Insight on the Role of RKIP in Cancer through Key Protein Partners and Cellular Protrusions

FRANÇOISE SCHOENTGEN

*Institute of Mineralogy, Materials Physics and Cosmochemistry (IMPMC), Sorbonne University,
UPMC Univ Paris 06, CNRS UMR 7590, MNHN, IRD UMR 206, Paris, France*

Abstract

Previously, by comparing the cellular processes in which RKIP is implied and by analyzing the function of numerous molecular partners, we have suggested that RKIP may participate in regulating actin reorganization and membrane remodeling. Here, based on the role of RKIP in cancer, we describe the cellular function of five important partners of RKIP (GRK2, IQGAP, MT1-MMP, LC3 and Rab8). By their own activity, they may explain, at least in part, the multiple functions of RKIP as they are key actors in diverse cellular processes. All these proteins play a role in the formation or maturation of cellular protrusions such as primary cilium, lamellipodia, filopodia or invadopodia. Considering the close relationship between actin cytoskeleton and membrane during cell migration, we suggest that a major role of RKIP in cells is likely to associate with main molecular assemblies, in order to drive the cortical actin/membrane complex in response to signaling pathways. In cancer cells, because of the lack of RKIP, this important regulation is lost.

Keywords: RKIP, PEBP1, GRK2, IQGAP1, MT1-MMP, LC3, Rab8, protrusions, primary cilium, invadosomes

Abbreviations

GRK2	G protein-coupled receptor kinase 2
IQGAP	IQ motifs and GTPase activating protein (GAP) related domains 1
LC3	microtubule-associated protein 1A/1B-light chain 3
MT1-MMP	membrane type 1-matrix metalloprotein
PEBP	Phosphatidylethanolamine binding protein
Rab8	Ras-related protein Rab8
RKIP	Raf kinase inhibitory protein

Address all correspondence to

Françoise Schoentgen, IMPMC, Case 115, Sorbonne Université, UPMC, 4 Place Jussieu, F- 75005 Paris, France. Phone +33 144 277 205. Email, francoise.schoentgen@upmc.fr

Conflict of interest

No potential conflicts of interest were disclosed

Introduction

During these last few years, many studies have described RKIP as a multifunctional protein implied in numerous cell processes such cell cycle, cytokinesis, proliferation, differentiation, apoptosis, autophagy, adhesion and motility. In parallel, RKIP was shown to play a critical role in important diseases as cancer, Alzheimer disease and ciliopathies [1]. On a molecular point of view, RKIP was demonstrated to interact with a large number of molecular partners, as nucleotides, lipids and proteins [2]. Among the proteins interacting with RKIP, several kinases of signaling pathways and GTPases were identified to be inhibited by RKIP [3, 4].

Considering the scattered vision given by all these data on the biological role of RKIP, we have previously compared the cell processes in which RKIP was implied and we have analyzed the biological role of several RKIP's partners. Doing this work, it appeared that various protein partners of RKIP are involved in diverse cell processes. Moreover we have noted that, to achieve all the considered processes, the actin reorganization in close relationship with the membrane remodeling was needed, suggesting that, in response to cell signaling, RKIP may be a regulator of the intertwined actin and membrane set up [5]. Assuming that RKIP plays a role in membrane and actin changes, it seems likely that RKIP participates in cell homeostasis. Thus, it is not surprising that its role is primarily obvious in some pathologies, when cell homeostasis is disrupted.

Studies of cancer disease in patients and in cancer cells, revealed the implication of RKIP in signaling modulation, cell cycle, proliferation, adhesion, motility and resistance to drugs [3, 5]. Meanwhile, RKIP was described to be progressively lost in tumor cells of many types of cancer and its downregulation was shown to drive metastases development. This important feature led up to consider RKIP as a metastasis suppressor [6, 7]. In the following paragraphs, we mention the main signaling pathways regulated by RKIP, then, we consider some proteins interacting with RKIP in cancer and normal cells such GRK2, IQGAP, LC3 and Rab8. We also discuss MMPs whose expression is regulated by RKIP and that degrade the extracellular matrix during cell migration. Among the numerous known partners of RKIP, we chose to discuss these proteins as they are key actors in cancer and because, additionally, they allow us to understand the apparent multi-functionality of RKIP. Finally, the proteins presented here are regulators of cellular protrusions. Consequently, we consider cell protrusions modulated by RKIP and implied in cell cycle such primary cilia, and also in cell motility and adhesion, namely lamellipodia, filopodia and invadopodia. The primary cilium is particularly crucial to lead important cellular mechanisms in cell cycle, signaling, sensing, adhesion and motility. The participation of RKIP in primary cilium assembly and disassembly agrees with the broad implication of RKIP in cell fate. Among the cell protrusions, invadopodia are found in migratory cancer cells. Because RKIP is generally lacking in cancer cells, there is no direct indications of its role in invadopodia, but the mechanisms of formation and function of these protrusions give a useful insight of the potential role of RKIP in cells.

I- Brief overview of signaling protein kinases regulated by RKIP

RKIP belongs to a family of PhosphatidylEthanolamine Binding Proteins (PEBPs) implicated in numerous cellular processes in a large number of living organisms such as bacteria, yeasts, parasites, plants, insects and animals. In mammals, the main expressed isoform was firstly discovered and characterized in brain [8-11], where it was associated with membranes and phospholipids, particularly with phosphatidylethanolamine (PE) [12], then designated PEBP1. Few years later, one major discovery was the inhibitory effect of PEBP1 toward Raf1, a kinase of the signaling pathway Raf/MEK/ERK [13]. This

discovery prompted the authors to define PEBP1 as a Raf Kinase inhibitory protein (RKIP). Since then, RKIP was described to regulate many other protein kinases implicated in various signaling pathways such as NF- κ B, GPCRs, STAT3, GSK3 [14,15], PI3K/Akt/mTOR [16], Wnt [14], p38 [17] and Notch1 [18]. Thus, today RKIP is admitted to interplay with many, if not all, pivotal intracellular signaling cascades that control cellular growth, proliferation, division, differentiation, motility, apoptosis, genomic integrity, and therapeutic resistance [3]. In most cases, RKIP was demonstrated to regulate the phosphorylation cascades by inhibiting the kinases implicated in the pathway. The inhibition is due to physical interaction of RKIP with the kinases, preventing them to interact with their cascade targets. However, one exception was noted with GSK3 β which is activated by RKIP [19]. The regulation of signaling pathways appeared to depend on the cell type, revealing the ability of RKIP to regulate the pathways that are actually activated in a given cell at a given time [20].

II – Some key partners of RKIP implicated in cancer : GRK2, IQGAP, MMPs, LC3, Rab8

Previously, based on a large amount of literature, we have discussed the features and functions of about 50 proteins known to be partners of RKIP in various cellular mechanisms and in diverse normal or pathologic cell types [5]. Among them, a selection of some key proteins appeared to be representative of the main cellular functions of RKIP and are sufficient to explain its critical roles in cancer cells. In brief, GRK2 appeared to be critical in many cellular mechanisms and alone, may be responsible of the cellular effects described for RKIP. IQGAP interacting with RKIP and several targets, modulates actin network. MMPs are essential in migration and invasion of cancer cells by degrading the extracellular matrix. LC3 regulates autophagy and primary cilium formation. At last, Rab8 is a GTPase protein implicated in primary cilium formation and elongation. We discuss below the essential functionalities for each of them.

GRK2

RKIP was demonstrated to inhibit Raf-1 by direct interaction, preventing the kinase to target its downstream substrates. It was demonstrated that the PKC isoforms $-\alpha$, $-\beta$ I, $-\beta$ II, $-\gamma$, and atypical PKC ζ phosphorylate RKIP at Ser153 [3]. The phosphorylated RKIP releases from Raf1 and then binds to G protein-coupled receptor kinase 2 (GRK2) inhibiting its activity. The inhibition of GRK2 by the phosphorylated form of RKIP results in blocking the G-protein coupled receptors (GPCRs) internalization [21]. Thus, RKIP appeared to act in the control of GPCRs, a large family of membrane receptors involved in various signaling cascades. The nearly 1000 GPCRs encoded by the human genome regulate virtually all physiological functions and induce signals at the cell surface [22].

GRK2 phosphorylates activated receptors which causes them to dissociate from G proteins and leads to GPCRs internalization, a process that results in the transfer of receptors from the plasma membrane to membranes of the endosomal compartment. But, GRK2 is not just implicated in GPCRs fate and phosphorylates diverse non-GPCRs substrates displaying a complex network of functional interactions with proteins involved in many cell processes [23]. Via its complex network of connections with other cellular proteins, GRK2 contributes to the modulation of basic cellular functions such as cell proliferation, survival, or motility and is involved in metabolic homeostasis, inflammation, or angiogenic processes. Thus, given its functional connections with the most relevant signaling networks required for the viability of the cell, GRK2 has emerged as an oncomodulator able to modulate carcinogenesis in a

cell-type specific way [24]. For instance, in different breast cancer cell lines, the stimulation of estrogen or EGFR receptors, the Ras-HER2, and the hyperactivated PI3K-AKT cascades, converge in promoting enhanced GRK2 expression in transformed breast epithelial cells [25]. Moreover, GRK2 upregulation markedly favors anchorage-independent growth of luminal MCF7 or MDA-MB-231 basal cancer cells and increases their competence to trigger tumor growth *in vivo* [25]. Other available data on breast tumorigenesis indicated downregulation of GRK2 in endothelial cells and human breast cancer vessels [26].

Interestingly, inhibition of RKIP by locostatin induced cell death and reduced migration capacity of chronic lymphocytic leukemia cells (CLL) via its interaction with GRK2 [27]. Locostatin, a cell migration inhibitor, was demonstrated to bind RKIP [28]. It was shown that locostatin disrupted interactions of RKIP with Raf-1 and also with GRK2. After binding RKIP, part of locostatin is slowly hydrolyzed, leaving a smaller RKIP -butyrate adduct [29]. The use of locostatin as an inhibitor of RKIP revealed the important roles of RKIP in the regulation of cell adhesion, as it positively controls cell-substratum adhesion while negatively controls cell-cell adhesion [30]. In CLL cells, PKC is constitutively active and immuno-blotting and immuno-precipitation showed that RKIP is phosphorylated and highly expressed. It was suggested that constitutively active PKC may phosphorylate RKIP, driving its interaction with GRK2 rather than Raf-1. However, when locostatin is present, the binding of RKIP to GRK2 is inhibited as well as subsequent GRK2-mediated downregulation of ERK1/2 and Akt [27]. The effects of the RKIP inhibitor locostatin support the proposal that RKIP, via its binding to GRK2, has roles in CLL-cell migration, survival, and proliferation in the lymph node and bone marrow microenvironments by regulation of both ERK1/2 and AKT-mediated signaling.

In addition, GRK2 appeared to be a key integrative node in cell migration control and was identified as a modulator of diverse molecular processes involved in motility, such as gradient sensing, cell polarity or cytoskeletal reorganization. GRK2 dynamically associates with and phosphorylates HDAC6 to stimulate its α -tubulin deacetylase activity at specific cellular localizations such as the leading edge of migrating cells, promoting local tubulin deacetylation and enhanced motility [31]. Thus, GRK2 can play an effector role in the organization of actin and microtubule networks and in adhesion dynamics, by means of substrates and transient interacting partners, such as the GIT1 scaffold or the cytoplasmic HDAC6. The overall effect of altering GRK2 levels or activity on chemotaxis would depend on how such different roles are integrated in a given cell type and physiological context [32].

In fact, the increasing complexity of GRK2 interactome, leads to focus on the roles of this kinase in cell migration and cell cycle progression. Interestingly, GRK2 has been described to interact with various partners of RKIP, among them are PI3K, Akt, MEK, ezrin (an ERM protein), adenomatous polyposis coli (APC) [23], and HDAC6 [31]. Consistently, recent evidence suggests that GRK2 plays an important and intricate role in epithelial and immune cell migration. Moreover, GRK2 interactome is involved in the modulation of cell cycle progression by interacting with diverse regulatory networks acting at specific stages of the cell cycle [32]. Indeed, in response to both extrinsic and intrinsic cues, GRK2 protein plays a critical role in driving cell progression through G1/S and G2/M transitions in a kinase-dependent and independent manner. GRK2 is part of an intrinsic pathway that ensures timely progression of cell cycle at G2/M by means of its functional interaction with CDK2/cyclinA and Pin1 [33]. Another study [34] showed that GRK2 regulates membrane protrusion and cell migration during wound closure in Madin Darby canine kidney (MDCK) epithelial cell monolayers, at least partly, through activating phosphorylation of radixin (an ERM protein). Evidence was provided that GRK2 regulates membrane protrusion and collective migration of a cell sheet during wound closure in MDCK cell monolayers at

least partly through phosphorylation of ERM proteins, in particular radixin. In this study, the GRK2-knockdown cells migrated more slowly than control cells during wound closure. It was observed that the level of phosphorylated ERM was low in the unwounded monolayer and just after wounding [34]. However, phosphorylation began to increase by 15 minutes post-wounding. High levels of phosphorylated ERM proteins were observed 1 h, 3 h and 6 h after wounding, when MDCK cells extend membrane protrusions and actively migrate into the open area left by the wound [34].

Furthermore, GRK2 can play a effector role in the organization of actin and microtubule networks and in adhesion dynamics, by means of novel substrates and transient interacting partners, such as the GIT-1 scaffold or the cytoplasmic α -tubulin deacetylase histone deacetylase 6 (HDAC6) [31]. GRK2 also interacts with and phosphorylates the ERM proteins ezrin and radixin in response to serum or muscarinic receptor activation [32]. By bridging the plasma membrane and actin filaments at the leading edge in a phosphorylation dependent manner, ERMs contribute to local F-actin polymerization-dependent membrane protrusion. Consistently, GRK2 stimulated cortical actin reorganization and migration in an ERM-mediated manner [32]. Furthermore, besides PI3K, Akt, MEK, HDAC6 and ERM proteins which are known to interact with GRK2 [23] on the one hand and with RKIP [3, 5] on the other hand, it is of interest to mention also adenomatous polyposis coli (APC) which is considered a tumor suppressor [23, 35]. APC participates in several fundamental cellular processes. It inhibits classical Wnt signaling through forming complexes with both GSK3 β and axin to promote beta-catenin degradation [36]. APC is also involved in other vital processes including cellular adhesion and migration, organization of actin in relation with microtubule skeleton network, spindle formation, and chromosome segregation [37]. Mutations in APC gene are commonly responsible for sporadic colon cancer, and FAP, an autosomal dominantly inherited disease [38]. APC mutations result in loss of C-terminal regions and the expression of N-terminal fragments. It was suggested that a feedback loop of beta-catenin/PI3K/AKT/GSK-3 β regulates N-terminal APC fragment-induced primary cilia defects. In this loop, both beta-catenin and GSK-3 β act to up-regulate HDAC6 which leads to decreased acetylated tubulin levels, thereby causing primary cilia defects. Interestingly, these data provided an insight into the relationship between APC mutations and disease caused by primary cilia abnormality [39].

In conclusion, GRK2 regulates and phosphorylates various substrates, leading to a large number of functions in the cell. Among them are the control of the cell cycle as well as implication in cell migration by acting with HDAC6 and ERM proteins. Indeed, GRK2 contributes to microtubule networks and to local F-actin polymerization-dependent membrane protrusion, by targeting HDAC6 and ERM proteins, respectively.

IQGAP1

The Ras GTPase-activating-like protein IQGAP1 is a scaffold protein involved in the regulation of various cellular processes ranging from organization of the actin cytoskeleton, transcription and cellular adhesion to cell cycle control. Despite its name, IQGAP1 is not a GAP but stabilizes Rac1 and Cdc42 in their active GTP-bound forms and consequently modulates the cytoskeleton indirectly. Over-expression of IQGAP1 was associated with increased migration and invasion in the human breast epithelial cancer cell line MCF-7 [40, 41]. IQGAP1 may also be involved in the deregulation of proliferation and differentiation through its modulation of the MEK/ERK pathway [42].

Mapping the interactome of overexpressed RKIP in a gastric cancer cell line (SGC7901 cells) was of particular interest as a total of 72 RKIP-interacting proteins were identified by MS/MS [43]. Among the 72 proteins, 35 proteins were found to have existing interactions with RKIP as first and second level

neighbors. Previously, we have discussed the main properties of each of the 35 close partners [5]. Seven proteins were identified as first level neighbors of RKIP and among them we have noted several scaffold proteins such HSP90, 14-3-3 protein and IQGAP1, capable of forming complex assemblies implicated in various cell processes. Our attention was particularly drawn to IQGAP1 as it was shown to be involved in cancer biology, cell motility and actin remodeling [44]. Interestingly, IQGAP interacts with several proteins such as Raf1, MEK1/2, ERK1/2, PKCepsilon (which is able to phosphorylate IQGAP1 and RKIP), Akt, mTOR, Ezrin, vimentin, Filamin A, and also N-methyl-D-aspartate receptor NMDAR and APC [23, 45], all known to be also partners of RKIP [3, 5, 35, 43]. As RKIP, several interactants of IQGAP are known to be downregulated in cancer. For instance, the protein levels of RKIP and E-cadherin were significantly lower in lung squamous cell carcinoma than in the surrounding normal tissues. Both RKIP and E-cadherin are tumor suppressors and their low expression levels may be associated with initiation, invasion and/or metastasis [46]. Thus, even if the direct interaction of RKIP with IQGAP1 is not fully demonstrated, all these data suggest that they are members of common molecular assemblies. Particularly APC, interacting with RKIP and with IQGAP1, suggests that the 2 proteins are implied in protein assemblies that control actin cytoskeletal binding with microtubules [47].

In cancer, over-expression of IQGAP1 was associated with increased migration and invasion in the human breast epithelial cancer cell line MCF-7. In these cells, IQGAP1 mutant constructs and a chemical inhibitor suggested that actin, Cdc42/Rac1, and the MAPK pathway contribute to the mechanism by which IQGAP1 increases cell invasion [40, 41]. The expression of IQGAP1 in different pancreatic cancer cell lines was examined and it was found that IQGAP1 level is highly correlated with the degree of malignancy of pancreatic cancer cell metastasis. Mechanistic analysis indicated that Cdc42/Rac1 pathway might contribute to IQGAP1-mediated-pancreatic cell proliferation and tumorigenesis [48]. In pancreatic cells, IQGAP1 seems to be involved in tumor progression, since its maximum expression is at the growing front of tumor, just at the apical part of the expanding cells. These observations suggest a role for IQGAP1 in cell cycle-associated nuclear envelope assembly/disassembly and in survival, cell growth, cytokinesis, and cell migration, all key processes in tumorigenesis and carcinogenesis [48]. Furthermore, other data showed that cancer cells in which the RAF/MEK/ERK pathway is activated are particularly sensitive to the disruption of IQGAP1 function [49].

IQGAP was found to be required for the degradation activity of invadosomes. Indeed, invadosomes are characterized by an antagonist behavior, combining intense actin polymerization and adhesion to the ECM with local degradation caused by the delivery of metalloproteases such as membrane type 1 metalloproteinase (MT1-MMP). One of the mechanisms involved in degradation is the control of local delivery of MT1-MMP at the plasma membrane by the exocyst complex, through its recruitment by IQGAP1 [50]. A role for IQGAP1 in regulating early S phase replication events has also been proposed. Indeed, authors observed that the nuclear IQGAP1 localization was low in asynchronous cells, but was significantly increased in cells arrested in G1/S phase, suggesting that IQGAP1 enters the nucleus at G1/S phase and exits in late S phase [51]. Arresting cells at the G1/S boundary significantly increases the fraction of cells with nuclear IQGAP1, and knockdown of IQGAP1 delayed the ability of arrested cells to progress beyond the G1/S phase, suggesting that IQGAP1 may participate in cell cycle regulation [52].

In Vero cells (a monkey kidney epithelial line) authors showed a molecular connection between the actin cross-linking protein IQGAP1 and the microtubule-stabilizing protein APC that impacts the cells ability to migrate into a wound. Their work suggested IQGAP1 might link actin to microtubules in the leading edge of migratory cells. Remarkably, IQGAP1 and APC also bind activated forms of Rac1 and Cdc42. Consistent with these protein interactions, IQGAP, APC, Rac1, and Cdc42 all accumulate at the

leading edge of Vero cells during migration into a wound. There are several important implications of this study. First, it suggests that IQGAP1 contributes to local actin assembly at the leading edge of migrating cells. Second, it provides a means for recruiting APC to a region of actin crosslinking. Finally, it ties regional actin reorganization to microtubule-stabilizing activities [53].

In summary, IQGAP1 is involved in diverse cellular processes including cell migration and adhesion. In cancer, over-expression of IQGAP1 is associated with increased migration and invasion in the human breast epithelial cancer cell line MCF-7. Indeed, IQGAP1 is required for the exocyst dependent delivery at the cell surface of MT1-MMP and for the ECM degradation activity of invadosomes. Furthermore, IQGAP1 is an important player in the coordination between actin polymerization and MT1-MMP secretion in invadosomes. IQGAP1 is also implicated in migration of non-cancerous cells. Rac and Cdc42 facilitate the function of IQGAP1 in actin-crosslinking and determine its subcellular localization, such as lamellipodia at the front of migrating cells or intercellular adhesion sites in epithelial cells [53]. Finally, IQGAP1 localization was low in asynchronous cells, but was significantly increased in cells arrested in G1/S phase, suggesting that IQGAP1 enters the nucleus at G1/S phase and exits in late S phase [53].

Matrix metalloproteinases

Independently of direct interaction with protein partners, RKIP acts also on cellular motility in cancer through the regulation of the expression levels of other proteins. It is the case for matrix metalloproteinases (MMPs). They are calcium dependent and zinc containing endopeptidases. Collectively, they are capable of degrading all kinds of extracellular matrix proteins but also various non-matrix proteins. MMPs play a major role in normal and cancer cells by acting on proliferation, migration, differentiation, angiogenesis, apoptosis and host defense. To date, 23 MMPs have been identified in humans, 17 of them are soluble MMPs. The others, being anchored to the membrane, are named membrane-type matrix metalloproteinases (MT-MMPs) [54]. Among MT-MMPs, MT1-MMP (MMP-14) is the most extensively investigated enzyme, and its role in cancer progression has been especially highlighted. Many of the soluble MMPs are extensively characterized biochemically, but *in vivo* function of those soluble MMPs have been difficult to understand because active enzymes in tissue cannot be easily detected. Since many of them are secreted as inactive zymogens, they need to be activated extracellularly to carry out their function. The membrane anchored nature of MT-MMPs separates them from the other soluble MMPs as the cell surface is an interface between the extracellular environment and the intracellular compartment, and MT-MMPs are modifiers of the immediate cellular microenvironment [54].

Migration and invasion of cells include cytoskeletal-reorganizations and degradations of extracellular matrix (ECM) [55]. Normally, degradations of ECM are primary steps of cancer cell invasions and matrix metalloproteinases (MMPs) may be the mediators of these courses [56]. There are numerous examples of the downregulation of MMPs expression by RKIP. RKIP is especially known to inhibit cell invasion and metastasis through downregulation of several MMPs expression. In glioma cells U87, overexpression of RKIP diminished MMP2 and MMP9 expression [56]. In Cholangiocarcinoma cells that originate in the biliary epithelium, RKIP inhibited the invasive and metastatic ability of the cholangiocarcinoma cell line RBE, by downregulating MMP-9 and upregulating the tissue inhibitor of metalloproteinases (TIMP-4) mRNA expression [57]. RKIP inhibited the invasive and metastatic abilities of esophageal cancer cell line TE-1 by downregulating mRNA expression of LIN28 and MT1-MMP [58]. In a panel of breast cancer, colon cancer and melanoma cell lines, RKIP negatively regulated the invasion

of the different cancer cells through extracellular matrix barriers by controlling the expression of various MMPs, particularly MMP-1 and MMP-2 [59]. In MDA-MB-231 breast cancer cells, MMP-1, MMP-2, MMP-9, MMP-11, MMP-13, MMP-14, MMP-16, and MMP-19 were enhancers of cancer cell invasiveness. The results indicated that each upstream cancer-progression determinant reaches these MMP expressions through different sets of signaling pathways. Particularly, RKIP could be involved in the control of the expression of MMP-1, -3, -7, -11, -13 and -14 (MT1-MMP) by modulating the Raf/MEK/ERK pathway that, in turn, regulates NF- κ B, p53, cJun/cFos, PEA3 and CREB pathways [60, 61]. It was observed that the expression of c1, MMP-1, MMP-3, MMP-10 and MMP-13 are negatively correlated with RKIP expression in various breast cancer cell lines (MDA-MB231, 4T1 and 168 FARN) and clinical samples. Since expression of MMPs by cancer cells is important for cancer metastasis, it was hypothesized that RKIP may mediate suppression of breast cancer metastasis by inhibiting multiple MMPs [62]. Moreover, it was shown that the expression signature of RKIP and MMPs is better at predicting high metastatic risk than the individual gene [62]. In conclusion, the results described above suggest that RKIP is probably involved in the control of the expression of various soluble MMPs and of MT1-MMP by modulating different signaling pathways.

As a membrane-type matrix metalloproteinase, MT1-MMP required special attention. MT1-MMP (MMP-14) was the first MT-MMP to be discovered and it was characterized as a cell surface proMMP-2 activator. MT1-MMP is tethered to the plasma membrane through transmembrane domain. MT1-MMP degrades fibrillar collagens including types I, II, and III, but it does not degrade type IV collagen, a major component of basement membranes. Collagens are the most abundant ECM component and act as a major structural component and barrier matrix in tissues. Fibrillar collagens, including type I collagen, is a major barrier during cellular migration, and it is resistant to many proteinases at neutral pH except collagenases that belong to MMP family, including MMP-1, MMP-2, MMP-8, MMP-13 and MT1-MMP. Notably, MT1-MMP is the only pericellular collagenase that promotes cellular invasion in 3D-collagenous matrices. This cell invasion promoting activity requires its membrane-anchored nature, as soluble mutant MT1-MMP cannot promote cellular invasion [54]. The MT1-MMP also promotes cell migration by processing cell adhesion molecules. The role of MT1-MMP during angiogenesis is likely to be promoting invasion of endothelial cells by degrading pericellular ECM components. The cell surface localization of MT1-MMP is particularly noteworthy. MT1-MMP is the only MMP that can directly promote cellular invasion in collagen-rich matrices. It has been shown that MT1-MMP is localized in membrane structures that represent leading edge of cells including lamellipodia, filopodia and invadopodia. Localization of MT1-MMP to lamellipodia was shown to be due to association with CD44, a hyaluronan receptor. This allows MT1-MMP to indirectly associate with cytoskeletal F-actin through CD44 [63]. Vesicle transport is a major step regulating MT1-MMP secretion. It was reported that transport of MT1-MMP-containing vesicles towards a collagen matrix was triggered by binding of β 1 integrin with the collagen matrix in a Rab8GTPase-dependent manner in invasive breast cancer cells, MDA-MB231. In macrophage, MT1-MMP secretion to podosomes was found to be regulated by a set of Rab GTPases, including Rab5a, Rab8a and Rab14 [54].

Importantly, it was found that MT1-MMP is the major enzyme that express ECM-degrading activity in invadopodia structures that are protrusions responsible of cancer cells migration. The MT1-MMP is now established as one of the essential components of invadopodia. The MT1-MMP was also shown to localize at podosomes, which are invadopodia-related membrane structures found in endothelial cells, macrophages, dendritic cells and src-transformed fibroblasts [54]. Additionally, MT1-MMP may localize also to focal adhesions due to association of the MT1-MMP cytoplasmic domain with a focal adhesion

kinase (FAK)-p130Cas. During invadopodia formation, SCC61 cancer cells transduced with shRNA targeting MT1-MMP were analyzed for invadopodia and adhesion ring formation and ECM degrading ability in fixed cell assays. It was found that podosome-like adhesion rings surround invadopodia shortly after their formation and their presence is highly correlated with invadopodium activity. Integrins and ILK were critical for adhesion ring formation, localization of the vesicular adaptor protein IQGAP and the transmembrane proteinase MT1-MMP to invadopodia, and subsequent ECM degradation. All these data indicated that adhesion signaling is critical for invadopodium maturation [64]. Finally, it should be noted that MT1-MMP was also implicated in primary cilia (**Figure 1**) in the case of injured skeletal muscle regeneration. Upon injury, muscle-resident fibro/adipogenic progenitors (FAPs) proliferated and gave rise to adipocytes. Blocking FAP ciliation enhanced myofiber regeneration after injury and reduced myofiber size decline in a muscular dystrophy model. It was proposed that ciliary Hh signaling induces TIMP3 to restrict MT1-MMP activity, thereby inhibiting adipogenesis [65].

In sum, MMPs and particularly MT1-MMP are important components of various protrusions such as lamellipodia, filopodia, invadopodia and podosomes. Moreover, in the particular case of injured skeletal muscle regeneration, MT1-MMP was shown to act at primary cilia of fibro/adipogenic progenitors (FAPs) to give rise to adipocytes.

LC3

RKIP was demonstrated to bind with MAP1LC3B/LC3B (microtubule-associated protein 1 light chain 3 β) (LC3) by direct interaction. In the membranes, LC3 is conjugated with phosphatidylethanolamine (PE), then designated LC3-II. LC3 regulates initiation of autophagy through interaction with many autophagy-related proteins possessing an LC3-interacting region (LIR) motif (WXXL). RKIP interacts through the LIR motif ⁵⁵WDGL⁵⁸ that is situated in an exposed external loop of RKIP and mutation of the LIR motif disrupted its interaction with LC3. RKIP was observed specifically bound to PE-unconjugated LC3 in cells, preventing starvation-induced autophagy. These data indicated that RKIP could associate with LC3 to regulate autophagy before inclusion of LC3-II proteins within autophagosomes [66]. Upon the activation of autophagy, kinases (such as PKC isoforms) may phosphorylate RKIP to liberate LC3, leading to PE conjugation which is a critical step necessary for autophagosome biogenesis. Thus, overexpression of RKIP significantly prevented starvation-induced autophagy, while, knockdown of RKIP expression induced high levels of autophagy via stimulation of LC3-lipidation and Akt/mTORC1 activation [66].

In cancer, autophagy promotes the progression of gastric cancer at an early clinical stage (stage I) by LC3, Beclin and p62. The prognosis of the patients with LC3-positive, Beclin 1-positive, p62-positive, and autophagy-positive cancer was significantly worse than that of patients negative for the respective marker. Autophagy was associated with aggressive clinical behavior, such as vessel invasion, lymph node disease, and hepatic metastasis in gastric cancer [67]. Correlations between increased expression of LC3 and cancer development were also found in other types of cancer such as colon carcinomas [68] and oral squamous cell carcinoma [69]. On the contrary, decreased expression of LC3 was found in triple-negative breast cancer (TNBC). The results indicated that during the progression and development of TNBC, autophagy of cancer stem cells (CSCs) is low and LC3 deficiency may restrain TNBC in mature tumor cells and CSCs [70]. Decreased expression of LC3 and Beclin-1 was also described in human lung cancer [71].

It appeared that interaction between the LC3-interacting region of the cargo receptor NBR1 and LC3 proteins promotes the targeting of autophagosomes to focal adhesions which leads to sequestration of

focal adhesion proteins [72]. The resulting focal adhesion disassembly drives turnover of cell–matrix adhesions and promotes cell migration [73]. Clearly, autophagy contributes to the destabilization and turnover of cell–matrix contacts by locally capturing focal adhesion proteins. Tension induces autophagy, as shown by an increase in autophagosome-incorporated LC3 proteins coinciding with increased tension, suggesting that localized tension at focal adhesions could be one mechanism through which autophagy is spatially and temporally regulated [74].

LC3 plays a key role in primary cilium growth. Maximum activation of autophagy in response to nutrient deprivation requires the strategic location of components of the autophagic machinery at the ciliary base in an IFT and Hh signaling-dependent manner. LC3 was localized as discrete puncta along the ciliary axoneme (**Figure 1**). The presence along the cilium axoneme of the pre-autophagosomal Atg16L and integral autophagosome membrane LC3 and GABARAP as well as their IFT-dependent enrichment at the plasma membrane suggest that cilia-mediated autophagy may induce autophagosome formation from this location, making the ciliary pocket, characterized by high vesicular activity, an attractive place for autophagosome formation. While abrogation of ciliogenesis partially inhibits autophagy, blockage of autophagy enhances primary cilia growth and cilia-associated signaling during normal nutritional conditions. It was proposed that basal autophagy regulates ciliary growth through the degradation of proteins required for intraflagellar transport [75].

Branched actin networks assembled within phagophore membranes are required for generating the autophagosome membrane shape and movement. Hu et al., recently showed that during autophagy, LC3 recruits Junction-mediating and regulatory protein (JMY) to the phagophore and promotes its actin nucleation activity. Moreover, in an *in vitro* reconstitution system, it was demonstrated that membrane-bound LC3 is sufficient to recruit JMY and stimulate JMY-mediated actin filament assembly [76, 77]. LC3 interacts with microtubules via the microtubule-associated proteins MAP1A, MAP1B or MAP1S and during starvation, studies have shown that early autophagosomal structures move along the microtubules. However, more recent studies have shown that actin and LC3 co-localize under autophagy conditions [78] and that actin and LC3 co-localization occurred at the isolation membrane (vesicle's outer membrane).

In sum, LC3 is implicated in autophagosomes formation, focal adhesion disassembly and primary cilium, where it is localized with the axoneme and also at the ciliary pocket where it directs autophagy.

Rab8 GTPase

In ciliopathies, the study of photoreceptor degeneration mediated by Cep290 revealed the direct interaction of RKIP with Cep290 and an aberrant accumulation of RKIP. Moreover, in zebrafish and cultured cells, ectopic accumulation of RKIP led to defective cilia formation, an effect mediated by its interaction with the ciliary GTPase Rab8A. Overexpression of RKIP revealed mislocalization of Rab8A in about 40% of the cells and it was shown that RKIP directly interacted preferentially with the GDP-locked mutant of Rab8A while little interaction was detected with the GTP-locked mutant of Rab8A [79]. Moreover, the GTPase Rab8A is a critical component of the protein trafficking machinery of photoreceptors and studies showed that overexpression of the GDP-locked variant of Rab8A resulted in mistrafficking of rhodopsin and photoreceptor degeneration [80]. Whereas the GDP-locked variant Rab8A disrupted cilia formation, expression of the GTP-locked variant Rab8A promoted ciliogenesis. As RKIP interacts preferably with the GDP-bound form of Rab8A in the retina and accumulation of RKIP due to Cep290 mutation is associated with retinopathy, it was proposed that the RKIP-Rab8A (GDP) complex may need to dissociate for the release of Rab8A-GDP and subsequent conversion to Rab8A-GTP for

appropriate ciliary transport [79]. Finally, Rab8 was recognized as a shared regulator of ciliogenesis and immune synapse assembly [81].

Rab8 was also described to be implicated in autophagy. Numerous Rab proteins have been shown to be involved in various stages of autophagy. Rab1, Rab5, Rab7, Rab9A, Rab11, Rab23, Rab32, and Rab33B participate in autophagosome formation, whereas Rab9 is required in non-canonical autophagy. Rab7, Rab8B, and Rab24 have a key role in autophagosome maturation. Rab8A and Rab25 are also known to be involved in autophagy [82]. Rab8 activation increased Rac1 activity, whereas its depletion activated RhoA, which led to reorganization of the actin cytoskeleton. Rab8 was also associated with focal adhesions, promoting their disassembly in a microtubule-dependent manner. This Rab8 effect involved calpain, MMP14 and Rho GTPases. Moreover, data revealed that Rab8 drives cell motility by mechanisms both dependent and independent of Rho GTPases, thereby regulating the establishment of cell polarity, turnover of focal adhesions and actin cytoskeleton rearrangements, thus determining the directionality of cell migration [83].

In cancer, it is clear that Rabs, although not usually considered oncogenic products, could profoundly affect the proliferation, survival and invasiveness of cancer cells. Rabs' loss or gain-of-function resulting from deletion or gene amplification could therefore promote cell transformation and/or enhance tumor cell motility. Through modulation of integrin traffic and MT1-MMP activity, Rabs may act on tumor cell adhesion, migration and invasion [84]. As described above, MT1-matrix metalloproteinase (MT1-MMP) is one of the most critical factors in the invasion machinery of tumor cells. Subcellular localization to invasive structures is key for MT1-MMP proinvasive activity. It was reported that polarized exocytosis of MT1-MMP occurs during MDA-MB-231 adenocarcinoma cell migration into collagen type I three-dimensional matrices. Polarized trafficking of MT1-MMP is triggered by β 1 integrin-mediated adhesion to collagen, and is required for protease localization at invasive structures. Localization of MT1-MMP within VSV-G/Rab8-positive vesicles suggests the involvement of the exocytic traffic pathway. Furthermore, constitutively active Rab8 mutants induce MT1-MMP exocytic traffic, collagen degradation and invasion, whereas Rab8-knockdown inhibited these processes. Thus, it was concluded that MT1-MMP delivery to invasive structures, and therefore its proinvasive activity, is regulated by Rab8 GTPase [85]. Later on, further studies revealed that Rab8 activation significantly induced Rac1 and Tiam1 to mediate cortical actin polymerization and RhoA-dependent stress fiber disassembly. Indeed, Rab8 activation increased Rac1 activity, whereas its depletion activated RhoA, which led to reorganization of the actin cytoskeleton. In the metastatic breast carcinoma cell line MDA-MB-231, Rab8 knocking down significantly induced stress fiber assembly and reduced cortical actin levels, while promoting a reorganization of focal adhesions, which appeared larger and more abundant at central cellular areas [83]. In MDA-MB-231 cells, Rab8 was critical for sustaining cell polarization and migration directionality. Taken together, the results revealed that Rab8 mediates directional migration by regulating the homeostasis of Rho GTPases (Rac1 and RhoA), thereby reorganizing the actin cytoskeleton and focal adhesions. Additionally, the results defined a mechanism of Rab8-regulated focal adhesion disassembly mediated by the proteases calpain and MT1-MMP independently of Rho GTPases [83].

Activation of Rab8 is linked to the formation of filopodia, lamellipodia, ruffles, and primary cilia, whereas inhibition of Rab8 affects negatively their appearance. All these structures contain actin [86]. Rab8 was also associated with focal adhesions, promoting their disassembly in a microtubule-dependent manner. This Rab8 effect involved calpain, MT1-MMP and Rho GTPases. Moreover, the role of Rab8 was also demonstrated in the cell migration process. The data revealed that Rab8 drives cell motility by mechanisms both dependent and independent of Rho GTPases, thereby regulating the establishment of

cell polarity, turnover of focal adhesions and actin cytoskeleton rearrangements, thus determining the directionality of cell migration [83]. In HeLa cells, Rab8 Wild Type induced formation of multiple local actin-based cell protrusions, whereas the constitutively active Rab8Q67L mutant promoted widespread cortical actin rearrangements over the cell perimeter, suggesting that the promotion of cellular protrusions is dependent on Rab8 activation occurring locally at the cell surface. Rab8 overexpression and activation induced cortical polymerized actin and decreased actin fibrillary structures in central (i.e. inner) areas of the cell. Thus, it appeared that Rab8 is involved not only in actin cytoskeleton organization – affecting both cortical actin and the formation of stress fiber – but also in the size and distribution of focal adhesions [83].

These data reveal that Rab8 drives cell motility by mechanisms both dependent and independent of Rho GTPases, thereby regulating the establishment of cell polarity, turnover of focal adhesions and actin cytoskeleton rearrangements, thus determining the directionality of cell migration. It is clear that Rab8, although not usually considered oncogenic products, could profoundly affect the proliferation, survival and invasiveness of cancer cells. Rab8 loss or gain-of-function resulting from deletion or gene amplification could therefore promote cell transformation and/or enhance tumor cell motility. Rab8 possible influence on tumor cell adhesion, migration and invasion through modulation of integrin traffic and MT1-MMP activity were shown [86].

In conclusion, activation of Rab8 is linked to the formation of filopodia, lamellipodia, protrusions, ruffles, and primary cilia, whereas inhibition of Rab8 affects negatively their appearance. Thus, one main role of Rab8 is essentially to contribute to primary cilium formation and elongation. Rab8 is also implied in autophagosome maturation and focal adhesion.

The functional partners of RKIP discussed above may explain by their own activity the multifunctionality of RKIP. Particularly, GRK2 and RKIP are implicated in the same cellular mechanisms. Interestingly, the five proteins described here, present relationships between them, for instance, GRK2 and IQGAP share the common interactant APC and are implied in cell migration and motility. MT1-MMP and IQGAP are both localized at the adhesion ring of invadopodia. In invasive breast cancer cells, transport of MT1-MMP-containing vesicles was triggered by binding of $\beta 1$ integrin in a Rab8GTPase-dependent. Then LC3 and Rab8 are both implicated in autophagy and in primary cilium formation. Furthermore, all these partners of RKIP participate in the formation of diverse cellular protrusions and in cell migration.

III- Cellular protrusions driven by RKIP and its partners: primary cilium, lamellipodia, filopodia and invadopodia

It must be recalled at this point, that to date, more than 80 proteins were described to interact directly or indirectly with RKIP. By searching the known properties of numerous RKIP interactants, we have previously noticed that a large part of them are multifunctional proteins, involved in molecular assemblies that control cytoskeletal architecture and transmit signaling pathways through the cell [5]. Strikingly, several partners of RKIP appeared to participate in cortical actin reorganization in close coordination with membrane or in connection with microtubules and intermediate filaments [5].

Here, based on the observation that RKIP is considered a suppressor of metastases in numerous cancer types, we focus on some protein partners that can explain the main features of RKIP such control of cell cycle, environment sensing, cell motility and invasion. To this end, in the preceding paragraphs, we mention kinases of signaling pathways and we discuss the functional role of GRK2, IQGAP, MMPs, LC3,

and Rab8. Interestingly, these protein partners of RKIP drive the formation and the function of diverse cell protrusions involved in cell motility and invasion. To highlight the relationships between them, these main cell protrusions are described below; they are primary cilia, lamellipodia, filopodia, and invadopodia and are found in various wild type cells and in cancer cells. Primary cilia are key cellular substructures leading cell cycle and various cell processes, lamellipodia and filopodia are implied in cell migration and invadopodia govern migration and invasion of cancer cells. For their formation, maturation and action, all these protrusions require membrane remodeling in close relationship with actin cytoskeleton reorganization.

The primary cilium

The primary cilium is interesting because it drives cell mechanosensing, signal transduction and cell cycle, all important processes in which RKIP participates. So we take up space in the following paragraphs to detail the structure of primary cilium and its major roles played in cells.

RKIP and primary cilium

Using the Cep290 mutant mouse retinal degeneration (rd16), it was shown that Cep290-mediated photoreceptor degeneration is associated with aberrant accumulation of RKIP, and that Cep290 physically interacts with RKIP. For the first time, these data suggested that RKIP prevents cilia formation and is associated with Cep290-mediated photoreceptor degeneration [79]. RKIP co-localized with Cep290 at the transition zone of the photoreceptors, which is the region between the basal body and the axoneme. RKIP revealed to be present in the apical inner segment, transition zone, and to a lesser extent, at the basal body and centriole of the photoreceptors. The concentrated signal of RKIP at these sites and its ability to interact with Rab8A suggested its involvement in the regulation of docking and transport of membrane protein-containing vesicles in photoreceptors [79]. The accumulation of RKIP led to defective cilia formation in zebrafish and cultured cells, this effect being mediated by the interaction of RKIP with the ciliary GTPase Rab8A. RKIP preferentially associated with Rab8A in the presence of GDP, suggesting that the RKIP-Rab8A (GDP) complex may need to dissociate for subsequent conversion to Rab8A-GTP and appropriate ciliary transport. These results suggested that RKIP modulates the localization of a distinct set of proteins required for cilia assembly and maintenance (**Figure 1**). Moreover, RKIP levels appeared to be critical for cilia formation, injection of mRNA encoding RKIP into wild-type zebrafish embryos resulting in defective ciliogenesis. Finally, the accumulation of RKIP seemed to be due to increased stability of the protein. The phosphorylation of RKIP at Ser153 did not affect its ability to reduce cilia formation [79]. To complete these results, the study of double knockout mice *Cep290^{rd16}; RKIP^{ko/ko}* showed that retinal degeneration in the *Cep290^{rd16}* mice could be delayed by downregulating the expression of RKIP [87]. The data also suggested a role of CEP290 in modulating RKIP levels for normal photoreceptor development and survival. Moreover, as it was previously shown that RKIP could be degraded *via* the ubiquitin-proteasome system (UPS) [88], and given the involvement of cilia and basal body proteins in regulating the UPS [89], it seemed conceivable that CEP290 targets RKIP to the UPS [87].

Very interestingly, most of the main roles and functions of primary cilium strongly overlap with those of RKIP. The following paragraphs describe some functional areas of action known for both primary cilia and RKIP. In relation to cancer, we particularly discuss below the main implications of primary cilium in the cell cycle, signaling and sensing, extracellular matrix, autophagy, migration and invasion.

Structure of the primary cilium

The primary cilium is a single antenna-like projection of the apical membrane, it is found in nearly every human cell type. This organelle plays important roles in numerous physiological phenomena, including tissue morphogenesis, signal transduction, determination of left-right asymmetry during development, and adult neurogenesis [89]. It is also involved in the cell cycle. The cilium is formed as a membrane-bound microtubule extension, known as the ciliary axoneme, protruding from the distal end of the mother centriole at the apical surface of the cell [90]. The barrier between the cilium and the cytosol is maintained at the transition zone of the cilium, the region of the organelle just proximal to the axoneme. The primary cilium is nucleated at its base by the basal body, consisting of the eldest centriole in the cell (named the mother centriole) with associated appendage proteins that dock it to the plasma membrane [91-93] (**Figure 1**).

The primary ciliary pocket is defined as the membrane domain starting from the transition fiber to the region where ciliary sheath emerges into extracellular environment. In photoreceptors, the ciliary pocket is part of the connecting cilium/transition zone (CC/TZ) where CEP290 and RKIP were colocalized [79] (**Figure 1**). The ciliary pocket was detected in various types of cells, playing a key role in membrane trafficking and endocytosis [94]. In addition, the ciliary pocket is connected to the actin cytoskeleton and probably serves as an interface between the primary cilium and the actin cytoskeleton [95].

Primary cilium and cancer

Numerous studies have demonstrated that various types of cancer cells fail to express cilia. Notably, it has been indicated that a number of renal carcinogens induce a significant loss of cilia in renal epithelial cells. Taking all this evidence together, it can be considered that primary cilia, through their absence or dysfunction, contribute to the development of cancer via interference in well-established cancer signaling pathways, including Wnt, hedgehog and ERK/MAPK [96]. Other ciliary signalling proteins, such as Smo (a key regulator of Hh signalling) or platelet-derived growth factor receptor (PDGFR), are proto-oncogenes that are aberrantly activated at cilia in cancer [97]. Aurora A and B play a crucial role in the mitosis and cell cycle, they are overexpressed in many carcinomas, such as hepatocellular, ovarian, esophageal, bladder, breast, prostate, pancreas and lung cancers [98]. It was observed that the cancer cells with elevated activated Aurora A rarely possess a primary cilium [99]. As a consequence, the loss of primary cilia may render multiple regulatory signals incapable of tumor growth suppression [95].

Primary cilium and RKIP in the cell cycle

Primary cilia assemble specifically when cells exit the cell cycle and become quiescent or differentiate in G0 phase (**figure 2**), the cells are also competent to form cilia in G1. Only the mother centriole can initiate ciliogenesis. During the process of ciliogenesis, an axoneme is assembled, consisting of a microtubular structure, then it is disassembled as cells progress towards S phase, concomitant with remodelling of the distal end of the basal body. During S phase, centrosomes commence duplication, at which point cilia have largely disassembled. After mitosis, centrosomes are again competent to assemble primary cilia, either in G0 or in early G1 phase [97].

Using HEK-293 RKIP depleted cells (termed HEK-499) and Flp-In T-Rex-293 RKIP inducible cell lines combined with whole transcriptome analysis, it was shown that RKIP silencing accelerates DNA

synthesis and G1/S transition entry by inducing the expression of *cdc6*, several minichromosome maintenance (or MCM) proteins, *cdc45L*, cyclins D1, D2, E2, SKP2 and the downregulation of p21. Moreover, RKIP depletion accelerates the time from nuclear envelop breakdown (NEB) to anaphase markedly, while the upregulation of RKIP shortened the NEB to anaphase time. In particular, RKIP depletion induced the expression of NEK6, a molecule known to enhance G2/M transition, and down-regulated G2/M checkpoint molecules like Aurora B, cyclin G1 and sertuin that slow the G2/M transition time (**figure 2**). Particularly Aurora B is known to be inhibited by RKIP at the mitotic spindle checkpoint (G2/M). In sum, RKIP appears to control cell cycle checkpoints and prevent primary cilium disassembly [100]. Additionally, it was shown that RKIP depletion enhances cellular motility by inducing the expression/stabilization of β -catenin, vimentin, MET and PAK1. Overall, these data suggested that regulation of the cell cycle checkpoints and motility by RKIP may be fundamental to its metastasis suppressive function in cancer [100]. Other studies demonstrated that RKIP levels are critical for cilia formation as injection of mRNA encoding RKIP into wild type zebrafish embryos resulted in defective ciliogenesis. Numbers of cilia were decreased by 60% and cilia length was reduced by 45–50% compared with control cells. In RPE cells, a dose-dependent effect of RKIP levels was observed on ciliogenesis. A 1.9-fold increase in the levels of RKIP over endogenous RKIP resulted in 76% decrease in the number of ciliated cells compared with controls, whereas a 0.7-fold increase RKIP levels was associated with only a 10% decrease in the number of ciliated cells [79].

When observing RKIP activity during the cell cycle of cultured mammalian cells, hyperactivation of the MAPK pathway in RKIP-deficient cells displayed decreased localization of phosphorylated and active Aurora B to kinetochores, thus indicating RKIP activity in spindle checkpoint regulation [101]. Indeed, during G1/S and G2/M transitions, RKIP influenced cell cycle kinetics as its overexpression was found to reduce cell growth and proliferation rate, therefore suggesting a tumor suppressive role for the endogenous RKIP [100]. It is to note that the influence of RKIP on G2/M transition agrees with its implication in controlling the mitotic spindle checkpoint as during the process of cell division, the spindle checkpoint prevents separation of the duplicated chromosomes until each chromosome is properly attached to the spindle apparatus.

Primary cilium in cell signaling and sensing

Primary cilium has important sensory roles affecting multiple cellular processes, with a large proportion of G-protein coupled receptors, ion channels, and downstream effector molecules sequestered and confined to its membrane and lumen. To perform specialized sensory tasks, cilia are enriched with various receptors, for example, odorant receptors and rhodopsin to establish olfaction and vision [102]. Furthermore, the primary cilium plays an active role in multiple signaling pathways in which receptors traffic into/out of the ciliary axoneme during signal transduction [103]. A diverse array of signaling pathways have been linked to the cilium, including Hedgehog, Wnt, Notch, Hippo, GPCR, PDGF, mTOR, and TGF β and it is clear that the vast majority of signaling pathways in vertebrates function through the primary cilium. This has led to the adoption of the term “the cells’s antenna” as a description for the primary cilium [104].

Beyond its function as cell cycle checkpoint organelle, the pivotal function of cilia is sensing the cellular microenvironment and transmitting specific signals into the interior of the cell, shifting the attention to the plasma membrane that covers cilia. In mammalian cells, actin-binding proteins were identified as mechanosensitive components of cilia that might have important functions in cilia membrane dynamics. Moreover, actin networks that transmit tension via the centrosome might also link any

mechanical stimuli from the cilium to the actin cytoskeleton in the cell and might therefore be a link between mechanical ciliary bending and polarity signaling [105]. The primary cilium is sensing mechanical and chemical cues provided in the cellular environment and in some tissue types, ciliary orientation to lumens allows response to fluid flow; in others, such as bone, ciliary protrusion into the extracellular matrix allows response to compression forces [106]. Organelles sensing chemical cues, primary cilia of cholangiocytes (epithelial cells lining the biliary tree in the liver) can detect nucleotides, particularly ATP. Therefore, the effect of ATP on cell migration, invasion, and proliferation was explored. The data suggest that ATP inhibits migration and invasion in a cilia-dependent manner, with an inhibitory effect in the presence of primary cilia but a stimulatory effect in the absence of primary cilia [107].

Primary cilium and autophagy

Nutrient deprivation is a stimulus shared by both autophagy and the formation of primary cilia. Pampliega et al. showed that part of the molecular machinery involved in ciliogenesis also participates in the early steps of the autophagic process. Signaling from the cilia, such as that from the Hedgehog pathway, induces autophagy by acting directly on essential autophagy-related proteins strategically located in the base of the cilium by ciliary trafficking proteins. While abrogation of ciliogenesis partially inhibits autophagy, blockage of autophagy enhances primary cilia growth and cilia-associated signaling during normal nutritional conditions. The data suggested that basal autophagy regulates ciliary growth through the degradation of proteins required for intraflagellar transport [75].

The role of LC3, a partner of RKIP, in primary cilium autophagy has largely been discussed in the first part of this paper, thus we just recall here that the presence along the cilium axoneme of integral autophagosome membrane LC3 and GABARAP and their IFT-dependent enrichment at the plasma membrane suggest that cilia-mediated autophagy may induce autophagosome formation from this location, making the ciliary pocket, characterized by high vesicular activity, an attractive place for autophagosome formation [75].

Actin and membrane role in primary cilium

Ciliogenesis represents a dynamic process where actin, microtubule, and membrane dynamics work in concert to generate a signaling organelle. Cdc42 localizes to the basal body to regulate Hh signaling and actin dynamics, restricting the frequency of ciliated cells and axoneme length. Cdc42 recruits both the aPKC–Par6a complex and MIM to the basal body, where they regulate the activity of Src to maintain appropriate levels of actin polymerization, ciliogenesis, axoneme length, and Hh signaling [108]. On the other hand, actin dynamics orchestrate several processes that are necessary for ciliogenesis. Transporting the mother centriole (future basal body) to the appropriate cortex of the cell is an actin-dependent process. Once there, focal adhesion proteins anchor the basal body to the underlying actin cytoskeleton [108]. Drummond et al. found that regulation of actin polymerization controls primary cilia and Hh signaling. Disrupting actin polymerization, or knockdown of N-WASp/Arp3, increases ciliation frequency, axoneme length, and Hh signaling. Cdc42, a potent actin regulator, recruits both atypical protein kinase C ι /lambda and Missing-in-Metastasis (MIM) to the basal body to maintain actin polymerization and restrict axoneme length [108].

In addition, cytoplasmic F-actin negatively regulates ciliogenesis and ciliary length while depolymerization of cytoplasmic F-actin induces ciliogenesis and cilia elongation. Otherwise, actin-binding proteins (ABPs) were identified as integral components of cilia, being part of the membrane-

associated protein network. This is suggestive for a role of ABPs in mature cilia and during dynamic regulation of ciliary length. Ciliary ABPs might serve to push and pull the ciliary membrane during elongation and shortening in parallel to dynamic alteration of the microtubule core [105].

Very interestingly, to promote the cell antennae function of primary cilia, signal transduction proteins including GPCRs and their downstream effectors, are enriched within ciliary membranes, where they detect light, odorants, and secreted molecules, among other signals. Using fast tracking of single quantum dot–labeled GPCRs and a two-photon super-resolution fluorescence recovery, ciliary membranes appeared to be partitioned into highly fluid membrane nanodomains that are delimited by filamentous actin, and intrinsic ciliary membrane proteins diffused rapidly within these highly fluid local membrane domains. Moreover, data showed that the apparent confinement of the GPCRs was not caused by binding to stationary objects [109]. The obtained results showed that lipids and the μ opioid receptor underwent hop diffusion between plasma membrane domains delimited by F-actin. Data strongly indicated that ciliary membrane corrals are delimited, at least in part, by F-actin and that the dynamics of F-actin may regulate the permeability of the corral boundaries. Altogether, the studies suggest that the primary cilium is divided into functional domains, perhaps to segregate signaling cascades, as evident from enrichment of subregions of the ciliary membrane with specific proteins. The confinement of the GPCRs within corrals may dramatically increase the likelihood of a given GPCR to encounter other proteins, such as a G protein, within the same corral, and would reduce the probability of encountering one outside of the corral. Thus, the corrals may help to generate local signaling nanodomains and could conceivably play regulatory roles in ciliary signaling by limiting the number of interactions between cascade components on the time scale of signal transduction [109].

In conclusion, evidence continues to amass in support of the idea that the cilia plays an important role in cellular homeostasis, based on its ability to integrate chemical cues and flow and compression forces. Disruptions in cilia deregulate cell growth and polarity, and produce an extracellular environment, and frequent fibrosis. In turn, disruptions in the extracellular environment alter the signals received by cilia, again influencing cell growth properties [106]. Interestingly, recent studies have demonstrated that mechanical stimuli such as cell shape, contractility and extracellular matrix rigidity are major determinants of ciliogenic activity. Cell spatial confinement, low contractility or soft extracellular substrate facilitates ciliogenesis in quiescent cells. It is remarkable that actin cytoskeleton architecture is fully responsible for the regulation of ciliogenesis by these physical parameters [110].

Beside primary cilia, the other cellular protrusions: lamellipodia, filopodia, podosomes and invadopodia

Cell migration entails a plethora of activities combining the productive exertion of protrusive and contractile forces to allow cells to push and squeeze themselves through cell clumps, interstitial tissues or tissue borders. Cellular protrusions vary in their names as well as in their microstructural compositions and precise function(s) in tumor cells, starting from their positions in the tumor cells to their roles in the invasion process. Four main types of these cellular structures are distinctly identified. They are, lamellipodia, filopodia, podosomes and invadopodia. Podosomes and invadopodia are collectively known as invadosomes. Formation of these structures is driven by spatially- and temporally-regulated actin polymerization at the leading edge of the migratory cells. Lamellipodia and filopodia are implicated in cell migration, they appear on the leading edges of migrating cells and function to command the direction of the migrating cells. Invadopodia and podosomes are specialized in matrix degradation. They are special F-

actin-rich structures that arise on the ventral surface of the cell membrane. Invadopodia are found in a variety of carcinomatous cells including squamous cell carcinoma of head and neck region whereas podosomes are found in normal highly motile cells of mesenchymal and myelomonocytic lineage [111]. Invadopodia are long-lived, stable structures that last up to a few hours, as opposed to podosomes, whose lifespans is in the order of few minutes. Invadopodia and podosomes also differ in their degree of penetration into the ECM: invadopodia were shown to penetrate deep into the matrix and form filament-like processes, while podosomes display shallow appearance and, fail to form long membrane protrusions. Both podosomes and invadopodia degrade the ECM by local secretion of proteolytic enzymes [111].

Lamellipodia

Lamellipodia are defined as flat broad membranous protrusions located at the leading edge of the migrating cells. Lamellipodia determine the cell movement direction by protrusive force that arises from the actin network. This network is formed as a result of actin filament polymerization against the plasma membrane. The complex organization within the lamellipodia consists of orthogonal arrays of actin filaments with branched actin filaments near the leading edge [111]. In various cell types, formation of lamellipodia is intimately linked to the presence of functional Arp2/3 complex [112]. All filaments in the lamellipodial network are positioned with their barbed ends facing forwards and mother filament generation could be required, in principle, during all stages of lamellipodium protrusion. A dominance of branching over elongation should reduce protrusion speed of lamellipodia, but increase their persistence, and *vice versa* if elongation dominates. At the structural level, this would coincide with high network density in the high branching regime, and low network density in the low branching. Thus, the latter case corresponds to reduced protrusion stability and high elongation regime. Finally, the lamellipodium could be considered as branched actin network supported by filaments or filament bundles of Arp2/3-independent origin that can be switched on and off by Rho GTPase-triggered signalling and that is continuously turning over by assembly at the front and disassembly throughout the structure [112].

It seems that Lamellipodia may constitute initial protrusions in cancer cells. During the migratory processes, one of the most important steps is the formation of lamellipodia, which are broad, flat protrusions at the leading edge of cells that have the ability to sense the surrounding environment, and drive and guide cell locomotion. Lamellipodia formation requires the assembly of the actin cytoskeleton and the motility of membranes. Lipid rafts are liquid-ordered plasma membrane microdomains, in principle well suited to serve major roles in regulating membrane motility and besides, when the integrity of lipid rafts is disrupted, the migration of multiple cancer cells is inhibited. In melanoma cells A375 recent data indicated that lipid rafts recruit $\beta 1$ and $\beta 3$ integrin to the leading edge, thereby facilitating lamellipodia formation, in a manner dependent on the actin cytoskeleton. These findings provide insight into the association between lipid rafts and lamellipodia formation [113].

Actin polymerization is thought to be an initial step in the cancer cell motility cycle which drives the formation of cell protrusions and defines the direction of migration. Cofilin, an actin-regulating molecule, regulates the migration of cancer cells by the formation of lamellipodia and filopodia. A study demonstrated that aPKC inhibition impedes the metastasis of colorectal cancer cells by increasing phospho-cofilin and changing actin organization [114].

Filopodia

Filopodia are thin, finger-like projections formed by parallel bunches of actin filaments. As with lamellipodia, filopodia appear as spike-like processes at the leading edge of the migrating cells. Similar to lamellipodia, filopodia function to command the direction of the migrating cells and contribute to cancer cell invasion [111]. Filopodia that polymerize beyond the edges of lamellipodia display less bundled arrays that are called microspikes than the lamellipodia-like structures.

Mounting *in vitro*, *in vivo* and clinical evidence suggest an important role for filopodia in driving cancer cell invasion. Filopodia are involved in substrate tethering and environment sensing *in vivo*. They are transient and extend out from the advancing lamellipodium, whereas invadopodia are more stable, localize beneath the cell body and possess substrate degradation properties. There is an increasing body of evidence demonstrating that cells use filopodia or filopodia-like protrusions, in 3D or *in vivo*, for ECM and environment sensing. As cells migrate, filopodia are likely to not only probe and/or modify the surrounding ECM but also to interact with other cells and therefore to facilitate intercellular communication. Within a filopodium, actin-bundling proteins, such as fascin, villin or α -actinin, tightly pack parallel actin filaments into bundles with their barbed ends facing toward the plasma membrane. This unidirectional organization allows molecular motors, such as myosin-X (MYO10), to transport various proteins, including transmembrane receptors, along actin filaments to the tips of filopodia to participate in filopodia maintenance and stabilization, environment and cue sensing, establishment of cell-cell junctions and long-distance transport. Filopodia are classically associated with proteins such as myosin-X and fascin, while invadopodia contain proteins such as cortactin and the ECM-degrading protease MT1-MMP. Precisely, in filopodia it was shown that fascin over-expression leads to increased migration and invasion both in 3D and in animal models, whereas fascin gene silencing leads to reduced invasiveness [115]. In another study, using a high-throughput microscopic-based drug screen, FDA-approved calcium channel blockers were identified as potent inhibitors of filopodia formation in cancer cells. It was discovered that L-type calcium channels are functional and frequently expressed in cancer cells suggesting a previously unappreciated role for these channels during tumorigenesis. At filopodia, L-type calcium channels are activated by integrin inside-out signaling, integrin activation and Src. Moreover, L-type calcium channels promote filopodia stability and maturation into talin-rich adhesions through the spatially restricted regulation of calcium entry and subsequent activation of the protease calpain-1. It was proposed that cycles of filopodia stabilization, followed by maturation into focal adhesions, direct cancer cell migration and invasion. A major conclusion of this study is that L-type calcium channel blockers inhibit filopodia formation [116].

Podosomes

Current views suggest podosomes are structures found in both normal and pathologic cells. Podosomes have also been demonstrated in a variety of cell types that were subjected to stimulation under cell stress or matrix receptor. They are small protrusions measuring only 0.4 μm in length and 1 μm in diameter. These cellular structures are specific F-actin rich protrusions created on the inner cell membrane of the migrating cells at points of cell-extracellular matrix contacts [111].

Podosomes are actin-based dynamic structures of the plasma membrane of metazoan cells that represent sites of attachment to, and degradation of, the extracellular matrix. Key proteins in these structures include the actin regulators cortactin and (N)-WASP, the adaptor proteins Tks4 and Tks5, and the metalloprotease MT1-MMP. Podosome proteins have been implicated in human developmental and immune disorders and dysregulation of podosome formation is associated with atherosclerosis. Podosomes are also found in vascular smooth muscle and endothelial cells, as well as cells derived from

monocyte lineages and their presence correlates with migratory ability. Many cell types elaborate these structures, including invasive cancer cells, vascular smooth muscle and endothelial cells, and immune cells such as macrophages and dendritic cells [117]. These small and unstable structures are less well studied than the invadopodia described in the following paragraph.

Invadopodia

To make a connection with the preceding paragraph, it is to note that invadopodia and podosomes, often referred to, collectively, as “invadosomes,” are actin based membrane structures. Invadopodia are F-actin protrusions that arise on the internal surface of cell membrane and are closely located to the extracellular matrix (ECM). They are finger-like ventral membrane protrusions form by cancer cells during migration [111]. Given the importance of invadopodia in cancer metastasis, we provide below details concerning their structure and function. Interestingly, several proteins guiding structural or functional features of invadopodia are known to interact with RKIP. Invadopodia penetrate into ECM, and enzymatically degrade it, facilitating matrix remodeling and cell invasion across tissues, processes that occur under specific physiological conditions such as bone remodeling, as well as under pathological states such as bone, immune disorders, and cancer metastasis. Invadopodia in cancer cells are considered to possess three functional domains responsible for the metalloproteinase-based degradation of the ECM, the cytoskeleton-based mechanical penetration into the matrix, and the integrin adhesome-based adhesion to the ECM. We describe here the structural and molecular organization of each domain.

The proteolytic domain

The matrix degradation capacity of invadopodia is attributed to a variety of proteases, belonging to the metalloproteinase (MMPs), ADAM, and serine protease families. Among these, the MMPs were shown to be the most functionally significant proteases associated with invadopodia activity in cancer cells [118]. Soluble MMP9 and MMP2 are secreted around invadopodia, and promote local ECM degradation. The membrane-bound form, MT1-MMP (or MMP14), was shown to be not just an invasion-promoting component, but also a master regulator of invadopodia development whose recruitment promotes invadopodia maturation. Polarized exocytosis and transport of vesicles of MT1-MMP to sites of invadopodia is regulated by Rab8 GTPase in breast cell carcinoma.– Exocytosis was also shown to be involved in MT1-MMP regulation via IQGAP, a key regulator of cell polarity, which connects the actin cytoskeleton to microtubules. Upon activation of RhoA and CDC42, IQGAP is localized to invadopodia with sec8, a component of the exocytotic machinery; together, they regulate the docking of MT1-MMP vesicles to the plasma membrane [119].

Interestingly, five of the components of the proteolytic domain, namely MT1-MMP, MMP2, MMP9, Rab8 and IQGAP, are partners of RKIP and three of them (MT1-MMP, Rab8 and IQGAP) are described in the preceding paragraphs. Given that RKIP is lost or strongly down-regulated in most of the cancer cells, it is tempting to propose that in normal cells, RKIP participates in the control of cell migration across the ECM, preventing invadopodia formation and migration but allowing correct cell migration to assume tissue development and differentiation.

The invasive domain

Local matrix invasion, driven by invadopodia, is a highly coordinated process, whereby enzymatic ECM degradation and mechanical cytoskeletal “pushing” occur at approximately the same time and in the same area, ultimately leading to effective penetration of the cell protrusion into the matrix. The temporal sequence of events that eventually leads to invadopodia formation is not fully understood; yet it has been

proposed that this process is initiated by ligand-induced activation of receptor tyrosine kinases, such as the EGF receptor. This signaling event triggers the activation of c-src, PKC isoforms, and eventually, the adaptor protein TKS5 [119]. Actin polymerization in the core of invadopodia is regulated by small Rho-family GTPases, mostly, CDC42, which can directly activate the N-WASP-WIP complex, which, in turn, drives actin polymerization by the Arp2/3 complex. Arp 2/3 actin nucleation is promoted by the severing activity of cofilin. The actin bundle, thus formed, is further stabilized and mechanically reinforced by cortactin and fascin, producing a stable “invasive protrusion” that pushes against the ventral cell membrane, promoting its penetration into the ECM [119]. Besides actin network, other cytoskeletal systems, namely microtubules and intermediate filaments, are believed to play either direct or indirect roles in invadopodia formation and function. Transport of proteins toward the leading edge of migrating fibroblasts requires intact, functioning microtubules, a process that is thought to be important for the polarized transport of MMPs to invadopodia. Intermediate filaments were shown to interact with actin filaments, notably, vimentin intermediate filaments was shown to play a role in the invadopodia elongation [119]. To summarize, various proteins of the actin machinery work together in order to form a dense web of a dendritic actin network and long parallel bundles, which, together, apply mechanical force that pushes the plasma membrane toward and into the ECM [119].

The Adhesive Domain

Tissue cells grow and function in tight association with diverse components of the ECM. These adhesive interactions play a central role in tissue morphogenesis and remodeling, as well as in physiological and pathological cell migration. During the migration process, for example, the forward extension of lamellipodia or filopodia, driven by local actin polymerization, is guaranteed by the presence of focal adhesions or focal complexes at the posterior aspects of these protrusions [119]. In fact, it was reported that the adhesive domain of invadopodia is formed soon after the formation of the core F-actin bundle, showing an oscillatory behavior, and occasionally, disappears at later stages, after stabilization of the structure. Nevertheless, it was shown that integrin-mediated adhesion regulates invadopodia formation by activation of Rho GTPase family members and various kinases, together with recruitment of adhesion molecules. Like other adhesion structures, invadopodia are highly regulated by the chemical and physical properties of the ECM. Thereby, matrix rigidity directly increases both the number and activity of invadopodia. This mechanosensing is regulated by the contractile machinery of the cells, particularly myosin II and Rho kinase.

The special property of these three functional domains of invadopodia is their spatial organization and temporal coordination, which enables them to act in concert. Such tactics require fine-tuned crosstalk between the proteolytic (enzymatic), invasive (cytoskeletal), and adhesive domains. According to this view, the sequence of events must be the following. First, the cell utilizes its integrin adhesion machinery to probe the ECM and bind to it. Upon appropriate stimulation, global or local, actin nucleation is initiated at the cytoplasmic aspects of these adhesions, forming an initial actin core. Subsequently, the proteolytic domain is activated, presenting MT1-MMP on the ventral cell surface, and releasing MMP2 and MMP9. Next, rigorous actin polymerization in the core of the invasive domain pushes against the plasma membrane, driving the penetration into the “softened” matrix [119].

All the cell protrusions discussed here give evidence that cell migration entails a plethora of activities combining the productive exertion of protrusive and contractile forces to allow cells to push and squeeze themselves through cell clumps, interstitial tissues or tissue borders. All these activities require the generation and turnover of actin filaments that arrange into specific, subcellular structures. The diverse protrusions need also the transport of proteins to the membrane such as MT1-MMP in migratory cells and

GPCRs in primary cilium. The vesicles which contain proteins move along actin filaments, microtubules and intermediate filaments. Their traffic is, in part, regulated by Rab8 activity. By blocking Rab8 in its inactive form, RKIP may likely play a crucial role in the formation and functionality of the various cellular protrusions.

Conclusion

RKIP is considered as a multifunctional protein implicated in numerous cellular processes [5]. Several studies have shown that RKIP binds to diverse other proteins depending on the actual signals and mechanisms present in the cell. Thus, over the years, we have gained a scattered and sometimes contradictory view of the functional role played by RKIP. The RKIP partners, discussed in the preceding paragraphs, are themselves considered as multifunctional. They are engaged in key molecular complexes leading to diverse cell protrusions which drive cell cycle, adhesion, motility, invasion or autophagy. In fact, the apparent multiple functions of RKIP and partners are due to their participation in molecular complexes that conduct several important processes in cells.

With respect to the role of RKIP in cancer, the implication of its partners in proliferation, adhesion, migration and invasion seems to be particularly relevant. Here, we describe four proteins which function is modulated by a direct interaction with RKIP, namely GRK2, IQGAP, LC3 and Rab8. In brief, I) GRK2 contributes to the modulation of key cellular processes such as cell proliferation, survival and motility. Via its binding to RKIP, it has roles in chronic lymphocytic leukemia-cell migration, survival, and proliferation in the lymph node and bone marrow microenvironments [27]. Similarly to RKIP, GRK2 is also involved in cell cycle by modulating cell progression through G1/S and G2/M transitions. II) IQGAP enhances actin polymerization and colocalizes with actin in lamellipodia. As well, it was found in podosomes/invadosomes of glioblastoma cells. Over-expression of IQGAP1 is associated with increased migration and invasion in the human breast epithelial cancer cell line MCF-7 [40, 41]. III) LC3 is a critical player in autophagy and participates in primary cilium growth. It was localized as discrete puncta along the ciliary axoneme, suggesting that cilia-mediated autophagy may induce autophagosome, making the ciliary pocket, an attractive place for autophagosome formation. IV) Rab8 plays a role in autophagy and autophagosome maturation and is also recognized as a regulator of ciliogenesis. Interestingly, Rab8 controls the traffic of vesicles which contain the proteins intended to achieve the plasma membrane such as MT1-MMP in invadopodia and GPCRs in primary cilia. The transport of these proteins at the correct place in the membrane is crucial and, by blocking the inactive form of Rab8, RKIP possibly plays an important role in primary cilia mechanosensing and in migration/invasion of invadopodia. V) Finally, RKIP is not in direct contact with MMPs, but acts on cellular motility in cancer through the regulation of the expression levels of MMPs. MT1-MMP is particularly remarkable, being localized at the leading edge of cell protrusions including lamellipodia, filopodia and invadopodia.

All together, these proteins are mostly sufficient to explain the main properties of RKIP, notably its implication in cell cycle regulation, autophagy and, primarily, in cell motility. In reference to cancer cells, in which RKIP acts as a metastasis suppressor, it is to note that GRK2, MMPs, LC3, IQGAP and Rab8 are implied in protrusion formation and cell motility. Among the various protrusions, primary cilium in cell cycle and invadopodia in cell invasion, are particularly important and strongly agree with RKIP roles in cancer. Studies of invadopodia, in which RKIP is generally lost, give an idea of the role that RKIP may play, in association with other proteins, to interfere with migration and invasion of cells. In invadopodia, several known interactants (namely MT1-MMP, MMP2, MMP9, Rab8 and IQGAP) of RKIP were found

to be major actors, especially in the proteolytic domain. Because the leading edge of the protrusions in migratory cells is constituted by cell membrane underspined by actin networks, it seems necessary to consider the particular association between membrane and actin filaments. Indeed, the vast majority of forward or rearward movements of the plasma membrane are not thinkable without the dynamic turnover of actin filaments and a fine-tuned coordination between cortical actin and membrane is needed.

It is noteworthy that the actin architecture beneath the membrane, in addition to regulate the cell shape, is able to modulate the local and global composition of the membrane. Proteins and lipids into the membrane display free diffusion in small local regions. Occasionally, they jump into another area, where they again display free diffusion. These areas of free diffusion coincide with the mesh size of the underlying actin network. This leads to the conclusion that actin network, in connection with actin-membrane linker proteins, contributes to the plasma membrane compartmentalization [120]. Furthermore, membrane tension and cortical actin organization influence each other, suggesting that local changes in membrane composition may act on membrane tension, while changes in tension may lead to modification in membrane composition. Thus, it seems that the plasma membrane and actin cortex have to be considered together as a single composite that can change its composition locally and temporally. Importantly, in invadopodia, the branched actin cortex architecture may influence the shape and composition of the membrane [120]. Based on molecular and other features similarities between the diverse cellular protrusions, some authors have suggested that, despite differences in external appearances, all the regions of protrusions are just the same superstructure that appears in different locations and cells. They hypothesized that this superstructure represents an overlooked new type of organelle that they call plasma membrane-associated superstructure (PMAS) [121]. In an experimental study, the dynamics of cell shape and the distributions of actin and myosin in cells exhibiting cortical density traveling waves were investigated. A remarkable periodicity of the waves propagation, by repeated cycles of cortical compression (folding) and dilation (unfolding) that lead to periodic protrusions (oscillations) of the cell boundary, was observed. The overall shape transformation and distribution of actomyosin density were repeated from cycle to cycle even though the characteristics of the shape transformation vary significantly for different regions of the cell. During traveling, wave propagation, cortical actin and plasma membrane densities were tightly coupled at each point along the cell periphery and the major protrusion appears at the wave trailing edge just after the actin cortex density has reached a maximum. These interesting results agree with the idea that, at least in the compression/dilation traveling wave, the actin cortex and plasma membrane are tightly coupled. It could be anticipated that the quantification of boundary dynamics will lead to insights in other cell motility contexts in which periodicity is not necessarily as overt as was demonstrated in cells exhibiting cortical density traveling waves [122].

Thus, the idea that actin cortex and plasma membrane are tightly coupled is emerging. Cellular processes are intricacy and among them migration is a very complex, spatiotemporally regulated process. It is probable that several molecular assemblies are used by the cell to precisely regulate actin cortex and membrane accordingly to cell needs and cell environment. We suggest that a key role of RKIP may be to participate in molecular assemblies regulating the actin/membrane intertwined complex in response to signaling pathways.

On a molecular point of view, RKIP appears to regulate several cellular mechanisms by preventing key proteins to associate with their targets. Thereby RKIP inhibits several signaling pathways by physical interaction with their kinases, preventing them to phosphorylate their substrates. This was well demonstrated for Raf/MEK/ERK [123] and TAK/NIK/NF- κ B [124, 125]. Direct interactions of RKIP were also found with proteins such GRK2 [126], GSK3 [19], STAT3 [126] and Notch1 [18], modulating

their binding to their targets. In another system, Cep290-mediated photoreceptor degeneration was associated with aberrant accumulation of RKIP, and Cep290 physically interacted with RKIP. The concentrated signal of RKIP at the apical inner segment, transition zone, and to a lesser extent, at the basal body and centriole of the photoreceptors, together with its ability to interact with Rab8A suggested its involvement in the regulation of docking and transport of membrane protein-containing vesicles in photoreceptors [79]. In another process, RKIP delays mitosis by controlling the mitotic spindle checkpoint. Loss of RKIP accelerates G1/S and leads to the bypass of mitotic checkpoint [126]. At the kinetochore, Aurora B phosphorylates (and thereby inactivates) the microtubule-destabilizing proteins mitotic centromere-associated kinesin and stathmin, which promotes microtubule growth around kinetochores [127]. By inhibiting Aurora B, RKIP blocks the mitotic spindle and prevents microtubule growth around kinetochores. Finally, in a last system, RKIP blocks autophagy initiation by sequestering LC3 and thus preventing its association with phosphatidylethanolamine [18].

Thus, in signaling pathways, primary cilium, mitosis and autophagy, RKIP delays the initiation of molecular mechanisms to allow their initiation at the correct time and location. In all these processes, RKIP appears to protect the inactive form of its partners, preventing their activation. It seems that the association/dissociation of RKIP from its targets is controlled by post-translational modifications of RKIP, especially phosphorylation. In cancer cells, the downregulation of RKIP may promote the deregulation of major cellular mechanisms such signaling, mechanosensing, autophagy and migration, all processes that are in touch with the functioning of the actin-membrane tightly coupled complex.

To reverse RKIP lost in cancer cells, it would be advisable to act on membrane composition and subsequently actin network, in order to restore a sufficient level of RKIP expression. This could be obtained by the use of natural or synthetic compounds. In this regard cancer prevention is a unique tool as several flavonoids and polyphenols were found to act on the expression levels of RKIP [5, 128-130].

Acknowledgments

We would like to acknowledge the support received from colleagues of IMPMC. Particularly, we sincerely thank Isabelle Callebaut and Slavica Jonic for helpful comments and fruitful remarks and we are grateful to William Sacks and Alain Mauger for their interest in our work. We thank also the members of the “Canceropôle du grand Ouest” for stimulating and constructive discussions throughout these last few years.

References

- 1- Keller ET, Fu Z, Brennan M. The Role of Raf Kinase Inhibitor Protein (RKIP) in Health and Disease. *Biochem Pharmacol.* 2004;68(6):1049-1053.
- 2- Bucquoy S, Jollès P, Schoentgen F. Relationships Between Molecular Interactions (Nucleotides, Lipids and Proteins) and Structural Features of the Bovine Brain 21-kDa Protein. *Eur J Biochem.* 1994;225(3):1203-1210.
- 3- Al-Mulla F, Bitar MS, Taqi Z, Yeung KC. RKIP: Much More than Raf Kinase Inhibitory Protein. *J Cell Physiol.* 2013;228(8):1688-1702.
- 4- Liu S, Li X, Lin Z, Su L, Yan S, Zhao B, Miao J. SEC-Induced Activation of ANXA7 GTPase Suppresses Prostate Cancer Metastasis. *Cancer Lett.* 2018;416:11-23.
- 5- Schoentgen F, Jonic S PEBP1/RKIP: From Multiple Functions to a Common Role in Cellular Processes. *ArXiv.* 2018;arXiv:1802.02378[q-bio.SC].
- 6- Keller ET, Fu Z, Yeung K, Brennan M. Raf Kinase Inhibitor Protein: A Prostate Cancer Metastasis Suppressor Gene. *Cancer Lett.* 2004;207(2):131-137.
- 7- Lamiman K, Keller JM, Mizokami A, Zhang J, Keller ET. Survey of Raf Kinase Inhibitor Protein (RKIP) in Multiple Cancer Types. *Crit Rev Oncog.* 2014;19(6):455-468.
- 8- Bernier I, Jollès P. Purification and Characterization of a Basic 23 kDa Cytosolic Protein From Bovine Brain. *Biochim Biophys Acta.* 1984;790(2):174-181.
- 9- Schoentgen F, Saccoccio F, Jollès J, Bernier I, Jollès P. Complete Amino Acid Sequence of a Basic 21-kDa Protein From Bovine Brain Cytosol. *Eur J Biochem.* 1987;166(2):333-338.
- 10- Seddiqi N, Bollengier F, Alliel PM, Périn JP, Bonnet F, Bucquoy S, Jollès P, Schoentgen F. Amino Acid sequence of the Homo sapiens Brain 21-23-kDa Protein (Neuropolypeptide h3), Comparison with its Counterparts From *Rattus norvegicus* and *Bos taurus* Species, and Expression of its mRNA in Different Tissues. *J Mol Evol.* 1994;39(6):655-660.
- 11- Grandy DK, Hanneman E, Bunzow J, Shih M, Machida CA, Bidlack JM, Civelli O. Purification, Cloning, and Tissue Distribution of a 23-kDa Rat Protein Isolated by Morphine Affinity Chromatography. *Mol Endocrinol.* 1990;4(9):1370-1376.
- 12- Bernier I, Tresca JP, Jollès P. Ligand-Binding Studies with a 23 kDa Protein Purified From Bovine Brain Cytosol. *Biochim Biophys Acta.* 1986;871(1):19-23.
- 13- Yeung K, Seitz T, Li S, Janosch P, McFerran B, Kaiser C, Fee F, Katsanakis KD, Rose DW, Mischak H, Sedivy JM, Kolch W. Suppression of Raf-1 Kinase Activity and MAP Kinase Signalling by RKIP. *Nature.* 1999;401(6749):173-177.
- 14- Shin SY, Rath O, Zebisch A, Choo SM, Kolch W, Cho KH. Functional Roles of Multiple Feedback Loops in Extracellular Signal-Regulated Kinase and Wnt Signaling Pathways that Regulate Epithelial-Mesenchymal Transition. *Cancer Res.* 2010;70(17):6715-6724.
- 15- Rajkumar K, Nichita A, Anoor PK, Raju S, Singh SS, Burgula S. Understanding Perspectives of Signalling Mechanisms Regulating PEBP1 Function. *Cell Biochem Funct.* 2016;34(6):394-403.
- 16- Lin X, Bai F, Nie J, Lu S, Lu C, Zhu X, Wei J, Lu Z, Huang Q. Didymin Alleviates Hepatic Fibrosis Through Inhibiting ERK and PI3K/Akt Pathways via Regulation of Raf Kinase Inhibitor Protein. *Cell Physiol Biochem.* 2016;40(6):1422-1432.
- 17- Lai R, Gu M, Jiang W, Lin W, Xu P, Liu Z, Huang H, An H, Wang X. Raf Kinase Inhibitor Protein Preferentially Promotes TLR3-Triggered Signaling and Inflammation. *J Immunol.* 2017;198(10):4086-4095.
- 18- Noh HS, Hah YS, Ha JH, Kang MY, Zada S, Rha SY, Kang SS, Kim HJ, Park JY, Byun JH, Hahm JR, Shin JK, Jeong SH, Lee YJ, Kim DR. Regulation of the Epithelial to Mesenchymal Transition and Metastasis by Raf Kinase Inhibitory Protein-Dependent Notch1 Activity. *Oncotarget.* 2016;7(4):4632-4646.
- 19- Al-Mulla F, Bitar MS, Al-Maghrebi M, Behbehani AI, Al-Ali W, Rath O, Doyle B, Tan KY, Pitt A, Kolch W. Raf Kinase Inhibitor Protein RKIP Enhances Signaling by Glycogen Synthase Kinase-3 β . *Cancer Res.* 2011;71(4):1334-1343.
- 20- Gavilán E, Sánchez-Aguayo I, Daza P, Ruano D. GSK-3 β Signaling Determines Autophagy Activation in the Breast Tumor Cell Line MCF7 and Inclusion Formation in the Non-Tumor Cell Line MCF10A in Response to Proteasome Inhibition. *Cell Death Dis.* 2013;4:e572. doi: 10.1038/cddis.2013.95.
- 21- Corbit KC, Trakul N, Eves EM, Diaz B, Marshall M, Rosner MR. Activation of Raf-1 Signaling by Protein Kinase C Through a Mechanism Involving Raf Kinase Inhibitory Protein. *J Biol Chem.* 2003;278:13061-13068.
- 22- Calebiro D, Godbole A. Internalization of G-Protein-Coupled Receptors: Implication in Receptor Function, Physiology and Diseases. *Best Pract Res Clin Endocrinol Metab.* 2018;32(2):83-91.

- 23- Penela P, Murga C, Ribas C, Lafarga V, Mayor F Jr. The Complex G Protein-Coupled Receptor Kinase 2 (GRK2) Interactome Unveils New Physiopathological Targets. *Br J Pharmacol*. 2010;160(4):821-832.
- 24- Nogués L, Reglero C, Rivas V, Neves M, Penela P, Mayor F Jr. G-Protein-Coupled Receptor Kinase 2 as a Potential Modulator of the Hallmarks of Cancer. *Mol Pharmacol*. 2017;91(3):220-228.
- 25- Nogués L, Reglero C, Rivas V, Salcedo A, Lafarga V, Neves M, Ramos P, Mendiola M, Berjón A, Stamatakis K, Zhou XZ, Lu KP, Hardisson D, Mayor F Jr, Penela P. G Protein-Coupled Receptor Kinase 2 (GRK2) Promotes Breast Tumorigenesis through a HDAC6-Pin1 Axis. *EBioMedicine* 2016;13:132–145.
- 26- Rivas V, Carmona R, Muñoz-Chápuli R, Mendiola M, Nogués L, Reglero C, Miguel-Martín M, García-Escudero R, Dorn GW 2nd, Hardisson D, Mayor F Jr, Penela P. Developmental and Tumoral Vascularization is regulated by G Protein-Coupled Receptor Kinase 2. *J Clin Invest*. 2013;123(11):4714-4730.
- 27- Crassini K, Pyke T, Shen Y, Stevenson WS, Christopherson RI, Mulligan SP, Best OG. Inhibition of the Raf-1 Kinase Inhibitory Protein (RKIP) by Locostatin Induces Cell Death and Reduces the CXCR4-Mediated Migration of Chronic Lymphocytic Leukemia Cells. *Leuk Lymphoma*. 2018;59(12):2917-2928.
- 28- Bement WM. A Role for RKIP in Cell Motility. *Chem Biol*. 2005;12(9):953-954.
- 29- Beshir AB, Argueta CE, Menikarachchi LC, Gascón JA, Fenteany G. Locostatin Disrupts Association of Raf Kinase Inhibitor Protein With Binding Proteins by Modifying a Conserved Histidine Residue in the Ligand-Binding Pocket. *For Immunopathol Dis Therap*. 2011;2(1):47-58.
- 30- Mc Henry KT, Montesano R, Zhu S, Beshir AB, Tang HH, Yeung KC, Fenteany G. Raf Kinase Inhibitor Protein Positively Regulates Cell-Substratum Adhesion While Negatively Regulating Cell-Cell Adhesion. *J Cell Biochem*. 2008;103(3):972-985.
- 31- Lafarga V, Mayor F Jr, Penela P. The Interplay Between G Protein-Coupled Receptor Kinase 2 (GRK2) and Histone Deacetylase 6 (HDAC6) at the Crossroads of Epithelial Cell Motility. *Cell Adh Migr*. 2012;6(6):495-501.
- 32- Penela P, Nogués L, Mayor F Jr. Role of G Protein-Coupled Receptor Kinases in Cell Migration. *Curr Opin Cell Biol*. 2014;27:10-17.
- 33- Penela P, Rivas V, Salcedo A, Mayor F Jr (2010). G Protein–Coupled Receptor Kinase 2 (GRK2) Modulation and Cell Cycle Progression. *Proc Natl Acad Sci U S A*. 2010;107: 1118–1123.
- 34- Kahsai AW, Zhu S, Fenteany G. G Protein-Coupled Receptor Kinase 2 Activates Radixin, Regulating Membrane Protrusion and Motility in Epithelial Cells. *Biochim Biophys Acta*. 2010;1803(2): 300–310.
- 35- Yesilkanal AE, Rabe DC, Tiwari P, Frankenberger C, Johnson GL, Rosner M. A Novel Approach for Antimetastatic Therapies Against TNBC Utilizing a Physiologic Suppressor [abstract]. In: *Proceedings of the American Association for Cancer Research Annual Meeting* 2018;78(13 Suppl):Abstract nr 4179
- 36- Cobas M, Wilson A, Ernst B, Mancini SJ, MacDonald HR, Kemler R, [Radtke F](#). Beta-Catenin is Dispensable for Hematopoiesis and Lymphopoiesis. *J Exp Med* 2004;199:221–229.
- 37- Aoki K, Taketo MM. Adenomatous Polyposis Coli (APC): a Multi-Functional Tumor Suppressor Gene. *J Cell Sci* 2007;120:3327–3335.
- 38- Groden J, Thliveris A, Samowitz W, Carlson M, Gelbert L, Albertsen H, Joslyn G, Stevens J, Spirio L, Robertson M. Identification and Characterization of the Familial Adenomatous Polyposis Coli Gene. *Cell* 1991;66:589–600.
- 39- Song L, Jia Y, Zhu W, Newton IP, Li Z, Li W. N-Terminal Truncation Mutations of Adenomatous Polyposis Coli are Associated with Primary Cilia Defects. *Int J Biochem Cell Biol*. 2014;55:79-86.
- 40- White CD, Brown MD, Sacks DB. IQGAPs in Cancer: a Family of Scaffold Proteins Underlying Tumorigenesis. *FEBS Lett*. 2009;583(12):1817-1824.
- 41- Jadeski L, Mataraza JM, Jeong HW, Li Z, Sacks DB. IQGAP1 Stimulates Proliferation and Enhances Tumorigenesis of Human Breast Epithelial Cells. *J Biol Chem*. 2008;283(2):1008-1017.
- 42- Brown MD, Sacks DB. IQGAP1 in Cellular Signaling: Bridging the GAP. *Trends Cell Biol*. 2006;16(5):242-249.
- 43- Gu H, Zhan X, Zhang G, Yan L, Cho WC, Li M, Liu T, Chen Z. Mapping the Interactome of Overexpressed RAF Kinase Inhibitor Protein in a Gastric Cancer Cell Line. *BMC Cancer*. 2013;13:536. doi: 10.1186/1471-2407-13-536.
- 44- Rotoli D, Morales M, Ávila J, Maeso MDC, García MDP, Mobasher A, Martín-Vasallo P. Commitment of Scaffold Proteins in the Onco-Biology of Human Colorectal Cancer and Liver Metastases after Oxaliplatin-Based Chemotherapy *Int J Mol Sci*. 2017;18(4): 891. doi: 10.3390/ijms18040891
- 45- Hedman AC, Smith JM, Sacks DB. The Biology of IQGAP Proteins: Beyond the Cytoskeleton. *EMBO Rep*. 2015;16(4):427-446.

- 46- Zhu C, Wang Q, Xie J, Shi J, Zhou X, Li D, Xiong F, Zhang L. Expression and Significance of RKIP and E-Cadherin in Lung Squamous Cell Carcinoma. *Pathol Oncol Res.* 2013;19(1):19-26.
- 47- Iwase T, Ojika K, Matsukawa N, Nishino H, Yamamoto T, Okada H, Fujimori O, Ueda R. Muscarinic Cholinergic and Glutamatergic Reciprocal Regulation of Expression of Hippocampal Cholinergic Neurostimulating Peptide Precursor Protein Gene in Rat Hippocampus. *Neuroscience.* 2001;102(2):341-352.
- 48- Jin Y, Lv X, Zhou J, Chen J. Potential Involvement of IQGAP1 in Proliferation and Metastasis of Human Pancreatic Cancer. *Front Biosci (Landmark Ed).* 2016;21:1076-1083.
- 49- Sanchez-Laorden B, Viros A, Marais R. Mind the IQGAP. *Cancer Cell.* 2013;23(6):715-717.
- 50- Petropoulos C, Oddou C, Emadali A, Hiriart-Bryant E, Boyault C, Faurobert E, Vande Pol S, Kim-Kaneyama JR, Kraut A, Coute Y, Block M, Albiges-Rizo C, Destaing O. Roles of Paxillin Family Members in Adhesion and ECM Degradation Coupling at Invadosomes. *J. Cell Biol.* 2016;213(5):585-599.
- 51- Rotoli D, Pérez-Rodríguez ND, Morales M, Maeso MD, Ávila J, Mobasher A, Martín-Vasallo P. IQGAP1 in Podosomes/Invadosomes Is Involved in the Progression of Glioblastoma Multiforme Depending on the Tumor Status. *Int J Mol Sci.* 2017;18(1). pii: E150. doi: 10.3390/ijms18010150.
- 52- White CD, Erdemir HH, Sacks DB. IQGAP1 and Its Binding Proteins Control Diverse Biological Functions *Cell Signal.* 2012; 24(4): 826–834.
- 53- Tirnauer JS. A New Cytoskeletal Connection for APC: Linked to Actin through IQGAP. *Dev Cell.* 2004;7(6):778-780.
- 54- Itoh Y. Membrane-Type Matrix Metalloproteinases: Their Functions and Regulations *Matrix Biol.* 2015;44-46:207-223.
- 55- Cheng X, Lu SH and Cui Y. ECRG2 Regulates ECM Degradation and uPAR/FPRL1 Pathway Contributing Cell Invasion/Migration. *Cancer Lett* 2010; 290: 87-95.
- 56- Lei X, Chang L, Ye W, Jiang C, Zhang Z. Raf Kinase Inhibitor Protein (RKIP) Inhibits the Cell Migration and Invasion in Human Glioma Cell Lines in vitro. *Int J Clin Exp Pathol.* 2015;8(11):14214-14220.
- 57- Ma J, Shi J, Zhao D, Cheng L, Wang W, Li F, Jiang X, Jiang H. Raf Kinase Inhibitor Protein Inhibits Cholangiocarcinoma Cell Metastasis by Downregulating Matrix Metalloproteinase 9 and Upregulating Tissue Inhibitor of Metalloproteinase 4 Expression. *Oncol Lett.* 2015;9(1):15-24.
- 58- Zhao D, Ma J, Shi J, Cheng L, Li F, Jiang X, Jiang H. Raf Kinase Inhibitor Protein Inhibits Esophageal Cancer Cell Invasion through Downregulation of Matrix Metalloproteinase Expression. *Oncol Rep.* 2013;30(1):304-312.
- 59- Beshir AB, Ren G, Magpusao AN, Barone LM, Yeung KC, Fenteany G. Raf Kinase Inhibitor Protein Suppresses Nuclear Factor- κ B-Dependent Cancer Cell Invasion through Negative Regulation of Matrix Metalloproteinase Expression. *Cancer Lett.* 2010;299(2):137-149.
- 60- Delassus GS, Cho H, Park J, Eliceiri GL. New Pathway Links from Cancer-Progression Determinants to Gene Expression of Matrix Metalloproteinases in Breast Cancer Cells. *J Cell Physiol.* 2008;217(3):739-744.
- 61- Delassus GS, Cho H, Hoang S, Eliceiri GL. Many New Down- and Up-Regulatory Signaling Pathways, from Known Cancer Progression Suppressors to Matrix Metalloproteinases, Differ Widely in Cells of Various Cancers. *J Cell Physiol.* 2010;224(2):549-558.
- 62- Datar I, Feng J, Qiu X, Lewandowski J, Yeung M, Ren G, Aras S, Al-Mulla F, Cui H, Trumbly R, Arudra SK, De Las Casas LE, de la Serna I, Bitar MS, Yeung KC. RKIP Inhibits Local Breast Cancer Invasion by Antagonizing the Transcriptional Activation of MMP13. *PLoS One.* 2015;10(8):e0134494. doi: 10.1371/journal.pone.0134494.
- 63- Mori H, Tomari T, Koshikawa N, Kajita M, Itoh Y, Sato H, Tojo H, Yana I, Seiki M. CD44 Directs Membrane-Type 1 Matrix Metalloproteinase to Lamellipodia by Associating with its Hemopexin-Like Domain. *EMBO J.* 2002;21(15):3949–3959.
- 64- Branch KM, Hoshino D, Weaver AM. Adhesion Rings Surround Invadopodia and Promote Maturation. *Biol Open.* 2012;1(8):711-722.
- 65- Kopinke D, Roberson EC, Reiter JF. Ciliary Hedgehog Signaling Restricts Injury-Induced Adipogenesis. *Cell.* 2017;170(2): 340–351.
- 66- Noh HS, Hah YS, Zada S, Ha JH, Sim G, Hwang JS, Lai TH, Nguyen HQ, Park JY, Kim HJ, Byun JH, Hahm JR, Kang KR, Kim DR. PEBP1, a RAF Kinase Inhibitory Protein, Negatively Regulates Starvation-Induced Autophagy by Direct Interaction with LC3. *Autophagy.* 2016;12(11):2183-2196.
- 67- Masuda GO, Yashiro M, Kitayama K, Miki Y, Kasashima H, Kinoshita H, Morisaki T, Fukuoka T, Hasegawa T, Sakurai K, Toyokawa T, Kubo N, Tanaka H, Muguruma K, Masaichi O, Hirakawa K. Clinicopathological Correlations of Autophagy-related Proteins LC3, Beclin 1 and p62 in Gastric Cancer. *Anticancer Res.* 2016;36(1):129-136.

- 68- Park JM, Huang S, Wu TT, Foster NR and Sinicrope FA. Prognostic Impact of Beclin 1, p62/Sequestosome 1 and LC3 Protein Expression in Colon Carcinomas from Patients Receiving 5-fluorouracil as Adjuvant Chemotherapy. *Cancer Biol Ther.* 2013;14:100-107.
- 69- Tang JY, Hsi E, Huang YC, Hsu NC, Chu PY and Chai CY. High LC3 Expression Correlates with Poor Survival in Patients with Oral Squamous Cell Carcinoma. *Hum Pathol.* 2013;44: 2558-2562.
- 70- Chang SJ, Ou-Yang F, Tu HP, Lin CH, Huang SH, Kostoro J, Hou MF, Chai CY, Kwan AL. Decreased Expression of Autophagy Protein LC3 and Stemness (CD44+/CD24-/low) Indicate Poor Prognosis in Triple-Negative Breast Cancer. *Hum Pathol.* 2016;48:48-55.
- 71- Jiang ZF, Shao LJ, Wang WM, Yan XB, Liu RY. Decreased Expression of Beclin-1 and LC3 in Human Lung Cancer. *Mol Biol Rep.* 2012;39(1):259-67.
- 72- Kenific CM, Wittmann T, Debnath J. Autophagy in Adhesion and Migration. *J Cell Sci.* 2016;129(20):3685-3693.
- 73- Xu Z, Klionsky DJ. Autophagy Promotes Cell Motility by Driving Focal Adhesion Turnover. *Autophagy.* 2016 Oct 2;12(10):1685-1686
- 74- Ulbricht A, Eppler FJ, Tapia VE, van der Ven PF, Hampe N, Hersch N, Vakeel P, Stadel D, Haas A, Saftig P, Behrends C, Fürst DO, Volkmer R, Hoffmann B, Kolanus W, Höfeld J. Cellular Mechanotransduction Relies on Tension-Induced and Chaperone-Assisted Autophagy. *Curr Biol.* 2013;23(5):430-435.
- 75- Pampliega O, Orhon I, Patel B, Sridhar S, Díaz-Carretero A, Beau I, Codogno P, Satir BH, Satir P, Cuervo AM. Functional Interaction Between Autophagy and Ciliogenesis. *Nature.* 2013;502(7470):194–200.
- 76- Hu X, Mullins RD. LC3 and STRAP Regulate Actin Filament Assembly by JMY During Autophagosome Formation. *J Cell Biol.* 2019 7;218(1):251-266.
- 77- Liu X, Klionsky DJ (2019) Regulation of JMY's Actin Nucleation Activity by TTC5/STRAP and LC3 During Autophagy. *Autophagy.* 2019;15(3):373-374
- 78- Mi N et al (2015) CapZ Regulates Autophagosomal Membrane Shaping by Promoting Actin Assembly Inside the Isolation Membrane. *Nat Cell Biol* 17(9):1112–1123.
- 79- Murga-Zamalloa CA, Ghosh AK, Patil SB, Reed NA, Chan LS, Davuluri S, Peränen J, Hurd TW, Rachel RA, Khanna H. Accumulation of the Raf-1 Kinase Inhibitory Protein (Rkip) is Associated with Cep290-Mediated Photoreceptor Degeneration in Ciliopathies. *J Biol Chem.* 2011;286(32):28276-28286.
- 80- Deretic D, Huber LA, Ransom N, Mancini M, Simons K, Papermaster DS. Rab8 in Retinal Photoreceptors May Participate in Rhodopsin Transport and in Rod Outer Segment Disk Morphogenesis. *J Cell Sci.* 1995;108 (Pt 1):215-224.
- 81- Patrussi L, Baldari CT. The Rab GTPase Rab8 as a Shared Regulator of Ciliogenesis and Immune Synapse Assembly: From a Conserved Pathway to Diverse Cellular Structures. *Small GTPases.* 2016;7(1):16-20.
- 82- Ao X, Zou L, Wu Y. Regulation of Autophagy by the Rab GTPase Network. *Cell Death Differ.* 2014;21(3):348-358.
- 83- Bravo-Cordero JJ, Cordani M, Soriano SF, Díez B, Muñoz-Agudo C, Casanova-Acebes M, Boullosa C, Guadamillas MC, Ezkurdia I, González-Pisano D, Del Pozo MA, Montoya MC. A Novel High-Content Analysis Tool Reveals Rab8-Driven Cytoskeletal Reorganization through Rho GTPases, Calpain and MT1-MMP. *J Cell Sci.* 2016;129(8):1734-1749.
- 84- Tang BL, Ng EL. Rabs and Cancer Cell Motility. *Cell Motil Cytoskeleton.* 2009;66(7):365-370.
- 85- Bravo-Cordero JJ, Marrero-Diaz R, Megías D, Genís L, García-Grande A, García MA, Arroyo AG, Montoya MC. MT1-MMP Proinvasive Activity is Regulated by a Novel Rab8-Dependent Exocytic Pathway. *EMBO J.* 2007;26(6):1499-510.
- 86- Peränen J. Rab8 GTPase as a Regulator of Cell Shape. *Cytoskeleton (Hoboken).* 2011;68(10):527-539.
- 87- Subramanian B, Anand M, Khan NW, Khanna H. Loss of Raf-1 Kinase Inhibitory Protein Delays Early-Onset Severe Retinal Ciliopathy in Cep290rd16 Mouse. *Invest Ophthalmol Vis Sci.* 2014;55(9):5788-5594.
- 88- Qu Y, Dang S, Hou P. Gene Methylation in Gastric Cancer. *Clin Chim Acta.* 2013;424:53-65.
- 89- Huang S, Hirota Y, Sawamoto K. Various Facets of Vertebrate Cilia: Motility, Signaling, and Role in Adult Neurogenesis. *Proc Jpn Acad Ser B Phys Biol Sci.* 2009;85(8):324-36.
- 90- Qin H. Regulation of Intraflagellar Transport and Ciliogenesis by Small G Proteins. *Int Rev Cell Mol Biol.* 2012;293:149-168.
- 91- Seeley ES, Nachury MV. The Perennial Organelle: Assembly and Disassembly of the Primary Cilium. *J Cell Sci.* 2010;123(Pt 4):511-518.
- 92- Kim S, Dynlacht BD. Assembling a Primary Cilium. *Curr Opin Cell Biol.* 2013;25(4):506-511.
- 93- Broekhuis JR, Leong WY, Jansen G. Regulation of Cilium Length and Intraflagellar Transport. *Int Rev Cell Mol Biol.* 2013;303:101-138.

- 94- Molla-Herman A, Ghossoub R, Blisnick T, Meunier A, Serres C, Silbermann F, Emmerson C, Romeo K, Bourdoncle P, Schmitt A, Saunier S, Spassky N, Bastin P, Benmerah A. The Ciliary Pocket: an Endocytic Membrane Domain at the Base of Primary and Motile Cilia.. *J Cell Sci.* 2010;123(Pt 10):1785-1795. doi: 10.1242/jcs.059519.
- 95- Ke YN, Yang WX. Primary Cilium: an Elaborate Structure that Blocks Cell Division? *Gene.* 2014;547(2):175-185.
- 96- Higgins M, Obaidi I, McMorrow T. Primary Cilia and their Role in Cancer. *Oncol Lett.* 2019;17(3):3041-3047.
- 97- Sánchez I, Dynlacht BD. Cilium Assembly and Disassembly *Nat Cell Biol.* 2016; 18(7): 711–717
- 98- Wakahara K, Ohno T, Kimura M, Masuda T, Nozawa S, Dohjima T, Yamamoto T, Nagano A, Kawai G, Matsushashi A, Saitoh M, Takigami I, Okano Y, Shimizu K. EWS-Fli1 Up-Regulates Expression of the Aurora A and Aurora B Kinases. *Mol Cancer Res.* 2008;6(12):1937-1945.
- 99- Yuan K, Frolova N, Xie Y, Wang D, Cook L, Kwon YJ, Steg AD, Serra R, Frost AR. Primary Cilia are Decreased in Breast Cancer: Analysis of a Collection of Human Breast Cancer Cell Lines and Tissues. *J Histochem Cytochem.* 2010;58(10):857-870.
- 100- al-Mulla F, Bitar MS, Taqi Z, Rath O, Kolch W. RAF Kinase Inhibitory Protein (RKIP) Modulates Cell Cycle Kinetics and Motility. *Mol Biosyst.* 2011;7(3):928-941.
- 101- Shvartsur A, Givechian KB, Garban H, Bonavida B. Overexpression of RKIP and its Cross-Talk with Several Regulatory Gene Products in Multiple Myeloma. *J Exp Clin Cancer Res.* 2017;36(1):62. doi: 10.1186/s13046-017-0535-z.
- 102- Singla V, Reiter JF. The Primary Cilium as the Cell's Antenna: Signaling at a Sensory Organelle. *Science.* 2006;313(5787):629-633.
- 103- Conduit SE, Dyson JM, Mitchell CA. Inositol Polyphosphate 5-Phosphatases; New Players in the Regulation of Cilia and Ciliopathies. - *FEBS Lett.* 2012;586(18):2846-2857.
- 104- Wheway G, Nazlamova L, Hancock JT. Signaling Through the Primary Cilium. *Front Cell Dev Biol.* 2018;6:8. doi: 10.3389/fcell.2018.00008.
- 105- Kohli P, Höhne M, Jüngst C, Bertsch S, Ebert LK, Schauss AC, Benzing T, Rinschen MM, Schermer B. The Ciliary Membrane-Associated Proteome Reveals Actin-Binding Proteins as Key Components of Cilia. *EMBO Rep.* 2017;18(9):1521-1535.
- 106- Seeger-Nukpezah T, Golemis EA. The Extracellular Matrix and Ciliary Signaling. *Curr Opin Cell Biol.* 2012; 24(5): 652–661.
- 107- Mansini AP, Peixoto E, Jin S, Richard S, Gradilone SA. The Chemosensory Function of Primary Cilia Regulates Cholangiocyte Migration, Invasion, and Tumor Growth. *Hepatology.* 2019;69(4):1582-1598.
- 108- Drummond ML, Li M, Tarapore E, Nguyen TTL, Barouni BJ, Cruz S, Tan KC, Oro AE, Atwood SX. Actin Polymerization Controls Cilia-Mediated Signaling. *J Cell Biol.* 2018;217(9):3255-3266.
- 109- Lee S, Tan HY, Geneva II, Kruglov A, Calvert PD. Actin Filaments Partition Primary Cilia Membranes into Distinct Fluid Corrals. *J Cell Biol.* 2018;217(8):2831-2849.
- 110- Kim J, Jo H, Hong H, Kim MH, Kim JM, Lee JK, Heo WD, Kim J. Actin Remodelling Factors Control Ciliogenesis by Regulating YAP/TAZ Activity and Vesicle Trafficking. *Nat Commun.* 2015;6:6781. doi: 10.1038/ncomms7781.
- 111- Alblazi KM, Siar CH. Cellular Protrusions - Lamellipodia, Filopodia, Invadopodia and Podosomes - and their Roles in Progression of Orofacial Tumours: Current Understanding. *Asian Pac J Cancer Prev.* 2015;16(6):2187-2191.
- 112- Rottner K, Schaks M. Assembling Actin Filaments for Protrusion. *Current Opinion in Cell Biology* 2019;56:53–63.
- 113- Bi J, Wang R, Zeng X. Lipid Rafts Regulate the Lamellipodia Formation of Melanoma A375 Cells via Actin Cytoskeleton-Mediated Recruitment of $\beta 1$ and $\beta 3$ Integrin. *Oncol Lett.* 2018;16(5):6540-6546.
- 114- Islam SMA, Patel R, Bommarreddy RR, Khalid KM, Acevedo-Duncan M. The Modulation of Actin Dynamics via Atypical Protein Kinase-C Activated Cofilin Regulates Metastasis of Colorectal Cancer Cells. *Cell Adh Migr.* 2019;13(1):106-120.
- 115- Jacquemet G, Hamidi H, Ivaska J. Filopodia in Cell Adhesion, 3D Migration and Cancer Cell Invasion. *Current Opinion in Cell Biology* 2015;36:23–31.
- 116- Jacquemet G, Baghirov H, Georgiadou M, Sihto H, Peuhu E, Cettour-Janet P, He T, Perälä M, Kronqvist P, Joensuu H, Ivaska J. L-Type Calcium Channels Regulate Filopodia Stability and Cancer Cell Invasion Downstream of Integrin Signaling. *Nat Commun.* 2016;7:13297. doi: 10.1038/ncomms13297.
- 117- Murphy DA, Courtneidge SA. The 'Ins' and 'Outs' of Podosomes and Invadopodia: Characteristics, Formation and Function. *Nat Rev Mol Cell Biol.* 2011;12(7):413-426.

- 118- Castro-Castro A, Marchesin V, Monteiro P, Lodillinsky C, Rossé C, Chavrier P. Cellular and Molecular Mechanisms of MT1-MMP-Dependent Cancer Cell Invasion. *Annu Rev Cell Dev Biol.* 2016;32:555-576.
- 119- Revach OY, Geiger B. The Interplay Between the Proteolytic, Invasive, and Adhesive Domains of Invadopodia and Their Roles in Cancer Invasion. *Cell Adh Migr.* 2014;8(3):215-225.
- 120- Köster DV, Mayor S. Cortical Actin and the Plasma Membrane: Inextricably Intertwined. *Curr Opin Cell Biol.* 2016;38:81-89.
- 121- Rodríguez-Fernández JL, de Lacoba MG. Plasma Membrane-Associated Superstructure: Have We Overlooked a New Type of Organelle in Eukaryotic Cells? *J Theor Biol.* 2015;7;380:346-358.
- 122- Driscoll MK, Losert W, Jacobson K, Kapustina M. Spatiotemporal Relationships Between the Cell Shape and the Actomyosin Cortex of Periodically Protruding Cells. *Cytoskeleton (Hoboken).* 2015;72(6):268-281.
- 123- Yeung K, Janosch P, McFerran B, Rose DW, Mischak H, Sedivy JM, Kolch W. Mechanism of Suppression of the Raf/MEK/Extracellular Signal-Regulated Kinase Pathway by the Raf Kinase Inhibitor Protein. *Mol Cell Biol.* 2000;20(9):3079-3085.
- 124- Yeung KC, Rose DW, Dhillon AS, Yaros D, Gustafsson M, Chatterjee D, McFerran B, Wyche J, Kolch W. Raf Kinase Inhibitor Protein Interacts with NF-kappaB-Inducing Kinase and TAK1 and Inhibits NF-kappaB Activation. *Mol Cell Biol.* 2001;21(21):7207-7217.
- 125- Tang H, Park S, Sun SC, Trumbly R, Ren G, Tsung E, Yeung KC. RKIP Inhibits NF-kappaB in Cancer Cells by Regulating Upstream Signaling Components of the IkappaB Kinase Complex. *FEBS Lett.* 2010;584(4):662–668.
- 126- Yousuf S, Duan M, Moen EL, Cross-Knorr S, Brilliant K, Bonavida B, LaValle T, Yeung KC, Al-Mulla F, Chin E, Chatterjee D. Raf Kinase Inhibitor Protein (RKIP) Blocks Signal Transducer and Activator of Transcription 3 (STAT3) Activation in Breast and Prostate Cancer. *PLoS One.* 2014;9(3):e92478. doi: 10.1371/journal.pone.0092478.
- 127- Eves EM, Shapiro P, Naik K, Klein UR, Trakul N, Rosner MR. Raf Kinase Inhibitory Protein Regulates Aurora B Kinase and the Spindle Checkpoint. *Mol Cell.* 2006;23(4):561-574.
- 128- King ML, Murphy LL. American ginseng (*Panax quinquefolius* L.) extract alters mitogen-activated protein kinase cell signaling and inhibits proliferation of MCF-7 cells. *J Exp Ther Oncol.* 2007;6(2):147-155.
- 129- Farooqi AA, Li Y, Sarkar FH. The biological complexity of RKIP signaling in human cancers. *Exp Mol Med.* 2015;47:e185. doi: 10.1038/emm.2015.70.
- 130- Hussain SA, Sulaiman AA, Alhaddad H, Alhadidi Q. Natural Polyphenols: Influence on Membrane Transporters. *J Intercult Ethnopharmacol.* 2016;27;5(1):97-104.
- 131- Finetti F, Baldari CT. Compartmentalization of signaling by vesicular trafficking: a shared building design for the immune synapse and the primary cilium. *Immunol Rev.* 2013;251(1):97-112.

Figure 1. Schematic view of primary cilia structure. The ciliary skeleton is composed of axoneme and basal body. The transition zone is indicated by Y-shaped bridges extending from microtubule doublets to ciliary membrane and distal appendages, it corresponds to the transition between microtubule triplets (basal body and distal appendages) to microtubule doublets (axoneme). RKIP (PEBP1), CEP290 and Rab8 were described to interact at the transition zone [79]. LC3 form puncta along the axoneme. GPCRs and MT1-MMP are localized at the membrane. Primary ciliary pocket is defined to be membrane domain starting from distal appendages to the region where ciliary sheath emerges to extracellular environment. Primary cilia pocket plays a role in endocytosis. Incoming vesicles (from Golgi complex) dock at membrane junction at distal appendages and keep balances between ciliary membrane addition and removal [95]. One pathway for protein targeting to the ciliary membrane is led by the BBSome and its interactor Arl6, which recruits Rab8 and its guanine nucleotide exchange factor Rabin8 at the base of the cilium. Rabin8 is transported to this location in Rab11⁺ vesicles. Some membrane receptors destined for the ciliary membrane are transported from the trans-Golgi network apparatus to Rab8⁺ vesicles with the assistance of the adapter AP-1 [131]. In this figure, the general drawing of the primary cilium is based on [95] and the transport of Rab proteins is inspired from [131].

Figure 2. Primary cilia in the cell cycle. During G0 and G1 primary cilia are assembled and function as important signaling hubs. When the cell enters the S phase, ciliary disassembly begins with the resorption of the cilium. This allows the centrioles to detach from the basal body. In M phase, the mother and daughter centrioles are duplicated and can move to the spindle poles where they become centrosomes and the mitotic spindle, and can now carry out their role in the cell cycle. RKIP controls the cell cycle in the two interphases G1/S and G2/M. This figure is inspired from [96].