Cell Polarity and Migration: Emerging Role for the Endosomal Sorting Machinery

The endosomal sorting complex required for transport (ESCRT) machinery has been implicated in the regulation of endosomal sorting, cell division, viral budding, autophagy, and cell signaling. Here, we review recent evidence that implicates ESCRTs in cell polarity and cell migration, and discuss the potential role of ESCRTs as tumor suppressors.

Many cells polarize in response to a specific signal, and this is characterized by the reorganization of the cytoskeleton and the redistribution of certain organelles. This is clearly of importance in epithelial cells, where apical and basolateral surfaces need to be established, as well as during directed cell migration, where front-rear polarization enables the asymmetric morphology of cells migrating toward an extracellular signal.

Endocytosis is an important regulator of cell polarization and cell migration since it regulates the trafficking of adhesion and polarity proteins. The fate of endocytic cargo is either recycling through intracellular organelles back to the plasma membrane, retrograde transport to the trans-Golgi network (TGN), or degradation in the lysosome. Adherens junctions (AJs) provide anchorage to the actin cytoskeleton through the cytoplasmic catenins and mediate cell-cell contact. E-cadherin is a type I transmembrane protein and an important component of AJs. Its recycling is thought to contribute to maintaining cell-cell junction integrity and is dependent on Rab8 and Rab11 (15, 99) (FIGURE 1, LEFT). Adhesion sites are sites of engagement between the extracellular matrix (ECM) and the cell surface. Here, integrins, type I transmembrane glycoproteins made up of α- and β-chains (40), cluster and link the ECM to the actin cytoskeleton. Integrin recycling contributes to cell migration (7), regulated by the activities of the small GTPases Rab4, Rab11, Rab25, and Arf6 (8) (FIGURE 1, RIGHT).

Recent studies have revealed that, in addition to being recycled, cadherins and integrins are degraded during cell migration (56, 66) (FIGURE 1, RIGHT). A prerequisite for lysosomal degradation of transmembrane proteins is ubiquitination of their cytoplasmic tail. This results in recognition of the protein by the endosomal sorting complex required for transport (ESCRT), which sorts the ubiquitinated receptor into intraluminal vesicles (ILVs) of multivesicular endosomes (MVEs), where the cytoplasmic tail is sequestered from the cytosol and can therefore not signal any longer. Next, the MVE fuses with a lysosome, resulting in the degradation of the ILVs and their content by lysosomal lipases and proteases. The best characterized examples of cargoes sorted by the ESCRTs are the receptor tyrosine kinases (RTKs) and, in particular, the epidermal growth factor receptor (EGFR). The components that make up the ESCRT machinery are presented in FIGURE 2. So far, ESCRTs have been found to have a role in inward endosomal membrane budding and scission, sorting of ubiquitinated receptors, viral budding, autophagy, and cytokinesis (38). Recently, several studies have shown that ESCRTs also regulate apicobasal polarity (60, 85, 89) as well as cell migration (56, 66). Although the mechanisms are not clear, this reflects that the ESCRT machinery controls a variety of cellular processes. In this review, we focus on cell polarization and cell migration and discuss the potential mechanisms that could explain the involvement of ESCRTs in these processes.

ESCRTs as Positive Regulators of Cell Migration

Regulation of Cell Migration During Drosophila Melanogaster Development

Several studies have independently found the ESCRT machinery to regulate cell migration by regulating the activity of RTKs. The ESCRT-0 component Stam is required for tracheal cell migration in the air sac primordium (ASP) of the fruit fly Drosophila melanogaster (10). In tracheal cells, the fibroblast growth factor (FGF) ligand Branchless binds and activates its receptor Breathless, thereby triggering cell migration. This raises the question whether other ESCRT-0 components might be involved in regulating FGFR signaling (44, 82). Indeed, tracheal cell migration is strongly affected by deletion of the ESCRT-0 subunit hrs, and only a few cells are able to colonize the distal tip of the ASP (9). Since tracheal cell migration requires FGFR signaling, these results suggest that ESCRT-0 is required for FGF-dependent tracheal cell migration during larval
development and therefore acts as positive regulator of tracheal cell migration by regulating the signaling of the RTK FGFR.

During *Drosophila* oogenesis, border cells migrate directionally from the anterior follicular epithelium to the oocyte, thereby providing an excellent model to study directed cell migration in vivo. The platelet-derived growth factor receptor and vascular endothelial growth factor receptor (PVR) and EGFR act as guidance receptors in this context (18, 19), providing an example of the importance of RTK signaling for polarized migration. Interestingly, the signaling from these RTKs is locally maintained by receptor endocytosis and is required for cell migration (41). Additionally, it has been observed that some hrs mutant border cells show impaired migration to the oocyte (41). Hrs therefore appears to positively regulate border cell migration by regulating PVR/EGFR signaling.

There are three nuclear migration events during the preblastoderm period in *Drosophila* (92). The second event, termed “axial expansion” since the nuclei spread along the anterior-posterior axis in the early syncytial embryo, requires a functional cytoskeleton (92). The ESCRT-I component dVps28 regulates axial expansion of nuclei, since an irregular distribution of nuclei in the embryo is observed in *dyps28* mutants (78). Other phenotypes that also require a functional actin cytoskeleton are observed in the mutants, including formation of transient furrows during divisions in the cortex as well as cellularization, a process that allows the syncytial blastoderm to be separated into individual cells (78). Furthermore, aberrant F-actin distribution can be observed in blastoderm embryos where *dyps28* has been deleted (78). These results suggest that mutation or loss of *dyps28* results in the disruption of the actin cytoskeleton, thereby leading to the phenotypes observed. Aberrant actin

**FIGURE 1. Trafficking of E-cadherin, Connexin 43, and α5β1 integrin**

Left: in epithelial cells, E-cadherin is internalized and recycles back to the basolateral plasma membrane constitutively. Upon EGF or TPA treatment, gap junctions are ubiquitinated and internalized into connexosomes, and Connexin 43 is then trafficked to early endosomes on its way to degradation in lysosomes. This trafficking is also valid for RTKs upon growth factor stimulation (not shown). A connexon is a hexamer of connexins. Right: during epithelial–mesenchymal transition (EMT), E-cadherin is ubiquitinated and trafficked to the lysosome in an ESCRT-dependent manner, resulting in the loss of cell-cell junction and apicobasal polarity. Integrin recycling contributes to cell migration and occurs constitutively. In contrast, integrin degradation is a triggered process that occurs in fibroblasts upon binding to the extracellular matrix (ECM). The sorting step requiring the ESCRT machinery is indicated. One ubiquitin molecule is indicated, but E-cadherin, Connexin 43, and α5β1 integrin are most probably mono- and poly-ubiquitinated. AJ, adherens junction; GJ, gap junction; EE, early endosome; PNRE, perinuclear recycling endosome; MVE, multivesicular endosome; LY, lysosome.
distribution is also observed upon disruption of erupted/Tsg101, dvps25, dvps22, dvps2 (91) and the ESCRT-III regulator dvps4 (74). Interestingly, these defects do not seem to be linked to the role of ESCRTs in the biogenesis of multivesicular endosomes, since MVEs are still observed in ESCRT mutant cells (78). Since the regulation of the cytoskeleton is such a critical determinant of cell migration, it will be interesting to determine whether other ESCRT components involved in the regulation of the actin cytoskeleton (Vps22, Vps2, and the ESCRT-III regulator Vps4) can also regulate cell migration.

**ESCRTs and cell migration in mammalian cells**

Recent evidence shows that deletion of the ESCRT-I component Tsg101 leads to reduced cell migration in mouse embryonic fibroblasts (MEFs), thereby suggesting that TSG101 acts a positive regulator of fibroblast migration (87). Localization of the active form of the protein tyrosine kinase Src at focal adhesions (FAs) requires TSG101, since pY416 Src accumulates at late endosomes (LEs) in the absence of TSG101 and is unable to traffic to FAs. This leads to the inability of Src to activate focal adhesion kinase (FAK) and STAT3 in the absence of TSG101 (87). Interestingly, activation of c-Src upon adhesion to fibronectin is not affected by Tsg101 deletion. Since localization of active Src to FAs is required for FA turnover (24) and since Src activity is required for cell motility (45), it has been proposed that TSG101 regulates cell migration by regulating active Src localization. Furthermore, it seems that the LE/lysosomal compartment plays a role in determining Src localization. Indeed, disruption of late endosomal function by expressing dominant-negative (dn) VPS4 results in the same phenotype as the one observed upon deletion of Tsg101, namely a decrease of active Src recruitment to FAs. Overexpression of dnRAB7, which disrupts membrane fusion between LEs and lysosomes, displays similar phenotypes. Surprisingly, although downstream signaling is affected, shown by a decrease in FAK Y397 and Y925, ERK phosphorylation is not affected by Tsg101 deletion. The biological significance of this inhibition...
of downstream signaling therefore remains to be investigated.

Since active Src is ubiquitinated by the ubiquitin ligase Cbl (101), that active Src accumulates at LEs/lysosomes raises the question of whether Src is degraded via an ESCRT-dependent lysosomal pathway. Although proteasomal degradation of Src has been reported (30, 34), conclusions drawn from studies using proteasomal inhibitors can be ambiguous since prolonged exposure to these can lead to depletion of the free ubiquitin pool, leading to the inhibition of all ubiquitin-dependent processes (14). Therefore, it remains a possibility that active Src is downregulated by the ESCRT machinery. Furthermore, since active Src cannot localize to FAs in the absence of TSG101, it is possible that TSG101 regulates the transport step from LE/lysosome to FAs.

These results are interesting in the light of recent results showing that integrins are degraded in an ESCRT-dependent manner (56). The signaling pathways described (87) (FAK, Src) are in fact all initiated by integrins upon engagement by the ECM at FAs. It is possible that TSG101-deleted cells show decreased activation of these pathways due to defective integrin trafficking itself, which could thereby affect integrin signaling. Alternatively, it has been suggested that integrin degradation might be required to attenuate integrin signaling (39). Interestingly, an initial increase in active Src has been observed upon Tsg101 deletion, followed by a decrease (87). This could suggest that integrin signaling cannot be silenced in the early stages of Tsg101 deletion; however, on accumulation of integrin at endosomal structures (56), integrins are unable to initiate activation of Src.

Integrin degradation was recently shown to be required for cell migration of human fibroblasts (54) (FIGURE 1, RIGHT). Ubiquitination of α5β1 integrin is required for proper sorting and degradation in lysosomes, together with its ligand fibronectin. The proposed mechanism is that fibronectin-integrin complexes need to be degraded, since, if recycled, these may lead to the formation of nonfunctional adhesion sites. However, it is possible that integrin is trafficked to the lysosome for the sole purpose of fibronectin degradation, since this is also required for cell migration (72, 79). Integrin accumulates upon HRS and TSG101 depletion in human fibroblasts (56), and this is also the case on expression of dn-dVps4 in Drosophila (74). Since these results were obtained in fibroblasts in a wound-healing context, it will be interesting to determine whether integrins are also ubiquitinated and degraded in confluent cultures. The collective data, therefore, suggest that, through their regulation of integrin trafficking, ESCRTs regulate cell migration.

Connexin 43 (Cx43) is the most abundant protein in gap junctions (GJ), and has a role in regulating the transport of ions, metabolites, and cell signaling molecules between cells. The turnover of gap junctions is quite rapid (22) and is thought to control intercellular communication. The outcome of internalization of connexins is proteasomal or lysosomal degradation (43), and HRS and TSG101 are involved in the sorting of Cx43 to the lysosome (48) (FIGURE 1). Connexin degradation is triggered by EGF or the tumor-promoting phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA), which is a potent activator of protein kinase C (PKC) (49, 50). Through their role in adhesion, gap-junction proteins are unsurprisingly able to influence cell migration (61). Several studies have found that Cx43 deletion impairs neural crest migration in the cortex and the heart (21, 26, 37). Directed cell migration is also impaired upon deletion of Cx43 in cardiac neural crest cells (97) and in epicardially derived cells (73). This suggests a role of connexins in cell polarity. Since levels of connexins seem critical in determining cell migration, it is an attractive idea that ESCRTs might regulate cell migration by controlling connexin levels, and through degradation they allow the cell to migrate away from its neighbors.

HRS was found to interact with MERLIN in schwannoma (nerve sheath tumor) cells (29, 77). Merlin is a tumor suppressor gene that acts as a contact inhibition regulator and is found at cell-cell junctions. Overexpression of HRS and MERLIN show similar effects and inhibited growth, motility, and results in abnormal cell spreading (29). However, overexpression of HRS alone is not enough to lead to conclusions about its role, since it is known to have dominant effects (70). It is therefore quite possible that HRS is in fact a positive regulator of cell migration, and not the opposite. The mechanism behind this regulatory role of HRS in motility in schwannoma cells has not been investigated, but it is interesting that MERLIN and HRS are both involved in downregulating EGFR signaling (13), albeit through different mechanisms. Furthermore, that integrin-mediated adhesion triggers the phosphorylation of MERLIN (64), thereby resulting in its open conformation and inactivating it, and that HRS interacts with MERLIN in this conformation (29) fit well with the recently described data presenting HRS as a regulator of integrin trafficking.

**ESCRTs in the Regulation of Epithelial Polarity**

**Regulation of E-Cadherin**

Polarized epithelial cells form an efficient barrier between tissues, and this is made possible by AJs and tight junctions (TJs), which have
an important role in regulating the transport of solutes across the epithelial barrier, as well as establishing polarity in epithelial cells. Epithelial to mesenchymal transition (EMT) is characterized by the downregulation of AJ proteins, resulting in loss of cell-cell contacts and apical-basal polarity, acquisition of mesenchymal characteristics, and increased migration. EMT occurs during normal embryonic development (11, 62); however, when this occurs in the adult, it usually results in cancer progression (28, 84). The localization of E-cadherin is a key determinant in distinguishing epithelial from mesenchymal cells (66). E-cadherin is internalized and recycles back to the plasma membrane (FIGURE 1, LEFT). However, on induction of EMT, intracellular E-cadherin is ubiquitinated by the ubiquitin ligase Hakai (25) and trafficked to the lysosome for degradation (66) (FIGURE 1, RIGHT). This sorting requires the ESCRT-0 component HRS and is presumably mediated by the ESCRT machinery. This pathway accounts for the observed downregulation of E-cadherin during EMT (66) and comes as a new mechanism responsible for the downregulation of E-cadherin during EMT, since loss of E-cadherin on the cell surface has often been linked to suppression of E-cadherin transcription by a variety of transcription factors Snail, Slug, SIP, E12/47 (5, 6, 68, 100). Interestingly, downregulation of E-cadherin can also occur in a matrix metalloproteinase (MMP)-dependent manner. Upon EGFR activation in ovarian cancer cells, MMP-9 is upregulated, resulting in E-cadherin degradation (12). Therefore, although E-cadherin trafficking does not exclusively occur in an ESCRT-dependent manner, the three pathways possibly all contribute to E-cadherin downregulation, thereby contributing to EMT. Although the degradation of E-cadherin directly affects polarity, it also indirectly affects cell migration, since loss of apicobasal polarity enables the cell to lose contact with its neighboring cells and thereby promotes its ability to migrate.

**ESCRT Mutants and Apicobasal Polarity in Drosophila Melanogaster**

Genetic screens in *Drosophila* have enabled the discovery of many endocytic regulators. Interestingly, mutations in genes involved in early endocytic trafficking (internalization at the cell surface) or later (sorting into MVEs) result in a common defect in cell polarity (90). Mutation in *dvps25*, an ESCRT-II component, results in epithelial disorganization. Apicobasal polarity is lost, as seen by the mislocalization of the peripheral membrane-localized atypical PKC (aPKC), and an expansion of the apical membrane can be observed (85, 89). Mutation in the ESCRT-I component *erupted* (*Drosophila* ortholog of Tsg101) results in the mislocalization of the polarity marker Crumbs (Crb) in mutant eye disc cells, a marker of the zonula adherens, from the apical surface to a subapical domain (60). Loss of Crb is associated with loss of TJs and increased migration and metastasis (42). However, how the mislocalization of this marker leads to loss of epithelial polarity remains unclear. Loss of apicobasal polarity is a hallmark in the development of cancer. Overgrowth phenotypes have been observed upon disruption of ESCRT proteins (described below), leading to the discussion of whether ESCRTs can act as tumor suppressors.

**ESCRTs as Tumor Suppressors**

**ESCRTs as Neoplastic and Non-autonomous Tumor Suppressors**

Proteins that behave as negative regulators of signaling pathways can act as tumor suppressors, and *Drosophila* genetic mosaic screens have been a powerful tool to identify these. Tissue overgrowth can happen because the mutant tissue grows faster than the surrounding wild-type cells and/or because the mutant tissue grows for a longer time than the wild-type tissue, which has stopped growing (33). Interestingly, most of the genes involved in endocytic trafficking that act as tumor suppressor genes are involved in cargo sorting at the MVE.

In *Drosophila*, these encode the ESCRT-I proteins eruupted/TSG101 (60) and Vps28 (91), the ESCRT-II components Vps25 (35, 85, 89, 91), Vps22 (36, 91), and Vps36 (36), and the ESCRT-III components Vps20, Vps32, and Vps2 (91). Interestingly, none of the ESCRT-0 components have been described as tumor suppressors, and these are not conserved across the range of eukaryotic taxa (52). Mutation in some of these genes leads to a loss in epithelial polarity (*dvps25, erupted/Tsg101*) and overproliferation (*dvps25*), characteristics of neoplastic tumors. However, most of the genes stimulated proliferation in a non-autonomous manner; proliferation was stimulated in the surrounding wild-type cells (*erupted/Tsg101*, Vps22, Vps25, Vps28, Vps20, Vps2, Vps32) (60, 91). Interestingly, most of these mutant cells are very sensitive to apoptosis (35, 85, 89). The pro-apoptotic signaling pathways Hippo, JNK (c-Jun NH2-terminal kinase), and Hid are activated in *dvps25* clones (35). Expression of the caspase inhibitor p35 in *vps25* mutant cells restores cell growth and results in massive overgrowth (85), suggesting that several mutations are required to promote overgrowth. Blocking apoptosis by expressing mutants of *ark*, an essential component of the apoptotic pathway, or *diap1* (*Drosophila* inhibitor of apoptosis protein 1), results in overgrowth of the *dvps25* mutant tissue (35). Since *Drosophila* ESCRT mutants in general...
require apoptosis inhibition to yield a cell-autonomous tumor phenotype, ESCRT proteins cannot be classified as tumor suppressors in the classical sense.

**Mechanisms by Which ESCRT Mutations Stimulate Non-autonomous Overgrowth and Apoptosis in Drosophila Melanogaster**

Characteristics of most of the ESCRT mutants in *Drosophila* are loss of apicobasal polarity in epithelial cells, apoptosis of mutant cells (35, 85, 89), and also stimulation of overproliferation of the surrounding wild-type cells. FIGURE 3 represents the pathways involved in mosaic *Drosophila* epithelia. Studies in *Drosophila* have shed light on the main regulators of epithelial polarity; the three main complexes being aPKC-containing Par polarity complex, Scribble polarity complex, and Crumbs polarity complex. Interestingly, all of these components act as tumor suppressors, underlying the link between loss of epithelial polarity and tumor development (83). Early stage tumors often show loss of epithelial polarity markers (17, 96), and disruption of polarity complexes results in increased epithelial proliferation (3, 105). However, the exact mechanisms by which this occurs remain elusive. A possible mechanism by which loss of epithelial polarity may lead to overproliferation is the loss of contact inhibition of proliferation (90). Contact inhibition of proliferation constrains epithelial growth, and the loss of this is a hallmark of cancer (32). Another possible mechanism could be through Cdc42/Par6/aPKC, which has recently been described as having a role during apoptosis-induced compensatory proliferation (94). This term coins the ability of epithelial cells to compensate for cell loss in response to stress or injury-induced apoptosis (23). The working model is that tissue damage may disrupt the proper localization of

**FIGURE 3.** Mechanisms that govern overgrowth and apoptosis on mutation of ESCRTs in *Drosophila melanogaster*

In ESCRT mutant cells, apicobasal polarity is lost, as can be seen by localization of apical aPKC throughout the epithelial cell, as well as intracellular accumulation of Crumbs. JNK, Hid, and Hippo signaling are activated in these cells and lead to apoptosis. Thickveins, receptor of Dpp, accumulates at endosomes in ESCRT mutant cells and secretes Dpp ectopically, which leads to overproliferation of neighboring wild-type cells. Notch accumulates at aberrant endosomes and leads to the secretion of Upd, which binds to a receptor on wild-type cells and leads to JAK-STAT signaling, also leading to overproliferation. Additionally, Notch leads to an increase in the inhibitor of apoptosis Diap1, thereby promoting cell survival. Question marks indicate hypothetical connections. aPKC, atypical protein kinase C; Crb, Crumbs; JNK, c-Jun NH2-terminal kinase; JAK-STAT, Janus kinase signal transducer and activator of transcription; IAP, inhibitor of apoptosis; Tkv, thickveins; Dpp, depaptogenic; Upd, unpaired.
Cdc42/Par6/aPKC, thereby promoting the proliferation of surrounding cells and promoting apoptosis in the damaged cells through JNK signaling. The disruption of this particular polarity complex is unique in its effect on compensatory proliferation since disruption of Scribble/Dlg polarity complex only resulted in JNK-dependent apoptosis. JNK is an important component in apoptosis-induced compensatory proliferation (76). JNK signaling is in fact activated in dvps25 mutant cells (35) as well as cells expressing dn-dVps4 (74). However, apoptosis-induced compensatory proliferation was not shown to contribute significantly to the overgrowth observed in dvps25 mutant mosaics (35). Results are different if the whole tissue is mutant. In this case the cells are not apoptotic but overproliferate in a neoplastic manner (36). They still activate JNK signaling, since a transcriptional downstream target of JNK, the matrix metalloprotease MMP1 (88), accumulates in ESCRT mutant tissues (74, 91). The fact that JNK signaling is activated but does not lead to apoptosis suggests that accumulation of MMP1 might be through a JNK-independent mechanism.

MMP1 is in fact upregulated in tissue mutant for known neoplastic tumor suppressors (59). MMPs are zinc-dependent endopeptidases that have an important role in tissue remodeling, development (65) and inflammation (67), and also in pathological processes including cancer (20). They regulate cell-cell and cell-ECM interactions, processes that if deregulated lead to tumorigenesis. MMPs can mediate ECM degradation by hydrolyzing the structural proteins present in the ECM proteins fibronectin, collagen, laminin, elastin, vitronectin, and fibrinogen (80). Interestingly, PAR-1, a protease-activated receptor, is activated by MMP-1, generating PAR-1-dependent migration in breast cancer cells (4). MMP1 upregulation in ESCRT mutant tissue could therefore represent another potential mechanism by which the mutant cells affect their environment as well as cell migration, and thereby contribute to tumor progression.

The Delta/Notch signaling pathway is evolutionarily conserved and is involved in a variety of processes during development and adult life. Endocytosis of the cleaved Notch receptor is required for its activation and function as a transcription factor. In dvps25 and erupted/Tsg101 mutant cells, Notch accumulates intracellularly at Rab7-positive structures, resulting in the secretion of the cytokine-like molecule Unpaired (Upd), which stimulates proliferation of the surrounding non-mutant cells via the Janus kinase (JAK)-STAT (signal transducer and activator of transcription) pathway (60, 89). Additionally, dvps25 mutant cells show accumulation of the Decapentaplegic (Dpp) (ortholog of mammalian transforming growth factor β) receptor Thickveins in the mutant cells of the leg imaginal discs, resulting in enhanced secretion of the Dpp ligand. This ectopic expression of Dpp results in overproliferation, leading to ventral leg outgrowths (85). Therefore, Notch and Dpp signaling are sufficient to induce overproliferation and thereby hyperplasia in the surrounding tissue of dvps25 mutant cells, although additional factors contributing to overgrowth are probably involved.

The Hippo signaling pathway, which plays an important role in organ size control, has been shown to have an important role as a tumor suppressor. Upon high cell density in culture, the Hippo pathway kinase cascade is activated and leads to repression of proliferation and promoting apoptosis, thereby regulating cell number (106). Dysregulation of Hippo signaling has been linked to tumorigenesis (16). Inappropriate Hippo signaling is observed in dvps25 mutant clones (35) by using the marker Expanded, an upstream component of the Hippo pathway, which correlates inversely with the level of Hippo activity (31). Thus low levels of Expanded are observed in dvps25 mutant clones, suggesting an increase in Hippo signaling (35). This pathway was shown to control apoptosis in dvps25 mutant clones, since vps25 hippo double mutants blocked Caspase-3 activation. However, how or why Hippo signaling is increased in these mutant cells remains unclear. A possibility is that a putative receptor controlling Hippo signaling may be deregulated and thus trigger Hippo signaling. Interestingly, Merlin acts upstream of core components of the Hippo pathway (31). Furthermore, mutations in components of the Hippo pathway suppress Notch signaling in follicle cells during Drosophila oogenesis (27, 58, 69, 104).

Are ESCRTs Tumor Suppressors in Mammals?

Overactivation of RTK signaling pathways and their link to cancer has been established and well studied (51); however, the idea that impaired downregulation of RTK signaling can also lead to cancer is quite recent. In this context, since ESCRTs are involved in the downregulation of RTKs, which are themselves involved in the regulation of cell migration, proliferation, differentiation, survival, metabolism, and cell-cycle control (51), it seems intuitive that ESCRTs might act as tumor suppressors. Consistent with this, ESCRT depletion results in a delay in EGFR degradation (1, 2, 71), and signaling is sustained (2, 57) or increased (41). Accumulation of ubiquitinated receptors also accumulate in Drosophila ESCRT mutant cells (36, 41, 60, 85, 89, 91).

Tsg101 was identified through a random mutagenesis screen as a novel tumor suppressor in mouse NIH 3T3 cells (54). However, this result has
not been verified upon generation of a conditional knockout of Tsg101 in mice (47, 93). Consistent with results obtained in Drosophila, TSG101 is essential for cell growth, cell survival, and normal function of embryonic and adult mouse tissues (93). Cells deleted of Tsg101 show defective cell cycle regulation and increased cell death (75, 93), as well as inhibition of fibroblast migration (87). Additionally, TSG101 overexpression, instead of inhibiting tumor growth, rather shows mild oncogenic properties (63), suggesting that TSG101 is a positive regulator of growth and migration. The reason why erupted/TSG101 acts as a tumor suppressor in Drosophila but not in mammals remains poorly understood. The ESCRT-I protein VPS37A/HCRP1 has been identified as a potential tumor suppressor in humans since it acts as a negative regulator of growth factor receptor signaling (1) and is downregulated in hepatocellular carcinoma (98). In contrast, TSG101 is in fact overexpressed in a variety of cancers (46, 55, 63, 102, 103), and its upregulation is associated with poor prognosis (103). CHMP1A, a member of the ESCRT-III-associated complex, has a lower expression in human embryonic kidney and ductal pancreatic tumor cells (53), and seems to act as a tumor suppressor, especially in the pancreas. VPS24 was shown to induce neuroendocrine differentiation in prostate cancer cells, which correlates with advanced prostate cancer (95). Depletion of HRS results in inhibition of cell proliferation, anchorage-independent growth, and metastatic potential, whereas its overexpression increases these phenotypes (86). This suggests that HRS is a positive regulator of growth and proliferation and does therefore not act as a tumor suppressor. The observed decrease in motility, cell growth, and abnormal cell spreading on overexpression of HRS (29) should be interpreted with caution since HRS overexpression results in a dominant-negative phenotype (70). The finding that HRS is required for the tumor suppressor activity of the contact inhibition regulator MERLIN (81) could nevertheless suggest a potential tumor suppressor role, possibly dependent on the context.

Conclusions and Perspectives

Most studies involving the ESCRT machinery in the regulation of cell migration and cell polarity suggest that ESCRTs are positive regulators of these processes, since depletion or mutation of ESCRT components results in the inhibition of cell migration or the inhibition of apicobasal polarity. However, the fact that ESCRT mutant cells stimulate overproliferation in surrounding tissue and that ESCRT mutant cells develop into tumors if apoptosis is inhibited would rather suggest a role of ESCRT proteins as tumor suppressors. How can these data be reconciled?

First, it needs to be mentioned that ESCRTs, even in Drosophila, do not act as conventional tumor suppressors. ESCRT mutant cells are in fact very sensitive to apoptosis, and only once apoptosis is inhibited do mutant cells overproliferate (35, 85). Therefore, it is possible that Tsg101-null cells in mice might die of apoptosis, thereby hindering their tumorigenic potential. Inhibiting apoptosis in Tsg101-null cells might therefore be worth investigating. Furthermore, a situation where whole tissues are deleted for Tsg101 in mice might not be the correct environment for apoptosis-induced compensatory proliferation to occur, since no wild-type cells are surrounds the Tsg101-deleted cells. The generation of genetic mosaics in D. melanogaster is a good model mimicking cancer development, since carcinogenesis is thought to arise from normal cells that acquire different hallmarks of cancer and are surrounded by healthy cells. Loss of apicobasal polarity is a hallmark of cancer, but invasion of tumor cells is also required. Since ESCRTs seem to be positive regulators of cell migration, the fact that some ESCRTs are overexpressed in cancers could thereby promote the metastatic potential of these cells. Surprisingly, on transplantation of mutant vps25 discs where apoptosis had been blocked, 2 of 15 discs showed metastatic potential (85), suggesting that even loss of ESCRT function may lead to invasive properties under certain circumstances. The increase in MMP expression in some ESCRT mutant cells in Drosophila is consistent with increased migration and tumorigenicity, since MMPs allow cells to degrade their ECM, thereby contributing to EMT. However, whether this accumulation occurs in mammalian cells remains to be investigated.

In conclusion, the ESCRTs are involved in the regulation of several processes that are dysregulated in cancer-cell proliferation, apoptosis, migration, and polarity. Future studies should determine whether ESCRTs act as genuine tumor suppressors in mammals, since at this stage this still remains unclear.

V. H. Lobert is a PhD student funded by the Norwegian Cancer Society. H. Stenmark is the recipient of an Advanced Grant from the European Research Council. This work was also supported by the Research Council of Norway.

No conflicts of interest, financial or otherwise, are declared by the author(s).

References


12. Fincham VJ, Frame MC. The catalytic activity of Src is dispensable for translocation to focal adhesion substrates in a variety of these structures during cell motility. EMBO J 17: 81–92, 1998.


24. Feng C, Frame MC. The catalytic activity of Src is dispensable for translocation to focal adhesion substrates in a variety of these structures during cell motility. EMBO J 17: 81–92, 1998.


