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Lise Rodat-Despoix, Mohamed Chamlali, Halima Ouadid-Ahidouch

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## **Biochimica et Biophysica Acta - Reviews on Cancer**

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**Tentative title:** Ion channels as key partners of cytoskeleton in cancer disease

**Authors:** Lise RODAT-DESPOIX<sup>1\*</sup>, Mohamed CHAMLALI<sup>1</sup>, Halima OUADID-AHIDOUCH<sup>1</sup>

**Affiliations:** <sup>1</sup>Laboratoire de Physiologie Cellulaire et Moléculaire (UR 4667), Université de Picardie Jules Verne, UFR des Sciences, 33 Rue St Leu, 80039 Amiens, France.

\* Corresponding author: Dr Lise Rodat-Despoix

E-mail address: [lise.despoix@u-picardie.fr](mailto:lise.despoix@u-picardie.fr)

### **Abstract:**

Several processes occur during tumor development including changes in cell morphology, a reorganization of the expression and distribution of the cytoskeleton proteins as well as ion channels. If cytoskeleton proteins and ion channels have been widely investigated in understanding cancer mechanisms, the interaction between these two elements and the identification of the associated signaling pathways are only beginning to emerge. In this review, we summarize the work published over the past 15 years relating to the roles played by ion channels in these mechanisms of reorganization of the cellular morphology, essential to metastatic dissemination, both through the physical interactions with elements of the cytoskeleton and by intracellular signaling pathways involved.

**Abbreviations:**

ADP: Adenosine diphosphate

AURKA: Aurora kinase A

Ca<sup>2+</sup>: Calcium ion

[Ca<sup>2+</sup>]<sub>i</sub>: Cytosolic calcium concentration

CAM: Cell adhesion molecules

Cdc42: Cell division control protein 42 homolog

CDK1: Cyclin-dependent kinase 1

Cl<sup>-</sup>: Chloride

ClC: Chloride intracellular channel

EB1: Microtubule plus-end-binding protein

ECM: Extracellular matrix

ER: Endoplasmic reticulum

ERM: Ezrin, radixin and moesin proteins

EVH1: Enabled/VA sodilator-stimulated phosphoprotein homology 1

FAK: Focal adhesion kinase

FERM: 4.1R, ezrin, radixin, moesin

GTPase: Guanosine triphosphate hydrolase

H<sub>2</sub>O<sub>2</sub>: Hydrogen peroxide

IP<sub>3</sub>R: Intositol triphosphate receptor

K<sup>+</sup>: Potassium

kDa: KiloDaltons

L-type calcium channel: Long-lasting type calcium channel

MAP: Microtubules associated proteins

MAPK: Mitogen-activated protein kinases

mAR: Membrane androgen receptor

mDIA: Diaphanous-related formin

Mg<sup>2+</sup>: Magnesium

NCX: Sodium/calcium exchanger

NED: Neuroendocrine differentiation

NHERF: Na<sup>+</sup>/H<sup>+</sup> exchanger regulatory factor

PDAC: Pancreatic ductal adenocarcinoma cells

PI3K: Phosphoinositide 3 kinase

PLC: Phospholipase C

PLK1: polo-like kinase 1

PM: Plasma membrane

SICE: Store-independent  $\text{Ca}^{2+}$  entry

SOC: Store-operated channel

SOCE: Store-operated calcium entry

STIM: Stromal interaction molecule

TAC: Testosterone albumin conjugates

Tg: Thapsigargin

TRP channel: Transient receptor potential channel

TRPC: Transient receptor potential canonical channel

TRPM: Transient receptor potential melastatin channel

TRPV: Transient receptor potential vanilloid channel

UTP: Uridine triphosphate

VDAC: Voltage-dependent anion channel

VGCC: Voltage-gated calcium channel

YAP: Yes-associated protein

$\text{Zn}^{2+}$ : Zinc ion

## I- Introduction:

Despite significant progress in terms of screening and treatment, cancer remains a major public health problem because of their incidence and more particularly their mortality (World Health Organization, 2020). It is well-established that cancer cells hijack more or less the whole-cell machinery to sustain cell proliferation, promote cell migration and invasion, and evade apoptosis and immune system. All these processes define the hallmarks of cancer [1]. Changes in the extracellular microenvironment, such as pH or the composition of the extracellular matrix (ECM), also help to allow cancer cells to deviate cellular metabolism to their advantage [2-4]. Ionic imbalance, whether reflected in changes in ionic homeostasis or ion fluxes is another essential parameter of tumorigenesis. Numerous studies have thus contributed to considering ionic transporters, whether they are pumps, channels or receptors, as diagnostic, prognostic or therapeutic targets for cancers [5-7]. During carcinogenesis, cells are subject to specific modifications such as a loss of control of cell cycle or apoptosis. Moreover, cancer cells can reorganize their intracellular architecture, also known as cytoskeleton, to enable cell architecture deformations to migrate and invade surrounding tissues. In the past three decades, an interplay between cytoskeleton and intercellular as intracellular ion flows has been discovered. It is thus well established that the calcium ( $\text{Ca}^{2+}$ ) signaling, as well as potassium ( $\text{K}^+$ ), magnesium ( $\text{Mg}^{2+}$ ), and chloride ( $\text{Cl}^-$ ) flow impacts cytoskeletal dynamics [8-11].

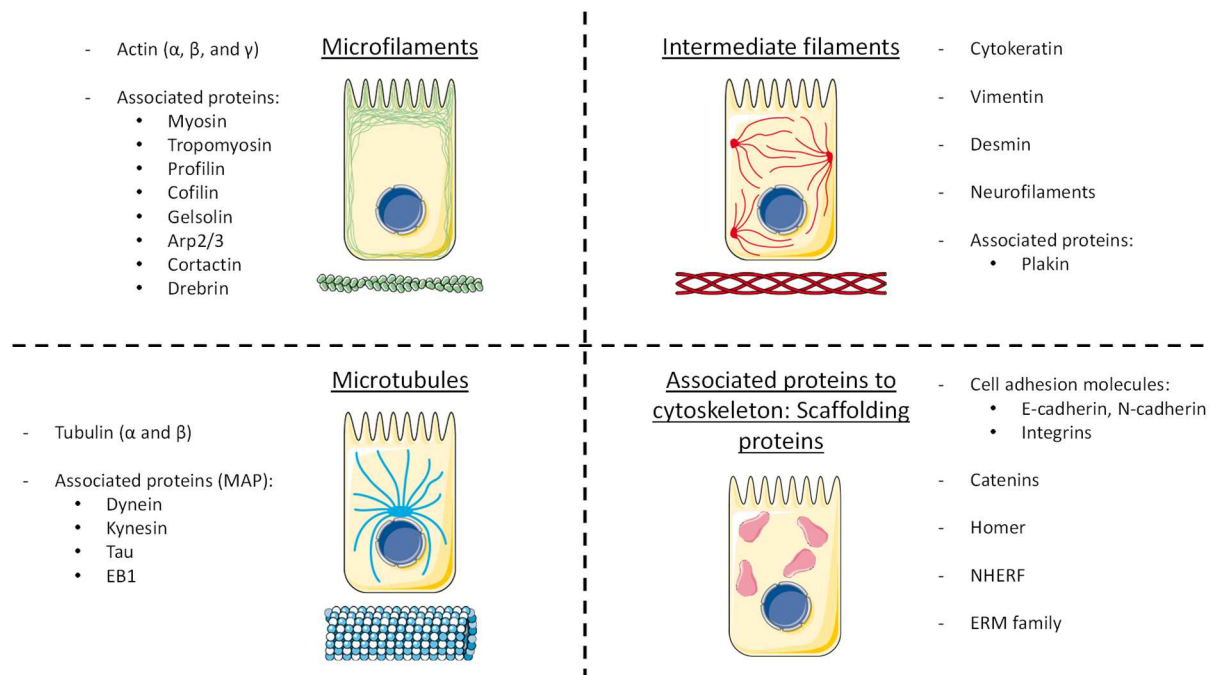
If most of these studies, conducted using drugs that selectively stabilize or destabilize microfilaments or microtubules (**Table 1**), described the impact on channel function, they could not demonstrate direct binding of specific cytoskeletal proteins to individual channel proteins [12]. This lack has been largely filled by technical advances in biochemistry and molecular biology, making it possible to dissociate the direct or indirect interactions of these channels with the components of the cytoskeleton. A relevant collection of the most significant advances on the influences between cell cytoskeleton and membrane channels, receptors, and transporters has been proposed in a very interesting issue edited by Jean-Claude Hervé [13]. However, unlike axonal growth processes, mechanical stress transduction, or cardiovascular disease for which the link between cytoskeleton and ion channels has been widely described, the identification of such connection in cancer processes is emerging.

This review discusses recent advances in our understanding of the direct and indirect interactions between ion channels and the cytoskeleton, and their roles in tumorigenesis.

## II- Cytoskeleton: an organized network

### A. Cytoskeletal filaments:

The cytoskeleton groups together a complex network of filaments ensuring the three-dimensional organization of cells. This network is made up of three main families of protein filaments; namely *microfilaments*, *microtubules*, and *intermediate filaments*; and confers mechanical, dynamic, and biological properties on cells. The assembly and disassembly of these filaments, offer to the cells the ability to maintain or remodel their internal architecture, modulating their interactions with their environment, trafficking internal organelles, or moving around one place to another (**Figure 1**).



**Figure 1:** Cytoskeletal organizational chart. *Actin and tubulin are the main representatives of microfilament and microtubule families respectively. Intermediate filaments are a more heterogeneous sub-class as well as the scaffolding proteins section.*

Filaments of the cytoskeleton with the smallest diameter (7 nm) microfilaments are formed of a central globular protein of 42 kiloDaltons (kDa): actin. Actin is the most conserved protein in eukaryotes with a much-conserved sequence broken down into 3 main isoforms:  $\alpha$ ,  $\beta$ , and  $\gamma$ -actin [14]. Associated with this central protein, actin-binding proteins or partner

proteins such as myosin [15], gelsolin [16], profilin [17], alpha-actinin [18], actin related protein 2/3 (ARP-2/3) [19], cofilin [20], allow the control of various cellular functions.

As the largest cytoskeleton filaments, with a 25 nm diameter, microtubules share also a common constitution: a globular protein of 50 kDa organized into dimers of tubulin, formed of  $\alpha$ - and  $\beta$ -subunits [21]. These heterodimers combine into polarized protofilaments, and 13 protofilaments constitute a hollow cylinder called a microtubule. To strengthen their stability, microtubules are associated with other proteins: Microtubules Associated Proteins (MAP), dyneins, and even kinesins [22, 23] which play the role of protein engines making it possible to move organelles and vesicles into the cell. The interaction between microtubules and the actomyosin cytoskeleton (under the control of Rho GTPases) is essential to cancer processes [24-27]. Several chemical products and natural or synthetic compounds have been purified and are extensively used for their specific effect on these filaments (**Table 1**).

	<b>Chemical agent</b>	<b>Impact on filament</b>	<b>Mechanism of action</b>	<b>Origin</b>	<b>References</b>
<b>ACTIN</b>	Phalloidin	Stabilize	binds to actin filaments and opposes their depolymerization	Death cap mushroom	[28]
	Jasplakinolide	Polymerize	induces an amorphous polymerization with a nucleation every 3 actin-G: the filament is then disorganized.	<i>Jaspis johnstoni</i> sponge	[29]
	Latrunculin A	Depolymerize	binds to the G-actin monomer and inhibits filament polymerization.	<i>Latrunculia Magnifica Keller</i> sponge	[30]

	Cytochalasin B	Depolymerize	covers the plus ends of the filaments	<i>Drechslera dematoidea</i> fungus	[31]
<b>MICROTUBULES</b>	Taxol®	Stabilize	attaches along the filaments	<i>Taxus brevifolia</i> Yew	[32]
	Nocodazole	Depolymerize	Binds to tubulin subunits	Synthetic	[33]
	Colchicine	Depolymerize	covers the ends of the filament	Autumn hooks	[34]

**Table 1:** Chemical agents affecting cytoskeleton organization.

While actin and tubulin have been highly conserved during evolution, families of intermediate filaments are much extended. There are various forms of intermediate, tissue-specific filaments in the cytoplasm of animal cells, including keratin filaments of epithelial cells, neurofilaments of nerve cells, and desmin filaments of muscle cells. Their main function is to provide mechanical resistance. With a 10 nm diameter, their role is thus mainly mechanical by constituting stable support for the microtubules to ensure the maintenance of the cell shape [35]. However, the proteins that make up these filaments are described as full-fledged molecular actors. Thereby, the expression of vimentin ensures mechanical stability of the cell but also appears essential for migratory and invasive properties, especially in breast cancer [36, 37]. In addition, vimentin stabilizes the Focal Adhesion Kinase (FAK) protein *via* activation of Rac1 in lung cancer [38].

### **B. Cytoskeletal associated proteins**

Cell morphology is also shaped by proteins serving as anchors to other proteins, thus facilitating their interaction. The name “scaffold” implies the formation of a stable complex, a notion further reinforced by their highly specific localization [39]. Scaffolding proteins bring together two or more proteins in a relatively stable configuration, facilitating their concerted interactions and functions. By establishing a close proximity between these proteins, they play critical roles in cellular signaling pathways (for example Mitogen-activated protein kinases (MAPK) signaling [40] or photosensory reception in *Drosophila* signaling [41]), are involved in the stabilization, sorting, recycling, and localization of cell surface receptors [42-



45] as well as in cell polarity [46]. Scaffolding proteins have thus been the subject of numerous studies, exponentially increasing the number of their members (**Figure 1**). Cell Adhesion Molecules (CAM) are major representatives of these proteins, including E- and N-cadherins as well as integrins, which determining role in the cancerous process is largely described. Indeed, E- and N-cadherins, transmembrane proteins of 120 to 140 kDa forming adherent junctions or desmosomes, are particularly involved in the epithelial-mesenchymal transition. Integrins are heterodimers made up of an alpha subunit and a beta subunit. Most of them bind to molecules of the extracellular matrix (ECM) (through their extracellular region) and actin microfilaments *via* several binding proteins that associate with their intracellular region. The activation of integrins can induce rearrangements of the actin cytoskeleton allowing the cells to adapt their morphology to the triggered processes (proliferation, migration, invasion ...). In that context, actin filaments form stress fibers that adhere to the substrate *via* the focal adhesion complexes and integrins, and which play an important role in the migratory, morphogenesis, and cellular trafficking processes [47].

Among the scaffolding proteins involved in the regulation of ion channels, Homer proteins are by far the most described. Members of the Homer family are predominantly expressed in the nervous system and are also present in peripheral tissues at low levels. Homer are adaptor proteins consisting of three members in mammals (Homer1, Homer2, and Homer3) classified into long (constitutively expressed) and short (expressed in an activity-dependent manner) forms [48]. The long Homer form contains an amino-terminal target-binding domain, which includes an Enabled/VA-sodilator-Stimulated Phosphoprotein (Ena/VASP) Homology 1 (EVH1) domain, and a carboxy-terminal self-assembly domain containing a coiled-coil domain, which allows the formation of homo- and heterocomplexes of Homer proteins, and two leucine zippers [49]. The short Homer forms lack the carboxyterminal domain. Members of the Homer family share a conserved amino-terminal EVH1-like domain, present in different Ca<sup>2+</sup>-handling proteins such as phospholipase C $\beta$  (PLC $\beta$ ), inositol triphosphate receptors (IP<sub>3</sub>Rs), ryanodine receptors, Transient receptor potential canonical (TRPC) channels, the voltage-gated calcium channel (VGCC) Cav1.2 and stromal interaction molecule 1 (STIM1) [49].

With increasing interest in oncology research these last years, the Na<sup>+</sup>/H<sup>+</sup> exchanger regulatory factor (NHERF) family of scaffolding proteins has been identified as a link between ion channels and the actin-based cytoskeleton [50]. The NHERF family is composed of four

members that possess either two or four PDZ domains. NHERF1 (EBP50) and NHERF2 (E3KARP) have two PDZ domains, whereas NHERF3 (PDZK1, CAP70, NaPi CAP-1) and NHERF4 (IKEPP, NaPi CAP-2) have four PDZ domains [51]. This PDZ domain is a common structural domain of 80-90 amino-acids playing a key role in the formation and function of signal transduction complexes [52]. Moreover, NHERF1/EBP50 and NHERF2/E3KARP display two PDZ domains and an additional Ezrin-Radixin-Moesin (ERM)-binding domain (EB) protein-interacting domain conferring the property of connecting cell surface proteins to the intracellular actin cytoskeleton underlying the plasma membrane [53]. Interestingly, a dual role of NHERF1 in tumorigenesis has been shown, depending on its subcellular localization: when NHERF1 is located at the plasma membrane, it acts as a tumor suppressor [54]; whereas, cytoplasmic localization or loss of NHERF1 membrane expression prevents NHERF1 from acting as a tumor suppressor and has been implicated in the invasive phenotype [54, 55].

In closed connection with NHERF members, ERM family has been identified as key regulators of cell signaling as well as cytoskeleton organization during cancer progression [56]. The ERM protein family consists of three closely related proteins that crosslink actin filaments with plasma membranes: Ezrin (originally isolated from chicken intestinal epithelial brush borders), Radixin (isolated from rat hepatocyte cell junctions), and Moesin (first isolated from the bovine uterus). The mammalian ERM proteins are 70–75 kDa in molecular weight and are very similar to one another, having around 72–80% amino acid identity [57]. Although there is some overlap in the involvement of ERM proteins in tumor progression, each of these three proteins exerts its role in the cancer process individually. Thus, the expression levels of ERM proteins regulate the functional activity of the protein they anchor at the cell membrane, but the relevant regulatory scaffold protein differs depending upon the transporter, and also upon the tissue in which it is expressed [56, 58]. Many studies have thus widely described the involvement of Ezrin in the cancerous process, where its expression is associated with a poor prognosis [59, 60]. If the role of Radixin and Moesin in tumor development is less described, their expression has nevertheless also been correlated with cancer progression [61].

### **III- Ion channels in direct association with cytoskeleton:**

If the organization of the cytoskeleton in healthy cells is a pledge for the cell to maintain its morphology and its anchoring in its environment, its rearrangement during tumorigenesis promotes the formation of tumors, survival, and metastasis. This reorganization is closely linked to changes in expression, localization, or even function of the ion channels with which certain components of the cytoskeleton interact.

### **1) K<sup>+</sup> channels and cytoskeleton: actin and microtubules**

Several earlier studies have shown the interaction between the cytoskeletal elements, including actin and microtubules, and ion channels in several cell types [12]. Studies over the last 25 years have also clearly deciphered the contribution of K<sup>+</sup> channels to the proliferation and migration/invasion of cancer cells, leading to consider them as "new markers" in oncological research. These cellular processes require changes in the cytoskeleton organization that directly impact the function and recruitment of channels in the plasma membrane.

#### ***Modulation of K<sup>+</sup> channels expression and activity by the cytoskeleton***

Kv10.1 (also known as Eag1, KCNH1) is a member of the voltage-gated potassium channel family and was the first identified ion channel showing oncogenic properties [62]. Its expression in normal tissues is mainly restricted to the brain [63]. However, it is overexpressed in over 70 % of human tumors and correlated with a worse prognosis [64]. Recently, Ouadid-Ahidouch group reported evidence that Kv10.1 interacts functionally with the Ca<sup>2+</sup> channel Orai1 and regulates breast cancer cell survival and migration by regulating a store-independent Ca<sup>2+</sup> entry (SICE) [65-67]. Kv10.1 is regulated during the cell cycle and it shows reduced current in the M phase [9, 68]. This regulation process is induced by alterations of its interaction with the cytoskeleton [9, 69]. Moreover, cortactin, an actin-binding protein involved in cytoskeletal architecture and frequently overexpressed in cancer [70], directly interacts with Kv10.1 in cervical cancer cell lines [71]. Indeed, cortactin binds to the carboxy terminus (C-ter) region of Kv10.1 and controls its stability at the plasma membrane. Silencing of cortactin leads to the internalization of Kv10.1. The role of cortactin in the regulation of the Kv10.1 membrane fraction is essential for its activity. Indeed, K<sup>+</sup> flow through Kv10.1 hyperpolarizes the membrane potential (V<sub>m</sub>), which increases the driving force for Ca<sup>2+</sup> [64].

#### ***Modulation of the cytoskeleton by K<sup>+</sup> Channels***

Kv10.1 is also reported to regulate proliferation by regulating cell cycle progression in several cancer types [64, 72]. Silencing of Kv10.1 induces an accumulation of cells in the G2/M phase strengthening its role in the cell cycle. However, the mechanism of action remained not determined. Recently, Movsisyan and Pardo [73] reported, how Kv10.1 participates in G2/M regulation by modulating the microtubule dynamics during mitosis in cancer cells (Hela cells). Kv10.1 has a dual effect on the mitotic microtubule dynamics through its conducting and non-conducting properties. By promoting the  $\text{Ca}^{2+}$  entry through Orai1, Kv10.1 regulates the cytosolic  $\text{Ca}^{2+}$  concentration  $[\text{Ca}^{2+}]_i$  and the frequency of cytosolic  $\text{Ca}^{2+}$  oscillations. The inhibition of Orai1 or Kv10.1 activity decreases both the  $[\text{Ca}^{2+}]_i$  and the frequency of cytosolic  $\text{Ca}^{2+}$  oscillations along with a reduction in growth speed/length as well as microtubule dynamicity. Interestingly, the blockade of the two channels has a non-additive effect demonstrating that these channels share the same pathways in regulating microtubule growth. However, silencing of Kv10.1 showed an opposite effect with an increase in  $[\text{Ca}^{2+}]_i$  and in  $\text{Ca}^{2+}$  oscillations frequency along with an increase in the dynamicity of mitotic microtubules. This suggested that Kv10.1 regulates the microtubule dynamics independently of its conducting pathway likely by interfering with the activation of kinases Cyclin-dependent kinase 1 (CDK1), Aurora kinase A (AURKA), or the polo-like kinase PLK1; even if it has not been demonstrated.

Several studies have also been led on  $\text{K}_v$  11.1 (also known as hERG 1), which is found to be overexpressed in several types of cancer and regulates different carcinogenesis processes [74, 75]. Moreover, this channel constitutes a sensor of the tumoral microenvironment [76]. Kv11.1 regulates cell migration of pancreatic ductal adenocarcinoma cells (PDAC), through a reorganization of F-actin in stress fibers and modulation of filopodia formation and dynamics [77]. Kv11.1 maintains stress fibers in a less organized arrangement in cells cultured on fibronectin that showed high levels of focal adhesion. Moreover, Kv11.1 activity determined the organization of cortical F-actin into sparse and long filopodia, and allowed F-actin polymerization in cells cultured in hypoxic conditioning medium of pancreatic stellate cells. Kv11.1 was mainly found co-localized with  $\beta$ 1-integrin in both focal adhesions and filopodia. Although the mechanism of action has not been shown in PDAC, the authors suggest the involvement of Kv11.1– $\beta$ 1-integrin-downstream signaling proteins, as paxillin, in the alteration of actin organization that regulate migration [77]. This mechanism seems to be

calcium-dependent since E4031, a specific Kv11.1 blocker, decreases intracellular  $\text{Ca}^{2+}$  concentration. The same authors also suggested that Kv11.1 could modulate cell migration by non-conductive mechanisms through a direct link to F-actin.

## 2) TRP channels

Regarding the role of the transient receptor potential (TRP) family in the regulation of the cytoskeleton, interest can be brought to the TRP Vanilloid1 (TRPV1) and TRP Melastatin 7 (TRPM7). In 2011, Caprodossi *et al* showed that the loss of TRPV1 expression in urothelial cancer cell lines led the capsaicin-treated cells to acquire a more invasive phenotype. In fact, cells treated with capsaicin have been shown to exhibit rounded morphology as well as  $\alpha$ -tubulin disassembly possibly due to increased granzyme A activity [78]. Considering the TRPM7 channel, it has been shown to participate in the formation of invadosomes as in the regulation of the cytoskeleton in N1E-115 mouse neuroblastoma cells [79]. Indeed, inhibition of TRPM7 by waixenicin-A induces the contraction of actomyosin filaments, leading to a decrease in the number of invadosomes [79]. Besides, TRPM7 regulates the contractility of the actomyosin cytoskeleton of the N1E-115 neuroblastoma cell line, through a  $\text{Ca}^{2+}$ -dependent mechanism and the activity of myosin IIA *via* the TRPM7 kinase domain [80]. Thus, treatment with bradykinin activates TRPM7 and increases the number and size of focal points. Moreover, TRPM7 activation by bradykinin induces podosome formation led by a redistribution of F-actin filaments and myosin IIA. It has also been demonstrated that the formation of podosomes induced by TRPM7 requires the activation of the kinase domain. In that context, the expression of dominant dead-kinase TRPM7 does not allow the formation of podosomes nor myosin IIA phosphorylation [80]. TRPM7 also interacts physically with cytoskeletal proteins such as myosin II (A, B, C), drebrin  $\alpha$ -actinin (1 and 4), and tropomodulin at the level of invadosomes to form a complex promoting the progression of neuroblastoma [81]. In breast cancer, TRPM7 confers a migrating phenotype by regulating actin architecture and formation of focal adhesions in the DA-MB-231 cell line. In that context, the loss of expression of TRPM7 allows the cell to acquire a contractile phenotype exhibiting in parallel an increased focal adhesion formation *via* paxilline phosphorylation [82]. In addition, TRPM7 activity helps to regulate the activation of myosin IIA fibers in breast cancer, as TRPM7 silencing reduces myosin IIA phosphorylation by 40% in MDA-MB-231 and MDA-MB-468 cell lines [83].

### 3) Other channels

Orai channels family, known as store-operated channels (SOC) and activated for the most part by stromal interaction molecule (STIM) has been characterized almost 2 decades ago. It has been found that Orai channels are overexpressed in many types of cancer such as lung, breast, liver, colorectal, and cervical cancers, where they play a critical role in many cell processes [84]. Among the Orai channels, Orai1 is the most studied SOC type channel and regulates a wide variety of physiological and pathophysiological processes. Recently, it was discovered that the Orai1 activation by STIM1 requires interaction with the cytoskeleton. Thus, it has been shown, in the context of cervical cancer and using normal cervical cells, primary cervical cancer cells, and cervical cancer cell lines, that STIM1 acquires a translocation capacity at the endoplasmic reticulum (ER) – plasma membrane (-PM) junction *via* its association with the cytoskeleton. Indeed, the activation of the STIM1 protein, by the depletion of the calcium stores of the ER, leads to its trafficking to the plasma membrane through a mechanism involving its association with the microtubule plus-end-binding protein EB1 [85-88].

Cytoskeletal filaments have also been identified to directly interact with intra-cellular channels such as the mitochondrial voltage-dependent anion channel (VDAC). This well-characterized mitochondrial outer-membrane channel regulates the permeability of the mitochondrial membrane as well as the flux of ions into the mitochondria [89].  $\alpha\beta$ -heterodimers of tubulin interact with VDAC and close the channel at nano-molar concentration [90]. The closure of VDAC by tubulin leads to a decrease in mitochondrial membrane potential of cancer cells, impacting the mitochondrial metabolism and promoting the Warburg effect [91]. Moreover, the direct interaction of gelsolin, an actin-associated protein, with VDAC enhances its closure, leading to the inhibition of the mitochondria-dependent apoptotic pathway [92].

Regarding the reticulum, Thillaiappan and collaborators detected, using total internal reflection fluorescence microscopy (TIRFM) and single-particle tracking that IP<sub>3</sub>R1, in HeLa cells, moved within the ER by diffusion or by a directional movement that depends in part on kinesin and dynein interaction. Moreover, by combined red fluorescent Ca<sup>2+</sup> indicator and TIRFM approach, they establish that Ca<sup>2+</sup> puffs are generated by immobile IP<sub>3</sub>Rs that are segregated from the more abundant pool of mobile IP<sub>3</sub>Rs [93].

#### **IV- Ion channels in indirect interaction with the cytoskeleton through scaffolding proteins:**

Allowing indirect association of epithelial ion channels with the actin-based cytoskeleton, scaffolding proteins stabilize and cluster channels at the membrane. By physically tethering proteins, scaffolds locally concentrate, compartmentalize and position transporters/receptors or enzymes in close vicinity of their substrates or regulatory proteins, avoiding background and nonspecific interactions [54].

##### **1) K<sup>+</sup> channels and cell-extracellular matrix elements: the integrins connection**

The best known K<sup>+</sup> channel interacting with cell-ECM elements is Kv11.1 through integrins (see reviews [76, 94-97]). It was shown that Kv11.1 and  $\beta$ 1-integrin co-localize in intracellular and surface membrane compartments [98]. The activation of Kv11.1 activates  $\beta$ 1-integrin. Indeed, several studies reported the involvement of the N-terminus of hERG for  $\beta$ 1-integrin binding [96, 99, 100]. Recently, He *et al.* [74], by using molecular docking, identified an amino acid residue within the hERG1 S4 voltage sensor as a potential interacting site for  $\beta$ 1-integrin. Interestingly, hERG1 is largely expressed in the heart and overexpressed in cancer but it interacts with  $\beta$ 1-integrin only in cancer cells and not in the heart. The role of the  $\beta$ 1-integrin-hERG1 complex and its signaling pathways have been extensively studied in different processes of several cancer types including leukemic, colorectal, or pancreatic cancers [77, 98, 101]. The stimulation of hERG1 current by ECM (mostly fibronectin) is accompanied by hERG1 binding to  $\beta$ 1-integrin that phosphorylates FAK and therefore regulates the adhesion receptor signaling. Recently, Crottes *et al.* [102] have identified a Sigma 1 receptor as a key regulator in the ECM-induced formation of hERG1-  $\beta$ 1-integrin complex in leukemia and colorectal cancer cells. Indeed, by increasing cell surface expression of hERG1, the Sigma1 receptor promotes the formation of a hERG1/  $\beta$ 1-integrin complex that leads to invasion, angiogenesis, and extravasation *via* the PI3K/AKT pathway. In colorectal cancer cells seeded on collagen 1, Kv11.1 forms also a macromolecular complex with  $\beta$ 1-integrin and Na<sup>+</sup>/H<sup>+</sup> exchanger 1 (NHE1) leading to intracellular pH regulation that, in turn, regulates cell motility triggered by  $\beta$ 1 integrin-dependent adhesion [103].

##### **2) Mechanosensitive channels**

Firstly characterized by Coste *et al.* [104] PIEZO channels are mechanosensitive channels composed of 2 isoforms named PIEZO 1 and 2. In the regulation of the cell architecture, the PIEZO2 isoform has been shown, in the MDA-MB-231 BrM2 cell line, to regulate the organization of the actin cytoskeleton in aggressive breast cancer cells. Indeed, it has been demonstrated that the calcium influx through PIEZO2 allows activation of the Rho/mDIA signaling pathway, promoting thus an architectural organization of actin into stress fibers *via* the transcription factor Yes-Associated Protein (YAP) [105].

Some TRP channels like TRPV4 can also be activated by a mechanical stimulus. TRPV4 has been shown to regulate the migration of breast cancer cells by reducing cell stiffness through cytoskeletal regulation. Indeed, TRPV4 overexpression causes a 5 to 10% decrease of the ERM family proteins phosphorylation [106]. In contrast, the combination with hypo-osmotic shock exacerbates this decrease by 10 to 20%, indicating a weaker binding of F-actin to transmembrane proteins. The overexpression of TRPV4 also decreases the levels of cofilin phosphorylation, the expression of E-cadherin, and increases the G-actin/F-actin ratio [106].

### **3) SOC channels**

Among the Orai channels, Orai1 is the most studied SOC type channel. In 2009, the study led by Yang *et al.* has shown that Orai1, in combination with STIM1, is required for cell migration as well as metastasis in breast cancer [107]. Indeed, they have shown that the functional invalidation of Orai1 and STIM1 leads to an increase in the size of the focal adhesions in the MDA-MB-231 breast cancer cell line. In addition, pharmacological inhibition of Orai1 by SKF96365 prevents the formation of new focal complexes, at the level of cell protrusions, and decreases the rate of focal adhesions disassembly. This result demonstrates the involvement of Orai1 in the regulation of the cytoskeleton *via* the small GTPases [107].

The actin cytoskeleton may also play a role in the regulation of calcium influx. This is the case, for example, in glioma. It has been shown that the dismantling of the actin cytoskeleton causes a decrease in the calcium signal activated by ADP or UTP and reduces the calcium entry into C6 glioma cells. In contrast, no difference was shown in the calcium signal induced by thapsigargin (Tg) [108]. Recently, it has been discovered that Orai1 expression could be regulated by a mechanism involving the cytoskeleton. Indeed, in the breast cancer cell line MCF-7, the activation of membrane androgen receptors (mARs) by testosterone albumin conjugates (TAC) triggers a strong transient increase of Orai1 at both



the transcriptional and protein level. The Orai1 overexpression required activation of Rac1 GTPase and reorganization of the actin cytoskeleton. Indeed, both the inhibition of Rac1 and the polymerization of actin prevent the effect on the expression of Orai1 [109]. In prostate cancer, it has also been found that SOC activity can be regulated by the cytoskeleton. In fact, pharmacological actin disassembly following cytochalasin D treatment has shown a decrease of the store-operated calcium entry induced by Tg. Moreover, the level of differentiation of cancer cells can also play a role in the regulation of store-operated calcium entry (SOCE): Vanoverberghe et al. [110] demonstrated that neuroendocrine differentiation (NED) of prostate cancer cells induced by androgen deprivation resulted in a decrease of SOCE due to cortical F-actin over-polymerization, whereas actin polymerization enhances Tg-induced  $\text{Ca}^{2+}$  stores depletion in undifferentiated LNCaP cells. The explanation could be the fact that when neuroendocrine differentiation occurs, over-polymerization of F-actin is observed, which may be a key point in the loss of SOCE induced by Tg in these cells. The cytoskeletal reorganization could be a signaling pathway used by cancer cells exhibiting neuroendocrine differentiation to downregulate SOC activity in prostate cancer cells [110].

#### **4) Other channels**

Interestingly, the function of TRPC1 has been shown to be modulated through an interaction with the scaffolding protein Homer in skeletal muscle sarcolemma [111]: the lack of its interaction promoting the constitutive activity of TRPC1 [112, 113]. Moreover, Homer expression was found to facilitate a physical association between TRPC1 and the  $\text{IP}_3\text{R}$  that is necessary for responses to G-protein-coupled receptor activation [114]. In human epidermoid carcinoma cells A431, Homer proteins are endogenously expressed and can specifically bind to  $\text{IP}_3\text{R1}$  and interact with components of the store-operated influx system, preventing constitutive channel activation [115].

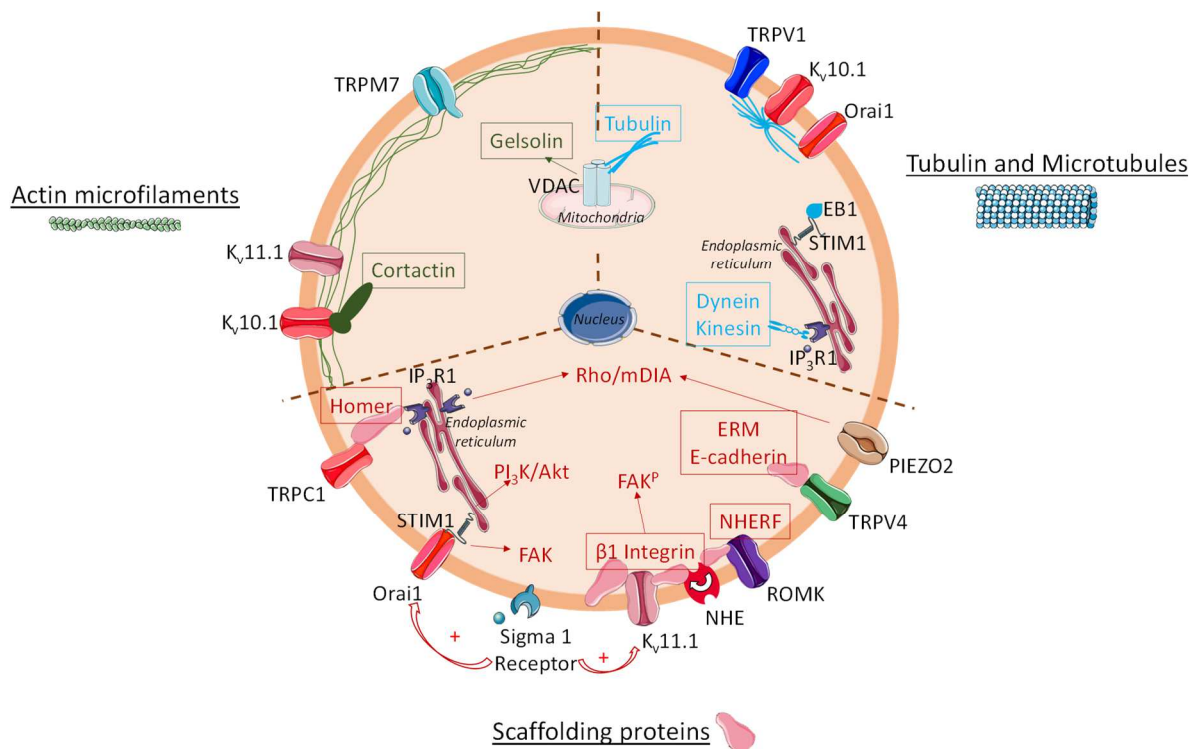
Additionally to the interaction of scaffolding protein Homer to assemble the TRPC1- $\text{IP}_3\text{R}$  complex, Vautrin-Glabik *et al.* established in breast cancer cell lines, the close link between the level of expression of  $\text{IP}_3\text{R3}$  and the remodeling of profilin cytoskeleton organization [116]. By silencing strategy, they demonstrated that  $\text{IP}_3\text{R3}$  silencing induces rounding shape and decreases adhesion in invasive breast cancer cell lines MDA-MB-231 and 435s. Moreover,  $\text{IP}_3\text{R3}$  silencing decreases Rho-GTPase activating-protein 18 (ARHGAP18) expression, RhoA activity, cell division control protein 42 homolog (Cdc42) and mDIA1

expression, and <sup>Y861</sup>FAK phosphorylation. Interestingly, IP<sub>3</sub>R3 was able to regulate profilin remodeling, without inducing any myosin II reorganization. Such a result could not be observed with IP<sub>3</sub>R1 or IP<sub>3</sub>R2 silencing, highlighting the specificity of the interaction between IP<sub>3</sub>R3 and profilin in breast cancer cell lines MDA-MB-231 and -435s [116].

Chloride channels are also linked to cytoskeletal rearrangements in metastasis processes. Indeed, Zhan *et al.* showed, in nasopharyngeal carcinoma (CNE-2Z) cells treated with 10 μM paclitaxel for 3h, an up-regulation of the sub-type 3 of chloride intracellular channel (ClC-3) proteins that are partially overlapped with the microtubules assembled from α-tubulins. Moreover, considering that the chloride currents were increased by paclitaxel, the authors speculated that the microtubule cytoskeleton may participate in the trafficking of ClC-3 proteins while the microtubule cytoskeleton in the membrane may regulate ClC-3 channel activities [117]. Channels related to cell volume regulation are also fine interacting partners of scaffold proteins, especially with the NHERF scaffolding proteins family. NHE1 has thus been proposed to facilitate cell migration through cell volume regulation, actin polymerization, collagen-integrin adhesion, and degradation of the extra-cellular matrix [118]. Moreover, the human breast cancer cell lines MCF-7 and MDA-MB-435 have higher NHE1 activity than normal human breast cells under serum deprivation conditions, a common environment in tumor tissue [119]. NHE-1 is also known to regulate invadopodium-mediated matrix degradation due to its recruitment by Talin. Indeed, Beaty *et al.* demonstrated that Talin, a member of 4.1R, ezrin, radixin, moesin (FERM) superfamily protein, is able to recruit the moesin–NHE-1 complex to invadopodia through its C-ter domain, and thus promoting mammary tumor metastasis [120]. Another ion transporter that interacts with scaffolding proteins is the Na<sup>+</sup>/Ca<sup>2+</sup> exchanger (NCX), whose knocking-down of type 1 sub-type induces an epithelial-to-mesenchymal transition in kidney epithelial cells [121]. In this study, the authors demonstrate that NCX1 knockdown does not affect the structure of the actin cytoskeleton but changes the morphology of the cell through the interruption of the connection with E-cadherin that is tightly linked to the actin cytoskeleton.

## CONCLUSION

Over the past decade, published work in oncology has helped to establish that there is a bidirectional interaction between the cytoskeleton and ion channels (Cf **Figure 2** for illustration). Indeed, the interaction of the cytoskeleton with the channels directly impacts the functioning of the latter, just as the functioning of the ion channels modulates the arrangement of the components of the cytoskeleton. It is now clearly established that this partnership between cytoskeleton and ion channels modulates the cancer process.



**Figure 2:** Schematic representation summarizing the major direct and indirect interaction of ion channels with the cytoskeleton network in cancer processes.

Ion channels have always been pharmacological targets of interest due to their location on the plasma membrane for the most part. A broad range of existing inhibitors is already in clinical use in cardiology [122] or in neurology [123]. Nevertheless, most of these inhibitors act as much on healthy cells as they do on cancer cells, with a risk that certain blockers may have off-target effects on normal physiological functions [7]. With the partially unanswered question of whether ion conductivity is needed for cancer development and progression, ion channels represent actually attractive diagnostic marker for cancer progression [3, 6, 7, 124] as chemotherapeutic resistance [125]. Aside from the potential use of ion channels as

anticancer drug targets, current clinical assays suggest using ion channels as tumor-specific targets for the delivery of toxins in a combinatorial treatments strategy [7, 126].

By disrupting mitosis, cytoskeleton-specific agents are currently used in chemotherapy since fifty years ago [127]. The potential and limitations of targeting various classes of cytoskeletal protein for therapeutic are remarkably exposed in the recent review of Ong *et al.* [91]. Pharmacological tools developed essentially target the microtubules and can be subdivided into two main classes: microtubule polymerization-inhibiting or microtubule-stabilizing agents [128, 129]. These include well-known drugs, such as taxanes and epothilones that stabilize the structure of the polymerized microtubules. Moreover, the targeting of cytoskeletal-associated proteins, can also affect cell adhesion or motility and inhibit cancer progression [91]. Nevertheless, drug resistance processes occur to microtubule-binding anti-cancer chemotherapeutics due to tubulin overexpression or interaction with other proteins disturbing drug delivery and metabolism [130]. This lead to develop pharmacological agents targeting intermediate filaments or cytoskeletal-associated protein such as integrins or tropomyosin whose inhibition impact cell morphology and tumor progression [91].

Another therapeutic strategy relies on specifically targeting cytoskeletal components, such as actin-targeting drugs like cytochalasin or jasplakinolide (*Cf Table 1*). However such a drug lacks specificity to cancer cells and induces lethality or cardiac and renal toxicity. Targeting intermediate filaments appear more suitable as they are specifically overexpressed in cancer cells vs healthy cells, limiting side effects and toxicity.

Over the past decade, special attention has also been paid to the nuclear cytoskeleton, whose closed interaction with the nuclear pore complex (NPC) has been established. The NPCs form selective channels that function together to guide protein and RNA trafficking between the nucleus and cytoplasm. NPC is also a linker of nucleoskeleton and cytoskeleton. In that context, changes in NPC-lamin interactions alter nuclear morphology in numerous cancer types [131], and alterations in nuclear plasticity may promote invasion and metastasis [132]. In conclusion, more and more studies tend to establish that ion channels can no longer only be considered as local regulators of ionic flow in biological processes but must be integrated as hubs connected with the intracellular network. In order to be as close as possible to the physiopathological reality, it seems opportune to develop new pharmacological tools taking into account these interactions between ion channels and the cytoskeleton.

**Conflict of interest statement**

All authors declare no conflict of interest

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