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Abstract. Ca^{2+}, the main second messenger, is central to the regulation of cellular growth. There is increasing evidence that cellular growth and proliferation are supported by a continuous store-operated Ca^{2+} influx. By controlling store refilling, the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) also controls store-operated calcium entry and thus cell growth. In this review, we discuss data showing the involvement of SERCA in the regulation of proliferation and hypertrophy. First, we describe the Ca^{2+}-related signalling pathways involved in cell growth. Then, we present evidence that SERCA controls proliferation of differentiated cells and hypertrophic growth of cardiomyocytes, and discuss the role of SERCA isoforms. Last, we consider the potential therapeutic applications of increasing SERCA activity for the treatment of cardiovascular diseases and of modulating SERCA and SR content for the treatment of cancer.

Keywords: Sarcoplasmic Reticulum Calcium Transporting ATPases, cell growth processes; calcium signalling.

Abbreviations. ATP – adenosine triphosphate; Ca^{2+} - ion calcium; CCE – capacitative Ca^{2+} entry; CDK4 – cyclin-dependent kinase 4; CRAC – calcium-release activated channel; CRACM1 - calcium-release activated channel molecule 1; IP3 –inositol-1,4,5-trisphosphate; IP3R – inositol-1,4,5-trisphosphate receptor; NFAT – nuclear factor of activated T-lymphocytes; PDGF – platelet derived growth factor; PKC – protein kinase C; PMCA – plasma membrane Ca^{2+} ATPase; Rb – retinoblastoma protein; ROC – receptor operated calcium channels; RyR –ryanodine receptor; SR/ER: sarco/endoplasmic reticulum; SERCA: sarco/endoplasmic reticulum Ca^{2+} ATPase; SOC – store-operated calcium channels; SRF – serum response factor; STIM1 – stromal interaction molecule 1; TRPC – transient receptor

potential channels; VEGF – vascular endothelial growth factor; VOCC – voltage operated calcium channels; WT – wild type.

Introduction.

The calcium ion, Ca^{2+}, is a ubiquitous second messenger controlling a broad range of cellular functions including growth and differentiation. The plasticity and diverse effects of this signal are based on extensive spatio-temporal compartmentalization. Spatial patterning defined by the amplitude, frequency and duration of the Ca^{2+} signal, is essential for appropriate intracellular function. There is increasing evidence that cellular growth and proliferation are supported by continuous store-operated Ca^{2+} influx. Different store-sensitive Ca^{2+} channels can be mobilized in different cell types, leading to activation of kinases or phosphatases which regulate the activity of transcription factors. One of these factors, the Ca^{2+}-regulated transcription factor NFAT (nuclear factor of activated T lymphocytes), is required for proliferation and hypertrophy. Several data highlight the colocalization of Ca^{2+} channels, pumps and transducers (protein kinases and phosphatases) with their targets, transcriptions factors, which are essential for proliferation (rev in [66]). Recent data pointed to the essential role of store sensitive Ca^{2+} entry in proliferation. These Ca^{2+} channels are activated by a decrease in the sarcoplasmic reticulum Ca^{2+} load. The sarcoplasmic reticulum Ca^{2+} ATPase, SERCA, controls SR refilling, and thereby also controls cellular growth. We begin with a brief overview of different types of Ca^{2+} events observed in quiescent cells and during induction of proliferation and/or hypertrophic growth. Then, we compile available information concerning the activation of signalling pathways controlled by SERCA, and discuss the physiological role of Ca^{2+} pumps in the control of cell proliferation and hypertrophy in different cell types. We conclude with a brief consideration of potential therapeutic developments for treatment of hypertropic and proliferative diseases, including cancer.

1. Ca^{2+}-related signalling pathways controlling proliferation and /or hypertrophy: the role of SR Ca^{2+} content.

In quiescent cells, the Ca^{2+} signal consists of a sudden increase in the concentration of cytosolic free Ca^{2+} ions following the opening of Ca^{2+} channels either on the cell surface: the voltage-operated Ca^{2+} channels (VOCC), receptor-operated channels (ROC) and store-operated channels (SOC), or on the sarco/endoplasmic reticulum membranes : the inositol-1,4,5-trisphosphate receptors (IP3Rs) and the ryanodine receptors (RyRs). The free Ca^{2+} concentration can be rapidly reduced by the Ca^{2+} pumps on the plasma membrane (plasma membrane Ca^{2+} ATPase, PMCA) or those on the internal store (sarco/endoplasmic reticulum Ca^{2+} ATPase, SERCA). Na^{+}/Ca^{2+} exchangers also contribute to Ca^{2+} efflux. These pumps and exchangers ensure that cytosolic Ca^{2+} remains low and that the stores are loaded with signal Ca^{2+}. There are various isoforms of all the Ca^{2+}-transporting channels and pumps and they are differentially expressed depending on cell type and proliferation state (reviewed by [6, 66, 82]).

In most quiescent cells, the major increase in cytosolic free Ca^{2+} is provided by the internal store; the role of extracellular Ca^{2+} influx being limited to a trigger for intracellular calcium release. However, it appears that proliferation is the consequence not of a sudden increase in the intracellular Ca^{2+} concentration but of a continuous store-operated Ca^{2+} influx, that corresponds to increased permeability of the plasma membrane to Ca^{2+}.

Recent studies have led to the identification of the key transcriptional Ca^{2+}-regulated pathway controlling proliferation in different cell types. Stimulation of phosphoinositide-coupled receptors by mitogens (hormones, growth factors, signalling molecules) is linked to generation of inositol-1,4,5-trisphosphate (IP3), activation of IP3R leading to Ca^{2+} release from intracellular stores and, subsequently, capacitative Ca^{2+} entry resulting in sustained

cytosolic Ca^{2+} increases (rev. in [66]) (Fig. 1). A long-lasting increase in cytosolic Ca^{2+} (at least 1-2h) is required for activation of the transcription factor NFAT – the mediator of proliferation in almost all cell types (rev. [13, 66]). Several factors are involved in generating this type of Ca^{2+} signal: IP3R; the sarcoplasmic reticulum Ca^{2+} release channel; the sensor molecule STIM1 (Stromal interaction molecule 1), that links store depletion to store-operated channels (CRAC, Orai, TRPC or others channels depending on cell types); and SERCA, that controls store refilling and thus the amplitude and propagation of the Ca^{2+} signal (Fig.1). Two functions have been attributed to STIM1: 1) the sensor function that initially detects the reduction of Ca^{2+} content in the lumen of the reticulum; 2) the messenger function provided by STIM1 translocation to the plasma membrane to activate store-operated channels [13]. Ablation of STIM1 inhibited thapsigargin-evoked Ca^{2+} entry without altering resting Ca^{2+} levels, Ca^{2+} release transients or the membrane potential [56, 106]. Furthermore, ablation of STIM1 neither inhibited SERCA activity nor prevented Ca^{2+} store refilling when cells were stimulated with physiological agonists [56]. These findings suggest that Ca^{2+} ions can be directly transferred from SOC to SERCA. Ablation of SERCA inhibits thapsigargin-evoked Ca^{2+} entry, suggesting that abnormally low Ca^{2+} store content or elevated level of cytosolic Ca^{2+} inhibit store-operated Ca^{2+} entry [139]. These various observations suggest the existence of microdomains containing SOC channels on the plasma membrane, STIM proteins on the SR/ER, SERCA pumps and elements of the calcineurin/NFAT signalling pathway (Fig. 1). Interestingly, stimulation of vascular smooth muscle cells with phosphoinositide-coupled agonist for a few hours resulted in prolongation of cytosolic Ca^{2+} clearance after Ca^{2+} release from the ATP-sensitive pool, suggesting inhibition of Ca^{2+} pump activity during induction of proliferation [69].

The sustained increase in cytosolic Ca^{2+} due to activation of SOC is necessary to activate calcineurin, a Ca^{2+}/calmodulin-dependent phosphatase, which dephosphorylates

many proteins; one such protein is the transcription factor NFAT and its dephosphorylation results in its rapid import into the nucleus and increased intrinsic DNA binding activity [28, 102]. In the nucleus, NFAT binds to the promoter of various genes as a homodimer or as a heterodimer with other transcription factors including SRF, Fos-Jun, and GATA [5, 41, 74]. Of the five different isoforms, four (NFATc1 to c4) are regulated by calcineurin, but they may well have opposite effects on cell proliferation (rev [47, 66]): NFATc2 exerts tumour suppressor properties, whereas NFATc1, NFATc3 and NFATc4 appear to function as inducers of proliferation/hypertrophy. There is some evidence for an inhibitory role of NFATc2 in the regulation of cellular growth: it represses the expression of the key cell cycle regulatory kinase, cyclin-dependent kinase 4 (CDK4) [4] , and expression of cyclins A2, B1, E and F [12]. By contrast, NFATc1 and NFATc3 favour cell cycle progression by induction of the cell cycle-related genes cyclin D1, cyclin D2, retinoblastoma protein (Rb) and c-myc, which are required for passage through the G1/S checkpoint [65, 89].

In summary, proliferation is associated with a sustained increase in cytosolic Ca^{2+} due to 1.) enhanced excitability of IP3Rs after IP3 binding; 2.) decreased store refilling probably due to inhibition of SERCA and 3.) enhanced store-operated Ca^{2+} entry. This sustained increase in cytosolic Ca^{2+} favours activation of the calcineurin/NFAT complex leading to induction of a genetic programme of proliferation/hypertrophy remodelling.

3. Effect of different SERCA isoforms on cellular growth and proliferation.

SERCA is encoded by three different genes (*ATP2A1, ATP2A2 and ATP2A3*), each gene giving rise to various isoforms by alternative splicing at the 3' ends of the mRNA [6]. The SERCA isoforms differ mainly by their affinity for Ca^{2+} (2b>2a=1>>3) [73] and their Ca^{2+} transport turn-over rates, SERCA2b having the lowest transport capacity of all SERCAs [73, 130]. In many cells, at least two isoforms are expressed but the role of each isoform in

growth and proliferation is not well understood. For example, in skeletal and smooth muscle, SERCA2a and SERCA2b are present in quiescent differentiated cells, whereas in proliferating cells only the SERCA2b protein is present [21, 65, 69, 125].

SERCA2b differs from SERCA2a by an extension of 46 amino acids that forms an additional transmembrane domain placing the C-terminus of SERCA2b in the ER lumen. In overexpressing systems, the C-terminal domain of SERCA2b interacts with calnexin and calreticulin. This could control the activity of SERCA2b and account for the functional differences in terms of Ca^{2+} wave properties between SERCA2a and SERCA2b when overexpressed in Xenopus oocytes [55, 104].

There is no available data concerning specific association of SERCA2a or SERCA2b with components of SOC/NFAT signalling. Nevertheless, studies involving gene transfer clearly demonstrated that SERCA2a and SERCA2b are not equivalent in terms of signal transduction. SERCA2a is lost from proliferating VSMC and we have shown that restoring SERCA2a expression to VSMC inhibited VSMC proliferation and neointima formation in rats [65]. Other groups have demonstrated that overexpression of wild-type SERCA2b has no effect on VSMC migration [135]. Both differences in SERCA isoform and in the mechanisms controlling proliferation and migration may explain these results. Unfortunately, there is currently no information available about the effect of SERCA2b overexpression on VSMC proliferation or of SERCA2a on migration.

Data from transgenic mice also clearly demonstrate differences between SERCA2a and SERCA2b in terms of hypertrophic growth of cardiomyocytes [127-129]. Wuytack et coll. have produced transgenic mice where SERCA2a was replaced by the high Ca^{2+} affinity SERCA2b isoform, resulting in cardiac dysfunction and hypertrophy [129]. In the SERCA2a-deficient animals, expression of phospholamban (PLN) was increased. In SERCA2a/PLN double knock-out mice the phenotype was even more severe with a high risk of cardiac death

after beta-adrenergic stimulation, so an increase in the PLN level may be an adaptation mechanism to lower Ca^{2+} affinity. The total level of SERCA was lower in SERCA2a-deficient mice and, in the initial study, cardiac hypertrophy could be interpreted as a consequence of down-regulation of total SERCA [129]. However, increasing the cardiac SERCA2b level in these mice did not prevent hypertrophy [127, 128]. Interestingly, SERCA2b/WT heterozygotes in which the natural SERCA2a isoform is the major isoform, do not present hypertrophy.

These results demonstrate that 1) the SERCA2a and SERCA2b isoforms are not equivalent in terms of growth signal transduction in cardiac and vascular myocytes; 2) having, at baseline, a low SERCA2a level or having a SERCA pump with a much higher Ca^{2+} affinity may be detrimental for the heart, and 3) replacing SERCA2a, which has low affinity for Ca^{2+}, with the isoform with high affinity, SERCA2b, results in cardiac dysfunction and alteration of Ca^{2+} signalling pathways.

3. Alterations of Ca^{2+} signalling during proliferation/hypertrophy in various cell types: the role of SERCA isoforms.

An increase in cytosolic Ca^{2+} concentration — either oscillatory or sustained depending on cell type — is required for activation of NFAT transcriptional activity. In pathological situations or under the influence of various growth stimuli, the intracellular Ca^{2+} signal is altered in such a way that a new Ca^{2+}-regulated transcription pathway is activated. Decrease in SERCA expression and/or activity, reported in various growing cells, may play a role of support for sustained activation of store-operated Ca^{2+} entry in proliferating cells (Table. 1). This occurs in excitable and non excitable cells but the pathways differ between cell types.

3.1. Non-excitable cells. In non-excitable cells, stimulation with phosphoinositide-coupled agonists can result in complex global Ca^{2+} signals organized as regenerative waves. The Ca^{2+} wave propagation sites are rich in the ER proteins SERCA, calreticulin and IP3Rs (rev in [66]). This oscillatory signal is prevented by inhibition of PKC, SERCA or CCE, or by external Ca^{2+} removal showing the involvement of IP3R Ca^{2+} release, SR Ca^{2+} uptake and Ca^{2+} entry through SOC in the generation of these waves [80]. Overexpression of SERCA in Xenopus oocytes increases the frequency of IP3-induced waves and narrows the width of individual calcium waves by relieving the inhibitory effect of high Ca^{2+} on IP3R [14]. These various observations indicate that in non excitable cells SERCA controls the kinetics of Ca^{2+} wave propagation, and thereby finely regulates Ca^{2+}-dependent transcription pathways. Interestingly, during activation of T-lymphocytes, SERCA3 expression decreases by 90%, whereas SERCA2b expression approximately doubles [64]. These isoforms have very different Ca^{2+} sensitivities and may trigger new cellular processes.

In non excitable cells such as lymphocytes, 3T3-L1 preadipocytes, endothelial cells, epithelial cells, and pancreatic beta cells, proliferation is driven by the calcium-dependent calcineurin/NFAT pathway [30, 46, 89, 101, 108, 131, 136]. Also, in non dermal epithelia cells, induction of proliferation by VEGF requires NFAT activation via store-operated STIM-mediated Ca^{2+} entry [131]. The most complete description of this signalling pathway has been in lymphocytes, in which the importance of all signalling molecules — SERCA, STIM1, a pore subunit named Orai1 (CRACM1) and NFAT — has been validated by ablation, patch clamp experiments and reporter-promoter assays [13, 31, 98, 106, 115, 116, 134, 139, 140].

Together, these findings demonstrate that proliferation in non-excitable cells is driven by NFAT activated by a long-lasting oscillatory store-operated Ca^{2+} signal. SERCA, controlling the amplitude and the kinetics of Ca^{2+} wave propagation, also can finely regulate Ca^{2+}-dependent transcription pathways.

3.2. Cardiomyocytes. In cardiomyocytes, depolarization-induced Ca^{2+} cycling controlling myocyte contraction has no effect on activation of NFAT signalling pathway. By contrast, stimulation with phosphoinositide-coupled agonists induces a slow increase in resting Ca^{2+} due to the activation of IP3Rs and hypertrophic growth.

A decrease in SERCA activity, associated with a decrease in SERCA2a expression, was described in cardiac hypertrophy in the early 1990s [22, 60, 77, 86]. Since then, numerous papers have been published on the topic. Not all of the authors agreed about decreased expression and discordance between expression of SERCA2a at the mRNA and protein levels has been reported [112]. The decrease in the SERCA2a level is related to the intensity and duration of cardiac overload, but most authors now agree that SERCA2a is down regulated in severe heart failure [2]. Reduced SERCA2a activity and SR Ca^{2+} uptake lead to abnormal Ca^{2+} handling in failing cardiomyocytes and this involves an increase in diastolic Ca^{2+}, an abnormally long time course of Ca^{2+} transients, and a decrease in SR Ca^{2+} release [20, 42, 43]. Furthermore, reduced SR Ca^{2+} stores and increased expression of transient receptor potential channels (TRPC) in failing heart favour capacitative Ca^{2+} entry and sustain activation of calcineurin [63, 88].

Results from transgenic mice reveal a primordial role for SERCA2a dysfunction in induction of signalling pathways leading to cardiac hypertrophy and failure. Indeed, deletion of the SERCA2 gene (*ATP2A2*) is lethal but heterozygous mice are viable and develop cardiac hypertrophy [97]. Increasing the cardiac load by aortic banding resulted in faster heart failure in SERCA 2+/- mice than in WT controls [111]. However, in human there is no evidence for cardiac hypertrophy in patients carrying a mutation of the *ATP2A2* gene (Darier's disease)[76, 122]: up regulation of the normal allele is a possible explanation for the absence of cardiac hypertrophy [121].

During phenylephrine-induced hypertrophy in neonatal rat cardiac myocytes, the early and prominent feature of hypertrophic remodelling is the reduction of the abundance of the SERCA2 transcript [99]. The consequence of SERCA2a down-regulation on Ca^{2+} signalling is compensated by alternate Ca^{2+} transport mechanisms, and this contributes to the induction of a genetic programme of hypertrophic remodelling. Indeed, reduction of SERCA2a expression by RNA silencing in cardiac myocytes resulted in activation of the calcineurin-dependent complex leading first to increased expression of prohypertrophic transcription factors Sp1, MEF2 and NFATc4, and, subsequently, to up-regulation of Ca^{2+} handling proteins including the Na^{+}/ Ca^{2+} exchanger and TRPC [113]. Calcineurin was identified as a central prohypertrophic signalling molecule for the myocardium [45] and its target, NFAT, is necessary and sufficient for mediating pathological skeletal myocyte and cardiac hypertrophy [45, 81, 85, 132].

These studies suggest that hypertrophic stimuli induce a sustained increase in resting Ca^{2+} in cardiomyocytes due to inhibition of SERCA2a activity. This resting Ca^{2+} leads to activation of calcineurin/NFAT signalling resulting in hypertrophic remodelling of cardiomyocytes. Furthermore, modulation of expression of Ca^{2+} transporters in hypertrophic cardiomyocytes leads to functional abnormalities in Ca^{2+} cycling and in the long term to impairment of cardiac contractile function.

3.3. Smooth muscle. In vascular smooth muscle cells (VSMC) phosphoinositide-coupled agonists induce a sustained increase of cytosolic Ca^{2+} due to the generation of repetitive Ca^{2+} waves [40, 53], inhibition of activity of Ca^{2+} pumps [69] and increased capacitative Ca^{2+} entry (CCE) [38, 39].

Proliferation of VSMC is associated, in the rat and rabbit, with loss of the cardiac isoform of the sarcoplasmic reticulum (SR) Ca^{2+} pump, SERCA2a, and of the SR Ca^{2+}

channel, the ryanodine receptor, RyR [69, 75, 125]. Loss of SERCA2a suggests a decrease in store refilling and this would favour activation of store-operated Ca^{2+} influx. Indeed, the expression of the store-operated Ca^{2+} channels TRPC1, TRPC4 or TRPC6 is increased in proliferating VSMC [30, 39, 118, 138]. The ER calcium sensor STIM1 regulates store-operated Ca^{2+} entry via interaction with TRPC1 in VSMC, and ablation of STIM1 by siRNA inhibits VSMC proliferation [119]. The number of T-type VOC channels is increased in proliferating VSMC [62, 103] and T-type channels are able to replenish the depleted Ca^{2+} store [34]. Mibefradil, a selective T-type channel blocker, inhibits proliferation of VSMC [68, 105, 110] and NFAT transcriptional activity [68]. Alterations of the Ca^{2+}-handling proteins may be part of the VSMC dedifferentiation process, and has been described for many proteins [92]. It is also plausible that under a growth stimulus, alterations in Ca^{2+} handling may be a trigger for the activation of new Ca^{2+}-regulated transcription pathways.

The main Ca^{2+}-regulated transcription pathway described in VSMC involves the calcineurin/NFAT pathway. Many receptor tyrosine kinase and G-protein coupled receptor agonists, such as angiotensin II, endothelin 1, and platelet derived growth factor (PDGF-BB), and also very low density lipoproteins, induce VSMC proliferation or migration through activation of the NFAT transcription pathway [40, 41, 69, 71, 117, 133, 137]. NFATc3 is induced by endothelin-1 and ablation of this isoform also inhibits VSMC proliferation [40, 90]. In animal models, restenosis was shown to be prevented by restoring normal SR Ca^{2+} handling using SERCA2a gene transfer [65] and also by inhibiting the NFAT transcription pathway [72, 137].

These findings indicate that VSMC proliferation is driven by NFAT activation following a sustained increase in cytosolic Ca^{2+} which is due to inhibition of SERCA activity and increased voltage-independent Ca^{2+} entry.

3.4. Cancer cells. The calcium-dependent calcineurin/NFAT signalling pathway is increasingly recognized as a central player in the development of a number of very different malignancies (rev. [9, 82]). Variation in expression of Ca^{2+} pumps and channels, most frequently an increase in TRPCl expression and decrease in SERCA expression, have been described in numerous cancers (rev. [82]). Furthermore, NFATc1 is commonly overexpressed in pancreatic, breast and colon carcinomas and enhances the malignant potential of tumour cells [10, 54]. Loss of SERCA activity and expression has been detected in many different malignancies. Indeed, changes in SERCA3 expression have been observed in colon cancer: the protein was either absent or present in much lower abundance in colon carcinoma than normal tissue, consistent with a loss of differentiation in tumour cells [8, 36]. Next, SERCA2b expression is very substantially decreased in oral cancers (squamous cell carcinoma), and in thyroid cancer, where it is the major isoform [29, 93]. Alternatively, somatic and germline mutations in lung cancer and germline mutations in colon cancer in the *ATP2A2* gene result in the loss or reduction of SERCA2 expression [61]. The direct evidence linking a deficiency of SERCA to tumour genesis has been provided by development of heterozygous SERCA2 mice ($ATP2a2^{+/-}$). These mice are sensitized to the development of squamous-cell carcinoma, which arises directly as a result of SERCA2 haploinsufficiency [70, 97, 100]. The keratinocytes from Darier's disease patients, deficient in SERCA2b, as well as normal keratinocytes in which SERCA2b was silenced by siRNA, showed enhanced proliferation supported by up-regulation of the store-operated TRPC1 channel [94]. These observations suggest that haploinsufficiency of the SERCA 2 gene is involved in growth and proliferation of specific tumor cells.

Thus, there is increasing evidence that loss of SERCA activity and store depletion induces proliferation in various normal and cancer cell types via store-operated Ca^{2+} entry and

NFAT activation. Some groups have reported that in cancer cell lines an increase in SERCA2b expression and in sarco/endoplasmic reticulum Ca^{2+} content are required for induction of proliferation, but the molecular pathway involved has not been identified [17, 18, 50]. Crepin et al [18] reported that the increased rate of proliferation of immortalized epithelial prostate cells (PNT1A) induced by prolactin is associated with an increase in the SR calcium content and SERCA2b expression. Silencing of SERCA2b expression by siRNA leads to reduction of PNT1A cell proliferation. These observations led the authors to conclude that SERCA2b overexpression is a protagonist of prolactin-induced proliferation. However, in primary human prostate cancer epithelial cells (hPCE), store-operated Ca^{2+} entry and NFAT activation was identified as the main proliferation pathway [123].

In conclusion, there is a large body of data demonstrating that in various cell types store-operated Ca^{2+} entry is necessary to activate NFAT signalling, although the exact mechanism of this process is not yet completely understood. SERCA, by controlling store refilling, plays a primordial role in the control of cell growth and proliferation.

4. SR/ER-based therapeutic strategies.

The endoplasmic reticulum is not only a Ca^{2+} reservoir but is also the site for protein synthesis and folding. This has consequences for the design of any Ca^{2+} cycling-based therapeutic strategies: the SR/ER Ca^{2+} load has to be finely tuned to maintain cell integrity. With this in mind, two types of strategy have been designed with SERCA as a target: one aims to restore normal SR load and SERCA activity and is used in cardiovascular diseases. The other consists of inducing ER stress to cause apoptosis; this strategy is designed to kill cancer cells.

4.1-Increasing SERCA2a activity

Two approaches have been proposed to increase SERCA2a activity: SERCA2a overexpression; and phospholamban (PLN) ablation. They have been particularly well studied in the heart where abnormal Ca^{2+} cycling is the main determinant of contractile dysfunction and heart failure [67].

SERCA overexpression. Transgenic animals with cardiac-specific expression of either SERCA2a or SERCA1a showed improved contractility under baseline conditions and after pressure overload [3, 84, 120]. The mortality rate after aortic banding in animals overexpressing SERCA2a was identical to that in the WT controls [84]. However, in the same model, Chen et al reported increased mortality after myocardial infarction in association with an increased frequency of arrhythmias [16]. Less persistent arrhythmias were observed after post-ischemic injury in SERCA1a-overexpressing hearts from transgenic rats as well as in hearts overexpressing SERCA2a from an adenoviral vector [24, 120]. The data from transgenic animals indicate that having a high basal level of SERCA (2a or 1a) can improve cardiac function and prevent heart failure.

The enhanced contractility associated with SERCA2a overexpression has been reported to be protective against both heart failure and cardiac hypertrophy [19, 51, 79, 84, 87, 124]. Adenovirus-mediated gene transfer of SERCA2a restored the Ca^{2+} transient in cardiomyocytes isolated from failing human hearts[23], improved cardiac haemodynamics and increased survival in animal models of heart failure [19, 25]. SERCA2a rescues depressed contractility and survival without adverse effects on energy metabolism [19, 79] or cardiac arrhythmia in animal models [24] but definite proof of SERCA2a gene transfer efficiency in human await the on-going clinical trials.

We have shown that normalization of Ca^{2+} handling by SERCA2a gene transfer prevents injury-induced vascular remodelling in rats [65]. Thus, preventing SERCA2a loss by

gene transfer is a novel potential strategy for treating restenosis. Coronary restenosis is a major complication of percutaneous coronary balloon angioplasty. It is characterized by neointimal hyperplasia due to proliferation of VSMC. Although the use of drug-eluting stents (DES) limits neointimal hyperplasia, recent data suggest that their use may be associated with adverse clinical effects [7, 35, 126]. Thus, there is a need to discover novel mechanisms governing VSMC proliferation and this information could be used to develop new modalities for treating restenosis. Rapamycin and taxol, used in drug-eluting stents, were designed to induce cell death in proliferating VSMC. The strategy based on SERCA2a gene transfer should preserve cell integrity and prevent loss of the "differentiated/contractile phenotype" of VSMCs .

The same mechanism governs the beneficial effect of SERCA2a overexpression in heart failure and in proliferative vascular diseases: by lowering cytosolic Ca^{2+}, SERCA2a expression inhibits calcineurin activity and the activation of the NFAT pathway [65]. This explains why SERCA2a gene transfer inhibits hypertrophic, hyperplastic and apoptotic signalling pathways mediated by calcineurin. Another reason for the beneficial role of SERCA overexpression might be that it reduces oxidative stress. Indeed, high levels of oxygen-derived free radicals are generated during myocardial ischemia/reperfusion and this damages SERCA2a, potentially contributing to cellular Ca^{2+} overload and myocardial injury. Similarly, in atherosclerosis, cysteine 674 from SERCA2 is irreversibly oxidized due to prolonged oxidative stress, and consequently the NO-induced S-glutathiolation, activation of SERCA and arterial relaxation are impaired [1].

Phospholamban ablation. Phospholamban (PLN) is a 52-amino-acid protein which controls the affinity of SERCA for Ca^{2+}. It is expressed mainly in cardiac, slow skeletal, smooth muscle cells where SERCA2a is also present, and at low level in endothelial cells. In

its unphosphorylated form, PLN inhibits SERCA activity and phosphorylation by various protein kinases: PKA, PKG, and Ca^{2+}/Calmodulin kinase relieve this inhibition. Decreasing the inhibitory effect of PLN is another way of enhancing SERCA activity. This has been studied in great detail in heart failure and most findings now indicate that, in hypertrophy and failure, the level of PLN is unchanged or slightly decreased but that PLN is hypophosphorylated [112]. Both an increase in the PLN-to-SERCA ratio and the presence of unphosphorylated PLN should increase the inhibitory function of PLN. Suppressing the inhibitory effect of phospholamban is a promising approach to improving cardiac function. Indeed, the ablation of PLN completely prevents the spectrum of heart failure phenotypes in a mouse model of dilated cardiomyophy [78]. Furthermore, chronic inhibition of PLN using a pseudo-phosphorylated mutant results in favourable changes in cardiac haemodynamics in rat and sheep models of heart failure and prevents cardiomyopathy in a myopathic hamster model [49, 52, 58]. PLN ablation has also been shown to rescue depressed contractile function of calsequestrin-overexpressing hearts [107] and in a mouse line overexpressing a mutant myosin heavy chain [33]. In addition, normalization of the Ca^{2+} transient and restoration of cell contractility have been reported in cardiomyocytes isolated from failing human hearts [23]. The excitement generated by these studies has been tempered by the discovery of mutations in PLN, leading to a super-inhibitory PLN, which has been suggested to be causative of human dilated cardiomyopathy [44, 109]. Furthermore, in other genetic models, PLN ablation rescued cardiomyocyte dysfunction but did not prevent ventricular remodelling leading to heart failure [114].

Therapeutic strategies based on normalization of the SR Ca^{2+} load and of cytosolic Ca^{2+} by increasing SERCA activity seem promising for preventing hypertrophic growth and vascular proliferative disease. SERCA2a gene therapy for treatment of heart failure is now undergoing clinical trials in USA [67], and prevention of post-injury restenosis by SERCA2a

gene transfer is at the preclinical study stage [65]. The use of pseudophosphorylated PLN in the treatment of heart failure is also being considered in preclinical studies [58]. Furthermore, the development of small molecules for enhancing Ca^{2+} cycling has now appeared on the horizon and may offer new hope for treatment of Ca^{2+}cycling defects in cardiovascular disease.

4.2 Use of SERCA as a target to induce cell death

The issue of Ca^{2+} and cancer has been covered recently in a very comprehensive review by G. Monteith [82]. Here we focus on manipulating SERCA and SR Ca^{2+} load as methods for anticancer therapy. Two strategies could be used: either inducing a general ER stress by depletion of the ER Ca^{2+} store, or targeting particular SERCA isoforms that are induced or repressed in cancer cells.

An example of the first strategy is provided by the thapsigargin 'prodrug' approach to the treatment of prostate cancer [26]. Thapsigargin is a general inhibitor of SERCA, it induces complete SR/ER Ca^{2+} depletion and apoptosis. To target cancer cells selectively, the drug has been coupled to a peptide to produce an inactive "prodrug" that is only activated by prostate cancer-specific proteases such as the serine protease prostate-specific antigen [26]. However, thapsigargin resistance has been clearly demonstrated [91] and may hamper the efficacy of this approach. ER stress can also be obtained by blockade of the voltage-independent Ca^{2+}-channels which normally refill the ER after store depletion. Carboxyamido-triazole (CAI), a low molecular weight compound, inhibits these types of channels and is undergoing clinical validation for use as an anticancer agent [82]. CAI acts as an antiangiogenic and antimetastatic agent because it inhibits endothelial cell proliferation [59].

There is a multiplicity of isoforms of SERCA and especially SERCA3 [6] and the various SERCA3 isoforms may have different functions: SERCA3b and 3f have different

roles in cell adhesion and ER stress [15]. Interestingly, SERCA3 is repressed in highly neoplastic colon cancer cells [8]; consequently, overexpressing SERCA3 in colon cancer to preserve normal ER Ca^{2+} levels may be of therapeutic value. SERCA2b expression is decreased in skin disorders related to Darier's disease [11, 27, 121] and in thyroid cancer [93] but because SERCA2b is the ubiquitous isoform it might be difficult to target precisely.

6. Conclusions and future directions

In the past decade, there has been increasing evidence of the role of SERCA in diseases and especially in cardiovascular diseases. As a consequence, SERCA2a gene therapy has now progressed to clinical trials. Furthermore, the development of small molecules to increase SR Ca^{2+} cycling provides the hope of treatment for Ca^{2+} cycling defects. The reasons for and consequences of the existence of multiple SERCA isoforms, and especially of SERCA3 isoforms, remain to be elucidated, as does their importance in pathologies such as cancers. Exploitation of siRNA technology to knock-down protein production should help identify the role of these isoforms.

Store-operated calcium channels are increasingly being recognized as central players in the control of hyperthrophic growth and proliferation in almost all cell types including diverse malignancies. The enigma of the cross-talk between depletion of the ER store and refilling by calcium influx through store-operated calcium channels has been highlighted with the discovery of the sensor protein, STIM1, but studies of the role of both STIM1 and SOC in diseases are at an early stage.

The importance of the Ca^{2+}-regulated transcription factor NFAT, which links alteration in Ca^{2+} cycling to pathological growth and proliferation, has been documented but other Ca^{2+}-regulated pathways may also be involved and remains to be described. Furthermore, new mechanisms of control of gene expression have been uncovered with the

discovery of micro RNAs. Micro RNAs play an important role in pathological growth but the trigger for induction of specific miRNAs as well as the relationship with previously described alterations in Ca^{2+} signalling remains to be elucidated.

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Figure legend.

Figure 1. Schematic representation of calcium-regulated signalling pathways controlling proliferation in various cell types. A – quiescent cell, B – proliferating cell. CaM – calmodulin; GPCR – G-protein coupled receptor; PLC – phospholipase C; PP2B – protein phosphatase 2B (calcineurin); NFAT –nuclear factor of activated T lymphocytes; P – phosphate; IP3 –inositol -1,4,5-trisphosphate, IP3R –inositol -1,4,5-trisphosphate receptor; S –SERCA, sarco/endoplasmic Ca^{2+} ATPase; SR/ER sarco/endoplasmic reticulum; St- STIM1, Stromal interaction molecule 1.

Table 1. Variation of SERCA isoforms expression in different tissue under growth.

(expression of various isoforms of SERCA has not been tested systematically in all tissue)

Tissue	SERCA isoform regulation	Effect on growth	Comments	References
Non excitable cells				
T lymphocytes	SERCA3↓ SERCA2b↑	activation, protein synthesis	activation of T-lymphocytes by phorbol myristate acetate and ionomycin	[64]
Epithelial cells	SERCA2↓	enhanced proliferation, hyperkeratinosis	mutation of ATP2A2 gene (+/-) (Darier's disease)	[94]
Endothelial cells	SERCA3↓	proliferation	loss of SERCA3a in proliferating cells	[83]
Muscle				
Cardiomyocytes	SERCA2a↓	hypertrophic remodeling, impairment of contractile function	over 180 references including human and experimental animal hypertrophy, transgenic mice and protein ablation	reviewed by [32, 67, 95, 96]
Cardiomyocytes	SERCA2a↑ overexpression	prevention of hypertrophic remodeling	over 40 references including *in vivo*, *in vitro* gene transfer and transgenic mice	reviewed by [37, 48, 57, 67]

Cardiomyocytes	SERCA2b↑ replacement of SERCA2a by SERCA2b	hypertrophic remodeling, impairment of contractile function	transgenic mice	[127-129]
Vascular smooth myocytes	SERCA2a↓	induction of proliferation	loss of SERCA2a *in vivo* and *in vitro* in proliferating VSMC	[65, 68, 69, 125]
Vascular smooth myocytes	SERCA2a↑ overexpression	blockade of proliferation in G1 phase of cell cycle	gene transfer of SERCA2a prevents proliferation of VSMC *in vitro* and *in vivo*	[65, 69]
Cancer				
Oral cancer	SERCA2↓	squamous cell carcinoma	patient tissue samples and cell lines	[29]
Oral cancer	SERCA2↓	squamous cell carcinoma	transgenic mice ATP2A2(+/-)	[70, 97, 100]
Colon and lung cancer	SERCA2↓	somatic and germline mutation in lung cancer germline mutation in colon cancer	mutation of ATP2A2 gene may predispose to lung and colon cancer	[61]
Colorectal cancer	SERCA2↑	enhances the malignant potential	patient tissue samples (50 patients)	[17]
Thyroid cancer	SERCA2↓	enhances the malignant potential	cell lines	[93]

Colon cancer/ Colonic epithelial cells	SERCA3↓	enhances the malignant potential	expression of SERCA3 is inversely correlated with differentiation state of adenocarcinomas	[8, 36]

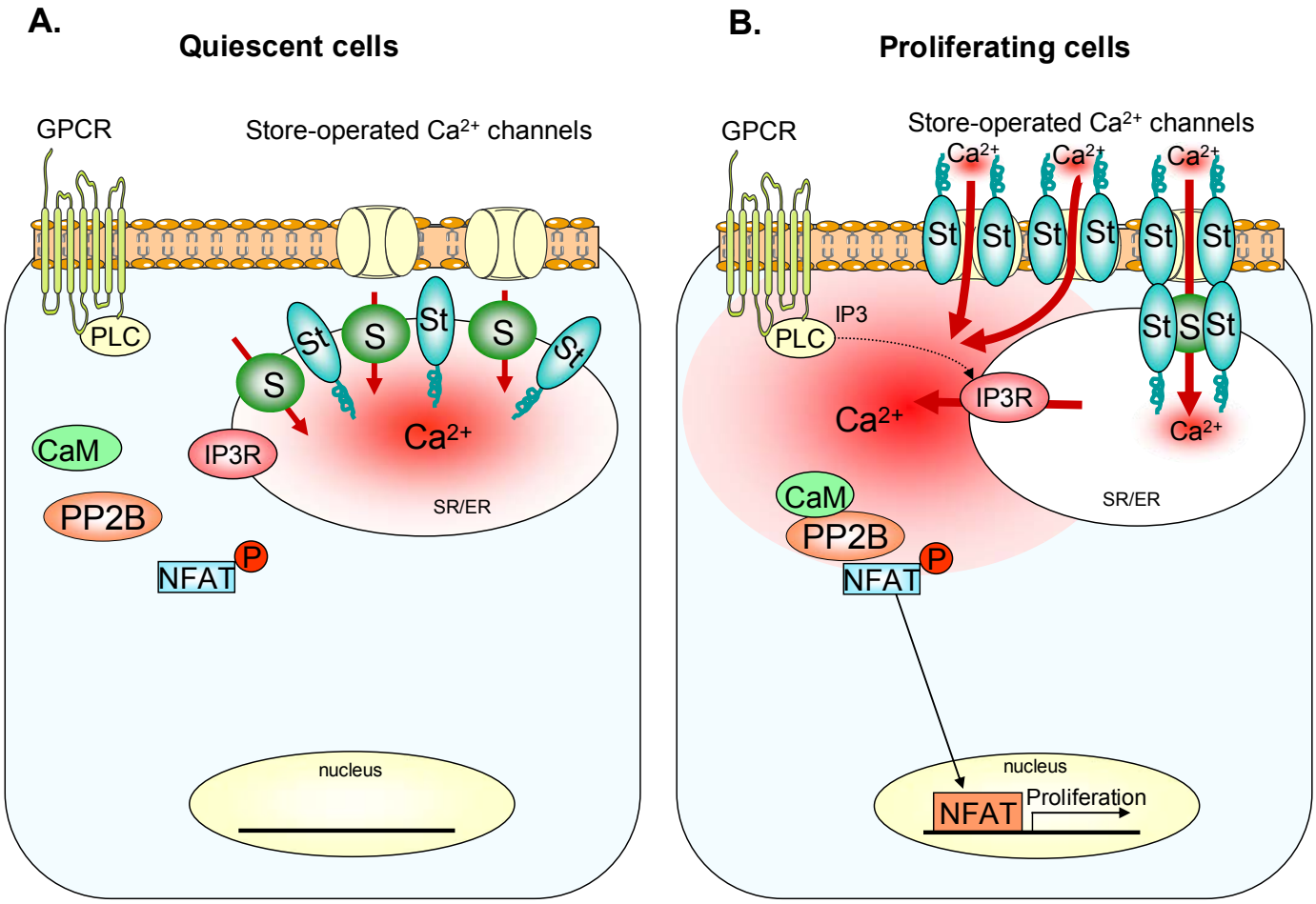

Figure 1.