Regulation of Cancer Cell Behavior by the Small GTPase Rab13

The members of the Rab family of GTPases are master regulators of cellular membrane trafficking. With ~70 members in humans, Rabs have been implicated in all steps of membrane trafficking ranging from vesicle formation and transport to vesicle docking/tethering and fusion. Vesicle trafficking controls the localization and levels of a myriad of proteins, thus regulating cellular functions including proliferation, metabolism, cell-cell adhesion, and cell migration. It is therefore not surprising that impairment of Rab pathways is associated with diseases including cancer. In this review, we highlight evidence supporting the role of Rab13 as a potent driver of cancer progression.

The Biology of Rab13

As for all small GTPases, Rab13 cycles between an active, GTP-bound state and an inactive, GDP-bound state. Activation of Rab13 is mediated by DENND2B and DENND1C/connecdenn 3, guanine nucleotide exchange factors (GEFs) that interact with GDP-bound Rab13 and facilitate the exchange of GDP for GTP (3–5). These proteins each bear a differentially expressed in normal and neoplastic cells (DENN) domain, an evolutionarily ancient protein module that encodes GEF activity for a large number of Rab GTPases (6–8). Rab13 is activated by the GTPase-activating protein (GAP) Akt substrate 160 (AS160)/TBC1D4, which enhances the ability of Rab13 to hydrolyze GTP (3–5). In its active form, Rab13 binds to several effectors including MICAL-L1, MICAL-L2, and PAK (9, 10).

Rab13, which is ubiquitously expressed, is thought to have diverged from its closest homologue Rab8, early in the vertebrate lineage (11). Similar to Rab8, Rab13 has been implicated in both biosynthetic and endosomal recycling pathways. Rab13 is found at the trans-Golgi network, on recycling endosomes, on late endosomes, and at the plasma membrane (12–14). Disruption of Rab13 function inhibits the delivery of cargo to the plasma membrane via endosomes from either the biosynthetic or the endocytic recycling pathways. Thus, Rab13 is thought to control membrane trafficking at the level of endosomes. Interestingly, Rab13 can traffic on endosome-derived vesicles in its GDP-bound form (14, 15). Strikingly, C-terminal prenylation is not required for Rab13 to associate with these vesicles, and the protein is resistant to extraction by GDP dissociation inhibitor (GDI) (3, 15). A FRET-based activation biosensor revealed that Rab13 is activated selectively at the plasma membrane where its GEFs DENND2B and connecdenn 3 are located (3, 15). Thus, Rab13 dysfunction likely alters the cell surface delivery of cargo locally at the plasma membrane, perhaps by playing a role in docking or fusion of membrane carriers derived from endosomes. However, it cannot be ruled out that activation of Rab13 at endosomes or even the trans-Golgi network to levels below the sensitivity of the biosensor by unidentified GEFs could contribute to the repertoire of Rab13-mediated cargo delivery.

Rab13 controls the trafficking and cellular localization of multiple proteins implicated in cancer cell behavior. These include the trafficking of integrins during cell migration, the surface delivery of the glucose transporter GLUT4 in response to insulin signaling, and the trafficking of VEGFR during angiogenesis (4, 5, 16). Rab13 is also implicated in the regulation of the actin cytoskeleton as both its GEFs, DENND2B and connecdenn 3, are localized on actin and all of its effectors indirectly promote actin polymerization. Through these activities, Rab13 controls the formation of membrane protrusions that contribute to the migratory capacity of cells in vitro and the metastasis of cancer cells in vivo (3). In addition, Rab13 regulates the proliferative capacity of cancer cells in vitro and the growth of tumors in vivo (3). Therefore, Rab13 activity regulates several pathways important for the behavior of cancer cells and the progression of the disease.

Regulation of Rab13 Expression

Consistent with a role for Rab13 in cancer progression, Rab13 is up-regulated in multiple cancer types including diffuse large B-cell lymphoma and glioblastoma (17, 18). In fact, Rab13...
is significantly amplified in the majority of cancers (Fig. 1A) (19, 20), and Rab13 levels inversely correlate with patient prognosis in cancers such as basal type breast cancer (Fig. 1B) (21). Moreover, Rab13 expression levels are elevated in cancer cell lines resistant to radiotherapy when compared with those that are sensitive to radiotherapy (22). Therefore, Rab13 expression correlates with cancer progression.

A key question relates to the mechanisms driving enhanced Rab13 expression in various cancers. p53 is one of the best studied genes in cancer biology, and p53 is the most commonly mutated gene in human cancer (23). Although multiple cancers have been associated with loss-of-function mutations in p53, several studies identified mutations in p53 that promote oncogenesis in a gain-of-function manner (24, 25). In fact, mice expressing gain-of-function mutations in p53 have more aggressive and metastatic cancers than p53 null mice (26, 27). Gain-of-function mutations in p53 have been reported to upregulate multiple genes implicated in cancer progression including key signaling receptors (28). Interestingly, Rab13 is one of several transcriptional targets of p53, and p53 promotes Rab13 expression (29, 30). Furthermore, ionizing radiation up-regulates Rab13 mRNA in neuroblastoma cells, and this up-regulation is blocked by inhibiting p53 activation induced by DNA damage (31, 32). Mutant p53 drives cancer cell invasion in part by promoting recycling of both integrin and epidermal growth factor receptor (EGFR) to the cell surface, and as discussed in detail below, Rab13 controls recycling of integrins and was suggested to do the same for EGFR (4, 13, 33). Therefore, up-regulation of Rab13 could contribute to the potent oncogenic effect of gain-of-function p53 mutations in human cancer.

There are multiple mechanisms in addition to p53 mutations that contribute to the up-regulation of Rab13 in cancer. For example, Rab13 expression is inhibited by hsa-miR-125b, and this microRNA is down-regulated in primary hepatocellular carcinoma, correlating with a poor patient prognosis (34). Up to 15% of all human cancers are associated with inflammation, and chronic inflammation is an important risk fac-
Role of Rab13 in Cellular Phenotypes Related to Cancer Progression

Cell Metabolism

Alterations in cell metabolism are an important consideration in cancer progression as cells within a tumor must adapt to various environmental conditions, such as nutrient deprivation, while continuing to proliferate (42). For example, multiple myeloma is one of many cancers with enhanced glucose utilization, and multiple myeloma cells exhibit increased glucose sensitivity due to an accumulation of the glucose transporter type 4 (GLUT4) on the plasma membrane (43). Interestingly, Rab13 functions in the translocation of GLUT4 to the cell surface in response to insulin (5). Insulin stimulation increases the pool of active Rab13, which binds to the Rab13 effector MICAL-L2, relieving MICAL-L2 auto-inhibition and thus allowing MICAL-L2 to recruit various actin regulatory proteins including actinin-4 (Fig. 2) (5, 44, 45). A complex involving Rab13, MICAL-L2, and actinin-4 binds to GLUT4 in an insulin-dependent manner, allowing docking/tethering of GLUT4 vesicles at the cell surface and thus enabling their fusion (46).

Rab13 is inactivated by its GAP, AS160, and AS160 is inactivated by phosphorylation (Fig. 2), thereby enhancing insulin-induced GLUT4 translocation to cell surface (47–49). Interestingly, Akt is constitutively active in multiple myeloma tumor cells, and not surprisingly, AS160 is hyper-phosphorylated in myeloma cell lines as well as in breast cancer tumors (50, 51). Thus, inhibition of AS160 by phosphorylation in cancer could enhance Rab13 activity to increase GLUT4 on the plasma membrane, increasing sensitivity to glucose and tumor growth.

Cell Proliferation

Perhaps the key feature of cancer cells is enhanced proliferation. The EGFR is a well characterized member of the ErbB family of receptor tyrosine kinases. EGFR is a key factor in tumor growth, and activating mutations, gene amplifications, and protein overexpression of EGFR have been observed in numerous cancer types (52, 53). Interestingly, overexpression of the Rab13 GEF DENND2B enhances signaling downstream of EGFR and promotes cell growth. DENND2B undergoes alternative RNA splicing, yielding three isoforms: p70, p82, and p126 (we refer to the p126 isoform as DENND2B). All three isoforms contain the DENN domain, and presumably, all isoforms activate Rab13, although this has only been directly demonstrated for the longest form. DENND2B was originally named suppression of tumorigenicity 5 as it mapped to human chromosome 11p15 and this locus mediates tumor suppression in various cell lines (54). However, only the p70 isoform had reduced mRNA levels in tumorigenic cell lines, suggesting that only this isoform functions as a tumor suppressor (55). Indeed, DENND2B promotes contact-independent cellular growth similar to transformed cells, whereas p70 inhibits this increased growth (56). DENND2B is thought to stimulate cell growth by mediating EGFR signaling. DENND2B expression enhances activation of ERK, and this effect is lost when the two proline-rich domains of DENND2B are deleted, suggesting that protein interactions with these motifs are required for this effect (57). The smaller isoforms, p82 and p70, do not contain the proline-rich domains and do not promote ERK activation. Instead, p70 inhibits DENND2B from activating ERK (57). Thus, in addition to activating Rab13, DENND2B plays a role in a growth-signaling pathway by linking EGFR stimulation to ERK activation, and this signaling is inhibited by the p70 isoform.

Rab13 and DENND2B may also influence EGFR signaling by regulating EGFR trafficking. Stimulation of EGFR by EGF induces receptor dimerization and internalization into signaling endosomes with eventual sorting to lysosomes for degradation. However, a fraction of EGFR recycles back to the plasma membrane, enhancing EGFR signaling (58).
Therefore, increases in EGFR recycling pathways enhance EGFR protein levels, signaling, and oncogenic potential. Trafficking of the EGFR is regulated in part by the Rab13 effector MICAL-L1. Overexpression of MICAL-L1 results in the accumulation of EGFR in late endosomes, whereas loss of MICAL-L1 increases degradation of EGFR, implying that MICAL-L1 promotes EGFR recycling (13). The ability of MICAL-L1 to promote EGFR recycling may be mediated by Rab13 as MICAL-L1 recruits active Rab13 to tubular recycling endosomes, which relieves MICAL-L1 auto-inhibition (13). Therefore, recruitment of active Rab13 could activate MICAL-L1 to enhance recycling of EGFR to the plasma membrane. However it is unclear whether this effect is specific to Rab13, as it has not been tested whether Rab13 influences EGFR signaling directly, and MICAL-L1 is known to bind other active Rabs including Rab8, Rab35, and Rab36 (59).

**Cell-Cell Adhesion**

In epithelial and endothelial cells, cell-cell adhesion is initiated by the formation of adherens junctions followed by tight junctions (60). Cadherins and nectins act as cell adhesion molecules at adherens junctions, whereas Claudins and occludins serve as cell adhesion molecules at tight junctions (Fig. 3). Loss of cell-cell adhesion is an important component of cell transformation in cancer, as cells must dissociate from each other to become more migratory. This is particularly important for single cell metastasis, but also in collective cell migration where cells must detach from the original tissue, and single cells may also detach from the collectively moving cell mass (61). Furthermore, weakening of tight junctions in vascular endothelial cells allows malignant cells to escape the vasculature as part of the metastatic process (62, 63). Rab13 may promote the invasiveness of cancer cells by negatively regulating the integrity of tight junctions.

One mechanism by which Rab13 inhibits tight junction formation is through regulation of its effector PKA (64). A notable substrate of PKA is vasodilator-stimulated phosphoprotein (VASP), an actin-binding protein that promotes actin polymerization. Phosphorylation of VASP by PKA abolishes its actin-polymerizing activity (10, 65). However, phosphorylation of VASP by PKA results in VASP relocation to tight junctions and is required for the establishment of tight junctions (66). In this way, PKA is proposed to contribute to tight junction recovery by allowing structural relaxation of the cortical actin rim (66). Active Rab13 binds the α-catalytic subunit of PKA, inhibits PKA-dependent phosphorylation of VASP, and prevents VASP association with intracellular junctions (10) (Fig. 3). Therefore, active Rab13 inhibits tight junction integrity by negatively regulating PKA and VASP phosphorylation. Given the importance of tight junction disassembly in tumorigenesis, it is not surprising that PKA and VASP have been implicated in cancer progression, although their roles in this process are complex. For example, PKA activity has been reported to both stimulate and inhibit cancer cell growth (67). Similarly, both down-regulation and overexpression of VASP result in the loss of contact inhibition and enhanced tumor growth (68). The contribution of VASP to tumorigenesis may depend on which of its residues are phosphorylated. Phosphorylation by PKA at Ser239 impairs F-actin bundling, whereas phosphorylation by PKD at Ser157 and Ser322 promotes VASP recruitment and actin bundling at the leading edge of cells (69). Therefore, the PKA/VASP axis is an important downstream target of Rab13 regulation (Fig. 3); however, the involvement of these proteins in cancer cell behavior is complex.

Loss of either Rab13 or MICAL-L2 inhibits surface levels of the junctional proteins claudin-1 and occludin, and for this reason, Rab13 was initially proposed to promote tight junction formation (70). However, this interpretation is inconsistent with several findings described below demonstrating that Rab13 and MICAL-L2 promote cell migration in part by disrupting cell adhesion. Furthermore, active Rab13 mutants disrupt claudin-1 and occludin at tight junctions, which suggests that Rab13 activity disrupts tight junction formation, whereas studies with inactive Rab13 mutants were conflicting as some studies found defective delivery of claudin-1 to tight junctions, whereas others found no effect on surface levels of claudin-1 (71, 72). Therefore, it is clear that Rab13 localizes to and regulates tight junctions, but it remains elusive how it does this.

**Cell Migration and Scattering**

Following disruption of cell-cell adhesion, metastatic cells become more migratory and invade into surrounding tissue. Rab13 is required for the scattering and migration of epithelial cells (3, 73). A common method for dissociating or scattering epithelial cells is treatment with 12-O-tetradecanoylphorbol-13-acetate (TPA). TPA-induced cell scattering requires activation of Rab13 and regulation of the actin cytoskeleton by the Rab13 effector MICAL-L2 (73). Active Rab13 relieves MICAL-L2 auto-inhibition, allowing MICAL-L2 to recruit various actin regulatory proteins. Not surprisingly, MICAL-L2 is up-regu-
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FIGURE 4. Rab13 activation drives cell migration. A, inactive Rab13 traffics on vesicles from a perinuclear pool to the cell periphery. Upon reaching the cell periphery, Rab13 is activated by its GEF DENND2B in a complex with its effector MICAL-L2 and can associate with the plasma membrane. This couples the recruitment of actin-bundling proteins such as filamin to the protruding or leading edge of migrating cells. B, Mst1 phosphorylates (indicated by circled red P) both the actin-bundling protein VASP to redistribute it to the cell periphery and DENND1C to promote its GEF activity toward Rab13. This coordinates the delivery of integrins to the cell surface with rearrangement of the actin cytoskeleton by VASP, thereby promoting cell migration.

lated in ovarian cancer tissue, and knockdown of MICAL-L2 suppresses the proliferation, migration, and invasive capacity of ovarian cancer cells in culture (74). One actin-bundling protein that is recruited by activated MICAL-L2 is filamin. Filamin is responsible for the cytoskeletal changes that occur during cell scattering and migration in epithelial cells (75, 76). Consistently, filamin levels also correlate with invasiveness of melanoma cell lines, and high filamin levels are predictive of poor prognosis in melanoma patients (77).

Interestingly, in the absence of Rab13, MICAL-L2 and actinin-4 promote cell-cell adhesion by stimulating thick actin bundling at the cell periphery (44, 45). Consistently, high levels of actinin-4 are associated with decreased cell motility (78, 79). However, upon activation of Rab13, MICAL-L2 binds actinin-4 and suppresses its cross-linking activity and thereby weakening cell-cell adhesion (80).

Rab13 activation by DENND2B also induces cell scattering, and DENND2B is required for cell invasion through a basement membrane matrix (3). Interestingly, DENND2B localizes on actin at the cell periphery and binds directly to MICAL-L2, thus coordinating activation of Rab13 and MICAL-L2 function on the actin cytoskeleton (3). Rab13 is delivered to the cell periphery on vesicles where it is activated locally, subsequently binding to MICAL-L2 to relieve MICAL-L2 auto-inhibition and promote actin remodeling at the leading edge of migrating cells (3) (Fig. 4). DENND2B also binds c-Abl, a non-receptor tyrosine kinase that is associated with several cancer types including, but not limited to, chronic myeloid leukemia, non-small cell lung cancer, and breast cancer. Interestingly, c-Abl is enriched in pseudopodial protrusions, the same structures where DENND2B and Rab13 co-localize (3, 81). Furthermore, active c-Abl promotes cell migration and membrane ruffling, cancer-associated phenotypes that are also induced by activation of Rab13 (82). Therefore, DENND2B may coordinate signaling of c-Abl and Rab13 at localized regions of the cell.

Important cargoes for cell migration such as integrins are under the control of Rab13. Integrins are cell surface receptors that mediate adhesion with the extracellular matrix, and recycling of integrins to the leading edge of cells is required for cell migration. Thus, integrins play a key role in the ability of cancer cells to metastasize from solid tumors (83). Rab13 functions in the trafficking of integrins to the leading edge of cells, contributing to cell migration (Fig. 4) (4). Presumably, integrin trafficking on vesicles that carry inactive Rab13, and these vesicles likely fuse with the plasma membrane following localized activation of Rab13 at the cell periphery (3). Consistent with this idea, both active and inactive Rab13-positive vesicles contain the SNARE tetanus neurotoxin-insensitive vesicle-associated membrane protein (T1-VAMP) (14), and T1-VAMP-mediated fusion of vesicles with the plasma membrane is required for integrin activation, whereas disruption of T1-VAMP impairs cell migration (84, 85). Although Rab13 has been found on both recycling and late endosomes, T1-VAMP mediates an exocytic pathway originating from late endosomes (86). Indeed, integrin recycling from late endosomes drives cancer cell invasion and metastasis (87). Enhanced integrin trafficking observed in p53 gain-of-function mutants also depends on the activity of diacylglycerol kinase α to tether Rab-coupling protein (RCP) to the tips of invasive protrusions (33, 88). Further studies are needed to determine whether Rab13 regulates the delivery of integrin-containing vesicles by interacting with Rab-coupling protein.

Rab13 can regulate integrin trafficking by directly binding the serine/threonine kinase mammalian Ste20-like 1 (Mst1) (4). Mst1 is required for chemokine-induced trafficking of integrins to the cell surface to mediate cell migration in lymphocytes (89). Mst1, an orthologue of Drosophila Hippo, controls organ size by regulating cell proliferation during development (90), and it functions as a tumor suppressor, because Mst1/Mst2 mutant mice develop multiple large tumors in the liver (91). Mst1 phosphorylates DENND1C, enhancing its GEF activity toward Rab13 (4, 8, 92). Active Rab13 then facilitates the delivery of the T lymphocyte integrin LFA-1 to the cell's leading edge, stimulating cell migration (Fig. 4) (4). Consistently, inhibiting Rab13 reduced lymphocyte adhesion and migration (4). However, Mst1 has been reported as a negative regulator of migration in epithelial cancer cell lines, as has the Mst1/Hippo orthologue KrsB in Dictyostelium discoideum (93, 94). These differences may be attributed to cell type-specific regulation of Mst1 and...
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whether Mst1 is acting within the classical Hippo pathway or a Hippo-independent pathway with Rab13 (95).

Cell Migration and Angiogenesis

Angiogenesis contributes to tumor progression in two notable ways. First, tumors require vascular support to grow and survive. Early work demonstrated that anti-angiogenic treatment of mice with islet cell carcinoma reduced tumor growth and increased apoptosis of the tumor cells (96). Secondly, cancer cells require access to blood vessels in order to metastasize to other tissue. When compared with mature blood vessels, tumor angiogenesis creates blood vessels that are immature and highly permeable and have less basement membrane as well as fewer intercellular junctions (97). Thus, tumor cells can enter these vessels more easily than mature blood vessels (97). Angiogenesis requires directional cell migration of endothelial cells in response to chemotactic cues such as VEGF (98). Furthermore, anti-angiogenic therapy targeting VEGFR can shrink tumors, albeit only temporarily as tumors inevitably acquire resistance to the drugs and resume growth (99).

Interestingly, Rab13 controls VEGF-directed cell migration and angiogenesis by regulating the spatiotemporal activation of RhoA (16). Both Rab13 and the RhoA GEF Syx are required for VEGF-induced directional cell migration. VEGF stimulates the recruitment of both RhoA and its GEF Syx onto Rab13-positive vesicles, which then traffic to the cell’s leading edge. Furthermore, Rab13 indirectly associates with Grb2, an endocytic adaptor protein that binds to activated VEGFR (100). Thus, Rab13-positive vesicles carrying RhoA/Syx associate with regions of the plasma membrane containing activated VEGFR, inducing the formation of the leading edge (16). Furthermore, Rab13 is required for the angiogenesis and growth of intersegmental vessels in zebrafish (16). Therefore, reducing Rab13 could help limit the growth of tumors by reducing VEGF-induced cell migration and angiogenesis.

Conclusions

Over the last 25 years, great progress has been made in understanding how defects in Rab GTPases cause disease. Here, we provide a detailed summary of how Rab13 regulates cell behaviors associated with cancer progression. Rab13 functions in the delivery of cargo to the plasma membrane, disruption of cell-cell adhesions, and rearrangement of the actin cytoskeleton coupled to surface delivery of selective proteins to drive cell migration. However, several aspects of Rab13 function remain to be studied and may shed additional insight into the physiology of cancer. For example, future studies should aim to elucidate the mechanisms regulating the fusion of Rab13-positive vesicles with the plasma membrane, determine whether Rab13 works in concert with other Rabs in the form of a Rab cascade, and determine how Rab13 associates with vesicles in its inactive form. Furthermore, a more detailed understanding of how Rab13 regulates cell proliferation will likely prove important in understanding tumor growth. Finally, given the high sequence homology of the Rab GTPase family, it is likely difficult to target Rab13 specifically. However, a thorough understanding of Rab13 regulators and effectors may uncover promising new targets for therapies.

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