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ESCRT proteins

Double-edged regulators of cellular signaling

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ESCRT pathway proteins play a key role in sorting ubiquitinated membrane receptors towards lysosomes providing an important mechanism for attenuating cell surface receptor signaling. However, recent studies point to a positive role of ESCRT proteins in signal transduction in multiple species studied under physiological and pathological conditions. ESCRT components such as Tsg101 and Hrs are overexpressed in human cancers and Tsg101 depletion is detrimental for cell proliferation, survival and transformed phenotype of tumor cells. However, the mechanisms underlying the positive contributions of ESCRT pathway to surface receptor signaling have remained unclear. In a recent study, we showed that Tsg101 and Vps4 are essential for translocation of active Src from endosomes to focal adhesion and invadopodia, thereby revealing a role of ESCRT pathway in promoting Src-mediated migration and invasion. We discuss the implications of these and other recent studies which together suggest a role for the ESCRT pathway in recycling of endocytic cargo proteins, aside from its role in lysosomal targeting, potentially explaining the positive roles of ESCRT proteins in signal transduction.

Key words: endocytotic trafficking, recycling, ESCRT proteins, Src, Signal transduction, cancer, lysosomal degradation, receptor tyrosine kinase

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Endocytosis of cell surface receptors is a fundamental cell biological process. Upon their entry into early endosomal compartments, internalized receptors undergo a sorting process that determines their alternate itineraries. For example, activated receptor tyrosine kinases (RTKs) internalized upon stimulation by growth factors can be alternatively targeted to the lysosome where they are degraded or recycled back to the cell surface through the recycling endocytic pathway for further rounds of growth factor binding and signaling. Thus, the endocytic route of internalized receptors can control the duration and strength of activation signals. Therefore, mechanisms that control the alternative lysosomal versus recycling fates of internalized receptors have received substantial attention in recent years. Ubiquitination has emerged as a key mechanism to target cell surface receptors for lysosomal degradation. In a mechanism that is conserved from yeast to mammals, the sorting of ubiquitinated receptors is orchestrated by a series of protein complexes collectively known as Endosomal Sorting Complexes Required for Transport or ESCRT proteins (reviewed in ref. 1). The components and the arrangement of the currently described four ESCRT complexes, ESCRT-0, -I, -II and -III, as well as their associated proteins are highly conserved. The heterotetrameric ESCRT-I complex includes the

proteins Vacuolar sorting protein (Vps) 23 (known as Tumor suppressor gene 101 or Tsg101 in mammals), Vps28, Vps37 and Multivesicular body protein (MVB) 12. Tsg101 plays a key role in ESCRT-I function as it can bind to ubiquitinated receptors and mediates interactions with other ESCRT complexes via its ubiquitin-conjugating enzyme E2 variant (UEV) domain.

Since the lysosomal targeting of activated cell surface receptors is now a well accepted mechanism of signal attenuation,+ much attention has naturally focused on the negative regulatory roles of the ESCRT pathway especially in the context of RTK signaling in mammalian cell systems. Yet, a large number of studies, especially those done in organismic contexts, have revealed essential positive roles of ECSRT proteins. Analyses of Tsg101 provide the clearest example of these diametrically opposite functional roles.

While initial identification of Tsg101 as a transformation suppressor apparently mutated in breast cancers was consistent with the negative regulatory role of ESCRT complexes in receptor signaling, later studies did not confirm these findings.[2] Remarkably, several independent studies using genetic ablation approaches showed that Tsg101 was required for mouse embryonic development and viability of adult tissues and cells.[3-5] A similar essential role of Tsg101 homologs and other ESCRT components in embryonic development is shown by studies in model organisms such as *C. elegans* and Drosophila. More recent work has shown that Tsg101 is overexpressed rather than lost in a subset of breast,[5] thyroid,[6] ovarian[7] and colon[8] cancers. Furthermore, depletion of Tsg101 was found to impair tumorigenicity of several cancer cell lines.[9,10]

ESCRT proteins are increasing recognized as versatile proteins functioning in diverse membrane abscission processes. Extensive studies for example have shown the requirement of ESCRT functions for retroviral budding as well as orchestrating several cellular processes such as autophagy and cytokinesis (reviewed by ref. 1). These findings have painted a more complex picture of the roles played by Tsg101 as well as other ESCRT proteins.

While the seemingly positive roles in signal transduction have been defined most clearly for Tsg101, other ESCRT proteins are likely to behave in a similar manner. For example, the ESCRT-0 component Hepatocyte growth factor receptor substrate (Hrs) was found to be essential for cell proliferation, anchorage-independent growth, tumorigenesis and metastatic potential of HeLa cells and mouse fibroblasts in vitro and in vivo.[11] In addition, overexpression of Hrs was found to be associated with advanced malignancy and poor prognosis in human cancers.[11] Interestingly, both positive and negative[12,13] roles of Hrs in the signaling have been reported in Drosophila and mammalian cells, suggesting that the exact role of Hrs could be cell type- and context-dependent.

The ability of Tsg101, and perhaps other ESCRT proteins, to serve positive as well as negative roles in cellular homeostasis necessitate a better understanding of their complex biochemical and cellular functions. In this regard, Tsg101 and other ESCRT proteins share some features of the so-called "two-faced" proteins that are being increasingly recognized in many fields and can regulate biological functions in seemingly opposite ways under different physiological scenarios. As a notable example of this expanding group of proteins, TGFβ can exhibit an oncogenic role in one context but an exactly opposite tumor suppressor role in a different context.

The question of how ESCRT proteins such as Tsg101 and Hrs serve positive roles in cellular signaling has remained largely unanswered, until recently. In one potential scenario, the seemingly opposite functions of ESCRT proteins could relate to their distinct complexes or subcellular pools, although there is no clear evidence for this idea at this time. Alternatively, distinct ESCRT-mediated functions may reflect their diverse endosomal sorting roles in the context of a broad range of molecular targets that have vastly different roles in cellular homeostasis. For example, ESCRT pathway has been shown to promote lysosomal proteolysis of anti-proliferative proteins such as E-cadherin[11] and GSK-3,[14] which can translate into positive cellular roles of the ESCRT pathway.

Emerging evidence that ESCRT proteins promote sorting of endosomal cargo proteins to destinations other than lysosomes could also underlie their positive roles in cellular signaling. Recently, we demonstrated that Tsg101 and Vps4, two well-studied ESCRT proteins, are required for the translocation of active c-Src tyrosine kinase (Src) from late endosomal/lysosomal compartment to focal adhesions, where Src functions to promote cell motility and to activate downstream effectors such as FAK and STAT3.[15] Additional findings (our unpublished observations) indicate that Tsg101 is also critically required for the formation of Src-dependent structure called invadopodia, membrane protrusions that are known to mediate matrix invasion by highly invasive tumor cells (reviewed in ref. 16). Thus, Tsg101 (and ESCRT complexes) appears to be essential for Src activity and function, by ensuring the dynamic trafficking of Src to its sites of function at focal adhesions and invadopodia.

These findings have several important implications. First, this study revealed a novel connection between ESCRT pathway and the Src tyrosine kinase, the latter a participant in a broad range of cellular responses such as survival, proliferation and migration in normal cells as well as during oncogenesis. Src activity is frequently elevated in a number of human cancers, cooperating with other oncoproteins to confer more invasive characteristics to cancer cells. These findings may explain in part why Tsg101 plays a positive role in cellular signal transduction and why its overexpression rather than reduced expression is common in highly malignant cancers. Obviously, additional studies in other experimental settings will be necessary to validate the functional interaction we have shown between Src and ESCRT pathway, and to test the potential role of this new interaction in oncogenesis. Should the latter be validated, ESCRT pathway could emerge as a potential target to develop new anticancer therapeutic agents.

Secondly, the demonstration that Tsg101 and Vps4 are required for the trafficking of active Src out of late endosomes instead of targeting it for degradation expands the functional roles of ESCRT

proteins. Other recent data further point to a general involvement of the ESCRT pathway in the recycling of endosomal cargo. Vps4, which is required for dynamic cycling of ESCRT complexes between the endosomal membrane and the cytoplasm,[17] has been shown to be essential for trafficking of transferrin receptor, mucin-like receptor endolyn[18] and low density lipoprotein receptor (LDLR) between endosomes and other membrane compartments.[19] Even in the case of classical RTKs whose lysosomal sorting is facilitated by ESCRT proteins, further work and reinterpretation of previous findings may be necessary. For instance, previous studies showing an important role of the ESCRT pathway in RTK dynamics utilized the persistence of labeled ligands such as EGF at endosomes as evidence for a selective defect in lysosomal degradation in cells with dysfunctional ESCRT pathway.[20,21] However, if recycling of EGFR was still operational when the ESCRT machinery was rendered defective, one would expect a larger fraction of EGFR at the plasma membrane; however, this has not been documented. Thus, the phenotypes observed in ESCRT-defective cells may be better explained by a combined block of lysosomal degradation and recycling. This idea is supported by our unpublished data as well as a recent study which showed that recycling of EGFR triggered by stimulation with amphiregulin is reduced upon Tsg101 depletion.[22]

Accumulating evidence indicates that other ESCRT complexes are also implicated in recycling or endocytic cargo proteins. Alix, an ESCRT protein interact with Tsg101 and ESCRT III complex, is required for endocytic recycling of specific basolateral cargo in the *C. elegans*.[23] Hrs has been shown to mediate endocytic recycling of G-protein coupled receptors such as β2 adrenergic receptor,[24] protease-activated receptor 2 (PAR2) and calcitonin receptor-like receptor (CLR).[25] Importantly, this role seems not to be limited to mammalian cells or G-protein coupled receptors. In Drosophila, mutations in Hrs and another ESCRT-0 component STAM have been shown to sequester fibroblast growth factor receptor (FGFR) in aberrantly enlarged early endosomes, in contrast to its normal localization at the plasma membrane (PM).[13] Overexpression of ESCRT III complex component CHMP6 has been shown to cause the retention of transferrin receptor at endosomes.[26] Recently, ESCRT-I function was shown to be essential for transport of cargo proteins from early endosomes to the limiting membrane of melanosomes in melanocytes.[27] These data further boost the notion that ESCRT machinery may be equally important for sorting of endosomal cargoes to inner vesicles of the MVB and other endocytic destinations. This notion is consistent with the emerging idea that different sorting domains coexist in a multifunctional endosomal sorting compartment with ESCRT proteins facilitating sorting of cargo towards multiple destinations.[18,21]

Obviously, further investigations are needed to confirm and extend our knowledge regarding the dual roles of ESCRT proteins in endosomal trafficking. How is endosomal recycling being facilitated by ESCRT complexes? Given our findings using Tsg101-null cells,[15] a reconstitution approach should allow structure-function analysis of Tsg101 to address which domains are required for endosomal recycling, thereby pointing to potential mechanisms. What cargos might be sorted for recycling rather than lysosomal delivery, and what might determine the ratio of degradation versus recycling for a given protein that could be sorted to either destination? It is possible that accessory factors and/or post-translational modifications of cargo proteins in part dictate the destination of the sorted cargo. In this regard, while ubiquitin-modified receptors are targeted by ESCRT complexes for delivery into inner vesicles of the MVB for delivery to the lysosome, it is possible that ESCRT proteins may help sort unmodified receptors back to the cell surface. Consistent with such a scenario, ESCRT-0 complex STAM has been shown to interact with deubiquitinases and this interaction has been demonstrated to help avert the degradation fate of EGFR.[28,29] In addition, the expression of SCAMP3, a protein that interacts with Tsg101 and Alix, was shown to favor EGFR recycling at the expense of lysosomal degradation.[30] Studies using ESCRT-null or -depleted cells should help test these ideas further. Perhaps most importantly, it is pertinent to ask to what extent the ESCRT-facilitated transport of proteins from endosomes to non-lysosomal destinations contributes to the positive roles of ESCRTs in signal transduction? Answers to this question could help reconcile some of the more puzzling observations in ESCRT and endocytosis fields especially as it relates to cellular signaling receptors.

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