Tom1L1在细胞信号转导及受体内吞中的研究进展

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摘要　　Tom1L1(Tom1 like 1)参与并调节细胞信号转导及受体运输通路。在不同细胞中Tom1L1对信号转导具有不同的调节作用。Tom1L1-CHC(clathrin heavy chain)复合物减少Src蛋白在小窝(caveolae)处富集, 从而阻碍Src蛋白与血小板衍生因子(platelet derived growth factor, PDGF)受体的结合, 抑制PDGF受体介导的有丝分裂和转化信号传导。活化的表皮生长因子受体(epidermal growth factor receptor, EGFR)通过Src家族蛋白激酶(src family kinase, SFK)磷酸化Tom1L1, 磷酸化的Tom1L1通过Grb2和Shc的桥梁作用与EGFR结合, 介导EGFR的内吞进程。Tom1L1和Hrs(hepatocyte growth factor regulated tyrosine kinase substrate)、TSG101(tumor susceptibility gene 101)的相互作用表明, 它也可能参与了泛素化蛋白分选入多泡体的过程。该文就其在细胞信号转导通路及受体内吞/分选过程的作用作一综述。

关键词　　Tom1L1; SFK; EGFR内吞; 受体分选

Progresses and Perspectives on the Role of Tom1L1 in Signal Transduction and Endocytosis Pathways

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Abstract　　Tom1L1 (Tom1 like 1) participates in cell signal transduction pathways and receptors' endocytosis. It has different effects on cell signal transduction depending on cell types. Moreover, Tom1L1-CHC (clathrin heavy chain) complex represses mitogenic and transforming signals by reducing the level of Src in caveolae. EGFR (epidermal growth factor receptor) stimulates transient tyrosine phosphorylation of Tom1L1 by the SFK (src family kinases), and pTom1L1 (phosphorylated Tom1L1) promotes endocytosis of EGFR bridged by Grb2 and Shc. Tom1L1 also interacts with Hrs (hepatocyte growth factor regulated tyrosine kinase substrate) and TSG101 (tumor susceptibility gene 101), which suggests that it might play a role in sorting of ubiquitinated proteins into MVB (multivesicular body). This review will focus on the specific role of Tom1L1 in signal transduction pathways and the endocytosis/sorting of receptors.

Key words　　Tom1L1; SFK; EGFR endocytosis; sorting of receptor

Tom1蛋白家族是一组真核细胞蛋白, 包括Tom1、Tom1L1和Tom1L2, 由氨基末端的VHS结构域、中间的GAT结构域及羧基末端组成[1]。在Tom1蛋白家族中, Tom1和Tom1L2有59%的同源性, 而Tom1L1与Tom1、Tom1L2分别只有30%和31%的同源性, 其羧基末端与Tom1、Tom1L2的差异最大。其
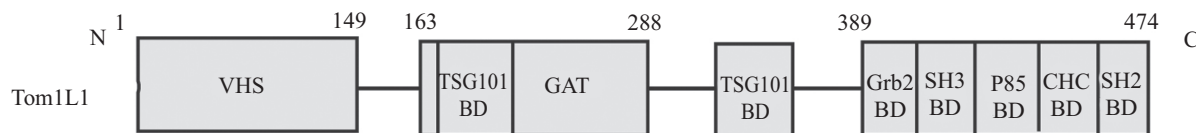
BD: 结合区域; CHC: clathrin重链。Tom1L1中有与TSG101结合的序列[179]PTAP和[310]PSAP、与clathrin重链结合的序列[447]FDPL, 羧基末端有与SFK的SH3结合的序列[420]LPPLP、与SFK的SH2结合的序列[457]YEEI、与Grb2的SH2结构域结合的序列[392]YDNF、与PI3K p85的SH2结构域结合的序列[441]YEVM。

BD: binding domain; CHC: clathrin heavy chain. Tom1L1 interacts with TSG 101 via its [179]PTAP sequence and [310]PSAP sequence. Also its [447]FDPL sequence interacts with the clathrin heavy chain. Its motifs [420]LPPLP and [457]YEEI interact with the SH3 and SH2 domains of SFK, respectively. Moreover, its motif [392]YDNF interacts with the SH2 domain of Grb2; Motif [441]YEVM interacts with the SH2 domains of PI3K p85.

图1 Tom1L1结构示意图(根据参考文献[1]修改)
Fig.1 Structure of Tom1L1 (modified from reference [1])

中, Tom1L1蛋白中的GAT结构域可通过泛素结合基序(ubiquitin-interacting motif, UIM)与泛素结合[2]; 羧基末端中还有与SFK的SH3、SH2结构域以及Grb2和PI3K p85亚基的SH2结构域相结合的序列[3](图1)。

1 在信号转导中的调节作用

SFK属于非受体酪氨酸激酶家族, 在调控细胞形态、增殖和生长方面起着关键作用。SFK通过SH2和SH3结构域与自身羧基端磷酸化的酪氨酸(pY527)及链接区的多聚脯氨酸结构域相结合, 形成无活性的"封闭"结构[4]。表皮生长因子(epidermal growth factor, EGF)通过SFK磷酸化Tom1L1, 活化的Tom1L1与SFK中的SH2和SH3结构域结合, 解除SFK的封闭结构, 进一步激活内源性的SFK(图2)。因此, Tom1L1不仅是SFK的磷酸化底物, 还可促进SFK的自身磷酸化, 调节SFK的信号转导。

Tom1L1被SFK磷酸化激活后, 通过SH2结构域与Grb2和p85结合。Grb2和p85作为重要的信号转导调节分子, 在RAS-MAP蛋白激酶和Akt信号转导途径中具有重要的调节作用[5]。

1.1 细胞信号转导中的调节

SFK是表皮细胞生长和分化的重要调节因子。角化细胞中表达三种SFK, 包括Src、Fyn和Yes。研究表明, 表皮生长因子促进Tom1L1的磷酸化, 使Tom1L1高表达, 进而激活内源性的Fyn, 而高表达的
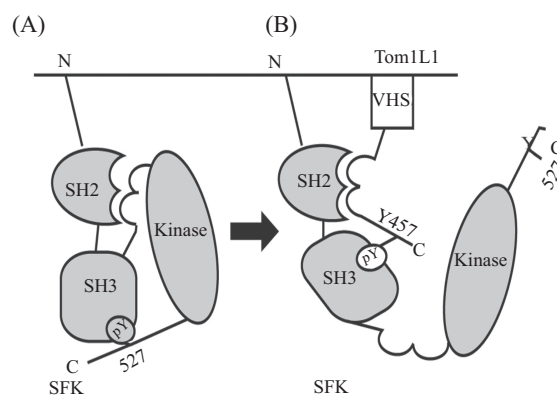
A: SFK的自我封闭结构; B: Tom1L1介导的SFK激活及其自身的磷酸化。

A: closed configuration of the SFK; B: phosphorylated Tom1L1 stabilizes the active conformation of SFK.

**图2 Tom1L1对于SFK的调节作用(根据参考文献[5]修改)**
Fig.2 The Mechanism of SFK activation regulated by Tom1L1 (modified from reference [5])

Fyn促使角化细胞从增殖向分化的转化[6]。在皮肤肿瘤中表现为SFK的过度表达和Tom1L1的表达水平下降, 表明Tom1L1具有抑制细胞增殖的功能[7]。同时有实验研究表明, 磷酸化的Tom1L1可在表皮组织和K14-Fyn Y528F/K14-Srcasm双重转基因小鼠中降低Fyn的表达水平, 抑制Fyn诱发的增生过多现象, 使表皮生长恢复正常[8](表1)。因此, Tom1L1可通过调节SFK活性来影响角化细胞的增殖和分化。

另外, 在食管癌中, 通过比较食管鳞状细胞癌与正常细胞中Tom1L1的表达水平, 齐宇等[10]发现在

表1 不同表达水平的Tom1L1和SFK对角化细胞的影响(根据参考文献[9]修改)
Table 1 The effect of different levels of Tom1L1 and SFK on the state of keratinocyte (modified from reference [9])

Tom1L1表达水平 Tom1L1 levels	SFK表达水平 SFK levels	SFK活性 SFK activity	角化细胞 Keratinocyte
Relatively high	Physiological	Increased	Differentiation
Relatively high	Elevated	Downregulated	Differentiation
Relatively low	Physiological or elevated	Increased	Proliferation
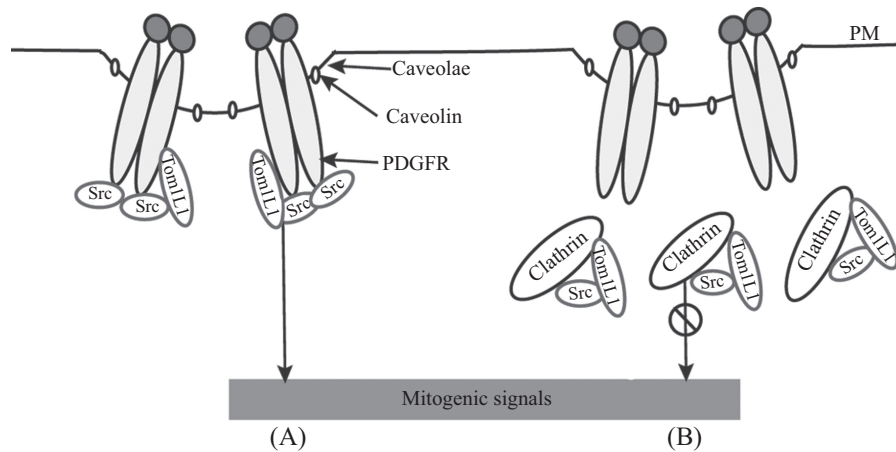
A: 当Tom1L1不与CHC结合时, Src在穴样内陷中聚集并与PDGF受体结合; B: 当Tom1L1与CHC形成复合物后导致小窝内Src减少, 从而影响Src与PDGF受体结合。PM: 细胞膜; PDGFR: 血小板衍生因子受体。

A: Src associates with the PDGF receptor in absence of Tom1L1-CHC complex in caveolae; B: the complex of Tom1L1-CHC re-localizes Src from the caveolae, thus preventing its association with PDGF receptors. PM: plasma membrane; PDGFR: platelet derived growth factor receptor.

图3 Tom1L1- CHC复合物介导Src在细胞膜的分区

Fig.3 The Tom1L1-CHC complex regulates membrane partitioning of Src

食管鳞状癌细胞中存在Tom1L1表达水平下降的现象。这种Tom1L1在食管鳞状细胞癌组织中低表达而在正常食管黏膜组织中相对高表达的现象, 提示Tom1L1可能在食管鳞状细胞癌的发生发展中起负调控作用。然而在小鼠的异种移植实验中, 却发现Tom1L1协同Src促进人结肠癌细胞的增长[11]。

1.2 PDGF信号转导的调节

小窝, 即细胞膜穴样内陷, 是一种特化的富含胆固醇的细胞质膜脂筏结构, 主要由蛋白质和脂类组成, 其中小窝蛋白(caveolin)是其主要结构和调节成分。小窝不仅参与物质的跨膜转运, 还调控细胞信号分子富集和信号转导, 因此参与细胞分化、增殖等多种病理生理过程。PDGF诱导DNA合成需要Src在小窝内激活并与PDGF受体结合[12]。而Tom1L1-CHC复合物减少Src在小窝中的富集, 进而阻止Src与PDGF受体在该结构区域中结合, 因此Tom1L1可通过抑制Src的聚集, 调节有丝分裂和转化信号(图3)。

2 作为衔接蛋白参与EGFR内吞作用

EGF与EGFR胞外结构域结合后激活EGFR, 使两个EGFR单体形成二聚体, 激活其自身的酪氨酸激酶活性, 诱导细胞下游激酶的级联反应, 从而实现EGFR在细胞内的信号转导[13]。EGF同时还可触发EGFR的内吞过程: EGF与胞膜上的EGFR结合后形成包被小泡, 进入细胞后与早期内体融合形成胞内体。在胞内体膜上, 泛素化的EGFR被分选进入多泡体, 通过多泡体被转运到溶酶体进行降解, 从而维持细胞的正常代谢和生理功能。这是EGFR信号转导途径中重要的负反馈作用, 提高了信号转导的精确性[14]。

Clathrin介导的细胞表面受体的内吞作用, 需要衔接蛋白来连接目的蛋白[15], 从而参与有被小泡的形成(图4)。EGF激活细胞后, 细胞膜上的EGFR通过SFK磷酸化Tom1L1。磷酸化的Tom1L1通过Grb2和Shc与活化的EGFR结合, 被吸引到细胞质膜上, 从而参与到EGFR的内吞作用中。Tom1L1调节EGFR内吞作用的能力依赖于它与活化的EGFR和Clathrin的衔接作用[16]。因此, EGFR内吞作用与EGFR的激活、Tom1L1的磷酸化、磷酸化的Tom1L1与EGFR的结合以及Tom1L1羧基末端与Clathrin重链羧基末端的结合密切相关。

3 Tom1L1与转运必需内体分选复合物(endosomal sorting complex required for transport, ESCRT)的相互作用

胞内体作为重要的蛋白分选站, 决定着蛋白是通过循环内体回到细胞质膜, 还是被传送到溶酶体被降解[17]。ESCRT在胞内体分选泛素化标记的目的蛋白过程中发挥主要作用[18]。ESCRT由ESCRT-
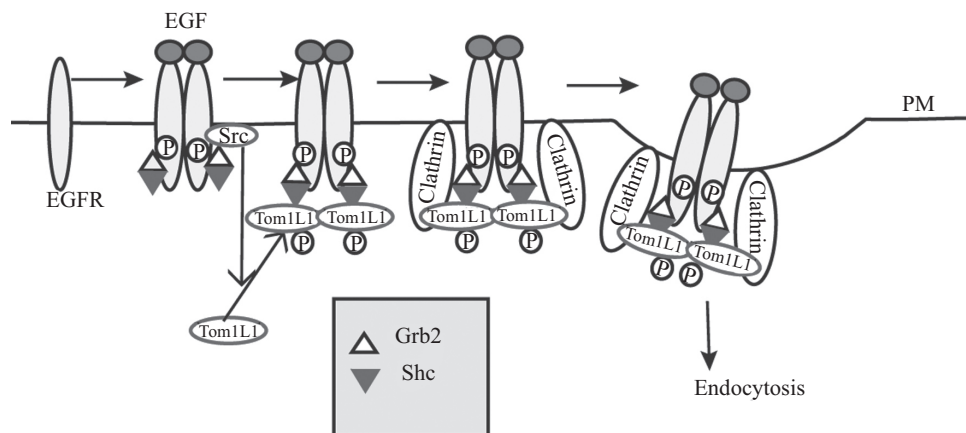
PM: 细胞膜; EGF: 表皮生长因子; EGFR: 表皮生长因子受体。EGFR通过SFK磷酸化Tom1L1, 磷酸化的Tom1L1通过Grb2和Shc与活化的EGFR结合, 促进EGFR的内吞作用。

PM: plasma membrane; EGF: epidermal growth factor; EGFR: epidermal growth factor receptor. Activated EGFR phosphorylates Tom1L1 via SFK, and pTom1L1 promotes endocytosis of EGFR bridged by Grb2 and Shc.

图4　Tom1L1介导的内吞作用(根据参考文献[11]修改)

Fig.4　The role of Tom1L1 in endocytosis (modified from reference [11])
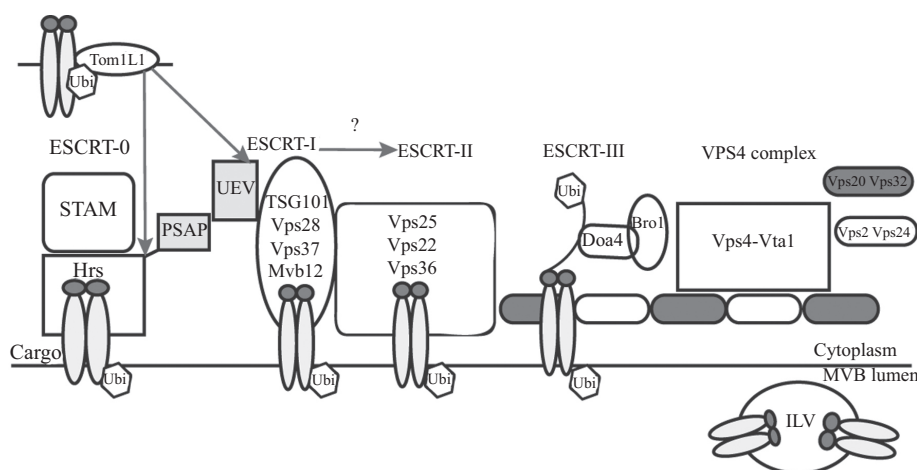
MVB: 多泡体; ILV: 管腔内小泡。Tom1L1通过与TSG101、Hrs的结合被招募到胞内体膜上。但在蛋白分选过程中, Tom1L1与ESCRT的作用顺序仍有待进一步的研究。

MVB: multivesicular body; ILV: intraluminal vesicals. Tom1L1 is recruited to the endosome membrane through interaction with TSG101 and Hrs. Meanwhile, its role in protein sorting remains elusive.

图5　Tom1L1在受体分选中的作用(根据参考文献[25]修改)

Fig.5　The role of Tom1L1 in receptor's sorting (modified from reference [25])

0、-I、-II、-III和一些辅助组分构成[19-21]。ESCRT-0、ESCRT-I和ESCRT-II复合体都含有能与泛素蛋白结合的亚基, 因此它们都能直接与泛素化的目的蛋白结合, 并且按顺序依次发挥作用。最终, 胞内体形成多胞体与溶酶体融合, 将目的蛋白降解[22]。ESCRT在细胞分裂[23]、胞吐[24]中也发挥重要作用。另外, ESCRT还参与病毒的出芽繁殖[25]。

在ESCRT中, ESCRT-0由Hrs和STAM组成, Hrs通过FYVE锌指结构与胞内体脂质磷脂酰肌醇-3磷酸

盐(phosphatidylinositol-3-phosphate, Ptdins-3-P)结合而被招募到胞内体膜上[26], 同时有研究表明, ESCRT-0可作为衔接蛋白介导蛋白的内吞作用[27]。ESCRT-I复合物由TSG101、Vps28、Vps37和Mvb12四个亚单位组成, Hrs中的PSAP序列通过与TSG101的UEV结构域结合, 而将ESCRT-I复合物募集到胞内体膜上[28]。

存在于细胞质中的Tom1L1分别通过PSAP和PTAP序列与TSG101中的UEV结构域结合[29]、VHS结构域与Hrs结合[29-30], 而被招募到胞内体膜上。另外,

Tom1L1还可通过GAT结构域与泛素化蛋白结合, 所以Tom1L1很有可能参与到ESCRT的分选机制中。但是Tom1L1是与Hrs和STAM一起发挥作用, 还是可以完全替代Hrs和STAM, 仍需要进一步的实验验证(图5)。

4 结语与展望

综上所述, Tom1L1在角化细胞Src信号转导及EGFR的内吞中都发挥重要的调节作用, 并与ESCRT的亚基及泛素修饰蛋白相互作用。但还有很多问题仍待进一步的实验证明, 如: 在细胞信号转导中具体如何调节Tom1L1的功能和表达水平? Tom1L1是否介导其他酪氨酸激酶受体的内吞作用? 以及Tom1L1是否与ESCRT共同介导胞内体的分选机制? 通过对Tom1L1的研究, 我们将深入了解胞内体蛋白分选和细胞的调控机理, 进而加深对肿瘤病理生理机制的认识, 为今后的临床应用打下良好的基础。

参考文献 (References)

1　Wang T, Liu NS, Seet LF, Hong W. The emerging role of VHS domain-containing Tom1, Tom1L1 and Tom1L2 in membrane trafficking. Traffic 2010; 11(9): 1119-28.

2　Akutsu M, Kawasaki M, Katoh Y, Shiba T, Yamaguchi Y, Kato R, *et al*. Structural basis for recognition of ubiquitinated cargo by Tom1-GAT domain. Febs Lett 2005; 579(24): 5385-91.

3　Franco M, Furstoss O, Simon V, Benistant C, Hong WJ, Roche S. The adaptor protein Tom1L1 is a negative regulator of Src mitogenic signaling induced by growth factors. Mol Cell Biol 2006; 26(5): 1932-47.

4　Xu W, Doshi A, Lei M, Eck MJ, Harrison SC. Crystal structures of c-Src reveal features of its autoinhibitory mechanism. Mol Cell 1999; 3(5): 629-38.

5　Seykora JT, Mei L, Dotto GP, Stein PL. 'Srcasm: A novel Src activating and signaling molecule. J Biol Chem 2002; 277(4): 2812-22.

6　Li W, Marshall C, Mei L, Dzubow L, Schmults C, Dans M, *et al*. Srcasm modulates EGF and Src-kinase signaling in keratinocytes. J Biol Chem 2005; 280(7): 6036-46.

7　Meulener MC, Ayli EE, Elenitsas R, Seykora JT. Decreased Srcasm expression in hyperproliferative cutaneous lesions. J Cutan Pathol 2009; 36(3): 291-5.

8　Zhao L, Li W, Marshall C, Griffin T, Hanson M, Hick R, *et al*. Srcasm inhibits Fyn-induced cutaneous carcinogenesis with modulation of Notch1 and p53. Cancer Res 2009; 69(24): 9439-47.

9　Li W, Marshall C, Mei L, Gelfand J, Seykora JT. Srcasm corrects Fyn-induced epidermal hyperplasia by kinase down-regulation. J Biol Chem 2007; 282(2): 1161-9.

10　Qi Y, Li X, Zhao L, Seykora JT. Decreased Srcasm expression in esophageal squamous cell carcinoma in a Chinese population. Anticancer Res 2010; 30(9): 3535-9.

11　Sirvent A, Vigy O, Orsetti B, Urbach S, Roche S. Analysis of SRC oncogenic signaling in colorectal cancer by stable isotope labeling with heavy amino acids in mouse xenografts. Mol Cell Proteomics 2012; 11(12): 1937-50.

12　Collin G, Franco M, Simon V, Benistant C, Roche S. The Tom1L1-clathrin heavy chain complex regulates membrane partitioning of the tyrosine kinase Src required for mitogenic and transforming activities. Mol Cell Biol 2007; 27(21): 7631-40.

13　Shankaran H, Resat H, Wiley HS. Cell surface receptors for signal transduction and ligand transport: A design principles study. PLoS Comput Biol 2007; 3(6): e101.

14　Roepstorff K, Grovdal L, Grandal M, Lerdrup M, van Deurs B. Endocytic downregulation of ErbB receptors: Mechanisms and relevance in cancer. Histochem Cell Biol 2008; 129(5): 563-78.

15　Sorkin A, Goh LK. Endocytosis and intracellular trafficking of ErbBs. Exp Cell Res 2008; 314(17): 3093-106.

16　Liu NS, Loo LS, Loh E, Seet LF, Hong W. Participation of Tom1L1 in EGF-stimulated endocytosis of EGF receptor. Embo J 2009; 28(22): 3485-99.

17　Jovic M, Sharma M, Rahajeng J, Caplan S. The early endosome: A busy sorting station for proteins at the crossroads. Histol Histopathol 2010; 25(1): 99-112.

18　Piper RC, Luzio JP. Ubiquitin-dependent sorting of integral membrane proteins for degradation in lysosomes. Curr Opin Cell Biol 2007; 19(4): 459-65.

19　Wollert T, Yang D, Ren X, Lee HH, Im YJ, Hurley JH. The ESCRT machinery at a glance. J Cell Sci 2009; 122(Pt 13): 2163-6.

20　Winter V, Hauser MT. Exploring the ESCRTing machinery in eukaryotes. Trends Plant Sci 2006; 11(3): 115-23.

21　Hurley JH, Emr SD. The ESCRT complexes: Structure and mechanism of a membrane-trafficking network. Annu Rev Biophys Biomol Struct 2006; 35: 277-98.

22　Raiborg C, Rusten TE, Stenmark H. Protein sorting into multivesicular endosomes. Curr Opin Cell Biol 2003; 15(4): 446-55.

23　Caballe A, Martin-Serrano J. ESCRT machinery and cytokinesis: the road to daughter cell separation. Traffic 2011; 12(10): 1318-26.

24　de Saint BG, Menasche G, Fischer A. Molecular mechanisms of biogenesis and exocytosis of cytotoxic granules. Nat Rev Immunol 2010; 10(8): 568-79.

25　Mageswaran SK, Dixon MG, Curtiss M, Keener JP, Babst M. Binding to any ESCRT can mediate ubiquitin-independent cargo sorting. Traffic 2014; 15(2): 212-29.

26　Bache KG, Raiborg C, Mehlum A, Stenmark H. STAM and Hrs are subunits of a multivalent ubiquitin-binding complex on early endosomes. J Biol Chem 2003; 278(14): 12513-21.

27　Mayers JR, Wang L, Pramanik J, Johnson A, Sarkeshik A, Wang Y, *et al*. Regulation of ubiquitin-dependent cargo sorting by multiple endocytic adaptors at the plasma membrane. Proc Natl Acad Sci USA 2013; 110(29): 11857-62.

28　Lu Q, Hope LW, Brasch M, Reinhard C, Cohen SN. TSG101 interaction with HRS mediates endosomal trafficking and receptor down-regulation. Proc Natl Acad Sci USA 2003; 100(13): 7626-31.

29　Yanagida-Ishizaki Y, Takei T, Ishizaki R, Imakagura H, Takahashi S, Shin HW, *et al*. Recruitment of Tom1L1/Srcasm to endosomes and the midbody by Tsg101. Cell Struct Funct 2008; 33(1): 91-100.

30　Puertollano R. Interactions of TOM1L1 with the multivesicular body sorting machinery. J Biol Chem 2005; 280(10): 9258-64.