Review Article
The dual functions of Rab11 and Rab35 GTPases-regulation of cell division and promotion of tumorigenicity

Paulius Gibieža, Vilma Petrikaitė

Laboratory of Drug Targets Histopathology, Institute of Cardiology, Lithuanian University of Health Sciences, Kaunas, LT-50162, Lithuania

Received January 28, 2021; Accepted March 1, 2021; Epub May 15, 2021; Published May 30, 2021

Abstract: The broad studies of cancer have led researchers to the creditable understanding of biological and environmental factors that make benign cells to become malignant, as well as the developmental aspects of the tumour cells, known as the “hallmarks of cancer”. However, additional research is needed to uncover the features of cancer biology, which would allow to design new and more effective treatment strategies for cancer patients. Since RabGTPases and their effectors are frequently altered in cancer, their role in a regulation of cell division leading to the acquisition of cancer cell-like phenotype has drawn a lot of attention from different research groups in recent years. Both, Rab11 and Rab35 belong to a superfamily of small monomeric GTPases that regulate a diverse array of cellular functions. Lately, Rab11 and Rab35 were declared as oncogenic, and because of their association with abundant cellular functions, a linkage to the induction of cancer, has been proposed. Although the clear connection between the improper regulation of Rab11 or Rab35 and the initiation of tumorigenicity has only beginning to emerge, in this review we will discuss the newest findings regarding the participation of RabGTPases in a control of cell division and promotion of tumorigenesis, trying to link the actual function to the cancer causality.

Keywords: Rabs, endocytic transport, actin, furrow, cytokinesis, migration, invasion, cancer, tumorigenesis

Introduction

RabGTPases have evolved from the Ras, which is a protein superfamily of small GTPases. Based on the structure, sequence and function, small GTPases are further subdivided into five main subfamilies, including Ras, Rho, Ran, Arf and Rab GTPases [1]. There are about 70 different Rab proteins identified in humans [2, 3]. In cells, Rabs cycle between an active guanosine triphosphate (GTP)-bound state and an inactive guanosine diphosphate (GDP)-bound state. The activation of Rabs is carried out by guanine nucleotide exchange factors (GEFs), which catalyse the exchange of GDP by GTP. This is followed by Rab interaction to a diverse array of effector proteins, and subsequent binding and positioning of Rab recycling endosomes (REs) to the specific membrane-bound compartments called organelles, where Rabs serve their function. Upon deactivation, the GTP is hydrolysed by GTPase-activating proteins (GAPs) back into GDP, which makes Rab endosomes to separate from their designated organelles and disperse in cell cytoplasm until the next functional cycle [1]. Interestingly, despite of structural and biochemical similarities, Rab proteins possess multiple, but very different roles, and are responsible for almost all fundamental cellular processes. These include acting as molecular switches and controlling signal transduction from outside of the cells into intracellular signalling networks that regulate a huge variety of intracellular processes, necessary for the support of homeostasis. Such cellular processes include proliferation, migration, differentiation, adhesion, apoptosis, polarity, survival, morphology, cell maintenance and cell division. Thus, it is not surprising that alterations of RabGTPases have direct and inevitable consequences on cellular functions that are vital to human health. There is plenty of data showing that Rabs dysfunction causes pathogenesis of various human diseases,
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Figure 1. Localisation of recycling endosomes and secretory vesicles during the distinct steps of cytokinesis. The scheme depicts the progression of cellular processes that take place during cytokinesis: furrow ingression followed by intercellular bridge formation, where secondary ingression defines the site of abscission. Distinct recycling endosomes (Rab11-FIP3, Rab35 and Rab8), secretory and endocytic vesicles are indicated in different colours. Microtubules are marked as straight blue lines and F-actin as black mesh.

Cells do not simply stand apart during cytokinesis, and so abscission requires cross-interaction between complex cellular pathways, including actin/microtubule cytoskeleton reorganisation, and transport of endocytic membranes, as well as various secretory vesicles to the furrow (Figure 1) [18]. In more detail, an intracellular membrane trafficking takes place, where different molecules required for the cleavage of microtubules and actin are brought to the secondary constriction site (also known as secondary ingression) at the ICB during cytokinesis (Figure 1). Since the first evidence of membrane trafficking in animal cells during cytokinesis dates back to 2000 [19], so far, there has been a lot of research done on this subject, and the most recent studies suggest the cross-talk between various small GTPases and their effector proteins that play an important role during cytokinetic abscission [10, 20, 21].

It has been discussed in many reviews that the role of vesicles fusion and membrane remodel-
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Figure 1. The Rab11 subfamily in mammals is composed of Rab11a, Rab11b and Rab25 (or Rab11c), which share a high sequence homology (61%-91% among three Rab11 proteins in mouse) [30]. Even though the different roles of Rab11 isoforms remain vague, Rab11 proteins were shown to be involved in a huge variety of cellular trafficking pathways, being responsible for the regulation of cell polarity, ciliogenesis, integrin recycling, neuritogenesis, oogenesis, receptor/adhesion protein recycling and cytokinesis [31-34]. When cell undergoes mitotic division, Rab11 accumulates around the centromeres during metaphase, while as cell progresses into telophase, it moves via central spindle microtubules towards the cleavage furrow and occupies the region around the MB at the ICB during late telophase (Figure 1). The role of Rab11 during cytokinesis have been studied extensively, providing reliable and meaningful data, which demonstrates that Rab11 regulates REs transport and is the main driving factor for the ICB abscission and ensures a successful cellular division. All Rab-GTPases exert their function by binding to a diverse array of effector proteins. Indeed, Rab11 have several effector proteins (FIP1, FIP2, FIP3, FIP4 and FIP5), but exclusively, binding specifically to FIP3, drives the machinery prerequisite for the delivery of REs to the ICB [25, 35]. Importantly, Rab11-FIP3 endosomes deliver SCAMP2/3 and p50RhoGAP to the ICB, which then inhibits RhoGTPase activity, and in turn, reduces F-actin polymerisation, which further narrows the ICB at the abscission site (Figure 2) [36]. In this way, the FIP3-endosome-provoked secondary ingression allows the endosomal sorting complex required for transport (ESCRT)-III to step in, and by binding to microtubule-severing enzyme spastin via its component CHMP1B, to ultimately disintegrate microtubule bundles at the secondary ingression site and complete the final stage of cytokinesis-abscission (Figure 2) [36-39]. Of note, that FIP3-endosome-dependent delivery of cytokinesis regulators for the depolymerization of the cortical F-actin are most likely not limited only to SCAMP2/3 and p50RhoGAP [36]. In addition, FIP3 directly co-operates with Arf6 GTPase, which also promotes abscission [40]. Rab11-FIP3 endosomes are docked to the ICB via a coupling to Sec15 on the exocyst or via an interaction with Arf6, which then interacts with the exocyst component Sec10 [40]. Next, FIP3 binds to the central spindle complex Cyk4, which is enriched at the centre of the ICB and allows for the FIP3 REs recruitment at the abscission site [41].

Rab11 regulates cytokinetic abscission

The fusion of Rab11-FIP3 to Rab35 endosomes to the ICB turned out to be an important step in preparing the plasma membrane for the abscission [10]. The dual role of Rab11 and Rab35 is to clear the F-actin off the cleavage furrow, which would subsequently activate the ESCRT-III-mediated microtubule fibre depolymerisation, resulting in a final cleavage of the ICB at the abscission site (Figure 2). In addition to the role of Rab11-FIP3 endosomes, regulating the process of cytokinesis, as discussed earlier, Rab35-GTPase was also shown to perform F-actin clearance at the abscission site by reducing the amount of phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P₂) in the ICB, whose main function is to promote actin polymerisation during late cytokinesis (Figure 2). Therefore, the PtdIns(4,5)P₂ is a crucial component for maintaining the ICB stability during cytokinesis [42]. For this reason, phosphoinositides must be hydrolysed during post-furrowing steps, which is prerequisite for the success of abscission [43]. Indeed, Rab35 functions by...
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recruiting its effector Oculo-Cerebro-Renal syndrome of Lowe (OCRL) lipid phosphatase to the ICB (Figure 2) [44]. Upon local hydrolysis of the PtdIns(4,5)P$_2$ by the OCRL, the F-actin oligomerization is limited in such a way that it would allow to keep actin levels in the ICB low enough to complete normal cytokinetic abscission [23, 27]. The second mechanism by which Rab35 mediates F-actin clearance during cytokinesis is via binding to another effector MICAL1, which in turn catalyses the oxidation of methionine residues (Met44 and Met47) of F-actin [45], and thus activates disassembly/depolymerisation of actin filaments at close proximity to the abscission site (Figure 2) [46-48]. This then leads to the recruitment of ESCRT-III, and subsequential depolymerisation of microtubule helices, allowing for the abscission at the secondary ingression in the ICB to take place (Figure 2) [47].

Improper reorganisation of cytoskeleton during cytokinesis gives rise to cancer

There are many important cellular pathways and various molecular regulators that are essential for a successful completion of cytokinesis, and if these get interfered, it can cause tumorigenicity [15, 49, 50]. However, in this review we focus only on RabGTPase-regulated molecular machinery that is directly responsible for the regulation of cytokinesis. A number of studies showing F-actin accumulation upon the depletion of F-actin depolymerising proteins or their effectors suggest that there is a tightly regulated balance between an actin polymerisation and depolymerisation in the ICB during the final abscission step, and that an imbalance there can cause abnormal levels of F-actin, which will subsequently disrupt cytokinesis [23, 40, 48, 51]. It is well defined that an aberrant F-actin levels can make the ICBs unstable, resulting in lagging chromosomes, which altogether leads to a formation of binucleated cells and aneuploidy after repeatedly failed cytokinesis during abnormal mitosis, and provoke cytokinesis regression [47, 52]. Indeed, aneuploidy is one of the major contributors of tumour formation and oncogenic transformation, where most of the solid tumours are either aneuploids or made of cells that experienced various chromosomal aberrations, also known

Figure 2. Mechanisms regulating cytoskeleton reorganization in the post-furrowing events of cytokinesis. The clearance of F-actin and microtubules from the secondary ingression site mediated by Rab11-FIP3, Rab35 and their effectors is necessary for the recruitment of ESCRT-III that ensures successful abscission. Central spindle microtubules are labelled as grey straws, F-actin as grey mesh and ESCRT-III complex as green spirals.
as chromosomal instability [53, 54]. In addition, while the newest report by Baudoin et al. [55] suggests that asymmetric clustering of the supernumerary centrosomes can forejudge the development of normal cells into tetraploid cells, tumours genome analysis provided reliable evidence displaying that almost 40% of all cancers sampled, were tetraploid [56]. Correspondingly, tetraploidy was shown to have a causal relationship with tumorigenesis [15, 57]. Therefore, understanding of molecular mechanisms regulating cytokinesis would allow to better grasp of cellular defects that underly carcinogenesis. Since the most studied role of Rab11 and Rab35 GTPases in human diseases is the involvement in cancer, the next paragraphs of this review will focus on identifying oncogenic functions of these proteins and their effectors during the induction of tumorigenesis.

Rab11 functions as oncogene

Taking into account the important roles that Rab proteins play in membrane trafficking, cell signalling, maintaining homeostasis and cell division, it is very likely that dysregulation of these tightly regulated GTPases is associated with various human diseases, such as neurodegenerative disorders, infections, immune diseases and cancer [4, 58-60]. As the main focus of this review, alterations of these small GTPases in cancer have attracted a lot of attention in recent years [5, 61, 62].

Functional mis-regulation of Rab proteins due to mutations or post-translational modifications, disturbing the regulatory circuit of vesicle and endosome transport within the cell have been linked to tumorigenesis and cancer. The same Rab can be up-regulated or down-regulated in several different tumours. Indeed, different members of Rab11 subfamily are up-regulated and modulate the aggressiveness and metastasis of multiple cancers, including gastric cancer [63], lung cancer [64], renal cell carcinoma [65] and ovarian cancer [66]. In many cancers, Rab11c-induced proliferation, invasion, and migration of cancer cells is related to the ability of Rab11 to couple with its effector Rab-coupling protein (RCP). This coupling enhances the endosomal transport of integrins, which is required for cells to bind to the extracellular matrix (ECM), force generation and cytoskeleton remodelling (Figure 3), and thus is prerequisite for the induction of an invasive cancerogenic phenotype [67], which is mostly associated with epithelium-based cancers [68]. Surprisingly, Rab11c is down-regulated in colorectal adenocarcinomas [69] and triple-negative breast cancer [70], which is related to poor prognosis and shows tumour suppressor properties [71]. Whether Rab11c functions as a tumour promoter or a tumour inhibitor depends on the involvement of the chloride intracellular channel protein 3 (CLIC3) in Rab11c-regulated integrin recycling [72] and cell invasion [73] (Figure 3). In cell lines lacking CLIC3, Rab11c may designate integrins to lysosomal degradation, and therefore inhibit the invasion of cells, acting like a tumour suppressor. While in cell lines possessing high levels of CLIC3, Rab11c may circulate integrins to stimulate cell invasion and migration (Figure 3) [73]. When considering structural difference in sequences, Rab11c has a unique GTP-binding motif, which is different from the GTP-binding motif sequences of the remaining Rabs [74]. This feature allows for the inhibition of GTP hydrolysis of Rab11c, and results in a constitutive activation of Rab11c, which is associated with an aggressive metastasis in cancers where Rab11c is overexpressed.

Abnormal expression of Rab GTPases is often related to various cancers, particularly when Rabs are directly associated to the recycling of adhesion molecules and endocytosis, required for cell invasion, migration, cellular signalling and division [62]. It was shown that in tumour cells, hypoxia regulates these important cellular processes via a modulation of Rab11 membrane protein recycling and translocation from trans-Golgi network to the plasma membrane (Figure 3). Under hypoxic conditions, tumour cells experience an increased Rab11-mediated integrin trafficking and expression on the cell surface, which in turn promotes hypoxia-induced invasion of cancer cells (Figure 3) [75]. Also, several studies have shown that Rab11 effector, Rab11 family interacting protein 2 (Rab11-FIP2), is upregulated in colorectal cancer (CRC) tissue, and that an overexpression of Rab11-FIP2 induce colorectal cancer metastasis [76, 77]. In fact, Rab11-FIP2 promotes invasion and migration of colorectal carcinoma cells through an activation of phosphatidylinositol 3'-OH kinase (PI3K) and protein kinase B
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(Akt), which then upregulates MMP7 expression, and thus initiates the ECM turnover in CRC cells (Figure 3) [77]. In addition, a study by Yun et al. [78] demonstrated that overexpression of another Rab11 effector Rab11-FIP4, contributes to pancreatic tumour progression, and is associated with poor clinical outcomes. Another study by Prekeris et al. [79] showed that Rab11-FIP3 recycling endosomes regulate Arf6 localisation at the plasma membrane of MDA-MB-231 cells, and subsequently activate the Rac1-mediated actin polymerisation in membranous protrusions at the cell edges, known as lamellipodia (Figure 3). In particular, the migratory cells use these actin-rich lamellipodia to degrade ECM proteins, generate force, as well as penetrate the basement membrane and vasculature during an invasion of metastatic cancer cells (Figure 3) [80]. Importantly, endosomal targeting to the cleavage furrow during cytokinesis was shown to be directly dependent on Rab11-FIP3 acting as a complex, as depletion of FIP3 by RNAi resulted in the increase of binucleate and multinucleate cells (Figure 3) [25]. Since binucleate cells can form via two processes, either from a failure to furrow or via a regression of the furrow when cellular abscission fails, Rab11-mediated trafficking of REs with various effector and motor proteins plays a crucial role in successful cytokinesis. Importantly, a number of reports demonstrated that polyploid/multinucleated giant...
cells possess a more aggressive and metastatic phenotype, than compared to parental cells [81, 82], and that they can even facilitate epithelial to mesenchymal transition (EMT) [83-85]. Since the EMT was shown to be responsible for the induction of metastasis and progression of cancer, as well as resistance to various therapeutic agents [83], an application of treatment strategy, targeting a single or complex cytokinesis regulators could potentially be an effective treatment strategy for patients with aggressive and metastatic cancers.

Rab35 functions as oncogene

Rab35 is involved in many cellular processes, and the aberrant regulation of some of them, such as the regulation of cell polarity, phagocytosis, immunity, exosome secretion, cytokinesis and membrane trafficking, may be the cause of cancer [23, 86-88]. Indeed, membrane trafficking is often altered in cancer cells, which leads to the upregulation of growth factor receptors on the cell surface. The latest studies showed that cells with activating mutations of Rab35 possess oncogenic phenotypes. The PI3K/Akt signalling is a major pathway, regulating cell growth, proliferation and survival, and is usually activated in various cancers via binding of growth factors to the receptor tyrosine kinase (RTK) (Figure 3) [89]. Recently, a study by Wheeler et al. [61] demonstrated that two somatic mutations (A151T and F161L) of Rab35, a well-described cytoskeleton organization regulator, are responsible for the growth factor-induced activation of PI3K and Akt in human tumours (uterus, lymphoid and lung tissues). This subsequently promotes cancer cell survival in human tumours, displaying a phenotype similar to mutations in KRAS alleles, turning them oncogenic. Surprisingly, the expression of GTP-bound Rab35 and a constitutively activated mutant of Rab35 (Q67L) was sufficient to activate the PI3K/Akt signalling, even in the absence of growth stimuli (Figure 3) [61]. Thus, it suggests that these with Rab35 mutations-associated gain-of-functions enable tumour cells to survive in the absence of growth factor signalling and significantly promote tumorigenesis.

Another way by which Rab35 promotes tumorigenicity is via a suppression of p53-related protein kinase (PRPK)-induced p53 transcriptional activity. The pS3 is a tumour suppressor, and inhibition of p53 allows the cells to bypass apoptosis checkpoints, and thus promotes the development of tumour cells (Figure 3) [90].

The depletion of Rab35, one of the major cytokinesis regulators, results in the accumulation of endocytic markers on cytoplasmic vesicles in cells that failed cytokinesis, and an increase in binucleate cells (Figure 3) [27]. In addition, it is tempting to speculate that because of the inhibition of Rab35 effector proteins OCRL and MICAL1, and subsequent accumulation of F-actin at the ICB that was previously reported [23, 48], the cytokinesis could also fail, thus enabling the formation of multinucleate cells (Figure 3). Accordingly, it was established that cytokinesis failure, leading to a formation of binucleated tetraploid cells can promote tumorigenesis and that this abnormal karyotype is found in a number of different cancers [56, 91]. In addition, epidermal growth factor (EGF)-mediated activation of Rab35 induces the activation of MICAL1, which then stimulates the generation of reactive oxygen species (ROS). As a consequence, produced ROS leads to a phosphorylation of Akt signalling pathway, and thus promotes invasion of breast cancer cells [92].

Supplementing the Rab35 GTPase and its effectors role in clearing the F-actin in cytokinesis, Rab35 was also shown to link cytokinesis and initiate apical polarity and lumen positioning during cyst development. A study by Klinkert et al. [86] showed that by gathering vesicles containing important apical proteins (aPKC, Crumbs3, Cdc42 and lumen promoting factor Podocalyxin) at the secondary ingression site, Rab35 delivers them to the foreseen apical membrane initiation site (AMIS). Whereas Rab35 depletion by any means (RNAi and S22N) inhibits AMIS, and subsequent lumen formation, which leads to an inversion of apico-basal polarity in vitro [86]. Since the changes in apico-basal polarity are assumed to initiate cancer development, the loss of Rab35 function might stimulate tumorigenesis. In fact, Rab35 is downregulated in several tumours [93].

Cells constantly adjust the levels of integrins and cadherins occupying the plasma membrane during cytokinesis, as well as cancer cell migration and invasion. It is well known that activation of Arf6 induces integrin and epidermal growth factor receptor (EGFR) recycling to
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the cell surface and subsequent signalling, which leads to the induction of integrin-mediated cell migration (Figure 3) [94, 95]. Since Rab35 is a negative regulator of Arf6-mediated integrin recycling, Rab35 depletion was shown to increase integrin levels at the cell surface, and thus to promote cell migration (Figure 3) [93].

Cancer cells are characterised by the transformed membrane trafficking that have a potential to upregulate the EGFR and adhesion molecules (integrins and cadherins) at the cell surface. This in turn stimulates the EMT and the invasiveness of cancer cells during tumour progression [96, 97]. As previously reported, Rab35 is downregulated in many cancers, which leads to an increased EGFR recycling and signal transduction, this being a hallmark of various cancers (Figure 3) [98-100]. Interestingly, in light of manipulations of endocytic trafficking of these receptors, a study by Rush et al. [101] demonstrated that artificially induced accumulation of ligand-activated EGFR in intracellular endosomes promotes apoptosis in MDA-MB-468 breast cancer cells, which implies that targeting of endocytic trafficking could be one of many approaches in suppressing the cancerogenic phenotype.

Concluding remarks and future perspectives

Since RabGTPases are emerging as important regulators of metastasis - a multistep process that is characterised by uncontrolled angiogenesis, migration, invasion, and cell division, more research should be devoted to clarifying the exact roles that Rab proteins play in a control of these important aspects of tumorigenesis. Due to the lack of knowledge in molecular machinery that leads to an aberrant cytokinesis and subsequent initiation of cancer, identification of overlapping signalling pathways and targeting factors that facilitate cytokinesis failure and formation of polyploidy should be identified. Most importantly, this would allow for the new drug targets in polyploid tumour cells to be generated. For this reason, the molecular machinery that drives Rab11- and Rab35-mediated cytokinesis under normal and tumorigenic conditions should be investigated extensively, as these proteins are the master regulators of cell division, and moreover, were shown to cause cancer and induce tumorigenesis. Also, there is not enough data about the interplay between Rab11 and Rab35 in a control of cell division, and therefore, additional studies would “shed more light” into these mysterious, and so far, only partly solved signalling pathways that can give rise to cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Paulius Gibieža, Laboratory of Drug Targets Histopathology, Institute of Cardiology, Lithuanian University of Health Sciences, Sukilėlių pr. 13, Kaunas, LT-50162, Lithuania. Tel: (+370) 629 10490; E-mail: paulius.gibieza@lsmuni.lt

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