CURRENT CANCER TREATMENT

NOVEL BEYOND CONVENTIONAL APPROACHES

Edited by **Öner Özdemir**

INTECH

Current Cancer Treatment – Novel Beyond Conventional Approaches
Edited by Öner Özdemir

Published by InTech
Janeza Trdine 9, 51000 Rijeka, Croatia

Publishing Process Manager Masa Vidovic
Technical Editor Teodora Smiljanic
Cover Designer InTech Design Team
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First published November, 2011
Printed in Croatia

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Current Cancer Treatment – Novel Beyond Conventional Approaches,
Edited by Öner Özdemir
p. cm.
ISBN 978-953-307-397-2

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

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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-APTases), 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 (Wilkens, 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).

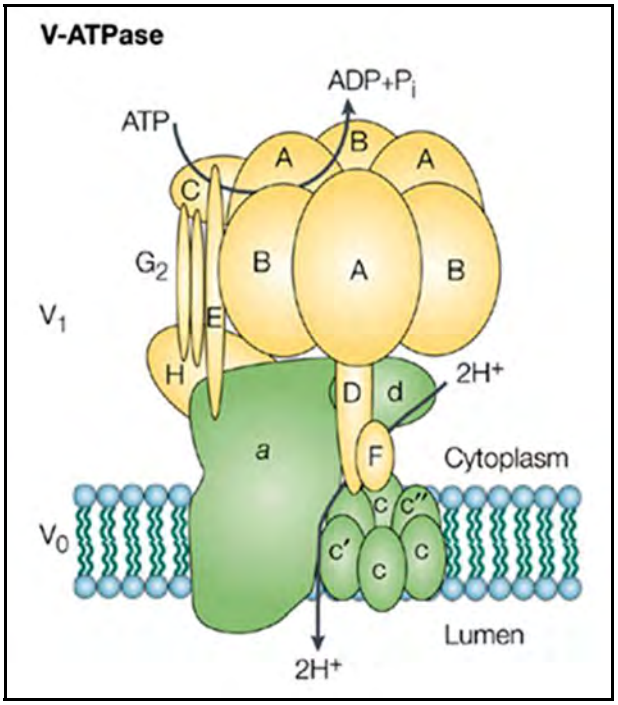

Fig. 1. Diagram of V-ATPase. The cytosolic domain (in yellow) is formed by three A subunits, three B subunits, three G subunits, and one C, D, E, F, and H subunit. The V0 transmembrane domain is formed by five subunits: a, c, c', c'' and d. The V1 domain contains the catalytic unit (Nishi & Forgac 2002).

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 salicylihalamide (Erickson et al. 1997), lobatamide A and B (Galinis et al. 1997), apicularen (Kunze B., Janse R., Sasse F., Höfle G. and Reichenbach H. 1998), indolyls (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	*Streptomyces*	Unknown	V-ATPases inhibition Ionophoric properties
Benzolactone enamides	Salicylihamide A	Macrocyclic salicylate	Sponge *Haliclona* sp.	VO complex	Animal V-ATPases inhibition Cytotoxin
	Apicularens	Lactone ring	*Chondromyces*	VO complex	Highly toxic for human and animal cell
	Lobatamides	Substitution of enamide NH, salicylate, and phenyl salicylate	*Tunicate Aplidium lobatum*	VO complex	Animal and mammalian V-ATPases inhibition
	Oximidines	Lactone ring	*Pseudomonas* sp.	VO complex	Animal and mammalian V-ATPases inhibition
	Cruentaren	Lactone ring	*Byssovorax cruenta*	VO complex	Cytotoxicity on mammalian and fungal cells at mitochondrial F-ATPases
Archazolid		Macrocyclic lactone ring with a thiazole side	*Archangium gephyra Cystobacter violaceus*	VO subunit c	Cytotoxicity on mammalian cell line
Indolyls		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 (TBTCl), 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).

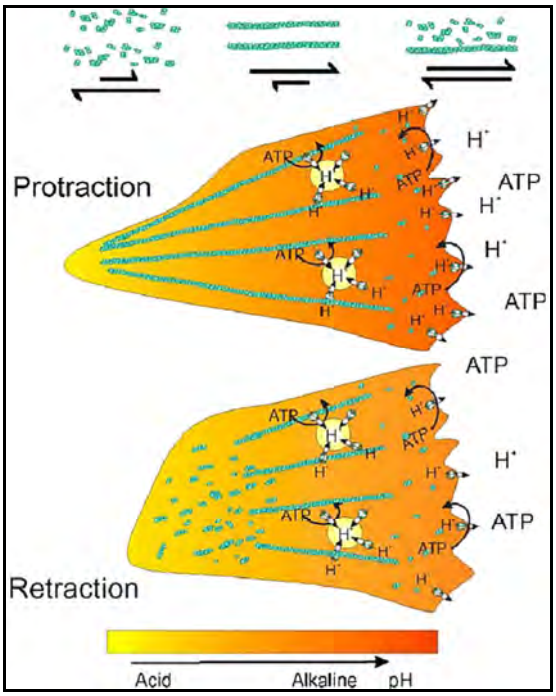

Fig. 2. Proposed mechanism by which overexpression of pmVATPase at the leading edge of the cell modulates cell migration/invasion. The proposed model should be viewed as a framework to explain how pmV-ATPases determine the acquisition of an invasive phenotype needed for angiogenesis and metastasis. Changes in pHcyt 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 & Kempski 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 1α (HIF-1α) (Zhong et al. 1999). The interaction of bafilomycin with HIF-1α 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).
ancer 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).

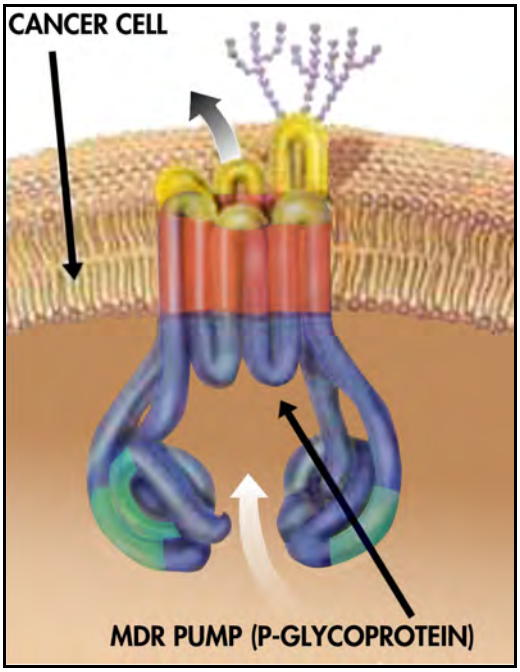

Fig. 3. Pgp resides in the cell membrane, where it may act to pump toxins out of the cell. The painting shows a model of the protein's structure that is based on its known sequence of amino acids. The protein chain is thought to snake back and forth 12 times across the lipid bilayer of the membrane forming 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. sugesting 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 Milito, 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). Becellli 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 Milito, 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

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