TRP Channels and Small GTPases Interplay in the Main Hallmarks of Metastatic Cancer

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Transient Receptor Potential (TRP) cations channels, as key regulators of intracellular calcium homeostasis, play a central role in the essential hallmarks of cancer. Among the multiple pathways in which TRPs may be involved, here we focus our attention on the ones involving small guanosine triphosphatases (GTPases), summarizing the main processes associated with the metastatic cascade, such as migration, invasion and tumor vascularization. In the last decade, several studies have highlighted a bidirectional interplay between TRPs and small GTPases in cancer progression: TRP channels may affect small GTPases activity *via* both Ca^{2+}-dependent or Ca^{2+}-independent pathways, and, conversely, some small GTPases may affect TRP channels activity through the regulation of their intracellular trafficking to the plasma membrane or acting directly on channel gating. In particular, we will describe the interplay between TRPC1, TRPC5, TRPC6, TRPM4, TRPM7 or TRPV4, and Rho-like GTPases in regulating cell migration, the cooperation of TRPM2 and TRPV2 with Rho GTPases in increasing cell invasiveness and finally, the crosstalk between TRPC1, TRPC6, TRPM8, TRPV4 and both Rho- and Ras-like GTPases in inducing aberrant tumor vascularization.

Keywords: transient receptor potential channels, small guanosine triphosphatases, cancer hallmarks, metastasis, molecular signaling, migration, invasion, tumor vascularization

INTRODUCTION

Ca^{2+} signaling plays a central role in the regulation of many important cellular functions and indeed, not surprisingly, a dysregulation of Ca^{2+} homeostasis has been observed in various pathological conditions, including tumorigenesis (Monteith et al., 2017). Changes in Ca^{2+}

Abbreviations: EC, endothelial cell; ECM, extracellular matrix; EGF, epidermal growth factor; EMT, epithelial to mesenchymal transition; ER, endoplasmatic reticulum; FA, focal adhesion; FAK, focal adhesion kinase; GAP, GTPases activating protein; GEF, guanine exchange factor; GDI, guanine-nucleotide dissociation inhibitors; GDP, guanosine diphosphate; GTP, guanosine triphosphate; GTPase, guanosine triphosphatases; MLC, myosin light chain; MMP, membrane-matrix metalloproteinases; PCa, prostate cancer; PI3K, phosphoinositide 3-kinase; PFGF, platelet-derived growth factor; TEC, tumor endothelial cell; TRP, transient receptor potential; VEGF, vascular endothelial growth factor.

signaling leading to dysfunctions in cancer cells are due to alterations in the so-called "Ca^{2+}-signaling toolkit" (Berridge et al., 2003) which includes, among others, ion channels, responsible for altered fluxes within the cell, and several Ca^{2+}-dependent effectors, which mediate various signal transduction pathways in response to altered Ca^{2+} homeostasis.

In recent years, the involvement of Ca^{2+}-permeable channels in neoplastic diseases has been extensively investigated and a direct correlation between their dysregulation and cancer development has been shown (Monteith et al., 2017). Among them, Transient Receptor Potential (TRP) channels have revealed an important involvement in the regulation of many signaling pathways associated with tumor progression (Yang and Kim, 2020). One of the key features of TRP channels is their polymodal activation mechanism and their involvement in different signal transduction pathways. Among the multiple pathways TRPs are signaling through, the ones involving specific intracellular messengers belonging to the family of small guanosine triphosphatases (GTPases) are emerging as essential in tumorigenesis in the last decade. Therefore, in this review, we will focus on the interplay between TRPs and small GTPases, and its role in several aspects of the metastatic process (Aspenström, 2004; Clayton and Ridley, 2020).

The metastatic cascade leads to spread of malignant cells from the primary tumor through the lymph or blood circulation to establish secondary growth in a distant organ; it is a multistep process involving highly complex structural and functional alterations within cancer cells. Primary tumor cells are primed for dissemination by the process of epithelial-mesenchymal transition (EMT), during which they assume a more aggressive mesenchymal-like phenotype. This phenotypic switch allows them to detach from the primary tumor mass and to acquire migratory and invasive properties, in order to move from their original location, migrate and invade the extracellular matrix (ECM) and endothelium to spread to secondary sites and form metastases (Roche, 2018). All the steps involved in metastatic cascade (EMT, cell migration, invasion and tumor vascularization) are regulated by the intracellular Ca^{2+} concentration (Berridge et al., 2003) and specifically by the TRP-mediated calcium influx (Fels et al., 2018), as well as by the most important small GTPases (Rho-like and Ras-like) involved in cytoskeletal dynamics and cell polarity (Ungefroren et al., 2018). Here, we discuss TRPs and small GTPases contribution in cancer progression, focusing on signaling pathways involving a direct interplay between TRPs and small GTPases in three main metastatic cancer hallmarks: migration, invasion and tumor vascularization.

TRP Channels

TRP channels mainly act as signal transducers by altering membrane potential or intracellular Ca^{2+} concentration in response to various environmental stimuli, including physical-chemical stimuli, such as temperature, pH changes, osmolarity, and pressure as well as endogenous and exogenous ligands (Nilius and Owsianik, 2011). TRP channels have been shown to play a central role in carcinogenesis as well as in various late stages of tumor progression. In particular, it has been shown that changes in

the expression of TRP channels are correlated with the progression of different types of cancer. To date, most changes involving TRP proteins do not involve mutations in the TRP gene but rather dysregulation of the wild-type TRP protein expression levels, depending on the stage of cancer (Gkika and Prevarskaya, 2011; Lehen'kyi and Prevarskaya, 2011; Bernardini et al., 2015; Shapovalov et al., 2016). Moreover, several recent studies have highlighted a direct correlation between cancer patient survival and TRP channel expression. Tumor differential expression of the main TRP channels discussed in this review and its correlation with patients' survival are summarized in **Table 1**.

These observations strongly indicate that TRPs play a significant role in cancer progression and more specifically in many processes underlying the metastatic cascade, making them promising candidates as both molecular biomarkers and therapeutic targets in various types of cancer (Gkika and Prevarskaya, 2011; Fiorio Pla and Gkika, 2013; Bernardini et al., 2015; Lastraioli et al., 2015; Litan and Langhans, 2015; Fels et al., 2018). It has been shown that TRP-mediated effects on metastatic cancer cell behavior are mainly associated to their Ca^{2+} permeability. Indeed, through the regulation of intracellular Ca^{2+} concentration, both in the cytosol and within subcellular organelles, TRP channels play a key role in many Ca^{2+}-dependent signaling pathways, including those associated with the metastatic cascade such as EMT, migration, invasion and tumor vascularization (Iamshanova et al., 2017). In response to different environmental challenges during the metastatic cascade, like hypoxic, acidic and mechanical cues, cancer cells "re-program" TRPs expression and "misuse" their functions in order to put in place and sustain a more aggressive, metastatic phenotype. However, although most of the TRP-mediated pathways involved in cancer progression are due to alteration of Ca^{2+} homeostasis, it has been also demonstrated an involvement of these channels independent from their Ca^{2+} permeability. As an example, TRPM7 regulation on cell migration is mainly due to Mg^{2+} influx through the channel (Abed and Moreau, 2009; Su et al., 2011). Similarly, the Na^+-selective TRPM4 channel has been implicated in cancer migration, although it is inherently Ca^{2+}-impermeable (Vennekens and Nilius, 2007; Cáceres et al., 2015; Holzmann et al., 2015). On the other hand, TRPs' involvement in the metastatic cascade may also be pore-independent, extending the interest in TRPs beyond the field of ion channels (Vrenken et al., 2015). Indeed, TRPM7 promotes many of its biological effects through its peculiar intrinsic kinase activity (Desai et al., 2012; Faouzi et al., 2017; Cai et al., 2018). Moreover, we recently demonstrated the role of TRPM8 in inhibiting vascular endothelial cell migration, which is independent from the pore function of the channel (Genova et al., 2017).

It is therefore evident that, despite the countless advances made in recent years in the study of TRP channels, there are still many aspects to be explored in order to better characterize the main TRP-mediated pathways involved in tumor progression and thus be able to develop new cancer therapies that use TRPs as therapeutic targets.

Small GTPases

The family of small GTPases is composed of a large group of structurally and functionally related proteins, subgrouped into

TABLE 1 | TRPs expression in cancer and correlation with patient survival prognosis.

Channel	Cancer Type	Expression			Prognosis	References
		Healthy/ Benign	Tumor	Invasive		
TRPC1	Breast basal/lymph nodes	Yes	↑	↑	poor	Azimi et al., 2017;
	Breast ductal adenocarcinoma	yes	↑			Dhennin-duthille et al., 2011
TRPC5	Colon	yes	↑	↑	poor	Chen et al., 2017a
TRPC6	Prostate	yes	↑	↑		Yue et al., 2009
	Glioblastoma	yes	↑	↑		Chigurupati et al., 2010
	Esophageal squamous cell carcinoma	yes	↑	↑	poor	Zhang et al., 2013
	Breast/Invasive ductal adenocarcinoma	yes	↑			Guilbert et al., 2008; Dhennin-duthille et al., 2011
TRPM2	Breast	yes	↑	↑	Depends on subtype	Sumoza-Toledo et al., 2016
TRPM4	Prostate	yes	↑			Ashida et al., 2004; Singh et al., 2006
	Cervical	yes	↑			Narayan et al., 2007
TRPM7	Breast	yes	↑	↑	poor	Middelbeek et al., 2012; Meng et al., 2013
	Invasive ductal adenocarcinoma/lymph nodes	yes	↑	↑		Dhennin-duthille et al., 2011; Guilbert et al., 2013
	Pancreatic ductal adenocarcinoma/lymph nodes	yes	↑	↑	poor	Rybarczyk et al., 2012; Yee et al., 2015
	Nasopharyngeal	no	↑	↑	poor	Chen et al., 2015
	Ovarian; Prostate; Melanoma; Sarcoma			↑		Meng et al., 2013
TRPM8	Breast ductal adenocarcinoma	yes	↑	↑		Dhennin-duthille et al., 2011
	Prostate	yes	↑	loss		Tsavaler et al., 2001; Fuessel et al., 2003; Bidaux et al., 2005
	Urothelial carcinoma of bladder	yes	↑	↑	poor	Xiao et al., 2014
	Lung	Very low level	↑			Tsavaler et al., 2001
	Colon	no	↑			Tsavaler et al., 2001
	Melanoma	no	↑			Tsavaler et al., 2001
	Osteosarcoma	yes	↑	↑	poor	Zhao and Xu, 2016
TRPV2	Prostate	no		↑		Monet et al., 2010
	Bladder	yes (full isoform)	↑	↑		Caprodossi et al., 2008
		(short isoform)	↓	Loss		
	Esophageal squamous cell carcinoma	yes	↑	↑	poor	Zhou et al., 2014
	Gastric	yes	↑	↑	poor	Zoppoli et al., 2019
	Breast (Triple negative/basal subtype)	yes	↑		better	Elbaz et al., 2018
TRPV4	Breast	yes	↑	↑	poor	Lee et al., 2016; Lee et al., 2017
	Gastric		↑		poor	Lee et al., 2016
	Ovarian		↑		poor	Lee et al., 2016
	Glioma	yes	↑	↑	poor	Ou-yang et al., 2018

↑: increment; ↓: reduction.

six families: Ras, Rho, Arf, Rab, Ran, and RGK. Among them, Ras-like and Rho-like GTPases are the most well characterized. Mechanistically, small GTPases are molecular switches that cycle between an active GTP- bound form and an inactive GDP-bound form. More specifically, these enzymes, once bound to GTP, can catalyse its hydrolysis to GDP and this reaction then results in a conformational change which causes the inactivation of the proteins (Vetter and Wittinghofer, 2001; Cherfils and Zeghouf, 2013). The cycling between GTP- and GDP-bound states is tightly regulated by specific GTPases activating protein (GAPs), which act as negative regulators, promoting the GDP-bound state by increasing the hydrolysis activity of small GTPases, and by guanine exchange factors (GEFs), which act as positive regulators, guiding the replacement of the hydrolysed GDP for a GTP, thus promoting the enzyme active state (Mishra and Lambright, 2016). Moreover, a third family of regulatory proteins called guanine-nucleotide dissociation inhibitors (GDIs) inhibits small GTPases activity by controlling their intracellular localization: GDIs bind to GTPases in their inactive GDP-bound state and sequester them in the cytosol, thus preventing their translocation to intracellular membranes, where activation occurs (Mishra and Lambright, 2016). Indeed, it has been shown that spatial and temporal distributions of the different small GTPases, as well as of their regulators, are important determinants in signaling by small GTPases, determining many aspects of cell behavior (Yarwood et al., 2006; Cherfils and Zeghouf, 2013; Moissoglu and Schwartz, 2014).

The conformational changes following the binding to GTP allow the association of small GTPases with a large number of potential effector proteins such as enzymes and scaffold proteins, which mediate the specific biological effects of each GTPases. Thanks to their ability to interact with a wide number of downstream targets and to co-ordinately activate several molecular processes required for a particular cellular response, small GTPases function as signaling switches in numerous cellular processes. In general, it

has been found that Ras-like GTPases are mainly implicated in regulating cell cycle, differentiation and proliferation, (Shields et al., 2000), whereas Rho-like GTPases are mainly involved in cell morphology, cytoskeletal dynamics and cell polarity (Ridley, 2001). Moreover, Rab GTPases play a key role in many cellular functions, by controlling intracellular trafficking between organelles through vesiculotubular carriers and thus ensuring the spatiotemporal regulation of vesicle traffic (Stenmark, 2009). Considering the central role of Ca^{2+} signaling in many of these processes, it is not surprising that small GTPases activity was found to be strongly related to Ca^{2+} homeostasis (Aspenström, 2004). More specifically, their activation/inactivation may occur through Ca^{2+}-dependent mechanisms acting on specific GEF/GAP proteins or directly on them. Moreover, some GTPases have revealed a direct influence on calcium signaling by regulating the activity of certain calcium channels, including TRPs, by itself or through their effectors (Koopman et al., 2003; Aspenström, 2004; Iftinca et al., 2007; Correll et al., 2008). Finally, several small GTPases collaborate with calcium signaling through the activation of specific Ca^{2+}-related effectors involved in cellular processes, such as cell adhesion, cell migration and exocytosis (Cullen and Lockyer, 2002; Aspenström, 2004; Bader et al., 2004).

Small GTPases expression results dysregulated in several tumors and has been correlated with the progression of the disease (Sahai and Marshall, 2002; Svensmark and Brakebusch, 2019). Without a doubt, Ras is still the most studied small GTPase in cancer, due to the role played by mutated ras genes in the pathogenesis of human tumors (Bos, 1989; Hobbs et al., 2016; Li et al., 2018). Indeed, oncogenic mutations on H-ras, K-Ras, and N-Ras genes have been detected in several tumor types, although the incidence varies greatly (Bos, 1989; Prior et al., 2012; Li et al., 2018). Moreover, some members of the Ras superfamily like Rap1, resulted involved in cancer hallmarks such as migration and angiogenesis, due to their key role in integrin-mediated "inside-out" signaling events (Chrzanowska-wodnicka et al., 2008; Boettner and Van Aelst, 2009; Carmona et al., 2009). As regards the Rho superfamily, its role in many aspects of the metastatic cascade, including EMT, cell migration, invasion and angiogenesis, has been well established (Bryan and D'Amore, 2007; Ungefroren et al., 2018; Clayton and Ridley, 2020).

TRPs-Small GTPases Interplay in Metastatic Cancer

Migration

One of the key steps in the metastatic cascade involves the acquisition of motility by cancer cells. This results from a complex coordination between cytoskeleton dynamics, cellular contractility and cell adhesion rearrangements. Cell migration is a dynamic process characterized by the cycling of four principal steps: after an initial phase in which cell spreading increases about twice with the generation of protrusions at the leading edge and cell adhesion to the ECM increases, then spreading is reduced and thanks to the generation of traction forces cell compacts and detaches at the trailing edge, allowing for cellular movement (Haws et al., 2016). Due to their role as key regulators of

cytoskeletal dynamics and cell polarity, it is not surprising that Rho GTPases play a central role in controlling cell migration (Clayton and Ridley, 2020). This process is triggered by cell acquisition of a front-rear end polarity due to the formation of protrusive structures, called filopodia and lamellipodia, at the front edge, opposite to a retracting trailing edge (Llense and Etienne-Manneville, 2015). Filopodia consist of actin filaments organized as long parallel bundles and their formation is dependent on Rho GTPase Cdc42 activity, whereas lamellipodia result from the subsequent Rho GTPase Rac1-mediated branching of actin filaments. The extension of actin-based protrusions is accompanied by the formation at the leading edge of new adhesions that link integrins on the plasma membrane to the F-actin cytoskeleton through talin, vinculin, and focal adhesion kinases (FAKs) and which can mature into focal adhesions (FAs) in a process Rho GTPase RhoA- dependent. Contemporary, RhoA also mediates cytoskeletal rearrangements that lead to the formation of stress fibers, structures composed of bundles of actin and myosin II that have a high contractile capability. The force necessary to pull the cell body forward is engendered by the association of mature FAs with the end of stress fibers and by the actin-myosin cytoskeleton contraction. Finally, the disassembly of FAs in the rear of the migrating cells supports cell retraction at the trailing edge, allowing for cell detachment and movement.

A direct involvement of TRP channels activity in all processes associated with cell migration, including cytoskeletal rearrangement, FA turnover and cellular contractility, has been well established (Fiorio Pla and Gkika, 2013; Canales et al., 2019). Therefore, it is not surprising that several studies have highlighted a tight interplay between TRP channels and Rho GTPases in controlling cell migration during cancer progression. As depicted in **Figure 1**, this interplay is characterized by a bidirectional communication, in which TRP channels promote actin cytoskeleton reorganization through a cation-dependent activation of Rho GTPases, and, conversely, some small GTPases cause changes in TRP channel location, protein–protein interactions and channel gating, thereby modulating their function. More specifically, as described in the following paragraphs, TRPC5, TRPC6, TRPC1, TRPM7, TRPM4, and TRPV4 have been found to affect cell migration through a direct interaction with some small GTPases belonging to the Rho family.

Indeed, TRPC5 and TRPC6 channels have been identified as antagonist regulators of actin remodelling and cell motility in fibroblast and kidney podocytes, mediating the activation of Rac1 and RhoA, respectively (Tian et al., 2010). It has been shown that TRPC5 and TRPC6 trigger antagonistic and mutually inhibitory pathways associated with the maintenance of the balance between contractility and motility. In particular, TRPC5-mediated Ca^{2+} influx induces Rac1 activation, thereby enhancing motility and cell migration. Conversely, TRPC6-mediated Ca^{2+} influx stimulates an increase in RhoA activation, thereby promoting stress fibers formation, cell contractility and the subsequent inhibition of cell migration (Tian et al., 2010) or the disruption of podocytes architecture in glomerular renal diseases, which may lead to proteinuria (Jiang et al., 2011). Interestingly, the functional coupling
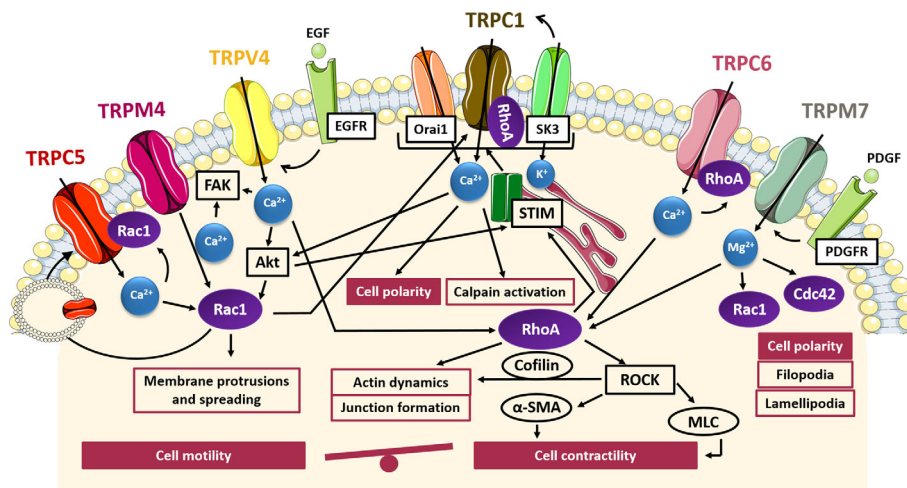
FIGURE 1 | TRP- small GTPases signaling pathways interplay in cell migration. Cartoon depicting TRP channels signaling pathways affecting cell motility and contractility through GTPases. TRPC5, TRPM4 and TRPV4 induce the formation of protrusions and spreading *via* Rac1 activation in a Ca²⁺-dependent manner and at the same time Rac1 promote the translocation of TRPC5 into the plasma membrane; Rac1 and RhoA through SOCE activation induce TRPC1-mediated cell polarization for directional cell migration; TRPM7 control polarized cell movement through the regulation of Rac1 and Cdc42 in a Mg²⁺-dependent way; TRPM7, TRPV4, and TRPC6 contribute to actin dynamics and cell contractility through the Mg²⁺- or Ca²⁺-mediated activation of RhoA/ROCK pathways. FAK, focal adhesion kinase; Akt, protein-kinase B; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; Orai1, calcium release-activated calcium channel; SK3, small conductance calcium-activated potassium channel 3; STIM, stromal interaction molecule 1; ROCK, Rho-associated protein kinase; α-SMA, alfa-smooth muscle actin; MLC, myosin light chain.

between TRPC5 and TRPC6 with Rac1 and RhoA respectively, gives rise to two distinct molecular complexes predominantly localized to discrete membrane compartments, as detected by co-immunoprecipitation and immunofluorescence (Tian et al., 2010). Of note, the presence of constitutively active Rac1 has been shown to affect TRPC5 channel localization, leading to an increase in plasma membrane abundance of TRPC5 with respect to TRPC6, which, conversely, is predominant in cells expressing constitutively active RhoA. In this regard, RhoA seemed to have no effect on the dynamics of TRPC6 insertion into the membrane, whereas Rac1 was found to promote translocation of TRPC5 into the plasma membrane, according to other findings (Bezzerides et al., 2004). This close interdependence between TRPC5 and Rac1 could suggest a positive feedback mechanism in which the Rac1-mediated TRPC5 insertion from a vesicular pool into the cell membrane leads to enhanced TRPC5-mediated Ca²⁺ influx, which in turn triggers the activation of Rac1 and the subsequent migratory phenotype. Although a direct correlation between TRPC5/TRPC6 and Rac1/RhoA has not yet been established in cancer models, it is possible to speculate that a similar interplay may also occur in the regulation of cancer cells migration, taking into account the evidence for direct involvement of TRPC5 and TRPC6 in the increased migratory potential of several cell types (Greka et al., 2003; Xu et al., 2006; Rampino et al., 2007), including some tumors such as colon cancer and glioblastoma (Chigurupati et al., 2010; Chen L. et al. 2017; Chen Z. et al., 2017). However, whether these effects on tumor migration are dependent on the impact of TRPC5 and TRPC6 activity on cell contractility and motility remains to be clarified.

Similarly, TRPC1 has shown a correlation with an increased migratory phenotype in some tumors, such as glioblastoma, osteosarcoma, thyroid, pancreatic and colon carcinoma (Dong et al., 2010; Asghar et al., 2015; Huang et al., 2015; Guéguinou et al., 2016; Lepannetier et al., 2016). One of the key steps in cell migration is the establishment of a functional and morphological polarity along the axis of movement. In this context, it has been demonstrated that TRPC1 is localized to lipid raft domains at the leading edge of migrating cells and plays a role in determining their polarity and directionality (Fabian et al., 2008; Bomben et al., 2011; Huang et al., 2015). For instance, it has been shown that silencing of TRPC1 in transformed renal epithelial Madin–Darby Canine Kidney-Focus (MDCK-F) cells leads to a decrease in migration associated with a failure of cell polarization and an impaired lamellipodia formation. This effect is due to the partial loss of the local Ca²⁺ gradient at the front edge, needed to establish and maintain the axis of movement in migrating cells (Fabian et al., 2008). The same results were also shown in U2OS bone osteosarcoma cells, in which TRPC1 inhibition or knockdown correlates with a decrease in the percentage of polarized cells and the consequent reduction in cell migration (Huang et al., 2015). However, the link between TRPC1-mediated Ca²⁺ gradients and actin dynamics, as well as the possible involvement of TRPC1-mediated Rho GTPases activation in these processes has not been yet fully characterized. Nonetheless, a direct interaction between TRPC1 and RhoA has been characterized in intestinal epithelial cells (IECs) and in pulmonary arterial endothelial cells (Mehta et al., 2003; Chung et al., 2015). Indeed, TRPC1-RhoA interaction

regulates TRPC1-mediated Ca^{2+} influx through SOCE, stimulating IECs migration after wounding (Chung et al., 2015). A reduction of RhoA/TRPC1 complexes, induced by downregulation or inactivation of either small GTPase or TRP channel, is associated with an inhibition of Ca^{2+} influx after store depletion and the decrease in wound healing after injury (Chung et al., 2015). Besides RhoA, a mechanism involving TRPC1 and Rac1 in promoting colon cancer cell migration has recently been proposed (Guéguinou et al., 2016). Indeed, in HCT-116 colon cancer cells TRPC1 and Rac1 are involved in a complex positive feedback loop in which EGF-induced SOCE activates both Rac1 and STIM1 through Akt pathway; in turn, STIM1 activation promotes translocation of TRPC1 and Orai1 into lipid rafts where SK3 is located and thereby triggers SOCE mediated by the complex SK3/TRPC1/Orai1. At the same time, Akt-mediated Rac1 activation enhances SOCE and thereby SOCE-dependent cell migration through Akt pathway, with subsequent lamellipodia formation and calpain activation. Taken together these data suggest a direct interplay between TRPC1 and Rho GTPases in controlling cell polarity and actin rearrangements during cancer cell migration.

TRPM7 is involved in directional migration in different cell types including migrating fibroblasts, osteoblasts, astrocytes and endothelial cells (ECs) (Abed and Moreau, 2009; Wei et al., 2009; Baldoli et al., 2013; Zeng et al., 2015). Similarly to TRPC1, TRPM7 has been shown to be involved in Ca^{2+} gradient formation, contributing to cell polarization and directional migration (Wei et al., 2009). In particular, TRPM7 is positively correlated with platelet-derived growth factor (PDGF)-induced Mg^{2+} influx as well as high-calcium microdomains "Ca^{2+} flickers", most active at the leading lamella of migrating cells (Abed and Moreau, 2009; Wei et al., 2009). Interestingly, several data have shown that the effects of TRPM7 in controlling cytoskeleton and polarized cell movement are independent of its kinase activity and are associated with its channel function. This has been clearly demonstrated in fibroblasts and neuroblastoma cells where re-expression of TRPM7, as well as a kinase-inactive mutant of TRPM7 on knock out cells, reverted phenotypic changes in cell polarization enhancing cell spreading and adhesion (Clark et al., 2008; Su et al., 2011). In particular, it has been shown that Mg^{2+} plays a central role in TRPM7-mediated control of directional migration in fibroblasts and osteoblasts (Abed and Moreau, 2009; Su et al., 2011). The effects observed by TRPM7 depletion in cell morphology, disruption of actin filaments and myosin fibers and a decrease in the number of FAs, correlates with decreased activity of RhoA GTPase, suggesting a role for TRPM7 in RhoA regulation (Su et al., 2011). Besides its interaction with RhoA, TRPM7 is also functionally coupled with Rac1 and Cdc42: indeed, TRPM7 knockdown prevents Rac1 and Cdc42 activation, with a subsequent deficiency in their ability to form lamellipodia and impaired polarized cell movements (Su et al., 2011). Although Rho GTPases, differing from Ras and Rab proteins, do not require Mg^{2+} for high-affinity nucleotide binding, it has been shown that Mg^{2+} plays a role in regulating nucleotide binding and hydrolysis kinetics in the GEF- and GAP-catalyzed reactions

of Rho family GTPases (Zhang et al., 2000). In particular, RhoGAPs exploit Mg^{2+} to achieve high catalytic efficiency and specificity and, conversely, RhoGEFs are negatively regulated by free Mg^{2+}, since the presence of Mg^{2+} significantly decreases the intrinsic dissociation rates of the nucleotides. This finding may suggest that one role of GEFs is to displace bound Mg^{2+} from Rho proteins in order to efficiently perform their function and dissociate nucleotide from Rho GTPases (Zhang et al., 2000). Taken together this data revealed an interesting and so far little investigated interplay between TRP channel and Rho GTPases in controlling cell migration mediated by Mg^{2+} homeostasis.

More recently, another member of the TRPM subfamily, TRPM4, has been recognized as the first TRP channel to be part of the adhesome that is the set of protein components of FAs required for migration and contractility (Cáceres et al., 2015). Indeed, it has been shown that TRPM4 localises to FAs in different cell types and that its suppression impaires FAs relocation and lamellipodia formation, leading to a reduced cellular spreading and migration in mouse embryonic fibroblasts. Actually, FA turnover plays a key role in cell migration, contributing to the generation of the traction forces necessary for cellular movements. TRPM4-mediated effects on cell migration are at least partially due to the activation of Rac1 GTPase. Indeed, it has been observed that upon silencing TRPM4 the serum-induced activation of Rac1 and lamellipodial distribution are significantly reduced, suggesting a direct cooperation between TRPM4 and Rac1 in the regulation of cellular spreading. Moreover, it has been demonstrated that TRPM4 pharmacological inhibition caused retarded skin wound healing *in vivo*, affecting cell contractility (Cáceres et al., 2015). TRPM4 has been found to affect the migratory behaviour of many cell types, including prostate cancer cells (Holzmann et al., 2015). Although TRPM4 itself is Ca^{2+}-impermeable, its contribution to cell migration through the regulation of intracellular Ca^{2+} signaling has been well established (Vennekens and Nilius, 2007). For instance, in androgen-insensitive prostate cancer cells, it has been shown that TRPM4 acts as an important negative feedback regulator of SOCE, thus promoting cell migration (Holzmann et al., 2015). However, further investigations are needed to deepen the knowledge of the molecular mechanism underlying the pro-migratory effect of TRPM4 on prostate cancer cell migration.

To date, several experimental pieces of evidence have revealed a critical role of TRPV4 in regulating the migratory properties of many tumors, including liver, breast and gastric cancer (Vriens et al., 2004; Lee et al., 2016; Xie et al., 2017). However, little is known about the molecular mechanism driving this process. Nonetheless, it has been shown that TRPV4 is involved in the dynamics of trailing adhesions, likely through an interplay with other cation channels or proteins present at the FA sites (Mrkonjić et al., 2015). A direct correlation between TRPV4 and RhoA/ROCK pathways has been revealed in cardiac fibroblast remodelling and myofibroblast contraction. In particular, it has been shown that after stimulation with growth factors, TRPV4 contributes to cell contractility by increasing the actin protein α-SMA expression and incorporation

into stress fibers, through the Ca^{2+}-mediated activation of RhoA/ROCK pathways (Tomasek et al., 2006; Adapala et al., 2013; Thodeti et al., 2013). Another study reported a role of TRPV4 in the modulation of adherent-junctions, mediated by the TRPV4-dependent activation of Rho GTPases, thereby promoting actin fibers organization and junctions formation (Sokabe and Tominaga, 2010). Furthermore, the exogenous up-regulation of TRPV4 in breast cancer has been shown to aid actin dynamics and lead to higher activation of cofilin, a downstream protein effector of RhoA/ROCK pathways that promotes actin filaments depolymerisation, thus conferring cellular "softness" and promoting transendothelial migration (Lee et al., 2016). Accordingly, TRPV4 knockdown reduced migration, invasion and transendothelial migration in breast cancer cells (Lee et al., 2016). Finally, a recent study of Ou-yang and co-worker (Ou-yang et al., 2018) has described the Akt/Rac1 signaling pathway through which TRPV4 promotes migration and invasion in glioma cancer cells (Ou-yang et al., 2018). Mechanistically, agonist-mediated TRPV4 activation promoted the activation of Rac1 by targeting Akt for phosphorylation, thus enhancing glioma cell migration and invasion (Ou-yang et al., 2018). Accordingly, in gastric cancer, TRPV4-mediated Ca^{2+} influx promotes cell migration through the activation of the downstream Akt/β-catenin pathways (Xie et al., 2017). Collectively, these data support a direct interplay between TRPV4 and small GTPases in controlling cytoskeletal remodeling aimed to confer migratory phenotypes.
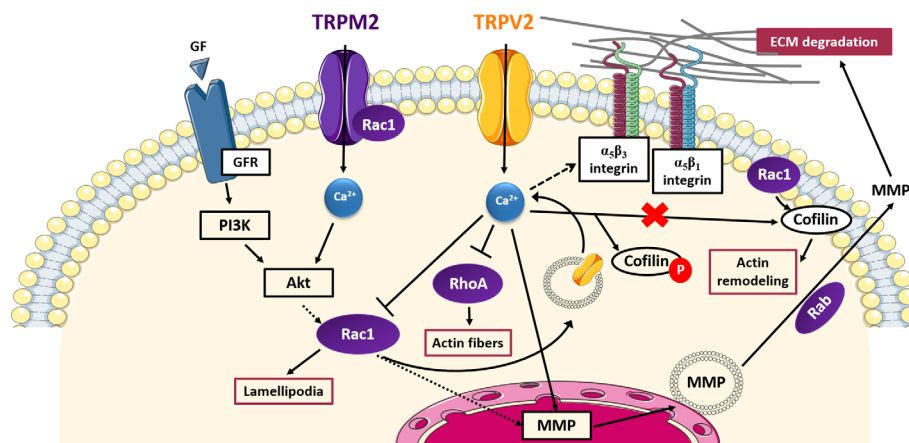
Invasion

In order to reach lymph- and bloodstreams and to colonize sites distant from the primary tumor, cancer cells have to acquire, beyond migratory phenotype, the ability to degrade ECM. Consequently, invasion is another key step of the metastatic cascade. Invasiveness of cancer cells comes from their ability to produce special protrusions called invadopodia, which are actin-rich protrusions of the plasma membrane with proteolytic activity. Once matured, invadopodia recruit proteolytic enzymes such as membrane-matrix metalloproteinases (MMPs), which are endopeptidases able to locally degrade ECM, allowing cell invasion. Among them, MMP-2 and MMP-9 are considered the most important in metastasis, since they were often aberrantly expressed in tumors (Jabłońska-trypuć et al., 2016). Both TRP channels and small GTPases have been implicated in increased tumor invasiveness through the induction of MMPs expression (Betson and Braga, 2003; Yang and Kim, 2020). Indeed, TRPM2, TRPM7, TRPM8, TRPV2, and TRPC1 have shown to cause upregulation of MMP9 in a Ca^{2+}-dependent manner in gastric, bladder, oral squamous, prostate and thyroid cancer cells respectively (Monet et al., 2010; Okamoto et al., 2012; Asghar et al., 2015; Cao et al., 2016; Chen L. et al. 2017; Chen Z. et al., 2017; Almasi et al., 2019). Additionally, TRPM2, TRPM7, and TRPC1 activity have been also correlated with MMP2 production in gastric, lung, pancreatic and thyroid cancer (Asghar et al., 2015; Rybarczyk et al., 2017; Liu et al., 2018; Almasi et al., 2019). On the other hand, Rho and Rac GTPases activation has been correlated with increased MMPs expression in different cancer cell types

(Zhuge and Xu, 2001; Abécassis et al., 2003; Santibáñez et al., 2010; Jacob et al., 2013).

TRPV2 and TRPM2 have been shown to affect cell invasiveness through signaling pathways involving small GTPases, which are summarized in **Figure 2**.

In particular, TRPV2 has been found to positively correlate with prostate cancer (PCa) invasiveness, promoting PCa progression to the aggressive castration-resistant stage (Monet et al., 2010). More specifically, it has been shown that siRNA-mediated silencing of TRPV2 leads to a decrease in MMP-2 and MMP-9 expression, reducing growth and invasive properties of PC3 prostate tumors established in nude mice xenografts (Monet et al., 2010). The mechanism by which the Ca^{2+} influx mediated by TRPV2 is linked with the up-regulation of MMPs has not been characterized. However, another study has revealed that TRPV2 trafficking to the plasma membrane correlates with enhanced cell migration and invasion in PCa, *via* the phosphoinositide 3-kinase (PI3K) pathway (Oulidi et al., 2013). Taking into account evidence of a PI3K-mediated activation of Rac1 in several tumor models (Haws et al., 2016; Ungefroren et al., 2018) and considering that Rac1 has been found to regulate TRPV2 intracellular trafficking in fibrosarcoma cells (Nagasawa and Kojima, 2015), it is possible to speculate that in PCa a PI3K-mediated Rac1 activation may allow the translocation to the plasma membrane of the "de novo" expressed TRPV2 in PC3 cells, thus giving rise of the TRPV2-mediated increase of cytosolic Ca^{2+} concentration responsible for MMPs overexpression. Confirming this hypothesis, Rac1 has been related to the up-regulation of MMP-2 and MMP-9 in fibrosarcoma (Zhuge and Xu, 2001) and transformed keratinocytes (Santibáñez et al., 2010), respectively. Unlike in PCa, TRPV2 has been found to suppress the invasiveness of fibroblast-like synoviocytes (FLS), which have an aggressive and invasive behaviour resembling that of cancer cells (Laragione et al., 2015). TRPV2 activation has been associated both *in vitro* and *in vivo* with reduced cell invasiveness and a down-regulation of the IL-1β-induced expression of MMP-2 and MMP-3 (Laragione et al., 2015). More recently, the cell signaling events mediating this TRPV2 suppressive activity have been characterized (Laragione et al., 2019). Interestingly, a direct interplay between TRPV2 channel function and RhoA/Rac1 GTPases activity in suppressing FLS cell invasion has been proposed (Laragione et al., 2019). In particular, it has been shown that, upon stimulation with the commercially-available TRPV2-specific agonist O1821, the channel nearly disappears from the plasma membrane, as well as integrins αv, β1, and β3, involved in cell binding to ECM. Concomitantly, a decrease in cell adhesion and the number of thick actin filaments and a reduction in lamellipodia formation are observed. Mechanistically, it has been found that O1821-induced TRPV2 activation causes a decrease in both RhoA and Rac1 activation, giving rise to the observed inhibition of actin filaments and lamellipodia formation, respectively. Moreover, TRPV2 activation significantly increases levels of phosphorylated (inactive) cofilin and affects the localization of active cofilin keeping it in the cytosol away from cell protrusions and lamellipodia, in which normally it exerts its function on actin remodeling, upon activation by Rac1. Considering the active involvement of Rac1 in TRPV2 trafficking found by Nagasawa and Kojima (2015), Rac1

FIGURE 2 | TRP- small GTPases signaling pathways interplay in cell invasion. Cartoon depicting TRP channels signaling pathways affecting cell invasiveness through GTPases. Rac1 promotes the translocation of TRPV2 into the plasma membrane and thus TRPV2-mediated increased in MMPs expression; TRPV2 affects also cell adhesion and invasion interfering with integrin-mediated signalling and inhibiting Rac1, RhoA, and cofilin activation by Rac1 in a Ca^{2+}-dependent manner; TRPM2 and Rac1 physically interact with each other, mutually influencing their activity and lead to an increase in MMPs production; MMPs exocytosis is mediated by the Rab superfamily of small GTPases. GF, growth factor; GFR, growth factor receptor; PI3K, phosphoinositide-3 kinase; Akt, protein-kinase B; MMP, membrane-matrix metalloproteinase; ECM, extracellular matrix.

inhibition through TRPV2 described by Laragione *et al.* might also explain the observed reduction of TRPV2 expression on the plasma membrane after channel stimulation, suggesting an intriguing negative feedback loop between TRPV2 and Rac1 regulation. Indeed, TRPV2 activation on the plasma membrane may inhibit in a Ca^{2+}-dependent manner Rac1 activation, which in turn resulted in a decrease of TRPV2 expression on the cell surface. Taken together these data account for a possible two-sided interplay between Rac1 and TRPV2, based on which TRPV2 may exert both pro- and anti-invasiveness activities depending on cell type. In the first case Rac1 is activated by the PI3K pathway, thus allowing the overexpression of the constitutively active TRPV2 on the plasma membrane and the increased calcium flow responsible for MMPs overexpression and the increased cell invasiveness (Nagasawa and Kojima, 2015); in the second case TRPV2, upon activation by external stimuli, causes Rac1 inactivation and the inhibition of pro-invasive intracellular pathways as well as the expression of TRPV2 in the membrane through a negative feedback mechanism (Laragione et al., 2019). Therefore, in an interesting interchangeable relationship, TRPV2 may act as either modulator or effector of Rac1, depending on cell type, thus reflecting the bivalent activity showed by the channel in cell invasiveness.

TRPM2 offers another potential field of investigation on TRP-small GTPase relationship in cancer cells' ability to invade surrounding tissues. In fact, it has been recently proven that TRPM2 causes an increase in MMP-9 production in gastric cancer (Almasi et al., 2019). As in the case of TRPV4, the function of TRPM2 appears to be regulated by the Akt pathway, known to regulate the activity of several GTPases including Rac1 (Ho et al., 2010), whose interaction with TRPM2 has been already well established in response to oxidative stress (Gao et al., 2014) (**Figure 2**). In this regard, it has been demonstrated that TRPM2 and Rac1 physically interact

with each other, mutually influencing their activity, similarly to what was observed between TRPC5 and Rac1 in podocytes previously described (Bezzerides et al., 2004; Tian et al., 2010). Thus, we could speculate a TRPM2-Akt-Rac1 axis in the modulation of MMP-9 expression in gastric cancer.

Tumor Vascularization

Tumor vascularization, is a critical step in the metastatic cascade since the formation of new blood vessels is crucial not only to provide sufficient oxygen and nutrients and thus promoting the continuous growth of tumors but also to drive tumor spread and metastasis. Tumor vascularization, promoted by the tumor cells themselves through the secretion of several growth factors, results aberrant and leads to the formation of new vessels characterized by abnormal morphology, irregular blood flow, and distribution, non-uniform pericyte coverage and hyper-permeability (Carmeliet and Jain, 2000; Carmeliet and Jain, 2011). TRP channels are widely expressed within vascular ECs and several data correlate aberrant TRP channels expression and/or activity with tumor vascularization, thanks to their high sensitivity to both pro-angiogenic signals and subtle changes in the local microenvironment (Brossa et al., 2019; Negri et al., 2020; Scarpellino et al., 2020). TRP channels have been related to critical steps of tumor vascularization, including cell adhesion, cell migration, enhanced permeability and *in vitro* angiogenesis (Fiorio Pla and Gkika, 2013). Similarly, several Rho and Ras small GTPases, thanks to their contribution to actin dynamics, cell contractility and integrin-mediated "inside-out" signaling events, have been found to be dysregulated during tumor vascularization (Carmona et al., 2009; Chrzanowska-Wodnicka, 2010). Some common pathways involving both TRP channels and small GTPases in determining aberrant tumor vascularization have been described, such as those

concerning TRPV4, TRPM8, TRPC1, and TRPC6, depicted in **Figure 3**.

TRPV4 has been the first TRP channel to be clearly implicated in tumor angiogenesis, although it can have both pro-angiogenic and anti-angiogenic effects depending on tumor type. In particular, TRPV4-mediated Ca^{2+} signals are implicated in tumor-derived endothelial cells (TECs) migration *via* a membrane-stretch activated arachidonic acid release and subsequent actin remodeling and TRPV4 insertion in the plasma membrane (Fiorio Pla et al., 2012). These data are in accordance with previous results showing that TRPV4 is required for shear stress EC reorientation in an integrin-dependent manner (Thodeti et al., 2009). However, in different TECs models, TRPV4 results to be down-regulated as compared to normal tissues. Moreover, this down-regulation is correlated with aberrant mechano-sensitivity of TECs towards ECM stiffness and subsequently with enhanced cell motility and abnormal angiogenesis. Subcutaneous injection of Lewis Lung Carcinoma cells in TRPV4 knockout mice leads to increased vascular density, higher vessel diameter, and reduced pericyte coverage, overall resulting in enhanced tumor growth (Adapala et al., 2016). More recently, it has also been shown by the same group that TRPV4 silencing causes a significant decrease in VE-cadherin expression at cell-to-cell junctions, with the subsequent increase in vascular leakage (Cappelli et al., 2019). Accordingly, overexpression or pharmacological stimulation of TRPV4 with GSK1016790A has been shown to lead to a "normalization" of the vascular endothelium, enhancing the permeability of chemotherapeutic drugs, and basically blocking tumor growth (Adapala et al., 2016). In particular, TRPV4-mediated "normalization" of aberrant capillary-like tubules *in vitro* is achieved by restoring the mechano-sensitivity of ECs toward ECM stiffness through the blockade of basal Rho activity (Adapala et al., 2016). In this contest, it was previously shown that the aberrant TECs mechano-sensitivity of TECs in response to ECM stiffness and cyclic strain results, at least partially, due to constitutively high level of Rho/ROCK basal activities (Ghosh et al., 2008). Moreover, ECs isolated from TRPV4 knockout (KO) mice display higher basal Rho activity as compared to EC WT and the inhibition of the Rho/ROCK pathway in TRPV4KO mice results in vessel normalization, confirming the role of TRPV4 as an important modulator of Rho activity also *in vivo* (Thoppil et al., 2016).

In agreement with other recent findings which have highlighted non-conducting functions of TRP channels in many processes, including the regulation of cytoskeletal dynamics (Vrenken et al., 2015), our recent study (Genova et al., 2017) has unveiled that TRPM8 inhibits ECs migration in a Ca^{2+}-independent manner. TRPM8 is mainly localized in the endoplasmatic reticulum (ER) in ECs and its expression results remarkably down-regulated in breast TECs (BTECs) as compared to healthy human microvascular ECs (HMECs) and human umbilical vein ECs (HUVECs) (Genova et al., 2017). Mechanistically, TRPM8 inhibits ECs migration and spheroid sprouting by trapping Rap1 intracellularly, thereby preventing its relocation toward the plasma membrane, which is required to activate β_1-integrin signaling. Inactive Rap1 (GDP- bound) physically interacts with the N-terminal tail of TRPM8 and interestingly, its retention within the cytosol occurs also in the presence of a TRPM8 pore mutant, demonstrating that TRPM8 inhibitory effects on ECs migration and adhesion by trapping Rap1 are independent from its channel function (Genova et al., 2017). Curiously, TRPM8 activation through specific channel agonists (icilin and menthol) or endogenous activators such as
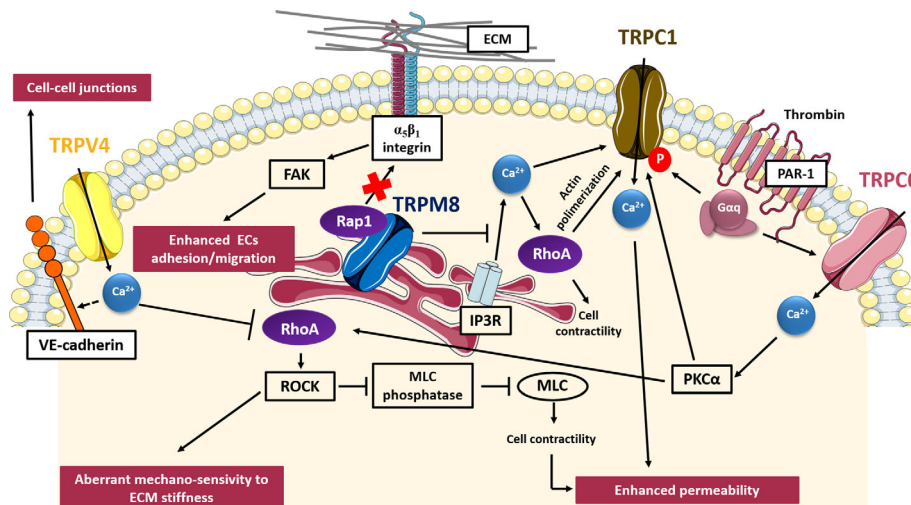
FIGURE 3 | TRP- small GTPases signaling pathways interplay in aberrant tumor vascularization. Cartoon depicting TRP channels signaling pathways affecting tumor vascularization through GTPases. TRPV4 induces aberrant mechano-sensitivity to ECM through the Ca^{2+}-dependent inhibition of Rho/ROCK pathway; TRPC1 and TRPC6 enhance vessels permeability affecting cell contractility through a Ca^{2+}-mediated regulation of RhoA; on the contrary, TRPM8 exerts a protective role in tumor vasculature permeability, inhibiting the store-operated RhoA activation and subsequent cell contraction; TRPM8 also inhibits ECs adhesion and migration, impairing activation of β_1-integrin through the intracellular retention of Rap1. VE-cadherin, vascular E-cadherin; FAK, focal adhesion kinase; ROCK, Rho-associated protein kinase; MLC, myosin light chain; PKCα, protein kinase C alpha; IP3R, inositol trisphosphate receptor; PAR-1, protease-activated receptor-1.

prostate-specific antigen (PSA), significantly reduces the amount of active Rap1-GTP bound and enhanced its inhibitory effect on migration, thus raising questions about the possible effects of agonists on TRPM8 besides pore gating. TRPM8-mediated inhibition of Rap1 cytoplasm–plasma membrane trafficking impairs the activation of inside-out signaling pathway known to trigger the conformational activation of β_1-integrin and, consequently, cell adhesion and migration. Indeed, icilin-stimulated TRPM8 significantly inhibits active-β_1 integrins as revealed by immunofluorescence assays. In addition, agonist-induced TRPM8 stimulation leads to a significant decrease in FAK phosphorylation, suggesting the involvement of FAK as a downstream effector of the β_1-integrin pathway affected by TRPM8 (Genova et al., 2017). This study has highlighted how TRP channels may regulate GTPases activity not only by the generation of local Ca^{2+} fluxes, but also acting through physical interactions which affect their intracellular localization and thus their activity. Indeed, as well as TRP channels, also small GTPases are used for spatial and temporal control of cell behavior, as reported by several studies (Yarwood et al., 2006; Wang et al., 2012; Moissoglu and Schwartz, 2014). Another evidence of a TRPM8 interplay with small GTPases in controlling ECs behavior comes from a study of Sun and coworkers (Sun et al., 2014), which has revealed a role of TRPM8 in vasoconstriction and hypertension through attenuating RhoA/Rho kinase pathway. In particular, the authors have shown that TRPM8 activation by menthol attenuates vasoconstriction by inhibiting the RhoA/ROCK pathway in wild-type mice, but not in TRPM8KO mice (Sun et al., 2014). TRPM8 effect is associated with inhibition of intracellular calcium release from the sarcoplasmic reticulum, thus reducing Ca^{2+}-mediated activation of RhoA/ROCK and ECs contraction. Since several experiments have correlated ECs contraction with vessel permeability (Hicks et al., 2010), this study could suggest a protective role of TRPM8 not only in ECs migration, but also in tumor vasculature permeability.

The increased permeability shown by tumor vessels may be induced by several factors, including hypoxia and inflammatory signals, such as thrombin. In this regard, TRPC6 and TRPC1 have been implicated in thrombin-induced hyper-permeabilization of ECs through the RhoA/ROCK signaling pathway. Thrombin is a serine-protease which binds specifically to the protease-activated receptor-1 (PAR-1) on endothelial cell surface, inducing a signaling cascade, which results in an increase in ECs contraction, changes in cell shape and finally, in the development of tiny inter-endothelial junctional gaps that lead to increased endothelial permeability. Interestingly, TRPC6 activation is promoted by thrombin stimulation through the α subunit of G protein-coupled receptors ($G\alpha_q$) (Singh et al., 2007). Then, TRPC6-mediated Ca^{2+} influx leads to the activation of PKCα and thereby induces RhoA activity and ECs contraction, with subsequent cell shape changes, inter-endothelial gaps formation and increase in EC permeability (Singh et al., 2007). Indeed, upon thrombin stimulation it has been shown that RhoA activates its downstream effector ROCK, which in turn promotes myosin light chain (MLC) phosphatase regulatory subunit phosphorylation, reducing its phosphatase activity (Birukova et al., 2004). TRPC6 have also been found to

play a central role in determining the angiogenic potential of glioma cells, since its inhibition affected EC tube formation *in vitro* by reducing the number of branch points (Chigurupati et al., 2010). Moreover, TRPC6 has been shown to exert pro-angiogenic effects by affecting vascular endothelial growth factor (VEGF)-induced calcium influx in ECs. Although there are not robust data on GTPases-mediated TRPC6 functions in angiogenesis, recently Zahra and coworkers defined an important role of RhoA in ECs proliferation, migration, invasion and sprouting triggered by important angiogenesis inducers, including VEGF (Zahra et al., 2019), thus suggesting a possible interplay between TRPC6 and RhoA in VEGF-induced angiogenesis.

Similarly to TRPC6, also TRPC1 has been found to have a role in increasing ECs permeability through RhoA activation in response to thrombin stimulation. In this regard, an intriguing mechanism depicting RhoA as a TRPC1 modulator in human pulmonary arterial endothelial cells has been proposed by Mehta and co-worker (Mehta et al., 2003). They showed that RhoA, upon thrombin-induced activation, forms a complex with IP_3R and TRPC1, which then translocates to the plasma membrane, where TRPC1 can mediate a store depletion-induced Ca^{2+} entry and the resultant increase in endothelial permeability. Moreover, it has been demonstrated that RhoA-induced TRPC1-IP_3R association is dependent on actin filament polymerization, since its inhibition hinders both the association and Ca^{2+} entry (Mehta et al., 2003). Moreover, TRPC1 serine/threonine phosphorylation by PKCα is crucial for inducing Ca^{2+} influx and consequently EC permeability (Ahmmed et al., 2004). However, the mechanism by with PKCα integrates with RhoA in ECs to trigger TRPC1-mediated Ca^{2+} influx remained to be characterized. Nonetheless, considering the previously described role of PKCα as a downstream effector of TRPC6 (Singh et al., 2007), it is possible that the PKCα-dependent RhoA-induced TRPC1 activation gives rise to a positive feedback loop that overall leads to a persistent increase endothelial permeability. This study provides an example of pathway in which small GTPases do not act as TRP Ca^{2+}-dependent effectors, but rather as modulators of TRP channel activity, influencing protein-protein interactions and channel gating, and thus corroborating the bidirectional nature of this complex interplay.

Conclusions and Perspectives

TRPs and small GTPases show a direct interplay in cancer progression, characterized by a bidirectional communication, whereby TRP channels have been shown to affect small GTPases activity *via* both Ca^{2+}-dependent or Ca^{2+}–independent pathways, and conversely some small GTPases may affect TRP channels activity through the regulation of their intracellular trafficking to the plasma membrane or acting directly on channel gating (**Table 2**). In most cases, TRP-GTPase interaction is mediated by Ca^{2+} signals, triggered by TRP-mediated Ca^{2+} influx through the plasma membrane induced by growth factors, specific ligands or mechanical stimuli. In some specific cases, such as TRPM7, TRP-mediated small GTPases activation/inhibition may be supported by Mg^{2+} rather than Ca^{2+} homeostasis regulation. Moreover, also Ca^{2+}-impermeable TRP channels like TRPM4 have been found to

TABLE 2 | TRPs-small GTPases relationship in metastatic cancer hallmarks.

Hallmark	TRP Channel		GTPase	Biological Effect	Reference
Migration	TRPC1	Ca^{2+} ←	RhoA	↑	(Mehta et al., 2003; Chung et al., 2015)
		Ca^{2+} ↔	Rac1	↑	(Guéguinou et al., 2016)
	TRPC5	Ca^{2+} ↔	Rac1	↑	(Bezzerides et al., 2004; Tian et al., 2010)
	TRPC6	Ca^{2+} →	RhoA	↓	(Tian et al., 2010)
	TRPM4		Rac1	↑	(Cáceres et al., 2015)
	TRPM7	Mg^{2+} →	RhoA	↑	(Su et al., 2011)
		Mg^{2+} →	Rac1 Cdc42		(Su et al., 2011)
	TRPV4	Ca^{2+} →	RhoA	↑	(Tomasek et al., 2006; Adapala et al., 2013; Thodeti et al., 2013; Lee et al., 2016)
		Ca^{2+} →	Rac1	↑	(Ou-yang et al., 2018)
Invasion	TRPM2	Ca^{2+} ↔	Rac1	↑	(Gao et al., 2014)
	TRPV2	←	Rac1	↑	(Nagasawa and Kojima, 2015)
		Ca^{2+} ⊣	RhoA/ Rac1	↓	(Laragione et al., 2019)
Aberrant tumor vascularization	TRPC1	←	RhoA	↑	(Mehta et al., 2003)
	TRPC6	Ca^{2+} →	RhoA	↑	(Singh et al., 2007)
	TRPM8	⊣	Rap1	↓	(Genova et al., 2017)
		⊣	RhoA	↓	(Sun et al., 2014)
	TRPV4	⊣	Rho	↓	(Thoppil et al., 2016; Adapala et al., 2016)

→: TRP enhances GTPase activity; ←: GTPase enhance TRP activity; ↔: bidirectional activation; ⊣: TRP inhibits GTPase activity; ↑: increase; ↓: decrease.

regulate small GTPases activity, probably through an indirect control on other Ca^{2+}-signaling pathways. Finally, alternative regulatory pathways that go beyond the canonical ones involving cation homeostasis have been characterized for TRP-mediated small GTPases regulation. For instance, it has been shown that some TRPs, such as TRPM8 in ECs, may act similarly to a GDI-like protein, inhibiting small GTPases activity by physically trapping and restraining them within a specific cellular compartment and thus preventing their switch to the active form that generally occurs at the plasma membrane. Although in most cases small GTPases act as TRP effectors, there are some evidence about the role of small GTPases as modulators of TRP channel activity. Indeed, it has been shown that some small GTPases may affect TRPs channel activity by influencing channel trafficking, gene expression, protein-protein interactions and channel gating. In some cases, positive feedback loop mechanisms, wherein TRP channels activate small GTPases which in turn increase TRPs insertion to the plasma membrane, have also been described. In addition, some studies have highlighted that effector/modulator roles may be interchangeable between TRPs and small GTPases depending on cell type. For instance, it has been shown that Rac1 may act as either modulator or effector of TRPV2 activity, thus determining opposite effects on cell invasion, depending on cell type.

In addition to the examples of a direct TRP-small GTPases interaction in metastatic cancer hallmarks reported in this review, many studies suggest other possible signal transduction pathways associated with tumor progression involving both TRPs and small GTPases. For instance, they have both been implicated in the regulation of MMPs production through pathways like IP3K/Akt and Hsp90α-uPA-MMP2 that offer many points of contact in between them (Koike et al., 2000; Zhuge and Xu, 2001; Yang et al., 2015; Luo et al., 2016; Rybarczyk et al., 2017; Liu et al., 2018). However, to date, a direct correlation between TRPs and small GTPases in these signal transduction pathways has not yet been established but remains to be deeper clarified. Moreover, several studies have reported a role of both these two superfamilies of molecules in promoting another key step of the metastatic cascade that is EMT. In response to the same growth factor/cytokines-induced signaling pathways both TRPs and small GTPases are able to induce the up-regulation of mesenchymal-like markers such as vimentin and the down-regulation of epithelial-like markers such as E-cadherin through the direct regulation of transcriptional factors including STAT3, Snail and Twist (Simon et al., 2000; Davis et al., 2014; Liu et al., 2014; Yang et al., 2015; Chen L. et al., 2017; Kim et al., 2018 Liu et al., 2019; Seiz et al., 2020). However, a synergistic cooperation between TRPs and Rho GTPases during the early stage of growth-factor induced EMT is still only speculation, since to the best knowledge of the authors a direct correlation between TRPs and small GTPases effects on EMT has not yet been characterized. In conclusion, the interplay between

TRP channels and small GTPase in cancer progression is only partially investigated to date and further investigations are required to shed light on many other aspects of this interesting crosstalk in cancer not well known.

AUTHOR CONTRIBUTIONS

DG conceived the study. GC provided wrote and edited the manuscript and designed the figures. AP revised the manuscript. All authors contributed to the article and approved the submitted version.

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