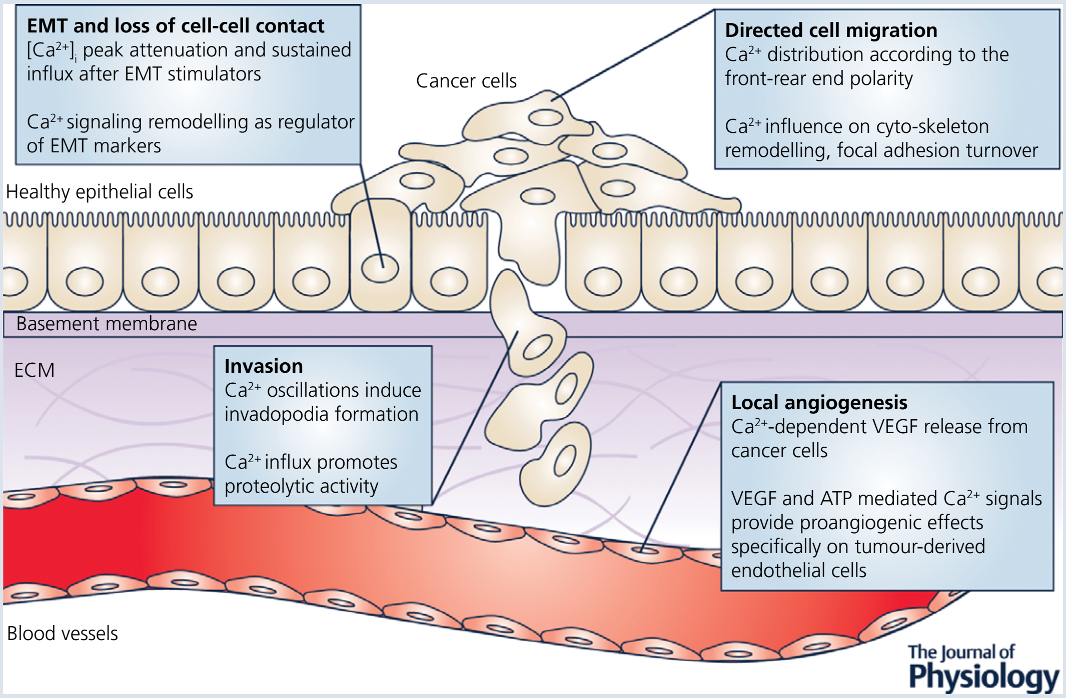
J Physiol 595.10 (2017) pp 3063–3075

SYMPOSIUM REVIEW

Molecular mechanisms of tumour invasion: regulation by calcium signals

Oksana Iamshanova[1], Alessandra Fiorio Pla[1,2] and Natalia Prevarskaya[1]

[1] *Inserm U1003, Laboratory of Excellence, Ion Channels Science and Therapeutics, Equipe Labellisée par la Ligue Nationale Contre le Cancer, SIRIC ONCOLille, University of Lille, 59656 Villeneuve d'Ascq, France*
[2] *Department of Life Science and Systems Biology, University of Torino, Torino, Italy*



Abstract Intracellular calcium (Ca^{2+}) signals are key regulators of multiple cellular functions, both healthy and physiopathological. It is therefore unsurprising that several cancers present a strong Ca^{2+} homeostasis deregulation. Among the various hallmarks of cancer disease, a particular role is played by metastasis, which has a critical impact on cancer patients' outcome. Importantly,

Oksana Iamshanova is a PhD student at the University of Lille 1 (France) in the Laboratory of Cell Physiology headed by Natalia Prevarskaya. Investigating the role of ion channels in tumorigenesis is one of her major research interests. **Alessandra Fiorio Pla** is associate professor at the University of Torino (Italy) and visiting professor at the University of Lille 1 (France). Her research activity is focused on Ca^{2+} channels characterization, in particular TRP channels, and their involvement in angiogenesis and tumour vascularization/progression by combining Ca^{2+} imaging and live cell imaging techniques with cell biology. **Natalia Prevarskaya** is full-time professor of physiology at the University of Lille 1 (France), director of the Laboratory of Cell Physiology and head of the team 'Ca^{2+} signatures of prostate cancer' certified by the National League Against Cancer. Her scientific interests are the function and regulation of ion channels, the role of ion channels and Ca^{2+} signalling in carcinogenesis, Ca^{2+} signalling in proliferation, apoptosis, migration and differentiation, prostate cancer and prostatic diseases.

This review was presented at "Advances and Breakthroughs in Calcium Signaling", which took place in Honolulu, Hawaii, 7–9 April 2016.

DOI: 10.1113/JP272844

Ca^{2+} signalling has been reported to control multiple aspects of the adaptive metastatic cancer cell behaviour, including epithelial–mesenchymal transition, cell migration, local invasion and induction of angiogenesis (see Abstract Figure). In this context Ca^{2+} signalling is considered to be a substantial intracellular tool that regulates the dynamicity and complexity of the metastatic cascade. In the present study we review the spatial and temporal organization of Ca^{2+} fluxes, as well as the molecular mechanisms involved in metastasis, analysing the key steps which regulate initial tumour spread.

(Received 20 November 2016; accepted after revision 20 January 2017; first published online 17 March 2017)
Corresponding author N. Prevarskaya: Inserm U1003, Laboratory of Excellence, Ion Channels Science and Therapeutics, Equipe Labellisée par la Ligue Nationale Contre le Cancer, SIRIC ONCOLille, University of Lille, 59656 Villeneuve d'Ascq, France. Email: natacha.prevarskaya@univ-lille1.fr, http://phycell.univ-lille.fr/

Abstract figure legend Model of the specific patterns of Ca^{2+} signals that are associated with different steps of cancer progression. It is important to note that most of the findings on Ca^{2+} signalling have been generated *in vitro*. Therefore, it is possible that in tissues, Ca^{2+} signals do not simply follow the same patterns.

Abbreviations AA, arachidonic acid; EC, endothelial cell; ER, endoplasmic reticulum; EGF, epidermal growth factor; EMT, epithelial–mesenchymal transition; ECM, extracellular matrix; KCa, Ca^{2+}-activated potassium channels; MMP, matrix metalloproteinase; MT1-MMP, membrane type 1 matrix metalloproteinase; MLCK, Ca^{2+}-dependent myosin light chain kinase; myo II, myosin II; NCX, Na^+/Ca^{2+} exchanger; NFAT, nuclear factor of activated T-cells; ORAI, Ca^{2+} release-activated Ca^{2+} channel protein; PMCA, plasma membrane ATPase; SOCE, store-operated Ca^{2+} entry; STIM, stromal interaction molecule; TEC, tumour-derived endothelial cell; TRP, transient receptor potential; TRPA, transient receptor potential ankyrin channel; TRPC, transient receptor potential canonical channel; TRPM, transient receptor potential melastatin channel; TRPV, transient receptor potential vanilloid channel; VEGF, vascular endothelial growth factor; VGCC, voltage-gated Ca^{2+} channel.

Introduction

Calcium (Ca^{2+}) is a ubiquitous second messenger which is involved in the tuning of multiple fundamental cellular functions (Berridge *et al.* 2000). Due to its multifaceted roles, it is not surprising that deregulated Ca^{2+} homeostasis has been observed in various disorders, including tumourigenesis (Monteith *et al.* 2007; Prevarskaya *et al.* 2011). Among the various manifestations of cancer, a particular role is played by metastasis, which has a critical impact on cancer patients' outcome (Hanahan & Weinberg, 2011). Tumour spread is a highly regulated process that usually starts with the loss of cell–cell contact and the epithelial–mesenchymal transition (EMT) (Kalluri & Weinberg, 2009). During metastasis, cancer cells also acquire enhanced directional movement and activate molecular pathways that enable the proteolysis of an extracellular matrix (ECM) as well as local angiogenesis. As a result, cancer cells enter the body's circulation systems and disseminate to distinct sites around the organism. Importantly, Ca^{2+} signalling has been reported to control multiple aspects of the adaptive metastatic cancer cell behaviours, including EMT, migration, local angiogenesis induction and intravasation (Chen *et al.* 2013). In this context, it is considered to be a substantial intracellular tool that regulates the dynamicity and complexity of the metastatic cascade. Intracellular free Ca^{2+} concentration is highly controlled by the fine regulation of 'ON' and 'OFF' mechanisms that ultimately generate Ca^{2+} signals with various amplitudes and frequencies. As regarding the ON mechanisms, cytosolic Ca^{2+} can either be delivered from extracellular space due to the activity of Ca^{2+}-permeable channels and transporters in plasma membrane, or occur as a result of a release from Ca^{2+}-containing organelles (e.g. endoplasmic reticulum) (Berridge *et al.* 2000). In order to maintain low resting Ca^{2+} concentration, cells remove Ca^{2+} using an energy-dependent mechanism, such as plasma membrane ATPases (PMCAs), or the Na^+/Ca^{2+} exchanger (NCX). Moreover Ca^{2+} is sequestered intracellularly into Ca^{2+}-containing organelles, primarily endoplasmic reticulum (ER), by means of mechanisms which require either ATP hydrolysis (e.g. a sarco/endoplasmic reticulum Ca^{2+}-ATPase pump), or a favourable electrochemical gradient. In this review we will overview the spatial and temporal organization of Ca^{2+} fluxes as well as those molecular mechanisms involved in metastasis, analysing the key steps which regulate tumour spread.

Epithelial–mesenchymal transition and loss of cell–cell contact

EMT is a cellular process during which epithelial cells acquire a fibroblast-like morphology. This process involves changes in cellular shape, a loss of epithelial polarized organization and cell–cell contacts like tight and adherens junctions. Accordingly, one of the most recognized features of cells undergoing EMT, is a suppression of multiple epithelial markers (e.g. E-cadherin, claudins, occludins) and an overexpression of mesenchymal markers (e.g. N-cadherin, vimentin, integrins) (Fig. 1).

Of note, EMT and the disruption of cell–cell contact is one of the key events in tumour progression. This can be induced by various effectors like growth factors, hypoxia and inflammation (Diepenbruck & Christofori, 2016). Interestingly, the remodelling of Ca^{2+} signals during EMT processes has been reported for a variety of cancer cells. For example, in breast cancer cells, the potency of ATP-mediated cytosolic Ca^{2+} transients exhibits significant changes after epidermal growth factor (EGF) and hypoxia-induced EMT (Davis *et al.* 2011; Azimi *et al.* 2016). Specifically, an attenuation of the cytosolic Ca^{2+} peak and a sustained phase of Ca^{2+} influx in the response to ATP have been attributed to the activity of G-protein-coupled purinergic receptors (P2Y family) and ligand-gated Ca^{2+} channels (P2X family) (Davis *et al.* 2011; Azimi *et al.* 2016). Another study reveals that an inhibition of P2X5 reduces the expression of the EMT marker vimentin, whereas its increased expression correlates with breast cancer cells that are associated with a more mesenchymal phenotype (Davis *et al.* 2011). Moreover, the chelation of free cytosolic Ca^{2+} suppresses the production of mesenchymal markers like vimentin, N-cadherin and CD44, after the exposure of breast cancer cells to EGF and hypoxia (Davis *et al.* 2013; Stewart *et al.* 2015). Similar findings have been reported for hepatic cancer cells, where chelation of intracellular Ca^{2+} reversed doxorubicin-induced EMT (Wen *et al.* 2016). Furthermore, the EMT of colon cancer cells may be regulated by the small conductance calcium-activated channel, subfamily N, such as KCNN4, through Ca^{2+}-dependent mechanisms (Lai *et al.* 2013). Regarding the store-operated Ca^{2+} entry (SOCE), the data are ambiguous. On one hand, SOCE and basal Ca^{2+} influx are reduced after the EGF-induction of EMT in the MDA-MD-468 breast cancer cell line (Davis *et al.* 2012). On the other hand, transforming growth factor β1 (TGF-β1)-induced EMT is associated with enhanced SOCE in the MCF-7 breast cancer cell line (Hu *et al.* 2011).

It is now clear that the remodelling of Ca^{2+} signalling is a prominent feature of EMT in various cancer types. Therefore, a deregulation of Ca^{2+}-permeable channels could subserve as an important EMT regulator during carcinogenesis. Indeed, silencing and pharmacological inhibition of transient receptor potential melastatin channels (TRPM) such as TRPM7 and TRPM8 reduce the expression of a variety of mesenchymal markers in breast cancer cells (Davis *et al.* 2013; Liu *et al.* 2014). In the MCF-7 breast cancer cell line that exhibits a more epithelial-like phenotype, the overexpression of TRPM8 leads to EMT induction as indicated by the profile of markers expressed (Liu *et al.* 2014). Consistent with this data, TRPM8 has been found to be upregulated in breast cancer tumour tissues, when compared to adjacent non-tumour tissues, thereby suggesting the role of TRPM8 as a determinant of EMT transition (Liu *et al.* 2014). Moreover, in breast cancer cells, EGF-induced EMT significantly increases the mRNA level of Ca^{2+} release-activated Ca^{2+} channel protein 1 (ORAI1) and leads to altered Ca^{2+} signalling, possibly due to the involvement of transient receptor potential canonical channel type 1 (TRPC1) (Davis *et al.* 2012). In hepatic cancer cells, another member of the transient receptor potential canonical channel family, TRPC6, has been shown to affect the expression of EMT markers after doxorubicin induction (Wen *et al.* 2016).

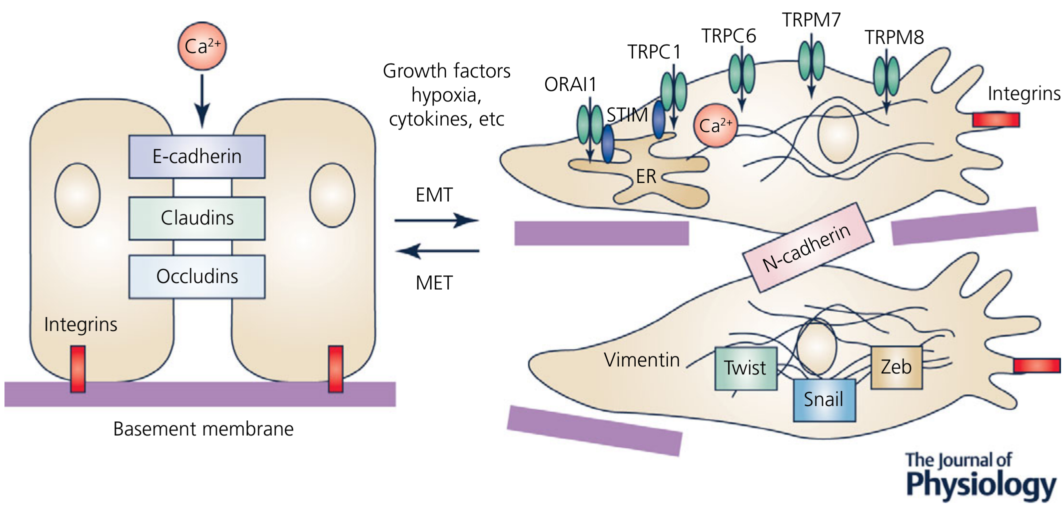


Figure 1. Epithelial-to-mesenchymal transition (EMT), loss of cell–cell contacts and downregulation of proteins such as E-cadherin, claudin or occludin
EMT transition is accompanied by the changes in Ca^{2+} signals due to several factors such as growth factors, cytokines and hypoxia. The most studied Ca^{2+}-permeable channels, which are associated with EMT, are indicated.

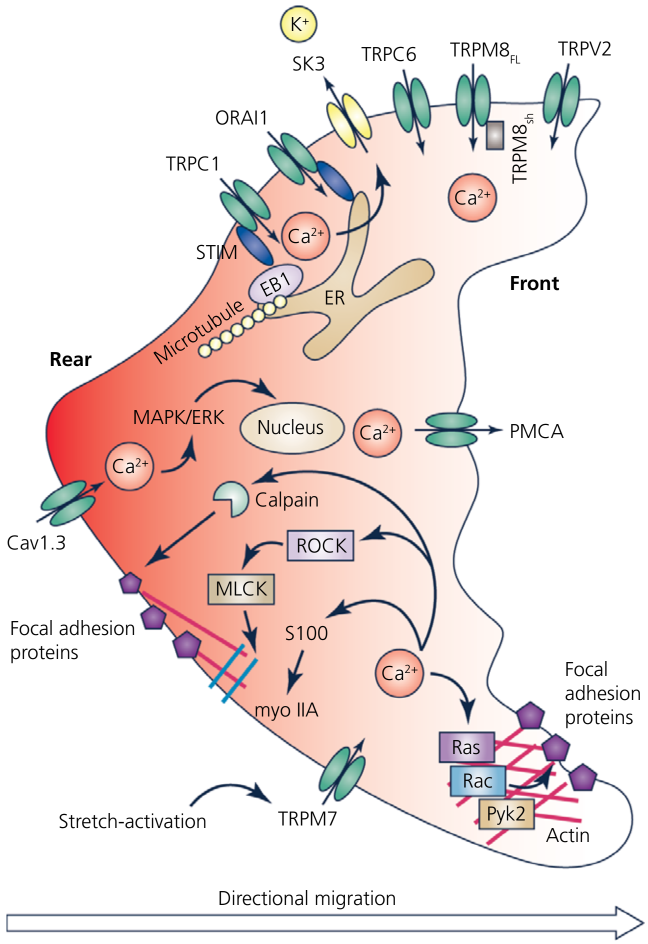
Overall, the studies of Ca^{2+} signalling and Ca^{2+}-permeable channels using various cancer models and EMT effectors have defined a critical role for the Ca^{2+} signal in the EMT process during tumourigenesis.

Cell migration

The principal component of cancer cell motility is directional migration, which is due to front–rear end polarity (Mayor & Etienne-Manneville, 2016). Typically, the leading edge is represented by flat cell membrane extensions with directed actin polymerization and nascent attachment sites, whereas at the rear of the cell, adhesions are disassembled and the trailing edge is contracted (Mayor & Etienne-Manneville, 2016). Interestingly, global cytosolic Ca^{2+} is generally higher at the rear end, whereas Ca^{2+} flickers are enriched near the front edge (Evans *et al.* 2007; Wei *et al.* 2009; Tsai & Meyer, 2012). It is suggested that such Ca^{2+} distribution is involved in controlling directed cellular locomotion (Brundage *et al.* 1991).

Of note, migration is a complex and multistep process that involves coordination between cytoskeleton remodelling, cell-substrate adhesion/detachment and cellular protrusion/contraction (Gardel *et al.* 2010; Parsons *et al.* 2010). Importantly, several key molecular components and signalling events of the cellular migration machinery are Ca^{2+} sensitive (Fig. 2). For example, the myosin II (myo II)-based actomyosin contraction is mainly mediated through the activity of Ca^{2+}-dependent myosin light chain kinase (MLCK) (Clark *et al.* 2007). The focal adhesion turnover is also highly dependent on Ca^{2+} signalling. On the one hand, the disassembly of cell adhesions is achieved due to the cleavage of focal adhesion proteins, such as integrins, talin, vinculin and focal adhesion kinases, by the Ca^{2+}-sensitive protease, calpain (Franco & Huttenlocher, 2005). On the other hand, Ca^{2+} is important for the modulation of nascent focal adhesion sites by activating proline-rich tyrosine kinase 2 (Pyk2), and small GTPases like Ras and Rac (Lysechko *et al.* 2010; Selitrennik & Lev, 2015). S100 proteins, a subgroup of the EF-hand Ca^{2+}-binding protein family, regulate a variety of cellular processes via an interaction with different target proteins (Bresnick *et al.* 2015). In particular, their influence on F-actin polymerization and myo II–actin assembly has been suggested as governing cell migration due to cytoskeletal structural remodelling (Gross *et al.* 2014) (Fig. 2). Overall, it is now clear that cell migration can be considered as a Ca^{2+}-dependent process. Importantly, Ca^{2+}-permeable channels are responsible for the cytosolic Ca^{2+} delivery from external and internal cellular stores. Therefore, their activity would define the occurrence of those sustained and transient Ca^{2+} changes which are important for the orchestration of cellular migration.

Interestingly, in migrating erythrocytes and human umbilical vein endothelial cells, the low basal Ca^{2+} levels at the leading edge are maintained due to the activity of PMCA and the inhibition of PMCA leads to an abrogated front-to-rear Ca^{2+} gradient and decreased migration (Pérez-Gordones *et al.* 2009; Tsai *et al.* 2014). Similar mechanisms could be utilized by the metastatic cells, since the expression of PMCA has been found to directly correlate with the tumourigenicity of breast cancer cells (Lee *et al.* 2005) (Fig. 2). At the same time, in the front end of ER, low local Ca^{2+} concentration provokes high sensitivity to SOCE (Tsai *et al.* 2014). Indeed, the



The Journal of Physiology

Figure 2. Global cytosolic Ca^{2+} is generally higher at the rear (marked in red), whereas Ca^{2+} flickers are enriched near the front edge of migrating cell
The key molecular components and signalling events of the cellular migration machinery are Ca^{2+} dependent. The most studied Ca^{2+}-permeable channels, which are associated with directional migration, are indicated. ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; MLCK, myosin light chain kinase; Pyk2, proline-rich tyrosine kinase 2; ROCK, Rho-associated protein kinase; STIM, stromal interaction molecule.

ER residual Ca^{2+} sensor of SOCE, stromal interaction molecule (STIM), has been found to be distributed along the polar axis in the leading edge of the migrating cell (Tsai *et al.* 2014). The STIM molecule responds to the ER Ca^{2+} depletion and provokes ion influx through the plasma membrane ORAI channel (Liou *et al.* 2005; Roos *et al.* 2005). Of note, STIM–ORAI proteins have been found to be significantly upregulated in various cancer types and SOCE-activated Ca^{2+} signalling is implemented in the mediation of actomyosin assembly and the focal adhesions required for efficient migration (Chen *et al.* 2011; Fiorio Pla *et al.* 2016; Jardin & Rosado, 2016) (Fig. 2).

Plasma membrane extensions and protrusions play the role of a mechanical stress and thus provide Ca^{2+} influx through stretch-activated channels at the front end of a migrating cell. Indeed, TRPM7 can be activated intracellularly through phospholipase C, or by a membrane stretch (Su *et al.* 2006; Wei *et al.* 2009; Gao *et al.* 2011; Middelbeek *et al.* 2012). Interestingly, TRPM7 is located in close proximity to calpain and myo II (Clark *et al.* 2007). Therefore, Ca^{2+} entry provided through TRPM7 modulates actomyosin cytoskeleton contraction, as well as the dynamics of the focal adhesion turnover required for directional cell migration (Clark *et al.* 2007). Indeed, the pro-migratory role of TRPM7 has been demonstrated for breast, lung, pancreatic and nasopharyngeal cancers (Visser *et al.* 2014). Moreover recently, the mechano-sensitive TRPC1 activation, located at the rear of the cells, has been shown to play a role in the formation of cell polarity of U2OS bone osteosarcoma cells and their directional migration (Huang *et al.* 2015). Similarly, several members of the TRP channel family have been implicated in cell migration in various cancer types (Fiorio Pla & Gkika, 2013). In particular most TRP channels have been associated with an increase in migration potential. This is the case for TRPC members such as TRPC1 and TRPC6 in glioma cells (Chigurupati *et al.* 2010; Bomben *et al.* 2011). In addition, the vanilloid subfamily TRPV2 has also been associated with increased cellular migration in prostate, bladder and breast cancer (Oulidi *et al.* 2013; Gambade *et al.* 2016). In contrast, full length TRPM8, has been reported to inhibit cell migration, thus suggesting a protective role for TRPM8 in prostate metastatic cancer progression (Gkika *et al.* 2010, 2015), whereas the short TRPM8 isoform could have a pro-metastatic potential (Peng *et al.* 2015; Bidaux *et al.* 2016).

Voltage-gated Ca^{2+} channels (VGCCs) present another pathway for Ca^{2+} influx that activates a downstream mitogen-activated protein kinase (MAPK)–extracellular signal-regulated kinase (ERK) signalling pathway and increases migration (Mertens-Walker *et al.* 2010). In particular, Cav1.3 has been found to be overexpressed in endometrial carcinoma and its knockdown has been shown to reduce migration (Hao *et al.* 2015). Indeed, at filopodia tips increased Ca^{2+} concentration provided through L-type VGCCs directs cancer cell migration due to calpain activation (Jacquemet *et al.* 2016).

Intracellular Ca^{2+} is an important regulator of Ca^{2+}-activated potassium channels (KCa). Furthermore, ORAI and TRPC1 channels may form complexes with small conductance KCa channel SK3 (Chantome *et al.* 2013; Guéguinou *et al.* 2016). Such an SK3–ORAI complex is crucial for the migratory function of breast and prostate cancer cells and has been found in bone metastasis (Chantome *et al.* 2013). Similarly, colon cancer cell migration is dependent on SOCE through the SK3–TRPC1–ORAI1 channel complex (Guéguinou *et al.* 2016).

Invasiveness and invadopodia formation

The invasiveness of cancer cells is due to their ability to degrade ECM and migrate into neighbouring connective tissues as well as the lymph- and bloodstreams. There, cancer cells spread throughout the organism and give rise to secondary tumour outbursts, metastases. Consequently, the understanding and hence prevention of the process of cancer cell invasion would remarkably improve the survival rate of cancer patients. Cancer cell invasion is achieved due to special structures – invadopodia, which are dynamic actin-enriched cell protrusions with proteolytic activity. Typically, the invadopodia formation process can be differentiated into the following steps: initiation, assembly and maturation (Fig. 3) (Jacob *et al.* 2015). The assembly of invadopodia is initiated in response to the focal generation of phosphatidylinositol-3,4-bisphosphate and the activation of the non-receptor tyrosine kinase Src (Mader *et al.* 2011; Pan *et al.* 2011; Yamaguchi *et al.* 2011). The matured invadopodia recruit proteolytic enzymes, such as membrane type 1 (MT1)-matrix metalloproteinase (MMP), MMP2 and MMP9, to facilitate the focal degradation of the extracellular matrix and allow cell invasion (Beaty *et al.* 2013).

Intriguingly, a particular pattern of Ca^{2+} signalling, Ca^{2+} oscillations, has been revealed as a predisposing factor for invadopodia formation and activity (Fig. 3) (Sun *et al.* 2014). For example, Ca^{2+} oscillations mediated through STIM1 and ORAI1 channels have been reported to activate Src kinase and hence facilitate the assembly of invadopodial precursors in melanoma cells (Sun *et al.* 2014). The proteolytic activity of invadopodia is predetermined by the incorporation of MMP-containing endocytic vesicles into the plasma membrane at the ECM degradation sites and can also be linked to Ca^{2+} signalling machinery (Bravo-Cordero *et al.* 2007). Indeed, the inhibition of SOCE-abrogated fusion of MMP-containing vesicles with the plasma membrane results in a constrained ECM degradation (Sun *et al.* 2014). Moreover, constitutively active TRPV2 engenders

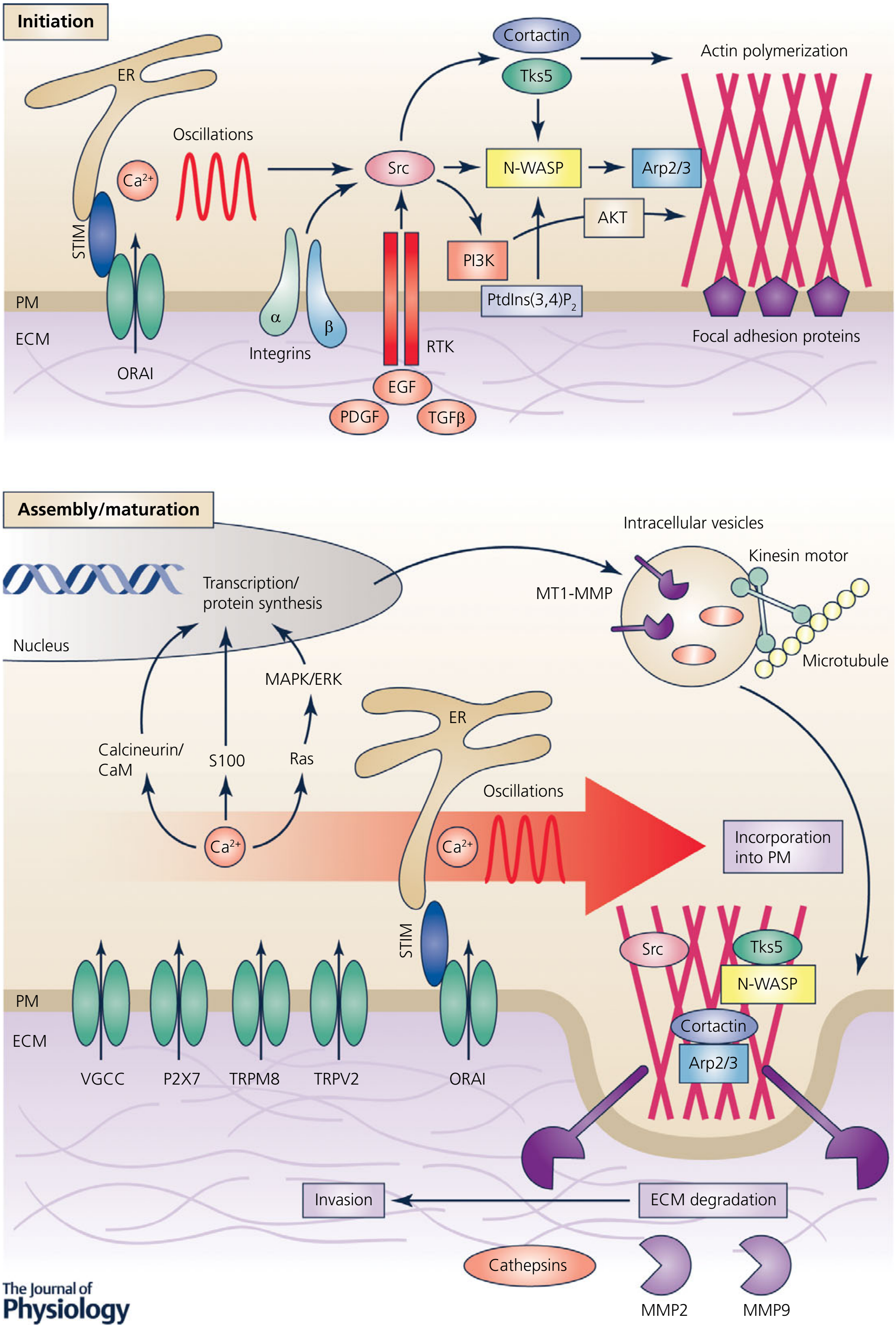


Figure 3. Ca^{2+} oscillations are required for the initiation of the invadopodia formation process, whereas Ca^{2+} influx activates the focal degradation of the extracellular matrix (ECM), in particular through upregulation of the proteolytic enzymes like matrix metalloproteinases (MMPs) and cathepsins

The most studied Ca^{2+}-permeable channels, which are associated with invasiveness, are indicated. PI3K, phosphoinositide 3-kinase; PM, plasma membrane; PtdIns(3,4)P_2, phosphatidylinositol-3,4-bisphosphate. N-WASP, neural Wiscott-Aldrich syndrome protein; PDGF, platelet-derived growth factor; RTK, receptor tyrosine kinase.

an intracellular Ca^{2+} increase and has been associated with an upregulation of MMP9 and the invasive potential of prostate cancer cells (Monet *et al.* 2010). In oral squamous carcinoma, TRPM8 activity directly correlates with MMP9 activity and the metastatic potential of cells (Okamoto *et al.* 2012). The downregulation of MMP9 might also be achieved after the inhibition of VGCCs (Kato *et al.* 2007). Furthermore, in the highly metastatic MDA-MB-435 human breast cancer cell line, the activity of the ATP-gated Ca^{2+}-permeable P2X7 receptor increases invasion by the release of gelatinolytic cysteine cathepsins (Jelassi *et al.* 2011). Therefore, in invadopodia, Ca^{2+} influx is required for the focal degradation of ECM, in particular through the upregulation of proteolytic enzymes like MMPs and cathepsins, whereas Ca^{2+} oscillations are required for the initiation of the invadopodia formation process (Fig. 3).

Induction of local angiogenesis

The first mechanical and functional interface between blood and tissues is blood vessels. Thus, in order to sustain metastatic dissemination, as well as providing sufficient metabolic support, tumour neovascularization is required. Indeed, tumour cells enable the 'activation' of the nearby endothelial cells (ECs) due to the secretion of specific molecules, such as vascular endothelial growth factor (VEGF). Hence, the complex and multistep process of angiogenesis is achieved due to the proliferation, migration, differentiation and stabilization of such tumour-derived ECs (TECs) in a new circulatory network (Carmeliet, 2005; Folkman, 2006).

It should be noted that in tumours of various origins, Ca^{2+} signalling has been shown to regulate the release of VEGF and hence modulate angiogenesis (Fig. 4). For example, plasma membrane Cav3.2 and ER-residing STIM1 proteins have been shown to promote angiogenesis *in vivo* due to the stimulation of VEGF secretion in both prostate and cervical cancers (Chen *et al.* 2011; Warnier *et al.* 2015). Moreover, the importance of TRPC6 as a modulator of angiogenic potential has been revealed in glioma cells (Chigurupati *et al.* 2010). According to this study, the number of EC branch points decreases after growing in a conditioned medium harvested from glioma cells, where hypoxia-induced TRPC6 overexpression and nuclear factor of activated T-cells (NFAT) activation are inhibited (Chigurupati *et al.* 2010). Of note, methyl syringate, which is suggested to be a highly specific and selective agonist of transient receptor potential ankyrin channel 1 (TRPA1) suppresses the hypoxia-induced migration, invasion and secretion of VEGF in human lung epithelial cells (Park *et al.* 2016).

Importantly, during tumour neovascularization, the remodelling of Ca^{2+} machinery has been associated not only with the angiogenic potential of cancer cells, but also with the distinct functions of the 'activated' endothelium cells (Fig. 4). Indeed, proangiogenic Ca^{2+} signals and their related pathways are significantly altered in TECs, compared with normal ECs (Fiorio Pla & Munaron, 2014). As an example, Ca^{2+} signals mediated by specific factors like VEGF and ATP and intracellular messengers such as arachidonic acid (AA), nitric oxide, or hydrogen sulfide and cyclic AMP are involved in pro-migratory effects in TEC, but not in normal ECs (Fiorio Pla *et al.* 2008, 2010, 2012*b*; Pupo *et al.* 2011; Avanzato *et al.* 2016).

Moreover, the role of intracellular Ca^{2+} increase has been investigated at length in endothelium (Fiorio Pla & Munaron, 2014). Both pro- and antiangiogenic molecules can induce an intracellular Ca^{2+} increase, often leading to different biological effects. For instance, Ca^{2+} entry triggered by VEGF, as well as by other proangiogenic factors, is often associated with an increase in vessel permeability, EC survival/proliferation, migration and *in vitro* tubulogenesis (Dragoni *et al.* 2011, 2015; Li *et al.* 2011). These outcomes can be achieved by the activation of a distinct intracellular mechanism, such as SOCE via ORAI and TRPC1 channels (Mehta *et al.* 2003; Paria *et al.* 2004; Jho *et al.* 2005; Abdullaev *et al.* 2008; Dragoni *et al.* 2011; Li *et al.* 2011; Fiorio Pla & Munaron, 2014), non-SOCE mechanisms via TRPC6 channels (Cheng *et al.* 2006; Hamdollah Zadeh *et al.* 2008), the specific engagement of the two-pore channel TPC2 subtype on acidic intracellular Ca^{2+} stores, resulting in Ca^{2+} release and angiogenic responses (Favia *et al.* 2014), or by reverse mode activation of NCX (Fig. 4) (Andrikopoulos *et al.* 2011). Of note, in a recent study VEGF-mediated Ca^{2+} signalling in individual endothelial cells has been investigated and shown to correlate with both stochastic and deterministic response characteristics to the selection of phenotype-associated angiogenesis. In particular, altering the amount of VEGF signalling in endothelial cells by stimulating them with different VEGF concentrations triggered distinct and mutually exclusive dynamic Ca^{2+} signalling responses, which correlated with different cellular behaviours such as cell proliferation (monitored by NFAT nuclear translocation) or cell migration (involving MLCK) (Noren *et al.* 2016). The *in vivo* role of Ca^{2+} signals has been recently studied in zebrafish, during angiogenic input by means of high-speed, three-dimensional time-lapse imaging to describe intracellular Ca^{2+} dynamics in ECs at single-cell resolution (Yokota *et al.* 2015; Noren *et al.* 2016). It may be noted that TRP Ca^{2+}-permeable channels have profound effects on the control of different steps of tumour angiogenesis. Besides their role in the VEGF-mediated Ca^{2+} signals previously described, several data clearly show their involvement Ca^{2+}-mediated signal transduction with a prominent roles in tumour angiogenesis. In this context, TRPV4 is an emerging player in angiogenesis as on ECs it acts as a mechano-sensor during changes in cell morphology, cell swelling and shear stress. TRPV4 plays a significant role in endothelial migration, (Fiorio Pla *et al.* 2012*b*) displaying a marked increase in ECs derived from human breast carcinomas, as compared with 'normal' ECs, leading to a greater Ca^{2+} entry that in turn activates migration in TECs (Fig. 4) (Fiorio Pla *et al.* 2012*b*). Moreover, TRPV4 has recently been described as an important player in tumour vasculature normalization, thereby potentially improving cancer therapies (Adapala *et al.* 2015; Thoppil *et al.* 2015, 2016). In addition, TRPM2 has been recently identified as mediating an H_2O_2-dependent increase in macrovascular pulmonary EC permeability (Fig. 4) (Hecquet *et al.* 2008; Mittal *et al.* 2015). TRPM7 inhibits human umbilical vein endothelial cell proliferation and migration, whereas its functions in human mammary epithelial cells seem to be the opposite (Fig. 4) (Inoue & Xiong, 2009; Baldoli & Maier, 2012; Baldoli *et al.* 2013; Zeng *et al.* 2015). Recently, TRPA1 has been found to have a role in the vasodilatation of cerebral arteries, via an increase in Ca^{2+} influx generated by the detection of reactive oxygen species, a process that requires the peroxidation of membrane lipids (Sullivan *et al.* 2015). Similarly, TRPV2 has been shown to be

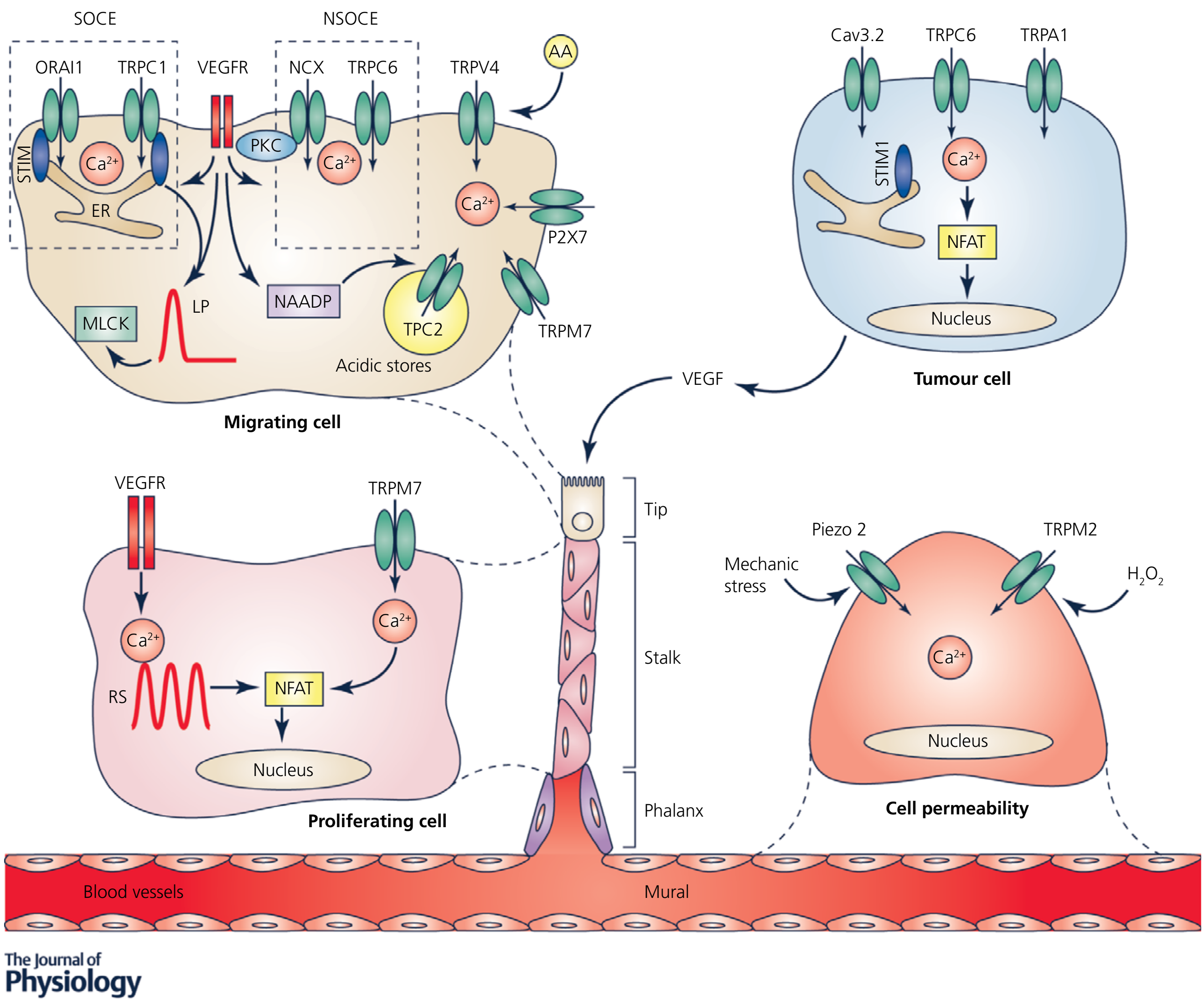


Figure 4. Induction of local angiogenesis by Ca^{2+} signalling remodelling
In tumour cells Ca^{2+} signals regulate the secretion of proangiogenic stimuli, like vascular endothelial growth factor (VEGF). A newly formed vessel can be differentiated into the following structures: tip – represented by the migrating edge of the vessel; stalk – mostly proliferating part of the vessel; phalanx – tightly apposed, regularly ordered ECs that provide perfusion and oxygenation; mural – functional preformed ECs. Interestingly, VEGF- and ATP-mediated Ca^{2+} signals provide proangiogenic effects specifically on tumour-derived tissue and not on healthy ECs. The most studied Ca^{2+}-permeable channels, which are associated with local angiogenesis, are indicated. NAADP, nicotinic acid adenine dinucleotide phosphate. LP, long persistent; RS, repeated spikes; NSOCE, non-store-operated Ca^{2+} entry.

expressed in aorta endothelium, but no clear functional data have been reported (Earley, 2011).

Finally, the emerging family of mechanosensitive Piezo channels has recently been described in vascular endothelial cells: Piezo2 knockdown is involved in glioma angiogenesis, both *in vitro* and *in vivo* by promoting abnormal intracellular Ca^{2+}, Wnt11/β-catenin signalling reduction, leading to altered angiogenic activity of endothelial cells (Fig. 4) (Yang *et al.* 2016).

In conclusion, due to its multifaceted role in the control of endothelium homeostasis, Ca^{2+} machinery is a potential molecular target for strategies against tumour neovascularization.

Conclusions

A remodelling of Ca^{2+} signalling plays an important role during tumourigenesis. Interestingly, there are some specific channel patterns through which such Ca^{2+} signals occur, at the different stages of cancer progression. This could be partially explained by the specificity of Ca^{2+} flux, its compartment localization and the proximity of downstream Ca^{2+}-dependent targets. Furthermore, some ion channels represent multimodal activity and are characterized not only as Ca^{2+}-permeable pore proteins, but also as possessing other functional domains. For example, the C-terminal end of TRPM7 is constituted by a serine/threonine protein kinase domain and hence, due to the phosphorylation of cytoskeletal components, regulates cellular migration (Clark *et al.* 2008).

Importantly, plasma membrane Ca^{2+} channels are easily and directly accessible via the bloodstream. Therefore, they are potential targets for a variety of therapeutic strategies, such as their regulation on a transcriptional and translational level, their trafficking to the plasma membrane or their stabilization at the plasma membrane (Gkika & Prevarskaya, 2009; Fiorio Pla *et al.* 2012*a*; Bernardini *et al.* 2015; Earley & Brayden, 2015).

References

Abdullaev IF, Bisaillon JM, Potier M, Gonzalez JC, Motiani RK & Trebak M (2008). Stim1 and Orai1 mediate CRAC currents and store-operated calcium entry important for endothelial cell proliferation. *Circ Res* **103**, 1289–1299.

Adapala RK, Thoppil RJ, Ghosh K, Cappelli HC, Dudley AC, Paruchuri S, Keshamouni V, Klagsbrun M, Meszaros JG, Chilian WM, Ingber DE & Thodeti CK (2015). Activation of mechanosensitive ion channel TRPV4 normalizes tumor vasculature and improves cancer therapy. *Oncogene* **35**, 314–322.

Andrikopoulos P, Baba A, Matsuda T, Djamgoz MBA, Yaqoob MM & Eccles SA (2011). Ca^{2+} influx through reverse mode Na^+/Ca^{2+} exchange is critical for vascular endothelial growth factor-mediated extracellular signal-regulated kinase (ERK) 1/2 activation and angiogenic functions of human endothelial cells. *J Biol Chem* **286**, 37919–37931.

Avanzato D, Genova T, Fiorio Pla A, Bernardini M, Bianco S, Bussolati B, Mancardi D, Giraudo E, Maione F, Cassoni P, Castellano I & Munaron L (2016). Activation of P2X7 and P2Y11 purinergic receptors inhibits migration and normalizes tumor-derived endothelial cells via cAMP signaling. *Sci Rep* **6**, 32602.

Azimi I, Beilby H, Davis FM, Marcial DL, Kenny PA, Thompson EW, Roberts-Thomson SJ & Monteith GR (2016). Altered purinergic receptor-Ca^{2+} signaling associated with hypoxia-induced epithelial-mesenchymal transition in breast cancer cells. *Mol Oncol* **10**, 166–178.

Baldoli E, Castiglioni S & Maier JAM (2013). Regulation and function of TRPM7 in human endothelial cells: TRPM7 as a potential novel regulator of endothelial function. *PLoS One* **8**, e59891.

Baldoli E & Maier JAM (2012). Silencing TRPM7 mimics the effects of magnesium deficiency in human microvascular endothelial cells. *Angiogenesis* **15**, 47–57.

Beaty BT, Sharma VP, Bravo-Cordero JJ, Simpson MA, Eddy RJ, Koleske AJ & Condeelis J (2013). β1 integrin regulates Arg to promote invadopodial maturation and matrix degradation. *Mol Biol Cell* **24**, 1661–1675.

Bernardini M, Fiorio Pla A, Prevarskaya N & Gkika D (2015). Human transient receptor potential (TRP) channel expression profiling in carcinogenesis. *Int J Dev Biol* **59**, 399–406.

Berridge MJ, Lipp P & Bootman MD (2000). The versatility and universality of calcium signalling. *Nat Rev Mol Cell Biol* **1**, 11–21.

Bidaux G, Borowiec A-S, Dubois C, Delcourt P, Schulz C, Vanden Abeele F, Lepage G, Desruelles E, Bokhobza A, Dewailly E, Slomianny C, Roudbaraki M, Héliot L, Bonnal J-L, Mauroy B, Mariot P, Lemonnier L & Prevarskaya N (2016). Targeting of short TRPM8 isoforms induces 4TM-TRPM8-dependent apoptosis in prostate cancer cells. *Oncotarget* **7**, 29063–29080.

Bomben VC, Turner KL, Barclay TTC & Sontheimer H (2011). Transient receptor potential canonical channels are essential for chemotactic migration of human malignant gliomas. *J Cell Physiol* **226**, 1879–1888.

Bravo-Cordero JJ, Marrero-Diaz R, Megías D, Genís L, García-Grande A, García MA, Arroyo AG & Montoya MC (2007). MT1-MMP proinvasive activity is regulated by a novel Rab8-dependent exocytic pathway. *EMBO J* **26**, 1499–1510.

Bresnick AR, Weber DJ & Zimmer DB (2015). S100 proteins in cancer. *Nat Rev Cancer* **15**, 96–109.

Brundage RA, Fogarty KE, Tuft RA & Fay FS (1991). Calcium gradients underlying polarization and chemotaxis of eosinophils. *Science* **254**, 703–706.

Carmeliet P (2005). Angiogenesis in life, disease and medicine. *Nature* **438**, 932–936.

Chantome A, Potier-Cartereau M, Clarysse L, Fromont G, Marionneau-Lambot S, Guéguinou M, Pagès JC, Collin C, Oullier T, Girault A, Arbion F, Haelters JP, Jaffrès PA, Pinault M, Besson P, Joulin V, Bougnoux P & Vandier C (2013). Pivotal role of the lipid raft SK3-orai1 complex in human cancer cell migration and bone metastases. *Cancer Res* **73**, 4852–4861.

Chen Y-F, Chen Y-T, Chiu W-T & Shen M-R (2013). Remodeling of calcium signaling in tumor progression. *J Biomed Sci* **20**, 23.

Chen Y-F, Chiu W-T, Chen Y-T, Lin P-Y, Huang H-J, Chou C-Y, Chang H-C, Tang M-J & Shen M-R (2011). Calcium store sensor stromal-interaction molecule 1-dependent signaling plays an important role in cervical cancer growth, migration, and angiogenesis. *Proc Natl Acad Sci USA* **108**, 15225–15230.

Cheng H-W, James AF, Foster RR, Hancox JC & Bates DO (2006). VEGF activates receptor-operated cation channels in human microvascular endothelial cells. *Arterioscler Thromb Vasc Biol* **26**, 1768–1776.

Chigurupati S, Venkataraman R, Barrera D, Naganathan A, Madan M, Paul L, Pattisapu JV, Kyriazis GA, Sugaya K, Bushnev S, Lathia JD, Rich JN & Chan SL (2010). Receptor channel TRPC6 is a key mediator of Notch-driven glioblastoma growth and invasiveness. *Cancer Res* **70**, 418–427.

Clark K, Langeslag M, Figdor CG & van Leeuwen FN (2007). Myosin II and mechanotransduction: a balancing act. *Trends Cell Biol* **17**, 178–186.

Clark K, Middelbeek J, Lasonder E, Dulyaninova NG, Morrice NA, Ryazanov AG, Bresnick AR, Figdor CG & van Leeuwen FN (2008). TRPM7 regulates myosin IIA filament stability and protein localization by heavy chain phosphorylation. *J Mol Biol* **378**, 790–803.

Davis F, Azimi I, Faville R, Peters A, Jalink K, Putney J, Goodhill G, Thompson E, Roberts-Thomson S & Monteith G (2013). Induction of epithelial–mesenchymal transition (EMT) in breast cancer cells is calcium signal dependent. *Oncogene* **33**, 2307–2316.

Davis FM, Kenny PA, Soo ETL, van Denderen BJW, Thompson EW, Cabot PJ, Parat MO, Roberts-Thomson SJ & Monteith GR (2011). Remodeling of purinergic receptor-mediated Ca^{2+} signaling as a consequence of EGF-induced epithelial-mesenchymal transition in breast cancer cells. *PLoS One* **6**, e23464.

Davis FM, Peters AA, Grice DM, Cabot PJ, Parat MO, Roberts-Thomson SJ & Monteith GR (2012). Non-stimulated, agonist-stimulated and store-operated Ca^{2+} influx in MDA-MB-468 breast cancer cells and the effect of EGF-induced EMT on calcium entry. *PLoS One* **7**, e36923.

Diepenbruck M & Christofori G (2016). Epithelial-mesenchymal transition (EMT) and metastasis: yes, no, maybe? *Curr Opin Cell Biol* **43**, 7–13.

Dragoni S, Pedrazzoli P, Rosti V, Tanzi F & Moccia F (2011). Vascular endothelial growth factor stimulates endothelial colony forming cells proliferation and tubulogenesis by inducing oscillations in intracellular Ca^{2+} concentration. *Stem Cells* **29**, 1898–1907.

Dragoni S, Reforgiato M, Zuccolo E, Poletto V, Lodola F, Ruffinatti FA, Bonetti E, Guerra G, Barosi G, Rosti V & Moccia F (2015). Dysregulation of VEGF-induced proangiogenic Ca^{2+} oscillations in primary myelofibrosis-derived endothelial colony-forming cells. *Exp Hematol* **43**, 1019–1030.

Earley S (2011). Vanilloid and melastatin transient receptor potential channels in vascular smooth muscle. *Microcirculation* **17**, 237–249.

Earley S & Brayden JE (2015). Transient receptor potential channels in the vasculature. *Physiol Rev* **95**, 645–690.

Evans JH, Falke JJ & Mcintosh JR (2007). Ca^{2+} influx is an essential component of the positive-feedback loop that maintains leading-edge structure and activity in macrophages. *Proc Natl Acad Sci USA* **104**, 16176–16181.

Favia A, Desideri M, Gambara G, Ruas M, Esposito B, Del Bufalo D, Parrington J, Ziparo E, Palombi F, Galione A & Filippini A (2014). VEGF-induced neoangiogenesis is mediated by NAADP and two-pore channel-2-dependent Ca^{2+} signaling. *Proc Natl Acad Sci USA* **111**, E4706–E4715.

Fiorio Pla A & Munaron L (2014). Functional properties of ion channels and transporters in tumour vascularization. *Philos Trans R Soc Lond B Biol Sci* **369**, 20130103.

Fiorio Pla A, Avanzato D, Munaron L & Ambudkar IS (2012*a*). Ion channels and transporters in cancer. 6. Vascularizing the tumor: TRP channels as molecular targets. *Am J Physiol Cell Physiol* **302**, C9–C15.

Fiorio Pla A, Genova T, Pupo E, Tomatis C, Genazzani A, Zaninetti R & Munaron L (2010). Multiple roles of protein kinase A in arachidonic acid-mediated Ca^{2+} entry and tumor-derived human endothelial cell migration. *Mol Cancer Res* **8**, 1466–1476.

Fiorio Pla A & Gkika D (2013). Emerging role of TRP channels in cell migration: from tumor vascularization to metastasis. *Front Physiol* **4**, 311.

Fiorio Pla A, Grange C, Antoniotti S, Tomatis C, Merlino A, Bussolati B & Munaron L (2008). Arachidonic acid-induced Ca^{2+} entry is involved in early steps of tumor angiogenesis. *Mol Cancer Res* **6**, 535–545.

Fiorio Pla A, Kondratska K & Prevarskaya N (2016). STIM and ORAI proteins: crucial roles in hallmarks of cancer. *Am J Physiol Cell Physiol* **310**, C509–C519.

Fiorio Pla A, Ong HL, Cheng KT, Brossa A, Bussolati B, Lockwich T, Paria B, Munaron L & Ambudkar IS (2012*b*). TRPV4 mediates tumor-derived endothelial cell migration via arachidonic acid-activated actin remodeling. *Oncogene* **31**, 200–212.

Folkman J (2006). Angiogenesis. *Annu Rev Med* **57**, 1–18.

Franco SJ & Huttenlocher A (2005). Regulating cell migration: calpains make the cut. *J Cell Sci* **118**, 3829–3838.

Gambade A, Zreika S, Guéguinou M, Chourpa I, Fromont G, Bouchet AM, Burlaud-Gaillard J, Potier-Cartereau M, Roger S, Aucagne V, Chevalier S, Vandier C, Goupille C & Weber G (2016). Activation of TRPV2 and BKCa channels by the LL-37 enantiomers stimulates calcium entry and migration of cancer cells. *Oncotarget* **7**, 23785–23800.

Gao H, Chen X, Du X, Guan B, Liu Y & Zhang H (2011). EGF enhances the migration of cancer cells by up-regulation of TRPM7. *Cell Calcium* **50**, 559–568.

Gardel ML, Schneider IC, Aratyn-Schaus Y & Waterman CM (2010). Mechanical integration of actin and adhesion dynamics in cell migration. *Annu Rev Cell Dev Biol* **26**, 315–333.

Gkika D, Flourakis M, Lemonnier L & Prevarskaya N (2010). PSA reduces prostate cancer cell motility by stimulating TRPM8 activity and plasma membrane expression. *Oncogene* **29**, 4611–4616.

Gkika D, Lemonnier L, Shapovalov G, Gordienko D, Poux C, Bernardini M, Bokhobza A, Bidaux G, Degerny C, Verreman K, Guarmit B, Benahmed M, de Launoit Y, Bindels RJM, Fiorio Pla A & Prevarskaya N (2015). TRP channel-associated factors are a novel protein family that regulates TRPM8 trafficking and activity. *J Cell Biol* **208**, 89–107.

Gkika D & Prevarskaya N (2009). Molecular mechanisms of TRP regulation in tumor growth and metastasis. *Biochim Biophys Acta* **1793**, 953–958.

Gross SR, Sin CGT, Barraclough R & Rudland PS (2014). Joining S100 proteins and migration: For better or for worse, in sickness and in health. *Cell Mol Life Sci* **71**, 1551–1579.

Guéguinou M, Harnois T, Crottes D, Uguen A, Deliot N, Gambade A, Chantôme A, Haelters JP, Jaffrès PA, Jourdan ML, Weber G, Soriani O, Bougnoux P, Mignen O, Bourmeyster N, Constantin B, Lecomte T, Vandier C & Potier-Cartereau M (2016). SK3/TRPC1/Orai1 complex regulates SOCE-dependent colon cancer cell migration: a novel opportunity to modulate anti-EGFR mAb action by the alkyl-lipid Ohmline. *Oncotarget* **7**, 36168–36184.

Hamdollah Zadeh MA, Glass CA, Magnussen A, Hancox JC & Bates DO (2008). VEGF-mediated elevated intracellular calcium and angiogenesis in human microvascular endothelial cells in vitro are inhibited by dominant negative TRPC6. *Microcirculation* **15**, 605–614.

Hanahan D & Weinberg RA (2011). Hallmarks of cancer: the next generation. *Cell* **144**, 646–674.

Hao J, Bao X, Jin B, Wang X, Mao Z, Li X, Wei L, Shen D & Wang J-L (2015). Ca^{2+} channel subunit a 1D promotes proliferation and migration of endometrial cancer cells mediated by 17b-estradiol via the G protein-coupled estrogen receptor. *FASEB J* **29**, 2883–2893.

Hecquet CM, Ahmmed GU, Vogel SM & Malik AB (2008). Role of TRPM2 channel in mediating H_2O_2-induced Ca^{2+} entry and endothelial hyperpermeability. *Circ Res* **102**, 347–355.

Hu J, Qin K, Zhang Y, Gong J, Li N, Lv D, Xiang R & Tan X (2011). Downregulation of transcription factor Oct4 induces an epithelial-to-mesenchymal transition via enhancement of Ca^{2+} influx in breast cancer cells. *Biochem Biophys Res Commun* **411**, 786–791.

Huang Y, Chang S, I-Chen Harn H, Huang H, Lin H, Shen M, Tang M & Chiu W (2015). Mechanosensitive store- operated calcium entry regulates the formation of cell polarity. *J Cell Physiol* **230**, 2086–2097.

Inoue K & Xiong ZG (2009). Silencing TRPM7 promotes growth/proliferation and nitric oxide production of vascular endothelial cells via the ERK pathway. *Cardiovasc Res* **83**, 547–557.

Jacob A, Prekeris R, Kaverina I & Roger S (2015). The regulation of MMP targeting to invadopodia during cancer metastasis. *Front Cell Dev Biol* **3**, 4.

Jacquemet G, Baghirov H, Georgiadou M, Sihto H, Peuhu E, Cettour-Janet P, He T, Perälä M, Kronqvist P, Joensuu H & Ivaska J (2016). L-type calcium channels regulate filopodia stability and cancer cell invasion downstream of integrin signalling. *Nat Commun* **7**, 13297.

Jardin I & Rosado JA (2016). STIM and calcium channel complexes in cancer. *Biochim Biophys Acta* **1863**, 1418–1426.

Jelassi B, Chantoe A, Alcaraz-Peez F, Baroja-Mazo A, Cayuela M, Pelegrin P, Surprenant A & Roger S (2011). $P2X_7$ receptor activation enhances SK3 channels- and cystein cathepsin-dependent cancer cells invasiveness. *Oncogene* **30**, 2108–2122.

Jho D, Mehta D, Ahmmed G, Gao X-P, Tiruppathi C, Broman M & Malik AB (2005). Angiopoietin-1 opposes VEGF-induced increase in endothelial permeability by inhibiting TRPC1-dependent Ca^{2+} influx. *Circ Res* **96**, 1282–1290.

Kalluri R & Weinberg RA (2009). The basics of epithelial-mesenchymal transition. *J Clin Invest* **119**, 1420–1428.

Kato Y, Ozawa S, Tsukuda M, Kubota E, Miyazaki K, St-Pierre Y & Hata RI (2007). Acidic extracellular pH increases calcium influx-triggered phospholipase D activity along with acidic sphingomyelinase activation to induce matrix metalloproteinase-9 expression in mouse metastatic melanoma. *FEBS J* **274**, 3171–3183.

Lai W, Liu L, Zeng Y, Wu H, Xu H, Chen S & Chu Z (2013). KCNN4 channels participate in the EMT induced by PRL-3 in colorectal cancer. *Med Oncol* **30**, 566.

Lee WJ, Roberts-Thomson SJ & Monteith GR (2005). Plasma membrane calcium-ATPase 2 and 4 in human breast cancer cell lines. *Biochem Biophys Res Commun* **337**, 779–783.

Li J, Cubbon RM, Wilson LA, Amer MS, McKeown L, Hou B, Majeed Y, Tumova S, Seymour VAL, Taylor H, Stacey M, O'Regan D, Foster R, Porter KE, Kearney MT & Beech DJ (2011). Orai1 and CRAC channel dependence of VEGF-activated Ca^{2+} entry and endothelial tube formation. *Circ Res* **108**, 1190–1198.

Liou J, Kim ML, Won DH, Jones JT, Myers JW, Ferrell JE & Meyer T (2005). STIM is a Ca^{2+} sensor essential for Ca^{2+}-store- depletion-triggered Ca^{2+} influx. *Curr Biol* **15**, 1235–1241.

Liu J, Chen Y, Shuai S, Ding D, Li R & Luo R (2014). TRPM8 promotes aggressiveness of breast cancer cells by regulating EMT via activating AKT/GSK-3β pathway. *Tumor Biol* **35**, 8969–8977.

Lysechko TL, Cheung SMS & Ostergaard HL (2010). Regulation of the tyrosine kinase Pyk2 by calcium is through production of reactive oxygen species in cytotoxic T lymphocytes. *J Biol Chem* **285**, 31174–31184.

Mader CC, Oser M, Magalhaes MAO, Bravo-Cordero JJ, Condeelis J, Koleske AJ & Gil-Henn H (2011). An EGFR-Src-Arg-cortactin pathway mediates functional maturation of invadopodia and breast cancer cell invasion. *Cancer Res* **71**, 1730–1741.

Mayor R & Etienne-Manneville S (2016). The front and rear of collective cell migration. *Nat Rev Mol Cell Biol* **17**, 97–109.

Mehta D, Ahmmed GU, Paria BC, Holinstat M, Voyno-Yasenetskaya T, Tiruppathi C, Minshall RD & Malik AB (2003). RhoA interaction with inositol 1,4,5-trisphosphate receptor and transient receptor potential channel-1 regulates Ca^{2+} entry. Role in signaling increased endothelial permeability. *J Biol Chem* **278**, 33492–33500.

Mertens-Walker I, Bolitho C, Baxter RC & Marsh DJ (2010). Gonadotropin-induced ovarian cancer cell migration and proliferation require extracellular signal-regulated kinase 1/2 activation regulated by calcium and protein kinase Cd. *Endocr Relat Cancer* **17**, 335–349.

Middelbeek J, Kuipers AJ, Henneman L, Visser D, Eidhof I, Van Horssen R, Wieringa B, Canisius SV, Zwart W, Wessels LF, Sweep FCGJ, Bult P, Span PN, Van Leeuwen FN & Jalink K (2012). TRPM7 is required for breast tumor cell metastasis. *Cancer Res* **72**, 4250–4561.

Mittal M, Urao N, Hecquet CM, Zhang M, Sudhahar V, Gao X, Komarova Y, Ushio-Fukai M & Malik AB (2015). Novel role of reactive oxygen species-activated *trp* melastatin channel-2 in mediating angiogenesis and postischemic neovascularization significance. *Arterioscler Thromb Vasc Biol* **35**, 877–887.

Monet M, Lehen'kyi V, Gackiere F, Firlej V, Vandenberghe M, Roudbaraki M, Gkika D, Pourtier A, Bidaux G, Slomianny C, Delcourt P, Rassendren F, Bergerat JP, Ceraline J, Cabon F, Humez S & Prevarskaya N (2010). Role of cationic channel TRPV2 in promoting prostate cancer migration and progression to androgen resistance. *Cancer Res* **70**, 1225–1235.

Monteith GR, McAndrew D, Faddy HM & Roberts-Thomson SJ (2007). Calcium and cancer: targeting Ca^{2+} transport. *Nat Rev Cancer* **7**, 519–530.

Noren DP, Chou WH, Lee SH, Qutub AA, Warmflash A, Wagner DS, Popel AS & Levchenko A (2016). Endothelial cells decode VEGF-mediated Ca^{2+} signaling patterns to produce distinct functional responses. *Sci Signal* **9**, ra20.

Okamoto Y, Ohkubo T, Ikebe T & Yamazaki J (2012). Blockade of TRPM8 activity reduces the invasion potential of oral squamous carcinoma cell lines. *Int J Oncol* **40**, 1431–1440.

Oulidi A, Bokhobza A, Gkika D, Vanden Abeele F, Lehen'kyi V, Ouafik L, Mauroy B & Prevarskaya N (2013). TRPV2 mediates adrenomedullin stimulation of prostate and urothelial cancer cell adhesion, migration and invasion. *PLoS One* **8**, e64885.

Pan YR, Chen CL & Chen HC (2011). FAK is required for the assembly of podosome rosettes. *J Cell Biol* **195**, 113–129.

Paria BC, Vogel SM, Ahmmed GU, Alamgir S, Shroff J, Malik AB, Tiruppathi C & Biman C (2004). Tumor necrosis factor-induced TRPC1 expression amplifies store-operated Ca^{2+} influx and endothelial permeability. *Am J Physiol Lung Cell Mol Physiol* **287**, L1303–L1313.

Park J, Shim MK, Jin M, Rhyu MR & Lee YJ (2016). Methyl syringate, a TRPA1 agonist represses hypoxia-induced cyclooxygenase-2 in lung cancer cells. *Phytomedicine* **23**, 324–329.

Parsons JT, Rick Horwitz A & Schwartz MA (2010). Cell adhesion: integrating cytoskeletal dynamics and cellular tension. *Nat Rev Mol Cell Biol* **11**, 633–643.

Peng M, Wang Z, Yang Z, Tao L, Liu Q, Yi L & Wang X (2015). Overexpression of short TRPM8 variantα promotes cell migration and invasion, and decreases starvation-induced apoptosis in prostate cancer LNCaP cells. *Oncol Lett* **10**, 1378–1384.

Pérez-Gordones MC, Lugo MR, Winkler M, Cervino V & Benaim G (2009). Diacylglycerol regulates the plasma membrane calcium pump from human erythrocytes by direct interaction. *Arch Biochem Biophys* **489**, 55–61.

Prevarskaya N, Skryma R & Shuba Y (2011). Calcium in tumour metastasis: new roles for known actors. *Nat Rev Cancer* **11**, 609–618.

Pupo E, Fiorio Pla A, Avanzato D, Moccia F, Avelino Cruz JE, Tanzi F, Merlino A, Mancardi D & Munaron L (2011). Hydrogen sulfide promotes calcium signals and migration in tumor-derived endothelial cells. *Free Radic Biol Med* **51**, 1765–1773.

Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, Safrina O, Kozak JA, Wagner SL, Cahalan MD, Veliçelebi G & Stauderman KA (2005). STIM1, an essential and conserved component of store-operated Ca^{2+} channel function. *J Cell Biol* **169**, 435–445.

Selitrennik M & Lev S (2015). PYK2 integrates growth factor and cytokine receptors signaling and potentiates breast cancer invasion via a positive feedback loop. *Oncotarget* **6**, 22214–22226.

Stewart TA, Azimi I, Thompson EW, Roberts-Thomson SJ & Monteith GR (2015). A role for calcium in the regulation of ATP-binding cassette, sub-family C, member 3 (ABCC3) gene expression in a model of epidermal growth factor-mediated breast cancer epithelial-mesenchymal transition. *Biochem Biophys Res Commun* **458**, 509–514.

Su LT, Agapito MA, Li M, Simonson WTN, Huttenlocher A, Habas R, Yue L & Runnels LW (2006). TRPM7 regulates cell adhesion by controlling the calcium-dependent protease calpain. *J Biol Chem* **281**, 11260–11270.

Sullivan MN, Gonzales AL, Pires PW, Bruhl A, Leo MD, Li W, Oulidi A, Boop FA, Feng Y, Jaggar JH, Welsh DG & Earley S (2015). Localized TRPA1 channel Ca^{2+} signals stimulated by reactive oxygen species promote cerebral artery dilation. *Sci Signal* **8**, ra2.

Sun J, Lu F, He H, Shen J, Messina J, Mathew R, Wang D, Sarnaik AA, Chang WC, Kim M, Cheng H & Yang S (2014). STIM1- and Orai1-mediated Ca^{2+} oscillation orchestrates invadopodium formation and melanoma invasion. *J Cell Biol* **207**, 535–548.

Thoppil RJ, Adapala RK, Cappelli HC, Kondeti V, Dudley AC, Gary Meszaros J, Paruchuri S & Thodeti CK (2015). TRPV4 channel activation selectively inhibits tumor endothelial cell proliferation. *Sci Rep* **5**, 14257.

Thoppil RJ, Cappelli HC, Adapala RK, Kanugula AK, Paruchuri S & Thodeti CK (2016). TRPV4 channels regulate tumor angiogenesis via modulation of Rho/Rho kinase pathway. *Oncotarget* **7**, 25849–25861.

Tsai FC & Meyer T (2012). Ca^{2+} pulses control local cycles of lamellipodia retraction and adhesion along the front of migrating cells. *Curr Biol* **22**, 837–842.

Tsai F-C, Seki A, Yang HW, Hayer A, Carrasco S, Malmersjö S & Meyer T (2014). A polarized Ca^{2+}, diacylglycerol and STIM1 signalling system regulates directed cell migration. *Nat Cell Biol* **16**, 133–144.

Visser D, Middelbeek J, Van Leeuwen FN & Jalink K (2014). Function and regulation of the channel-kinase TRPM7 in health and disease. *Eur J Cell Biol* **93**, 455–465.

Warnier M, Roudbaraki M, Derouiche S, Delcourt P, Bokhobza A, Prevarskaya N & Mariot P (2015). CACNA2D2 promotes tumorigenesis by stimulating cell proliferation and angiogenesis. *Oncogene* **34**, 5383–5394.

Wei C, Wang X, Chen M, Ouyang K, Song L-S & Cheng H (2009). Calcium flickers steer cell migration. *Nature* **457**, 901–905.

Wen L, Liang C, Chen E, Chen W, Liang F, Zhi X, Wei T, Xue F, Li G, Yang Q, Gong W, Feng X, Bai X & Liang T (2016). Regulation of Multi-drug Resistance in hepatocellular carcinoma cells is TRPC6/Calcium Dependent *Sci Rep* **6**, 23269.

Yamaguchi H, Yoshida S, Muroi E, Yoshida N, Kawamura M, Kouchi Z, Nakamura Y, Sakai R & Fukami K (2011). Phosphoinositide 3-kinase signaling pathway mediated by p110α regulates invadopodia formation. *J Cell Biol* **193**, 1275–1288.

Yang H, Liu C, Zhou R-M, Yao J, Li X-M, Shen Y, Cheng H, Yuan J, Yan B & Jiang Q (2016). Piezo2 protein: a novel regulator of tumor angiogenesis and hyperpermeability. *Oncotarget* **7**, 44630–44643.

Yokota Y, Nakajima H, Wakayama Y, Muto A, Kawakami K, Fukuhara S & Mochizuki N (2015). Endothelial Ca^{2+} oscillations reflect VEGFR signaling-regulated angiogenic capacity in vivo. *Elife* **4**, e08817.

Zeng Z, Inoue K, Sun H, Leng T, Feng X, Zhu L & Xiong Z-G (2015). TRPM7 regulates vascular endothelial cell adhesion and tube formation. *Am J Physiol Cell Physiol* **308**, C308–C318.

Additional information

Competing interests

The authors have no conflicts of interest to declare.

Author contributions

O.I. and A.F.P. collected information, conceived the concept, prepared figures and drafted the manuscript. N.P. was involved in supervising and editing the manuscript. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding

O.I. was funded by the ‘la Ligue Nationale Contre le Cancer’, France (GB/MA/CD 11813) and Laboratory of Excellence in Ion Channel Science and Therapeutics. The work of A.F.P. and N.P. was funded by Institut National du Cancer. The research in the authors’ laboratory is supported by INSERM, la Ligue Nationale Contre le Cancer, Le Ministere de l’Education Nationale, the Region Nord/Pas-de-Calais, la Fondation de Recherche Medicale, and l’Association pour la Recherche sur le Cancer.