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Abstract

Intracellular calcium (Ca^{2+}) signals are key regulators of multiple cellular functions: both healthy and physiopathological. It is therefore not surprising that several cancers present a strong deregulation of Ca^{2+} homeostasis. Among the different hallmarks of cancer disease particular role is played by metastasis which has a critical impact on cancer patients' outcome. Importantly, Ca^{2+} signaling has been reported to control multiple aspects of the adaptive metastatic cancer cell behavior, including epithelial-mesenchymal transition, cell migration, local invasion and induction of angiogenesis (Fig. 1). In this context Ca^{2+} signaling is considered being a substantial intracellular tool that regulates dynamicity and complexity of the metastatic cascade. In the present study we review spatial and temporal organization of Ca^{2+} fluxes as well as the molecular mechanisms involved in metastasis analyzing the different key steps regulating initial tumor spread.

Abbreviations

Calcium, Ca^{2+}; sodium, Na^{+}; epithelial-mesenchymal transition, EMT; extracellular matrix, ECM; plasma membrane ATPase, PMCA; Na^{+}-Ca^{2+} exchanger, NCX; endoplasmic reticulum, ER; sarco/endoplasmic reticulum Ca^{2+}-ATPase, SERCA; epidermal growth factor, EGF; store-operated Ca^{2+} entry, SOCE; transient receptor potential channel, TRP channel; transforming growth factor β1, TGF-β1; Ca^{2+} release-activated Ca^{2+} channel protein, ORAI; myosin II, myo II; Ca^{2+}-dependent myosin light chain kinase, MLCK; proline-rich tyrosine kinase 2, Pyk2; stromal interaction molecule, STIM; phospholipase C, PLC; voltage-gated Ca^{2+} channels, VGCCs; Ca^{2+} activated potassium channels, KCa; matrix metalloproteinase, MMP; endothelial cells, ECs; vascular endothelial growth factor, VEGF; arachidonic acid, AA; Nitric Oxyde, NO; sulfidric acid, H_2S; cyclic AMP, cAMP; two-pore channel, TPC; human umbilical vein endothelial cell (HUVEC); reactive oxygen species, ROS.

Introduction

Calcium (Ca^{2+}) is an ubiquitous second messenger which is involved in the tuning of multiple fundamental cellular functions (Berridge MJ, Lipp P, 2000). Due to its multifaceted roles, it is not therefore surprising that deregulated Ca^{2+} homeostasis has been observed in various disorders, including tumorigenesis (Monteith GR, McAndrew D, Faddy HM, 2007; Prevarskaya *et al.*, 2011). Among the different manifestations of cancer disease particular role is played by metastasis which has a critical impact on cancer patients' outcome (Hanahan & Weinberg, 2011). Tumor spread is highly regulated process that usually starts with loss of cell-cell contact and typical epithelial-mesenchymal transition (EMT) (Kalluri & Weinberg, 2009). During metastasis, cancer cells also acquire enhanced directional movement and activate molecular pathways that enable proteolysis of extracellular matrix (ECM) and local angiogenesis. As a result, cancer cells enter the body circulation systems and disseminate to the distinct sites of the organism. Importantly, Ca^{2+} signaling has been reported to control multiple aspects of the adaptive metastatic cancer cell behaviors, including EMT, migration, local angiogenesis induction and intravasation (Chen *et al.*, 2013). In this context is considered being a substantial intracellular tool that regulates 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 amplitude as well as frequency. As regarding the "ON" mechanisms, cytosolic Ca^{2+} can 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 release from Ca^{2+} containing organelles (e.g. endoplasmic reticulum) (Berridge MJ, Lipp P, 2000). In order to maintain low resting Ca^{2+} concentration, cells remove Ca^{2+} using energy-dependent mechanism such as plasma membrane ATPases (PMCA) or Na^{+}-Ca^{2+} exchanger (NCX); moreover Ca^{2+} is sequester intracellularly into Ca^{2+} containing organelles, primarily endoplasmic reticulum (ER), by meaning of mechanisms which requires either ATP hydrolysis (e.g. SERCA pump) or a favorable electrochemical gradient. In this review we will overview the spatial and temporal organization of Ca^{2+} fluxes as well as the molecular mechanisms involved in metastasis analyzing the different key steps regulating tumor spread.

Epithelial-mesenchymal transition and loss of cell-cell contact

EMT is a cellular process during which epithelial cells acquire fibroblast-like morphology. This process involves changes in cellular shape, 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 suppression of multiple epithelial markers (*e.g.* E-cadherin, claudins, occludins) and overexpression of mesenchymal markers (*e.g.* N-cadherin, vimentin, integrins) (Fig.2).

Of note, EMT and disruption of cell-cell contacts is one of the key events in tumor progression and it can be induced by various effectors like growth factors, hypoxia, and inflammation (Diepenbruck & Christofori, 2016). Interestingly, remodeling of Ca^{2+} signals during EMT processes has been reported for the variety of cancer cells. For example, in breast cancer cells the potency of ATP-mediated cytosolic Ca^{2+} transients exhibited significant changes after epidermal growth factor (EGF) and hypoxia induced EMT (Davis *et al.*, 2011; Azimi *et al.*, 2016). Specifically, attenuation of cytosolic Ca^{2+} peak and the 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 inhibition of $P2X_5$ reduces expression of the EMT marker vimentin and its increased expression correlates with breast cancer cells that are associated with a more mesenchymal phenotype (Davis *et al.*, 2011). Moreover, chelation of free cytosolic Ca^{2+} suppresses production of mesenchymal markers like vimentin, N-Cadherin and CD44, after 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, EMT of colon cancer cells may be regulated by KCNN4 through Ca^{2+}-dependent mechanisms (Lai *et al.*, 2013). Regarding the store-operated Ca^{2+} entry (SOCE), the data is ambiguous. On one hand, SOCE and basal Ca^{2+} influx are decreased after EGF induction of EMT in breast cancer cell line MDA-MD-468 (Davis *et al.*, 2012). On the other hand, transforming growth factor β1 (TGF-β1) induced EMT is associated with enhanced SOCE in breast cancer cell line MCF-7 (Hu *et al.*, 2011).

It is now clear that remodelling of Ca^{2+} signaling is a prominent feature of EMT in various cancer types. Therefore, deregulation of Ca^{2+}-permeable channels could subserve as an important regulator of EMT during carcinogenesis. Indeed, silencing and pharmacological inhibition of transient receptor potential melastatin channels (TRPM) such as TRPM7 and

TRPM8 decreased expression of a variety of mesenchymal markers in breast cancer cells (Davis *et al.*, 2013; Liu *et al.*, 2014). In MCF-7 breast cancer cell line that exhibit 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 upregulated in tumor breast cancer tissues, when comparing to the adjacent nontumor tissues, suggesting therefore on the role of TRPM8 as a determinant of EMT transition (Liu *et al.*, 2014). Moreover, in breast cancer cells EGF induced EMT significantly increases mRNA level of Ca^{2+} release-activated Ca^{2+} channel protein 1 (ORAI1) and provides altered Ca^{2+} signaling possibly due to involvement of transient receptor potential canonical channel type 1 (TRPC1) (Davis *et al.*, 2012). In hepatic cancer cells, another member of transient receptor potential canonical channel TRPC6 has been shown to affect expression of EMT markers after doxorubicin induction (Wen *et al.*, 2016).

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

Cell migration

Principal component of cancer cell motility is the directional migration which is realized due to the 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, the 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 implicated in controlling the directed cellular locomotion (Brundage RA, Fogarty KE, Tuft RA, 1991).

Of note, migration is complex and multistep process that involves coordination between cytoskeleton remodeling, cell-substrate adhesion/detachment and cellular protrusion/contraction (Gardel *et al.*, 2010; Thomas Parsons *et al.*, 2010). Importantly, several key molecular components and signaling events of the cellular migration machinery are Ca^{2+}-sensitive (Fig. 3). For example, the myosin II-based (myo II) 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+} signaling.

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 SJ, 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 interaction with different target proteins (Bresnick *et al.*, 2015). In particular, their influence on F-actin polymerization and myo II-actin assembly has been proposed to govern cell migration due to the cytoskeletal structural remodeling (Gross *et al.*, 2014) (Fig.3). Overall, it is clear now 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 sustained and transient Ca^{2+} changes important for orchestration of the 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 the 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 tumorigenicity of breast cancer cells (Lee *et al.*, 2005) (Fig. 3). At the same time, in the front end of ER low local Ca^{2+} concentration provokes high sensibility to SOCE (Tsai *et al.*, 2014). Indeed, ER residual Ca^{2+} sensor of SOCE, stromal interaction molecule (STIM), has been found polarly distributed in the leading edge of the migrating cell (Tsai *et al.*, 2014). STIM molecule responds to the Ca^{2+} ER depletion and provokes ion influx through plasma membrane ORAI channel (Liou *et al.*, 2005; Roos *et al.*, 2005). Of note, STIM-ORAI proteins have been found significantly upregulated in various cancer types and SOCE-activated Ca^{2+} signaling implemented in the mediatiaion of actomyosin assembly and focal adhesions required for efficient migration (Chen *et al.*, 2011; Fiorio Pla *et al.*, 2016; Jardin I, 2016) (Fig. 3).

Plasma membrane extensions and protrusions play role of a mechanical stress and thus provide Ca^{2+} influx through stretch-activated channels at the front end of migrating cell. Indeed, TRPM7 can be activated intracellularly through phospholipase C (PLC) or by membrane stretch (Su *et al.*, 2006; Wei *et al.*, 2009; Gao *et al.*, 2011; Middelbeek *et al.*, 2012). Interestingly, TRPM7 is localized in close proximity with calpain and myo II (Clark *et*

al., 2007). Therefore, Ca^{2+} entry provided through TRPM7 modulates actomyosin cytoskeleton contraction and dynamics of the focal adhesion turnover required for directional cell migration (Clark *et al.*, 2007). Indeed, pro-migratory role of TRPM7 has been demonstrated for breast, lung, pancreatic and nasopharyngeal cancers (Visser *et al.*, 2014). Moreover recently the mechanosensitive TRPC1 activation, localized at the rear end of the cells, was shown to play a role in the formation of cell polarity of bone osteosarcoma U2OS cells and their directional migration (Huang *et al.*, 2015). Similarly, several members of TRP channels has been implicated in cell migration in various caner types (Fiorio Pla & Gkika, 2013). In particular most of TRP channels has been associated with increase in migration potential. This is the case for TRPC members such as TRPC1, TRPC6 in glioma cells (Chigurupati *et al.*, 2010; Bomben *et al.*, 2011); and vanilloid subfamily TRPV2 which has also been associated with increased cellular migration in prostate, bladder and breast cancer (Oulidi *et al.*, 2013; Gambade *et al.*, 2016). On contrary, another subfamily of TRP channels melastatin, full length TRPM8, has been reported to inhibit cell migration thus suggesting a protective role for TRPM8 in prostate metastatic cancer progression, whereas short TRPM8 isoform could have pro-metastatic potential (Bidaux *et al.*, 2016). Voltage-gated Ca^{2+} channels (VGCCs) represent another pathway for Ca^{2+} influx that activates downstream MAPK/ERK signaling pathway and increases migration (Mertens-Walker *et al.*, 2010). In particular, Cav1.3 has been found overexpressed in endometrial carcinoma and its knockdown has been shown to reduce migration (Hao *et al.*, 2015).

Intracellular Ca^{2+} is important regulator of Ca^{2+} activated potassium channels (KCa). Interestingly, ORAI and TRPC1 channels may form complexes with small conductance KCa channel SK3 (Chantome *et al.*, 2013; Guéguinou *et al.*, 2016). Such SK3-ORAI complex is crucial for 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 trough SK3/TRPC1/Orai1 channel complex (Guéguinou *et al.*, 2016).

Invasiveness and invadopodia formation

Invasiveness of cancer cells is their ability to degrade ECM and migrate into neighboring connective tissues as well as lymph- and bloodstream. There, cancer cells are spread within the organism and give rise to the secondary tumors outbursts, metastases. Therefore, 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 the

special structures – invadopodia, which are dynamic actin-enriched cell protrusions with proteolytic activity. Typically, invadopodia formation process can be differentiated into following steps: initiation, assembly and maturation (Fig.4) (Jacob *et al.*, 2015). The assembly of invadopodia is initiated in response to the focal generation of phosphatidylinositol-3,4-biphosphate and the activation of the nonreceptor tyrosine kinase Src (Mader *et al.*, 2011; Pan *et al.*, 2011; Yamaguchi H & Sakai R, 2011). The matured invadopodia recruit the proteolytic enzymes, such as membrane type 1 (MT1)–matrix metalloproteinase (MMP), MMP2, and MMP9, to facilitate the focal degradation of extracellular matrix and cell invasion (Beaty *et al.*, 2013).

Interestingly, a particular pattern of Ca^{2+} signaling, Ca^{2+} oscillations, has been revealed to predispose the invadopodia formation and activity (Fig. 4) (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). Proteolytic activity of invadopodia is predetermined by the incorporation of MMP-containing endocytic vesicles to the plasma membrane at the ECM degradation sites and can also be linked to the Ca^{2+} signaling machinery (Bravo-Cordero JJ *et al.*, 2007). Indeed, inhibition of SOCE abrogated fusion of MMP-containing vesicles with the plasma membrane and as a result constrained ECM degradation (Sun *et al.*, 2014). Moreover, constitutively active TRPV2 provides intracellular Ca^{2+} increase and has been associated with upregulation of MMP9 and invasive potential of prostate cancer cells (Monet *et al.*, 2010)
In oral squamous carcinoma, TRPM8 activity directly correlates with MMP9 activity and metastatic potential of the cells (Okamoto *et al.*, 2012). The downregulation of MMP9 might be also achieved after inhibition of voltage-gated Ca^{2+} channels (Kato *et al.*, 2007). Furthermore, in the highly metastatic human breast cancer cell line MDA-MB-435 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 the proteolytic enzymes like MMPs and cathepsins, whereas Ca^{2+} oscillations are required for the initiation of the invadopodia formation process (Fig. 4).

Induction of local angiogenesis

Vascularization is a key step required for tumor metabolic support and sustaining metastatic dissemination. Tumor vascularization is promoted by the same tumor cells upon secretion of a number of growth factors. Vessel formation is a complex multistep process during which 'activated' endothelial cells (ECs), the first mechanical and functional interface between blood and tissues, proliferate, migrate, differentiate and are stabilized in a new circulatory network (Carmeliet, 2005; Folkman J, 2006).

Because of its multifaceted role in the control of endothelium homeostasis, the Ca^{2+} machinery is a potential molecular target for strategies against tumor neovascularization. Interestingly, several studies from our laboratory depict a distinct Ca^{2+} machinery in tumor derived EC (TEC) as compared with healthy ones (Fig. 5). Importantly, proangiogenic Ca^{2+} signals and their related pathways are significantly altered in TEC compared with normal EC (Fiorio Pla & Munaron, 2014). As an example, Ca^{2+} signals mediated by specific factors like vascular endothelial growth factor (VEGF) and ATP and intracellular messengers such as arachidonic acid (AA), Nitric Oxyde (NO), or sulfidric acid (H2S) and cyclic AMP (cAMP) are involved in promigratory effects in TEC, but not in normal EC (Fiorio Pla *et al.*, 2008, 2010, 2012*b*; Pupo *et al.*, 2011; Avanzato *et al.*, 2016).

Several studies highlighted the importance of agonist-stimulated Ca^{2+} signals in angiogenesis and the role of intracellular Ca^{2+} increase has been deeply investigated 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 to an increase of 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 activation of 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), 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. 5) (Andrikopoulos *et al.*, 2011). Of note, in recent study VEGF-mediated Ca^{2+} signaling in individual endothelial cells has been investigated and shown to correlate both stochastic and deterministic response characteristics to the selection of

phenotypes associated angiogenesis. In particular altering the amount of VEGF signaling in endothelial cells by stimulating them with different VEGF concentrations triggered distinct and mutually exclusive dynamic Ca^{2+} signaling responses that correlated with different cellular behaviors such as cell proliferation (monitored by NFAT nuclear translocation) or cell migration (involving MLCK) (Noren *et al.*, 2016). *In vivo* role of Ca^{2+} signals has been recently studied on zebrafish during angiogenic input by means high-speed, 3-dimensional (3D) time-lapse imaging to describe intracellular Ca^{2+} dynamics in ECs at single-cell resolution in zebrafish (Yokota *et al.*, 2015; Noren *et al.*, 2016). Of note, TRP Ca^{2+}-permeable channels have profound effects in the control of different steps of tumor angiogenesis (Fiorio Pla *et al.*, 2012*a*; Fiorio Pla & Gkika, 2013; Earley & Brayden, 2015). Besides their role in VEGF-mediated Ca^{2+}-signals previously described, several data clearly show their involvement Ca^{2+} mediated signal transduction with prominent roles in tumor angiogenesis. In this context TRPV4 is an emerging player in angiogenesis, on EC it acts as a mechanosensor during changes in cell morphology, cell swelling and shear stress (Vriens *et al.*, 2004; Hartmannsgruber *et al.*, 2007; Thodeti *et al.*, 2009; Everaerts *et al.*, 2010; Fiorio Pla & Munaron, 2014). We described the role of TRPV4 in endothelial migration, (Fiorio Pla *et al.*, 2012*b*) showing that TRPV4 display a significant increase in EC derived from human breast carcinomas (BTEC), as compared with 'normal' EC (HMVEC), leading to a greater Ca^{2+} entry that activates migration in TEC (Fig. 5) (Fiorio Pla *et al.*, 2012*b*). Moreover, TRPV4 has been recently described as important player in the tumor vasculature normalization therefore potentially improving cancer therapies (Adapala *et al.*, 2015; Thoppil *et al.*, 2015, 2016). In addition, TRPM2 has been recently identified to mediate H_2O_2-dependent increase in macrovascular pulmonary EC permeability (Fig. 5) (Hecquet *et al.*, 2008; Mittal *et al.*, 2015). TRPM7 inhibits HUVEC proliferation and migration, whereas its functions on HMEC seem to be opposite (Fig. 5) (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 of Ca^{2+} influx generating by sensing of ROS, process that requires the peroxidation of membrane lipids (Sullivan *et al.*, 2015). Similarly TRPV2 has been shown to be expressed in aorta endothelium but not clear functional data have been reported (Earley, 2011).

Finally, the emerging family of mechanosensitive Piezo channels has been recently described in vascular endothelial cells: Piezo2 knockdown is involved in glioma angiogenesis both *in vitro* as well as *in vivo* by promoting abnormal intracellular Ca^{2+}, Wnt11/β-catenin

signaling reduction, leading to altered angiogenic activity of endothelial cells (Fig. 5) (Yang *et al.*, 2016).

Conclusions

Remodeling of Ca^{2+} signaling plays important role during tumorigenesis advancement. Interestingly, there is some specific pattern of channels through which such Ca^{2+} signals are provided at the different stages of cancer progression. This could be partially explained due to the specificity of Ca^{2+} flux, its compartment localization, and proximity of the downstream Ca^{2+}-dependent targets. Furthermore, some ion channels represent multimodal activity and are characterized as not only Ca^{2+}-permeable pore proteins but also possess other functional domains. For example, C-terminal end of TRPM7 is constituted of 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 to the plasma membrane (Gkika & Prevarskaya, 2009; Fiorio Pla *et al.*, 2012*a*; Bernardini *et al.*, 2015; Earley & Brayden, 2015).

Competing interests

The authors have no conflicts of interest to declare.

Authors' contributions

Oksana Iamshanova and Prof Alessandra Fiorio Pla collected information, conceived the concept, prepared figures, and drafted the manuscript. Prof Natalia Prevarskaya is involved in drafting part of the manuscript. All of the authors read and approved the final manuscript.

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Figure legends

Fig. 1. Abstract figure. Specific patterns of Ca^{2+} signals that are associated with different steps of cancer progression.

Fig. 2. Epitnelial-to-mesenchymal transition (EMT) and loss of cell-cell contacts are accompanied by the changes of Ca^{2+} signals after epidermal growth factor (EGF) and hypoxia induced EMT. Specifically, attenuation of cytosolic Ca^{2+} peak and the sustained phase of Ca^{2+} influx in the response to ATP. The most studied Ca^{2+}-permeable channels, which are associated with EMT, are indicated.

Fig. 3. The global cytosolic Ca^{2+} is generally higher at the rear end, whereas Ca^{2+} flickers are enriched near the front edge of migrating cell. The key molecular components and signaling events of the cellular migration machinery are Ca^{2+}-dependent.

Fig. 4. Ca^{2+} oscillations are required for the initiation of the invadopodia formation process, whereas Ca^{2+} influx activate focal degradation of the extracellular matrix (ECM), in particular through the upregulation of the proteolytic enzymes like matrix netalloproteinases (MMPs) and cathepsins.

Fig. 5. Induction of local angiogenesis by Ca^{2+} signaling remodeling. Vascular endothelial growth factor (VEGF) and ATP mediated Ca^{2+} signals provide proangiogenic effects specifically on tumor derived endothelial cells (ECs).

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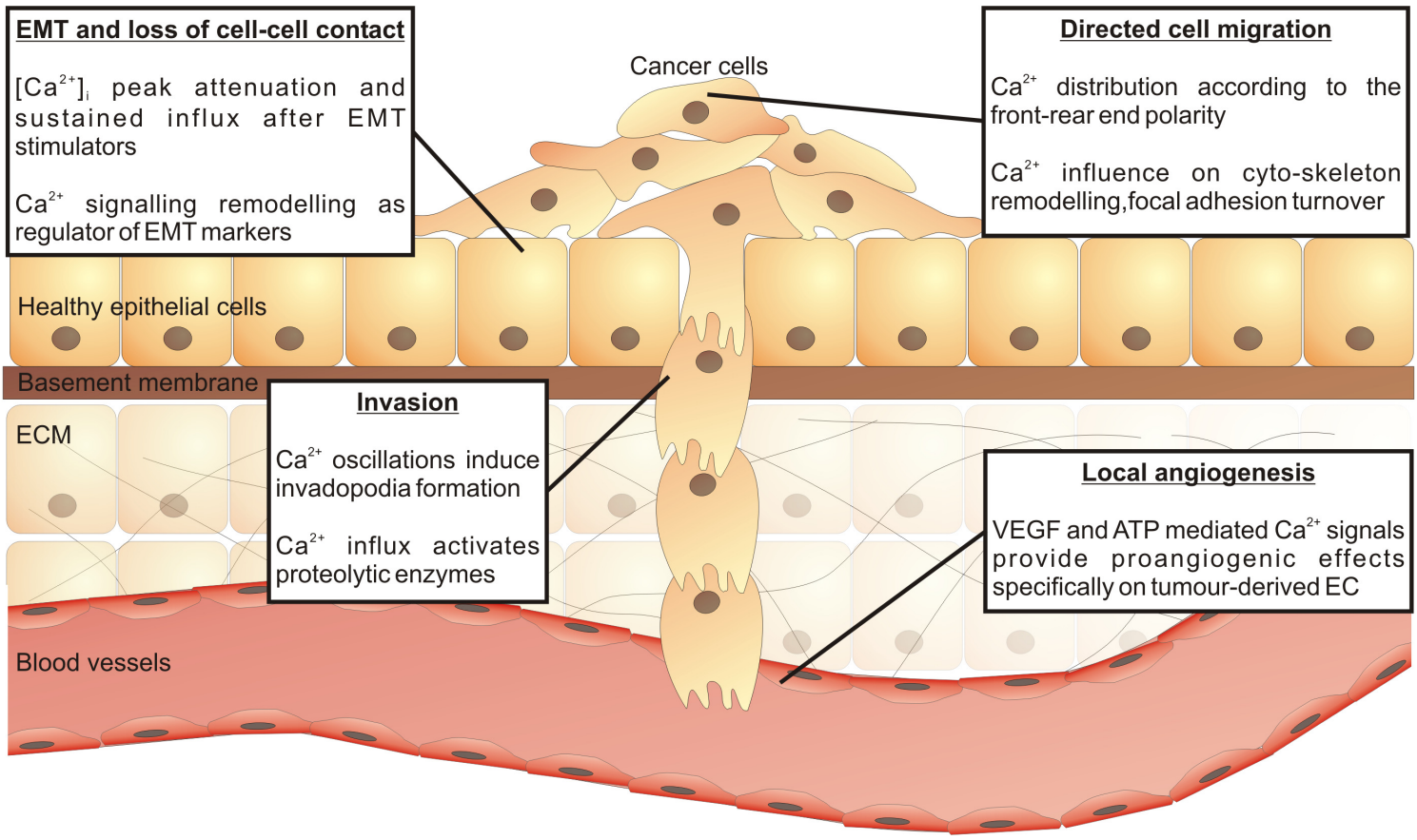
EMT and loss of cell-cell contact
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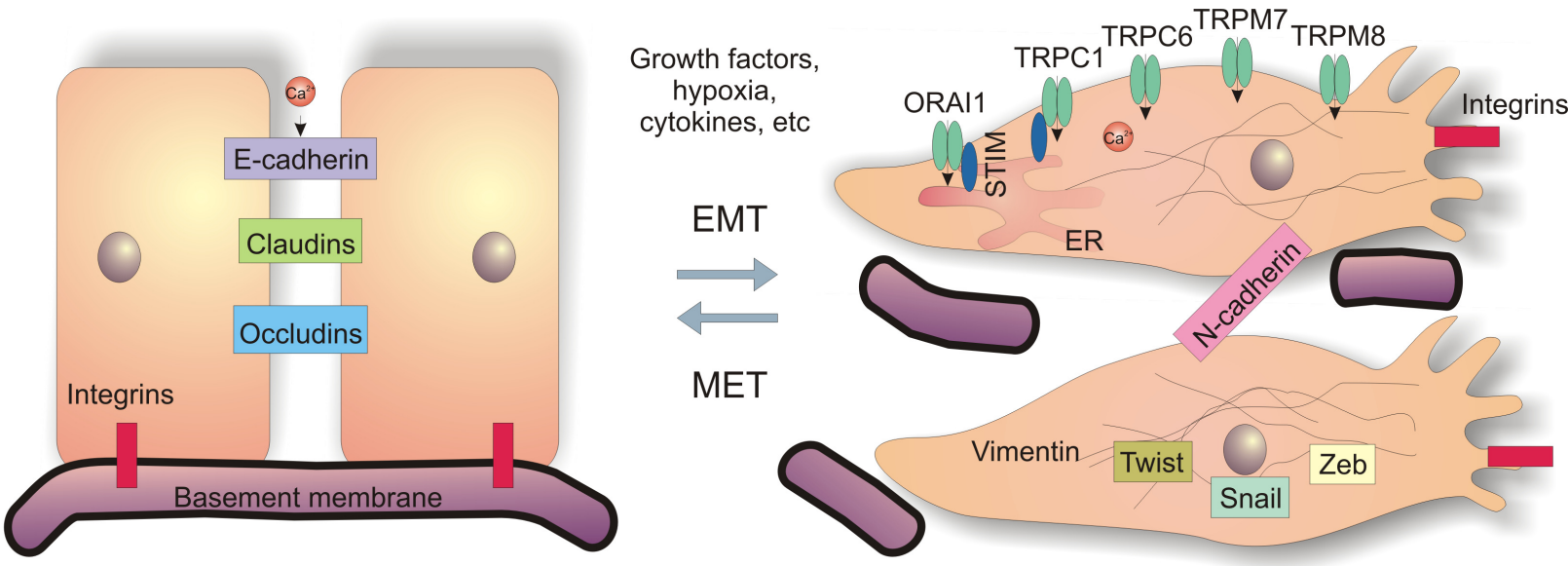
Ca2+ signalling remodelling as regulator of EMT markers
Cancer cells
Directed cell migration
Ca2+ distribution according to the front-rear end polarity
Ca2+ influence on cyto-skeleton remodelling,focal adhesion turnover
Healthy epithelial cells
Basement membrane
ECM
Invasion
Ca2+ oscillations induce invadopodia formation
Ca2+ influx activates proteolytic enzymes
Local angiogenesis
VEGF and ATP mediated Ca2+ signals provide proangiogenic effects specifically on tumour-derived EC
Blood vessels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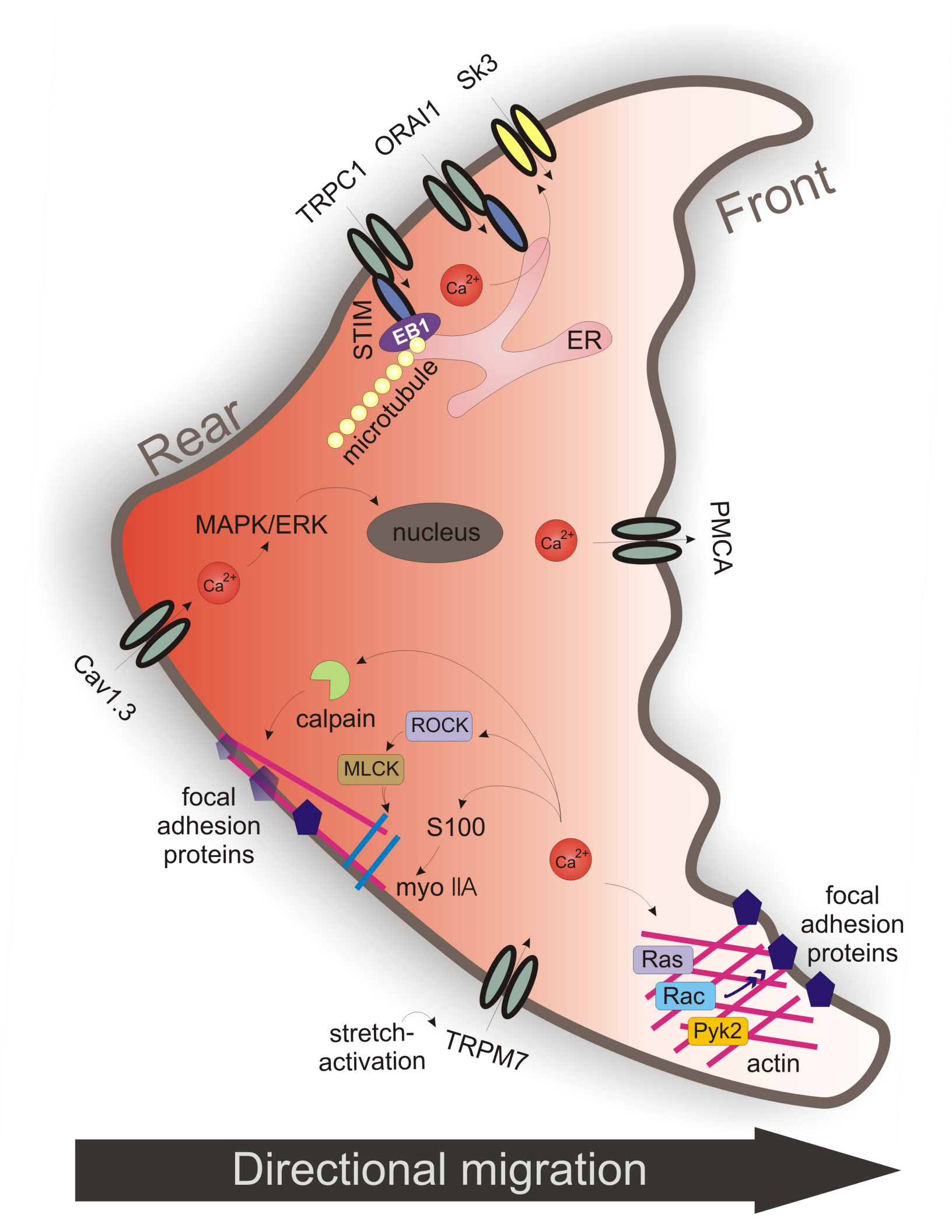
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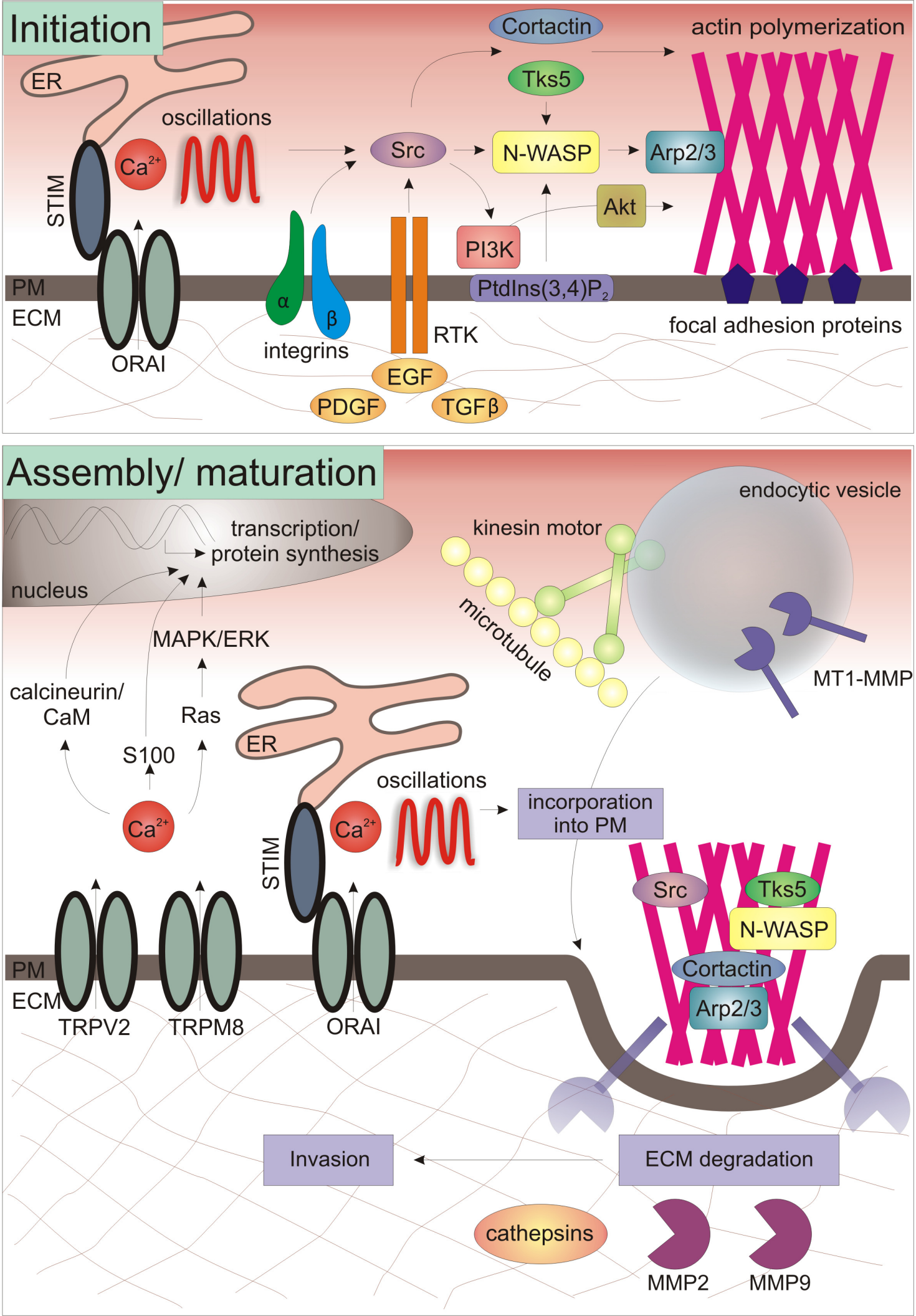
Initiation
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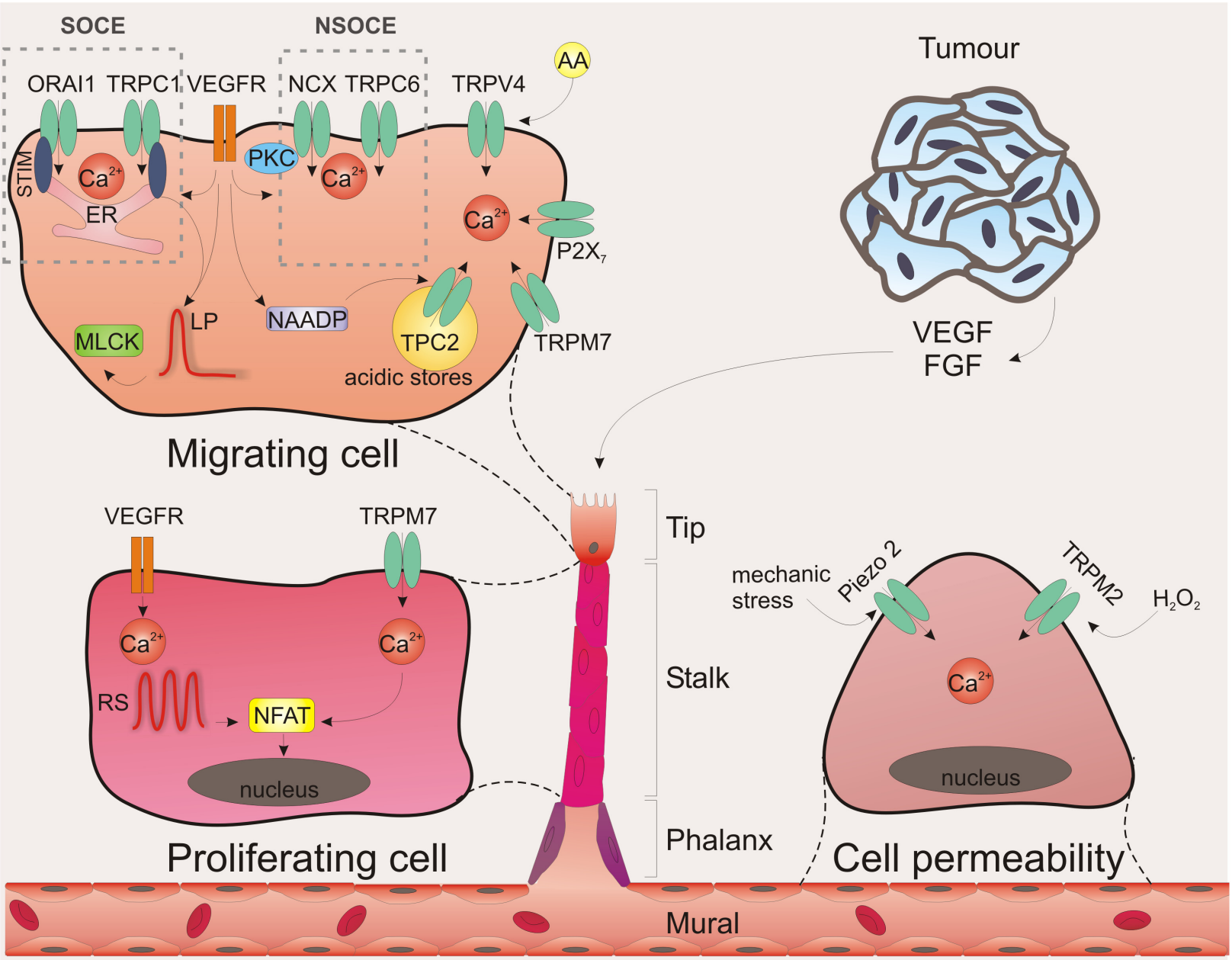
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Proliferating cell
Tip
Stalk
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mechanic
stress
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Cell permeability
Mural