Published in final edited form as:

Nat Rev Cancer. 2013 November ; 13(11): 813–820. doi:10.1038/nrc3601.

A central role for vesicle trafficking in epithelial neoplasia: Intracellular highways to carcinogenesis

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Abstract

Epithelial cell carcinogenesis involves the loss of polarity, alteration of polarized protein presentation, dynamic cell morphology changes, increased proliferation and increased cell motility and invasion. Elements of membrane vesicle trafficking underlie all of these processes. Specific membrane trafficking regulators, including Rab small GTPases, through the coordinated dynamics of intracellular trafficking along cytoskeletal pathways, determine cell surface presentation of proteins and overall function of both differentiated and neoplastic cells. While mutations in vesicle trafficking proteins may not be direct drivers of transformation, elements of the machinery of vesicle movement play critical roles in the phenotypes of neoplastic cells. Therefore, the regulators of membrane vesicle trafficking decisions are critical mediators of the full spectrum of cell physiologies driving cancer cell biology, including initial loss of polarity, invasion and metastasis. Targeting of these fundamental intracellular processes may provide important points for manipulation of cancer cell behaviour.

Introduction

The vast majority of the solid cancers in humans develop from the epithelial cells that line internal organs at the interface between the outside world and the internal milieu. These adenocarcinomas lose many of the characteristics of their normal counterparts, adopting less organized structures that promote local invasion and metastasis. Much of cancer research has focused on changes in the cell cycle underlying proliferation and cytoskeletal dynamics that might mediate the transformed phenotype. But, far less attention has been paid to the roles of intracellular vesicle trafficking pathways that are responsible for the correct distribution of membrane proteins inside cells and their targeting to plasma membrane surfaces. Indeed, the intracellular movement of vesicles along cytoskeletal highways likely mediates many of the aspects of cell transformation invasion and metastasis.

The intracellular trafficking of membrane vesicles is responsible for the maintenance and regulation of the components of the plasma membrane of all cells.¹ In normal epithelial cells with apico-basal polarity, the movement of membrane vesicles is coordinated through a

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highway of interconnecting and diverging transit pathways set up along microtubule and F-actin filament causeways. Proper vesicle trafficking establishes the compendium of proteins on the apical and basolateral surfaces and adherens and tight junction components required to maintain the polarized mucosa.^{2, 3} Alterations in these fundamental pathways responsible for accurate delivery of proteins to the cell surface can lead to losses in cellular polarity, which represent the earliest stages of carcinogenesis (Figure 1).^{4, 5} Furthermore, vesicle trafficking pathways in the transformed cell are central to the processes of invasion and metastasis, where membrane dynamics mediate the physical requirements for invasion. Indeed, changes in the presentation and degradation of key membrane receptors act as critical modulators of tumour cell growth and invasion. Imbalances in dynamic vesicle trafficking processes may play important roles in both the initiation of transformation as well as the process of tumour cell invasion.⁶⁻⁸ Thus, vesicle trafficking stands at a central point for understanding carcinogenesis and developing novel strategies to intervene in cancer cell behaviour (Figure 1). These vesicle trafficking pathways are not necessarily unitary the “drivers” of transformation, but rather act as mediators of the deleterious neoplastic phenotype that enables loss of polarity, invasion and metastasis. While most cancer research focuses on the read outs of cell transformation and invasion or cell proliferation, few studies have considered the intracellular vesicle trafficking pathways that functionally mediate many of these processes. This narrative seeks to highlight the potential contributions of vesicle trafficking to the induction of neoplasia, cell transformation, cell invasion and metastasis.

Polarity is fundamental to differentiated epithelia

The “identity” of the normal polarized epithelial cell is fundamentally tied to its ability, along with its neighbors, to establish an intact mucosal sheet with directional flow of ions, nutrients and receptor-dependent signals. At its most basic level, a polarized epithelium requires the maintenance of apical and basolateral membranes with distinct characteristics and segregation of functional channels, transporters, receptors and adhesion molecules in defined apical and basolateral zones separated by intercellular adherens and tight junctions^{9, 10}. Some epithelia, such as in the kidney, are relatively stable, but others, such as those lining the gut, are under constant renewal. Those epithelia that continually self-renew must maintain barrier function during renewal, which is supported by dynamic intracellular vesicle trafficking pathways that are responsible for the turnover of polarized membrane domains.

In general, membrane proteins find their way to their proper cell surface positions through the interactions of *de novo* synthesis and trafficking from the Golgi apparatus with the ongoing endocytic and recycling pathways (Figure 2).¹ Newly synthesized membrane proteins leave the Golgi apparatus in membrane vesicles and are sorted to the apical or basolateral membranes according to discrete motifs on their cytoplasmic domains (Figure 2A). Once located on these membrane surfaces, endocytosis can retrieve proteins back into the cell either constitutively or through ligand-induced internalization. As proteins are endocytosed, the cell must decide a protein’s eventual fate along several distinct pathways. Some internalized proteins are targeted for degradation through trafficking to the lysosome (Figure 2B). This mechanism obviously provides a means for down-regulation of surface

molecules as well as protein replacement. Other proteins will be recycled back to the membrane surface from where they were derived (Figure 2C). This mechanism provides a pathway for internalization of nutrients (e.g. transferrin)^{11, 12} as well as transmission of signals into the cytoplasm or termination of that signal (e.g. Epidermal Growth Factor receptor (EGFR)).¹³⁻¹⁵ Some proteins will be recycled back to the Golgi apparatus (Figure 2D), a mechanism that can potentially account for repair of damaged receptors (especially damaged glycosylation residues on membrane proteins).¹⁶ Finally, in polarized epithelial cells, internalized proteins may be transcytosed to the opposite surface (either basolateral to apical or apical to basolateral). These transcytotic pathways account for exchange of nutrient and critical proteins: e.g. apical to basolateral transport of maternal immunoglobulin Gs (IgGs) in the neonatal gut (Figure 2E)¹⁷ and basolateral to apical transport of IgA in many epithelia (Figure 2F).^{18, 19} The summation of all of these processes leads to the presentation of the correct compendium of pumps, channels and transporters that are necessary for maintaining all aspects of the normal epithelial physiology, including the resting short circuit, defined barrier functions and the presentation of nutrient absorptive enzymes and transporters. In addition, coordinated trafficking processes are necessary for the construction and maintenance of apical specializations such as microvilli and the primary cilium.²⁰⁻²³ All of these processes are critical for the consistent functioning of mucosal surfaces. Thus, the dynamic intracellular decisions in vesicle trafficking can radically impact the physiology of the epithelial cell.

Small GTPases specify dynamic membrane trafficking

Over the past 20 years increasing evidence has suggested that distinct classes of small GTPases are intimately involved in the intracellular decision processes that orchestrate the movement of membrane vesicles through defined trafficking fates.²⁴ These classes of Rab and Arf proteins associate with and often define distinct populations of membrane vesicles. In general, these small GTPases provide the anchors for the assembly of multiprotein complexes that mediate membrane vesicle tethering, membrane vesicle movement, and decisions in vesicle fate at branch points along the trafficking highway.^{25, 26} Their movements are in turn influenced by other small GTPases (e.g. Rac, cdc42, and Rho proteins) that modify the structure of cytoskeletal elements.²⁷ Thus, these small GTPases represent a compendium of GTP-driven timers²⁸ that are responsible for the direction of intracellular trafficking and the appropriate protein sorting to polarized cell surfaces. The activities of small GTPases are determined by their GTP-versus GDP-bound states (Figure 3A). The guanine nucleotide binding state for small GTPases is determined by their interaction with either GTPase activating proteins (GAPs) or guanine nucleotide exchange factors (GEFs). Since it is thought that small GTPase function requires cycling of the proteins between the GTP-bound and GDP-bound states,²⁹ the GAPs and GEFs control the relative amounts of active and inactive small GTPases. Our knowledge of the GAPs and GEFs for Rab proteins is relatively rudimentary. Proteins with TBC (Tre2-Bub2-Cdc16) domains are putative Rab GAPs.³⁰ These GAPs can markedly alter the dynamics of specific vesicle trafficking pathways. Each GTP cycle represents an opportunity for the cell to change trafficking decisions: to release a tether and facilitate trafficking, to release a motor protein and slow down trafficking or change tracks from actin to tubulin, or to hand-off trafficking between Rab-dependent regulators. A loss of GAP proteins or an overproduction

of GEFs would lead to accumulation of active GTP-bound small GTPases. This in turn would affect the dynamics of interactions and activation of down-stream effectors that associate with the GTP-bound form of the small GTPases. Recent investigations also suggest that one Rab protein may recruit the GEF or GAP for another Rab protein, a critical mechanism for sequential activation of Rab proteins during trafficking through membrane systems.²² While much of cancer research has focused on activating mutations in proteins, particularly in the small GTPase Ras,^{31, 32} It seems just as likely that, in the majority of cancer cells without such driver mutations, functional activation of Rab small GTPases provides a logical pathway for promoting the properties of neoplastic cells.

The diversity of interacting proteins for individual Rab proteins provides another level of complexity (Figure 3A). Small GTPases can interact with a wide range of scaffolding and motor proteins as part of their central functions in specific vesicle trafficking pathways. These interacting proteins include classes of molecular motors (e.g. myosins and kinesins) and scaffolding proteins that organize multiprotein complexes. The Rab-interacting proteins themselves can coordinate interactions with molecular motor proteins, associate with other small GTPases, and directly scaffold higher order complexes (Figure 3A).

Figure 3B illustrates an example of such an interaction network for the Rab11 family members. There are presently at least 10 effectors for GTP-bound Rab11 including seven Rab11-Family Interacting Proteins (Rab11-FIPs),^{33, 34} Rab11-binding protein (also known as Rabphilin-11),³⁵ Myosin Va and Myosin Vb,^{36, 37} Rabin-8,^{22, 38} and Sec15.³⁹⁻⁴² (Figure 3B). All of these proteins can be found in the same cells.^{37, 43} Thus, alterations in the abundance of individual small GTPase interacting effectors could shift the dynamics of trafficking towards different pathways. Rab11a binds Rabin-8, a GEF for Rab8a, and regulates local activation of Rab8a.^{22, 38} The coordination of trafficking systems is particularly relevant for molecular motors such as Myosin V and Kinesin II, which are responsible for the directed movement of vesicles along actin filaments and microtubules, respectively. In HeLa cells, over-expression of particular Rab11-FIPs can lead to changes in the morphology of the membrane recycling system.⁴⁴ Interestingly, co-expression of Rab11a with Rab11-FIP1b, Rab11-FIP1C or Rab11-FIP3 can limit structural tubulation of the recycling system, a manifestation of the slowing of the dynamic flow through the recycling system, which is seen when the individual Rab11-FIP proteins are over-expressed by themselves.⁴⁴ These results support the concept that individual Rab proteins may be in limiting concentrations within cells and effectors must either compete for Rab protein binding or array themselves along pathways that facilitate the orderly hand-off of vesicles between effectors.

As an added point for complexity, a number of effectors bind multiple Rab proteins or both Rabs and Arf proteins. These interactions can set up points of recruitment, as in the case of Rab11a activation of Rabin8, a GEF for Rab8a.³⁸ Alternatively, dual Rab binding may provide for trafficking transitions, as exemplified by Rabaptin-5, which binds both Rab5 and Rab4.⁴⁵ Finally, dual small GTPase binding may provide for effective intracellular trafficking decision making, as proposed for Rab11-FIP1C/RCP binding of both Rab11 and Rab14,^{46, 47} Myosin Va and Myosin Vb binding of multiple Rab proteins,^{37, 48-50} and Rab11-FIP3 binding to both Rab11 and Arf6.^{51, 52} If Rab proteins provide a “zip code” for

the allocation of vesicles to different pathways, proteins that can interact with more than one Rab protein can determine how the distribution of vesicles is achieved. Finally, Sec15 binds Rab11a and coordinates its association with the exocyst complex, which is responsible for basolateral exocytosis.^{39, 42, 53} Together this network of interactions provides a widely dynamic process for regulating the flow of cargoes through the recycling system. As a more general concept, these types of interactions with Rab proteins at each of the steps along exocytotic and endocytotic pathways also amplify the complexity of regulation that can alter the passage of trafficking cargoes towards and away from the cell surface.

Loss of polarity in early carcinogenesis

Alterations in the production or plasma membrane delivery of critical regulators of structural polarity, including components of the intercellular junctions or cell adhesion molecules, influence polarized epithelial cell identity. Aberrations in the correct delivery of pumps or channels to polarized surfaces can markedly impact the physiological phenotype of epithelial cells. Studies in *Drosophila* have demonstrated that dynamic vesicle trafficking pathways for endocytosis and recycling regulated by Rab5 and Rab11 are integral to orderly establishment of epithelial structures and planar cell polarity during development.⁵⁴⁻⁵⁸ Alterations in endocytic trafficking in *Drosophila* can lead to disruption of normal polarized cell and tissue development, leading to aberrant tumor formation.⁵⁸

While decrements in individual constituents usually do not induce transformation in organized epithelial tissues, multiple losses in the components of polarity are likely proximate events in early carcinogenesis. Much of cancer research in the past has focused attention on over-arching oncogenes which, when disrupted on their own, can lead to cancer. However, in epithelial cancers, the examples of such genes are relatively few. They include the effects of mutation of E-cadherin in familial gastric cancer,⁵⁹ APC mutations in familial polyposis⁶⁰ and BRCA1 mutations in breast cancer⁶¹. Yet even these familial cancer mutations often require further genetic or environmental perturbations to effect full penetrance. Still, individual losses in structural polarity components do not generally lead to carcinogenesis. Thus, loss of either β 1-integrin or Rab25 does not by itself lead to cancer, but the combination with other secondary perturbations in cell function can then reveal tumor suppressor function.^{62, 63} It appears that the epithelial monolayers have enough redundancy to maintain their functional integrity in the face of single protein dysfunctions under situations of normal homeostasis. Nevertheless, these mucosal cells may be susceptible to the influence of secondary perturbations including chronic inflammation, noxious viral or bacterial infection, or chronic injury. A slow or impaired response to injury may promote the loss of epithelial cell polarity and early carcinogenesis.

It is important to consider the impact of losses in polarity. First, inappropriate trafficking of proteins to apical or basolateral domains may allow aberrant signaling through misplaced receptors. Thus, for instance, a mis-located receptor tyrosine kinase such as the epidermal growth factor receptor (EGFR) may come in contact with an EGFR ligand that should only see the receptor if there is a breach in the mucosa.⁶⁴ This scenario might usually trigger a reparative response, but in the loss-of-polarity context this might cause inappropriate proliferation and invasion. In a similar scheme, mis-trafficking of a receptor ligand to

presentation or release at the incorrect cell surface may lead to inappropriate signaling. Thus, mis-trafficking of epiregulin, an EGF-receptor ligand, to the apical surface of polarized epithelial cells can induce transformation.⁶⁵ Second, inappropriate trafficking may cause the redistribution of cell adhesion molecules, such as integrins, within cells. Since integrin signaling is tied into the cell proliferative and motility responses, these changes can promote a transformed phenotype.⁶⁶⁻⁶⁹ Third, inappropriate or altered delivery of junctional components could elicit changes in the permeability of the mucosa as well as further redistribution of proteins normally segregated to apical or basolateral domains.^{8, 70-72} Disruption of the mucosal barrier can also facilitate exposure of the basolateral surface to luminal factors as well as influx of immune cells and inflammatory cytokines.⁷⁰ Losses in these junctional components can thereby promote the assumption of a transformed phenotype. Fourth, losses in polarity can lead to inappropriate delivery of degradative enzymes such as matrix metalloproteinases (MMPs) to cell surfaces, thereby promoting cell invasion and transformation.⁷³ Fifth, changes in polarity may lead to inappropriate or at least deleterious trafficking decisions within the cell. Without established polarity, cells may, for instance, aberrantly traffic post-Golgi or endocytotic vesicles to lysosomes or autophagosomes.⁶ These changes in trafficking can lead then to radical alterations in cell function. All of these scenarios indicate that vesicle trafficking pathways and vesicle trafficking decisions are central to the establishment of altered cell behaviors during early stages of epithelial cell transformation.

Membrane protein presentation during carcinogenesis

The fundamental change inherent in the loss of polarity is a deviation from complete segregation of apical and basolateral domains. These changes are manifested in multiple ways, all of which are mediated by alterations in vesicle trafficking pathways. The analysis of alterations in critical proteins in cancer has traditionally focused on regulation of transcription or translation. Indeed, several studies have documented changes in Rab protein expression, a number of which correlate with tumor aggression or metastasis (Table 1). Many of the changes seen in carcinogenesis are due to changes in the dynamic processing of proteins through vesicle trafficking pathways that influence protein delivery to the plasma membrane, protein endocytosis and the decisions to either recycle or degrade following endocytosis.⁷⁴ Increases in endocytosis without compensatory increases in recycling can deplete a protein from the cell surface. Similarly, losses in a cell surface protein will accrue from shunting of endocytosed proteins away from recycling and towards degradation in the lysosome.⁷⁵ In contrast, a loss of endocytosis or an increase in recycling will result in increased protein presentation at the cell surface. Furthermore, aberrant delivery of proteins to the wrong surface defines polarity loss and can lead to significant changes in cell adhesion and receptor-dependent signaling.⁶⁵

All of these trafficking pathways are highly dynamic. It is critical to recognize that many of the Rab proteins and other trafficking regulators are often expressed at limiting concentrations. Thus, competition of effectors for individual Rab proteins may regulate the dynamic morphology of the recycling system.⁴⁴ Furthermore, the roles of particular trafficking proteins in the differentiated epithelial cells may reflect expression of these proteins only in the highly differentiated state. Thus, in the case of Rab25 in CaCo-2 colonic

cells, protein expression increases in concert with maturation of differentiated polarity.⁸ Loss of Rab25 in Caco-2 cells leads to aberrant integrin presentation at the cell surface. Specifically, loss of Rab25 leads to a decrease in $\alpha 5$ -integrin transcription and a decrement of $\beta 1$ -integrin at the lateral cell surface. In this case of Caco-2 cells, this alteration promotes a more invasive phenotype with alterations in components of both adherens and tight junctions. Recent investigations suggest that a loss of Rab25 may also be associated with triple-negative breast cancer and head and neck cancers.^{76, 77} Nevertheless, other studies indicate that Rab25 over-expression is associated with invasion in ovarian cancers.^{78, 79} How can these disparate results be reconciled? Perhaps the key to this conundrum is that Rab25 should not be expressed in non-polarized cells. Thus, ovarian cancers typically show a poorly differentiated phenotype, where Rab25 expression would be considered inappropriate. Norman and colleagues have suggested that an association of Rab25 with lysosome-associated CLIC3 may account for tumor promoter effects in ovarian cancers through restructuring of trafficking towards degradative pathways.⁶ Aberrant expression of Rab25 in a non-polarized ovarian cell may lead to inappropriate protein interactions that promote mistrafficking of components such as $\alpha 5 \beta 1$ -integrin, in this case towards lysosomal degradation. Thus, the effects of these trafficking proteins on cell behavior may accrue from subtle alterations in the balance of Rab proteins and their effectors. Furthermore, it should be noted that the changes in net trafficking that may result from small alterations in Rab protein or effectors may be substantial. Since membrane recycling is highly dynamic and may be responsible for endocytosis and recycling of proteins at the rate of one cycle every 10-20 minutes or less, just a 5% change in the speed or partitioning of recycling could lead to large changes in cell surface presentation over the course of hours or days.

Vesicle trafficking can alter cell signaling

Alterations in the presentation of signaling receptors at the cell surface support a role for vesicle trafficking in transformation.^{74, 80} Overall, the presentation of critical signaling receptors at the cell surface reflects a series of dynamic processes including the rate of *de novo* synthesis, endocytosis, recycling and degradation. In particular, the decision point between recycling and degradation can markedly revise the net presentation of receptors at the cell surface and the longevity of receptor signaling. Thus, each cell must constantly regulate a number of decision points that determine a balance among internalization, recycling and degradation. These processes can be regulated by specific ligand interactions as in the case of the EGFR. Both EGF and TGF α binding to the EGFR lead to endocytosis of EGFR, but receptor-EGF complexes are predominantly trafficked for degradation in lysosomes, while TGF α -bound receptor is predominantly recycled back to the cell surface.⁸¹ Thus, changes in ligand-dependent activation can radically impact growth factor receptor signaling. In addition, alterations in internal trafficking decisions can also modulate receptor signaling. These can include decisions to recycle versus degrade through regulation of ubiquitinylation.⁸² Furthermore, changes in receptor recycling may manifest from changes in the dynamic regulation of vesicle trafficking processes. Thus, loss of a RabGAP for Rab5 and Rab4 leads to alteration in the levels of platelet derived growth factor receptor (PDGFR) activation.⁸³ Changes in the balance between Arf6 and Rab35 have recently been implicated in the dynamics of $\beta 1$ -integrin and EGFR recycling.⁸⁴ Overall, changes in trafficking that lead to deficits in receptor recycling and trafficking may manifest as cell

insensitivity to pro-apoptotic or differentiating signals, while augmentation of these trafficking pathways would account for enhanced pro-proliferative or anti-apoptotic signals. The net impact of such modifications will be a cell with a dysregulated proliferative phenotype.

Invasion: a vesicle trafficking problem

Perhaps the most obvious manifestation of transformation in an epithelial cell context is the adoption of active cell migration. By definition, cell migration through a matrix requires dynamic extension of cell processes. This behavior requires active turnover of cytoskeletal elements and movement of membrane into and out of cell extensions: a clear membrane trafficking problem similar to that observed in axonal sprouting. Indeed, many components of the endocytotic and recycling system machinery are concentrated in the leading extensions of invading cells.⁸⁵ In effect these invasive cells have adopted a different paradigm for “polarized” function dedicated to directed delivery and recycling of membrane to the invasive front. Knowledge of how this alteration occurs and is maintained by vesicle trafficking pathways, is fundamental to an understanding of the regulation of a cellular invasive phenotype that is central to the biology of tumor metastasis. Indeed, cell invasion requires the redistribution of adhesive elements as well as the directed secretion of matrix degrading enzymes. In the former case, integrins trafficked into cell extensions regulate the formation of filopodia and invadopodia.^{79, 86} Several investigations have highlighted the roles of Rab21^{87, 88} as well as Rab11-FIP1C/RCPRCP^{67, 89} in the regulation of cancer cell invasion. Alterations in integrin trafficking, activation and presentation on the surface of cells can markedly alter the invasive behavior of cancer cells.^{67, 68, 87} Alterations vesicle trafficking pathways affecting integrin trafficking can also lead to failure of cytokinesis and resulting genomic instability.^{90, 91} Over-expression of various Rab small GTPase and their regulators can lead to exaggerated membrane extensions and promotion of migration.^{92, 93} Similarly, defined vesicle trafficking pathways can modulate the release of MMPs and other proteases and thereby alter cell migration. Rab4A appears to regulate specifically the secretion of Procathepsin L in melanoma cells.⁹⁴ Rab8 and the vesicle SNARE protein VAMP-7 mediate the secretion of membrane type 1-MMP (MT1-MMP), which regulates the invasive phenotype of HeLa and osteosarcoma cells.^{73, 95-97} It is likely that these specific membrane trafficking vesicle populations are in turn tied to discrete interactions with microtubule and F-actin microfilament-directed movement.⁹⁸⁻¹⁰⁰ The net impact of these pathways would be the promotion of cell protrusions and extensions that are likely required for cell invasion. These investigations indicate a framework that can be applied to multiple pathways involved in the initiation of cell invasion and metastasis through marshaling of the coordinated vesicle trafficking machinery to define the enhanced migratory capacity in the transformed cell phenotype.

Vesicle trafficking in progenitor cells

Whether one ascribes to a cancer stem cell theory or a concept of derived cancer progenitor cells, the effects of polarized function and vesicle trafficking on stem cells remains unclear. In epithelial monolayers, these progenitor cell populations all appear to have distinct apical and basolateral domains,^{101, 102} and it seems likely that they sense the environment within mucosal-lined gut lumen to evaluate nutritional states and mucosal integrity. Thus, the

maintenance of polarized domains in these stem cell populations may be of special importance for the preservation of oriented adhesion molecules, growth factor receptors and luminal sensing molecules. Indeed, alterations in polarity and vesicle trafficking function could impact the fundamental behavior of epithelial stem cells.¹⁰³ Furthermore, the recent identification of Lrig1, a directly interacting negative regulator of EGFR function, in quiescent and active intestinal stem cells, suggests that issues of EGFR presentation likely have a prominent influence on stem cell function.¹⁰² Given that the proper influence of growth factor receptors by their ligands is intimately tied to growth factor function, one can expect that these pathways in stem cells will hold a position of special prominence. Future investigations will likely uncover the importance of specific membrane trafficking pathways in the ultimate ability of stem cells to produce an appropriate compendium of mucosal lineages as well as provide for mucosal repair and renewal. Similarly, the trafficking of critical receptors in cancer stem cells is likely governed by many of the same pathways that are utilized in normal stem cells.

Conclusion

Vesicle trafficking pathways and dynamics are intimately involved in all aspects of cell behavior associated with cell differentiation, loss of polarity, cell transformation, cell invasion and metastasis. These vesicle trafficking highways along the microtubules and microfilaments provide critical dynamic decision points within all cells for the proper targeting of proteins destined to cell surfaces. In the end, for all cells, you are what you traffic, and trafficking determines the faces that cells, normal or neoplastic, present to the world. Since these decision points are dynamic and at least partially redundant, individual perturbations in vesicle trafficking pathways usually do not lead to significant aberrations required for carcinogenesis. Coexistent alterations likely lead to many of the manifestations of the transformed phenotype including loss of polarity, adoption of an invasive transformed phenotype and increased proliferation. Thus, while individual vesicle trafficking regulators may not serve as actual drivers of transformation, they are integral to the downstream processes that are required for manifestation of the neoplastic phenotype. Thus, although many of the vesicle trafficking pathways involved are ubiquitous, selective targeting of therapeutic interventions to particular trafficking mechanisms may enable future pharmacological intervention in cancer cell behavior. In particular, strategies to reestablish normal trafficking pathways may provide a means to reverse transformation and elicit differentiation of neoplastic cells, causing arrest of tumour growth and metastasis.

Acknowledgments

Dr. Goldenring's work is supported by NIH National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) grants RO1 DK048370 and RO1 DK070856 and a VA Merit Review.

GLOSSARY OF TERMS

Apico-basal polarity

Separation in polarized epithelial cells between apical membrane surfaces that face the external environment and the basolateral membrane, which faces the internal milieu.

Tight junctions	Intercellular junctions that are composed of proteins such as ZO-1, occludins and claudins which are responsible for the tightness of the barrier between epithelial cells.
Adherens junctions	Intercellular junctions that lie deep to tight junctions and regulate actin filament insertion and act as a reference for basolateral trafficking.
Exocyst	Evolutionarily conserved (yeast to man) multiprotein complex that mediates exocytosis at the plasma membrane.
Glycosylation	Addition of sugar residues to the external regions of membrane proteins.
Resting short circuit current	The electrical manifestation of epithelial polarity manifested by junctional characteristics and directed ion pumps and channels.
Microvilli	Organized plasma membrane protrusions on the apical surface of cells that increase the surface area absorption and secretion.
Primary cilium	In mammalian cells, a specialized protrusion with sensory functions.
Integrins	Transmembrane cell adhesion molecules which are responsible for interaction with extracellular matrix components including collagen and fibronectin.
Autophagosomes	Specialized lysosomal vacuoles that are responsible for degradation of intracellular organelles and recycling of components for use in de novo synthesis.
TBC domains	Conserved motif present in many Rab-GTPase Activating Proteins.
Tubulation of the recycling system	Part of a spectrum of intracellular morphologies between small vesicles and tubules that indicates the process of trafficking dynamics.

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Figure 1.

Vesicle trafficking stands at the center of epithelial carcinogenesis. Vesicle trafficking is a central contributor to all stages in the evolution of epithelial cancers. The early loss of polarity is a critical factor in early dysplastic changes *in situ*. These include inappropriate trafficking of junctional proteins and cell adhesion molecules (e.g. integrins) as well as decrements in the apical trafficking of proteins involved in apical specializations. Similarly, progression towards a more invasive phenotype is associated with trafficking into membrane protrusions and relocation of integrins as well as targeted secretion of matrix metalloproteinases (MMPs) at the invasive front. Finally, metastatic lesions are associated with further alterations in vesicle trafficking that promote more dynamic cell motility and lead to decreased apoptosis.

A. De novo synthesis from Golgi apparatus **B. Endocytosis and degradation** **C. Apical and basolateral recycling** **D. Endocytosis and recycling to Golgi** **E. Apical to basolateral transcytosis** **F. Basolateral to apical transcytosis** **Figure 2.**

Paradigms for exocytic and endocytic trafficking in polarized epithelial cells. A. De novo trafficking from the Golgi apparatus.

TJ: tight junction; AJ Adherens junction. B. Endocytosis leading to degradation in the lysosome. BEE: basolateral early endosome; AEE: apical early endosome. C. Endocytosis with recycling to the same membrane surface. CRE: common recycling endosome; ARE: apical recycling endosome. D. Endocytosis with trafficking back to the Golgi apparatus. E. Apical to basolateral transcytosis. F. Basolateral to apical transcytosis. All of these pathways may be operating in most polarized epithelial cells. Together these pathways sum to define the presentation of proteins at the basolateral and apical membranes.

Figure 3.

The complex web of interactions regulating Rab activation and interaction with down-stream effectors. **A.** A general scheme Rab protein activation and interaction with downstream effectors. The activity of Rab proteins is determined by the GTP/GDP binding state as regulated by GAP and GEF proteins. Active GTP-bound Rabs interact with downstream primary interacting proteins. These interacting proteins may have direct functions, as in the case of molecular motors or GEFs and GAPs, or may function as scaffolding proteins for assembly of higher order complexes. **B.** The figure illustrates an example, using Rab11 family members, of a complicated network of protein interactions for regulation membrane trafficking through recycling endosomes. Primary Rab11 interacting proteins are shown in red. Motor proteins that have direct interactions with either Rab11 proteins themselves or their interactors are shown in green. The interaction of these motor proteins with Rab proteins likely defines the movement characteristics and trafficking pathways used, in this case, for membrane recycling. Also note the further interactions among Rab11-interacting proteins (e.g. Rab11-FIP2 with MYO5A and MYO5B) and the interaction of effectors with multiple other Rab proteins (shown in light blue). In the case of Rabin-8, Rab11a binding localizes the Rab8a GEF activity. Finally, interaction of Sec15a with Rab11a coordinates a higher order complex with the exocyst complex (dark blue). Together this network of interactions provides a rich matrix for decision processing that can dynamically regulate the protein composition of the plasma membrane.

Table 1

Alterations in Rab protein expression in human cancers.

Rab protein	Cancer	Expression	Associations	Reference
Rab1A	Tongue	Increased		104
Rab5A	Breast	Increased	Metastasis	105
Rab7	Lung	Increased		106
Rab14	Lung (Non-small cell)	Increased	Down-regulation of miR-451	107
Rab23	Bladder	Increased	FGFR3-non-mutated muscle-invasive tumors	108
	Gastric	Increased	Diffuse gastric cancer	109
Rab25	Ovarian	Increased	Rab25 gene amplified	78
	Breast	Decreased	Triple negative breast cancer	76, 110
	Breast	Increased	Metastatic ER/PR positive	111
	Colon	Decreased		63
	Head and neck	Decreased	Metastasis	77
Rab40b	Breast	Increased	Invasion	112