glycoprotein in drug disposition", *Therapeutic drug monitoring*, vol. 22, no. 1, pp. 137-140.
- Torigoe, T., Izumi, H., Ishiguchi, H., Uramoto, H., Murakami, T., Ise, T., Yoshida, Y., Tanabe, M., Nomoto, M., Itoh, H. & Kohno, K. 2002, "Enhanced expression of the human vacuolar H⁺-ATPase c subunit gene (ATP6L) in response to anticancer agents", *The Journal of biological chemistry*, vol. 277, no. 39, pp. 36534-36543.
- Uematsu, T., Hasegawa, T., Hiraoka, B.Y., Komatsu, F., Matsuura, T., Yamada, A.S. & Yamaoka, M. 2001, "Multidrug resistance gene 1 expression in salivary gland adenocarcinomas and oral squamous-cell carcinomas", *International journal of cancer. Journal international du cancer*, vol. 92, no. 2, pp. 187-194.
- van Hille, B., Vanek, M., Richener, H., Green, J.R. & Bilbe, G. 1993, "Cloning and tissue distribution of subunits C, D, and E of the human vacuolar H⁽⁺⁾-ATPase", *Biochemical and biophysical research communications*, vol. 197, no. 1, pp. 15-21.
- Volk, C., Albert, T. & Kempfski, O.S. 1998, "A proton-translocating H⁺-ATPase is involved in C6 glial pH regulation", *Biochimica et biophysica acta*, vol. 1372, no. 1, pp. 28-36.
- Wada, Y., Sun-Wada, G.H., Tabata, H. & Kawamura, N. 2008, "Vacuolar-type proton ATPase as regulator of membrane dynamics in multicellular organisms", *Journal of Bioenergetics and Biomembranes*, vol. 40, no. 1, pp. 53-57.
- Wang, X., Pavelic, Z.P., Li, Y., Gleich, L., Gartside, P.S., Pavelic, L., Gluckman, J.L. & Stambrook, P.J. 1997, "Overexpression and amplification of glutathione S-transferase pi gene in head and neck squamous cell carcinomas", *Clinical cancer*

- research : an official journal of the American Association for Cancer Research*, vol. 3, no. 1, pp. 111-114.
- Wilkens, S., Zhang, Z. & Zheng, Y. 2005, "A structural model of the vacuolar ATPase from transmission electron microscopy", *Micron*, vol. 36, no. 2, pp. 109-126.
- Wu, Y.C., Wu, W.K., Li, Y., Yu, L., Li, Z.J., Wong, C.C., Li, H.T., Sung, J.J. & Cho, C.H. 2009, "Inhibition of macroautophagy by bafilomycin A1 lowers proliferation and induces apoptosis in colon cancer cells", *Biochemical and biophysical research communications*, vol. 382, no. 2, pp. 451-456.
- Xie, Z.J., Yang, X.F., Gu, Z.Y. & Wu, Q.L. 2000, "P-glycoprotein expression in squamous cell carcinoma of the oral and maxillofacial region", *The Chinese journal of dental research : the official journal of the Scientific Section of the Chinese Stomatological Association (CSA)*, vol. 3, no. 1, pp. 23-26.
- Xu, J., Feng, H.T., Wang, C., Yip, K.H., Pavlos, N., Papadimitriou, J.M., Wood, D. & Zheng, M.H. 2003, "Effects of Bafilomycin A1: an inhibitor of vacuolar H (+)-ATPases on endocytosis and apoptosis in RAW cells and RAW cell-derived osteoclasts", *Journal of cellular biochemistry*, vol. 88, no. 6, pp. 1256-1264.
- Yajima, T., Ochiai, H., Uchiyama, T., Takano, N., Shibahara, T. & Azuma, T. 2009, "Resistance to cytotoxic chemotherapy-induced apoptosis in side population cells of human oral squamous cell carcinoma cell line Ho-1-N-1", *International journal of oncology*, vol. 35, no. 2, pp. 273-280.
- Yamagata, M., Hasuda, K., Stamato, T. & Tannock, I.F. 1998, "The contribution of lactic acid to acidification of tumours: studies of variant cells lacking lactate dehydrogenase", *British journal of cancer*, vol. 77, no. 11, pp. 1726-1731.
- 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. 2009, "Small interfering RNA targeting the subunit ATP6L of proton pump V-ATPase overcomes chemoresistance of breast cancer cells", *Cancer letters*, vol. 280, no. 1, pp. 110-119.
- Zhang, L., Xiao, Y. & Priddy, R. 1994, "Increase in placental glutathione S-transferase in human oral epithelial dysplastic lesions and squamous cell carcinomas", *Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology*, vol. 23, no. 2, pp. 75-79.
- Zhang, P., Zhang, Z., Zhou, X., Qiu, W., Chen, F. & Chen, W. 2006, "Identification of genes associated with cisplatin resistance in human oral squamous cell carcinoma cell line", *BMC cancer*, vol. 6, pp. 224.
- Zhong, H., De Marzo, A.M., Laughner, E., Lim, M., Hilton, D.A., Zagzag, D., Buechler, P., Isaacs, W.B., Semenza, G.L. & Simons, J.W. 1999, "Overexpression of hypoxia-inducible factor 1alpha in common human cancers and their metastases", *Cancer research*, vol. 59, no. 22, pp. 5830-5835.



Current Cancer Treatment - Novel Beyond Conventional Approaches

Edited by Prof. Oner Ozdemir

ISBN 978-953-307-397-2

Hard cover, 810 pages

Publisher InTech

Published online 09, December, 2011

Published in print edition December, 2011

Currently there have been many armamentaria to be used in cancer treatment. This indeed indicates that the final treatment has not yet been found. It seems this will take a long period of time to achieve. Thus, cancer treatment in general still seems to need new and more effective approaches. The book "Current Cancer Treatment - Novel Beyond Conventional Approaches", consisting of 33 chapters, will help get us physicians as well as patients enlightened with new research and developments in this area. This book is a valuable contribution to this area mentioning various modalities in cancer treatment such as some rare classic treatment approaches: treatment of metastatic liver disease of colorectal origin, radiation treatment of skull and spine chordoma, changing the face of adjuvant therapy for early breast cancer; new therapeutic approaches of old techniques: laser-driven radiation therapy, laser photo-chemotherapy, new approaches targeting androgen receptor and many more emerging techniques.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Mario Pérez-Sayáns and Abel García García (2011). V-ATPase Inhibitors in Cancer Treatment and Their Implication in Multidrug Resistance in Oral Squamous Cell Carcinoma, Current Cancer Treatment - Novel Beyond Conventional Approaches, Prof. Oner Ozdemir (Ed.), ISBN: 978-953-307-397-2, InTech, Available from: <http://www.intechopen.com/books/current-cancer-treatment-novel-beyond-conventional-approaches/v-atpase-inhibitors-in-cancer-treatment-and-their-implication-in-multidrug-resistance-in-oral-squamo>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen