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Research Article

Proton Pump Inhibitors and Melanocytes

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ABSTRACT
Melanocytic disorders comprise of vitiligo as well as melanoma both of which constitute the extreme ends of this spectrum. Also both of these have proven to be formidable challenges in terms of treatment. Hence a variety of drugs have undergone experimentation in this field and its benefits and side effects discussed. Proton pump inhibitors (PPI) are popular class of drugs used in gastroesophageal disorder management. These drugs have gained popularity due to their efficacy and minimal toxicity. This article is an attempt to elucidate the various mechanisms involving role of PPIs in the above mentioned melanocytic disorders.

Keywords: Chemoresistance, Melanocytes, Melanoma, PPI, Vitiligo.

INTRODUCTION

Melanocytes are present in the basal cells of epidermis which are specialized in the melanin synthesis. Melanosome is a lysosome related organelle which synthesizes melanin. Melanocytic disorders comprise of vitiligo as well as melanoma which constitute the extreme ends of the spectrum. Both of these conditions have been proven to be formidable challenges in terms of treatment. Hence, variety of drugs have undergone experimentation in this field to explore their benefits and side effects.

Proton Pump Inhibitors and Vitiligo

Drugs like Proton pump inhibitor (PPI) have been tried in the treatment of melanoma and other tumors to minimize chemoresistance and achieve enhanced therapeutic effect. PPI have also been pointed as a culprit for induction of vitiligo. Many other drugs when used therapeutically to treat certain diseases have been known to induce or worsen vitiligo as an untoward effect. Based on the analysis of published studies various classes of drugs have been correlated with vitiligo.

Meta analysis done by Curzytek et al in 2007[1] enumerated the drugs inducing vitiligo as follows

- Anticonvulsants – carbamazepine, valproic acid, flunazepam.
- Antimalarial drugs – chloroquine and quinine.
- Biological drugs – interleukin-2, interleukin-4, interferon alpha, infliximab.
- Drugs for parkinsons disease- tolcapone, levodopa.
- External drugs for alopecia – diphencyprone, squaric acid, dibutylester.
- Other drugs – fluphenazine, clofazamine, dopamine, hydroquinone, monobenzyl ether ester, gancyclovir, beta blockers, lispro insulin.

Pathophysiological mechanisms of drug induced vitiligo suggested in the reported cases are as follows

- Drug induced activation of cytotoxic T cells directed against melanocyte antigens like - MART-1/Melan A, gp 100, TRP-1, TRP-2.
- Neural hypothesis - melanocytic injury arising as a result of excess release of norepinephrine a malanocytotoxin from the autonomic nerve endings into microenvironment of melanocytes, injures the cells. Enzyme monoamino oxidase, a catecholamine degrading enzyme is induced by norepinephrine which causes hydrogen peroxide toxicity around the melanocytes.
- Direct, cytotoxic effects of drugs on melanocytes-apoptosis[1]
- Melanocytic damage due to altered melanosome membrane permeability which normally prevents diffusion of toxic melanin precursors in to cytoplasm and may cause melanocyte damage[2]. Defect either acquired or inherited in the membranes of melanosomes results into loss of melanocytes and cause vitiligo.
- Autoimmune mechanism – autoimmune loss of melanocytes from the involved area lead to vitiligo which is frequently associated with other autoimmune diseases like rheumatoid arthritis, type I diabetes mellitus, addisons disease and was correlated to the genetic susceptibility[2].

Apart from above mentioned drugs PPI have also been observed to induce or worsen vitiligo lesions in the genetically predisposed patients. They are promising and popular drugs for the treatment of gastro oesophageal disorders being more effective with negligible adverse effects. PPI are prodrugs which get activated in acidic pH. Recent evidence suggests that PPI are delivered not only

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to proton pumps in the gastric mucosa but also to the other acidic compartments like skin as in case of vitiligo patients[3]. Melanocytes have H+ K+ ATPase at cytoplasmic membrane and melanosomes have vacuolar H+ K+ ATPpase. Vitiligo is an acquired depigmentation disorder of skin characterized by whitish patches. Schalleurter and Rokos in 2007[4] and Holla et al in 2011[5] have reported four and one patients respectively of vitiligo who developed new depigmented lesions following oral administration of PPI despite successful treatment with UVB therapy. The mechanism attributed for this was the blockade of H+ K+ ATPase on melanocytic cytoplasmic receptors. Another more accepted explanation was apoptotic melanocytic loss. PPI make melanocytes prone for apoptosis by modifying pH gradient in melanocytes and enhancing oxidative stress in melanocytes. Melanogenesis is a complex process which involves multiple metabolic signals and enzymes in the human melanocytes. Melanin pigment is derived from hydroxylation of L- tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) by enzyme tyrosinase (TYR) with two copper ions at the active centre[6]. Melanin biosynthesis is modulated by expression of microphthalmia associated transcription factor (MITF) which is an integral transcription factor regulating melanocyte differentiation and pigmentation. MITF also regulates the transcriptional expression of the TYR, TYR related protein -1 (TRP-1) and TYR related protein-2(TRP-2) genes in melanocytes[7,8]. An in vitro and in vivo study was conducted by Shin et al in 2014 to assess the effect of PPI on melanogenesis. In vitro study it was observed by these authors that, PPI decreased melanin synthesis but they could not prove apoptosis as a mechanism which was seen in other studies as there was no change in apoptotic markers like bcl 2 and bax after PPI treatment. Similarly, they could not confirm the H+ K+ ATPase inhibition modulating melanogenesis. Thereby they support the theory of tyrosinase enzyme inhibition as an explaination for worsening of vitiligo[9]. The Esmoprazole (ESOM) a prototype of PPI can trigger vitiligo through an inhibitory effect on melanosome maturation and also add oxidative stress in melanocytes of vitiligo[3]. PPI also block MNK a P type ATPase which is localised in a transgolgi network and helps in transportation of copper to tyrosinaase which is synthesized within the secretory pathway[10]. But this did not explain satisfactorily depigmentation as a main pathology in vitiligo, as there is a loss of melanocyte rather than reduced melanogenesis, Hence, the later can be considered as an epiphenomenon[11]. But Schareuter K et al postulated that melanocyte are not immediately destroyed in vitiligo but are progressively suppressed by inhibition of melanisation which further induces death of melanocytes[12]. Matsui MS et al (2015) demonstrated that treatment with omeprazole reduced melanogenesis significantly by inhibiting ATP-7 gene expression as well as it increased the degradation of tyrosinase[13]. Baek et el (2015) studied the effect of PPI on melanogenesis. They concluded that PPI decreased melanogenesis. Rabeprazole and not other PPI strongly inhibited mushroom tyrosinase in dose dependent manner. All the studied PPI like rabeprazole, ESOM, lansoprazole and pantoprazole inhibited copper chelating activity. Treatment of melanin a cell with 100 µM concentration of PPI significantly reduced melanin content of melanin cells. But it did not manifest into cytotoxic effects. Rabeprazole strongly supressed the mRNA expression of each of melanogenesis associated genes (TYR, TRP-1, TRP-2 and MITF). Other PPI don't have effects of TYR expression levels which indicates they may be reducing melanogenesis by some other mechanism like via copper chelating activity and not via effects on TYR activity[14]. Rise in pH by V-ATPase in melanosomes enhances tyrosinase activity and facilitates melanogenesis. PPI activity on melanosome V-ATPase should stimulate melanogenesis as they raise melanosome pH[15]. On the contrary PPI are found as culprit for initiation and progression of the vitiligo by inhibiting melanogenesis. Hence other mechanisms like apoptosis of melanocytes, increase in oxidative stress and downregulation of genes responsible for melanogenesis (TYR, TRP-1, TRP-2, and MITF) may be playing role in reduced melanocyte activity leading to vitiligo. In vitro studies by Shin JM et al (2014) revealed decreased melanin content by inhibition or negative regulation of tyrosinase activity and tyrosinase related protein-l (TRP-1). Hence it is possible that despite rise in melanosome pH by PPI, they may be inhibiting tyrosinase activity directly irrespective of pH modulation[9].

Role of PPI in modifying chemoresistance in malignancy

Modification of pH of melanocytes by PPI has been proposed as a therapeutic tool in treating tumors like melanoma by overcoming chenoresistance. V-ATPase is large multiunit proton pumps distributed within plasma membrane and membranes of some organelles such as lysosomes, endosomes and secretory vesicles. Their housekeeping function consists of acidifying lysosomes, endosomes and phagosomes compartments and extracellular environment in certain specialized cells[16,17]. Mammalian V-ATPase are composed of 13 different subunits which are divided into 8 peripheral proteins called as catalytic V1 domain and 5 membrane intrinsic proteins also called as V0 domain[18,19]. V- ATPase is referred to formally as ATP-6[20]. V1 subunits are A, B, C, D, E, F and H and V0 subunits are a,b,c,d, and e, V1 is responsible for ATP hydrolysis and V0 provides transmembraneous transport of protons across cell membrane to extracellular milleu or across the organelle membrane to intracellular compartments making extracellular pH acidic, intracellular cytosolic pH alkaline around 7 and lysosomal pH acidic. This pH regulation is required for regulation of physiological functions of cell[16,17,19]. V-ATPase has close relation to several diseases and most important being tumor where it enhances carcinogenesis, malignant transformation, growth, proliferation, cancer progression invasion and metastasis. It also correlates to the acquired mutlidrug resistance. Hence V-ATPase inhibition is considered as effective target for anticancer therapy[21]. Agents altering tumor pH homeostasis, exert antitumor effect by inhibiting tumor growth, metastasis and by reverting drug resistance[22-25]. Subunit c of V- ATPase is

correlated with proliferation and metastasis of tumor cells. In vitro or in animal models metastasis was found to be supressed by inhibition of V- ATPase c subunit through si RNA[24]. Most cancer cells predominantly derive energy by high rate of glycolysis leading to lactic acid fermentation in the cytosol. This is in contrast with majority of the normal cells which have low rate of glycolysis followed by oxidation of pyruvate in the mitochondria. The Warberg effect is a result of damage to mitochondria in a cancer cells or could be an adaptation to the low oxygen environment in the tumor cells. In tumor cells there is a shift of energy production from oxidative phophorylation to aerobic glycolysis known as ‘’Warburg effect’ leading to intra cellular acidosis which is not conducive for their survival[26]. For cancer cells survival and to get protected from self digestion intracellular proton needs to get extruded to maintain cytosolic pH neutral to alkaline[27-29]. V- ATPases are most important among the four types of pH regulators. The other three being Na+ H+ exchanger, bicarbonate transporters, proton/lactate symporter. Proton pumps in the tumor cells are essential for V- ATPases to regulate cytosol pH[30]. Tumor cell needs to extrude excessive cytosolic acid for its survival and to protect from apoptosis. V- ATPase plays a major role by upregulation of proton pumps. Supression of this proton extrusion by inhibiting V- ATPase make the tumor cell more susceptible to cell death than normal cell. The inhibition of V- ATPase may induce apoptotic cell death in several human cancer cell lines like liver cancer[31] breast cancer[32], gastric cancer[33], B cell hybridoma cell[34,35]. The inhibition of V-ATPase will decrease cytosolic pH, increase lysosomal pH affecting lysosomal function. V- ATPase induced apoptosis is either through lysosome mediation or non lysosomal one. Lysosomal mediated apoptosis is due to increased lysosomal pH and permeability triggering release of cathepsin D and activation of caspase[33]. Inhibition of V- ATP ase is known to induce apoptosis by suppressing anti apoptotic bcl-2 or bcl–xl and facilitates the caspase independent apoptotic pathway[36]. Sometimes mitochondria and lysosomes are affected by V- ATPase inhibition resulting into apoptosis via caspase pathway or ROS dependent manner due to ROS accumulation[35,37]. It has also been reported that V- ATPase are known to regulate tumor associated m TOR (mammalian target of rapamycin)[38]. Notch[39,40] or Wnt[41,42]. Activation of Notch is involved in V- ATPase associated endosomal system[40,43]. V-ATPase activity is required for notch activation which is a common hallmark of increasing number of cancers[44,45]. Tumor metastasis initiates with breaking through basement membrane, degrading extracellular matrix, initiating angiogenesis and invasion of vascular system. The invasive phenotype is related to highly active V-ATPase causing activation of proteases and breaking down of extracellular matrix. Several types of tumors including melanoma[46], pancreatic cancer[47] and breast cancer[48,49] are reported to be correlated with the improper action of V-ATPase. Active V-ATPase enhances tumor invasion and metastasis in extracellular acidic milieu by activating proteases. The plasma membrane V-ATPase are responsible for extruding cytosol protons to the extracellular space resulting into low extracellular pH which is required for activation of several types of proteases including cathepsin, metalloproteases and gelatinases[50]. Activated extracellular cathepsin degrades extracellular matrix proteins and activates other secreted proteases like matrix metalloprotease causing invasion[51,52] and gelatinases[53]. The plasma membrane V-ATPase gets recruited at the proceeding edge of cancer cells by interaction with F-actin which produces acidic microenvironment at the edge[49]. Intracellular V ATPase also facilitates the invasion and metastasis possibly due to modulating proteolytic activity of cathepsin or matrix metalloproteases within lysosomes or secretory vesicles and shifting the proteases containing secretory vesicles to the cell surface to be extracytosed[49]. Thus cutting edge is conferred upon tumor cells by accumulation of acidic concentration of plasma membrane, V-ATPases and activated proteases crown on the proceeding surface of metastatic cells. Acquired multidrug resistance (MDR) limits the therapeutic potential and invites relapse. MDR is known to correlate with family of ATP binding cassette (ABC) proteins P glycoprotein (Pg). V ATPase is also known to play role in MDR in pg independent manner. Inhibition of V- ATPase not only suppresses tumor cells directly but also sensitizes tumor cells to chemotherapy[54]. It is documented that pre-treatment with PPI sensitised tumor cells for cisplatin, 5- fluorouracil and vinblastin. Increase in both external pH and pH of cytosomal organelle by PPI, enhanced cytosolic retention of these drugs. Oral pretreatment with omeprazole sensitised human solid tumors to cisplatin[55]. V ATPase produces multidrug resistance by neutralizing drug intracellularly and extracellularly, decreasing drug internalization, altering DNA repair and inhibition of apoptosis. Tumor microenvironment pH influence uptake of anticancer drugs. As extracellular pH in tumors is low and intracellular pH is neutral to alkaline, weakly basic drugs having acid dissociation constant of 7.5-9.5, such as doxorubicin, mitoxantrone, vinblastin and vincristine get protonated causing decreased cellular uptake[56-58]. In vitro or animal models studies indicate that substantial improvement takes place in therapeutic effectiveness of antitumor drugs by extra cellular alkalinization leading to the enhanced cellular drug uptake and cytotoxicity[57,27]. Reduced intracellular accumulation of antitumour drugs may also be due to the role of V-ATPase as a cooperating factor to ATP dependent membrane protein that functions as drug efflux pumps[56]. The drugs which are known to change tumor pH are prone to be having antitumor effects by reverting drug resistance and inhibiting tumor growth and metastatic progression. These have become suitable therapeutic adjuncts. Several studies have shown that PPI like omeprazole, esmoprazole, pantoprazole have anti neoplastic activity against human hematopoietic and solid tumors. PPI being prodrugs get activated in tumor cell acidic pH. PPI treatment changes tumor pH gradient which leads to drug retention and traffic of acidic vesicles in human melanoma and gastric carcinoma. PPI have shown to decrease chemoresistance in drug resistant tumors and can induce direct tumor cell killing[59,35,3]. Hence PPI have

been proposed as valid and feasible approach with relatively low toxicity and potential selectivity for tumor cells. Chemically modified omeprazole containing NAC molecule (NACO) to increase its bioavailability has been shown to induce apoptosis in human melanoma[60,61]. Previously PPI have been shown to induce caspase independent and partially ROS dependent cell death in human B cell tumors. PPI also induce caspase dependent cell death in human melanoma as a result of accumulation of ROS. Both mitochondrial and NADPH oxidase seems to be triggering ROS accumulation in ESOM treated cells. ESOM induced accumulation of hydrogen peroxide and superoxide radicals in melanoma cells as shown by DHR123 and HE fluorosence respectively in the study done by M L Marino et al. (2010)[61] ESOM dependent induced apoptosis is caspase dependent. It was also observed that inhibition of NADPH oxidase by diphenylene iodonium (DPI) induce a significant reduction of ESOM induced cell death in Me30966 cells. DPI is known to be pharmacological inhibitor of NADPH[61]. ESOM treated melanoma cells show massive vacuolization which was noted to occur in cells before apoptosis. ESOM have been shown to induce accumulation of autophagosomes in melanoma cells which is ROS mediated. This leads to decreased autophagic flux. Autophagy is a cellular catabolic pathway leading to lysosomal degradation and recycling of proteins and organelles and represents a defense mechanism in cancer cells under metabolic stress. It is an adaptive survival mechanism to overcome drug induced cellular stress and cytotoxicity and also change in pH homeostasis induced by PPIs. Pretreated melanoma cells with ESOM use autophagic pathway as a defense and adaptive mechanism; hence it is rational to combine PPI therapy and autophagy inhibitors for treatment of melanoma[61]. Recently the concept of targeting extracellular tumor Ph (pHe) as an antitumor therapeutic strategy was supported by several studies. Alkalization of tumor pHe with bicarbonate has shown to inhibit metastasis and enhance the response to chemotherapy[62,63,25]. The use of pH sensitive lytic peptides and nanotechnology was suggested to selectively target tumor tissues[3,64].

CONCLUSION

Considering the substantial available evidence regarding the role of PPI's in melanoma we suggest that this aspect needs to be explored further as it has a lot of potential as an adjunct in the treatment of chemoresistant melanomas and other tumors. It is also important to understand that clinicians today generously co-prescribe PPIs in order to counteract the hyperacidity caused due to other drugs. We would like to suggest that one needs to exercise caution while including PPIs in their treatment portfolio and should keep a track of the duration of treatment and inadvertent side effects if any observed especially among genetically predisposed patients.

REFERENCES

1. Curzytek K, Pietowska J, Spiewak R. Vitiligo induced by drugs: a meta-analysis of reported cases. Abstract 1280. Allergy 2007; 62 (Suppl 83: XXVI Congress of the European Academy of Allergology and Clinical Immunology): 447.
2. MR Namazi. Phenytoin as a novel anti-vitiligo weapon. Journal of Autoimmune Diseases 2005, 2:11.
3. De Milito A, Canese R, Marino M, Borghi M, Iero M, Villa A, Venturi G, Lozupone F, Iessi E, Logozzi M, Mina P, Santinami M, Rodolfo M, Podo F, Rivoltini L, Fais S. PH-dependent antitumor activity of proton pump inhibitors against human melanoma is mediated by inhibition of tumor acidity. International Journal of Cancer 2010; 127: 207-19.
4. Schallreuter KU, Rokos H. From the bench to the bedside: proton pump inhibitors can worsen vitiligo. The British Journal of Dermatology 2007; 156: 1371-3.
5. Holla A, Kumar R, Parsad D, Kanwar AJ. Proton Pump Inhibitor Induced Depigmentation in Vitiligo. Journal of Cutaneous and Aesthetic Surgery 2011; 4: 46-47.
6. Baek S, Ahn J, Nam S, Yoon C, Shin J, Lee S. S-(−)-10,11-Dihydroxyfarnesoic Acid Methyl Ester Inhibits Melanin Synthesis in Murine Melanocyte Cells. Int. J. Mol. Sci. 2014, 15, 12750-12763.
7. Kim JH, Baek SH, Kim DH, Choi TY, Yoon TJ, Hwang JS, Kim MR, Kwon HJ, Lee CH. Downregulation of melanin synthesis by haginin A and its application to in vivo lightening model. J Invest Dermatol. 2008; 128:1227-35.
8. Tuerxuntayi A, Liu Y, Tulake A, Kabas M, Eblimit A, Aisa H. Kaliziri extract upregulates tyrosinase, TRP-1, TRP-2 and MITF expression in murine B16 melanoma cells. BMC Complementary and Alternative Medicine 2014; 14: 166.
9. Shin JM, Lee JY, Lee DY, Yoon TY, Lee JC, Lim EH, Sohn KC, Lee YH, Im M, Seo YJ, Kim CD, Lee JH, Lee Y. Proton pump inhibitors as a possible cause of vitiligo: an in vivo and in vitro study. J Eur Acad Dermatol Venereol. 2014; 28:1475-9.
10. Petris MJ, Strausak D, Mercer JF. The Menkes copper transporter is required for the activation of tyrosinase. Hum Mol Genet. 2000; 9: 2845-51.
11. Namazi MR. Proton pump inhibitors may trigger vitiligo by rendering melanocytes prone to apoptosis. Br J Dermatol. 2008; 158: 844-5.
12. Schallreuter KU, Bahadoran P, Picardo M, Slominski A, Elassiuty YE, Kemp EH, Giachino C, Liu JB, Luiten RM, Lambe T, Le Poole IC, Dammak I, Onay H, Zmijewski MA, Dell'Anna ML, Zeegers MP, Cornall RJ, Paus R, Ortonne JP, Westerhof W. Vitiligo pathogenesis: autoimmune disease, genetic defect, excessive reactive oxygen species, calcium imbalance, or what else? Exp Dermatol. 2008; 17: 139-40.
13. Matsui MS, Petris MJ, Niki Y, Karaman-Jurukovska N, Muizzuddin N, Ichihashi M, Yarosh DB. Omeprazole, a gastric proton pump inhibitor, inhibits melanogenesis by blocking ATP7A trafficking. J Invest Dermatol. 2015; 135: 834-41.

14. Baek SH, Lee SH. Proton pump inhibitors decrease melanogenesis in melanocytes Biomed Rep. 2015; 3: 726-730.
15. Fuller BB, Spaulding DT, Smith DR. Regulation of the catalytic activity of preexisting tyrosinase in black and Caucasian human melanocyte cell cultures. Exp Cell Res. 2001; 262: 197-208.
16. Nishi T, Forgac M. The vacuolar (H+)-ATPases--nature's most versatile proton pumps.Nat Rev Mol Cell Biol. 2002; 3: 94-103.
17. Forgac M. Vacuolar ATPases: rotary proton pumps in physiology and pathophysiology. Nat Rev Mol Cell Biol. 2007; 8: 917-29.
18. Toei M, Saum R, Forgac M. Regulation and isoform function of the V-ATPases. Biochemistry. 2010; 49: 4715-23.
19. Muench SP, Trinick J, Harrison MA. Structural divergence of the rotary ATPases. Q Rev Biophys. 2011; 44: 311-56.
20. Smith AN, Lovering RC, Futai M, Takeda J, Brown D, Karet FE. Revised nomenclature for mammalian vacuolar-type H+ -ATPase subunit genes. Mol Cell. 2003; 12: 801-3.
21. Fais S, De Milito A, You H, Qin W. Targeting vacuolar H+-ATPases as a new strategy against cancer. Cancer Res. 2007; 67: 10627-30.
22. Martínez-Zaguilán R, Raghunand N, Lynch RM, Bellamy W, Martinez GM, Rojas B, Smith D, Dalton WS, Gillies RJ. pH and drug resistance. I. Functional expression of plasmalemmal V-type H+-ATPase in drug-resistant human breast carcinoma cell lines. Biochem Pharmacol. 1999; 57: 1037-46.
23. 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. Effect of proton pump inhibitor pretreatment on resistance of solid tumors to cytotoxic drugs. J Natl Cancer Inst. 2004; 96: 1702-13.
24. 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. The growth and metastasis of human hepatocellular carcinoma xenografts are inhibited by small interfering RNA targeting to the subunit ATP6L of proton pump. Cancer Res. 2005; 65: 6843-9.
25. Robey IF, Baggett BK, Kirkpatrick ND, Roe DJ, Dosescu J, Sloane BF, Hashim AI, Morse DL, Raghunand N, Gatenby RA, Gillies RJ. Bicarbonate increases tumor pH and inhibits spontaneous metastases. Cancer Res. 2009; 69:2260-8.
26. Subarsky P, Hill RP. The hypoxic tumour microenvironment and metastatic progression. Clin Exp Metastasis. 2003; 20: 237-50.
27. Trédan O, Galmarini CM, Patel K, Tannock IF. Drug resistance and the solid tumor microenvironment. J Natl Cancer Inst. 2007; 99: 1441-54.
28. Gillies RJ, Robey I, Gatenby RA. Causes and consequences of increased glucose metabolism of cancers. J Nucl Med. 2008; 49: 24S-42S.
29. Chiche J, Brahimi-Horn MC, Pouysségur J. Tumour hypoxia induces a metabolic shift causing acidosis: a common feature in cancer. J Cell Mol Med. 2010; 14: 771-94.
30. Torigoe T, Izumi H, Ishiguchi H, Uramoto H, Murakami T, Ise T, Yoshida Y, Tanabe M, Nomoto M, Itoh H, Kohno K. Enhanced expression of the human vacuolar H+-ATPase c subunit gene (ATP6L) in response to anticancer agents. J Biol Chem. 2002; 277: 36534-43.
31. Morimura T, Fujita K, Akita M, Nagashima M, Satomi A. The proton pump inhibitor inhibits cell growth and induces apoptosis in human hepatoblastoma. Pediatr Surg Int. 2008; 24:1087-94.
32. McHenry P, Wang WL, Devitt E, Kluesner N, Davisson VJ, McKee E, Schweitzer D, Helquist P, Tenniswood M. Iejimalides A and B inhibit lysosomal vacuolar H+-ATPase (V-ATPase) activity and induce S-phase arrest and apoptosis in MCF-7 cells. J Cell Biochem. 2010; 109: 634-42.
33. Nakashima S, Hiraku Y, Tada-Oikawa S, Hishita T, Gabazza EC, 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. J Biochem 134, 359-364.
34. Nishihara T, Akifusa S, Koseki T, Kato S, Muro M, Hanada N. Specific inhibitors of vacuolar type H(+)-ATPases induce apoptotic cell death. Biochem Biophys Res Commun. 1995; 212: 255-62.
35. De Milito A, Iessi E, Logozzi M, Lozupone F, Spada M, Marino ML, Federici C, Perdicchio M, Matarrese P, Lugini L, Nilsson A, Fais S. Proton pump inhibitors induce apoptosis of human B-cell tumors through a caspase-independent mechanism involving reactive oxygen species. Cancer Res. 2007; 67: 5408-17.
36. Sasazawa Y, Futamura Y, Tashiro E, Imoto M. Vacuolar H+-ATPase inhibitors overcome Bcl-xL-mediated chemoresistance through restoration of a caspase-independent apoptotic pathway. Cancer Sci. 2009; 100: 1460-7.
37. Ishisaki A, Hashimoto S, Amagasa T, Nishihara T. Caspase-3 activation during the process of apoptosis induced by a vacuolar type H(+)-ATPase inhibitor. Biol Cell. 1999; 91: 507-13.
38. O'Callaghan KM, Ayllon V, O'Keeffe J, Wang Y, Cox OT, Loughran G, Forgac M, O'Connor R. Heme-binding protein HRG-1 is induced by insulin-like growth factor I and associates with the vacuolar H+-ATPase to control endosomal pH and receptor trafficking. J Biol Chem. 2010; 285: 381-91.
39. Fortini ME, Bilder D. Endocytic regulation of Notch signaling. Curr Opin Genet Dev. 2009; 19: 323-8.
40. Vaccari T, Duchi S, Cortese K, Tacchetti C, Bilder D. The vacuolar ATPase is required for physiological as well as pathological activation of the Notch receptor. Development. 2010; 137: 1825-32
41. Cruciat CM, Ohkawara B, Acebron SP, Karaulanov E, Reinhard C, Ingelfinger D, Boutros M, Niehrs C. Requirement of prorenin receptor and vacuolar H+-

ATPase-mediated acidification for Wnt signaling. Science. 2010; 327: 459-63.
42. Buechling T, Bartscherer K, Ohkawara B, Chaudhary V, Spirohn K, Niehrs C, Boutros M. Wnt/Frizzled signaling requires dPRR, the Drosophila homolog of the prorenin receptor. Curr Biol. 2010; 20: 1263-8.
43. Yan Y, Denef N, Schüpbach T. The vacuolar proton pump, V-ATPase, is required for notch signaling and endosomal trafficking in Drosophila. Dev Cell. 2009; 17: 387-402.
44. Lucio M, Todd G, Barbara O. Notch signaling in cancer. Current Molecular Medicine 2006; 6: 905-918.
45. Roy M, Pear WS, Aster JC. The multifaceted role of Notch in cancer. Curr Opin Genet Dev. 2007; 17: 52-9.
46. Nishisho T, Hata K, Nakanishi M, Morita Y, Sun-Wada GH, Wada Y, Yasui N, Yoneda T. The a3 isoform vacuolar type H^+-ATPase promotes distant metastasis in the mouse B16 melanoma cells. Mol Cancer Res. 2011; 9: 845-55.
47. Chung C, Mader CC, Schmitz JC, Atladottir J, Fitchev P, Cornwell ML, Koleske AJ, Crawford SE, Gorelick F. The vacuolar-ATPase modulates matrix metalloproteinase isoforms in human pancreatic cancer. Lab Invest. 2011; 91: 732-43.
48. Sennoune SR, Bakunts K, Martínez GM, Chua-Tuan JL, Kebir Y, Attaya MN, Martínez-Zaguilán R. Vacuolar H+-ATPase in human breast cancer cells with distinct metastatic potential: distribution and functional activity. Am J Physiol Cell Physiol. 2004; 286: C1443-52.
49. Hinton A, Sennoune SR, Bond S, Fang M, Reuveni M, Sahagian GG, Jay D, Martinez-Zaguilan R, Forgac M. Function of a subunit isoforms of the V-ATPase in pH homeostasis and in vitro invasion of MDA-MB231 human breast cancer cells. J Biol Chem. 2009; 284: 16400-8.
50. Cotter K, Capecci J, Sennoune S, Huss M, Maier M, Martinez-Zaguilan R, Forga M. Activity of Plasma Membrane V-ATPases Is Critical for the Invasion of MDA-MB231 Breast Cancer Cells. J Biol Chem. 2015; 290: 3680–3692.
51. Joyce JA, Hanahan D. Multiple roles for cysteine cathepsins in cancer. Cell Cycle. 2004; 3: 1516-619.
52. Gocheva V, Joyce JA. Cysteine cathepsins and the cutting edge of cancer invasion. Cell Cycle. 2007; 6: 60-4.
53. Martínez-Zaguilán R, Seftor EA, Seftor RE, Chu YW, Gillies RJ, Hendrix MJ. Acidic pH enhances the invasive behavior of human melanoma cells. N Clin Exp Metastasis. 1996; 14: 176-86.
54. De Milito A, Fais S. Tumor acidity, chemoresistance and proton pump inhibitors. Future Oncol. 200; 1: 779-86.
55. 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. Effect of proton pump inhibitor pretreatment on resistance of solid tumors to cytotoxic drugs. J Natl Cancer Inst. 2004; 96: 1702-13.
56. Raghunand N, He X, van Sluis R, Mahoney B, Baggett B, Taylor CW, Paine-Murrieta G, Roe D, Bhujwalla ZM, Gillies RJ. Enhancement of chemotherapy by manipulation of tumour pH. Br J Cancer. 1999; 80: 1005-11.
57. Gerweck LE, Vijayappa S, Kozin S. Tumor pH controls the in vivo efficacy of weak acid and base chemotherapeutics. Mol Cancer Ther. 2006; 5:1275-9.
58. McCarty MF, Whitaker J. Manipulating tumor acidification as a cancer treatment strategy. Altern Med Rev. 2010; 15:264-72.
59. Yeo M, Kim DK, Kim YB, Oh TY, Lee JE, Cho SW, Kim HC, Hahm KB. Selective induction of apoptosis with proton pump inhibitor in gastric cancer cells. Clin Cancer Res. 2004; 10: 8687-96.
60. Hersey P, Watts RN, Zhang XD, Hackett J. Metabolic approaches to treatment of melanoma. Clin Cancer Res. 2009; 15: 6490-4.
61. Marino ML, Fais S, Djavaheri-Mergny M, Villa A, Meschini S, Lozupone F, Venturi G, Mina PD, Pattingre S, Rivoltini L, Codogno P, DeMilito A. Proton pump inhibition induces autophagy as a survival mechanism following oxidative stress in human melanoma cells. Cell Death Dis. 2010; 1: e87.
62. Raghunand N, Gillies RJ. pH and drug resistance in tumors. Drug Resist Updat. 2000; 3: 39-47.
63. Raghunand N, Mahoney B, van Sluis R, Baggett B, Gillies RJ. Acute metabolic alkalosis enhances response of C3H mouse mammary tumors to the weak base mitoxantrone. Neoplasia. 2001; 3: 227-35.
64. Lee ES, Gao Z, Bae YH. Recent progress in tumor pH targeting nanotechnology. J Control Release. 2008; 132: 164-70.