

# Novel functions for Rab GTPases in multiple aspects of tumour progression

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## Abstract

Rab GTPases are master regulators of intracellular trafficking and, in recent years, their role in the control of different aspects of tumour progression has emerged. In the present review, we show that Rab GTPases are dysregulated in many cancers and have central roles in tumour cell migration, invasion, proliferation, communication with stromal cells and the development of drug resistance. As a consequence, Rab proteins may be novel potential candidates for the development of anticancer drugs and, in this context, the preliminary results obtained with an inhibitor of Rab function are also discussed.

## Rab proteins as key regulators of intracellular trafficking

Rab proteins are part of the large Ras superfamily of small GTPases. There are more than 60 members in humans and each of them is specifically localized to a subcellular membrane compartment, of which it controls the intracellular traffic [1]. Rab proteins cycle between an inactive cytosolic GDP-bound form and an active membrane-associated GTP-bound form. When in their GTP-bound form, Rabs recruit effector proteins that mediate vesicle budding from donor organelles, transport along microtubules or actin filaments, and tethering and fusion to acceptor compartments [2]. In this way, Rab proteins control protein secretion, endocytosis, recycling and degradation, and are thus widely recognized as master regulators of intracellular trafficking. The complexity of combinations between the different Rabs and their myriad effectors is modulated further by two families of regulators: the GEFs (guanine-nucleotide-exchange factors), that promote GTP binding by Rabs, and the GAPs (GTPase-activating proteins), that trigger Rab GTPase activity and thus GTP hydrolysis to GDP. The specific localization of GEFs and GAPs regulate in a precise space- and time-specific manner the interaction between Rabs and their effectors and, as a consequence, they control Rab activity.

In recent years, a new role has been emerging for Rab proteins in the control of tumour progression. Several Rabs have been found to be deregulated in cancers and many have been described as central in tumour cell migration, invasion of the ECM (extracellular matrix), proliferation, signalling to and from stromal cells, and drug resistance. In the present

review, we will summarize the current knowledge on the role that some of these Rabs play in the aforementioned processes.

## Rabs are aberrantly expressed in tumours

Several Rabs have been found in transcriptomic studies to present a deregulated expression in cancer cells compared with normal tissues, but for many of them a clear function in the context of cancer progression awaits characterization. For example, some Rabs such as Rab1b, Rab4b, Rab10, Rab22a and Rab24 are overexpressed in hepatocellular carcinoma [3]. Rab1a is overexpressed in tongue squamous cell carcinoma [4], whereas Rab2 is overexpressed in peripheral blood mononuclear cells from patients with solid tumours [5] and is associated with lung tumour progression in mouse [6]. Rab3B is up-regulated in prostate cancer, where it promotes cancer cell survival [7]. Rab20 is overexpressed in exocrine pancreatic carcinoma [8] and high levels of Rab31 are associated with worse outcome in breast cancer patients [9]. Interestingly, recent studies have analysed the expression profile of miRNAs (microRNAs) in cancer samples and have found that down-regulation of several miRNAs is associated with high levels of expression of various Rabs: this is the case for *miR-9* and Rab34 [10] and *let-7a* and Rab40c [11] in gastric carcinoma, *miR-451* and Rab14 in non-small lung carcinoma [12], and *has-miR-373* and Rab22a in colon cancer [13]. Conversely, some Rab genes are hypermethylated and thus down-regulated in tumours, such as those encoding Rab32 in colon [14] and gastric [15] cancer, and Rab37 in metastatic lung cancer [16]. Furthermore, Rab38 is down-regulated in melanoma primary tumours and metastasis compared with melanocytes [17]. Interestingly, a recent study in bladder cancer describes aberrant expression of not only several Rabs but also some Rab effectors, GAPs and GEFs [18], thus extending the analysis to Rab-related genes.

The picture emerging from these studies highlights the possibility that Rab GTPases may play an important role in cancer progression. Indeed, the contribution of some Rabs

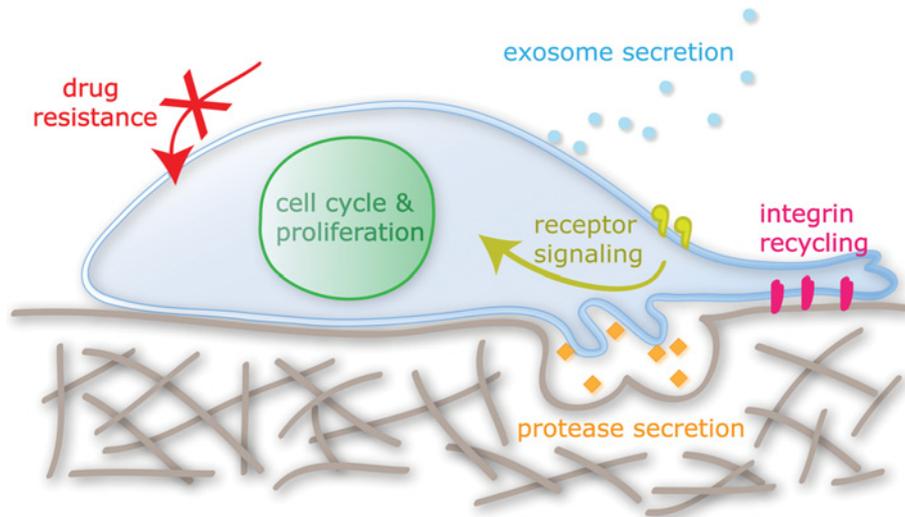
**Key words:** cancer, exosome, invasion, migration, Rab GTPase, tumour microenvironment.

**Abbreviations used:** CAF, cancer-associated fibroblast; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; GAP, GTPase-activating protein; GEF, guanine-nucleotide-exchange factor; MDR, multidrug resistance; miRNA, microRNA; MMP, matrix metalloproteinase; MT1-MMP, membrane-type 1 MMP; P-gp, P-glycoprotein; RabGGTase, Rab geranylgeranyltransferase; RCP, Rab-coupling protein; Shh, Sonic Hedgehog.

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**Figure 1 | Rab proteins have multiple functions in tumour cells**

The functions of Rab proteins in tumour cells include delivery and recycling of integrins at the front during cell migration, secretion of proteases to degrade the ECM and invade the surrounding tissues, secretion of exosomes that mediate the communication with stromal cells, trafficking of receptors and control of their signalling, impact on cell cycle and survival, and resistance to anticancer drugs.



to tumour progression has been investigated more in detail and is described in the following sections.

**Rabs direct integrin trafficking and cell migration**

A fundamental characteristic of metastatic cells is their ability to migrate and invade through the ECM to form metastases in other organs. In a series of well-documented studies, the family of Rab11 proteins (Rab11a, Rab11b and Rab25) and their effectors have been demonstrated to play a key role in the migration of cancer cells through the modulation of integrin recycling at the migration front (Figure 1). Rab11a had been described to regulate  $\alpha 6 \beta 4$  integrin transport at the cell surface during hypoxia-stimulated migration in breast cancer cells [19]. Rab25 is overexpressed in human ovarian cancers, where it correlates with poor prognosis, and increases cell survival *in vitro* and tumorigenesis *in vivo* [20]. In seminal works from Jim Norman's group, Rab25 has been shown to associate with  $\alpha 5 \beta 1$  integrin and promote its delivery at the pseudopodial tip of invading cells, thus driving cell migration in a three-dimensional environment [21]. Also, Rab25 collaborates with CLIC3 (chloride intracellular channel protein 3) to recycle integrins from late compartment to the back of migrating cells, a process necessary for the release of the cell rear during invasion [22]. Interestingly, the same group has also shown that the recycling of  $\alpha 5 \beta 1$  integrin is coupled to the recycling of EGFR (epidermal growth factor receptor) via the interaction with RCP (Rab-coupling protein), and this results in an increase in EGFR signalling to the survival factor Akt [23]. RCP is an effector of Rab11 and has been described as an oncogene that promotes breast cancer transformation

[24]. Furthermore, RCP-mediated recycling and signalling of both integrin and EGFR are located downstream of a mutant form of p53 that drives an increase in cancer cell invasiveness [25]. In this way, metastatic cells integrate and co-ordinate signalling pathways important for migration and proliferation through Rab-mediated vesicular trafficking. Of note, although Rab25 has been described clearly as an oncogene in certain types of cancer, it has also been shown to act as a tumour suppressor in others, such as colorectal cancers [26,27] and triple-negative breast cancers [28,29]. So the exact function of Rab25 in tumour progression is likely to be context-dependent. Another Rab involved in the control of integrin trafficking is Rab21. Rab21 binds to  $\alpha$  integrins in a GTP-dependent fashion and its level of expression correlates with integrin-mediated adhesion and motility in breast cancer cells [30]. Integrin internalization is also mediated by Rab5 and depletion of Rab5 reduces migration *in vitro* and tumour metastasis in neuroblastoma, downstream of caspase 8 signalling [31]. Furthermore, Rab5a expression is associated with higher-grade and lymph node metastasis in breast cancer patients [32], and it is overexpressed in cervical cancer, where again it promotes cell motility and invasion [33], and in hepatocellular carcinoma, where a dominant-negative mutant decreases cell migration [34].

**Rabs promote protease secretion and cell invasion**

Tumour cells secrete a variety of proteases, including MMPs (matrix metalloproteinases), which help the cells to breach the basal membrane and the ECM and migrate to distant organs. Some Rabs have been described to play a role in the context of MMP secretion or activation (Figure 1). In breast

cancer cells, for instance, Rab8 contributes to cell invasiveness by promoting the secretion of MT1-MMP (membrane-type 1 MMP) at peripheral invasive structures [35]. Rab7 has also been recently described as contributing to MT1-MMP secretion and a dominant-negative mutant of Rab7 impairs both cell migration and invasion [36]. Rab27b, which is associated with lymph node metastasis and differentiation grade in ER (oestrogen receptor)-positive human breast cancer patients, induces invasive growth of breast cancer cells via the secretion of Hsp90 $\alpha$  (heat-shock protein 90 $\alpha$ ), which is required for the extracellular activation of MMP2 [37]. Rab27a is overexpressed in lymph node metastases in breast cancer cells [38] and in primary hepatocellular carcinoma [39], it has been suggested to promote cell invasiveness [40] and we recently observed that it is required for secretion of MMP9 by mouse mammary carcinoma cells [41]. Another protease important for the modification of the tumour microenvironment is procathepsin-L and its secretion is controlled by Rab4 [42]. Indeed, overexpression of Rab4 in melanoma causes an increase in tumour mass, whereas depletion of Rab4 induces a reduction in tumour volume [42].

### **Rabs contribute to tumour-stromal cell communication and the modification of the tumour microenvironment**

In recent years, the rising importance of stromal cells in the creation of a tumour microenvironment favourable to cancer cell growth and metastasis has emerged. Important actors in this process are CAFs (cancer-associated fibroblasts) [43], which, among other functions, remodel the ECM. In this context, Rab21 has been shown to promote integrin accumulation at the plasma membrane and actomyosin contractility in CAFs, which results in collagen contraction and allows the invasion of squamous cell carcinoma through the CAF-modified matrix [44]. Other important mediators in intercellular communication between cancerous and stromal cells are tumour-derived microvesicles and exosomes [45] (Figure 1). In this context, Rab27a was shown to be required for secretion of exosomes by HeLa cells [46]. *In vivo*, Rab27a inhibition in melanoma cell lines reduced primary tumour growth and formation of lung metastases: this anti-tumour effect was due to a combination of impaired secretion of pro-angiogenic factors such as PlGF (placental growth factor), and decreased secretion of exosomes that 'educate' and recruit bone-marrow-derived cells to create a pro-metastatic niche [47]. Of note, Rab27a had been identified previously as a driver gene in melanoma [48]. In a similar manner, combined Rab27a-dependent secretion of MMP9 and exosomes contributes to the mobilization of a pro-tumoral neutrophil population and supports growth of a mouse mammary tumour and its metastasis to lungs [41].

### **Rabs affect cell-cycle progression**

The aberrant expression of some Rabs has been shown to affect cell-cycle progression. One example is Rab27b:

its overexpression promotes cell-cycle progression and proliferation in the breast cancer cell line MCF-7 and this is dependent on its GTP-loaded active state [37]. Another Rab described in this context is Rab6c, which is localized to the centrosome and whose overexpression results in G<sub>1</sub>-phase arrest and whose depletion generates tetraploid cells [49]. Furthermore, Rab21-mediated trafficking of integrins at the cleavage furrow is required for cytokinesis and, as a consequence, cells depleted of Rab21 present multinuclei and genomic instability [50].

### **Rab23 antagonizes the Shh (Sonic Hedgehog) pathway**

The morphogenic Shh pathway is essential in the embryonic dorsoventral development and is deregulated in many cancers. Rab23 was first identified as an antagonist of the Shh signalling pathway [51] and recently it has been shown to interact in its GTP-bound form with Su(Fu) and thus inhibit the Gli transcription factor that is normally activated downstream of Shh signalling [52]. However, Rab23 is overexpressed in diffuse-type gastric cancer, where it promotes cell invasion [53], and in hepatocellular carcinoma, where it supports cell growth [54], so its role in tumour progression might also be Shh-independent.

### **Rabs are involved in resistance to anticancer drugs**

MDR (multidrug resistance) in human cancers is often associated with the overexpression of P-gp (P-glycoprotein), a large transmembrane protein localized at the plasma membrane that extrudes anticancer drugs and thus decreases their concentration, and toxicity, inside the cells [55]. The recycling protein Rab4 interacts with P-gp, but it is underexpressed in MDR cells [56]. However, when Rab4 is overexpressed, it decreases P-gp levels at the plasma membrane, thus increasing drug sensitivity in MDR cells [56] (Figure 1). Similarly, Rab6c is underexpressed in MDR cells and its overexpression induces the intracellular accumulation of several anticancer drugs, although the mechanism underlying this phenotype has not yet been established [57]. Another Rab involved in resistance to a chemotherapeutic drug is the secretory Rab8: cancer cells resistant to the platinum compound cisplatin have high levels of Rab8 and overexpression of Rab8 in sensitive cells enhances resistance to cisplatin, possibly by increasing the secretion of the cisplatin-resistance-associated protein TMEM205 (transmembrane protein 205), with which it co-localizes [58].

### **A role for GAPs in cancer**

As mentioned above, the activity of Rab proteins is regulated by GEFs and GAPs, and some of them may well play a role in cancer. PRC17 (prostate cancer gene 17) (TBC1D3A) is a GAP for Rab5, it is overexpressed in metastatic

prostate cancer and its overexpression transforms NIH 3T3 fibroblasts [59]. Another GAP for Rab5 implicated in cell transformation is the regulatory subunit of class I PI3K (phosphoinositide 3-kinase) p85 $\alpha$ : if p85 $\alpha$  is impaired in its GAP function, it induces loss of contact inhibition, growth in soft agar and tumour formation in nude mice, owing to disrupted regulation of PDGFR (platelet-derived growth factor receptor) trafficking and increased downstream signalling to Akt [60]. Another GAP recently described in cancer is TBC1D7, a GAP for Rab17: it is overexpressed in lung cancer, where it is associated with poor prognosis, and promotes cell growth [61]. Also, TBC1D16 has been identified using a bioinformatic approach as a driver gene for melanoma and its depletion reduces melanoma cell growth [48]. However, its substrate Rab is not yet known.

## Rabs as therapeutic targets

Since Rab proteins are implicated in multiple aspects of tumour progression, they might represent new targets for anticancer therapies. Although no Rab-specific drug has been developed to date, it is important to mention encouraging results obtained with inhibitors of RabGGTase (Rab geranylgeranyltransferase). All Rabs are post-translationally modified mostly by the addition of two geranylgeranyl groups at their C-termini by RabGGTase and this modification is essential for the association of Rab proteins to membranes. The *gunmetal* mouse model, in which Rab prenylation is severely reduced owing to an 80% decrease in RabGGTase activity, presents only hypopigmentation and altered platelet biogenesis, showing that a decrease in Rab prenylation would be generally well tolerated [62]. RabGGTase is overexpressed in some tumours and some farnesyltransferase inhibitors that have a pro-apoptotic effect indeed target RabGGTase and can be mimicked by depletion of RabGGTase [63]. A bisphosphonate derivative, 3-PEHPC [3-(3-pyridyl)-2-hydroxy-2-phosphonopropanoic acid], which is highly specific for RabGGTase, has an anti-resorptive activity in osteoclasts [64], induces apoptosis of human myeloma cells *in vitro* [65] and prevents bone disease *in vivo* in a mouse model [66]. Furthermore, it inhibits metastatic cell invasion *in vitro* [67] and reduces skeletal tumour growth *in vivo* [68]. These findings highlight the potentiality of Rab inhibition as a therapeutic approach.

## Concluding remarks

Mutations in some Rabs or in regulators of the Rab GTP/GDP cycle had been identified as causative of some severe complex hereditary diseases [69]. However, the potential role of Rab proteins in the context of tumour progression has only begun to emerge in recent years. As presented above, some functions for Rab GTPases in cancer development have now been elucidated and the emerging picture points to the importance of intracellular trafficking as a co-ordinator of the cross-talk between signalling pathways promoting proliferation, migration and invasion. Also, Rabs

have been shown to direct the communication between cancerous and stromal cells, and are thus able to modulate the creation of a metastatic niche favourable to tumour growth, an emerging concept that is attracting much interest as a therapeutic target. Although inhibitors of specific Rab proteins have not yet been developed, they could represent a potential tool as novel anticancer drugs in the years to come.

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