

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

## Calcium signalling and angiogenesis

**This is a pre print version of the following article:**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1506826> since 2015-10-12T17:02:38Z

*Publisher:*

Bentham Science Publishers BV:PO Box 7917, Exec Ste Y 26, Saif Zone Sharjah Uae:011 971 6 5571132,

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)



# UNIVERSITÀ DEGLI STUDI DI TORINO

***This is an author version of the contribution published on:***

*Questa è la versione dell'autore dell'opera:*

*Topics in anti-cancer research vol3 pp 179-201, DOI:*

*10.2174/97816080590891140301*

***The definitive version is available at:***

*La versione definitiva è disponibile alla URL:*

*<http://ebooks.benthamscience.com/book/9781608059089/chapter/125646/>*

# CALCIUM SIGNALLING AND ANGIOGENESIS

Luca Munaron

<sup>1</sup>Department of Life Sciences & Systems Biology <sup>2</sup>Center for Complex Systems in Molecular Biology and Medicine (SysBioM) <sup>3</sup>Nanostructured Interfaces and Surfaces Centre of Excellence (NIS), University of Torino, Italy, Via Accademia Albertina 13, 10123 Torino, ITALY. email: [luca.munaron@unito.it](mailto:luca.munaron@unito.it)

**Running title:** Angiogenesis and calcium signals

**Keywords:** calcium signalling, calcium channels, TRP, calcium channel blockers, calcium entry, angiogenic factors, VEGF, angiogenesis, neovascularization, antiangiogenic factors, endothelial cells, tumor-derived endothelial cells, signal transduction, tyrosine kinase receptors, arachidonic acid, PLA2, eicosanoids, nitric oxide, NOS, calcium-dependent transcription factors.

## ABSTRACT

Proliferation and migration of vascular endothelial cells (ECs) are critical steps in angiogenesis and are strictly controlled by a number extracellular stimuli. Proangiogenic peptides binding to tyrosine kinase receptors (i.e. VEGFs and FGFs) are released by several cell types, including ECs and tumor cells. Proangiogenic intracellular signalling cascades involve many messengers working in a sort of network. In particular, in this review we describe the properties and functions of the intracellular calcium signals ( $Ca_i$ ), a universal, evolutionary conserved and highly versatile pathway involved in the regulation of EC proliferation and migration.

Angiogenic factors generate  $Ca_i$  rises *via* two mechanisms: entry from extracellular medium, through the opening of calcium-permeable channels in the plasmamembrane, or release from intracellular  $Ca^{2+}$  stores. Calcium entry, the main topic of this review, can be dependent on previously  $InsP_3$ -activated emptying of calcium stores (store-operated  $Ca^{2+}$

entry - SOCE), or independent on it (non store-operated  $\text{Ca}^{2+}$  entry - NSOCE). The intracellular pathways underlying endothelial  $\text{Ca}^{2+}$  entry involve, among the other pathways, arachidonic acid (AA) and nitric oxide (NO) metabolism. Even if some  $\text{Ca}^{2+}$  entry blockers are under clinical trial with encouraging results, a better knowledge about the molecular nature of proangiogenic  $\text{Ca}^{2+}$  channels and their intracellular regulation in healthy and pathological processes could lead to new and more powerful strategies in the therapeutical approaches aimed to interfere with altered tissue vascularization. Here, we discuss the state of the art in the field of calcium signaling and angiogenesis, the related recent literature and patents.

## **INTRODUCTION**

### **Role of $\text{Ca}^{2+}$ in the control of EC proliferation and migration**

Intracellular calcium ( $\text{Ca}_i$ ) signals are a highly conserved and ubiquitous mode for the control of cell survival, proliferation, motility, migration, apoptosis, and differentiation [1-4]. They are involved at different critical phases in the regulation of angiogenesis, both in healthy and in altered conditions [5-9].

Endothelial cells (EC) are the major actors of new blood vessels formation, and particular attention has been focused on them. During angiogenesis, ECs leave the preexisting vessel moving through the matrix, proliferate and finally stop their mitogenic activity and reorganize in a new tube [10-23]. Both migration and proliferation are strictly controlled by  $\text{Ca}_i$  dynamics, specifically modulated by extracellular proangiogenic agents such as vascular endothelial growth factors (VEGFs) and fibroblast growth factors (FGFs) [5-9, 24, 25].

Calcium entry from external medium is usually mediated by  $\text{Ca}^{2+}$ -permeable cationic channels in the plasmamembrane, which show varying degrees of selectivity and can support longer lasting signals (up to tens of minutes). While voltage-operated  $\text{Ca}^{2+}$

channels open following a simple, direct stimulus such as a depolarization step, the activation of voltage-independent channels, largely prevalent in endothelium, needs the involvement of metabolic pathways stimulated by receptor tyrosine kinases (RTKs) or G-protein-coupled receptors (GPCRs), leading to the production of second messengers that modulate channel activity [26, 27].

We usually distinguish between two major types of calcium entry: store-operated  $\text{Ca}^{2+}$  entry - SOCE, secondary to- and dependent on a previously activated depletion of intracellular stores, and non store-operated  $\text{Ca}^{2+}$  entry - NSOCE, carried by channels sensitive to intracellular messengers released upon receptor activation [28-30]. SOCE and NSOCE often coexist in the same cell, in some cases depending on agonist concentration or on the level of expression of the same channel. In some cell types, SOCE and NSOCE could cross-regulate [31-34].

SOCE has been extensively associated with  $\text{Ca}^{2+}$  influx related to cell proliferation and involves the association of the endoplasmic  $\text{Ca}^{2+}$  sensor STIM-1 with channel-forming proteins including members of TRP and Orai1 [28, 35-41].

Several other examples are related to proliferation dependent on NSOCE; for others, finally, the mechanism is not specified. Among the second messengers proposed to play a role in NSOCE activation, arachidonic acid (AA) and other lipidic molecules are the most relevant (see below). In particular, in bovine and human ECs from normal and tumoral tissues, AA opens calcium-permeable channels independently from store depletion and promotes  $\text{Ca}^{2+}$ -entry-dependent cell proliferation and migration [42-52]. A store-independent  $\text{Ca}^{2+}$  channel, activated by low concentrations of AA, has been observed in HEK293 cells: this channel, responsible for  $I_{\text{ARC}}$  (arachidonate-regulated  $\text{Ca}^{2+}$  current), is not associated with cell migration and proliferation and its biophysical properties are significantly different from endothelial AA-activated calcium entry [31-34, 53, 54].

It is now well accepted that members of transient receptor potential (TRP) superfamily of channels contribute to agonist-activated  $\text{Ca}^{2+}$  entry in all tissues in a great number of physiological and pathological events [27, 55-63]. TRPs are cationic channels, some of which are directly activated by intracellular messengers, including diacylglycerol and AA [42, 50, 64].

Endothelial TRP channels (19 different TRP members) regulate angiogenesis, vascular tone and permeability, EC proliferation, motility and differentiation. Moreover they are involved in the progression of a number of human cardiovascular diseases [24, 41, 56, 58, 60, 61, 63, 65-82].

Beside TRPs, other channels play a role in proangiogenic signalling. Orai1 and Stim1, the components of the so-called calcium release activated currents (CRAC) channels, have been proposed to mediate VEGF-mediated SOCE in HUVECs and *in vivo* [39, 83]. In addition to their activity on mature ECs, Both TRPs and Orai1 are components of SOCE in endothelial progenitor cells (EPCs) that concur to neovascularization in tumors [83, 84].

## **CALCIUM CHANNELS BLOCKERS**

Several  $\text{Ca}^{2+}$  channel blockers are available, with different chemical structure and variable selectivity and specificity [5, 6, 24, 25, 41, 72-82, 85-87]. Both voltage-dependent and independent  $\text{Ca}^{2+}$  channels are competitively blocked by inorganic ions, such as divalent transition ions ( $\text{Ni}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ) and lanthanides ( $\text{La}^{3+}$ ,  $\text{Gd}^{3+}$ ). The use of this ions in clinical protocols is severely limited by their toxicity, and, at least in some cases, by their aspecificity.

A number of organic compounds are known to interfere with endothelial  $\text{Ca}^{2+}$  channels and angiogenic process [88] (figure 1).

**CAI.** Carboxyamidotriazole (CAI, L651582, NSC 609974) is a synthetic  $\text{Ca}^{2+}$  influx blocker, extensively associated with inhibitory effects on cell proliferation [89-98]. The antiproliferative effects of CAI have been ascribed to its ability to inhibit intracellular pathways involving phospholipase- $\text{C}\gamma$ ,  $\text{InsP}_3$  and arachidonic acid release, as well as to block  $\text{Ca}^{2+}$  channels. CAI inhibits proliferation and invasive properties of several tumor cell lines *in vitro*, including prostate, glioblastoma, hepatoma, small cell lung and breast-derived cell lines. It also significantly reduces proliferation of several types of ECs (HUVECs, HAECs, BAECs) and affects angiogenesis induced *in vitro* by VEGF. The effect on angiogenesis is mediated by the block of calcium-mediated nitric oxide synthase-vascular endothelial growth factor pathway. In HUVECs, a reduction of NO release was observed, while no significant effect on VEGFR,  $\text{PLC}\gamma$ , ERK1/2, NFAT activities was detected, supporting the high specificity of the drug. In BAECs, CAI partially inhibits calcium entry activated by AA, critically involved in the control of proliferation. Thus, the use of calcium channel inhibitors, at least in some cell types including endothelial and smooth muscle cells, suggests a role for NO and AA in the control of cell proliferation and secondarily of physiopathological processes (see below): however, the insurgence of complex feedbacks of AA and NO on calcium homeostasis renders the experimental data difficult to be clearly interpreted.

On ECs, CAI-sensitive  $\text{Ca}^{2+}$  influx has been correlated to cell adhesion, spreading, proteolysis and migration, all processes involved in tumor invasion. Endothelial cell spreading on type IV collagen, but not on type I, is specifically regulated by CAI-sensitive  $\text{Ca}^{2+}$  influx, and the expression of metalloproteinase-2 is modulated by  $\text{Ca}^{2+}$  influx and down regulated by CAI. Inhibition of angiogenesis and metastasis has been detected in *in vivo* studies on several types of solid tumors. Administration of CAI, also in combination

with other drugs, in preclinical and clinical investigations stabilized solid tumors including carcinomas and melanomas.

**Imidazole derivatives.** Imidazole derivatives, such as clotrimazole, econazole, miconazole, ketoconazole, are antimycotic drugs widely used for the treatment of yeast infections [5, 99]. Imidazole antimycotics are potent inhibitors of many mammalian cytochrome P450-dependent reactions. Secondary to their effects on cytochrome P450 activity, they have also been extensively used as non-specific blockers of  $\text{Ca}^{2+}$  influx. Moreover, other effects on  $\text{Ca}^{2+}$  homeostasis have been described: miconazole, clotrimazole and econazole, in the  $\mu\text{M}$  range, promote  $\text{Ca}^{2+}$  entry in canine kidney cells; clotrimazole inhibits the activity of the sarcoplasmic reticulum  $\text{Ca}^{2+}$  pump in rabbit cardiomyocytes. Notably, some imidazole derivatives have been shown to affect the activity of kinases strongly involved in mitogen-induced intracellular signalling. The compound SK&F 96365 is widely used, and it is commonly suggested to act on TRPC channels [100]. However, it has been in turn classified as a specific blocker of SOCs, NSOCs, or as a non-specific blocker, acting also on VOCs. Moreover, other aspecific effects have been reported, both of activation and block of other types of channels. The isoquinoline derivative LOE-908 is an inhibitor of SOCE and NSOCE that interferes with endothelial permeability. It has been used as TRPC inhibitor [88, 100].

**Calcium Trifluoroacetate.** A recent report described the effects of Calcium Trifluoroacetate,  $\text{Ca}(\text{TFA})_2$ , a complex, poorly dissociated salt with low toxicity [101].  $\text{Ca}(\text{TFA})_2$  inhibits VEGF-induced ECs proliferation *in vitro* and angiogenesis *in vivo*. Interestingly, it triggers  $[\text{Ca}]_i$  signals in HMECs preventing  $[\text{Ca}]_i$  signaling induced by VEGF [102]. The detailed mechanism of through which  $\text{Ca}(\text{TFA})_2$  reduces the efficiency of signal transduction triggered by the growth factor has not been investigated. It could be able to activate calcium-permeable channels, as well as to regulate calcium extrusion systems and potassium channels.

## CALCIUM SIGNALS RELATED TO ANGIOGENESIS

**Proangiogenic factors.** Most angiogenic factors (FGFs, EGF, PDGF, VEGFs, IGF-I), exert their effect by the interaction with intrinsic tyrosine kinase receptors (RTKs); cytokines (such as interleukins, ILs) bind to receptors associated with cytosolic TKs, while other growth factors (such as bradikinin, ATP, oxytocin, colecystokinin, many neuropeptides) act *via* G-protein-coupled receptors (GPCRs) [6, 10, 103-111]. While each of these classes has its peculiarities, and involves specific cascades of intracellular events, the distinction is actually not so sharp, since in many instances crosstalks between different pathways have been described and may represent a general and physiological process. Moreover, a high degree of convergence on the same effector (a channel or an enzyme) is quite usual, suggesting that some signalling modules (among them calcium signals) are well conserved and are employed by different agonists in different contexts; this finding prompts another question, i.e. how such an interwoven web of signals can be reconciled with the evidence that different factors can exert specific and unique effects on the same cells (figure 2).

Calcium rises activated upon membrane receptor recruitment have been detected in virtually every type of normal and tumor-derived cell lines.  $Ca_i$  signals are very early events, starting typically after a delay of seconds from the agonist exposure. On the basis of the time course, we can distinguish four types of  $Ca_i$  increases: a single spike usually due to release from intracellular stores; a slower and more persistent  $Ca^{2+}$  signal dependent on  $Ca^{2+}$  entry from the extracellular medium; a biphasic  $Ca_i$  elevation resulting from the combination of the two mechanisms; finally,  $Ca_i$  oscillations.

In particular, a striking feature of proangiogenic  $Ca_i$  signalling is its heterogeneity. Both amplitude and time course of the response is highly variable from cell to cell. This observation can be ascribed to the differential expression of the receptors, the  $Ca^{2+}$

channels, or the intracellular machinery leading to the response. Vascular endothelium is a heterogeneous tissue: macro- and microvascular EC lines, as well as ECs obtained from different tissues, display biochemical and functional differences (see also concluding remarks).

**Angiogenic inhibitors: Angiostatin and Endostatin.** A number of endogenous inhibitors of angiogenesis are associated with tumors: the best known are angiostatin and endostatin [112-114]. Angiostatins are constituted by the first three, four, or five kringle domains of the plasminogen molecule: they are released by matrix metalloproteases secreted by tumor-infiltrating macrophages. Endostatin is a 20 kDa C-terminal fragment of collagen XVIII, probably generated by proteases and elastases activity [113, 115-117]. Endostatin and angiostatin inhibit EC migration and proliferation, and both induce EC apoptosis. Interestingly the antiproliferative effects of angiostatin seem specific for ECs while other cell types are not affected. Even if some studies have been performed by the analysis of acute intracellular effects of these peptides, several aspects of their mechanisms of action (including the putative membrane receptors) are unknown [112, 114, 115].  $Ca_i$  signalling related to angiostatin and endostatin stimulation has been described in BAECs, HMECs, and CPAECs [117]. The response is dependent on INSP3 release following PLC activation and is composed by an initial release from intracellular stores followed by a prolonged  $Ca^{2+}$  entry. Another interesting observation is that prolonged exposure to endostatin attenuates acute calcium signalling in response to subsequent treatment with VEGF and FGF. Kringle domains of urokinase, another antiangiogenic endogenous factor, are also able to promote calcium increases in HUVECs but not in other cell types.

## **ARACHIDONIC ACID, NITRIC OXIDE AND $Ca^{2+}$**

## **Arachidonic acid metabolism and $\text{Ca}^{2+}$**

In resting cells, AA is stored within the cell membrane, esterified to glycerol in phospholipids. Three enzymes (the phospholipases A2, C and D), with different sites of attack on the phospholipid backbone, mediate the deacylation reaction that releases the fatty acid [5, 51, 99]. While PLA2 releases arachidonate in a single-step reaction, PLC and phospholipase D (PLD) do not produce AA directly; rather, they generate lipid products containing arachidonate, respectively DAG and phosphatidic acid; the latter can be metabolized to DAG by phosphatidic acid phosphatase (PA-PH). From DAG, AA can be subsequently released by diacylglycerol lipase (DAG lipase) .

In mammalian cells, PLA2 is present in several isoforms, classified in 11 groups differing in structure, intracellular localization, regulation, calcium dependence and pharmacological inhibition [118-124]. One of them is the secretory PLA2 (sPLA2), a low molecular weight enzyme (14 kDa), whose activity is dependent on high calcium concentration (in the millimolar range); the cytosolic PLA2 (cPLA2, 85 kDa) is the form that has been investigated more extensively and is the enzyme stimulated mainly by growth factors through a MAPK-dependent phosphorylation on Ser-505. Its activation requires also a translocation to the membrane, in order to interact with its substrate, in a calcium-dependent fashion, at the physiological  $\text{Ca}^{2+}$  levels reached after agonist stimulation. Another form of PLA2 (iPLA2) is  $\text{Ca}^{2+}$ -independent and its functional roles are not well characterized; recently, in a colon carcinoma cell line (Caco-2 cells), evidence has been provided for its involvement in serum-induced AA release and cell proliferation.

In vascular ECs, bFGF receptor activation leads to the recruitment of several adapter proteins (FRS2, Grb2) followed by the indirect activation of ras and the mitogen activated protein kinase (MAPK) cascade. MAPK triggers a series of downstream events, including the activation of phospholipase A2 (PLA2): this enzyme in turn catalyzes the hydrolysis of phospholipids at the sn-2 position, where arachidonic acid (AA) is acetylated.

### *AA metabolites and angiogenesis*

Once released, free AA has different potential fates: direct regulation of several target proteins (ion channels, enzymes), diffusion outside the cell, reincorporation into phospholipids and metabolism [122, 125]. Metabolism of AA is carried out by three enzyme families: cyclooxygenases (COX), which generate prostaglandins, prostacyclins and thromboxanes, lipoxygenases (LOX), that produce leukotrienes, and cytochrome P450 monooxygenases, yielding a variety of epoxyeicosatrienoic and hydroperoxyeicosatetraenoic acids. All these compounds are collectively called eicosanoids, and act as autocrine or paracrine regulators of a variety of functions, particularly in inflammatory processes. Notably, several lines of evidence suggest their involvement in the control of EC proliferation and angiogenesis progression. In ECs from bovine adrenal cortex capillaries, AA release and the LOX pathway play a critical role in vascular cell proliferation induced by bFGF, PDGF and serum. In ECs isolated from bovine aorta, cPLA2 is activated by bFGF through p42 MAPK-dependent phosphorylation, triggering the release of AA. Moreover, in the same cell type, bFGF is a potent stimulator of proliferation through the LOX pathway of AA metabolism. Therefore, in general, LOX metabolites display mitogenic activity on ECs, while eicosanoids produced by the cyclooxygenase pathway are considered to be predominantly involved in the stimulation of migration. Some of the pathways cited above lead to other well known regulators of calcium-permeable channels, such as diacylglycerol (DAG) and InsP3.

### *AA and Ca<sup>2+</sup>*

AA itself and some of its metabolites are able to induce Ca<sub>i</sub> increases in ECs. The direct effect of AA, independent on its metabolites, has been revealed via two major strategies: the pharmacological inhibition of COX, LOX and P450 MO (using indomethacin, NDGA, econazole derivatives and other compounds, some of which highly aspecific) or the use of ETYA, an AA analogue not metabolized by the enzymes cited above, able to

mimic at least partially AA-dependent  $\text{Ca}^{2+}$  entry. In bovine aortic ECs (BAECs), human microvascular ECs, and tumor-derived human ECs (TECs, see below), AA induces a sustained NSOCE. Calcium entry is due to different  $\text{Ca}^{2+}$  channels, including TRPs, and regulates EC proliferation and migration. In the same cells, NSOCE is also activated by angiogenic factors (bFGF and VEGF) [42, 44, 45, 47-49, 51, 52, 126-128].

## **Nitric oxide metabolism and calcium**

### *NO and angiogenesis*

Several lines of evidence point to a relationship between NO release and angiogenesis progression [129, 130]. However, the selective role of NO in the different steps of endothelial biochemical modifications during angiogenesis is debated. Many angiogenic factors increase the expression of endothelial NO synthase (eNOS) and stimulate the release of endothelium-derived NO. VEGF augments the endothelial expression of NOS, and stimulates the biosynthesis of NO from cultured human umbilical venous ECs and vascular segments of rabbit thoracic aorta. Similarly, transforming growth factor beta ( $\text{TGF}\beta$ ) or bFGF trigger NO release in ECs.

A number of different approaches show the global involvement of NO on angiogenesis [129-136]. Stimulation of human umbilical venous ECs in a three-dimensional gel with bFGF or VEGF triggers NO production and let them to form capillary-like structures. This process is abolished by the NOS antagonist *N*<sup>w</sup>-nitro-L-arginine methylester (L-NAME). The same effect of L-NAME is observed in the rabbit cornea, another model of angiogenesis. NO appears to affect more than one step of angiogenesis process, acting as a freely diffusible pleiotropic factor on different cell types: in particular, this ability is clearly exerted on endothelial and smooth muscle cells. NO is an endothelial survival factor, inhibiting apoptosis. It increases proliferation on some EC types and reduces it in others. NO also promotes endothelial migration, possibly via the activation of podokinesis. In addition, NO

enhances matrix–endothelial cell interaction by inducing the expression of  $\alpha v\beta 3$  and by increasing disruption of the extracellular matrix *via* the bFGF-induced up regulation of urokinase-type plasminogen activator. Intriguing evidences point to the well known ability of NO to act as vasodilator: increased flow in the skeletal microcirculation has been observed to trigger endothelial cell proliferation and it is therefore possible that in addition to its direct effects on endothelial cell proliferation, NO may influence endothelial growth indirectly by increasing blood flow locally. Detailed mechanisms underlying this process are not known, but the effect of NO on endothelial mechanically gated calcium-permeable channels activated by shear stress could be a critical route. Finally, it should be noted that NO can induce the synthesis and release of VEGF from vascular cells, giving rise to a positive feedback mechanism.

#### *NO and Ca<sup>2+</sup>*

NO release is controlled by calcium elevation, due to eNOS calcium-sensitivity. Accordingly to its plasmamembrane association, eNOS has been proposed to be preferentially recruited by calcium entry more than by calcium release from intracellular stores in bovine aortic ECs. Moreover, prolonged capacitative calcium entry strongly activates eNOS. On the other hand, eNOS can be activated by calcium-independent additional mechanisms, such as PI3K-Akt signalling.

Nitric oxide affects endothelial calcium homeostasis in different ways [48, 49, 132]. Flow induced calcium entry, mediated by mechanically-gated calcium-permeable channels, is sensitive to a protein kinase G-activated conductance in rat aortic ECs. In porcine pulmonary artery ECs (PAECs) NO upregulates the expression of cyclic nucleotide gated channels and activate a cGMP-independent calcium entry. Moreover, in calf pulmonary artery ECs (CPAECs), NO inhibits CCE and enhances endoplasmic reticulum uptake of calcium. In these reports, NO is not explicitly associated with cell proliferation: nevertheless, due to the ability of several mitogens to release this messenger, it could play

a critical role in the control of calcium signals related to proliferative processes. Interestingly, NO has been recently suggested as a mediator of AA-induced calcium entry in smooth muscle cells and isolated mouse parotid cells.

Notably the pathways leading to proangiogenic  $Ca_i$  increase are  $Ca^{2+}$ -dependent: some members of the phospholipase A2 (PLA2) (that release AA), PLC (releasing DAG and InsP3), NOS and TK families are calcium-regulated. This may establish a non-linear positive loop contributing to the complexity of the signal.

## **SPECIFICITY OF CALCIUM SIGNALLING**

### **Spatial dynamics of calcium signals: from elementary to global events**

Technical improvements during the last decades revealed the existence of localized  $Ca_i$  signals (microdomains called blips, puffs, quarks) in different cell types, including ECs [48, 137-146]. When diffusional and regenerative mechanisms are triggered, these elementary events evolve to global  $Ca_i$  waves involving all the cell volume, including the nucleus. Calcium microdomains have been detected in several regions: near the plasmamembrane  $Ca^{2+}$  channels and close to ER releasing sites. Such signals can remain localized and activate and/or recruit effectors in the vicinity. In bovine vascular ECs, focal ATP stimulation results in spatially restricted  $Ca^{2+}$  release and SOCE. Mitochondria have been shown to play a critical role in the local regulation of capacitative  $Ca^{2+}$  entry and store refilling in HUVECs. Caveolae, special membrane microdomains, may play a relevant role in controlling the spatial and temporal pattern of  $Ca_i$  signalling. Endothelial caveolae include several components of intracellular signalling such as  $Ca^{2+}$  pumps, InsP3 receptor-like proteins, eNOS, PLC, PKC, and both GPCR and RTKs.

### **Temporal dynamics of calcium signals and gene expression**

Intracellular calcium signals trigger arrays of both rapid and sustained events, respectively ranging in seconds/minutes and hours/days. These responses are highly variable from a cell type to another. Moreover, single cell analysis points to the existence of a variability also in the same cell population, depending on qualitative differential expression of receptors, signalling molecules,  $\text{Ca}^{2+}$  channels and other elements of intracellular signalling.

The detailed mechanisms underlying the complex relationship between  $\text{Ca}^{2+}$  signals with different time courses and gene activation are only partially known [147]. In T and B lymphocytes maturation and activation, the induction of a small transient spike due to  $\text{Ca}^{2+}$  release from internal stores is sufficient to activate a specific pattern of signalling molecules and transcription factors such as NF- $\kappa$ B and JNK [148-153]. However this brief calcium event fails to activate other transcription factors, notably NFAT. In resting cells the phosphorylated form of this protein is located in the cytosol: after stimulation, it is dephosphorylated by the  $\text{Ca}^{2+}$ -dependent phosphatase calcineurin and translocates in the nucleus. As a result, only a long lasting  $\text{Ca}_i$  increase, mediated by  $\text{Ca}^{2+}$  entry, is able to sustain NFAT activation. DNA microarray analysis on T lymphocytes confirms these evidence providing further informations:  $\text{Ca}^{2+}$ -dependent signalling mediates both gene induction and gene repression by integration of inputs from calcium store depletion,  $\text{Ca}^{2+}$  entry, calcineurin activation and other downstream pathways. Some authors suggest an opposite role of CREB and NFAT transcription factors in the control of cell growth and proliferation. Even if these observations have been provided on a particular and highly specialized cell type, they may be useful as a working hypothesis on ECs. A relevant goal could be to identify the role of  $\text{Ca}_i$  signals in the switch between the different events involving ECs during angiogenesis: proliferation, migration and reorganization in a new vessel.

## CURRENT AND FUTURE DEVELOPMENTS

Several independent lines of evidences suggest that endothelial calcium signals play a role in physiological and pathological vascularization. In particular,  $\text{Ca}^{2+}$  entry mediated by proangiogenic  $\text{Ca}^{2+}$ -permeable channels regulates endothelial migration and proliferation. For these reasons, this event could be a target for the development of anti-cancer approaches aimed to inhibit tumor vascularization. However, despite the discovery of several natural or synthetic  $\text{Ca}^{2+}$  channels blockers, some relevant concerns limit their therapeutical applications. Firstly, a great amount of  $\text{Ca}^{2+}$  channel types are involved, activated or modulated by proangiogenic factors. In addition, while the same intracellular messenger can modulate different  $\text{Ca}^{2+}$  channels, some proangiogenic  $\text{Ca}^{2+}$  channels (i.e. TRPs) are co-regulated by a variety of intracellular pathways. Finally, the pattern of endothelial  $\text{Ca}^{2+}$  channels and their functional roles are strictly dependent on the tissue-specific microenvironment. This great variability has been well established for normal tissues, and particularly evident between macro- and microvasculature. More recently, this observation was extended to ECs obtained from human tumoral tissues.

Tumor neovascularization can be achieved by the recruitment of cell types other than ECs, including tumor cells, inflammatory cells and endothelial precursors, giving rise to very peculiar blood vessels [11, 104]. Tumor-derived human ECs (TECs) differ from normal ones for their functional behaviour and intracellular signalling [154, 155]. In particular, breast carcinoma- derived human ECs are more responsive to proangiogenic stimuli in terms of calcium signals. The expression and functional role of some TRPs is altered [6, 42, 44, 45, 47, 128, 156-162].

A broad proteomic approach, combined to high-throughput functional screening assays, would be required to provide a more detailed information on the expression and regulation of  $\text{Ca}^{2+}$  channels involved in vascularization in health and disease.

## **CONFLICT OF INTEREST**

The author(s) confirm that this article content has no conflicts of interest.

## **Disclosure**

The chapter submitted for Patent Series eBook entitled "Topics in Anti-Cancer Research", Volume **2** is an update of our article "**Intracellular Calcium, Endothelial Cells and Angiogenesis**", published in the journal 'Recent Patents on Anti-Cancer Drug Discovery', Volume 1, Number 1, January, 2006, Page 105 to 119 with modified title, additional text and references.

## Figure Legends

Figure 1

### **Structure of some widely used blockers of proangiogenic $\text{Ca}^{2+}$ entry.**

Carboxyamidotriazole (CAI), Calciumtrifluoroacetate ( $\text{Ca}(\text{TFA})_2$ ), SKF (SK&F 96365).

Figure 2

### **Interplay among AA, NO and $\text{Ca}^{2+}$ in endothelial cells.**

Tyrosine kinase receptors (RTKs), phospholipase D (PLD), phospholipase C (PLC), phospholipase A2 (PLA2), phosphatidic acid phosphatase (PA-PH), diacylglycerol lipase (DAG lipase), mitogen activated protein kinase (MAPK), cyclooxygenases (COX), lipoxygenases (LOX), cytochrome P450 monooxygenases (P450-MO), Guanylyl Cyclase (GC), endothelial Nitric Oxide Synthase (eNOS), protein kinase A (PKA), cytosolic free calcium concentration ( $[\text{Ca}]_i$ ).

Dashed lines show the feedback effects of  $\text{Ca}^{2+}$  on intracellular signalling.

## References

- [1] Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. *Nat Rev Mol Cell Biol* 2000 Oct 1;1(1):11-21.
- [2] Bootman MD, Lipp P, Berridge MJ. The organisation and functions of local Ca(2+) signals. *J Cell Sci* 2001 Jun 1;114(Pt 12):2213-22.
- [3] Clapham DE. Calcium signaling. *Cell* 2007 Dec 14;131(6):1047-58.
- [4] Jaiswal JK. Calcium – how and why? 2001:1-7.
- [5] Munaron L. Intracellular calcium, endothelial cells and angiogenesis. *Recent patents on anti-cancer drug discovery* 2006 Jan 1;1(1):105-19.
- [6] Munaron L, Fiorio Pla A. Endothelial calcium machinery and angiogenesis: understanding physiology to interfere with pathology. *Curr Med Chem* 2009;16(35):4691-703.
- [7] Munaron L, Scianna M. Multilevel complexity of calcium signaling: Modeling angiogenesis. *World journal of biological chemistry* 2012 Jul 26;3(6):121-6.
- [8] Munaron L, Tomatis C, Fiorio Pla A. The secret marriage between calcium and tumor angiogenesis. *Technol Cancer Res Treat* 2008 Aug 1;7(4):335-9.
- [9] Patton AM, Kassis J, Doong H, Kohn EC. Calcium as a molecular target in angiogenesis. *Curr Pharm Des* 2003;9(7):543-51.
- [10] Carmeliet P. Angiogenesis in life, disease and medicine. *Nature* 2005 Dec 15;438(7070):932-6.
- [11] Ferrara N, Kerbel RS. Angiogenesis as a therapeutic target. *Nature* 2005 Dec 15;438(7070):967-74.
- [12] Folkman J. Angiogenesis. *Annu Rev Med* 2006 Feb 1;57(1):1-18.
- [13] Hillen F, Griffioen AW. Tumour vascularization: sprouting angiogenesis and beyond. *Cancer and Metastasis Reviews* 2007 Jan 1.
- [14] Cai Y, Xu S, Wu J, Long Q. Coupled modelling of tumour angiogenesis, tumour growth and blood perfusion. *Journal of Theoretical Biology* 2011 Jun 21;279(1):90-101.
- [15] Munoz-Chapuli R. Evolution of angiogenesis. *The International journal of developmental biology* 2011;55(4-5):345-51.
- [16] Potente M, Gerhardt H, Carmeliet P. Basic and therapeutic aspects of angiogenesis. *Cell* 2011 Sep 16;146(6):873-87.
- [17] Jain RK, Carmeliet P. SnapShot: Tumor Angiogenesis. *Cell* 2012 Jul 08;149(6):1408-11.
- [18] Crabtree GR, Graef I, Chen F. Methods and compositions for modulating angiogenesis. US7323439 (2008).
- [19] Cuttitta F, Martinez A, Stetler-stevenson WG. Target for anti-angiogenesis therapy. US20090048170 (2009).
- [20] Jacoby DB, Kesavan K, Sentissi A, Egan ME. Inhibition of angiogenesis. US20110027177 (2011).
- [21] Li J, Shie J-I, Laham RJ. Methods and compositions for treating conditions involving abnormal angiogenesis. US7850959 (2010).
- [22] Waisman D, Kwon M. Anti-angiogenesis methods, compositions and uses therefor. US7250271 (2007).
- [23] Unknown. Oligonucleotides inhibiting cellular migration. EP2283870 (2011).
- [24] Ingber D, Thodeti C. Methods of modulating angiogenesis via TRPV4. US20110150894 (2011).
- [25] Lockyer P, Lockyer J. Methods For Detecting Calcium Ion Influx. US20080160536 (2008).
- [26] Nilius B, Viana F, Droogmans G. Ion channels in vascular endothelium. *Annu Rev Physiol* 1997;59:145-70.
- [27] Nilius B, Droogmans G. Ion channels and their functional role in vascular endothelium. *Physiol Rev* 2001 Oct 1;81(4):1415-59.
- [28] Várnai P, Hunyady L, Balla T. STIM and Orai: the long-awaited constituents of store-operated calcium entry. *Trends in Pharmacological Sciences* 2009 Mar 1;30(3):118-28.
- [29] Cahalan MD. STIMulating store-operated Ca(2+) entry. *Nat Cell Biol* 2009 Jun;11(6):669-77.
- [30] Holmes AM, Roderick HL, McDonald F, Bootman MD. Interaction between store-operated and arachidonate-activated calcium entry. *Cell Calcium* 2007 Jan 1;41(1):1-12.
- [31] Shuttleworth TJ. Arachidonic acid, ARC channels, and Orai proteins. *Cell Calcium* 2009 Jun 1;45(6):602-10.

- [32] Shuttleworth TJ, Thompson JL, Mignen O. STIM1 and the noncapacitative ARC channels. *Cell Calcium* 2007 Aug 1;42(2):183-91.
- [33] Mignen O, Thompson JL, Shuttleworth TJ. STIM1 regulates Ca<sup>2+</sup> entry via arachidonate-regulated Ca<sup>2+</sup>-selective (ARC) channels without store depletion or translocation to the plasma membrane. *J Physiol (Lond)* 2007 Mar 15;579(Pt 3):703-15.
- [34] Shuttleworth TJ, Thompson JL, Mignen O. ARC channels: a novel pathway for receptor-activated calcium entry. *Physiology (Bethesda, Md)* 2004 Dec 1;19:355-61.
- [35] Beech DJ. Orai1 calcium channels in the vasculature. *Pflugers Arch* 2012 Mar 9.
- [36] Zhang W, Trebak M. STIM1 and Orai1: novel targets for vascular diseases? *Science China Life sciences* 2011 Aug;54(8):780-5.
- [37] Li J, McKeown L, Stacey M, O'Regan D, Foster R, Porter KE, et al. Orai1 and CRAC channel dependence of VEGF-activated Ca<sup>2+</sup> entry and endothelial tube formation. *Circulation Research* 2011 Jun 13;108(10):1190-8.
- [38] Frischauf I, Schindl R, Derler I, Bergsmann J, Fahrner M, Romanin C. The STIM/Orai coupling machinery. *Channels (Austin, Tex)* 2008 Jan 1;2(4):261-8.
- [39] Abdullaev IF, Bisailon JM, Potier M, Gonzalez JC, Motiani RK, Trebak M. Stim1 and Orai1 mediate CRAC currents and store-operated calcium entry important for endothelial cell proliferation. *Circ Res* 2008 Nov 21;103(11):1289-99.
- [40] Whitten JP, Pei Y, Stauderman KA, Roos J. Benzylthiotetrazole inhibitors of store operated calcium release. *US20110257177 (2011)*.
- [41] Whitten JP, Pei Y, Stauderman KA, Roos J. Phenylpyrazole inhibitors of store operated calcium release. *US20110230536 (2011)*.
- [42] Fiorio Pla A, Ong HL, Cheng KT, Brossa A, Bussolati B, Lockwich T, et al. TRPV4 mediates tumor-derived endothelial cell migration via arachidonic acid-activated actin remodeling. *Oncogene* 2012 Jan 12;31(2):200-12.
- [43] Munaron L. Shuffling the cards in signal transduction: Calcium, arachidonic acid and mechanosensitivity. *WJBC* 2011 Jan 1;2(4):59.
- [44] Fiorio Pla A, Genova T, Pupo E, Tomatis C, Genazzani A, Zaninetti R, et al. Multiple roles of protein kinase a in arachidonic acid-mediated Ca<sup>2+</sup> entry and tumor-derived human endothelial cell migration. *Molecular Cancer Research* 2010 Nov 1;8(11):1466-76.
- [45] Antoniotti S, Fattori P, Tomatis C, Pessione E, Munaron L. Arachidonic acid and calcium signals in human breast tumor-derived endothelial cells: a proteomic study. *J Recept Signal Transduct Res* 2009 Jan 1;29(5):257-65.
- [46] Meves H. Arachidonic acid and ion channels: an update. *British Journal of Pharmacology* 2008 Sep 1;155(1):4-16.
- [47] Fiorio Pla A, Grange C, Antoniotti S, Tomatis C, Merlino A, Bussolati B, et al. Arachidonic acid-induced Ca<sup>2+</sup> entry is involved in early steps of tumor angiogenesis. *Mol Cancer Res* 2008 Apr 1;6(4):535-45.
- [48] Tomatis C, Fiorio Pla A, Munaron L. Cytosolic calcium microdomains by arachidonic acid and nitric oxide in endothelial cells. *Cell Calcium* 2007 Mar 1;41(3):261-9.
- [49] Mottola A, Antoniotti S, Lovisolo D, Munaron L. Regulation of noncapacitative calcium entry by arachidonic acid and nitric oxide in endothelial cells. *FASEB J* 2005 Dec 1;19(14):2075-7.
- [50] Watanabe H, Vriens J, Prenen J, Droogmans G, Voets T, Nilius B. Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. *Nature* 2003 Jul 24;424(6947):434-8.
- [51] Antoniotti S, Fiorio Pla A, Pregnotato S, Mottola A, Lovisolo D, Munaron L. Control of endothelial cell proliferation by calcium influx and arachidonic acid metabolism: A pharmacological approach. *J Cell Physiol* 2003 Oct 13;197(3):370-8.
- [52] Fiorio Pla A, Munaron L. Calcium influx, arachidonic acid, and control of endothelial cell proliferation. *Cell Calcium* 2001 Oct 1;30(4):235-44.
- [53] Mignen O, Thompson JL, Shuttleworth TJ. Both Orai1 and Orai3 are essential components of the arachidonate-regulated Ca<sup>2+</sup>-selective (ARC) channels. *J Physiol (Lond)* 2008 Jan 1;586(1):185-95.
- [54] Mignen O, Thompson JL, Shuttleworth TJ. Ca<sup>2+</sup> selectivity and fatty acid specificity of the noncapacitative, arachidonate-regulated Ca<sup>2+</sup> (ARC) channels. *J Biol Chem* 2003 Mar 21;278(12):10174-81.
- [55] Bodding M. TRP proteins and cancer. *Cell Signal* 2007 Mar;19(3):617-24.
- [56] Dietrich A, Kalwa H, Fuchs B, Grimminger F, Weissmann N, Gudermann T. In vivo TRPC functions in the cardiopulmonary vasculature. *Cell Calcium* 2007 Aug 1;42(2):233-44.

- [57] Hardie RC. TRP channels and lipids: from *Drosophila* to mammalian physiology. *J Physiol* 2007 Jan 1;578(Pt 1):9-24.
- [58] Inoue R, Jensen LJ, Shi J, Morita H, Nishida M, Honda A, et al. Transient receptor potential channels in cardiovascular function and disease. *Circ Res* 2006 Jul 21;99(2):119-31.
- [59] Kiselyov K, Soyombo A, Muallem S. TRPpathies. *J Physiol* 2007 Feb 1;578(Pt 3):641-53.
- [60] Kwan HY, Huang Y, Yao X. TRP channels in endothelial function and dysfunction. *Biochim Biophys Acta* 2007 Aug 1;1772(8):907-14.
- [61] Nilius B. TRP channels in disease. *Biochim Biophys Acta* 2007 Aug 1;1772(8):805-12.
- [62] Venkatachalam K, Montell C. TRP channels. *Annu Rev Biochem* 2007;76:387-417.
- [63] Watanabe H, Murakami M, Ohba T, Takahashi Y, Ito H. TRP channel and cardiovascular disease. *Pharmacol Ther* 2008 Jun 1;118(3):337-51.
- [64] Hardie RC. TRP channels and lipids: from *Drosophila* to mammalian physiology. *J Physiol (Lond)* 2007 Jan 1;578(Pt 1):9-24.
- [65] Inoue R, Hai L, Honda A. Pathophysiological implications of transient receptor potential channels in vascular function. *Curr Opin Nephrol Hypertens* 2008 Mar 1;17(2):193-8.
- [66] Antoniotti S, Fiorio Pla A, Barral S, Scalabrino O, Munaron L, Lovisolo D. Interaction between TRPC channel subunits in endothelial cells. *J Recept Signal Transduct Res* 2006 Jan 1;26(4):225-40.
- [67] Curry F, Glass C. TRP Channels and the Regulation of Vascular Permeability: New Insights From the Lung Microvasculature. *Circ Res* 2006 Sep 28;99(9):915-7.
- [68] Vriens J, Appendino G, Nilius B. Pharmacology of vanilloid transient receptor potential cation channels. *Mol Pharmacol* 2009 Jun;75(6):1262-79.
- [69] Abramowitz J, Birnbaumer L. Physiology and pathophysiology of canonical transient receptor potential channels. *FASEB J* 2009 Feb 1;23(2):297-328.
- [70] Trebak M. Canonical transient receptor potential channels in disease: targets for novel drug therapy? *Drug Discov Today* 2006 Oct 1;11(19-20):924-30.
- [71] Yao X, Garland CJ. Recent developments in vascular endothelial cell transient receptor potential channels. *Circ Res* 2005 Oct 28;97(9):853-63.
- [72] Eggenweiler H-m, Eiermann V. Pharmaceutical formulation comprising puyrazolo[4,-3-d]pyrimidines and antithrombotics, calcium antagonists, or prostaglandins or prostaglandin derivatives. US20040063730 (2004).
- [73] Helson L. Intravenous infusion of curcumin and a calcium channel blocker. US 20110117186 (2011).
- [74] Lee N, Chen J, Feder JN, Wu S, Lee LM, Blonar MA. TRP channel family member, LTRPC3 polypeptides. US7504481 (2009).
- [75] Muthuppalaniappan M, Viswanadha S, Varanasi KV, Merikapudi GS, Vakkalanka SKS. Novel modulators of calcium release-activated calcium channel and methods for treatment of non-small cell lung cancer. US20110112058 (2011).
- [76] Nishimura N, Norman MH, Tamayo N, Tang P, Bo YY. Vanilloid receptor ligands and their use in treatments. US20090082358 (2009).
- [77] Norman MH, Bo YY, Gore VK, Horne D, Kaller M, Ma VV, et al. Trp-m8 receptor ligands and their use in treatments. US20100261728 (2010).
- [78] Reynolds M, Polakis P. Trp-p8 active compounds and therapeutic treatment methods. US7893072 (2011).
- [79] Smith TJ. Regulation of human transient receptor potential channel. US20040115675 (2004).
- [80] Stewart JM. Peptide composition for cancer treatment by inhibiting trpv6 calcium channel activity. US20110071089 (2011).
- [81] Tymianski M, Garman JD, Belmares MP. Polypeptides for Modulating Binding of Trp Channel Proteins and Trp- Associated Proteins. US20080119412 (2008).
- [82] Xie Y, Holmqvist M, Mahiou J, Ono M, Sun L, Chen S, et al. Method for molulating calcium ion-release-activated calcium ion channels. WO2005009954 (2005).
- [83] Li J, Cubbon RM, Wilson LA, Amer MS, McKeown L, Hou B, et al. Orail and CRAC channel dependence of VEGF-activated Ca<sup>2+</sup> entry and endothelial tube formation. *Circ Res* 2011 May 13;108(10):1190-8.
- [84] Kuang CY, Yu Y, Wang K, Qian DH, Den MY, Huang L. Knockdown of Transient Receptor Potential Canonical-1 Reduces the Proliferation and Migration of Endothelial Progenitor Cells. *Stem Cells Dev* 2011 Apr 8.

- [85] Bourinet E, Escoubas P, Marger F, Nargeot J, Lazdunski M. Identification of novel antagonist toxins of T-type calcium channels for antagonist purposes. US20120040909 (2012).
- [86] Olson E, Bush E. Inhibition of TRP channels as a treatment for cardiac hypertrophy and heart failure. US20050182011 (2005).
- [87] Petty HR, Kindzelski AL, Huang J-b. Anti-metastatic ability of mibefradil and gadolinium. US20060003020 (2006).
- [88] Wolkowicz P, Huang J, Wolkowicz P, Huang J. Manipulation of calcium channels to regulate after-depolarization events in cardiac myocytes. US20130017198 (2013).
- [89] Guo L, Ye C, Chen W, Ye H, Zheng R, Li J, et al. Anti-Inflammatory and Analgesic Potency of Carboxyamidotriazole, a Tumorostatic Agent. *Journal of Pharmacology and Experimental Therapeutics* 2008 Jan 17;325(1):10-6.
- [90] Mikkelsen T, Lush R, Grossman SA, Carson KA, Fisher JD, Alavi JB, et al. Phase II clinical and pharmacologic study of radiation therapy and carboxyamido-triazole (CAI) in adults with newly diagnosed glioblastoma multiforme. *Investigational new drugs* 2007 Jun 1;25(3):259-63.
- [91] Mignen O, Brink C, Enfissi A, Nadkarni A, Shuttleworth TJ, Giovannucci DR, et al. Carboxyamidotriazole-induced inhibition of mitochondrial calcium import blocks capacitative calcium entry and cell proliferation in HEK-293 cells. *J Cell Sci* 2005 Dec 1;118(Pt 23):5615-23.
- [92] Oliver VK, Patton AM, Desai S, Lorang D, Libutti SK, Kohn EC. Regulation of the pro-angiogenic microenvironment by carboxyamido-triazole. *J Cell Physiol* 2003 Oct 1;197(1):139-48.
- [93] Hussain MM, Kotz H, Minasian L, Premkumar A, Sarosy G, Reed E, et al. Phase II trial of carboxyamidotriazole in patients with relapsed epithelial ovarian cancer. *J Clin Oncol* 2003 Dec 1;21(23):4356-63.
- [94] Faehling M, Kroll J, Föhr KJ, Fellbrich G, Mayr U, Trischler G, et al. Essential role of calcium in vascular endothelial growth factor A-induced signaling: mechanism of the antiangiogenic effect of carboxyamidotriazole. *FASEB J* 2002 Nov 1;16(13):1805-7.
- [95] Bauer KS, Cude KJ, Dixon SC, Kruger EA, Figg WD. Carboxyamido-triazole inhibits angiogenesis by blocking the calcium-mediated nitric-oxide synthase-vascular endothelial growth factor pathway. *J Pharmacol Exp Ther* 2000 Jan;292(1):31-7.
- [96] Kohn EC, Felder CC, Jacobs W, Holmes KA, Day A, Freer R, et al. Structure-function analysis of signal and growth inhibition by carboxyamido-triazole, CAI. *Cancer Res* 1994 Feb 15;54(4):935-42.
- [97] Kohn EC, Liotta LA, Felder CC. Signal transduction inhibitor compounds. US5359078 (1994).
- [98] Kohn EC, Liotta LA, Felder CC. 1,2,3-triazole and imidazole compounds and their antitumor use. EP0644880 (1995).
- [99] Munaron L, Antoniotti S, Fiorio Pla A, Lovisolo D. Blocking Ca<sup>2+</sup> entry: a way to control cell proliferation. *Curr Med Chem* 2004 Jun;11(12):1533-43.
- [100] Berrout J, Jin M, O'Neil RG. Critical role of TRPP2 and TRPC1 channels in stretch-induced injury of blood-brain barrier endothelial cells. *Brain Res* 2012 Feb 3;1436:1-12.
- [101] Gobbi R. Calcium Trifluoroacetate for Preparing Antiangiogenetic Medicaments. US20070260088 (2007).
- [102] Bussolati B, Ribatti D, Munaron L, Bartorelli A, Bussolati G. Anti-angiogenic properties of calcium trifluoroacetate. *Microvasc Res* 2009 Jul 23.
- [103] Buxton ILO, Yokdang N, Matz RM. Purinergic mechanisms in breast cancer support intravasation, extravasation and angiogenesis. *Cancer Letters* 2010 Jun 28;291(2):131-41.
- [104] Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature* 2000 Sep 14;407(6801):249-57.
- [105] Crawford Y, Ferrara N. VEGF inhibition: insights from preclinical and clinical studies. *Cell Tissue Res* 2009 Jan 1;335(1):261-9.
- [106] Cristofaro B, Emanuelli C. Possible novel targets for therapeutic angiogenesis. *Current opinion in pharmacology* 2009 Apr 1;9(2):102-8.
- [107] Griffioen A, Molema G. Angiogenesis: potentials for pharmacologic intervention in the treatment of cancer, cardiovascular .... *Pharmacological reviews* 2000.
- [108] Hillen F, Griffioen A. Tumour vascularization: sprouting angiogenesis and beyond. *Cancer and Metastasis Reviews* 2007.
- [109] Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling - in control of vascular function. *Nat Rev Mol Cell Biol* 2006 May;7(5):359-71.

- [110] Pocock TM, Williams B, Curry FE, Bates DO. VEGF and ATP act by different mechanisms to increase microvascular permeability and endothelial [Ca(2+)](i). *Am J Physiol Heart Circ Physiol* 2000 Oct 1;279(4):H1625-34.
- [111] Scianna M, Munaron L, Preziosi L. A multiscale hybrid approach for vasculogenesis and related potential blocking therapies. *Progress in biophysics and molecular biology* 2011 Aug;106(2):450-62.
- [112] Ribatti D. Endogenous inhibitors of angiogenesis: a historical review. *Leuk Res* 2009 May 1;33(5):638-44.
- [113] Shichiri M, Hirata Y. Antiangiogenesis signals by endostatin. *FASEB J* 2001 Apr 1;15(6):1044-53.
- [114] Tabruyn SP, Griffioen AW. Molecular pathways of angiogenesis inhibition. *Biochem Biophys Res Commun* 2007 Mar 30;355(1):1-5.
- [115] Shi H, Huang Y, Zhou H, Song X, Yuan S, Fu Y, et al. Nucleolin is a receptor that mediates antiangiogenic and antitumor activity of endostatin. *Blood* 2007 Oct 15;110(8):2899-906.
- [116] Zhang AY, Tegatz EG, Zou AP, Campbell WB, Li PL. Endostatin uncouples NO and Ca<sup>2+</sup> response to bradykinin through enhanced O<sub>2</sub><sup>\*</sup>- production in the intact coronary endothelium. *Am J Physiol Heart Circ Physiol* 2005 Feb 1;288(2):H686-94.
- [117] Jiang L, Jha V, Dhanabal M, Sukhatme VP, Alper SL. Intracellular Ca(2+) signaling in endothelial cells by the angiogenesis inhibitors endostatin and angiostatin. *Am J Physiol, Cell Physiol* 2001 May 1;280(5):C1140-50.
- [118] Valentín-Berríos S, González-Velázquez W, Pérez-Sánchez L, González-Méndez R, Rodríguez-Del Valle N. Cytosolic phospholipase A2: a member of the signalling pathway of a new G protein alpha subunit in *Sporothrix schenckii*. *BMC Microbiol* 2009 Jan 1;9:100.
- [119] Burke JE, Dennis EA. Phospholipase A2 structure/function, mechanism, and signaling. *The Journal of Lipid Research* 2009 Apr 1;50 Suppl:S237-42.
- [120] Winget JM, Pan YH, Bahnson BJ. The interfacial binding surface of phospholipase A2s. *Biochim Biophys Acta* 2006 Nov 1;1761(11):1260-9.
- [121] Schaloske RH, Dennis EA. The phospholipase A2 superfamily and its group numbering system. *Biochim Biophys Acta* 2006 Nov 1;1761(11):1246-59.
- [122] Dennis EA. Phospholipase A2 in eicosanoid generation. *Am J Respir Crit Care Med* 2000 Feb 1;161(2 Pt 2):S32-5.
- [123] Hallahan DE, Yazlovitskaya EM. Specific inhibition of cPLA2 enhances the efficacy of radiotherapy. *US20080262286 (2008)*.
- [124] Patierno SR, Manyak MJ. Inhibitors of phospholipase A2. *US20020151470 (2002)*.
- [125] Smith WL. The eicosanoids and their biochemical mechanisms of action. *Biochem J* 1989 Apr 15;259(2):315-24.
- [126] Munaron L, Fiorio Pla A. Calcium influx induced by activation of tyrosine kinase receptors in cultured bovine aortic endothelial cells. *J Cell Physiol* 2000 Dec 1;185(3):454-63.
- [127] Antoniotti S, Lovisolo D, Fiorio Pla A, Munaron L. Expression and functional role of bTRPC1 channels in native endothelial cells. *FEBS Lett* 2002 Jan 16;510(3):189-95.
- [128] Fiorio Pla A, Avanzato D, Munaron L, Ambudkar IS. Ion channels and transporters in cancer. 6. Vascularizing the tumor: TRP channels as molecular targets. *Am J Physiol Cell Physiol* 2012 Jan;302(1):C9-15.
- [129] Cooke J, Losordo D. Nitric oxide and angiogenesis. *Circulation* 2002.
- [130] Ziche M, Morbidelli L. Nitric oxide and angiogenesis. *J Neurooncol* 2000 Oct-Nov;50(1-2):139-48.
- [131] Ahmad S, Hewett PW, Wang P, Al-Ani B, Cudmore M, Fujisawa T, et al. Direct evidence for endothelial vascular endothelial growth factor receptor-1 function in nitric oxide-mediated angiogenesis. *Circ Res* 2006 Sep 29;99(7):715-22.
- [132] Berkels R, Suerhoff S, Roesen R, Klaus W. Nitric oxide causes a cGMP-independent intracellular calcium rise in porcine endothelial cells-a paradox? *Microvasc Res* 2000 Jan 1;59(1):38-44.
- [133] Brecht DS. Nitric oxide signaling specificity--the heart of the problem. *J Cell Sci* 2003 Jan 1;116(Pt 1):9-15.
- [134] Fukumura D, Kashiwagi S, Jain RK. The role of nitric oxide in tumour progression. *Nat Rev Cancer* 2006 Jul;6(7):521-34.
- [135] Li H, Poulos TL. Structure-function studies on nitric oxide synthases. *J Inorg Biochem* 2005 Jan 1;99(1):293-305.

- [136] Mancardi D, Pla AF, Moccia F, Tanzi F, Munaron L. Old and New Gasotransmitters in the Cardiovascular System: Focus on the Role of Nitric Oxide and Hydrogen Sulfide in Endothelial Cells and Cardiomyocytes. *Curr Pharm Biotechnol* 2011 Jan 11;Jan 11. [Epub ahead of print].
- [137] Pani B, Singh BB. Lipid rafts/caveolae as microdomains of calcium signaling. *Cell calcium* 2009;45(6):625-33.
- [138] Laude AJ, Simpson AWM. Compartmentalized signalling: Ca<sup>2+</sup> compartments, microdomains and the many facets of Ca<sup>2+</sup> signalling. *FEBS J* 2009 Apr 1;276(7):1800-16.
- [139] Parekh AB. Ca<sup>2+</sup> microdomains near plasma membrane Ca<sup>2+</sup> channels: impact on cell function. *J Physiol (Lond)* 2008 Jan 1.
- [140] Rizzuto R, Pozzan T. Microdomains of intracellular Ca<sup>2+</sup>: molecular determinants and functional consequences. *Physiol Rev* 2006 Jan;86(1):369-408.
- [141] Isshiki M, Mutoh A, Fujita T. Subcortical Ca<sup>2+</sup> waves sneaking under the plasma membrane in endothelial cells. *Circ Res* 2004 Aug 6;95(3):e11-21.
- [142] Isshiki M, Anderson RGW. Function of caveolae in Ca<sup>2+</sup> entry and Ca<sup>2+</sup>-dependent signal transduction. *Traffic* 2003 Nov 1;4(11):717-23.
- [143] Isshiki M, Ying YS, Fujita T, Anderson RGW. A molecular sensor detects signal transduction from caveolae in living cells. *Journal of Biological Chemistry*, 2002: 43389-98.
- [144] Isshiki M, Ando J, Yamamoto K, Fujita T, Ying Y, Anderson RGW. Sites of Ca(2+) wave initiation move with caveolae to the trailing edge of migrating cells. *J Cell Sci* 2002 Feb 1;115(Pt 3):475-84.
- [145] Isshiki M, Anderson RGW. Calcium signal transduction from caveolae. *Cell Calcium*, 1999: 201-8.
- [146] Isshiki M, Ando J, Korenaga R, Kogo H, Fujimoto T, Fujita T, et al. Endothelial Ca<sup>2+</sup> waves preferentially originate at specific loci in caveolin-rich cell edges. *Proc Natl Acad Sci USA* 1998 Apr 28;95(9):5009-14.
- [147] Boulware MJ, Marchant JS. Timing in cellular Ca<sup>2+</sup> signaling. *Curr Biol* 2008 Sep 9;18(17):R769-R76.
- [148] Feske S. Calcium signalling in lymphocyte activation and disease. *Nat Rev Immunol* 2007 Sep;7(9):690-702.
- [149] Feske S, Draeger R, Peter HH, Rao A. Impaired NFAT regulation and its role in a severe combined immunodeficiency. *Immunobiology* 2000 Aug;202(2):134-50.
- [150] Feske S, Giltneane J, Dolmetsch R, Staudt LM, Rao A. Gene regulation mediated by calcium signals in T lymphocytes. *Nat Immunol* 2001 Apr;2(4):316-24.
- [151] Feske S, Skolnik EY, Prakriya M. Ion channels and transporters in lymphocyte function and immunity. *Nat Rev Immunol* 2012 Jul;12(7):532-47.
- [152] Gwack Y, Feske S, Srikanth S, Hogan PG, Rao A. Signalling to transcription: store-operated Ca<sup>2+</sup> entry and NFAT activation in lymphocytes. *Cell Calcium* 2007 Aug;42(2):145-56.
- [153] Shaw PJ, Feske S. Physiological and pathophysiological functions of SOCE in the immune system. *Front Biosci (Elite Ed)* 2012;4:2253-68.
- [154] Baluk P, Morikawa S, Haskell A, Mancuso M, McDonald DM. Abnormalities of basement membrane on blood vessels and endothelial sprouts in tumors. *Am J Pathol* 2003 Nov;163(5):1801-15.
- [155] Bussolati B, Deambrosis I, Russo S, Deregibus MC, Camussi G. Altered angiogenesis and survival in human tumor-derived endothelial cells. *Faseb J* 2003 Jun;17(9):1159-61.
- [156] Pla AF, Avanzato D, Munaron L, Ambudkar IS. VASCULARIZING THE TUMOR: TRP CHANNELS AS MOLECULAR TARGETS. *AJP: Cell Physiology* 2011 Aug 10.
- [157] Pupo E, Pla AF, Avanzato D, Moccia F, Cruz JE, Tanzi F, et al. Hydrogen sulfide promotes calcium signals and migration in tumor-derived endothelial cells. *Free Radic Biol Med* 2011 Nov 1;51(9):1765-73.
- [158] Munaron L. Editorial: Intracellular Calcium Signaling: Holding the Balance between Health and Disease. *Current medicinal chemistry* 2012 Dec 01;19(34):5765-7.
- [159] Munaron L, Arcangeli A. Editorial: ion fluxes and cancer. *Recent patents on anti-cancer drug discovery* 2013 Feb 01;8(1):1-3.
- [160] Munaron L, Avanzato D, Moccia F, Mancardi D. Hydrogen sulfide as a regulator of calcium channels. *Cell calcium* 2013 Mar 01;53(2):77-84.
- [161] Munaron L, Genova T, Avanzato D, Antoniotti S, Fiorio Pla A. Targeting calcium channels to block tumor vascularization. *Recent patents on anti-cancer drug discovery* 2013 Feb 01;8(1):27-37.

[162] St. Croix B, Seaman S. Differential gene expression in physiological and pathological angiogenesis. US20110207141(2011).