

**Research Article**

**A Novel Alternative for Cancer Therapy:  
Pharmacological Modulation of Ca<sup>2+</sup> /cAMP  
Intracellular Signaling Interaction**

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**ABSTRACT**

Cancer is a major public health problem and the second leading cause of mortality around the world. Cancer therapy has been growing in an unprecedented fashion in the past two decades. Specific gene mutation, protein dysfunction, dysregulation of intracellular signaling pathways, and immune response had been targeted. It has been shown that the dysregulation of intracellular signaling pathways mediated by Ca<sup>2+</sup> and cAMP participates in the cancer initiation, tumor formation, tumor progression, metastasis, invasion and angiogenesis. Thereby, proteins involved in these pathways, such as Ca<sup>2+</sup> channels and cAMP-dependent protein kinase (PKA), represent potential drugs targets for cancer therapy. We recently discovered that the interaction between intracellular signaling pathways mediated by Ca<sup>2+</sup> and cAMP (Ca<sup>2+</sup>/cAMP signaling interaction) participates in the regulation of several cellular responses, including neurotransmitter/hormone secretion and neuroprotection. Due to importance of the Ca<sup>2+</sup>/cAMP signaling in the regulation of cellular proliferation, we have proposed that the pharmacological modulation of these signaling pathways could be a new strategy for cancer therapy.

**Keywords:** Ca<sup>2+</sup>/cAMP signalling interaction; Carcinogenesis; Cancer therapy.

**INTRODUCTION**

Cancer is a major public health problem and the second leading cause of mortality around

the world. Cancer therapeutics has been growing in an unprecedented fashion and has evolved rapidly in the past two decades. Specific gene mutation, protein dysfunction

and dysregulation, intracellular signaling pathways, and immune modulation have been targeted. These therapeutic advances came largely because of improved understanding of the pathobiology of cancer at the genetic and molecular levels. Accumulating data suggest that multi-targeted drugs may produce greater benefits than those observed with single-targeted therapies, which may have acceptable tolerability profiles, and may be active against a broader range of tumour types. Thus, regulation of intracellular signaling pathways is properly regarded as a composite of multiple component pathways involved in diverse aspects of tumour cell function.

Several cell functions are finely regulated by calcium ions ( $\text{Ca}^{2+}$ ) and 3',5'-cyclic adenosine monophosphate (cAMP)<sup>1-3</sup>. Then, dysregulation of intracellular signaling pathways mediated by these universal regulators of cell function had been implicated in cancer initiation, tumor formation, tumor progression, metastasis, invasion and angiogenesis<sup>1-3</sup>. Some studies showed that drugs able to interfere with the intracellular  $\text{Ca}^{2+}$  signaling such as selective  $\text{Ca}^{2+}$  channel blockers (CCB), as amlodipine, inhibit proliferative response in different cancer cells<sup>4,6</sup>. In addition, drugs able to increase the intracellular cAMP levels (cAMP-enhancer compounds), such as phosphodiesterase (PDE) 4 inhibitors, have been proposed as potential adjuvant, chemotherapeutic or chemopreventive agents in some cancer types, including hepatocellular carcinoma<sup>7</sup>. Then, the

pharmacological modulation of the intracellular signaling mediated by  $\text{Ca}^{2+}$  and cAMP in the cancer cells may represent a new therapeutic strategy for cancer progression.

Recently, we discovered that the functional interaction between intracellular signaling pathways mediated by  $\text{Ca}^{2+}$  and cAMP ( $\text{Ca}^{2+}$ /cAMP signaling interaction) plays an important role in the regulation of the several cellular responses, including neurotransmitter/hormone release and neuroprotection<sup>8-14</sup>. It is well established that the free  $\text{Ca}^{2+}$  in the cytosol regulates adenylate cyclase (AC) activity and consequently cAMP production<sup>9-13</sup>. The AC activity is reduced in response to increase of cytosolic  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_c$ ), decreasing the cytosolic cAMP concentration ( $[\text{cAMP}]_c$ )<sup>9-13</sup>. In contrast, the AC activity is increased in response to the reduction of  $\text{Ca}^{2+}$ , elevating  $[\text{cAMP}]_c$  due to degradation of ATP<sup>9-13</sup>.

We showed that  $\text{Ca}^{2+}$ /cAMP signaling interaction can be pharmacologically modulated by combination of drugs that reduce  $[\text{Ca}^{2+}]_c$ , such as  $\text{Ca}^{2+}$  channel blockers (CCB), such as nifedipine and verapamil, with drugs that increase  $[\text{cAMP}]_c$  (cAMP-enhancer compounds), such as Forskolin (AC activator) and Rolipram (phosphodiesterase (PDE) inhibitor)<sup>9-13</sup>. In response to the reduction of  $\text{Ca}^{2+}$  influx through L-type voltage-activated  $\text{Ca}^{2+}$  channels (VACC) produced by CCB, the AC activity and  $[\text{cAMP}]_c$  are increased<sup>9-13</sup>. These CCB-effects can be potentiated by

cAMP-enhancer compounds<sup>9-13</sup>. Figure 1 shows how the Ca<sup>2+</sup>/cAMP signaling interaction can be pharmacologically modulated by combined use of the CCB and cAMP-enhancer compounds.

This important discovery on the cellular role of Ca<sup>2+</sup>/cAMP signaling interaction, and its pharmacological modulation, emerged from numerous clinical studies performed since 1975 that reported that the use of L-type CCB during antihypertensive therapy decreased arterial pressure, but produced several adverse effects including sympathetic hyperactivity<sup>15</sup>. Despite these CCB-effects have been attributed to adjust reflex of arterial pressure by autonomic system, the molecular mechanisms involved in these effects remained unclear for decades.

To exclude the influence of adjusting reflex, the CCB effects on the autonomic system was studied in isolated tissues richly innervated by sympathetic nerves (rodent vas deferens)<sup>8,16-18</sup>. Studies performed since 1975 showed that responses mediated by sympathetic nerves were completely inhibited by L-type CCB in high concentrations (>1 µmol/L), but unexpectedly and paradoxically potentiated in concentrations below 1 µmol/L<sup>8,16-18</sup>. This paradoxical CCB-induced sympathetic hyperactivity remained unclear for decades, but in 2013, we discovered that this effect was caused by the increase of secretory response from sympathetic neurons, and adrenal chromaffin cells, stimulated by CCB due to its modulatory action on the

Ca<sup>2+</sup>/cAMP signaling interaction in these cells<sup>8</sup>. In addition, we discovered that the CCB-induced sympathetic hyperactivity was potentiated by cAMP-enhancer compounds, such as AC activators (Forskolin and IBMX) and PDE inhibitors (Rolipram)<sup>9-13</sup>. Our finding showed for the first time that the Ca<sup>2+</sup>/cAMP signaling interaction participates in the regulation of the transmitter/hormone from neurones and neuroendocrine cells<sup>9-13</sup>.

Our studies also showed that the cellular damage and death caused by cytosolic Ca<sup>2+</sup> overload can be prevented by pharmacological modulation of the Ca<sup>2+</sup>/cAMP signaling interaction, due probably to stimulation of cellular survival pathways mediated by cAMP-response element binding protein (CREB)<sup>10-13</sup>. Thus, the pharmacological modulation of the Ca<sup>2+</sup>/cAMP signaling interaction can produce elevation of [cAMP]<sub>c</sub> and attenuation of cytosolic Ca<sup>2+</sup> overload, stimulating cellular responses involved in the neuroprotection and cardioprotection<sup>9-14</sup>. We have proposed that the combined use of the L-type CCB and cAMP-enhancer compounds to pharmacologically modulate the Ca<sup>2+</sup>/cAMP signaling interaction could be used as a new therapeutic strategy for neurological and psychiatric disorders related to neurotransmission deficit, and neuronal death, such as Alzheimer's and Parkinson's diseases<sup>9-13</sup>. In addition, this pharmacological modulation could attenuate cardiac arrhythmias and myocardial lesions caused by ischemia and reperfusion in patients with acute myocardial infarction<sup>14</sup>.

Cancer therapy has been growing in an unprecedented fashion in the past two decades. Specific gene mutation, protein dysfunction, dysregulation of intracellular signaling pathways, and immune response had been targeted<sup>1-3</sup>. It has been shown that the dysregulation of intracellular signaling pathways mediated by  $\text{Ca}^{2+}$  and cAMP participates in the cancer initiation, tumor formation, tumor progression, metastasis, invasion and angiogenesis<sup>1-3</sup>. Thereby, proteins involved in these pathways, such as  $\text{Ca}^{2+}$  channels and cAMP-dependent protein kinase (PKA), represent potential drug targets for cancer therapy. Due to the important role of the dysregulation of intracellular signaling pathways mediated by  $\text{Ca}^{2+}$  and cAMP in the carcinogenesis, we have proposed that the pharmacological modulation of the  $\text{Ca}^{2+}$ /cAMP signaling interaction in cancer cells could be a new therapeutic strategy for cancer progression. In this review, we will discuss how the pharmacological modulation of the  $\text{Ca}^{2+}$ /cAMP signaling interaction could be a novel alternative for cancer therapy.

### **Role of the intracellular $\text{Ca}^{2+}$ signaling in cancer cells**

Since the beginning of life,  $\text{Ca}^{2+}$  ions play a vital role for living organisms.  $\text{Ca}^{2+}$  mediates the fertilization process and regulates the cell cycle events during the early developmental processes<sup>19,20</sup>. After the cells differentiate to perform specific functions,  $\text{Ca}^{2+}$  regulates numerous cellular processes, including energy transduction, secretion, neuronal synaptic plasticity,

muscle contraction, cell migration, chemotaxis, cell proliferation, gene transcription, apoptosis and others<sup>20</sup>.

Intracellular  $\text{Ca}^{2+}$  concentration in resting cell is usually maintained very low at about 100 nM, due to the toxicity related to excess of  $\text{Ca}^{2+}$  in cytosol<sup>20</sup>. Thus, transient increases in  $[\text{Ca}^{2+}]_c$  activates several intracellular signaling mediated by  $\text{Ca}^{2+}$ , while numerous mechanisms involved in cellular  $\text{Ca}^{2+}$  homeostasis act to restore the intracellular  $\text{Ca}^{2+}$  levels corresponding to the resting state of the cell<sup>20</sup>.

In the excitable cells, the  $\text{Ca}^{2+}$  enters into cell through plasma membrane voltage-activated  $\text{Ca}^{2+}$  channels (VACC, also named CaV) and transient receptor potential channels (TRP), and triggering numerous cellular responses<sup>20</sup>. Inside the cell,  $\text{Ca}^{2+}$  is stored in specific organelles, such as endoplasmic reticulum (ER) and mitochondria<sup>20</sup>. Several  $\text{Ca}^{2+}$  channels and transporters finely regulate intracellular  $\text{Ca}^{2+}$  concentration, including VACC, ER  $\text{Ca}^{2+}$  channels regulated by inositol-1,4,5-triphosphate (IP3R) and ryanodine (RyR) receptors, plasmalemmal (PMCA) or ER (SERCA)  $\text{Ca}^{2+}$ -ATPase,  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (NCX) and mitochondrial  $\text{Ca}^{2+}$  uniporter (MCU)<sup>20</sup>.

To utilize  $\text{Ca}^{2+}$  as an intracellular messenger, cells have devised an ingenious mechanism of signaling that has overcome the inherent problems associated with lower diffusion rates and cytotoxicity of  $\text{Ca}^{2+}$ , by presenting oscillations in  $\text{Ca}^{2+}$  concentration as brief

spikes which are often organized as regenerative waves<sup>20</sup>. In order to provide for a very fast and effective Ca<sup>2+</sup> signaling, the cells spend a great amount of energy to maintain almost 20,000-fold Ca<sup>2+</sup> gradient between their intra and extracellular concentrations<sup>20</sup>. Then, the cells chelate, compartmentalize, or remove Ca<sup>2+</sup> from the cytoplasm to maintain this Ca<sup>2+</sup> gradient<sup>20</sup>.

Intracellular Ca<sup>2+</sup> signaling in normal cells is needed for cell proliferation, whereas tumor cell lines show changed dependency on Ca<sup>2+</sup> to maintain cell proliferation<sup>21,22</sup>. Carcinogenesis is a biological process of non-lethal genetic injury that can be inherited in the germ line or can be acquired by the action of environmental agents<sup>22</sup>. This process implies in changes in proto-oncogenes, genes proapoptotic genes, and DNA repair genes<sup>22</sup>. Several antineoplastic chemotherapeutic agents act in cell division, affecting both normal and neoplastic cells. It is well established that carcinogenesis process is related with an increased expression, and abnormal activation, of proteins that participate in the intracellular Ca<sup>2+</sup> homeostasis, such as Ca<sup>2+</sup> channels, transporters and pumps<sup>23</sup>. Then, these structures can be important therapeutic targets for inhibiting cancer growth.

Both the genetic and epigenetic mechanisms have been proposed for the specific roles of intracellular Ca<sup>2+</sup> signaling in carcinogenesis<sup>24</sup>. Due to mutations, the normal cells can be transformed to cancer cells by acquiring cancer-specific properties, including uncontrollable proliferation, immortality, and self-sufficiency in growth

signals<sup>24-26</sup>. It was showed that the intracellular Ca<sup>2+</sup> waves in concert with other signal-transduction cascades regulate several cellular processes, such as gene expression<sup>27-29</sup>. The activation by intracellular Ca<sup>2+</sup> of the protein kinase, such as PKC, causes the phosphorylation of methyltransferases involved in DNA methylation<sup>30</sup>.

Numerous evidences indicated that an increased expression and function of proteins (Ca<sup>2+</sup> channels, transporters, pumps) participate in the dysregulation of intracellular Ca<sup>2+</sup> signaling, contributing to cancer initiation, tumor formation, tumor progression, metastasis, invasion and angiogenesis<sup>1-3</sup>. For example, the overexpression of IP3R Ca<sup>2+</sup> release channels that regulate Ca<sup>2+</sup> leakage from the ER, or reduced sequestration of Ca<sup>2+</sup> due to lower levels of SERCA2, could decrease apoptotic rates<sup>1</sup>. The nucleoplasmic reticulum releases Ca<sup>2+</sup> independently of signals produced by cytosolic Ca<sup>2+</sup>, microdomain<sup>31</sup> where Ca<sup>2+</sup> binds to specific DNA promoter regions, regulating the activity of transcription factors, gene expression and cellular activity<sup>32</sup>. Then, these molecules involved in the intracellular Ca<sup>2+</sup> signalling have been proposed as therapeutic targets for inhibiting cancer progression<sup>23</sup>.

In cancer cells, the intracellular Ca<sup>2+</sup> signaling pathways are remodeled, or deregulated, changing their physiology, and distinguish them from non-malignant cells<sup>24,33</sup>. This remodeling, or dysregulation,

provides means by which cancer cells can overcome systemic anticancer defense mechanisms. In addition, this remodeling or dysregulation can lead to genetic diversity found in cancerous tissues thereby providing effective cellular strategies to the selection pressure to acquire specific traits<sup>24,33</sup>.

It was showed that the drugs that interfere with the intracellular  $\text{Ca}^{2+}$  signaling, such as CCB (amlodipine, mibefradil and NNC-55-0396), inhibit the proliferative response in different tumoral cells<sup>4-6</sup>. In addition, it was showed that the L-type VACC can directly modify the transcription of genes and their products, e.g., the proteolytically cleaved 75 kDa C-terminal fragment of  $\text{CaV}1.2$ , a L-type VACC named  $\text{Ca}^{2+}$  channel associated transcriptional regulator (CCAT), which translocates to the nucleus altering the transcription of several genes, including Myc, Bcl-associated death promoter (Bad) and artemin<sup>34</sup>. It was showed that the nuclear CCAT levels increase or decrease in response to low and high intracellular  $\text{Ca}^{2+}$ , respectively<sup>34</sup>. These findings support that the pharmacological modulation of the intracellular  $\text{Ca}^{2+}$  signaling in the cancer cells could be a novel alternative for cancer therapy.

Intracellular  $\text{Ca}^{2+}$  signaling in normal cells is highly regulated spatially by ER, mitochondria and cytoskeletal elements, and temporally by the  $\text{Ca}^{2+}$  oscillations, and  $\text{Ca}^{2+}$  wave frequencies, amplitudes, and durations<sup>1,20</sup>. In contrast, the spatio-temporal regulation of intracellular  $\text{Ca}^{2+}$  signaling in cancer cells is significantly modulated in

terms of frequencies, amplitudes and duration of  $\text{Ca}^{2+}$  signals. The specific targeting ( $\text{Ca}^{2+}$  channels, transporter or pumps) with restricted tissue distribution, altered expression in cancer and/or a role in the regulation of tumorigenic pathways, could disrupt intracellular  $\text{Ca}^{2+}$  homeostasis in cancer cells. Treating both normal and cancer cells with agents that disrupt these pathways may kill the cancer cell<sup>33</sup>. The altered expression of the  $\text{Ca}^{2+}$  channel in cancer cells can increase the  $\text{Ca}^{2+}$  influx, leading to activation of cell death pathways and/or disruption of cell-cycle progression<sup>33</sup>. Then, the selective alterations in the activity of the  $\text{Ca}^{2+}$  channel could inhibit the  $\text{Ca}^{2+}$ -dependent tumorigenic pathways, including the cell proliferation<sup>33</sup>.

L-type VACC has been implicated in the development and progression of several tumors, and a recent meta-analysis of microarray datasets showed VACC mRNA gene profile of different types of cancers<sup>35</sup>. It was showed that the L-type VACC are significantly up-regulated in colon and esophageal cancer [36-40]. Novel splice variants of T-type VACC are commonly detected in human glioma, breast, ovarian, prostate colon and esophageal cancer cells<sup>36-40</sup>. For example, the  $\text{Cav}3.1a$  transcripts predominate in the normal adult brain, but human glioma and glioma cell lines contain  $\text{Cav}3.1bc$  as predominant splice and  $\text{Cav}3.1ac$  as a novel splice variant, which is absent in normal brain<sup>36-40</sup>.

Several drugs that interfere with the intracellular  $\text{Ca}^{2+}$  signaling, such as CCB,

inhibit proliferative response in different tumoral cells<sup>4-6,41</sup>. For example, the L-type VACC blocker amlodipine inhibited both in vitro and in vivo the growth of human epidermoid carcinoma A431 cells, via arresting cell cycle at G1 phase, and reducing phosphorylation of retinoblastoma protein, expression levels of cyclin D1 and cyclin dependent kinase<sup>4</sup>. In addition, the T- and L-type VACC blocker mibefradil reduced tumor size, to improve the survival rate in glioma animal model as well as in a patient derived pancreas xenograft animal model<sup>36,42</sup>. A novel mibefradil-derived compound NNC-55-0396 inhibited angiogenesis in cancer cells, becoming a promising chemotherapy drug<sup>6,36</sup>.

It is important to mention that the cancer therapy with drugs that interfere in the intracellular Ca<sup>2+</sup> signaling, such as CCB, could be useful to control growth of cancer with high rates of resistance to conventional radiotherapy and chemotherapy treatments, or in combination with immunotherapy, to decrease dose of monoclonal antibodies intravenously infused, and their adverse effects<sup>32</sup>. The use of these drugs in association with existing cancer therapy may reduce the doses and adverse effects generated by radiotherapy and chemotherapy, conferring better quality of life to patients, and increase of global survival rate of patients with cancer.

cAMP is a derivative of adenosine triphosphate (ATP) produced by enzymatic action of AC. This chemical messenger is used for intracellular signal transduction in

many different organisms, conveying the cAMP-dependent pathway. cAMP regulates a large variety of cell functions in response to activated G-protein coupled receptors. The increase of [cAMP]c activates cAMP-dependent protein kinase (PKA), stimulating various cellular process. The widespread expression of PKA subunit genes, and the myriad of mechanisms by which cAMP is regulated within a cell suggest that cAMP/PKA signaling is vital for cellular function involved in the regulation of a wide variety of cellular processes, including metabolism, ion channel activation, cell growth and differentiation, gene expression and apoptosis<sup>43</sup>. Since it has been implicated in the initiation and progression of tumors, PKA has been proposed as a novel biomarker for cancer detection, and as a potential molecular target for cancer therapy<sup>43</sup>.

cAMP exerts positive or negative effects on cell proliferation in different cell types. Several in vitro studies have shown that the cAMP is a mitogenic factor in somatotrophs and in other endocrine cells. Some evidences suggest that the mutations of genes coding for proteins that contribute to increases in the cAMP signaling cascade may cause endocrine tumor development. Although the role of intracellular cAMP signaling in cancer cells has been poorly investigated, the drugs that increase the intracellular cAMP concentration (cAMP-enhancer compounds), such as PDE 4 inhibitor Rolipram, have been proposed as potential adjuvant, chemotherapeutic or chemopreventive agents in hepatocellular

carcinoma<sup>7</sup>. It was showed that cAMP-enhancer compounds produce cytoprotective effect in cancer model rats<sup>44</sup>. The impairment of cAMP and/or cGMP generation by overexpression of PDE isoforms has been reported in various types of cancer<sup>45</sup>. The inhibition of selective PDE isoforms produces increase of the intracellular cAMP and/or cGMP levels, inducing apoptosis and cell cycle arrest in a broad spectrum of tumour cells<sup>45</sup>. In addition, the inhibition of selective PDE isoforms regulates the tumour microenvironment<sup>45</sup>. This strategy may offer promising insight into future cancer therapy. We have proposed that the development and clinical application of drugs that modulate intracellular signaling mediated by  $Ca^{2+}$  and cAMP may selectively restore normal intracellular signalling, providing antitumour therapy with reduced adverse effects. Thus, our recent discovery of the role of  $Ca^{2+}$ /cAMP signaling interaction in the regulation of several cellular responses<sup>9-13</sup> opened the possibility that pharmacological modulation of these signalings could be useful in the cancer therapy.

The regulation of cyclic nucleotide signaling is properly regarded as a composite of multiple component pathways involved in diverse aspects of cancer cell function. This 'pathway approach' targeted to cAMP has identified AC activators (e.g., AC7), PDE inhibitors (e.g., PDE7B) and/or activators or inhibitors of downstream mediators (PKA and Epac, respectively), which might be utilized therapeutically in chronic

lymphocytic leukemia<sup>46</sup>. Therapy directed at such targets may prove to be clinically useful, and may also provide a proof-of-principle of the utility of targeting cAMP signaling in other types of cancer<sup>46</sup>.

Intracellular cAMP signaling, through the PKA-dependent and/or-independent pathways, is very relevant to cancer and its targeting has shown a number of antitumor effects, including the induction of mesenchymal-to-epithelial transition, inhibition of cell growth and migration and enhancement of sensitivity to conventional antitumor drugs in cancer cells<sup>47</sup>. It was showed that the AC activator forskolin produces antitumor effects due to increase of [cAMP]<sup>47</sup>. The 8-Cl-cAMP, and the PKA I-selective cAMP analogs (8-piperidoadenosine-3',5'-cyclic monophosphate (8-PIP-cAMP) and 8-hexylaminoadenosine-3',5'-cyclic monophosphate (8-HA-cAMP) produced antiproliferative effect in human cancer cell lines<sup>48</sup>.

The anti-proliferative effect of the PKA I-selective cAMP analogs was attributed to growth arrest, while the 8-Cl-cAMP appears produce pro-apoptotic effect. It also observed that the PKA I-selective cAMP analogs, but not 8-Cl-cAMP, inhibited ERK phosphorylation, whereas 8-Cl-cAMP alone induced a progressive phosphorylation of the p38 mitogen-activated protein kinase (MAPK), via activation of AMPK by its metabolite 8-Cl-adenosine<sup>48</sup>. Pro-apoptotic effect of 8-Cl-cAMP appears to be prevented by pharmacological inhibition of



the p38 MAPK. These findings suggest that 8-Cl-cAMP and the PKA I-selective cAMP analogs could be used in human cancer therapy.

Interestingly, the 8-Cl-cAMP and PKA type I-selective cAMP analogs (8-PIP-cAMP and 8-HA-cAMP) also showed a potent antiproliferative effect in medullary thyroid cancer cell lines<sup>49</sup>. It was showed that the 8-Cl-cAMP significantly inhibited the transition of cell population from G2/M to G0/G1 phase and from G0/G1 to S phase<sup>49</sup>. In addition, the 8-Cl-cAMP induced apoptosis in medullary thyroid cancer cell lines<sup>49</sup>. This finding demonstrated that cAMP analogs, particularly 8-Cl-cAMP, significantly suppress cell proliferation in medullary thyroid cancer cell lines and provide rationale for a potential clinical use of drugs that interfere with cAMP/PKA signalling in the cancer therapy.

Pharmacological modulation of Ca<sup>2+</sup>/cAMP signalling interaction in cancer cells as a new therapeutic strategy of cancer. It is interesting to note that since the 1980s there was an increase in research identifying genetic and molecular targets, and in clinical trials, using biomarkers able to detect the presence of genetic or molecular markers in a patient's cancer to select appropriate targeted therapy. This advance in the diagnosis and therapy of cancer has been made possible by increased knowledge of the genetic pathogenesis of cancer, and by increased capacity to sequence genes and genomes in clinically useful timeframes. But, many challenges and pitfalls remain in

selecting optimal targets, designing effective targeted drugs and antibodies, and identifying appropriate combinations of therapies are required. Therefore, the current knowledge about regulation of intracellular Ca<sup>2+</sup> and cAMP signalling in cancer cells, and the search for new pharmacological strategies to control these intracellular messengers may contribute to the development of new pharmacological strategies that specifically alter tumor growth, angiogenesis and metastasis, without affecting normal cell physiology.

The control of the intracellular Ca<sup>2+</sup> signaling has been described as an important strategy to reduce the rate of cancer tumor proliferation<sup>31,32,50-52</sup>. In addition, the intracellular cAMP signaling is very relevant to cancer, and its targeting has shown a number of antitumor effects and the enhancement of sensitivity to conventional antitumor drug therapy<sup>47</sup>. In fact, several drugs that interfere with the intracellular signalings mediated by Ca<sup>2+</sup> and cAMP inhibit tumor growth, angiogenesis and metastasis in different tumoral cells<sup>4-7,36,42-50</sup>. Then, the pharmacological modulation of the Ca<sup>2+</sup>/cAMP signaling interaction in the tumoral cells may represent a new therapeutic strategy of cancer progression<sup>19</sup>. In combination with existing antitumor therapies, the pharmacological modulation of the Ca<sup>2+</sup>/cAMP signaling interaction may be able to reduce the doses and adverse effects generated by radiotherapy and chemotherapy, conferring better quality of life to patients, and increase of global survival rate of patients with cancer. This

combined therapy could be used to control growth of cancer tumors with high rates of resistance to conventional radiotherapy, and chemotherapy treatments<sup>51,52</sup>. In addition, the pharmacological modulation of the Ca<sup>2+</sup>/cAMP signaling interaction could be used in combination with immunotherapy to decrease dose of monoclonal antibodies intravenously infused, and their adverse effects<sup>53</sup>. It also important to mention that the CCB and cAMP-enhancer compounds used to modulate the Ca<sup>2+</sup>/cAMP signaling interaction are actually used in antihypertensive, and antidepressant, therapy with good tolerability by most patients.

We have proposed that the pharmacological modulation of the Ca<sup>2+</sup>/cAMP signalling interaction could be a more efficient therapeutic approach to reduce cancer tumor growth, angiogenesis and metastasis, without affecting normal cell physiology deserves special attention. It would not be a surprise the suggestion of using CCBs in combination with pharmaceuticals which increase cAMP to inhibit cancer progression<sup>8-13</sup>. Then, the pharmacological modulation of the Ca<sup>2+</sup>/cAMP signalling interaction could be an efficient therapeutic strategy to prevent cancer progression.

## CONCLUSION

Considering that the dysregulation of intracellular signaling pathways mediated by Ca<sup>2+</sup> and cAMP participates in the cancer initiation, tumor formation, tumor progression, metastasis, invasion and

angiogenesis, and proteins involved in these signaling represent potential drugs targets for cancer therapy, we have proposed that the pharmacological modulation of the Ca<sup>2+</sup>/cAMP signalling interaction could be an alternative strategy more efficient and safer for cancer therapy.

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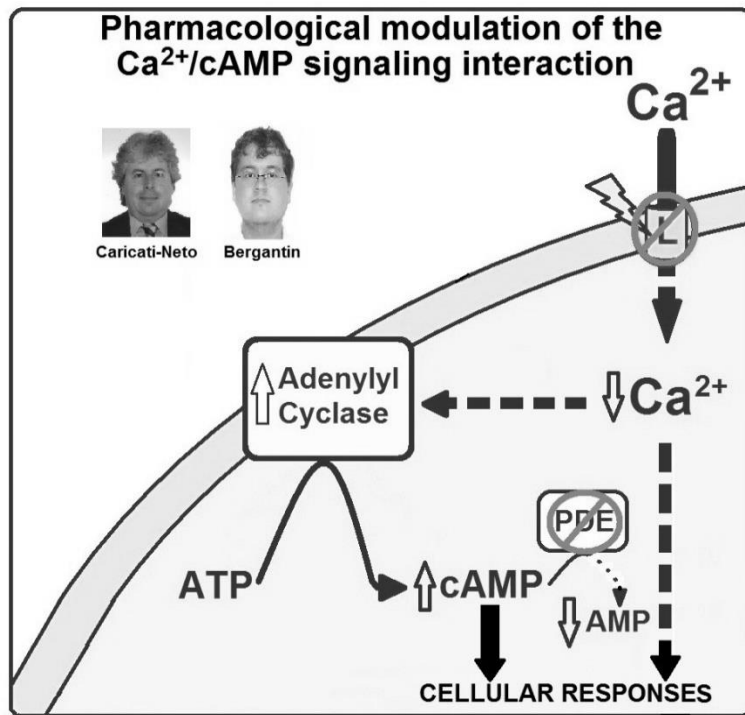
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**Figure 1.** Pharmacological modulation of the Ca<sup>2+</sup>/cAMP signaling interaction