# Clinical significance of P-class pumps in cancer (Review)

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**Abstract.** P-class pumps are specific ion transporters involved in maintaining intracellular/extracellular ion homeostasis, gene transcription, and cell proliferation and migration in all eukaryotic cells. The present review aimed to evaluate the role of P-type pumps [$Na^+/K^+$ ATPase (NKA), $H^+/K^+$ ATPase (HKA) and $Ca2^+$-ATPase] in cancer cells across three fronts, namely structure, function and genetic expression. It has been shown that administration of specific P-class pumps inhibitors can have different effects by: i) Altering pump function; ii) inhibiting cell proliferation; iii) inducing apoptosis; iv) modifying metabolic pathways; and v) induce sensitivity to chemotherapy and lead to antitumor effects. For example, the NKA β2 subunit can be downregulated by gemcitabine, resulting in increased apoptosis of cancer cells. The sarco-endoplasmic reticulum calcium ATPase can be inhibited by thapsigargin resulting in decreased prostate tumor volume, whereas the HKA α subunit can be affected by proton pump inhibitors in gastric cancer cell lines, inducing apoptosis. In conclusion, the present review highlighted the central role of P-class pumps and their possible use and role as anticancer cellular targets for novel therapeutic chemical agents.

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## 1. Introduction

The movement of ions across a biological membrane is a crucial physiological process necessary for maintaining cellular homeostasis. Such processes involve different types of membrane proteins which can move ions either down their concentration gradient or against their concentration gradient (1). The latter is considered an active transport mechanism, achieved by dephosphorylating ATP for each pump cycle, a mechanism by which a family of pumps known as P-type ATPases are known to operate with (Fig. 1) (2,3). Cancer treatments currently include different approaches such as surgery, chemotherapy, radiotherapy, immunotherapy or other processes that are characterized to be debilitating and traumatic for a patient with a direct effect on his quality of life (4). Therefore, specific characterized molecular targets could be used for the designing or isolation of novel chemical agents that could potentially be able to affect the tumor cell proliferation through manipulation of those specific molecular targets. P-type ATPases could be considered as such potential targets, as they are proteins regulated by different functional means such as phosphorylation, drug inhibition or activation and/or ion concentration sensitivity (2,3,5).

The P-type ATPases are divided into five families (P1 to P5) that can be further classified to one or more subgroups A, B, C, D (5,6). P-class pumps are proteins, present in all eukaryotic cells, as well as bacteria and are commonly integrated within the lipid bilayer of the cellular membranes (3,5). An essential segment of ATPases is the α-subunit that is known to be involved in the enzymatic activity of the pump but also the transport of ions across membranes. The pump expresses variations in structural confirmation depending on the factional stage of the protein found at any time during the transportation or translocation process of the involved ions (5,7). It is

well characterized that phosphorylation of the P site results in a conformational change exposing the enzymatic sites (8). Additional domains are also expressed in pumps such as the R (regulatory) domain involved in auto inhibition of the pump.

Three members of this family have been extensively studied *in vitro, in vivo* and in clinical studies, the sodium (Na+)/potassium (K+)-ATPase (NKA), sarcoendoplasmic reticulum calcium (Ca2+) ATPase (SERCA), and the proton (H+)/K+ ATPase (HKA) (3). NKA, HKA and SERCA are three among many transmembrane proteins that play a significant role in essential cellular functions, e.g., relaxation of muscle tissue or restoration of stomach acidity (9,10). A relationship on a molecular level, between pump behaviour and cancer development/inhibition was also reported. Such an effect could be justified by alterations (mutations) in genetic material, abnormal regulation of pump protein translation or stimulation of some cellular pathways and repression of others (11-16).

It is also known that cancer cells exhibit an acidic environment, extracellular build-up of H+ and production of lactic acid, creating numerous complications (10). These complications include changes in both metastatic and proliferation behaviour but can additionally result in resistance of cancer cells to chemotherapeutic drugs (17). Given the ability of P-type pumps to directly affect ion homeostasis, they are currently being explored as antineoplastic or antitumor targets/biomarkers as able to reverse the changes found in cancer cell environments (10).

This review aims to: (A) provide an understanding of the significance of P-Type ATPases in essential functions of a cell, (B) describe the outcomes when they are inhibited during pathogenesis and specifically cancer (Table I), (C) determine the use of those protein-pumps as potential biological cancer biomarkers and (D) probably propose future advancements.

## 2. Methodology

Evidence regarding P-class pumps was reviewed in *in vitro* and *in vivo* studies, as well as in clinical trials, in order to describe their quaternary structure, function, and if they communicate any anticancer effects. A literature search was performed in PubMed to identify relevant articles published from January 2010 until December 2020 and in ClinicalTrials. gov database for any relevant trials. The search was based on the following words and terms: 'Na+/K+ ATPase', 'SERCA', 'H+/K+ ATPase', 'P-type pumps' in association with the term 'cancer' (Fig. 2). The relevant papers found were then searched for discussing the structure, function and the potential involvement of the searched functional entities in cancer treatment. The references of the relevant articles were also accessed and reviewed. Additionally, expression data was collected from the UALCAN http://ualcan.path.uab.edu/ resource to evaluate the degree of gene expression of the pumps in 24 separate cell lines, comparing expression levels in cancer cells to healthy cells (18).

## 3. NKA

*NKA structure and function.* NKA is crucial for cell function, serving as a signal transducer involved in cell adhesion (14). Ions move against the concentration gradient in order to form

and protect the action potential which is vital for many physiological processes in a number of organs. NKA channel is present in the waste management in kidneys, sperm mobility and neuronal action potential production. The regulation of the neuron action potentials is also due to NKA maintaining the concentrations of Na+ and K+ at constant disequilibrium. When a neuron generates an impulse, a sudden shift is created from resting to active state due to a rapid movement of ions across the membrane.

For every NKA cycle an ATP is hydrolysed followed by three sodium ions binding to the pump to be exported from the cell in exchange of two potassium ions binding to be imported into the cytosol, resulting in a net positive charge extracellularly (19). The NKA transmembrane protein is composed of the α, β and FXYD subunits, which are subdivided into various isoforms (Fig. 1). Action potentials are affected by NKA exertions, through polarization/depolarization actions known to generate electrical signals (8,19,20). These subunits express in an heterologous manner and the isoform combinations vary in different tissues (21). The α subunit, as previously described, is the catalytic subunit (7). This structure has a mass of 110-kDa and it is transcribed by 1,000 amino acids and it spans the membrane ten times exhibiting functional domains intracellularly and extracellularly (e.g., A,N,P,R) (8). This subunit is comprised of four isoforms, α1-α4. The α1 subunit gene ATP1A, of NKA is expressed in most tissues as it plays a key role in signal transduction, cell/cell adhesion and tight junctions (Fig. 3A and B) (22). Its expression is prominent in the ependymal cells of the spinal cord and as well as in neurons of the ventral horn (23). The α2 (ATP1A2) subunit is highly expressed in cardiac T-tubules, skeletal and smooth muscle, lung, astrocytes and adipose tissue (24). The α3 (ATP1A3) subunit is mostly expressed in ventricular myocardial cells, axons and dendrites, while the α4 (ATP1A4) in the testes and epididymis (25-29). The β subunit has four separate isoforms (ATP1B1-ATP1B4), all highly glycosylated, situated mostly extracellularly (33-36 kDa) (Fig. 3) (30). The β subunits attract K+ and incorporate the binding site for cardiac glycosides (31). Expressed in skeletal muscle, heart muscle and human brain is the β1 subunit, which is also a constituent of cell-cell adhesion. The β2 (32 kDa) subunit is more commonly expressed in glial cells but expressed in myocardial cells and erythrocytes, and is additionally an essential enzyme in cell adhesion in the central nervous system (32,33). The third enzyme is the β3 subunit expressed predominately in oligodendrocytes of brain white matter and the optic nerve; it is also detected in testes (30,34). Finally, β4 varies from the other β isoforms, as it is situated mostly intracellularly (31,35,36) and is encountered in skeletal and cardiac muscle (37).

A third unit of NKA, FXYD, is a significant subunit, sometimes referred to as an accessory unit due to its scarce presence (16,20). This protein is transcribed by the FXYD genes and seven isoforms have been described thus far, FXYD1-FXYD7 (8). FXYD1 (phospholemman) is absent in cardiac tissue resulting in reduced NKA activity, indicating that FXYD1 regulates NKA in this tissue (38). FXYD2 (γ subunit) affects Na+ reabsorption in the thick ascending limb in the medulla of the kidney and has a low affinity for Na+ (39). FXYD3 (mammary tumor marker) is expressed in breast, lung, small intestine but also stomach, uterus and
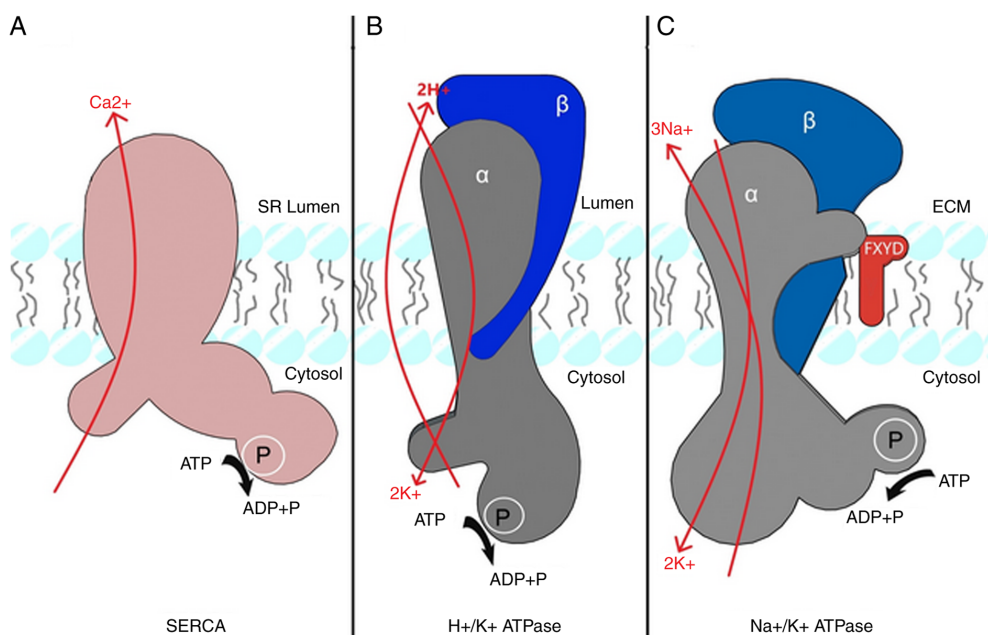
Figure 1. Illustration of the structure and function of P-class pumps created by authors. (A) SERCA pump transports $Ca^{2+}$ from the cytosol to the lumen. (B) The anti-transportation of $2H^+$ from the cytosol to the lumen and $2K^+$ from the lumen to the cytosol by HKA to regulate intracellular pH. (C) NKA is an anti-porter involved in moving $3 Na^+$ from the cytosol to the ECM for the charge of $2K^+$ being transported into the cytosol. All transportations take place in expense of ATP. ECM, extracellular matrix; SR, sarcoendoplasmic reticulum; SERCA, sarcoendoplasmic reticulum calcium ATPase; HKA, proton potassium ATPase; NKA, sodium potassium ATPase.

thymus (40). Additionally, FXYD3 decreases $K^+$ affinity, spans the membrane twice instead of once, affects β subunit glycosylation and its specificity is low as it is also associated with the β subunit of HKA (41,42). The final locus of $Na^+$ reabsorption in the kidneys is from the collecting duct where FXYD4 (channel-inducing factor) is expressed as part of NKA, instead of FXYD2 (43). FXYD4 has a high affinity to $Na^+$ therefore ensuring that $Na^+$ is reabsorbed into the bloodstream (42). Dysadherin (FXYD5) can double the volume of NKA activity (44). Phosphohippolin, also known as FXYD6, is transcribed in the cerebellum and the posterior lobe of the brain (lobules VI-IX) and is involved in brain neuronal excitability (45). It is additionally expressed in kidney, liver, lung and testis (45). Finally, FXYD7 affects NKA excitability in glial cells and neurons of the brain, by regulating $K^+$ movement (41,46). The combination in which the different isoforms of FXYD co-exist with the various isoforms of α and β, depends on the tissue in question (47).

*NKA significance in cancer.* The role of NKA in cancer has been supported by a growing number of studies, including *in vivo*, *in vitro* and genetic studies that have evaluated either the differential expression of NKA subunits, or the effects of different NKA modulators in malignancy.

Transcription and/or translation of the ion pump protein may either be increased or decreased as a response to the environmental settings created by cancerous cells. The gene expression of each NKA subunit in twenty-four different cancer types was compared to their expression in healthy tissue (Fig. 3A and B) (18). This data is constructive on two fronts: i) It denotes novel biomarkers; ii) It recognises potential treatment targets based on cancer geography. Expression of ATP1A1 in cancerous esophageal cells is significantly greater

than in healthy esophageal cells and the same was found true for the expression of this subunit in cervical squamous carcinoma, thymoma, paraganlgioma etc. When evaluating the expression of ATP1A1 in glioblastoma multiforme was found decreased compared to healthy cells. Ideally, these characteristic patterns in protein subunit expression can be manipulated for diagnostic purposes. For example, FXYD3 demonstrates overexpression in gastric adenocarcinomas, prostate carcinoma, pancreatic cancer and in breast tumors as opposed to their healthy counterparts (48). Inhibiting FXYD3 expression can result in down-regulation of cancer cell proliferation, without affecting the invasion rate or cellular apoptosis (49).

As previously reported mutations on specific genes can affect the expression and hence transcription of the NKA subunit, such as ATP1B2 deletions in breast cancer or the deletion of ATPA1A and ATP1B1 in epithelial-mesenchymal transition which can result in cancer and fibrosis (14,50). This was also true for the α3β1 subunit (isozyme) that was found to be abundant in colon cancer cells that metastasised to the liver, but absent in healthy liver tissue.

Expression is not the only factor affected in cancer cells. Intracellular localization of subunits has been recorded as well, where ATP1A1 is expressed near the peri-nuclear site in cancer environments, but in healthy tissue it it positioned on the basolateral side of cells instead (51). In normal cells, such as in the lung and colon, ATP1A3 is present close to the cytoplasmic membrane, in contrast to its peri-nuclear location in cancer cells of both tissue types. These modifications in intracellular localisation of the α1 and α3 can possibly serve as biomarkers and/or as possible treatment targets (52). As an example, the Oleandrin has been shown to express anti-proliferative activity promoting cell death in undifferentiated CaCo-2 cells (52).

Table I. Listing of clinical effects of P-class pumps in cancer, containing drug name and target, as well as the upregulated/downregulated pump.

| First author, year | Drug | Type of cancer | Pump outcome PETD | Cellular alterations PETD | (Refs.) |
|---|---|---|---|---|---|
| Yang *et al*, 2014 | Oleandrin | Human colorectal cancer (*in vitro*) | ↓NKA (α3) | ↑$K^+$ intracellularly ↓$Ca^{2+}$ intracellularly | (52) |
| McConkey *et al*, 2000 | Oleandrin | Prostate cancer (*in vitro*, *in vivo*) | ↓NKA | ↑$K^+$ intracellularly ↓$Ca^{2+}$ intracellularly | (133) |
| Newman *et al*, 2007 | Oleandrin | Pancreatic cancer (*in vitro*) | ↓NKA | ↓phospho Akt ↑phospho ERK ↓$G_2$/M cell | (11) |
| Li *et al*, 2011 | pNaktide (peptide) | Prostate cancer (*in vivo*) | ↑NKA (α1) | ↑$K^+$ intracellularly ↓$Ca^{2+}$ intracellularly ↓Src | (22) |
| Garcia *et al*, 2015 | Perillyl alcohol | Glioblastoma (*in vitro*) | ↓NKA (α1) | ↑$K^+$ intracellularly ↓$Ca^{2+}$ intracellularly ↓JNK | (53) |
| Alevizopoulos *et al*, 2016 | Istaroxime | Prostate cancer (*in vitro*, *in vivo*) | ↓NKA | ↑$K^+$ intracellularly ↓$Ca^{2+}$ intracellularly Caspase-3 ↓c-Myc expression | (128) |
| Li *et al*, 2011 | Bufalin (CG) | Hepatocallular carcinoma (*in vitro*) | ↓NKA (α3) | ↑Akt ↓FOXO3a ↑ERK ↑$K^+$ intracellularly ↓$Ca^{2+}$ intracellularly | (134) |
| Numazawa *et al*, 1994 | Bufalin (CG) | Leukaemia (*in vitro*) | ↓NKA (α3) | ↑$K^+$ intracellularly ↓$Ca^{2+}$ intracellularly | (135) |
| Sun *et al*, 2019 | Bufalin (CG) | Colorectal cancer (*in vitro*) | ↓NKA (α3) | ↑$K^+$ intracellularly ↓$Ca^{2+}$ intracellularly ↓NF-κB ↓HIF-1α ↓Cell cycle $G_2$/M | (136) |
| Yu *et al*, 2008 | Cinobufagin (CG) | Prostate cancer (*in vitro*) | ↓NKA | ↑$K^+$ intracellularly ↓$Ca^{2+}$ intracellularly ↑Caspase-3 Cytochrome c release | (137) |
| Ihenetu *et al*, 2007 | Digoxin (CG) | Leukemia (*in vitro*) | ↓NKA | ↑$K^+$ intracellularly ↓$Ca^{2+}$ intracellularly ↑FasL | (12) |
| Prassas *et al*, 2011 | Digitoxin (CG) | Pancreatic cancer (*in vitro*) | ↓NKA | ↑Src ↑MAPK signalling ↑$K^+$ intracellularly ↓$Ca^{2+}$ intracellularly | (55) |
| Hsu *et al*, 2016 | Digoxin (CG) | Ovarian clear cell carcinoma (*in vitro*, *in vivo*) | ↓NKA | ↓FXYD2 ↑$K^+$ intracellularly ↓$Ca^{2+}$ intracellularly | (62) |
| Kang *et al*, 2016 | Latonoside C (CG) | Colorectal cancer (*in vitro*) | ↓NKA | ↑$K^+$ intracellularly ↓$Ca^{2+}$ intracellularly Repair mitochondrial membrane potential | (138) |

Table I. Continued.

| First author, year | Drug | Type of cancer | Pump outcome PETD | Cellular alterations PETD | (Refs.) |
|---|---|---|---|---|---|
| Liu et al, 2013 | Ouabain (CG) | Lung cancer (in vitro) | ↓NKA | ↑$K^+$ intracellularly ↓$Ca^{2+}$ intracellularly | (56) |
| Huang et al, 2004 | Ouabain (CG) | Prostate cancer (in vitro) | ↓NKA (α) | ↑$K^+$ intracellularly ↓$Ca^{2+}$ intracellularly Restore mitochondrial membrane potential | (139) |
| Ono et al, 2016 | Ouabain (CG) | Brain glioblastoma (in vivo) | ↓NKA (α) | ↑$K^+$ intracellularly ↓$Ca^{2+}$ intracellularly | (140) |
| Li et al, 2019 | Digoxin (CG) | Glioblastoma (in vitro, in vivo) | ↓NKA (β2) | ↑$K^+$ intracellularly ↓$Ca^{2+}$ intracellularly Cell cycle arrest at $G_2$/M phase | (59) |
| Rocha et al, 2016 | 21-benzylidene digoxin (CG) | Cervical cancer and colon carcinoma (in vitro) | ↓NKA (α1) | DNA damage ↑p21 ↓Cyclin A, Bcl-2 and Bcl-XL | (141) |
| Denmeade and Isaacs, 2015 | Thapsigargin | Prostate cancer (in vitro, in vivo) | ↓SERCA | ↓$Ca^{2+}$ intracellularly Cytochrome c release | (142) |
| Mahalingam et al, 2016 | Mipsagargin | Hepatocellular carcinoma (phase i) | ↓SERCA | ↓$Ca^{2+}$ intracellularly | (81) |
| Fan et al, 2014 | Curcumin Analogue (F36) | Colorectal carcinoma (in vitro) | ↓SERCA | ↓$Ca^{2+}$ intracellularly | (143) |
| Denmeade et al, 2003 | PSA-Thapsigargin prodrug | Metastatic prostate cancer (in vitro, in vivo) | ↓SERCA | ↓$Ca^{2+}$ intracellularly | (83) |
| Jia et al, 2016 | Artemisin | Gallbladder cancer (in vitro, in vivo) | ↓SERCA | ↓ERK ↓CDK ↓Cyclin D1 ↓$Ca^{2+}$ intracellularly | (144) |
| Riganti et al, 2009 | Artemisin | Colon cancer (in vitro) | ↓SERCA | ↓$Ca^{2+}$ intracellularly ↓doxorubicin intracellularly ↑P-glycoprotein | (86) |
| Riganti et al, 2009 | Parthenolide | Colon cancer (in vitro) | ↓SERCA | ↓$Ca^{2+}$ intracellularly ↓doxorubicin intracellularly ↑P-glycoprotein | (86) |
| De Ford et al, 2016 | Clerodanee ditepene casearin J. | T-cell acute lymphoblastic leukemia (in vitro) | ↓SERCA | ↓$Ca^{2+}$ intracellularly ↓Oxidative stress ↑Notch1 | (87) |
| Madreiter-Sokolowski et al, 2016 | Resveratrol, Piceatannol and oligomycin A | Cervical adenocarcinoma (in vitro) | ↓SERCA | ↓$Ca^{2+}$ intracellularly Reverse Loss of mitochondrial membrane potential | (131) |

Table I. Continued.

| First author, year | Drug | Type of cancer | Pump outcome PETD | Cellular alterations PETD | (Refs.) |
|---|---|---|---|---|---|
| Wong *et al*, 2013 | Saikosaponin-d | Cervical cancer and breast cancer | ↓SERCA | ↓$Ca^{2+}$ intracellularly ↑CaMKKβ-AMP, (AMPK), (mTOR) signaling cascade ↑Unfolded protein responses | (13) |
| Jakab *et al*, 2014 | Sch28080 | Leukemia (*in vitro*) | ↓HKA (α1) | ↑$H^+$ intracellularly | (100) |
| Yeo, 2004 | Pantoprazole | Gastric cancer (*in vitro*, *in vivo*) | ↓HKA (α) | ↑$H^+$ intracellularly | (108) |
| Zhang *et al*, 2013 | Trametenolic acid B | Gastric cancer (*in vitro*, *in vivo*) | ↓HKA | ↑$H^+$ intracellularly | (107) |
| Gu *et al*, 2014 | Rabeprazole | Gastric cancer | ↓HKA | ↑$H^+$ intracellularly | (10) |
| Zhang *et al*, 2013 | Lansoprazole | Breast cancer | ↓HKA | ↑$H^+$ intracellularly | (107) |
| Lindner *et al*, 2014 | Esomeprazole | Esophageal cancer | ↓HKA | ↑$H^+$ intracellularly | (109) |

The arrows indicate downregulated or upregulated expression. CAMKKβ, calcium/calmodulin-dependent protein kinase kinase 2; CDK, cyclin-dependent kinase; FasL, Fas ligand; FOXO3a, Forkhead box O3; HIF-1α, hypoxia-inducible factor-1α; PETD, post-exposure to drug; CG, cardiac glycoside; SERCA, sarcoendoplasmic reticulum calcium ATPase; HKA, $H^+/K^+$ ATPase; NKA, $Na^+/K^+$ ATPase.

As we have previously discussed the α1 subunit is involved in multiple cell functions including the regulation effect on Src (a protein kinase that promotes cell proliferation and invasion) (22). These findings indicated that α1 expression decreases in prostate cancer and human metastatic prostate cell lines (Fig. 3A and B PRAD) while Src activity increased, favouring cancer progression. An *in vivo* study illustrated that administration of pNaktide peptide could restore the α1 expression with a consequent inhibition of Src action, that further decreased proliferation, migration and/or invasion (Table I) (22). Opposing evidence by *in vivo* experiments on glioblastoma cells displayed elevated expression of α1 which suppressed Src activity. However, when Src fails to phosphorylate JNK (protein Kinase) the glioblastoma cells proliferate freely, further supporting the influence of Src on cancer cell death. Administering perillyl alcohol, an NKA inhibitor, α1 is downregulated resulting in JNK phosphorylation and initiating glioblastoma cell death (53). Src was further reported able to affect MAPK and p38 proteins (53).

Cardiac glycosides (CGs) are natural compounds commonly used in treating heart failure and cardiac arrhythmias; display anticancer effects by decreasing $K^+$, thus increasing $Na^+$ and $Ca^{2+}$ intracellularly; and modify the behaviour of multiple cellular pathways, such as IL-8 and TNF-α/NF-Kb that are associated with angiogenesis, metastasis or apoptosis (54,55). Ouabain is a CG known to inhibit NKA. Treatment of A549 cells, a lung cancer cell line, with ouabain demonstrated low expression of both N-cadherin and vimentin, both known as epithelial-mesenchymal transition biomarkers, hence indicating decrease of A549 cell migration (56). It has also been shown that ouabain inhibits migration via an epidermal growth factor pathway that causes cytotoxicity in PC-3 (prostate cancer) cells by creating reactive oxygen species (ROS) or upregulating the prostate apoptosis response (56). CGs and their relationship with cancer are listed in Fig. 3A and B. It is also worth noting that the α1 subunit shows low affinity for ouabain which means that the specific drug is more effective when certain alternative are present (56,57).

In medulloblastoma, kidney carcinoma cells, colorectal cancer and liver metastases, tumorigenesis is favoured by inhibiting β1. The β1 subunit expression is additionally reported to inhibit tumorigenicity in MSV-MDCK cells and able to regulate the ERK pathway (regulating signalling molecules) activation (8,57). NKA β1 also affects cellular growth factors such as TGF-β2, as ion pump inhibition upregulates TGF-β2 (a growth factor cytokine), activity. Therefore the relationship between pump and disease are eminent, presenting it as a potential drug treatment target (58).

The expression of β2 subunits is greatly upregulated in cancer (59,60). *in vitro* down-regulation of β2 expression results in cell cycle arrest in glioblastoma multiforme (GBM) at the G2/M phase with increased $Ca^{2+}$ intracellularly, through the sodium/calcium exchanger, resulting in cell apoptosis (59).

The adhesion molecule on glia (β2/AMOG) is a glycoprotein that accompanies the β2 subunit. As β2/AMOG expression decreases in cancer cells the grade of malignancy increases, adhesion is lost and migration is increased (61). Alternatively, the degree of protein pump expression may be useful for prognosis,
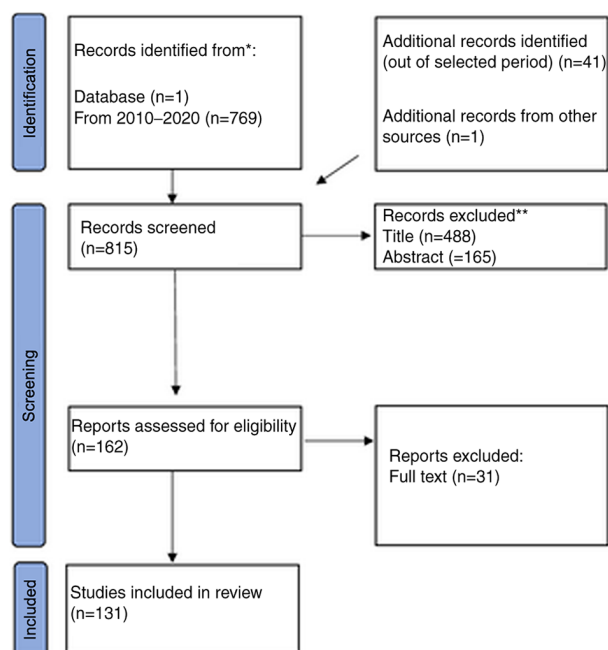
Figure 2. Flowchart of selected papers.

as β2 appears to be greatly expressed in primary GBM compared to secondary GBM (60). The opposite is revealed for β3, as it is more upregulated in secondary GBM than primary. This pattern may benefit as a prognostic marker (61).

The NKA protein and specifically the FXYD2, is significantly upregulated in ovarian clear cell carcinoma (OCCC) but is scarce to non-existent in surrounding healthy ovarian cells (62). This characteristic increase may be manipulated as a biomarker for OCCC prognostic purposes. Tumor growth is suppressed due to FXYD2 inhibition by digoxin and digitoxin as autophagy-mediated cell death is stimulated (62). Previous studies report a characteristic increase in FXYD3 expression in endometrial cancer, breast tumor, pancreatic and prostate cancer while in lung cancer the expression of the protein was significantly downregulated; possibly suggesting FXYD3 as a biomarker for the aforementioned cancer types (63,64). The same can be supported for FXYD6 in cholangiocarcinoma, while in hepatocellular carcinoma downregulating FXYD6 inhibited NKA activity resulting in a decrease in tumor volume, revealing the protein as a potential treatment target (65,66). In a case of ovarian cancer, FXYD5 involvement in cell adhesion is believed to drive cancer cell migration but may also be a useful mortality predictor (67).

## 4. SERCA pump

*SERCA pump structure and function.* The SERCA is a 110 kDa power-driven P-type pump that transports two $Ca^{2+}$ ions from the cytoplasm to the sarco/endoplasmic reticulum lumen per ATP dephosphorylated, balancing free $Ca^{2+}$ in both eukaryotes and prokaryotes (68). SERCA is also composed of a ten transmembrane α subunit segments (Fig. 1) (69). Three SERCA homologous genes transcribe to three subunits; SERCA1 (gene: ATP2A1), SERCA2 (ATP2A2), and SERCA3(ATP2A3) (70,71). The isoform which will be

expressed is determined by alternative splicing of genes, is cell-type dependent and they respond differently to drugs even though they are approximately 75% homologous (70). SERCA1 consists of two transcripts; 1a is mainly expressed in adult skeletal muscle whereas 1b is present in fetal skeletal muscle (68,72,73). SERCA2 also consists of two transcripts. The 2a transcript is found in adult skeletal and cardiac muscle. SERCA2b is abundant in all adult and fetal cardiac and skeletal muscles. SERCA3 is divided into three isoforms, SERCA3a-3c, all of which are expressed in non-muscle cells (68,70,73).

Signal transduction via intracellular free $Ca^{2+}$-concentration $[(Ca^{2+})_i]$ elevation is one of the fundamental events observed in cell regulation. Systems including muscle cells, neurons, immune system cells as well as plant cells and protozoa utilize $Ca^{2+}$-signalling.

Free calcium is an important second messenger for the cell. The ER acts as a reservoir and releases the ion into the cytosol upon signaling where it is involved in proliferation, cell death and gene regulation. In neurons, it is the most common intracellular messenger, it is activated by neurotransmitters dopamine and etc. whereas in T cells it is stimulated by the TCR pathway and promotes T cell immunity.

While $Ca^{2+}$ concentration is known to be greater extracellularly (cytosol) than intracellularly (lumen), a disordered homeostasis is indicative of pathological conditions, including cancer (68). This action is crucial for a number of cellular processes such as gene transcription, cell proliferation and cell death (68,74,75). Given that SERCA is found in fundamental structures of the cell and is involved in the regulation of cellular metabolism, any pump inhibitor recognised to have anti-tumor effects must be prescribed as a pro-drug to ensure only cancerous cells are affected and leaving healthy cells unharmed (72).

*SERCA significance in cancer.* The consequence of inhibiting SERCA results in depletion of the $Ca^{2+}$ concentration in mitochondria that can lead to the collapse of the electrochemical proton gradient, thus triggering numerous signalling pathways resulting in cell death (76). Use of SERCA pump inhibitors in cancer has been verified through *in vitro* and *in vivo* studies, with the most commonly used inhibitors being thapsigargin, artemisinin parthenolide, resveratrol and saikosaponin (13,77). In early stages of cancer, a noticeable decrease in SERCA3 is presented by breast cancer, colon cancer and lung adenocarcinoma rendering inhibitors impractical (71,78).

Thapsigargin is a sesquiterpene lactone isolated from *Thapsia garganica*, which naturally represses ATPase activity, induces endoplasmic reticulum (ER) stress by preventing $Ca^{2+}$ from leaving the cytosol to enter the ER but also exhibit anti-autophagic effects hence long-lived cytosolic proteins are not broken down (68). While SERCA transmembrane helices are in the E2 conformational state, Thapsigargin can bind to the pump and prevents its return to the initial E1 state capable of binding $Ca^{2+}$(more prominently binds to SERCA1b) (68). Therefore, Thapsigargin elevates intracellular free calcium $[(Ca^{2+})i]$, through inhibition of the endoplasmic reticulum Ca-ATPase which repossesses calcium from the cytosol (79,80). It has been reported that increase of $[Ca^{2+}]i$ in keratinocytes is associated with differentiation and can be inhibited by 3 nM of thapsigargin.
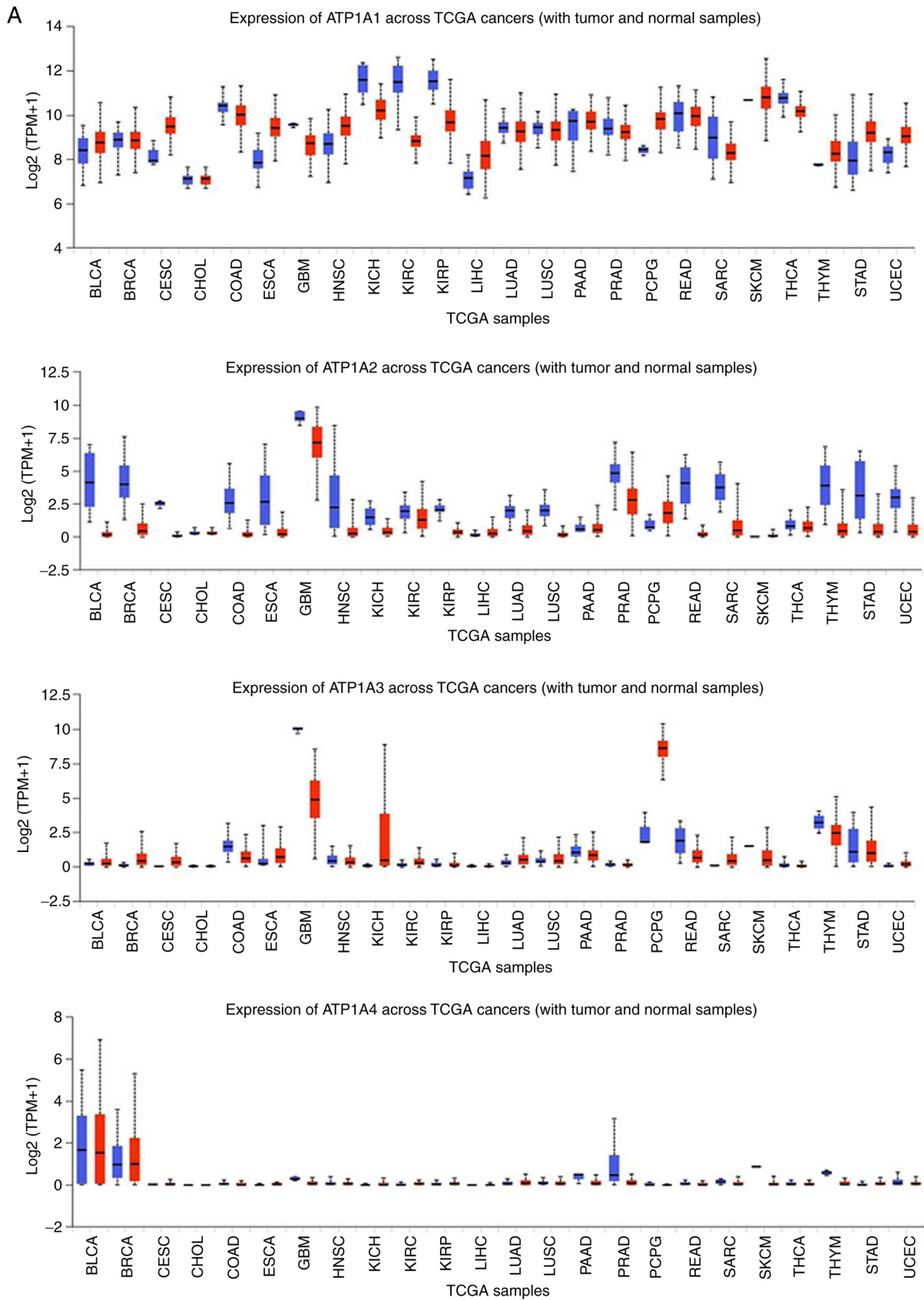
A



Figure 3. Continued.

Thapsigargin has been reported to promote apoptosis in human hepatoma cells via initiating apoptosis of cells, DNA fragmentation and chromatin condensation (80,81). Similarly, recent clinical studies with mipsagargin, a prodrug of thapsigargin, have supported its use against multiple solid tumors with strong evidence for treatment of hepatocellular carcinoma
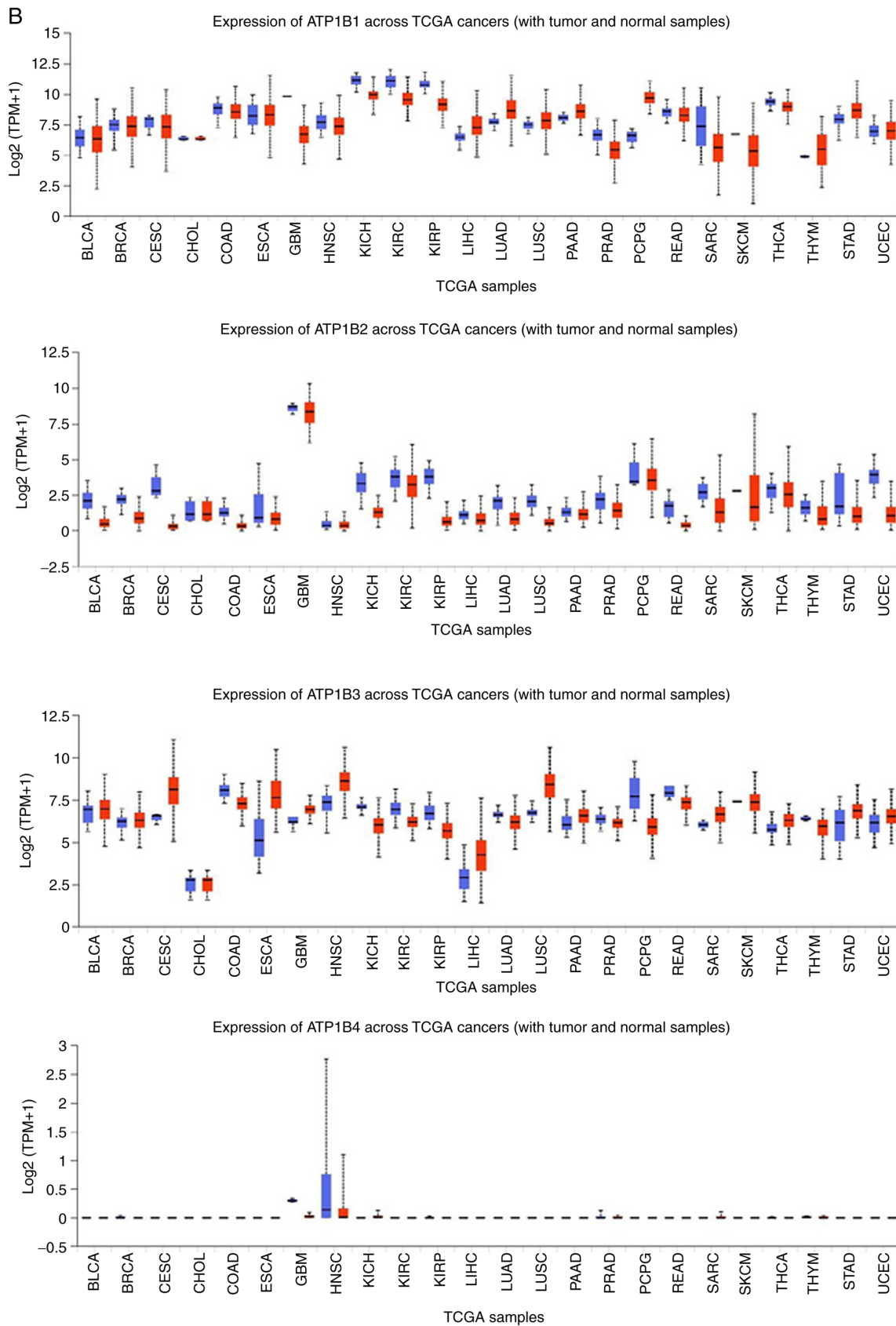
Figure 3. Expression levels of all eight NKA isoforms in 24 types of cancer (red) and their respective healthy tissues (blue). (A) ATP1A1, ATP1A2, ATP1A3 and ATP1A4. (B) ATP1B1, ATP1B2, ATP1B3 and ATP1B4. Data were collected and permitted for publication from the UALCAN resource. TPM, transcripts per million; TCGA, The Cancer Genome Atlas; NKA, Na$^+$/K$^+$ ATPase; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PAAD, pancreatic adenocarcinoma; PRAD, prostate adenocarcinoma; PCPG, pheochromocytoma and paragangliosarcoma; READ, rectal adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; THCA, thyroid carcinoma; THYM, thymoma; STAD, stomach adenocarcinoma; UCEC, uterine corpus endometrial carcinoma.

(Table I) (82). A PSA-Thapsigargin prodrug was administered to nude mice xenograft models diagnosed with prostate cancer and significantly reduced tumor size (81,82). However, a prodrug was used to ensure the effects of thapsigargin on SERCA would not be activated unless bound to prostate tissue, despite its location (83). Thapsigargin also inflicts tumor cell death more sufficiently when administered with a metabolic inhibitor of glucose, in *in vivo* parental cancer cells (84,85).

SERCA pump disregulation renders chemotherapeutic drugs useless due to the environmental conditions created inside a cell. Artemisinin is a plant-derived drug proven effective in triggering a pro-apoptotic pathway in colorectal adenocarcinoma cells and increases $Ca^{2+}$ levels intracellularly (86). Doxorubicin is a chemotherapy drug proven effective against HT29, Hep G2, MCF-7 and LoVo cells while expressing cytotoxic effects. Artemisin and parthenolide activate CaMKII which sets off a cascade of events resulting in increased resistance of cancer cells against Doxorubicin, therefore suggesting they should not be administered synergistically. Alternatively, a combination of parthenolide with a product known as clerodane diterpene casearin J, was effective in initiating T-cell acute lymphoblastic leukemia cell apoptosis by inhibiting simultaneously NF-κB and SERCA respectively (87). Parthenolides ability to inhibit SERCA, hence restoring $Ca^{2+}$ intracellularly, also assists the successful functioning of 5-fluorouracil (chemotherapy drug) (86,88).

ATP2A2 and ATP2A3 expression is suppressed in human breast cancer and human dermal fibroblast cell lines. Resveratrol, a polyphenol molecule, tested in both these cancer cell lines, is capable of reestablishing ATP2A3 expression therefore decreasing cytosol $Ca^{2+}$ and consequently decreasing proliferation and triggering apoptosis. Drugs that bind to pumps may be subunit specific, like resveratrol which restores ATP2A3 and not ATP2A2 (89).

As previously mentioned inhibiting pumps can lead to a cascade of cellular alterations. This was also observed when saikosaponin-d, a SERCA inhibitor that promoted autophagy via $Ca^{2+}$ build-up intracellularly induces ER stress and results in CaMKKβ-AMPK-mTORs signalling activation in cervical and breast cancer cells (13).

Alterations occur in gene expression of all three SERCA subunits when comparing cancer and healthy samples (Fig. 4). Analysing these gene expressions may indicate specific subunits to be used as biomarkers for tissue specific diagnosis of cancer as the previously discussing pumps. For instance, in liver cancer, ATP2A2 is highly expressed compared to healthy cells.

## 5. HKA

*HKA structure and function.* The HKA is a heterodimer composed of two subunits, α and β, which are expressed by two separate genes (ATP4A, ATP4B) (90). The ATP4A gene encodes for the gastric α subunit, composed of 10 transmembrane helices and housing the pump inhibitor-binding site intracellularly, known as cysteine 813. When referring to a separate α subunit located outside the gastric tissue, such as the prostate and skin, it is known as the ATP12A (110 kDA) (91). The ATP4B gene encodes for the β subunit (35 kDA) which is highly glycosylated transmembrane protein and protects the enzyme from the stomachs' acidic environment (92).

Apart from the parietal cells of the stomach, the HKA is also found in the distal nephrons of kidneys, prostate, skin and the placenta (93). ATP4A and ATP4B are highly expressed in stomach and esophageal tissue (Fig. 5), which is expected given the action of HKA in maintaining pH in both organs. However, both HKA subunits are scarce in other tissues, which may explain, at least in part, why studies on this ion pump are limited.

The HKA expresses characteristic structural changes once activated, known as E1 and E2 conformations, leading to the transportation of H+ into the lumen while $K^+$ is removed and released into the cytoplasm side of the enzyme (94-96). Therefore, when the protein is open to the cytoplasmic side then it is in the E1 phase, when it is open to the extracellular environment then it is in the E2 phase (92). At an almost neutral pH (~6), $2H^+$ and $2K^+$ are pumped whereas in acidic conditions (pH ~3), $1H^+$ and $1K^+$ are transported. For all this to happen, energy from dephosphorylation is required, giving HKA ion pumps the ability to succour the acidic environment in organs such as the stomach (97).

*H/K ATPase significance in cancer.* The behavior of HKA subunits suggests that ATP4A is more stable when its counterpart, ATP4B is present (98). The absence of ATP4A alone however, resulted in hyperplasia, metaplasia and an imbalance in growth factor in mice in chronic cases suffering from hypergastrinemia and achlorhydria (99). Ion pumps may not always have a direct effect on the pathology of the tissue. Pepsin expresses both inflammatory and carcinogenic effects in the hypopharynx and larynx when active, consequently an acidic environment created by HKA has been proven to activate pepsin freely present in the vicinity or stored in vesicles (98). In the case of rectal cancer, omeprazole improves efficacy of chemotherapy and relieves patients from chemotherapy side effects. As mentioned, these inhibitors bind to the α subunit causing conformational changes which cease ATPase action, hence the pump remains inactive (100). This favourable co-administration of proton pump inhibitor (PPI)-chemotherapeutic drug was noticeable within a human leukemia cell line (HL60 cells), where butyrate was administered inhibiting histone deacetylase (located on ATP12A) favouring apoptosis. However, SCH28080 was required first for inhibiting pump activity and restoring pH intracellularly (100).

As mentioned above, chemotherapy drugs are often rendered incompetent in eliminating cancer cells due to the greatly acidic environment expressed in tumors (101). Hence, PPIs such as omeprazole, pantoprazole and lansoprazole, serve to restore proton movement across cellular membranes, thus restoring the pH to levels where chemotherapy drugs can actively express their anticancer effects (91,102). In breast cancer cell lines doxorubicin was significantly more cytotoxic to when administered in combination with PPIs (103). The same was perceived when PPIs were synergistically administered with raloxifene to T47D cells (103), another breast cancer cell line, suppressing cell growth compared to the raloxifene alone. PPI administration was also evaluated in Prostate cancer patients, yet no significant results indicated their effectiveness as a cancer drug (104). HKA (coded by ATP4A, ATP4B and ATP12A) is expressed in pancreatic ducts and is responsible
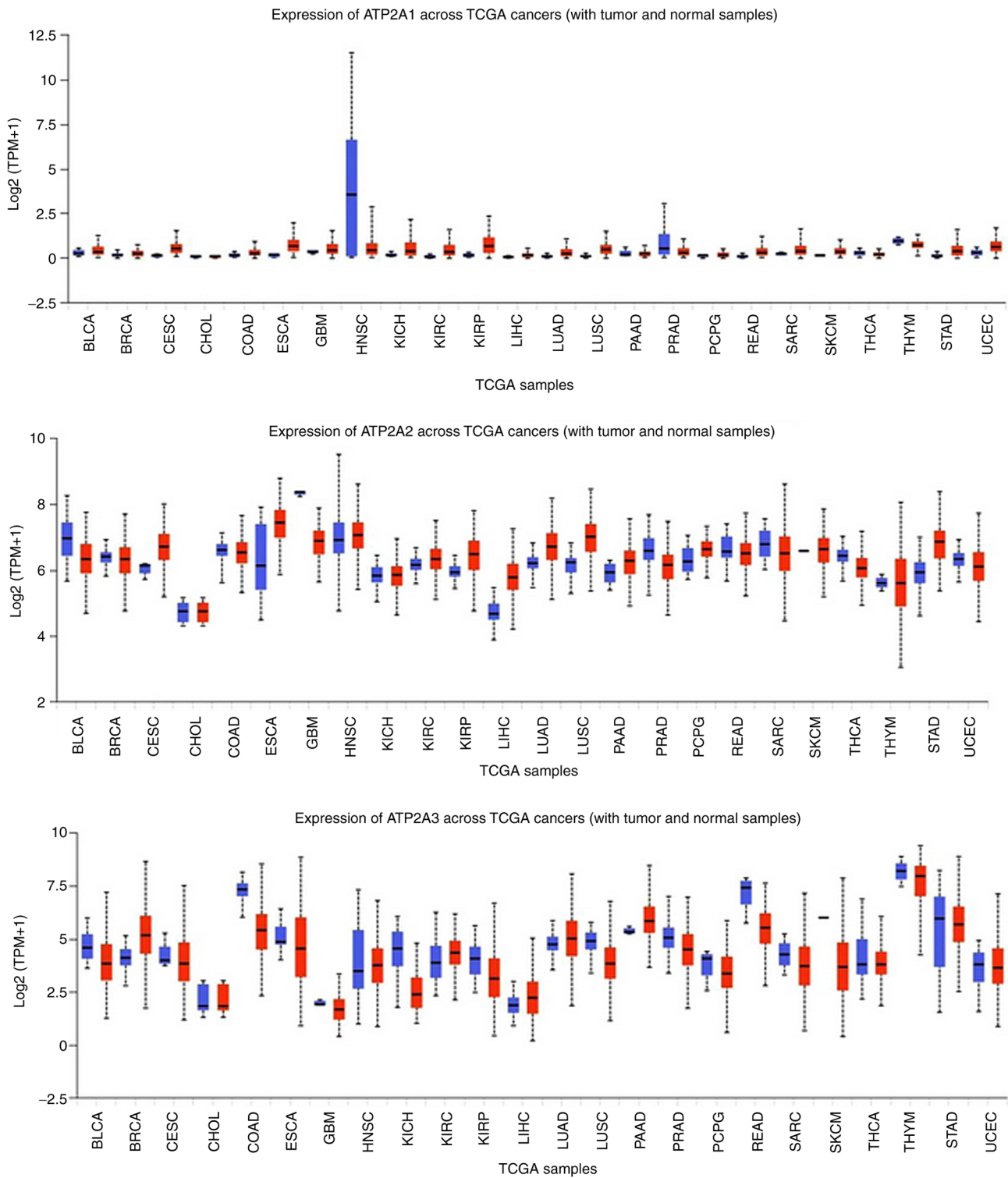
Figure 4. Expression levels of SERCA in 24 cancer tissues compared with in healthy tissues. Data were collected and permitted for publication from the UALCAN resource. TPM, transcripts per million; TCGA, The Cancer Genome Atlas; SERCA, sarcoendoplasmic reticulum calcium ATPase; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PAAD, pancreatic adenocarcinoma; PRAD, prostate adenocarcinoma; PCPG, pheochromocytoma and paragangliosarcoma; READ, rectal adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; THCA, thyroid carcinoma; THYM, thymoma; STAD, stomach adenocarcinoma; UCEC, uterine corpus endometrial carcinoma.

for regulating the secretion of pancreatic fluid but is also expressed in pancreatic cancer cells. Administrating PPIs to pancreatic ductal adenocarcinoma (PDA) and pancreatic stellate cells inhibited proliferation *in vitro* experiments while *in vivo* testing on PDA had brought the GO/G1 cell cycle at a halt, hence decreasing both tumor growth and fibrosis (105).
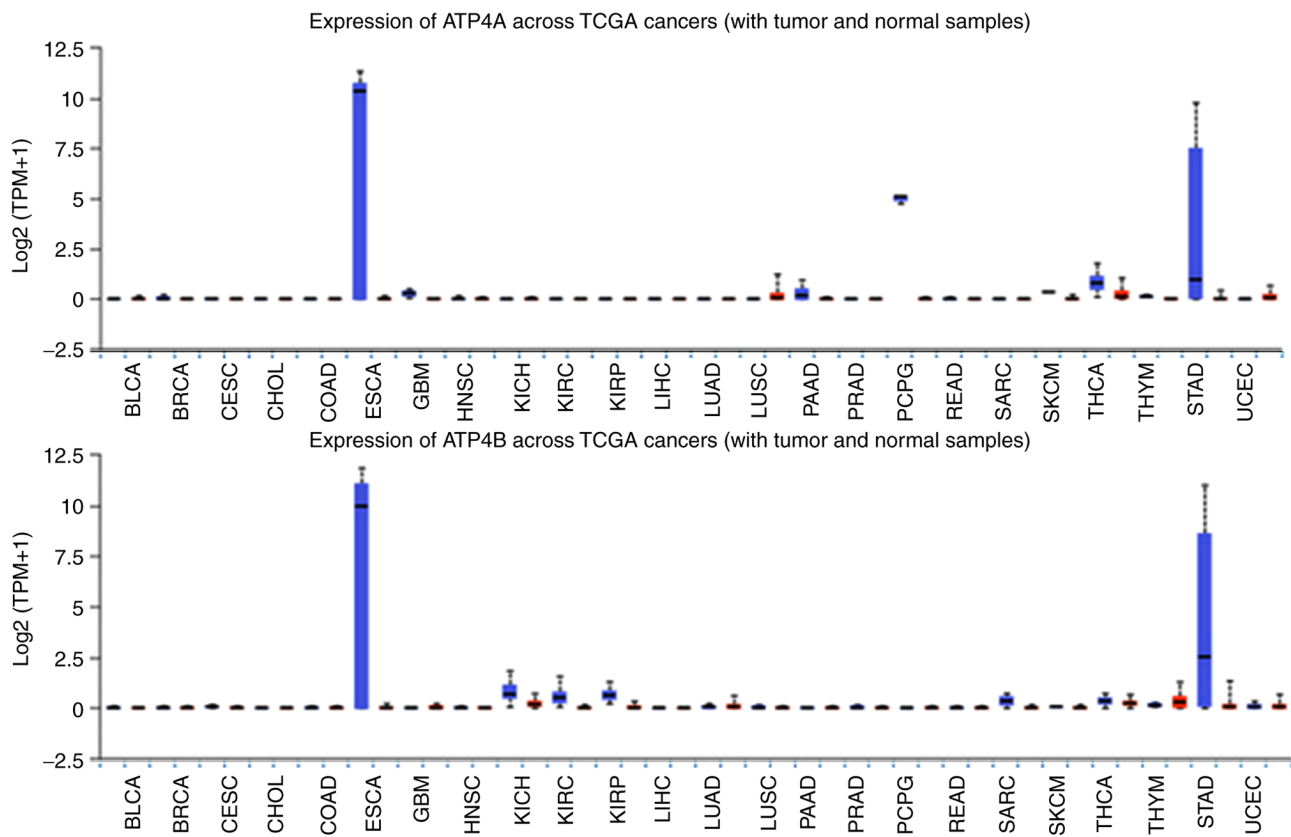
Figure 5. Expression levels of HKA in cancer compared with in healthy tissues across 24 types of cancer. Downregulation of HKA expression was markedly observed in esophageal carcinoma and stomach adenocarcinoma, and was less distinct in thyroid carcinoma. Overall expression in all other tissues appeared to be limited. Data were collected and permitted for publication from the UALCAN resource. TPM, transcripts per million; TCGA, The Cancer Genome Atlas; HKA, H$^+$/K$^+$ ATPase; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma; CHOL, chol-angiocarcinoma; COAD, colon adenocarcinoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PAAD, pancreatic adenocarcinoma; PRAD, prostate adenocarcinoma; PCPG, pheochro-mocytoma and paragangliosarcoma; READ, rectal adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; THCA, thyroid carcinoma; THYM, thymoma; STAD, stomach adenocarcinoma; UCEC, uterine corpus endometrial carcinoma.

Fig. 5 illustrates that ATP4B is exceedingly reduced in gastric cancer (GC) compared to its high expression in healthy gastric cells and this is also corroborated by the litera-ture (106). This often causes problems in terms of distorting chemotherapeutic drug behaviour. ATP4B can be restored using 5'-aza-2'-deoxycytidine (a DNA methyltransferase inhibitor) or trichostatin A (an histone deacetylase inhibitor), consequently reinstating the β subunit further restoring pH to homeostatic levels, hence creating a suitable environment for docetaxel (chemotherapeutic drug) to function (106).

Trametenolic acid B (TAB) targets HKA in healthy indi-viduals and rectifies pH in gastric environments reversing gastric ulcers diseases. The inhibitory effects of TAB also promote apoptosis in human gastric carcinoma (HGC-27 cells) and reduce cell viability both *in vitro* and *in vivo* (107). Model dose and pH need to be detailed for the drug to be effective without affecting surrounding healthy cells.

Another PPI is pantoprazole that is activated in acidic envi-ronments expressed in tumors, and has been reported capable of inducing apoptosis in MKN-45 cells and RGM-1 cells (108). Evaluation in a GC xenograft indicated suppressed tumor growth and promotion of apoptosis through covalent binding of pantoprazole to the cysteine residues on the α subunit of

HKA, manipulating the extracellular signal-regulated kinases pathway (108). Pantoprazole also proved to reduce chemo-resistance to fluorouracil (a chemotherapeutic drug) in both *in vitro* and *in vivo* studies in colorectal cancer cells (102). Rabeprazole on the other hand was found to be effective in treating gastric cancer, by preventing ERK 1/2 phosphoryla-tion and simultaneously inhibiting HKA, creating a less acidic environment (10).

Esophageal cancer cells express a sensitivity towards chemotherapeutics when exposed to esomeprazole (ESO) as tumor survival is reduced and metastasis is obstructed (*in vitro*) (109). ESO proved to alter the expression of miRNAs involved in chemo-resistance as it is a proton pump inhibitor preventing protons from entering the lumen of a cell, resulting in an intracellular basic state (109,110). This action also sensitizes human osteosarcoma cells to cisplatin, another chemotherapeutic drug (110). ESO has also proved effective when administered along with doxorubicin. Doxorubicin-esomeprazole combination was significantly more effective than either drug separately (111). Esomeprazole prevents H$^+$ from moving intracellularly, therefore causing a build-up of protons in the tumorigenic cells creating a toxic environment, hence favouring the action of doxorubicin (111).

Efavirenz, a reverse transcriptase inhibitor, is an antiviral and anti-tumor drug whose action is impaired in acidic conditions. Lansoprazole is a drug that is activated by the acidic environment in cancer cells and its function is to inhibit HKA. Once the HKA is inhibited then the acidic pH can be overturned to a state where efavirenz can perform. Efavirenz prevents migration of tumor cells and decreases proliferation in human melanoma cells (112). Lansoprazole is also effective in promoting apoptosis and inhibiting proliferation in breast cancer cell lines (MDA-MB-231), while inhibiting tumor growth *in vivo* (107).

Dexlansoprazole, tenatoprazole, revaprazan and vonoprazan are all PPIs used for treating esophageal reflux disease, with evidence suggesting faster HKA inhibition compared to other PPIs (113-115). However, to the best of our knowledge, they have yet to be evaluated as potential anticancer drugs. Rohitukine, on the other hand, expresses both antiulcer and anticancer properties, but it is uncertain if the anticancer effects are due to HKA pump inhibition (116). Maintaining HKA activity is crucial as additional studies point out that failure to treat disorders such as *Helicobacter pylori* infection, which renders HKA inactive, may lead to gastric cancer (64).

Compared to NKA and SERCA, HKA subunits expressed in normal and cancer cells have no significant alterations across the 24 cell lines studied using bioinformatic tools, with the exception of gastric and esophageal cells (Fig. 5). This pinpoints the specific subunits to be explored as potential targets for treatment or as biomarkers.

## 6. Clinical studies of P-class pump modulators

*Na/K ATPase*. Previous studies have shown that NKA expression is altered in various tumors, including drug-resistant tumors, marking it as a potential target by developing NKA modulators (97,117,118). For this reason, different NKA inhibitors, such as perillyl alcohol and cardiac glycosides, are currently being used in a number of clinical trials, to assess their effect on different cancers (63,119,120). Despite their narrow therapeutic window, cardiac glycosides have exhibited acceptable tolerance, so far. In addition to their direct cytotoxic effects and anti-proliferative properties, different NKA modulators can overcome multiple mechanisms of cancer cells that often lead to failure of existing chemotherapeutic agents (117).

In particular, in one study with biochemically-relapsed prostate cancer, digoxin was well-tolerated, but was not of additional benefit compared to that observed in controls reported in previous data (historical controls) (121). A study of patients with BRAF-wild type metastatic melanoma, showed a better response to trametinib when administered with digoxin, compared to studies of trametinib alone (119). In addition, effects of lapatinib on digoxin were assessed in a series of patients with HER2-positive breast cancer, showing that lapatinib significantly increased the absorption of digoxin, thus underscoring the necessity for dose adjustment. No effects on the tumor were reported (120).

*SERCA pump*. As $Ca^{2+}$ regulates different cellular functions, including proliferation and differentiation, disruption of $Ca^{2+}$ pathways may lead to the development of anti-cancer therapies. To this end, altered function of SERCA pumps have shown to contribute to cancer development (70). Among SERCA inhibitors, thapsigargin and its prodrug, mipsagargin, are used in different stages of clinical trials. A study of mipsagargin in patients with advanced solid tumors exhibited acceptable tolerability and safety, whereas cancer stabilization (defined as progression-free survival) was recorded, particularly in patients with hepatocellular carcinoma (81).

*H/K ATPase*. The clinical usefulness of the HKA is evident in the number of clinical trials that involve PPIs. PPIs can reduce tumor acidity, thus allowing other drugs to reach cancer cells, but also exhibit direct tumor cell toxicity themselves. Additionally, by affecting the acidic environment of the tumor, potentiate the pharmacokinetics of antitumor drugs (102). For this reason, most clinical studies include PPI in combinations for treating malignancies. Combination of high-dose PPIs and aspirin significantly improved composite end-point outcomes in patients with Barrett's esophagus (122). High dose PPI treatment enhanced antitumor effects (translated as time to progression) of chemotherapy in patients with metastatic breast cancer (102). Patients who received PPI during chemotherapy for colorectal cancer, had significantly lower rates of nausea and vomiting compared to patients who did not receive PPI (102). Although pantoprazole did not affect the pharmacokinetics of intravenous doxorubicin in patients with solid tumors, esomeprazole reduced the pharmacokinetic properties of pazopanib in patients with various solid tumors, suggesting decreased absorption of oral pazopanib when the gastric pH increases (106,108).

## 7. Concluding remarks and future perspectives

The aim of this review was to assess literature sources and determine if there is efficient evidence to support the implication that P-class pumps should be targeted in cancer treatments (Table SI). Overall, ion homeostasis can affect an organ's natural function, diseased or healthy. Research shows that cancer cell environments express ion imbalances (10) and further evidence, presented above, suggest one cause to be the involvement of p-class pumps. Subsequently, the functional roles of NKA, SERCA and HKA (ion pumps) as potential biomarkers but also as probable targets against tumorigenesis and progression were evaluated.

Two of the major setbacks of chemotherapeutic drugs is the hostile environments generated by cancer cells resulting in chemoresistance and toxicity created by the drugs themselves (110,111,117). Numerous reports present evidence of pump inhibitors administered synergistically with chemotherapy drugs overcome the hostile environment and result in inducing cell cycle arrest, decreasing proliferation and angiogenesis in various cancer types and primarily induce apoptosis. Nonetheless, other inhibitors administered in synergy with specific chemotherapeutic drugs have proven to have the unfavorable opposite effect. Some pump inhibitors administered do not express anti-cancerous effects per se but simply layout a more friendly or more hostile environment for the chemotherapeutic drug or cancer cells (100) respectively. Henceforth, further research is necessary to cross-examine which combinations of anticancer drugs to pump inhibitors express a favorable effect on which specific type of cancer cells (103).

Complication, besides some inconclusive outcomes, have surfaced through the literature where reports imply development of gastric cancer after long-term PPI treatment (123-125). One additional aspect to be considered is that PPIs are commonly metabolised by CYP-mediated isoforms that express drug-drug interactions, meaning other drugs may bind to the same CYP-active sites occupying them or even causing some alterations in the proteins structure. Such actions may render CYP unresponsive when PPIs try to attach, therefore concomitant medication should be considered when administering these drugs (126). Reference to toxicity was scarce throughout the literature, as the majority of reported studies were performed *in vitro*, however it is a significant feature to consider when selecting drugs for medical administration both short-term and long-term.

This review has also collected data indicating p-type pump expression is altered in tumor cells, affecting various cellular pathways which are involved in cell cycle, apoptosis, angiogenesis and metastasis, activation of MAPK, Caspase 3, ERK phosphorylation, downregulation/inhibition of IL-8, TNF-α/NF-κB, f and Bcl-2 (14,53,54,85,127-129). The main finding from the bioinformatics data collected (TCGA database) indicates that the differential expression of NKA, HKA and SERCA pump subunits is not following a specific expression pattern. These subunits expressed in a tissue specific manner with variations of high and low expression in cancer vs normal and vice versa.

Further evaluation of p-class pumps currently administered for other disorders (e.g., HKA in gastric reflux) may assist in determining the exact stage of cellular pathways affected by these pumps hence determining whether they can potentially contribute as novel cancer treatments (100,130). An additional gap identified in the literature is the absence of the exact pump subunits targeted by the drugs as this would help in better understanding and evaluating the behavior of the inhibitors. An example was presented with resveratrol established to specifically target the ATP2A3 subunit leading to its extensive evaluation and progression into clinical trials (89).

More detailed conclusions could have been drawn with more in-depth analysis of how the biochemical pathways involved are related to both cancer and the p-class pumps. Nevertheless, with this collection it is feasible to identify gaps which remain and directions in which to progress, such as the need for further studies to elucidate the safety and clinical impact of the above findings. The clinical studies section does refer to some additional drugs which are not analysed in detail in the review. There have been other collective reviews on p-class pumps (77,89,130-132). To the best of the authors knowledge no other review has brought together evidence from the literature on the behavior of P-class pumps *in-vivo*, *in-vitro* and the clinical environment. The progress made so far towards including p-class pumps as targets against cancer or as architects which utilize chemotherapeutic drugs has been depicted above.

## Acknowledgements

## Funding

## Availability of data and materials

Not applicable.

## Authors' contributions

ST and AY conceptualized the study. ST, AY, CT, AZ, EJ and IP collected and interpreted the data. ST, AY and CT drafted the manuscript. All authors revised the manuscript for important intellectual content, and read and approved the final manuscript. Data authentication is not applicable.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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