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Calcium-ATPases: Gene Disorders and Dysregulation in Cancer

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Abstract

Ca^{2+}-ATPases belonging to the superfamily of P-type pumps play an important role in maintaining low, nanomolar cytoplasmic Ca^{2+} levels at rest and priming organellar stores, including the endoplasmic reticulum, Golgi and secretory vesicles with high levels of Ca^{2+} for a wide range of signaling functions. In this review, we introduce the distinct subtypes of Ca^{2+}-ATPases and their isoforms and splice variants, and provide an overview of their specific cellular roles as they relate to genetic disorders and cancer, with a particular emphasis on recent findings on the secretory pathway Ca^{2+}-ATPases (SPCA). Mutations in human *ATP2A2*, *ATP2C1* genes, encoding housekeeping isoforms of the endoplasmic reticulum (SERCA2) and secretory pathway (SPCA1) pumps respectively, confer autosomal dominant disorders of the skin, whereas mutations in other isoforms underlie various muscular, neurological or developmental disorders. Emerging evidence points to an important function of dysregulated Ca^{2+}-ATPase expression in cancers of the breast, colon, lung and breast where they may serve as markers of differentiation or novel targets for therapeutic intervention. We review the mechanisms underlying the link between calcium homeostasis and cancer and discuss the potential clinical relevance of these observations.

1. Introduction to Ca^{2+} Transporters

Ionic calcium is a ubiquitous second messenger in the activation of signaling cascades [1–3]. Ca^{2+} signaling regulates a wide range of cellular and physiological processes, which include transcriptional activation, cell cycle control, muscle contraction, and lactation [4]. On the other hand, prolonged cytoplasmic elevation of free Ca^{2+} is toxic and triggers cell death [5]. Therefore, under normal circumstances, cells must tightly regulate cytoplasmic calcium levels between a resting (~100 nM) and an activated state (~500nM – 1μM). This sensitive balance is maintained by a cadre of membrane transport proteins that work in concert to move Ca^{2+} across membranes, in and out of the cell or intracellular storage organelles [3]. In terms of energetics and mechanism, Ca^{2+} transporters fall into the three broad classes of Ca^{2+} channels, exchangers, and pumps. Ca^{2+} channels are activated by a variety of chemical, mechanical or electrical signals: membrane voltage, ligand binding, mechanosensation and the endoplasmic reticulum (ER) store. In response to an activating signal, ion channels

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residing on cell or organellar membranes open, releasing a flood of Ca^{2+} down electrochemical gradients into the cytoplasm, resulting in signal transduction and amplification. Upon elevation of cytoplasmic Ca^{2+}, energy-dependent active transporters, including pumps and exchangers, work to refill stores, reset calcium levels to the resting state and re-establish transmembrane electrochemical Ca^{2+} gradients. Secondary active transporters exemplified by the plasma membrane Na^{+}/Ca^{2+} exchanger, couple to the sodium electrochemical gradient to rapidly expel the bulk of cytoplasmic Ca^{2+}. ATP-dependent Ca^{2+} pumps, discussed in this review, scavenge the remaining Ca^{2+} to establish low, nanomolar resting levels of this second messenger by translocating it out of the cell or by sequestering it in the ER, Golgi or secretory vesicles.

2. P-Type Ca^{2+}-ATPases

Calcium pumps belonging to the superfamily of P-Type ATPases (originally called E1E2-type) move ions across membranes, against their electrochemical gradient, by utilizing the energy from ATP hydrolysis [6–8]. Central to their mechanism is the formation of a phosphorylated reaction intermediate (E~P) that separates a series of distinct conformational states: E1 conformations display high Ca^{2+} affinity that bind the ion from the cytoplasmic side, and E2 conformations in which the affinity for Ca^{2+} has been reduced by ~1000-fold following ATP hydrolysis, thereby facilitating release of the ion(s) to the lumenal/extracellular side. Although related by sequence similarity, structural homology and common transport mechanism, there are three subtypes of calcium pumps that are phylogenetically distinct and marked according to their subcellular localizations: namely, the plasma membrane (*P*lasma *M*embrane *C*a^{2+}-*A*TPase or PMCA), endoplasmic reticulum (*S*arco/*E*ndoplasmic *R*eticulum *C*a^{2+}-*A*TPase or SERCA), and Golgi/Golgi-derived vesicles (*S*ecretory *P*athway *C*a^{2+}-*A*TPase or SPCA) [9]. The separation of these subtypes likely predates the emergence of eukaryotes since representative variants are found in archaea and eubacteria [9]. In humans, multiple isoforms and splice variants exist within each subtype, adding flexibility to tissue specific expression, regulation and kinetic characteristics to fine-tune both temporal and spatial Ca^{2+} signatures [10].

2.1 Sarco/Endoplasmic Reticulum Ca^{2+}-ATPases

The sarco/endoplasmic reticulum Ca^{2+}-ATPases (SERCA) are the best characterized of the three subtypes, being highly expressed in the specialized ER of muscle where they reach abundance of ~50% of membrane protein [11]. They are responsible for sequestering calcium in the ER, which is the most abundant and readily mobilized store for intracellular calcium [12, 13]. There are 3 genes (*ATP2A1-3)* coding the SERCA1-3 pumps, and all have varying expression levels and tissue distributions in the body [10, 14]. SERCA1 is expressed in skeletal muscle with 2 variants, SERCA1a, the adult form, and SERCA1b the neonatal form produced by alternate splicing of exon 22, which is expressed in the adult form only [15]. SERCA2 is ubiquitously expressed, with the SERCA2b variant serving an essential housekeeping function. SERCA2a is exclusively expressed in muscle and in neuronal cells, whereas SERCA2c and SERCA2d are expressed in the heart [16, 17]. These specific tissues are physiologically demanding for processes that require Ca^{2+} such as muscle contraction and propagation of action potentials in the nervous system. SERCA3 is the least

characterized isoform with high expression in specific hematopoietic-derived cells of the immune system and in other cell types. There are many variants of SERCA3, with 6 in humans (SERCA3a-f), 3 in mice (SERCA3a-c), and 2 in rats (SERCA3a,b/c), suggesting that these pumps may have a more widespread role in cellular Ca^{2+} homeostasis than has been appreciated thus far [18, 19]. Aided by natural abundance and years of intensive study, skeletal muscle SERCA1a isoform has been crystallized in multiple distinct conformations, revealing unprecedented insight into the transport mechanism [20–22].

2.2 Plasma Membrane Ca^{2+}-ATPases

There are four plasma membrane Ca^{2+}-ATPases [23], which are PMCA1-4 (gene names *ATP2B1-4*). Whereas PMCA1 and PMCA4 are widely distributed, PMCA2 and PMCA3 show more restricted tissue expression. PMCA1, the most ubiquitously expressed of the four, has 5 variants (PMCA1a-e) where PMCA1b is ubiquitously expressed and the other isoforms are expressed in the brain and in skeletal muscle. PMCA2 has 6 variants and PMCA3 has 3 variants, all of which are expressed in the brain and in tissues intimately connected to the nervous system [24, 25]. PMCA2 is expressed in the apical membranes of mammary gland acinar cells, where it is substantially induced during lactation for calcium secretion into milk. In mice lacking PMCA2, the milk contained 60% lower levels of Ca^{2+} relative to the control mice [26]. PMCA3 is exclusively found in the brain and in tissues that are closely associated with neuronal signals; interestingly, this isoform was shown to have a pre-synaptic distribution relative to PMCA2, which was found in post-synaptic regions of the cerebellar cortex [27]. Similarly to PMCA1, PMCA4 is ubiquitously expressed and has 8 variants, which are expressed in smooth muscle, the heart, and in the brain. PMCA4b is the variant that is ubiquitous and has been most widely investigated for function and regulation. [24, 25].

2.3 Secretory Pathway Ca^{2+}-ATPases

The most recent of the three subtypes to be discovered, the secretory pathway Ca^{2+}-ATPases (SPCA), comprise calcium pumps residing in the Golgi compartments and post-Golgi vesicles. The prototypic SPCA pump PMR1 (for *P*lasma *M*embrane ATPase-*R*elated) was discovered by homology cloning in the yeast *Saccharomyces cerevisiae* [28] and shown to have unique biochemical properties distinct from the SERCA and PMCA subtypes [29]. For example, PMR1 is insensitive to inhibition by nanomolar concentrations of thapsigargin, a classical hallmark of SERCA pumps. In addition to delivering Ca^{2+} into the secretory pathway where it is required for cargo sorting and protein processing, PMR1 also transports Mn^{2+} with very high (~20 nM) affinity into the Golgi lumen, serving a dual role in clearing excess Mn^{2+} via exocytosis and providing an essential co-factor for mannosyltransferases, required for protein glycosylation [30]. Null mutants (*Δpmr1*) thus have pleiotropic defects, and require ion-supplemented media for growth [30, 31].

Heterologous expression studies in yeast laid the groundwork for characterization of the mammalian orthologs [32], represented by SPCA1 and SPCA2, which are encoded by *ATP2C1* and *ATP2C2* respectively. It is interesting that invertebrates and lower vertebrates, including fish, have only one SPCA gene, as in yeast. A second gene appears in tetrapods, including modern amphibians, reptiles, birds and mammals [33]. SPCA1 is ubiquitously

expressed in all tissues, whereas SPCA2 is restricted to absorptive (intestinal) and secretory (pancreas, salivary and mammary glands) epithelia [34]. There are four splice variants of SPCA1, differing only at the C-terminus [35], and no known splice variants of SPCA2. SPCA2 has ~65% identity with SPCA1, differing largely at the N-terminus which is significantly longer than that of SPCA1 [33]. Both SPCA pumps share similar kinetic properties, with SPCA2 exhibiting lower apparent affinity for Ca^{2+} transport [36, 37].

In addition to the full-length ~103 kDa SPCA2 protein, Garside et al. [38] demonstrated the presence of a much smaller transcript that generates a ~20 kDa C-terminal fragment, expressed in pancreatic acinar cells under control of the MIST1 transcription factor (also known as *basic helix loop helix a15*, Bhlha15). Examination of other MIST1 expressing tissues (salivary, prostate and gastric glands, seminal vesicles) also revealed the presence of this shorter form, predicted to lack the majority of the membrane domains, as well as the essential phosphorylation and nucleotide-binding domains required for Ca^{2+} transport and ATP hydrolysis. Surprisingly, *Mist1*$^{-/-}$ mice lacking this fragment display defective Ca^{2+} signaling and secretion defects that suggest a functional role for the C-terminus, independent of Ca^{2+} pumping. An explanation for this “moon-lighting” function came from an independent study by Feng et al. [39] showing that the C-terminus of SPCA2 physically interacts with and restores trafficking of the calcium channel, Orai1, and rescues store-operated calcium entry (SOCE) in a SPCA2 knockdown model in mammary epithelial cells. Furthermore, the C-terminal domain is capable of store-independent activation of Ca^{2+} influx, mediated by Orai1 and possibly other channels. These studies assign a highly unusual and novel functional role of the SPCA2 C-terminus, independent of the Ca^{2+}-ATPase function, in chaperoning and activation of ion channels. Similarly, some ABC-type transporters are famously known to interact with and regulate ion channels [40, 41], and it remains to be seen if this function is shared by other P-type Ca^{2+}-ATPases.

In secretory and absorptive epithelia, Ca^{2+} transport processes must be coordinated at multiple membranes to affect Ca^{2+} transcytosis, as exemplified by movement from the blood to the lumen in the lactating mammary gland, resulting in accumulation of high millimolar concentrations of complexed calcium in milk [42]. During lactation, the mammary gland undergoes drastic morphological changes and a coordinated induction of Ca^{2+} transport proteins (dubbed CALTRANS), which support the increased demand for calcium transport into the milk [43]. Whereas SPCA2 expression is massively induced immediately prior to parturition, SPCA1 expression is relatively moderate and occurs during the mid phase of lactation [44, 45]. Upon involution of the mammary gland and cessation of lactation, both isoforms return to basal levels [46]. Using a three dimensional mammosphere model of lactation induction, SPCA2 was shown to mediate basolateral Ca^{2+} influx, in conjunction with Orai1 [44]. Taken together, these observations suggest that Ca^{2+} entering the polarized secretory cells of the mammary gland is pumped into vesicles by SPCA, packaged into casein-containing micelles and released into the lumen by exocytosis at the apical membrane, in addition to being pumped directly across the apical membrane by the PMCA2 isoform of the plasma membrane Ca^{2+}-ATPase [43]. A recent study investigating the role of Orai1 in lactation in both Orai1 null and conditional knockout mouse models in the mammary gland, found that mammary development was undisrupted, but milk delivery was

perturbed due to decreased SOCE and alveolar contractions [47]. These contractions, generated by the surrounding myoepithelial cells, require Orai1-mediated Ca^{2+} transport.

3. Gene Disorders of Ca^{2+} Pumps

Various genetic disorders have been associated with specific mutations of the Ca^{2+} pumps that show a wide range of different physiological phenotypes and clinical manifestations. Mouse models of Ca^{2+}-ATPase knockouts have been particularly insightful in modeling human disease phenotypes, although differences exist. Here, we summarize disease phenotypes and physiological consequences of disrupting Ca^{2+}-ATPase gene subtypes.

3.1 Gene disorders of PMCA

Mice homozygous for *pmca1*$^{-/-}$ null mutations were embryonic lethal whereas the heterozygous mice exhibited no apparent disease phenotype [48]. Since PMCA1 is ubiquitously expressed, it was concluded that it serves as a housekeeping gene in all tissue types. Okunade et al. also found that *pmca4*$^{-/-}$ null mice had no noticeable phenotype but that sperm motility was impaired, resulting in infertility. Furthermore, this study showed that in heterozygous *pmca1*$^{+/-}$ mice with homozygous *pmca4*$^{-/-}$ knockout, there was vascular contraction impairment in the smooth muscle, but only with one copy of the PMCA1 [48]. A genome-wide association study linked *ATP2B1*, the gene encoding PMCA1, to hypertension, and the conditional knockout of PMCA1 in smooth muscle cells resulted in increased blood pressure [49, 50]. Together, this provides evidence that PMCA1 has an important role in muscle contraction in the vascular system and that its loss may result in having a predisposition for hypertension.

Mutations or deletions resulting in the loss of function of PMCA2 lead to deafness phenotypes in both mice and humans [51–53]. Another interesting disease phenotype that has been associated with mutations in PMCA2 is the impaired ability to balance and walk normally, which was produced in the Wriggle Sagami mouse model [54]. The disorders associated with PMCA2 mutations result in impairment of organs and processes that are intimately connected with the nervous system. Disease phenotypes from PMCA3 mutations have a predominant effect on the nervous system due to its tissue distribution in the neurological organs. A knockout mouse model has not been developed, possibly consistent with an essential role for PMCA3 in early embryonic development.

3.2 Gene disorders of SERCA

Gene knockout studies of SERCA pumps in mouse models have revealed unexpected phenotypes, sometimes dissimilar to the loss of function gene effects in human [55]. There are two well-defined SERCA disorders, Brody and Darier disease, which are phenotypically quite different in their clinical manifestations. Brody disease is a rare autosomal recessive disorder affecting the skeletal muscle during exercise, with symptoms including painless cramps, slow muscle relaxation and stiffness [56]. This disorder was linked to the decreased uptake of Ca^{2+} in the sarcoplasmic reticulum and was later connected to the recessive mutations in *ATP2A1*, the gene encoding the SERCA1 isoform in Brody disease patients and in cultured muscle cells. Interestingly, some Brody disease patients do not harbor

mutations in *ATP2A1*, suggesting that the disease may be heterogeneous in origin [57–59]. Null mutants of SERCA1 in mouse are born normal, but develop respiratory insufficiency due to contractile defects in the diaphragm muscle that leads to cyanosis and death [60].

Darier disease is a rare autosomal dominant genetic skin disorder that is characterized by keratotic papules as a result of the loss of desmosomal proteins at the cell-to-cell junctions that bind keratin filaments. Lesions can form resulting in plaques in specific areas of the body such as the nails and the scalp. There have been over 100 mutations in *ATP2A2*, the gene encoding SERCA2, reported in patients, distributed through the gene. The only mutations that are specific to SERCA2 in Darier disease are those that are common in the SERCA2a and the SERCA2b isoforms [61, 62]. Patient-derived keratinocytes revealed lower Ca^{2+} concentrations in the ER, but cytosolic Ca^{2+} levels were compensated by SPCA1 in these patients [63]. Despite the compensated Ca^{2+} levels, the decreased ER concentrations could impact protein processing and the progression through the secretory pathway. This ER stress gives rise to the impaired sorting and trafficking of desmosomes to the plasma membrane [64]. Although heterozygous SERCA2 knockout mouse models do not recapitulate Darier disease, other phenotypes ranging from a selective predisposition to heart failure [65], to late developing squamous cell carcinoma [55] may be predictive of similar problems in Darier patients. Similarly, mouse models of *ATP2A3* knockout suggest subtle defects in vascular and tracheal smooth muscle contraction, as well as altered Ca^{2+} signaling in pancreatic β cells that potentially correlate with disease phenotypes in human [55]. This is partly corroborated by observations that SERCA3 sequence variants have been associated with genetic susceptibility to Type II diabetes [66].

3.3 Gene disorders of SPCA

Dysregulated expression or inactivating mutations in SPCA have been linked to two distinct disorders. Like SERCA2 (*ATP2A2*) mutations, heterozygous mutation of the ubiquitous SPCA isoform, *ATP2C1* results in a blistering and ulcerative skin disorder reminiscent of Darier disease, known as Hailey-Hailey disease [67, 68]. Haploinsufficiency, resulting from loss of function SPCA1 mutations [69], appears to disrupt the processing of desmosomal and cell-cell adhesion proteins in keratinocytes that may result in the blistering symptoms in these patients. Given the ubiquitous expression and essential role of both SERCA2 and SPCA1, it is unclear why these disorders of calcium pump haploinsufficiency are largely confined to skin. Presumably, there is adequate compensation by other pump isoforms in other tissues or the skin may be exquisitely and uniquely sensitive to perturbations of these intracellular calcium pump isoforms in ways that we do not yet understand. It has been suggested that skin phenotypes arise from lack of expression of the SERCA3 isoform in skin [70]. Consistent with its widespread distribution, SPCA1 has an essential housekeeping function: homozygous *atp2c1*$^{-/-}$ null mice show embryonic lethality, expansion of the Golgi and altered ER stress response, indicating that SPCA2 cannot adequately compensate for the loss [71]. Interestingly, heterozygous *atp2c1*$^{+/-}$ mice do not exhibit blistering phenotypes, similar to the *atp2a2*$^{+/-}$ mouse model. Instead, the mice displayed high propensity to develop squamous cell tumors in epithelia including skin and esophagus, as they matured into adulthood [71].

Genome wide association studies have linked deletions and variants in *ATP2C2* with speech language impairment (SLI) disorders [72, 73]. Risk genes in SLI disorders and in dyslexia have also been connected to children with autism spectrum disorders. SPCA2 has been detected in the brain, including hippocampal neurons [37], and the general idea is that risk associated variants elicit neurological disorders although the cellular and physiological basis for etiology of the disorder is unclear. A knockout mouse model for SPCA2 has not yet been reported, but we anticipate that this deletion will not be as detrimental due to its limited tissue expression.

4. Calcium and Cancer

There is increasing evidence for the importance of Ca^{2+} homeostasis in cancer [74]. A majority of the hallmarks of cancer [75], if not all, involve calcium signaling to mediate critical cellular processes, including transcriptional regulation which underlies the gene expression in a wide range of pathways crucial to tumorigenesis and metastasis, such as proliferation, angiogenesis, migration, cell cycle progression, immune system evasion, and bypass of apoptosis. To acquire tumorigenic potential, a cell must undergo many transformations before becoming malignant. The cellular demands for tumorigenesis make cancer a disease of many mutations and not the result of one specific genetic alteration. Calcium dysregulation is an example of a low input change that results in a large impact, disrupting homeostasis across the entirety of a cell. A cancer cell can hijack and transform calcium transients such that they fulfill the needs of a malignant tumor for a constitutively active and dividing state.

Many calcium transporters have altered expression levels in various cancers, which can accommodate this high demand for calcium movement within a cell. Within a cancer subtype, there are characteristic isoform specific alterations, as depicted in Figures 1–2, that contribute to the unique characteristics of each tumor. Patient derived tumor cell lines offer the opportunity to evaluate the role of individual Ca^{2+}-ATPases using knockdown and overexpression strategies, as has been reported for SPCA2 [39]. An interesting aspect of breast cancer is the development of microcalcifications, which are common radiographic signature that serve as a diagnostic of both benign and malignant tumors [43]. This phenomenon of excess calcium and phosphate depositions is not well understood at the mechanistic level but is a readily observable and common phenotype of calcium dysregulation in cancer [76–78]. Specific findings linking each of the three Ca^{2+}-ATPase subtypes to cancer are summarized below.

4.1 PMCA and Cancer

A common hallmark of cancer, the Warburg effect, is characterized by a preferential and high glycolytic rate relative to mitochondrial respiration. This phenomenon occurs in pancreatic ductal adenocarcinoma (PDAC) where glycolytic ATP fuels Ca^{2+} efflux via the PMCA pumps. Inhibition of glycolysis in PDAC led to irreversible intracellular Ca^{2+} overload and cell death, thus exposing a special vulnerability of cancer cells [79]. Altered expression of PMCA isoforms occurs in various types of cancer including lung, colon and breast. Certain breast cancer cell lines exhibit high expression of PMCA2, which is the

isoform that is predominantly expressed in mammary epithelia for the apical efflux of Ca^{2+} during lactation. In parallel with the SPCA2 pump, PMCA2 levels dramatically increase during lactation and return to basal levels upon reaching involution [80]. In these breast cancer cell lines, exemplified by ZR-75-1 where PMCA2 is constitutively expressed at levels as high as 100-fold over non-tumorigenic lines [81], lowered cytosolic Ca^{2+} levels bypass apoptosis by preventing increased uptake of Ca^{2+} into mitochondria and activation of cell death cascades. Conversely, in colon cancer PMCA4 is downregulated, increasing cytosolic Ca^{2+} due to the decreased Ca^{2+} efflux and increasing cell proliferation [82, 83]. Similarly, in human oral squamous cell carcinoma, epigenetic downregulation of PMCA1 was suggested to be an early event in malignancy that promotes cell proliferation [84]. Thus calcium straddles a fine line between promoting apoptosis in cell death versus cell proliferation through activation of cell cycle. This unique quality of Ca^{2+} supports the observation that cancer is not a uniform disease and that both up and down regulation of Ca^{2+}-ATPases can bypass normal cellular maintenance to promote tumorigenesis.

4.2 SERCA and Cancer

SERCA pumps have essential roles in calcium transport into the ER for replenishing stored calcium, promoting protein folding and maturation, and the synthesis of lipids and steroids. Within the ER lumen, calcium concentrations are tightly regulated to maintain sufficient stores for signaling, via release by calcium channels, and to activate calcium-dependent proteins such as the protein folding chaperones, calnexin and calreticulin. The importance of luminal Ca^{2+} is highlighted by the finding that SERCA inhibition preferentially impairs the maturation of leukemia-associated mutant NOTCH receptors, inducing a G0/G1 arrest [85]. Mutations and altered expression levels of SERCA isoforms have been implicated in many cancers, including colon, prostate, and in lung cancers. In colon adenocarcinoma cell lines, SERCA3 expression is induced during differentiation and appears to be progressively lost during multistage process of colon tumorigenesis [86]. Thus, it may be an important marker of colon cancer progression in patients that indicates the remodeling of calcium transients and ensuing morphological changes resulting in cancer differentiation [87]. Papp and colleagues have shown that SERCA3 expression is modulated during differentiation of colon and gastric carcinomas, choroid plexus tumors, and in myeloid leukemias [86–89]. SERCA3 is also downregulated during immortalization of B lymphocytes by the Epstein Barr virus, a human gammaherpesvirus involved in various malignancies including Burkitt's and other lymphomas, to shape the amplitude, intensity and duration of cytosolic calcium signals, and hence cell activation [90]. Conversely, SERCA2 overexpression in colorectal cancer cells drives proliferation and migration and is reversed by treatment with the curcumin analog, F36 [91]. Interestingly, although homozygous knockout of the SERCA2 gene in mouse is lethal, the heterozygous mutant develops high incidence of squamous cell tumors after a long latency, exemplifying the link between dysregulation of calcium homeostasis and cancer [92].

4.3 SPCA and Cancer

Of the two Secretory Pathway Ca^{2+}-ATPases, SPCA2 is frequently elevated in cancers of breast, prostate and colon, as exemplified in Figures 1–2. Patient data also showed that SPCA1 is highly expressed in basal-like breast cancers and has a low expression in the

luminal subtypes [93]. A good *in vitro* model cell line for basal cancers is MDA-MB-231, a claudin-low subtype, where *ATP2C1* knockdown slowed proliferation and had a modest effect in reducing thrombin/trypsin (PAR)-mediated cytosolic calcium transients. Knockdown of SPCA1 had the interesting phenotype of halting the proteolytic processing of the pro-IGF1R protein (insulin growth factor 1 receptor) [93]. The expression of pro-IGF1R has been correlated to poor prognosis in breast cancer suggesting that SPCA1 overexpression is a potential marker for basal-like breast cancers. Conversely, SPCA2 is significantly overexpressed in ERBB2/HER2-positive and luminal breast cancer cell lines. MCF7 cells had a significant overexpression of SPCA2 with low expression of SPCA1 (Figure 1), which served as a good model for Feng et al. [39] to probe the role of SPCA2 in breast cancer. Knockdown of SPCA2 resulted in attenuated growth as well as decreased colony formation of MCF7 cells in soft agar. In mouse xenograft experiments, SPCA2 knockdown drastically reduced tumor formation relative to the control. Furthermore, SPCA2 was able to confer increased proliferation as well as ability for colony formation in soft agar upon overexpression in MCF10A cells, a nonmalignant mammary epithelial cell line. SPCA2 knockdown and low calcium conditions conferred decreased activity in the ERK1/2 pathway, which may explain the decrease in proliferation. Rao and coworkers also showed that SPCA2 has the unique ability to elicit store-independent calcium entry (SICE) through its physical interaction and activation of Orai1. With its ability to pump calcium and its ability to activate SICE, SPCA2 may have an important role in the proliferative potential of cancer by enabling calcium entry for various calcium-dependent processes such as cell cycle progression. Mutations in the SPCA have not been linked to cancer but their constitutive levels of overexpression may have an important consequence in tumorigenesis.

5. Clinical Relevance

Despite clear evidence linking Ca^{2+}-ATPases and calcium signaling to tumorigenesis and malignancy, pharmacological targeting of calcium transporters is still at its infancy [94]. The most promising development in this regard is the exploitation of the potent and selective inhibition of SERCA pumps by thapsigargin. As the active ingredient in the ancient medicinal Mediterranean plant *Thapsia garganica*, thapsigargin is responsible for intense skin irritation upon contact, resulting from Ca^{2+}-induced histamine release from mast cells [95]. When bound to thapsigargin, SERCA is locked in an inactive E2-like conformation and calcium entry into the ER is abrogated, leaving the IP_3R calcium channel to leak calcium from the ER into the cytoplasm. Emptying of ER Ca^{2+} leads to organelle stress and induces programmed cell death pathways [96]. To avoid widespread cell death, thapsigargin may be targeted to cancer cells in the form of a prodrug: for example, a peptide-conjugated form of thapsigargin cannot enter cells to inhibit SERCA until the peptide is cleaved by a specific protease [95]. When targeted for prostate cancer, the carboxypeptidase protease is the prostate specific membrane antigen (PMSA) found in the vicinity of prostate cells. Currently, the prodrug compound (G202) is in Phase II clinical trials for glioblastoma [97], having successfully concluded Phase I [98].

The overexpression of Ca^{2+}-ATPase genes in breast cancer patients could have important clinical significance in defining specific cancer subtype. Subtype specific preference in expression has also been observed in cell culture lines derived from various cancers. The

majority of breast cancer patients are classified as either luminal A/B or ERBB2 (HER2), which are estrogen receptor (ER) positive and HER2 positive, respectively. These subtypes have treatment options that target the receptors to halt downstream activation of tumorigenic pathways. Analysis of patient tumor databases by Feng et al. [39] showed that SPCA2 was up regulated in ERBB2 subtype and down regulated in basal-like tumors. On the other hand, SPCA1 is overexpressed in basal-like tumors and down regulated in luminal subtypes [93]. Tumors derived from basal cells that lack receptor targets have a poorer prognosis than other subtypes due to fewer treatment options. Thus, expression levels of SPCA genes could be differential markers for basal-like tumors and ERBB2 tumors, respectively. This leads to the question of whether this inverse correlation of isoform expression and cancer subtype-specificity has functional relevance. A recent study found that high SPCA2 expression was correlated with epithelial genes in cancer cell lines, whereas SPCA2 expression was low in cell lines that exhibited mesenchymal phenotypes [99]. Epithelial gene expression is a hallmark of more differentiated cancers, whereas cancer cells that have acquired mesenchymal phenotypes are less differentiated and associated with metastasis. Patients with metastatic tumors have poor prognosis and may have developed drug-resistance. Epithelial-mesenchymal transition (EMT) is critical to initiate cancer metastasis, allowing cells of a primary tumor to detach from its neighbors and from the basement membrane, and migrate to form secondary tumors in distant organs, such as the brain, bone, or liver [100]. EMT should not be confused with mesenchymal-derived tumors, which are sarcomas that can develop in nonepithelial tissues such as the bone and muscle [101]. It remains to be determined whether SPCA2 down regulation facilitates EMT, allowing tumor cells to lose their epithelial characteristics and acquire markers of less differentiated or mesenchymal cells.

In summary, the role of SPCA and other calcium pump isoforms in breast and other cancers is an area of active investigation not only as biomarkers for characterizing tumors and their subtypes, but also as potential candidates for novel drug targeting.

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Highlights

- Three distinct subtypes of Ca^{2+}-ATPases maintain calcium homeostasis
- Gene mutations in *ATP2A-C* underlie disorders of skin, muscle and development
- Ca^{2+}-ATPases show isoform specific dysregulation in cancer subtypes
- SPCA2 (*ATP2C2*) is highly upregulated in epithelial cancers
- Ca^{2+}-ATPases may be markers of differentiation and drug targets in cancer therapy

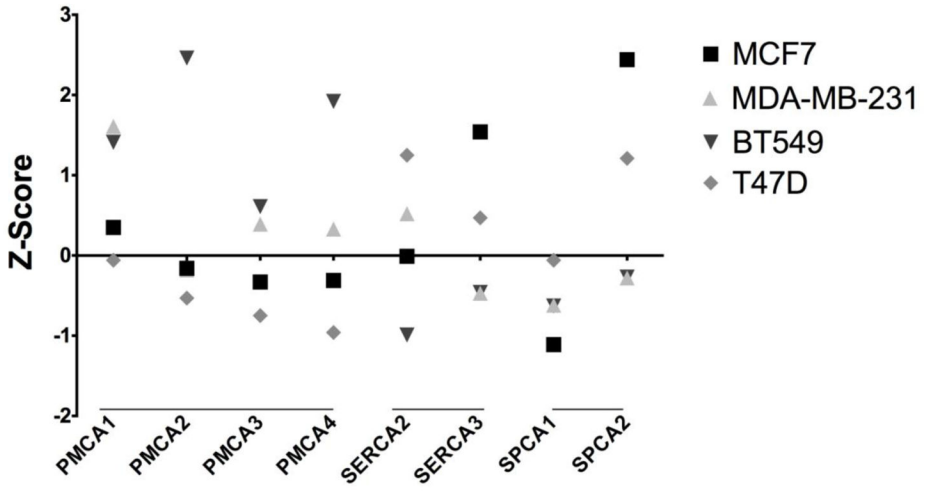

Figure 1.

Breast cancer cell lines from the NCI-60 human tumor cell line database [99] showing expression profiles for each of the Ca^{2+} pump isoforms depicted as Z score. Z-scores indicate how many standard deviations away a sample is from the mean expression value that was measured in a reference or control sample. Positive values reflect overexpression. The MCF7 and T47D cell lines are representative of the luminal A subtype and MDA-MB-231 and BT549 are representative of the claudin-low subtype. The database includes specific cell lines that are commonly utilized for cancer studies but is not comprehensive of all commercially available cancer cell lines. This database did not contain data for the SERCA1 isoform.

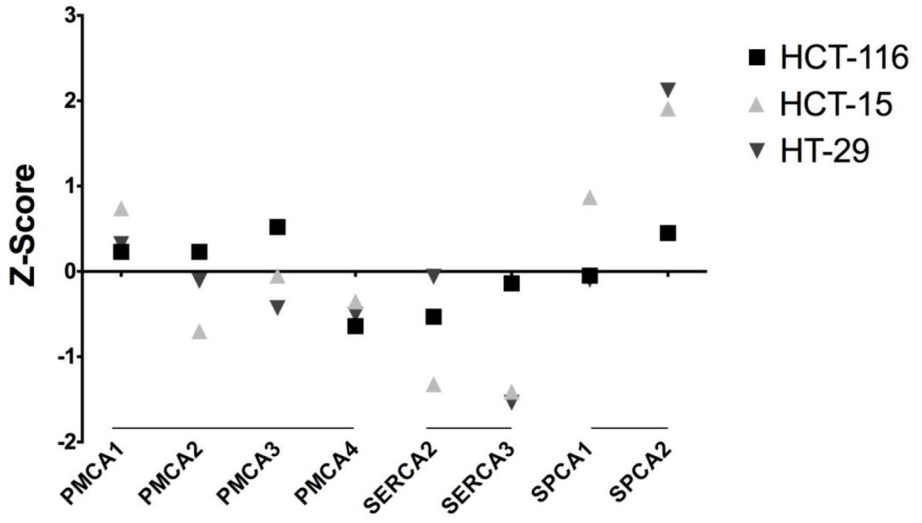

Figure 2.

Colon cancer cell lines from the NCI-60 human tumor cell line database [99] showing the expression profiles for each of the Ca^{2+} pump isoforms depicted as Z score. HCT-116 is a colorectal carcinoma cell line derived from a late stage primary tumor. HCT-15 and HT-29 are colorectal adenocarcinoma cell lines that have been derived from an advanced stage primary tumors.