Rab25 in cancer: a brief update

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Abstract

Detailed endocytosis is a hallmark of cancer. The endocytic pathway, as demonstrated by our laboratory, is a frequent target of genomic aberrations in cancer and plays a critical role in the maintenance of cellular polarity, stem cell function, bioenergetics, proliferation, motility, invasion, metastasis, apoptosis and autophagy. The Rab GTPases, along with their effectors, are critical regulators of this endocytic machinery and can have a huge impact on the cellular itinerary of growth and metabolism. Rab25 is an epithelial-cell-specific member of the Rab GTPase superfamily, sharing close homology with Rab11a, the endosomal recycling Rab GTPase. RAB25 has been implicated in various cancers, with reports presenting it as both an oncogene and a tumour-suppressor gene. At the cellular level, Rab25 was shown to contribute to invasiveness of cancer cells by regulating integrin trafficking. Recently, our laboratory uncovered a critical role for Rab25 in cellular energetics. Assimilating all of the existing evidence, in the present review, we give an updated overview of the complex and often context-dependent role of Rab25 in cancer.

Introduction

Rab25 (Rab11c, Catx8), identified by Goldenring et al. in 1993 [1] as a member of the Rab family of GTPases, shares 63% homology with the ubiquitous Rab11a. Rab25 contains a GTP-binding sequence, WDTAGLE, different from that of other Rab members, making it a constitutively active Rab GTPase, although this remains to be confirmed biochemically. At the cellular level, Rab25 function is thought to mirror that of Rab11a. Both of them are spatially and functionally associated with apical recycling and transcytosis pathways in polarized epithelial cells [2,3]. Loss of cell polarity being an essential hallmark of cancer implicates Rab25-related trafficking and its impact on the epithelial cell polarity programme [4,5] in cancer progression.

To date, the ~70 existing publications on Rab25 have built our understanding regarding the localization, trafficking and other functions of this epithelial-cell-specific GTPase. Most importantly, by uncovering the role of Rab25 in solid tumours, we are beginning to understand how abnormal vesicular trafficking affects cancer progression. Several reviews have been published updating the role of Rab25 and vesicular trafficking in cancer [6–9]. More recently, global interest in Rab25 has surged owing to the repeated appearance of Rab25 in multiple cancer screens across diverse studies in different parts of the world.

In the present review, we summarize the previous literature on Rab25 and its role in cancer, highlighting the mechanical insights that we have gained in the last few years.

Key words: cancer, endocytosis, endosomal recycling, integrin, Rab11a, Rab25.

Abbreviations used: CLIC3, chloride intracellular channel protein 3; CRE, cAMP-response element; CREB, CRE-binding protein; ER, oestrogen receptor; iPSC, induced pluripotent stem cell; KLF9, Krüppel-like factor 9; PPMD1, protein phosphatase magnesium D-element; TCGA, The Cancer Genome Atlas; TIMP1, tissue inhibitor of metalloproteinases 1; VEGF, vascular endothelial growth factor.

Context-dependent role of RAB25 in cancer: oncogene or tumour suppressor?

RAB25 as an oncogene

The first reported association of Rab25 with epithelial cancers came from a study by He et al. [10], where they characterized nine human GTPases showing variable gene expression in liver cancer. Rab25 was one of the six Rab proteins that were up-regulated in hepatocellular carcinoma [10]. However, the most exhaustive evidence for RAB25 as an oncogene came from a pioneering study by Cheng et al. [11], from our laboratory, where Rab25 was identified as the driver of the 1q22 amplicon, a region frequently amplified in ovarian cancers [11,12]. Rab25 was up-regulated at DNA copy number, mRNA and protein levels, and was associated with a poor outcome in clinical studies. Overexpression of Rab25 in ovarian cancer cell lines increased the quintessential hallmarks of cancer, namely anchorage-independent growth, cell proliferation and reduced apoptosis and anoikis, mostly in a PI3K (phosphoinositide 3-kinase)-dependent manner. Overall, both in vitro and in vivo evidence shows that Rab25 contributes to the aggressiveness of ovarian and breast cancers [11,12]. Subsequently, others also reported similar findings in ovarian cancer models [13], making RAB25 not only a key oncogene for ovarian cancers, but also an important biomarker. Rab25 expression varied on the basis of cellular lineage and facilitated differential diagnosis in cases presenting a highly heterogeneous disease [14]. Specifically, the RAB25 gene was a part of a signature that was up-regulated in primary peritoneal serous carcinoma, but not in highly metastatic diffused peritoneal malignant mesothelioma [15,16]. The broad range of Rab25 expression in ovarian cancers was also detectable in bioinformatics analysis of copy number variation and DNA methylation across primary serous ovarian tumour samples from TCGA (The Cancer Genome Atlas) project, suggesting that Rab25 is a sensitive
biomarker in addition to a potential therapeutic target for ovarian cancers [17].

In another study, exploiting the fact that androgens are implicated in the aetiology of ovarian cancers, Sheach et al. [18] subjected androgen-receptor-expressing ovarian cancer cell lines to androgen treatment and tested for changes in global gene expression profiles. Surprisingly, Rab25 and Rab35 were the most prominent hits both at the gene and protein level among the panel of 121 up-regulated genes.

Rab25 expression also correlated with histological grade in patients and emerged as an androgen-responsive gene in ovarian cancer, making Rab25 a clinical biomarker for androgen receptor function in ovarian cancers [18].

**Rab25 as a tumour suppressor**

Unlike ovarian cancers, the role of Rab25 is far more ambiguous in breast, colon and intestinal carcinomas, although it remains a major player in disease progression.

In a novel breast cancer cell line generated from human mammary epithelial cells by Rao’s group (by transducing the Q61L mutant Ras in these cells and immortalizing with the catalytic subunit of telomerase), mutations at the 1q22-23 chromosomal locus resulted in the loss of Rab25, leading to oncogenic transformation [19]. This was the first report of Rab25 as a tumour suppressor. The same group followed up their earlier work by showing that Rab25 is lost in triple-negative breast cancer, represented by the MDA-MB-231 cell line, and the introduction of Rab25 in these cells partially reverses oncogenic progression [20]. In their model, ectopic Rab25, in addition to enhancing apoptosis, also suppressed angiogenesis and invasion by regulating VEGF (vascular endothelial growth factor)-A and VEGFR1 (VEGF receptor 1) expression [20]. However, this finding is contradictory to what our laboratory has observed in various broad-spectrum analyses of breast cancer patients’ expression profiles as well as in cell lines where Rab25 is up-regulated and is associated with increased tumorigenicity and poor clinical outcome [11,12]. Recently, increased levels of RAB25 mRNA (using real-time PCR) and protein (using immunohistochemistry) were reported in tissues from invasive ductal carcinoma patients with lymphatic metastasis compared with those without lymph metastasis [21], again implicating RAB25 as an oncogene in breast cancer.

It is important to note that breast cancer is a highly heterogeneous disease, populated by diverse cell types [22,23]. Each molecular subtype has distinct gene expression profiles and overall outcome. Reconciling the conflicting evidence, it can be speculated that Rab25 is associated with specific cell lineages in breast cancer. Our unpublished work also strongly supports the notion that Rab25 is indeed an oncogene in hormone-receptor-positive luminal B subtype of breast cancers, whereas in subsets of basal subtypes (lacking hormone receptors), Rab25 is mostly lost, possibly playing a tumour-suppressive role (S. Mitra, K.W. Cheng and G.B. Mills, unpublished work). In fact, recently, patient data from the TCGA project presented the differential methylation status of Rab25 between luminal and basal subtypes of breast cancer with a significant increase in methylation in the latter group (S. Mitra, K.W. Cheng and G.B. Mills, unpublished work). Furthermore, with the re-evaluation of the molecular features of intrinsic subtypes of breast cancer and emergence of the new ‘claudin-low’ group within the basal category (exemplified by the MDA-MB-231 cell line), we found that Rab25 levels are concomitantly undetectable along with low claudin levels in these mesenchymal breast cancer subtypes [22,23]. So it is safe to conclude that, in breast cancers, the oncogenic role of Rab25 is context-dependent. In fact Yin et al. [21] provide evidence that the level of Rab25 is significantly higher in ER (oestrogen receptor)/PR (progesterone receptor)-positive patient samples than the ER/PR-negative ones, although the correlation of Rab25 with lymph metastasis holds true in both cases [21].

Interestingly, probing further into the molecular heterogeneity of breast cancers, Leth-Larsen et al. [24] performed an elegant study segregating the tumour-initiating cells from the CD44high population in triple-negative breast cancer cell lines. Many of the triple-negative cell lines are composed of at least two distinct phenotypic cell lineages. They found that there was a mixture of mesenchymal/basal B-type cells and epithelial or luminal/basal A-type cells. Unexpectedly, they found that the CD44high/C24low luminal/basal A-type cells retained stem-cell-like tumour-initiating mammosphere-forming and chemoresistant properties, instead of the mesenchymal subpopulation [24]. In their study, Rab25, along with Muc1 and a few other candidate genes, were positively correlated with tumour-initiating features and could potentially be used to predict distant metastasis in these ER-negative tumours [24].

Our laboratory is currently studying the context-dependent role of Rab25 in luminal compared with basal subtypes to gain mechanistic insights into how this trafficking protein serves both as a tumour suppressor and a tumour promoter. This dichotomy was addressed by Tang [8], and earlier by Mills et al. [7], offering insightful explanations. Ongoing work in our laboratory supports that the interaction of Rab25 with its effector, RCP (Rab-coupling protein) (also called Rab11Fip1), which itself is a potent oncogene driving the 8p11-12 amplicon, with high frequency of amplification in breast cancer [25], is of critical importance in understanding the clinical role of Rab25 in breast cancer (S. Mitra, K.W. Cheng and G.B. Mills, unpublished work).

In another instance of ambiguity, Rab25, reported previously to be present in colon carcinoma cell lines [1], was later shown to be lost in human colonic neoplasia [26,27], as well as in intestinal neoplasia in mice and humans [27]. Colorectal adenocarcinomas with low Rab25 correlated with shorter patient survival. Since Rab25 is essential for polarized trafficking to the cell surface, the authors suggested that loss of Rab25 alters cell polarity and induces transformation.

Thus, on the basis of the cases mentioned above, it is imperative for us to evaluate the role of Rab25 in a case-specific manner rather than view it as a global oncogene or tumour suppressor. Instead of using Rab25 alone as a
biomarker, it will be invaluable to generate a Rab25 signature with more powerful prognostic and predictive value.

**Frequent appearance of Rab25 in various cancer-screening studies**

As highlighted in the previous section, the role of Rab25 has been studied in greater detail in a handful of cancers providing significant mechanistic insights. However, Rab25 is gaining global prominence, as it features repeatedly in gene signatures of a wide variety of cancers. However, these studies did not analyse the functional role of Rab25 in the respective diseases, but they do open up the opportunity for groups focusing on Rab GTPases to gain more insights into the role of trafficking proteins in various cancers.

In Wilms's tumours, a gain in the 1q22 region housing Rab25 segregated histologically favourable tumours that eventually relapsed from those that remained relapse-free. RT (reverse transcription)–PCR validation of a recurring region of 1q, namely 1q22-q23.1, identified five candidate genes including Rab25 [28].

Another group studying frequent amplification and overexpression of PPMD1 (protein phosphatase magnesium D-8i) in cancers with poor prognosis, measured global gene expression changes in several ER-positive breast cancer cell lines with RNAi (RNA interference)-mediated down-regulation of PPMD1. Rab25 was noted in the shortlisted genes with maximum differential expression [29,30], supporting its key regulatory role in ER-positive breast cancers.

Rab25 featured in one study where the role of TIMP1 (tissue inhibitor of metalloproteinases 1) in tumour progression was assayed using triple-negative MDA-MB-231 cells overexpressing TIMP1 [31]. TIMP1, known to inhibit matrix metalloprotease-induced invasion, is often up-regulated in breast cancers and is associated with poor prognosis. The authors testing the effect of TIMP1 overexpression both in vitro and in vivo found different sets of gene networks were altered under each setting. Notably, Rab25 featured only in the in vivo xenograft panel comparing control with overexpressing cells [31]. Questions such as whether TIMP1 is a regulator of Rab25 transcription and/or protein expression arise from this study. Since the MDA-MB-231 cell line itself consists of heterogeneous cell populations, it raises the question of whether TIMP1 affects the epithelial/basal A cell population or some other cell populations.

Considering that Rab25 is reportedly a tumour suppressor in MDA-MB-231 cells, TIMP1 could be changing the pseudopodia tip during fibronectin-stimulated cell migration and simultaneously maintain a readily available pool of the same at the cell front [36].

**Mechanistic insights into the cellular function of Rab25 and its regulation**

One of the most powerful oncogenic effects of Rab25 is driving metastatic progression following the loss of cell polarity. Rab25 is implicated in cancer cell migration and invasion in multiple studies published from our laboratory and others [11,35]. The remarkable work of Jim Norman and his group pioneered our understanding of the molecular mechanisms of Rab25, especially how it affects metastasis. First, in a seminal piece of work, they showed a direct interaction between cytoplasmic end of β1 integrin and Rab25, promoting cell migration, involving long pseudopodia in a three-dimensional matrix [36]. Basically, Rab25-decorated vesicles transport α5β1 integrins to the plasma membrane at the pseudopodia tip during fibronectin-stimulated cell migration and simultaneously maintain a readily available pool of the same at the cell front [36].

A potential link between integrin recycling by Rab25 and the differential expression of Rab25 in TIMP1 overexpressing breast cancer cells grown in xenografts (as reported in the previous section [31]) remains to be tested, but may provide important clues to Rab25 function in triple-negative breast cancer.

Although Rab25 and Rab11 were originally identified in the apical recycling compartment and thought to be associated with long loop recycling (as opposed to short loop recycling via Rab4), a recent study using HeLa cells and MDA-MB-231 cells, found that ectopic Rab25
acts predominantly in the short-distance rapid recycling within cellular tips, whereas Rab11 propelled a rear-to-front long-distance transport in migrating cells [35]. This study also noted that overexpression of Rab25 and Rab11 increased the overall speed of cell migration [35].

Another fascinating discovery from Norman’s laboratory further elucidated Rab25-mediated integrin trafficking. Using photoactivation and biochemical approaches, the group showed that Rab25 rescues lysosomally targeted integrins from degradation by transporting them back to the plasma membrane [37]. The molecule that collaborates with Rab25 in achieving this retrograde transport is CLIC3 (chloride intracellular channel protein 3), which, not surprisingly, is up-regulated in Rab25-expressing cells [37]. CLIC3 itself is an independent marker of lymph node metastasis and poor prognosis in pancreatic cancers, suggesting that Rab25 and its effectors create a potent oncogenic network by deregulating vesicular trafficking of key signalling molecules that increases the aggressiveness of these cancers.

There is also an argument that the Rab25-associated invasive phenotype is not only related to actin-based cytoskeletal changes, but also profoundly alters microtubules, as observed during Rab25-induced transformation of rat intestinal epithelial cells [38].

The literature so far shows direct or indirect involvement of Rab25 in virtually all of the classic hallmarks of cancer proposed by Hanahan and Weinberg [39], including proliferation, survival, evading apoptotic signals, angiogenesis, invasion and migration. In the last few years, the field of oncology has developed renewed interest in cancer cell metabolism reviving Otto Warburg’s findings that cancer cells prefer aerobic glycolysis. In fact, the increased glucose uptake of cancer cells facilitated the development of [18F]fluorodeoxyglucose PET (positron-emission tomography) as a standard diagnostic tool in various solid tumours [40,41], enlisting altered metabolism as a new hallmark of cancer [42].

Recently, our laboratory showed, for the very first time, the profound effect of Rab25 on cellular energetics [43]. This is of immense importance because rapidly growing tumours frequently undergo metabolic stress arising from limited growth factors, nutrients and oxygen. The intercellular trafficking system is geared to rewire circulation of cellular resources to allow stressed cancer cells to survive. By conserving reserves, recycling glucose transporters and growth factors rather than degrading them, endosomal trafficking imparts a huge survival advantage. Rab25, as we showed in our recent work, regulates cellular bioenergetics and autophagy in cancer cells undergoing stress [43]. It does so by activating Akt, leading to increased glucose uptake. Unexpectedly, Rab25 also facilitates accumulation of glycogen, an additional energy reserve for cells. We found that Rab25-overexpressing ovarian cells maintained higher levels of basal cellular ATP under metabolic stress conditions, once again protecting them from energy catastrophe that would otherwise trigger apoptosis. Importantly, a wide array of bioenergetic targets were enriched in ‘Rab25-dependent’ patient profiles and this combined signature significantly improved identification of patients with poor prognosis [14]. Moreover, recently, another group also showed that loss of Rab25 in ovarian cancer cells inhibit cell proliferation and induce autophagy [14], suggesting a pivotal role for Rab25 in cellular energetics.

The big question remains how a gene like Rab25 with many critical pleotropic effects is regulated. To date, there is little understanding of it. But a recent breakthrough by Xue et al. [44] lays the foundation for future structural studies. By characterizing the human RAB25 promoter, the authors found a CRE (cAMP-response element)-binding CREB (CRE-binding protein) that binds to the RAB25 core promoter region and activates it. They also found that certain PKA (protein kinase A) activators could induce phosphorylation of CREB by exposing the CRE. CREB phosphorylation recruits cofactors and further opens up chromatic configuration, strengthening promoter activity and Rab25 expression [44].

**Conclusions**

Rab25 is a relatively new entrant in the field of Rab GTPases and we are only beginning to understand its role in disease progression, its various trafficking routes, the cargo it carries, its effectors and interactions, and how it is regulated. So far, its role as an oncogene is most convincing in ovarian cancers. Therefore its value as a clinical biomarker is also associated best with ovarian cancers [45]. In the case of breast cancers, Rab25 function is highly context-dependent. We believe this is also true for colon and intestinal carcinomas and other cancers with heterogeneous lineage. What will greatly benefit the field is the identification of Rab25 ‘interactomes’ in different cancers and also within specific subtypes in each cancer.

Lastly, development of a robust Rab25 activity assay is absolutely essential to improve functional studies involving this important trafficking protein.

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