J. Cell. Mol. Med. Vol 19, No 1, 2015 pp. 1-10

The role of the small GTPase Rab31 in cancer

Christelle En Lin Chua [a, b, *], Bor Luen Tang [a, b]

[a] *Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore*
[b] *NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore*

Received: April 20, 2014; Accepted: July 18, 2014

Abstract

Members of the small GTPase family Rab are emerging as potentially important factors in cancer development and progression. A good number of Rabs have been implicated or associated with various human cancers, and much recent excitement has been associated with the roles of the Rab11 subfamily member Rab25 and its effector, the Rab coupling protein (RCP), in tumourigenesis and metastasis. In this review, we focus on a Rab5 subfamily member, Rab31, and its implicated role in cancer. Well recognized as a breast cancer marker with good prognostic value, recent findings have provided some insights as to the mechanism underlying Rab31's influence on oncogenesis. Levels of Oestrogen Receptor α (ERα)- responsive Rab31 could be elevated through stabilization of its transcript by the RNA binding protein HuR, or though activation by the oncoprotein mucin1-C (MUC1-C), which forms a transcriptional complex with ERα. Elevated Rab31 stabilizes MUC1-C levels in an auto-inductive loop that could lead to aberrant signalling and gene expression associated with cancer progression. Rab31 and its guanine nucleotide exchange factor GAPex-5 have, however, also been shown to enhance early endosome-late endosome transport and degradation of the epidermal growth factor receptor (EGFR). The multifaceted action and influences of Rab31 in cancer is discussed in the light of its new interacting partners and pathways.

Keywords: cancer • mucin1 • Rab31 • membrane traffic

Introduction

There is an enormous flux of both membrane and soluble cargoes between intracellular membranous compartments in the eukaryotic cell. It is necessary for this flow of multidirectional traffic to be adequately regulated, and dysregulation at any point of the trafficking network could lead to disease states. A particularly important group of proteins regulating membrane traffic in eukaryotic cells are the Sar/Arf family and the Rab family of small GTPases [1]. Rabs (Ras-related proteins in brain) [2, 3] constitute the largest subfamily of the Ras superfamily of small GTPases [4], and has more than 60 genes encoded within the human genome. Translated as soluble, cytosolic proteins, Rabs acquire C-terminal prenylated lipid anchors (geranylgeranylation) and are localized rather specifically to different sub-cellular compartments for the regulation of particular membrane trafficking step(s) in the exocytic and endocytic pathways [5–7].

Analogous to the function of the proto-oncogene Ras in cellular signalling [8], Rab proteins are post-translationally modified and go through a cycle of guanine nucleotide exchange and hydrolysis to act as key switches in membrane traffic pathways [2, 9]. Rabs are kept in the cytosol by GDP dissociation inhibitors (GDIs) [10]. The Rab escort protein engages cytosolic Rab proteins to be presented to Rab geranylgeranyl transferase [11], allowing the Rab protein to be geranylgeranylated at its C-terminal cysteine residues before it is escorted

*Correspondance to: Christelle En Lin CHUA,
NUS Graduate School of Integrative Sciences and Engineering,
National University of Singapore, 8 Medical Drive, Singapore 117597.
Tel.: 65 6516 1040
Fax: 65 6779 1453
E-mail: g0901904@nus.edu.sg

doi: 10.1111/jcmm.12403

to the target membrane [12, 13]. The added lipid anchor facilitates the attachment of a Rab protein to the target membrane, but full activation requires its bound GDP to be exchanged for GTP, a process that is facilitated by guanine nucleotide exchange factors (GEFs) [14, 15]. Activated, GTP-bound Rabs engage effector molecules such as tethering complexes [16], motor proteins and motor adaptors [17], as well as components of the vesicle fusion machinery [18] to facilitate tethering and docking of vesicles to their target membranes. Rabs have also been shown to interact directly or indirectly with some cargo molecules [19]. Rab proteins are inactivated when the bound GTP is hydrolysed back to GDP. This will not occur spontaneously to any significant degree as Rabs have intrinsically weak GTPase activities. Instead, inactivation by GTP hydrolysis is assisted or regulated by GTPase activating proteins (GAPs) [14, 20].

Given the critical importance of regulating membrane traffic which impinges on tightly regulated processes like cell proliferation and cell migration, it is expected that Rab small GTPases' activities gone awry may directly or indirectly contribute to human diseases. Several Rab genes are associated to heritable monogenic diseases. Rab7 mutations underlie Charcot–Marie–Tooth type 2B neuropathy, a peripheral nervous system disorder that is believed to be due, in part, to the dysregulation of peripherin (a neuronal intermediate filament that has been shown to interact with Rab7) [21, 22]. Rab18 mutations [23] and that of a putative RabGAP TBC1D20 [24] causes Warburg micro-syndrome, a rare autosomal recessive genetic disorder characterized by microcephaly, defects in the visual system and mental retardation. Mutations in Rab23, which plays a role in the regulation of Sonic Hedgehog (Shh) signalling, underlie another autosomal recessive disorder, Carpenter's syndrome, characterized by craniosynostosis, polysyndactyly, obesity and cardiac defects [25, 26]. Rab27, which plays a role in melanosome transport *via* its effector myosin Va, has been implicated in Griscelli syndrome type 2, a recessive disorder in which patients exhibit pigmentation defects like partial albinism and immune deficiency [27]. Mutations in Rab39B, a Golgi-localized neuronal Rab that may play a role in synaptic maintenance, are responsible for X-linked mental retardation [28]. In addition, Rab38 [mutated in rat's Ruby (red eyed dilution; R) locus and the homologous mouse chocolate (cht) locus] has been implicated in the autosomal recessive disorder Hermansky–Pudlak syndrome (HPS) that is characterized by pigmentation and blood clotting disorders [29]. Recently, mutations in several HPS genes which encode components of Biogenesis of lysosome-related organelles complex-3 (BLOC-3), a Rab32 and Rab 38 GEF, have also been identified. It is postulated that the resulting defects in the biogenesis of lysosomal related organelles, of which Rab38 is believed to play a role, gives rise to some of the symptoms observed in HPS, including albinism and impaired platelet function [30]. Rab mutations or problems that are associated with aspects of Rab-mediated transport may indeed underlie a wider spectrum of neurological [31–33] and immune disorders [34].

As Rabs modulate membrane trafficking of growth factor receptors and cell adhesion molecules, it is also conceivable that dysregulation with regard to Rab-mediated endocytosis or recycling could lead to failure to control cell proliferation, adhesion and migration. A good number of Rabs have also been associated with human cancer [35, 36]. Interestingly, cellular transformation and invasion are linked largely to changes in expression levels of these Rabs, rather than their mutations. Rab31, a member of the Rab5 subfamily, has recently emerged as a membrane traffic modulator that has interesting associations with breast carcinoma as well as glioma, and is the focus of this review. We first take a broad overview at our current understanding of Rabs that have been implicated in cancer (see summary in Table 1).

Rabs in human cancer

Dysregulated expressions of multiple Rabs spanning the entire exocytic and endocytic pathways have been shown in transcription profiling analysis of various cancer tissues [35, 36]. Rab1A, which regulates ER-Golgi transport, is elevated in tongue squamous cell carcinomas [37] and melanoma [38]. Rab2, which also functions in the ER-Golgi boundary, is elevated in peripheral blood mononuclear cells (PBMCs) of tumour bearing patients [39, 40]. Rab5 isoforms (Rab5A, -B and -C) are key regulators of the early endocytic pathway, and are known to profoundly influence cell motility and invasion, possibly through the regulation of β1-integrin traffic [41, 42]. Rab5A has been shown to be up-regulated in non-small cell lung carcinoma [43], autonomous thyroid adenomas [44], hepatocellular carcinoma [45] and ovarian cancer [46]. Rab5B expression is elevated in melanoma cells [38]. Rab5C plays a role in enhancing EGF-induced invasion by breast cancer cells [47]. The key late endosomal Rab, Rab7, has been shown to be up-regulated in autonomous thyroid adenomas [44] and implicated in prostate cancer progression [48], possibly through its down-regulation of growth factor receptor signalling and its regulation of the movement of lysosomes, which carry proteinases that aid in cell motility. Rab20 is overexpressed in exocrine pancreatic carcinoma [49], and silencing of Rab20 reduced hypoxia-induced apoptosis [50]. Rab23 is overexpressed in a fraction of hepatocellular carcinoma [51] and diffuse-type gastric cancer [52]. Rab27B is involved in multiple aspects of breast cancer progression and is a prognosis marker. It may act through its regulation of the exocytosis of vesicles carrying Heat shock protein (HSP)-90α, which in turn activates matrix metalloproteases that aid in invasiveness [53–55].

Members of the Rab11 subfamily (Rab11A, Rab11B, and Rab25) are key regulators of endocytic recycling, including that of integrin, and their dysregulation are likely to affect aspects of cell transformation and migration [56]. Rab25 [57, 58], a member of the Rab11 subfamily that is highly expressed in the epithelial cells of the gastrointestinal tract, lungs and kidney, has in the past few years been implicated in cancers from multiple organs. These include breast [59, 60], ovarian [59], oesophageal [61], bladder [62] as well as head and neck squamous cell carcinoma [63]. The Rab coupling protein (RCP) or Rab11 family interacting protein 1 (Rab11FIP1), which is a Rab25 effector, is also well known as a breast cancer promoting gene [64]. Rab25's role in cancer is in some cases enigmatic as it could appear to act either as a cancer and metastasis promoter or a tumour suppressor, and we have previously suggested that its mode of action may depend on the availability of its effector RCP [65].

Table 1 A summary of studies implicating Rabs in cancer

Rab	Known physiological role	Implication in cancer	References
Rab1A	ER-Golgi transport, autophagy	Elevated in tongue squamous cell carcinomas and melanoma	[37,38]
Rab2	ER-Golgi transport	Elevated in peripheral blood mononuclear cells (PBMCs) of tumour bearing patients	[39,40]
Rab5A	Endocytosis	Elevated in non-small cell lung carcinoma, autonomous thyroid adenomas, hepatocellular carcinoma and ovarian cancer	[43–46]
Rab5B	Endocytosis	Elevated in melanoma cells	[38]
Rab5C	Endocytosis	A role in enhancing EGF-induced invasion by breast cancer cells	[47]
Rab7	Endo-lysosomal transport	Elevated in autonomous thyroid adenomas, associated with prostate cancer progression	[44, 48]
Rab8	Polarized exocytosis	Regulates exocytosis of MT1-matrix metalloproteinase	[76]
Rab20	Endocytosis/phagocytosis	Elevated in pancreatic carcinoma	[49]
Rab23	Modulation of Sonic hedgehog signalling	Elevated in hepatocellular carcinoma and diffuse-type gastric cancer	[51,52]
Rab25	Endosomal recycling	Associated with various aspects of breast, ovarian, oesophageal, and bladder cancers, as well as head and neck squamous cell carcinoma	[59–63]
Rab27B	Regulated secretion/exocytosis	Marker for breast cancer progression, invasiveness and metastasis	[53–55]
Rab31	EGFR endosomal trafficking, M6PR trafficking from TGN to late endosome	Elevated in breast cancer and influences breast cancer, cervical cancer and glioblastoma progression	[93–99]
Rab32	Melanosome transport, mitochondrial dynamics, autophagy	Tumourigenesis of neuroendocrine tumours	[66]

There are a number of other Rabs for which changed expression levels are associated with various types of cancers, although their mechanism of action has not yet been speculated upon. Rab32 dysregulation may be involved in tumourigenesis of neuroendocrine tumours [66]. Rab36 resides in a portion of chromosome 22q11, which is frequently deleted either heterozygously or homozygously in paediatric brain rhabdoid tumours [67].

Activities of Rabs in association with cancer have, in some cases, been shown to be epigenetically modulated. For example, down-regulation of Rab37 in metastatic lung cancer could be due to of promoter hypermethylation [68]. The micro-RNA (miR)-50 inhibits autophagy and tumour growth in colon cancer cells by suppressing, among other genes, the expression of Rab1B [69], the latter being an important factor in the initiation of autophagy [70]. Another miR, miR-451, has tumour suppressor functions in human non-small cell lung cancer, and could act by suppressing the expression of Rab14 [71]. On the other hand, miR-373 could be down-regulated by aberrant promoter methylation in colon cancer, with a concomitant up-regulation of its target, Rab22A [72]. miR-200b is a prognostic factor of breast cancer [73] and glioma [74], and it targets multiple Rabs including Rab3B, Rab18, Rab21 and Rab23.

Rab are not conventionally denoted as either oncogenes or tumour suppressors. However, abnormal expression of Rabs could conceivably drive several aspects of cellular transformation, particularly mitogenic signalling and cell migration/invasion. Multiple endocytic Rabs influence trafficking and signalling of cell surface growth factor receptors. Impaired or altered Rab regulation of the endocytic itineraries of these receptors could lead to impairment in receptor recycling or degradation, thus promoting mitogenic signalling that pre-disposes cells to oncogenic transformation [35, 75]. On the other hand, Rab-mediated endocytosis and recycling is linked to cell migration and invasion. Rab8, for example, regulates exocytosis of MT1-matrix metalloproteinase (MT1-MMP), a key metastatic factor, to invasive structures [76]. Rab25 appears to promote invasive migration in a 3-dimensional matrix by associating with and mediating the recycling of $\alpha5\beta1$ integrin and the epidermal growth factor receptor (EGFR), likely acting through RCP [77–79]. In the next section, we outline how Rab31 has been implicated in human cancers, and in the section after postulate the underlying mechanisms based on recent findings.

Rab31 and findings implicating Rab31 in human cancers

Rab31 was first cloned from human melanocytes and named Rab22b based on its close homology with Rab22 [80], but was also named Rab31 when subsequently cloned from human platelets [81]. Rat (rRab22b) [82] and mouse orthologues [83] were also subsequently reported. Structurally, Rab31 is homologous to Rab5 and is grouped

Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.

under the Rab5 subfamily [84, 85]. Larocca and colleagues first showed that Rab31 transcripts are enriched in brain oligodendrocytes, and demonstrated by video microscopy that Rab31 prominently labels tubulovesicular carrier structures originating from the trans-Golgi [82], and that Rab31 regulates transport of the cation-dependent mannose 6-phosphate receptor (CD-M6PR) from the Golgi to the endosome [86]. The authors also showed that Rab31 interacts with the Lowe oculocerebrorenal syndrome protein OCRL-1 (an Inositol polyphosphate 5-phosphatase) and that this interaction is required for trans-Golgi network (TGN) organization and transport carrier formation [87].

We have developed Rab31 antibodies that supported an enrichment of Rab31 protein in brain tissues and a functional role for Rab31 at the TGN [83, 88]. In addition, our findings also suggested that Rab31 has a role in regulating early endosome-late endosome transport, particularly of the epidermal growth factor receptor (EGFR) [88]. Other than M6PR and EGFR, Rab31 has also recently been shown to bind the signalling adaptor protein, phosphotyrosine interaction, pleckstrin homology (PH) domain, and leucine zipper-containing protein (APPL) 2 [89]. Two proteins GAPex-5 [90] and Rin3 [91], have been identified as GEFs for Rab31, and one of its confirmed effectors is early endosome antigen 1 (EEA1), which it shares with Rab5 and Rab22.

Several expression profiling analyses have implicated Rab31 in human cancers. A Serial Analysis of Gene Expression (SAGE) profiling found Rab31 to be among 11 genes that are robustly overexpressed in samples of Oestrogen Receptor α (ERα) positive breast carcinomas [92]. ERα is a transcription factor that is activated by oestrogens such as oestradiol, and regulates the transcription of target genes by binding to the oestrogen response element (ERE) upstream of the target genes. Rab31 transcripts were also found to be elevated in breast cancer cells expressing the urokinase-type tissue plasminogen activator (uPA)-receptor splice variant uPAR-del4/5 [93–95], and high Rab31 levels were significantly associated with distant metastasis-free survival and overall survival [96]. An analysis in advanced ovarian cancer samples did not, however, reveal any significant association with overall or progression-free survival [97]. Rab31 is also among the genes that associate with tumour progression in centrosomal protein transforming acidic coiled coil (TACC) 3 overexpressing HeLa cells as a model of cervical cancer [98].

Other than breast cancer, Rab31 is also identified as one of the cohort (race)-dependent associations with glioblastoma survival [99]. Meta-analysis of microarray studies using Bayesian network analysis also found Rab31 to be among 10 genes that are most influential in the development of glioblastoma multiforme [100].

Mechanisms underlying Rab31's role in cancer

Several recent findings have helped to shed light on the possible underlying molecular pathways and mechanisms linking Rab31 to cancer. These are outlined and discussed below, headed by key questions pertaining to Rab31's expression and pathophysiological roles.

Why are Rab31 levels elevated in cancer cells?

Rab31 levels are elevated in breast cancer cells, and recent findings offer two explanations for the phenomenon. One possibility is explained by a recent discovery that Rab31 transcripts are targets of HuR [101], an mRNA-binding and stabilizing protein of the ELAV-Hu family [102] which could thus stabilize Rab31 transcripts, resulting in their elevated levels (Fig. 1A). HuR itself is notably overexpressed in breast cancer tissues and has prognostic value [103, 104]. Another possibility is related to the observation that Rab31 is selectively elevated in ERα-positive breast cancer samples [92]. A new key finding in this regard is that the Rab31 promoter region has an ER responsive element [105], and could be thus regulated or deregulated in breast cancer cells by trans factors associating with the element. One such factor turned out to be mucin-1 (MUC1), an oncogenic glycoprotein that has been shown to be expressed in a large fraction of breast cancer samples [106].

MUC1 is a heterodimeric transmembrane protein consisting of MUC1-N (which harbours mucin-like repeats) and MUC1-C, which spans the cell membrane [107]. MUC1-C could be internalized by clathrin-based endocytosis [108] and imported into the nucleus [109], where it stabilizes and activates ERα [110]. Jin and colleagues demonstrated that MUC1-C forms a complex with ERα, and could activate Rab31 transcription in an oestradiol-dependent manner (Fig. 1B). Up-regulated Rab31 could in turn elevate MUC1-C levels, probably by reducing its lysosomal degradation through an as-yet-undefined mechanism (Fig. 1C). Rab31 expression in MCF10A cells could promote the formation of anchorage-independent cytospheric structures (mammospheres) in a MUC1-C-dependent manner [105]. MUC1-C and Rab31 therefore appear to form an auto-inductive loop that results in sustained over-expression of MUC1-C in breast cancer cells. Such an auto-inductive regulatory loop has also been previously reported between MUC1-C and the Signal transducer and activator of transcription 3 (STAT3), which is also a promoter of malignancy [111].

Is Rab31 a driver in cancer, and if so, how?

Interestingly, when Rab31 is overexpressed in breast cancer cell lines, it enhanced proliferation and diminished cell adhesion towards several extracellular matrix (ECM) components, as well as attenuated cell invasion through Matrigel [112]. When breast cancer cells moderately overexpressing Rab31 were xenografted onto nude mice, these exhibited significantly reduced lung metastasis compared to control cells. In invasive tumour lines at least, high levels of Rab31 appear therefore to switch these cells from an invasive to a more proliferative phenotype [112].

Could any of the factors that help elevate Rab31 levels described above attest to Rab31's oncogenic potential? Other than Rab31, HuR also stabilizes the transcript of uPA and its receptor (uPAR) [113]. uPA, localized by uPAR to the plasma membrane, cleaves plasminogen to give plasmin, which in turn cleaves and activates matrix metalloproteases (MMPs) that aid in degradation of ECM components. uPAR is elevated during inflammation and ECM remodelling, and is

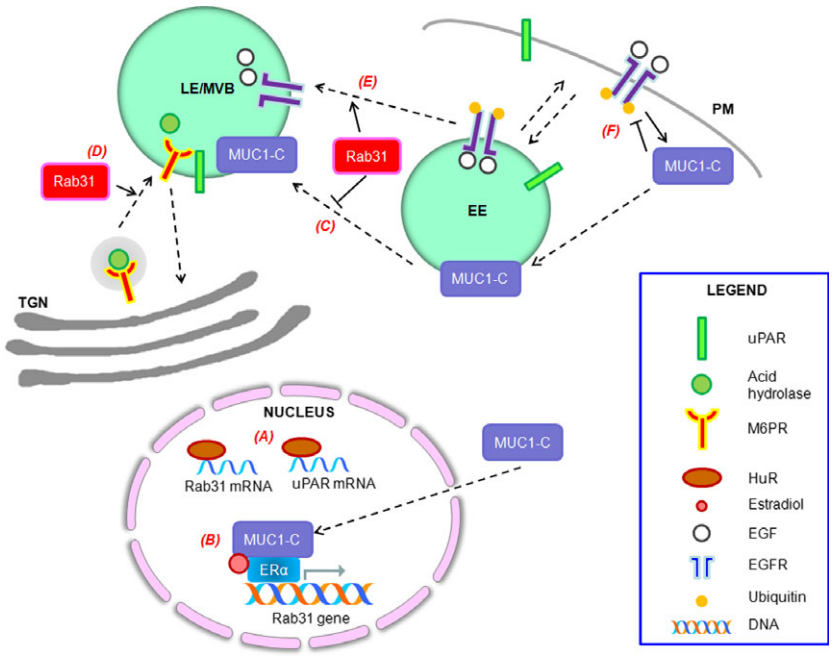

Fig. 1 Rab31 and the interactions implicated in its role in cancer. (A) HuR stabilizes transcripts including that of Rab31 and uPAR. (B) Transcription of Rab31 is regulated by an ER responsive element. MUC1-C stabilizes and activates ERα, which in turn activates Rab31 transcription in an estradiol-dependent manner. (C) Rab31 inhibits the lysosomal degradation of MUC1-C, *via* an as-yet-undefined mechanism, thus elevating MUC1-C levels. (D) Rab31 regulates the movement of M6PR from the TGN to endosomes. M6PR, in turn, interacts with uPAR and may be responsible for its movement to endosomes for degradation. (E) Rab31 participates in the trafficking of ligand-bound EGFR from early to late endosomes, thus enhancing the rate of degradation of ligand-bound EGFR. (F) MUC1-C is phosphorylated by ligand-bound EGFR, leading to enhanced interaction with downstream signalling components. In turn, MUC1-C inhibits the ubiquitination of EGFR, thus potentiating its signalling. Dashed lines represent movement of proteins. Solid arrows represent activating mechanisms of action; solid blocked arrows represent inhibiting mechanisms of action. TGN, trans-Golgi network; EE, early endosome; LE/MVB, late endosome/multivesicular body; PM, Plasma membrane.

usually associated with poor cancer prognosis [114]. uPAR could also activate a variety of intracellular signalling pathways such as the mitogen activated protein kinase (MAPK) pathway and the phosphatidylinositol 3-kinase (PI3K)-Akt pathway [115] through the engagement of co-receptors, such as integrins [116]. A splice variant of uPAR, uPAR-del4/5, which is unable to bind uPA, is a known prognostic marker for breast cancer [94, 96, 113] although phenotypically, *in vitro*, cells overexpressing this variant appear to have reduced invasive properties [95, 117], much like that observed for Rab31 [112]. While Rab31 transcripts have also been found in cancer cells with the uPAR splice variant, and both are stabilized by HuR, it is unclear if the two have a causal or functional relationship. An interesting connection with Rab31 in this regard is that uPAR interacts with CI-M6PR [118, 119], whose TGN-endosome transport is regulated by Rab31 [86, 87]. The interaction of uPAR with CI-M6PR may regulate the movement of uPAR to endosomes for degradation and thus modulate the levels of uPAR on the cell surface [118] (Fig. 1D). How this uPAR-CI-M6PR connection might relate to Rab31's role in cancer is yet unclear. It is, however, conceivable that Rab31 may modulate uPAR's activity in signalling as well as ECM modulation, *via* its regulation of M6PR trafficking dynamics, thus impacting on tumourigenesis and invasion.

Notably, the other modulator of Rab31 expression, MUC1-C, is a multifunctional oncoprotein [107]. MUC1-C overexpression in fibroblast is sufficient to induce anchorage-independent growth and tumour formation in nude mice [120]. In the nucleus, MUC1-C activates the Wnt/β-catenin [121], STAT3 [111] and NF-κB [122]- based transcription, all of which have been associated with tumourigenesis. At the plasma membrane, MUC1-C interacts with EGFR [123–125] and perhaps other members of the ErbB family to activate the MAPK and PI3K-Akt pathways. Furthermore, MUC1 could promote autophagy and survival responses of cancer cells to nutrient deprivation [126]. It was also recently shown to stabilize and activate hypoxia-inducible factor-1α (HIF-1α) to regulate hypoxic response in pancreatic cancer cells [127]. In view of the oncogenic potency of MUC1, it does appear that if Rab31 could effectively elevate and sustain functional levels of MUC1-C, it could help drive oncogenesis.

Rab31's modulation of EGFR trafficking – could it be tumour suppressive?

A twist to the general plot above came about from our findings that Rab31 directly modulates EGFR trafficking and possibly its signalling

Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.

[88, 128]. A hint that Rab31 may regulate EGFR trafficking first came from two earlier reports, the first from Stahl's laboratory which showed that GAPex-5, then newly identified as a Rab5 GEF, modulates EGFR ubiquitination, trafficking and degradation [129]. Another report indicated that GAPex-5 is also a GEF for Rab31, and it regulates insulin-stimulated Glut4 translocation to the plasma membrane in adipocytes [90]. We found that silencing of Rab31 inhibited, while overexpression enhanced, EGFR trafficking to the late endosomes (Fig. 1E), and the former observation phenocopied the effect of GAPex-5 silencing on EGFR trafficking. Interestingly, Rab31 is associated with EGFR in a GTP-dependent manner, and this association is dependent upon its effector early endosome antigen 1 (EEA1) as well as GAPex-5 (but not Rin3 [91], another Rab31 GEF) [128]. Rab31 may thus be recruited as part of an EGFR-containing membrane trafficking complex to regulate its transit from the early to late endosomes. Overexpression of Rab31 appeared to enhance the degradation of EGFR, and we have observed that, for A431 cells at least, this translates to a moderate decrease in the rate of cell proliferation [88].

On the face of it, our observations in A431 cells run counter to what might be expected for Rab31 overexpression in breast cancer cells. Given that Rab31 overexpression in breast cancer cell lines increased their proliferation [112], the discrepancy may be down to cell type differences. It may also simply be just another illustration of the complexity associated with cancer cells and tissues, where multiple factors act along with, or counter the action of one another in intersecting pathways. The cancerous phenotype, and its different dynamic manifestations as the cancer progresses, is thus a combinatorial sum of many. It is difficult at the moment to gauge quantitatively which one of the two apparently contrasting actions of Rab31, namely the auto-inductive loop that it is engaged with MUC1-C, or its effect on EGFR trafficking and signalling, would be a more important determinant of the cancer phenotype. It is conceivable though that one important determinant in the complex equation would be the availability and activity of its GEF GAPex-5, as well as it is yet to be identified GAP(s). In a way reminiscent to Rab25, both the availability of regulators and effector would be important variables in determining if the Rab would be oncogenic, or conversely tumour suppressive [65]. Furthermore, in breast cancer in particular, any moderating effect of Rab31 on EGFR signalling may well be completely muted by the fact that EGFR family receptor tyrosine kinase and their mutants are prevalent [130], or the activation of competing recycling pathways that will recycle endocytosed EGFR back to the surface [78].

On the other hand, one should also keep in view the complex relationship between Rab31, EGFR and MUC1-C. MUC1-C itself interacts with EGFR and is indeed a substrate of EGFR tyrosine kinase activity [123, 124], with the phosphorylation of MUC1-C by EGFR leading to enhanced interactions with its downstream components [123]. Conversely, MUC1-C was shown to inhibit ubiquitination of ligand-bound EGFR, thus reducing the degradation and enhancing the recycling of EGFR to the cell surface, thus potentiating its signalling [124] (Fig. 1F). At the moment it is unclear how elevated Rab31 levels may affect this EGFR-MUC1 interaction, but it is conceivable that the presence of Rab31 could alter EGFR signalling in this regard. Further work should determine if this influence is positive or negative. Given that overexpression of Rab31 in breast cancer cell lines actually enhanced proliferation [112], Rab31, when elevated in the presence of MUC1-C may enhance instead of retard EGFR signalling in these cells. Another point of contention that requires further clarification pertains to the effect of Rab31 on MUC-1C's expression. Jin and colleagues surmised that Rab31 could have diminished MUC-1C's lysosomal degradation as the lysosome inhibitor chloroquine increased MUC1-C levels in Rab31 silenced cells. How then does Rab31 diminish late endososome-lysosome targeting of MUC1-C while increasing the transport of EGFR? Answers to these and other questions await resolution by further work.

Epilogue and future perspectives

In this brief review, we have discussed how recent findings may explain Rab31's elevation in cancer, and how elevated Rab31 may influence cancer cell signalling through its effect on EGFR endosomal traffic. To fully understand the significance of Rab31 as a prognostic marker, we posit that assessment of the levels of its regulators such as GAPex-5 in various cancers would be important. It is far too early to postulate if Rab31 and its regulators would have therapeutic values. However, it is conceivable that in breast cancer cells overexpressing EGFR or other ErbB family members, using Rab31 to attenuate EGFR signalling may be a potentially useful adjunct therapy to anti-EGFR drugs. This may attenuate the selection pressure that would lead to the development of resistance against drugs targeting EGFR [131, 132].

Acknowledgement

The authors are supported by NUS Graduate School of Integrative Sciences and Engineering.

Conflict of interest statement

The authors confirm that there are no conflicts of interest.

References

1. **Mizuno-Yamasaki E, Rivera-Molina F, Novick P.** GTPase networks in membrane traffic. *Annu Rev Biochem.* 2012; 81: 637–59.
2. **Stenmark H.** Rab GTPases as coordinators of vesicle traffic. *Nat Rev Mol Cell Biol.* 2009; 10: 513–25.
3. **Kelly EE, Horgan CP, Goud B, *et al.*** The Rab family of proteins: 25 years on. *Biochem Soc Trans.* 2012; 40: 1337–47.

4. **Rojas AM, Fuentes G, Rausell A, *et al.*** The Ras protein superfamily: evolutionary tree and role of conserved amino acids. *J Cell Biol*. 2012; 196: 189–201.
5. **Seabra MC, Wasmeier C.** Controlling the location and activation of Rab GTPases. *Curr Opin Cell Biol*. 2004; 16: 451–7.
6. **Pfeffer S, Aivazian D.** Targeting Rab GTPases to distinct membrane compartments. *Nat Rev Mol Cell Biol*. 2004; 5: 886–96.
7. **Hutagalung AH, Novick PJ.** Role of Rab GTPases in membrane traffic and cell physiology. *Physiol Rev*. 2011; 91: 119–49.
8. **Karnoub AE, Weinberg RA.** Ras oncogenes: split personalities. *Nat Rev Mol Cell Biol*. 2008; 9: 517–31.
9. **Itzen A, Goody RS.** GTPases involved in vesicular trafficking: structures and mechanisms. *Semin Cell Dev Biol*. 2011; 22: 48–56.
10. **Pfeffer SR, Dirac-Svejstrup AB, Soldati T.** Rab GDP dissociation inhibitor: putting rab GTPases in the right place. *J Biol Chem*. 1995; 270: 17057–9.
11. **Shen F, Seabra MC.** Mechanism of digeranylgeranylation of Rab proteins. Formation of a complex between monogeranylgeranyl-Rab and Rab escort protein. *J Biol Chem*. 1996; 271: 3692–8.
12. **Gomes AQ, Ali BR, Ramalho JS, *et al.*** Membrane targeting of Rab GTPases is influenced by the prenylation motif. *Mol Biol Cell*. 2003; 14: 1882–99.
13. **Ali BR, Seabra MC.** Targeting of Rab GTPases to cellular membranes. *Biochem Soc Trans*. 2005; 33: 652–6.
14. **Barr F, Lambright DG.** Rab GEFs and GAPs. *Curr Opin Cell Biol*. 2010; 22: 461–70.
15. **Blümer J, Rey J, Dehmelt L, *et al.*** RabGEFs are a major determinant for specific Rab membrane targeting. *J Cell Biol*. 2013; 200: 287–300.
16. **Barrowman J, Bhandari D, Reinisch K, *et al.*** TRAPP complexes in membrane traffic: convergence through a common Rab. *Nat Rev Mol Cell Biol*. 2010; 11: 759–63.
17. **Horgan CP, McCaffrey MW.** Rab GTPases and microtubule motors. *Biochem Soc Trans*. 2011; 39: 1202–6.
18. **Novick P, Medkova M, Dong G, *et al.*** Interactions between Rabs, tethers, SNAREs and their regulators in exocytosis. *Biochem Soc Trans*. 2006; 34: 683–6.
19. **Aloisi AL, Bucci C.** Rab GTPases-cargo direct interactions: fine modulators of intracellular trafficking. *Histol Histopathol*. 2013; 28: 839–49.
20. **Fukuda M.** TBC proteins: GAPs for mammalian small GTPase Rab? *Biosci Rep*. 2011; 31: 159–68.
21. **Verhoeven K, De Jonghe P, Coen K, *et al.*** Mutations in the small GTP-ase late endosomal protein RAB7 cause Charcot-Marie-Tooth type 2B neuropathy. *Am J Hum Genet*. 2003; 72: 722–7.
22. **Spinosa MR, Progida C, De Luca A, *et al.*** Functional characterization of Rab7 mutant proteins associated with Charcot-Marie-Tooth type 2B disease. *J Neurosci*. 2008; 28: 1640–8.
23. **Bem D, Yoshimura SI, Nunes-Bastos R, *et al.*** Loss-of-function mutations in RAB18 cause Warburg micro syndrome. *Am J Hum Genet*. 2011; 88: 499–507.
24. **Liegel RP, Handley MT, Ronchetti A, *et al.*** Loss-of-function mutations in TBC1D20 cause cataracts and male infertility in blind sterile mice and Warburg micro syndrome in humans. *Am J Hum Genet*. 2013; 93: 1001–14.
25. **Jenkins D, Seelow D, Jehee FS, *et al.*** RAB23 mutations in Carpenter syndrome imply an unexpected role for hedgehog signaling in cranial-suture development and obesity. *Am J Hum Genet*. 2007; 80: 1162–70.
26. **Jenkins D, Baynam G, De Catte L, *et al.*** Carpenter syndrome: extended RAB23 mutation spectrum and analysis of nonsense-mediated mRNA decay. *Hum Mutat*. 2011; 32: E2069–78.
27. **Barral DC, Ramalho JS, Anders R, *et al.*** Functional redundancy of Rab27 proteins and the pathogenesis of Griscelli syndrome. *J Clin Invest*. 2002; 110: 247–57.
28. **Giannandrea M, Bianchi V, Mignogna ML, *et al.*** Mutations in the small GTPase gene RAB39B are responsible for X-linked mental retardation associated with autism, epilepsy, and macrocephaly. *Am J Hum Genet*. 2010; 86: 185–95.
29. **Oiso N, Riddle SR, Serikawa T, *et al.*** The rat Ruby (R) locus is Rab38: identical mutations in Fawn-hooded and Tester-Moriyama rats derived from an ancestral Long Evans rat sub-strain. *Mamm Genome*. 2004; 15: 307–14.
30. **Gerondopoulos A, Langemeyer L, Liang JR, *et al.*** BLOC-3 mutated in Hermansky-Pudlak syndrome is a Rab32/38 guanine nucleotide exchange factor. *Curr Biol*. 2012; 22: 2135–9.
31. **Cogli L, Piro F, Bucci C.** Rab7 and the CMT2B disease. *Biochem Soc Trans*. 2009; 37: 1027–31.
32. **Chua CEL, Tang BL.** Rabs, SNAREs and α-synuclein–membrane trafficking defects in synucleinopathies. *Brain Res Rev*. 2011; 67: 268–81.
33. **D'Adamo P, Masetti M, Bianchi V, *et al.*** RAB GTPases and RAB-interacting proteins and their role in the control of cognitive functions. *Neurosci Biobehav Rev*. 2014; pii: S0149–7634(14)00003–7.
34. **Krzewski K, Cullinane AR.** Evidence for defective Rab GTPase-dependent cargo traffic in immune disorders. *Exp Cell Res*. 2013; 319: 2360–7.
35. **Chia WJ, Tang BL.** Emerging roles for Rab family GTPases in human cancer. *Biochim Biophys Acta*. 2009; 1795: 110–6.
36. **Recchi C, Seabra MC.** Novel functions for Rab GTPases in multiple aspects of tumour progression. *Biochem Soc Trans*. 2012; 40: 1398–403.
37. **Shimada K, Uzawa K, Kato M, *et al.*** Aberrant expression of RAB1A in human tongue cancer. *Br J Cancer*. 2005; 92: 1915–21.
38. **Peinado H, Alečković M, Lavotshkin S, *et al.*** Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat Med*. 2012; 18: 883–91.
39. **Culine S, Honoré N, Tavitian A, *et al.*** Overexpression of the ras-related rab2 gene product in peripheral blood mononuclear cells from patients with hematological and solid neoplasms. *Cancer Res*. 1992; 52: 3083–8.
40. **Culine S, Honore N, Closson V, *et al.*** A small GTP-binding protein is frequently overexpressed in peripheral blood mononuclear cells from patients with solid tumours. *Eur J Cancer*. 1994; 30A: 670–4.
41. **Torres VA, Stupack DG.** Rab5 in the regulation of cell motility and invasion. *Curr Protein Pept Sci*. 2011; 12: 43–51.
42. **Mendoza P, Ortiz R, Díaz J, *et al.*** Rab5 activation promotes focal adhesion disassembly, migration and invasiveness in tumor cells. *J Cell Sci*. 2013; 126: 3835–47.
43. **Yu L, Hui-chen F, Chen Y, *et al.*** Differential expression of RAB5A in human lung adenocarcinoma cells with different metastasis potential. *Clin Exp Metastasis*. 1999; 17: 213–9.
44. **Croizet-Berger K, Daumerie C, Couvreur M, *et al.*** The endocytic catalysts, Rab5a and Rab7, are tandem regulators of thyroid hormone production. *Proc Natl Acad Sci USA*. 2002; 99: 8277–82.
45. **Fukui K, Tamura S, Wada A, *et al.*** Expression of Rab5a in hepatocellular carcinoma: possible involvement in epidermal growth factor signaling. *Hepatol Res*. 2007; 37: 957–65.
46. **Zhao Z, Liu XF, Wu HC, *et al.*** Rab5a overexpression promoting ovarian cancer cell proliferation may be associated with APPL1-related epidermal growth factor

Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.

signaling pathway. *Cancer Sci*. 2010; 101: 1454–62.

47. **Onodera Y, Nam JM, Hashimoto A, *et al.*** Rab5c promotes AMAP1-PRKD2 complex formation to enhance β1 integrin recycling in EGF-induced cancer invasion. *J Cell Biol*. 2012; 197: 983–96.
48. **Steffan JJ, Dykes SS, Coleman DT, *et al.*** Supporting a role for the GTPase Rab7 in prostate cancer progression. *PLoS ONE*. 2014; 9: e87882.
49. **Amillet JM, Ferbus D, Real FX, *et al.*** Characterization of human Rab20 overexpressed in exocrine pancreatic carcinoma. *Hum Pathol*. 2006; 37: 256–63.
50. **Hackenbeck T, Huber R, Schietke R, *et al.*** The GTPase RAB20 is a HIF target with mitochondrial localization mediating apoptosis in hypoxia. *Biochim Biophys Acta*. 2011; 1813: 1–3.
51. **Liu YJ, Wang Q, Li W, *et al.*** Rab23 is a potential biological target for treating hepatocellular carcinoma. *World J Gastroenterol*. 2007; 13: 1010–7.
52. **Hou Q, Wu YH, Grabsch H, *et al.*** Integrative genomics identifies RAB23 as an invasion mediator gene in diffuse-type gastric cancer. *Cancer Res*. 2008; 68: 4623–30.
53. **Hendrix A, Braems G, Bracke M, *et al.*** The secretory small GTPase Rab27B as a marker for breast cancer progression. *Oncotarget*. 2010; 1: 304–8.
54. **Hendrix A, Maynard D, Pauwels P, *et al.*** Effect of the secretory small GTPase Rab27B on breast cancer growth, invasion, and metastasis. *J Natl Cancer Inst*. 2010; 102: 866–80.
55. **Zhang JX, Huang XX, Cai MB, *et al.*** Overexpression of the secretory small GTPase Rab27B in human breast cancer correlates closely with lymph node metastasis and predicts poor prognosis. *J Transl Med*. 2012; 10: 242.
56. **Kelly EE, Horgan CP, McCaffrey MW.** Rab11 proteins in health and disease. *Biochem Soc Trans*. 2012; 40: 1360–7.
57. **Goldenring JR, Shen KR, Vaughan HD, *et al.*** Identification of a small GTP-binding protein, Rab25, expressed in the gastrointestinal mucosa, kidney, and lung. *J Biol Chem*. 1993; 268: 18419–22.
58. **Casanova JE, Wang X, Kumar R, *et al.*** Association of Rab25 and Rab11a with the apical recycling system of polarized Madin-Darby canine kidney cells. *Mol Biol Cell*. 1999; 10: 47–61.
59. **Cheng KW, Lahad JP, Kuo WL, *et al.*** The RAB25 small GTPase determines aggressiveness of ovarian and breast cancers. *Nat Med*. 2004; 10: 1251–6.
60. **Cheng JM, Volk L, Janaki DKM, *et al.*** Tumor suppressor function of Rab25 in triple-negative breast cancer. *Int J Cancer*. 2010; 126: 2799–812.
61. **Tong M, Chan KW, Bao JYJ, *et al.*** Rab25 is a tumor suppressor gene with antiangiogenic and anti-invasive activities in esophageal squamous cell carcinoma. *Cancer Res*. 2012; 72: 6024–35.
62. **Zhang J, Wei J, Lu J, *et al.*** Overexpression of Rab25 contributes to metastasis of bladder cancer through induction of epithelial-mesenchymal transition and activation of Akt/GSK-3β/Snail signaling. *Carcinogenesis*. 2013; 34: 2401–8.
63. **Amornphimoltham P, Rechache K, Thompson J, *et al.*** Rab25 regulates invasion and metastasis in head and neck cancer. *Clin Cancer Res*. 2013; 19: 1375–88.
64. **Zhang J, Liu X, Datta A, *et al.*** RCP is a human breast cancer-promoting gene with Ras-activating function. *J Clin Invest*. 2009; 119: 2171–83.
65. **Tang BL.** Is Rab25 a tumor promoter or suppressor–context dependency on RCP status? *Tumour Biol*. 2010; 31: 359–61.
66. **Hofsli E, Wheeler TE, Langaas M, *et al.*** Identification of novel neuroendocrine-specific tumour genes. *Br J Cancer*. 2008; 99: 1330–9.
67. **Zhou J, Fogelgren B, Wang Z, *et al.*** Isolation of genes from the rhabdoid tumor deletion region in chromosome band 22q11.2. *Gene*. 2000; 241: 133–41.
68. **Wu CY, Tseng RC, Hsu HS, *et al.*** Frequent down-regulation of hRAB37 in metastatic tumor by genetic and epigenetic mechanisms in lung cancer. *Lung Cancer*. 2009; 63: 360–7.
69. **Zhai H, Song B, Xu X, *et al.*** Inhibition of autophagy and tumor growth in colon cancer by miR-502. *Oncogene*. 2013; 32: 1570–9.
70. **Zoppino FCM, Militello RD, Slavin I, *et al.*** Autophagosome formation depends on the small GTPase Rab1 and functional ER exit sites. *Traffic*. 2010; 11: 1246–61.
71. **Wang R, Wang ZX, Yang JS, *et al.*** MicroRNA-451 functions as a tumor suppressor in human non-small cell lung cancer by targeting ras-related protein 14 (RAB14). *Oncogene*. 2011; 30: 2644–58.
72. **Tanaka T, Arai M, Wu S, *et al.*** Epigenetic silencing of microRNA-373 plays an important role in regulating cell proliferation in colon cancer. *Oncol Rep*. 2011; 26: 1329–35.
73. **Ye F, Tang H, Liu Q, *et al.*** miR-200b as a prognostic factor in breast cancer targets multiple members of RAB family. *J Transl Med*. 2014; 12: 17.
74. **Liu Q, Tang H, Liu X, *et al.*** miR-200b as a prognostic factor targets multiple members of RAB family in glioma. *Med Oncol*. 2014; 31: 859.
75. **Mellman I, Yarden Y.** Endocytosis and cancer. *Cold Spring Harb Perspect Biol*. 2013; 5: a016949.
76. **Bravo-Cordero JJ, Marrero-Diaz R, Megías D, *et al.*** MT1-MMP proinvasive activity is regulated by a novel Rab8-dependent exocytic pathway. *EMBO J*. 2007; 26: 1499–510.
77. **Caswell PT, Spence HJ, Parsons M, *et al.*** Rab25 associates with alpha5beta1 integrin to promote invasive migration in 3D microenvironments. *Dev Cell*. 2007; 13: 496–510.
78. **Caswell PT, Chan M, Lindsay AJ, *et al.*** Rab-coupling protein coordinates recycling of alpha5beta1 integrin and EGFR1 to promote cell migration in 3D microenvironments. *J Cell Biol*. 2008; 183: 143–55.
79. **Dozynkiewicz MA, Jamieson NB, Macpherson I, *et al.*** Rab25 and CLIC3 collaborate to promote integrin recycling from late endosomes/lysosomes and drive cancer progression. *Dev Cell*. 2012; 22: 131–45.
80. **Chen D, Guo J, Miki T, *et al.*** Molecular cloning of two novel rab genes from human melanocytes. *Gene*. 1996; 174: 129–34.
81. **Bao X, Faris AE, Jang EK, *et al.*** Molecular cloning, bacterial expression and properties of Rab31 and Rab32. *Eur J Biochem*. 2002; 269: 259–71.
82. **Rodriguez-Gabin AG, Cammer M, Almazan G, *et al.*** Role of rRAB22b, an oligodendrocyte protein, in regulation of transport of vesicles from trans Golgi to endocytic compartments. *J Neurosci Res*. 2001; 66: 1149–60.
83. **Ng EL, Wang Y, Tang BL.** Rab22B's role in trans-Golgi network membrane dynamics. *Biochem Biophys Res Commun*. 2007; 361: 751–7.
84. **Diekmann Y, Seixas E, Gouw M, *et al.*** Thousands of rab GTPases for the cell biologist. *PLoS Comput Biol*. 2011; 7: e1002217.
85. **Klöpper TH, Kienle N, Fasshauer D, *et al.*** Untangling the evolution of Rab G proteins: implications of a comprehensive genomic analysis. *BMC Biol*. 2012; 10: 71.
86. **Rodriguez-Gabin AG, Yin X, Si Q, *et al.*** Transport of mannose-6-phosphate receptors from the trans-Golgi network to endosomes requires Rab31. *Exp Cell Res*. 2009; 315: 2215–30.
87. **Rodriguez-Gabin AG, Ortiz E, Demoliner K, *et al.*** Interaction of Rab31 and OCRL-1

in oligodendrocytes: its role in transport of mannose 6-phosphate receptors. *J Neurosci Res*. 2010; 88: 589–604.

88. **Ng EL, Ng JJ, Liang F, *et al.*** Rab22B is expressed in the CNS astroglia lineage and plays a role in epidermal growth factor receptor trafficking in A431 cells. *J Cell Physiol*. 2009; 221: 716–28.
89. **King GJ, Stöckli J, Hu SH, *et al.*** Membrane curvature protein exhibits interdomain flexibility and binds a small GTPase. *J Biol Chem*. 2012; 287: 40996–1006.
90. **Lodhi IJ, Chiang SH, Chang L, *et al.*** Gapex-5, a Rab31 guanine nucleotide exchange factor that regulates Glut4 trafficking in adipocytes. *Cell Metab*. 2007; 5: 59–72.
91. **Kajiho H, Sakurai K, Minoda T, *et al.*** Characterization of RIN3 as a guanine nucleotide exchange factor for the Rab5 subfamily GTPase Rab31. *J Biol Chem*. 2011; 286: 24364–73.
92. **Abba MC, Hu Y, Sun H, *et al.*** Gene expression signature of estrogen receptor alpha status in breast cancer. *BMC Genom*. 2005; 6: 37.
93. **Luther T, Kotzsch M, Meye A, *et al.*** Identification of a novel urokinase receptor splice variant and its prognostic relevance in breast cancer. *Thromb Haemost*. 2003; 89: 705–17.
94. **Kotzsch M, Farthmann J, Meye A, *et al.*** Prognostic relevance of uPAR-del4/5 and TIMP-3 mRNA expression levels in breast cancer. *Eur J Cancer*. 2005; 41: 2760–8.
95. **Sato S, Kopitz C, Grismayer B, *et al.*** Overexpression of the urokinase receptor mRNA splice variant uPAR-del4/5 affects tumor-associated processes of breast cancer cells *in vitro* and *in vivo*. *Breast Cancer Res Treat*. 2011; 127: 649–57.
96. **Kotzsch M, Sieuwerts AM, Grosser M, *et al.*** Urokinase receptor splice variant uPAR-del4/5-associated gene expression in breast cancer: identification of rab31 as an independent prognostic factor. *Breast Cancer Res Treat*. 2008; 111: 229–40.
97. **Kotzsch M, Dorn J, Doetzer K, *et al.*** mRNA expression levels of the biological factors uPAR, uPAR-del4/5, and rab31, displaying prognostic value in breast cancer, are not clinically relevant in advanced ovarian cancer. *Biol Chem*. 2011; 392: 1047–51.
98. **Yim EK, Tong SY, Ho EM, *et al.*** Anticancer effects on TACC3 by treatment of paclitaxel in HPV-18 positive cervical carcinoma cells. *Oncol Rep*. 2009; 21: 549–57.
99. **Serão NVL, Delfino KR, Southey BR, *et al.*** Cell cycle and aging, morphogenesis, and response to stimuli genes are individualized biomarkers of glioblastoma progression and survival. *BMC Med Genom*. 2011; 4: 49.
100. **Kunkle BW, Yoo C, Roy D.** Reverse engineering of modified genes by bayesian network analysis defines molecular determinants critical to the development of glioblastoma. *PLoS ONE*. 2013; 8: e64140.
101. **Heinonen M, Hemmes A, Salmenkivi K, *et al.*** Role of RNA binding protein HuR in ductal carcinoma *in situ* of the breast. *J Pathol*. 2011; 224: 529–39.
102. **Simone LE, Keene JD.** Mechanisms coordinating ELAV/Hu mRNA regulons. *Curr Opin Genet Dev*. 2013; 23: 35–43.
103. **Heinonen M, Bono P, Narko K, *et al.*** Cytoplasmic HuR expression is a prognostic factor in invasive ductal breast carcinoma. *Cancer Res*. 2005; 65: 2157–61.
104. **Heinonen M, Fagerholm R, Aaltonen K, *et al.*** Prognostic role of HuR in hereditary breast cancer. *Clin Cancer Res*. 2007; 13: 6959–63.
105. **Jin C, Rajabi H, Pitroda S, *et al.*** Cooperative interaction between the MUC1-C oncoprotein and the Rab31 GTPase in estrogen receptor-positive breast cancer cells. *PLoS ONE*. 2012; 7: e39432.
106. **Rakha EA, Boyce RWG, Abd El-Rehim D, *et al.*** Expression of mucins (MUC1, MUC2, MUC3, MUC4, MUC5AC and MUC6) and their prognostic significance in human breast cancer. *Mod Pathol*. 2005; 18: 1295–304.
107. **Kufe DW.** MUC1-C oncoprotein as a target in breast cancer: activation of signaling pathways and therapeutic approaches. *Oncogene*. 2013; 32: 1073–81.
108. **Kinlough CL, Poland PA, Bruns JB, *et al.*** MUC1 membrane trafficking is modulated by multiple interactions. *J Biol Chem*. 2004; 279: 53071–7.
109. **Leng Y, Cao C, Ren J, *et al.*** Nuclear import of the MUC1-C oncoprotein is mediated by nucleoporin Nup62. *J Biol Chem*. 2007; 282: 19321–30.
110. **Wei X, Xu H, Kufe D.** MUC1 oncoprotein stabilizes and activates estrogen receptor alpha. *Mol Cell*. 2006; 21: 295–305.
111. **Ahmad R, Rajabi H, Kosugi M, *et al.*** MUC1-C oncoprotein promotes STAT3 activation in an autoinductive regulatory loop. *Sci Signal*. 2011; 4: ra9.
112. **Grismayer B, Sölch S, Seubert B, *et al.*** Rab31 expression levels modulate tumor-relevant characteristics of breast cancer cells. *Mol Cancer*. 2012; 11: 62.
113. **Tran H, Maurer F, Nagamine Y.** Stabilization of urokinase and urokinase receptor mRNAs by HuR is linked to its cytoplasmic accumulation induced by activated mitogen-activated protein kinase-activated protein kinase 2. *Mol Cell Biol*. 2003; 23: 7177–88.
114. **Han B, Nakamura M, Mori I, *et al.*** Urokinase-type plasminogen activator system and breast cancer. *Oncol Rep*. 2005; 14: 105–12.
115. **Smith HW, Marshall CJ.** Regulation of cell signalling by uPAR. *Nat Rev Mol Cell Biol*. 2010; 11: 23–36.
116. **Tang CH, Wei Y.** The urokinase receptor and integrins in cancer progression. *Cell Mol Life Sci*. 2008; 65: 1916–32.
117. **Grismayer B, Sato S, Kopitz C, *et al.*** Overexpression of the urokinase receptor splice variant uPAR-del4/5 in breast cancer cells affects cell adhesion and invasion in a dose-dependent manner and modulates transcription of tumor-associated genes. *Biol Chem*. 2012; 393: 1449–55.
118. **Nykjaer A, Christensen EI, Vorum H, *et al.*** Mannose 6-phosphate/insulin-like growth factor-II receptor targets the urokinase receptor to lysosomes *via* a novel binding interaction. *J Cell Biol*. 1998; 141: 815–28.
119. **Kreiling JL, Byrd JC, Deisz RJ, *et al.*** Binding of urokinase-type plasminogen activator receptor (uPAR) to the mannose 6-phosphate/insulin-like growth factor II receptor: contrasting interactions of full-length and soluble forms of uPAR. *J Biol Chem*. 2003; 278: 20628–37.
120. **Li Y, Liu D, Chen D, *et al.*** Human DF3/MUC1 carcinoma-associated protein functions as an oncogene. *Oncogene*. 2003; 22: 6107–10.
121. **Gnemmi V, Bouillez A, Gaudelot K, *et al.*** MUC1 drives epithelial-mesenchymal transition in renal carcinoma through Wnt/β-catenin pathway and interaction with SNAIL promoter. *Cancer Lett*. 2014; 346: 225–36.
122. **Ahmad R, Raina D, Joshi MD, *et al.*** MUC1-C oncoprotein functions as a direct activator of the nuclear factor-kappaB p65 transcription factor. *Cancer Res*. 2009; 69: 7013–21.
123. **Li Y, Ren J, Yu W, *et al.*** The epidermal growth factor receptor regulates interaction of the human DF3/MUC1 carcinoma antigen with c-Src and beta-catenin. *J Biol Chem*. 2001; 276: 35239–42.
124. **Pochampalli MR, el Bejjani RM, Schroeder JA.** MUC1 is a novel regulator of ErbB1 receptor trafficking. *Oncogene*. 2007; 26: 1693–701.
125. **Kato K, Lillehoj EP, Park YS, *et al.*** Membrane-tethered MUC1 mucin is phosphorylated by epidermal growth factor

receptor in airway epithelial cells and associates with TLR5 to inhibit recruitment of MyD88. *J Immunol*. 2012; 188: 2014–22.

126. **Yin L, Kharbanda S, Kufe D.** MUC1 oncoprotein promotes autophagy in a survival response to glucose deprivation. *Int J Oncol*. 2009; 34: 1691–9.

127. **Chaika NV, Gebregiworgis T, Lewallen ME, *et al.*** MUC1 mucin stabilizes and activates hypoxia-inducible factor 1 alpha to regulate metabolism in pancreatic cancer. *Proc Natl Acad Sci USA*. 2012; 109: 13787–92.

128. **Chua CEL, Tang BL.** Engagement of the small GTPase Rab31 and its effector, early endosome antigen 1, is important for trafficking of ligand-bound epidermal growth factor receptor from early to late endosome. *J Biol Chem*. 2014; 289: 12375–89.

129. **Su X, Kong C, Stahl PD.** GAPex-5 mediates ubiquitination, trafficking, and degradation of epidermal growth factor receptor. *J Biol Chem*. 2007; 282: 21278–84.

130. **Stern DF.** Tyrosine kinase signalling in breast cancer: ErbB family receptor tyrosine kinases. *Breast Cancer Res*. 2000; 2: 176–83.

131. **Jackman D, Pao W, Riely GJ, *et al.*** Clinical definition of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. *J Clin Oncol*. 2010; 28: 357–60.

132. **Hrustanovic G, Lee BJ, Bivona TG.** Mechanisms of resistance to EGFR targeted therapies. *Cancer Biol Ther*. 2013; 14: 304–14.