Open Journal of Immunology, 2016, 6, 34-48
Published Online March 2016 in SciRes. http://www.scirp.org/journal/oji
http://dx.doi.org/10.4236/oji.2016.61004

SNARE Protein in Cellular Membrane Trafficking, Its Regulation and as a Potential Target for Cancer Treatment

Mohammed Mehdi*, M. K. C. Menon

Department of Medical Biochemistry, School of Medicine, Faculty of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia
Email: *mohammed.mehdi@aau.edu.et, menakthmenon@gmail.com

Received 16 February 2016; accepted 20 March 2016; published 23 March 2016

Abstract

The role of SNARE [soluble NSF (N-ethylmaleimide-sensitive factor) accessory protein receptor] protein in cellular trafficking, membrane fusion and vesicle release in synaptic nerve terminals is described. The purpose of this review is to highlight the role of SNAREs in vital inflammatory conditions in maturing dendritic cells in order to retain the capacity to present new antigens together with altered cytokine secretory functions. This role of SNAREs can be used as novel targets for therapy in inflammatory diseases. The essential mechanism of SNAREs proteins for regulation of tumour formation through multiple signals and transportation pathways is also discussed. Finally, this review summarizes the current knowledge of SNARE proteins in regulating endocytosis in cancer cells and the possible therapeutic applications related to the pathogenesis of tumor formation.

Keywords

SNARE Protein, Inflammatory Diseases, Syntaxin, VAMP, SNAP-25, NSF, Tumour

1. Introduction

Fusion of plasma membrane is achieved by the cooperation of various proteins, such as SNARE proteins, Rab GTPases and their effectors, and SNARE chaperones. SNARE proteins bring two membrane lipid bilayers into close proximity, which induces the fusion of the two close positioned membranes [1]. These SNAREs proteins have a characteristic coiled-coil SNARE motif of approximately 70 amino acids comprising heptad repeats.

*Corresponding author.

How to cite this paper: Mehdi, M. and Menon, M.K.C. (2016) SNARE Protein in Cellular Membrane Trafficking, Its Regulation and as a Potential Target for Cancer Treatment. *Open Journal of Immunology*, **6**, 34-48.
http://dx.doi.org/10.4236/oji.2016.61004

Membrane trafficking of eukaryotic cell is necessary and it is driven by a SNARE protein complex. To accomplish this, transport vesicles bud off from an intracellular donor organelle and then target, dock and fuse with an acceptor organelle. In the nervous system, lipid bilayer fusion is the necessary step in chemical synaptic transmission because neurotransmitter packed pre-synaptic vesicles fuse in a calcium rely on with the lipid bilayer to release their content into the synaptic junction [2].

The SNARE hypothesis was first conceived in 1993, which suggested that a v-SNARE located on the vesicular membrane pair with cognate t-SNAREs on the target membrane and forms a complex that catalyzes the process of fusion [3]. The importance of this work in identifying SNARE complexes in immune function has culminated in a 2013 Nobel Prize in Physiology or Medicine to James E. Rothman, Randy W. Schekman and Thomas C. Südhof [4] [5]. Based on sequence homology and domain structure, the known mammalian SNAREs have been classified as members of the syntaxin (STX1) [6], VAMP (vesicle-associated membrane protein, also called synaptobrevin) [7] and SNAP-25 (25 kDa synaptosome associated protein) families [8].

In human cell, there are about 38 SNARE protein existed. Most of them contain one SNARE motif except four SNAREs (SNAP-23, SNAP-25, SNAP-29/GS32, and SNAP-47) contain two SNARE motifs. Both syntaxin and VAMP are attached to the membrane by a carboxy-terminal trans-membrane domain, whereas SNAP-25 is peripherally attached to the membrane by palmitoylation of four cysteine residues in the central region of the protein [9].

In addition to the SNARE motif, most SNAREs contain a C-terminal hydrophobic trans-membrane domain (TM). Seven of the 38 SNAREs do not have a TM domain and instead associate with membranes by various lipid modifications. SNAP-23, SNAP-25, SNAP-29, SNAP47, Stx9/19, and Stx11 are palmitoylated at multiple cysteine residues, whereas Ykt6 contains a C-terminal motif that mediates its prenylation (farnesylation) and subsequent palmitoylation. Moreover, many SNAREs contain N-terminal regulatory regions preceding the SNARE motif. These N-terminal regions regulate important properties of SNAREs [9].

When the ionic layer of SNARE complex showed in crystal structure comprises one arginine (R) (contributed by synaptobrevin) and three glutamine (Q) residues (contributed by Stx1A and SNAP-25) in the central position of the four-helix bundle. These results directed to the structural classification of R- and Q-SNAREs and functional SNARE complex generally comprises three Q-SNARE motifs and one R-SNARE motif. Most R-SNAREs act as v-SNAREs and most Q-SNAREs act as t-SNAREs [9] [10].

Similarly, the long in domain of Vamp7, an R-SNARE involved in both endocytic and secretory pathways, interacts with its SNARE motif via hydrophobic interactions. The N-terminal extension of VAMP4 contains a di-leucine motif and acidic clusters that mediate its recycling from the endosome to the trans-Golgi network (TGN) [9]. Syntaxin-16 has the H_{abc} region that can fold into a three-helix bundle and intra-molecularly interact with the SNARE motif, thereby preventing its assembly into a SNARE complex [11].

1.1. Different Types of SNARE Complex Configuration

The neuronal SNAREs (syntaxin-1, SNAP25, and VAMP2) assemble to form a ternary complex that bridges the vesicle and the plasma membrane at some point during exocytosis. To produce sufficient force for membrane fusion, the trans-SNARE assembly cannot be as tight as the cis-SNARE assembly. Indeed, the trans-SNARE complex can be reversibly disassembled. The trans-SNARE state maybe the initial configuration required for exocytosis [12].

The two types of the ternary SNARE complex, cis- and trans-, are showed in fusion process. Disassembly of cis-SNARE by NSF generates a “unitary-SNARE” state, in which each SNARE is not bound to other SNAREs [for details refer subtitle 1.2.2]. Subsequent assembly of t-SNAREs (syntaxin and SNAP25) generates a “binary-SNARE” complex, and then a v-SNARE (VAMP2) assembles with the binary complex to form a ternary trans-cSNARE complex linking the vesicle with the lipid bilayer [12]. If the trans-SNARE complex represents the initial state that triggers exocytosis, it needs to be protected from further SNARE assembly and fusion. In neuronal cell, synaptotagmin1 and complexin molecules have a clamping role for fusion of SNARE complex [13]. The two universally required components of the fusion machinery which play complementary functions in fusion are SNARE and SM (Sec1/Munc18-like) proteins. Vesicular (v-SNARE) and target membrane localized SNARE (t-SNARE) proteins can zipper-up and pull the membranes tightly together in α-helix bundle format [14]. When the merger of lipid bilayers takes place, through fusion, SNAREs are disassembled under energy consumption by the ATPase NSF, which becomes recruited to the SNARE complex through an adaptor called SNAP [15].

1.2. Non-Trans-SNARE Configurations

If exocytosis is initiated by non-trans-SNARE configurations, there are at least three possible initial states for exocytosis. These are binary-SNARE, unitary-SNARE, and cis-SNARE [for details refer subtitle 1.2.2, 1.2.3, and 1.2.4]. It is not easy to identify which of these states is used for the initial configuration, and more than one configuration may be utilized for a particular type of exocytosis [16]-[18].

1.2.1. Binary-SNARE Configurations

The binary t-SNARE complex of the two SNAREs (syntaxin and SNAP25) have been suggested that an intermediate stage of SNARE assembly. Auxiliary assembly of VAMP2 to form a trans-SNARE complex is the next stage of the fusion reaction. Binary-SNARE states are formed when the Munc18a/syntaxin1 complex binds to SNAP25 [16], a process catalyzed by Munc13. A binary-SNARE complex can efficiently induce trans-SNARE complex formation upon increases in $[Ca^{2+}]_i$ because synaptotagmins bind with the binary-SNARE complex, as well as with membrane phospholipids, in a Ca^{2+}-dependent manner. The binding of synaptotagmins with the plasma membrane supplies VAMPs within the vesicles to the binary complex in the plasma membrane. As a result VAMP2 can rapidly assemble with the binary complex [10] [19].

1.2.2. Unitary-SNARE Configurations

Unitary or free-SNAREs are a highly reactive form of SNARE complex and are an apparent candidate for the initial SNARE state. They named as unitary-SNAREs is to avoid the possible confusion that such SNAREs are completely free and not bound to other molecules, such as Munc18 [19].

Unassembled SNAREs can be produced from ternary- and binary-SNARE complexes. Thus unitary-SNAREs are dynamically maintained and may be generated immediately before exocytosis is triggered. Unassembled syntaxin can be chaperoned by Munc18a, which bundle SNARE motifs and H_{abc} for stabilization [10] [20].

Regardless of the clamping role of Munc18a, Proteoliposome experiments showed that Munc18a facilitates the formation of the binary-SNARE and trans-SNARE complexes. Munc13 encourages dissociation of Munc18 from closed syntaxin and the formation of binary- and ternary-SNARE complexes [17]. If vesicles contact with the plasma membrane after stimulation, VAMPs and synaptotagmins introduce intimate contacts with syntaxins and SNAP25, and facilitate SNARE assembly [21].

1.2.3. Cis-SNARE Complex Configurations and Its Disassembly

After exocytosis, cis-SNARE complex remains in the plasma membrane and this will be disassembled and recycled to another round of fusion events. Disassembly of the SNARE complex is facilitated by the NSF (Sec18p in yeast), and the contact between the SNARE complex and NSF requires SNAPs (Sec17p in yeast). NSF is a soluble ATPase, which contains an NH_2-terminal domain and two ATP-binding domains. The extreme stability of the SNARE complex forced that the NSF to use energy derived from ATP hydrolysis to disassemble it. Three SNAPs (α, β and γ) are recruited from the cytoplasm to everyone SNARE complex in the membrane, and the resulting SNAP/SNARE complex recruits a NSF hexamer, leading to the disassembly of the SNARE complex [22] [23]. Mutations in NSF block synaptic transmission due to accumulation of syntaxin and SNARE complexes in synaptic vesicles. Microinjection of NSF peptides that inhibit the ATPase activity of NSF also inhibited synaptic transmission [24] [25].

In contrast to SNAREs and atlastins, viral fusion proteins do not Trans-Oligomerizes in order to merge the two opposing membranes. Instead they bridge the two lipid bilayers via the formation of an extended intermediate that exposes a hydrophobic segment, termed fusion loop or fusion peptide, at the viral membrane distal end of the protein, such that it can insert into the target membrane. The extended intermediate then collapses into a hairpin that brings together fusion loop and viral transmembrane (TM) segments, thereby forcing the two membranes into close apposition [26]. SNAP-23 or NSF depletion and NSF-DN expression lead to defects in HIV-1 particle production. This defect correlates with a deficiency in Gag-membrane localization, suggesting a role for SNARE proteins in Gag accumulation at the Plasma Membrane [27].

2. Exocytosis

Proteoliposome studies had showed that a single trans-SNARE complex is capable of inducing exocytosis, whereas other studies report that 3 or between 5 and 11 are required. Smaller numbers might reduce the fusion rate.

In living cells, at least two SNARE complexes are involved in synaptic transmission, and three to five SNARE complexes are involved in exocytosis in PC12 cells and Ca^{2+}-primed chromaffin cells. Oligomers of SNAREs purified from the brain comprise three to five SNARE complexes [28]. On the other hand, cortical granule exocytosis (CGE) that is a calcium-regulated secretion during meiotic cell division of oocytes is thought to be mediated by the SNARE pathway [29].

2.1. Synaptic Vesicles Exocytosis

The molecular mechanism of SNARE mediated membrane fusion has been intensively studied in synaptic vesicle fusion. Neurotransmitters released by presynaptic neurons are recognized by their receptors on postsynaptic neurons. Ca^{2+} influx into the presynaptic cell then triggers membrane fusion between the presynaptic plasma membrane and synaptic vesicles, leading to the release of neurotransmitter [30].

Fusion of synaptic vesicle with plasma membrane done by three neuronal SNARE proteins these are syntaxin, SNAP25 and synaptobrevin (also referred to as VAMP). One SNARE motif from synaptobrevin (v-SNARE) on a synaptic vesicle and three SNARE motifs provided by syntaxin-1 and SNAP25 (t-SNAREs) from the plasma membrane assemble into a tight trans-SNARE complex that brings the two membranes into close apposition. This close apposition, in turn, induces lipid bilayers merging, thus releasing neurotransmitter into the synaptic cleft [1].

Other than the neuronal SNARE proteins, many other regulatory proteins, such as Munc18a and synaptotagmin, are required for synaptic vesicle fusion *in vivo*. Munc18a, a member of the Sec1/Munc18 (SM) protein family, appears to play a variety of roles in synaptic vesicle fusion. First, Munc18a binds to free syntaxin molecules and keeps them in a closed, inactive state, helping to prevent the formation of premature SNARE complexes. Additionally, Munc18a interacts with the syntaxin molecule within assembled t-SNARE complexes, guiding them in a manner conducive to productive trans-SNARE complex formation, which triggers membrane fusion. Neuronal synaptotagmin, anchored to synaptic vesicles, functions as a calcium sensor for synaptic vesicle fusion [31] [32].

Synaptic vesicles in the presynaptic terminals of neurons undergo exocytosis in four different modes: ultrafast [33], asynchronous [34], tonic [35], and spontaneous [36]. This suggests that the same types of vesicles show markedly different kinetics depending on the mode of exocytosis [34].

Constitutive exocytosis of neurotransmitters occurs without any particular external stimulation and carries membrane lipids and membrane proteins to the plasma membrane, which is necessary for cell survival. TIRF imaging of constitutive exocytosis reveals that it occurs in a manner similar to regulated exocytosis. Constitutive exocytosis is mediated by SNAREs, as is the case for regulated exocytosis, and some secretory cells appear to utilize the same set of SNAREs as are utilized for constitutive exocytosis [37] [38].

2.2. Fusion Pore Formation and Its Role

The vesicle has an aqueous compartment inside and its first connection with the extracellular fluid is through an exocytotic fusion pore. Measurements of progress in exocytosis can be made in terms of growth of the fusion pore. Tight temporal control by intracellular calcium concentrations control fast exocytosis of synaptic vesicles and this process differs from other membrane fusion reactions. Assembly of the fusogenic SNARE-complex is formed by controlling SNARE-dependent fusion pathway. The core fusion machinery consists of SNAREs, synaptotagmin, Munc18, and complexin. Initial SNARE-complex assembly and control of docking of vesicles is done by Munc18. On the other side, complexin and synaptotagmin cooperate in positioning the SNARE-complex in a release-ready state. The energy situation for fusion is calcium triggered and is promoted by the effects of complexin and synaptotagmin. Fusion pore formation and expansion are the final steps and this allows release of the water-soluble vesicle content. In the exocytosis pathway, the fusion pore formation is the most complicated part because of its short time existence [33].

Protein-protein and protein-lipid interactions lead to the fusion of vesicles with their target membranes. Vesicle fusion is guided by Rab 3 effectors, synaptotagmin III, nSecl, domains of the NSF chaperone and its adaptor SNAP. SNAREs are maintained in functional state by two chaperone systems HSC 70/αCSP/SGT and synuclein and any defects in these systems can lead to neuro-degeneration [39]. An auto-inhibitory closed confirmation of Syntaxin-1, binds to Munc18-1 and this protein is supposed to gate formation of SNARE complexes which has a role in membrane fusion. Many diverse presynaptic plasticity processes and diverse tethering factors depend on Ca^{2+} and RIM proteins for inducing fusion [40].

Two hypothesis/models are available currently to explain the formation of fusion pore which is an intermediate temporary structure. The first hypothesis claims that the fusion pore is composed of purely lipids of both membranes and SNARE-complex accelerates pore formation by bringing the membranes in close proximity [41]. The second model proposes a proteinaceous fusion pore and explains that transmembrane domains of SNARE proteins are incorporated into the pore [42]. It is also proved that at the transmembrane domain in the fusion pore, the vesicle SNARE (synaptobrevin-2) has a structural role with other two target plasma membrane SNAREs (Syntaxin and SNAP-25). These are able to draw the two membranes together in juxtaposition, and SNAREs remodel the lipid membranes. A contrasting hypothesis was proposed, which states that SNARE trans-membrane domains form a proteinaceous channel spanning both lipid bilayers [43] [44].

2.3. Trafficking of Glutamate Receptors in Neuronal Dendrites and Its Role in Regulating Inflammation

Activity dependent trafficking of glutamate receptors in postsynaptic dendrites is involved in the synaptic plasticity of glutamatergic synapses. Vesicles carrying glutamate receptors are found in the cytosol and undergo Ca^{2+} dependent exocytosis. Thus docking and fusion of these vesicles must be induced in a Ca^{2+} dependent manner to enable rapid activity dependent changes in synaptic functions. Since exocytosis is Ca^{2+} dependent, these vesicles may fall into the same class as enlargesomes [22].

2.4. Immune System and the Role of SNAREs in Immune Responses

Trafficking of molecules and membranes within cells is a prerequisite to all aspects of cellular immune function. Recycling of cell surface proteins and delivery are involved in processes such as secretion of immune mediators, ingestion of pathogens and activation of lymphocytes. All aspects of trafficking in innate and adaptive immune responses and its tight regulation is executed by SNAREs [45].

The link between innate and adaptive immunity is provided by the dendritic cells. Antigens, recognized by dendritic cells through pattern-recognition receptors, toll-like receptors are able to initiate a specific immune response. The nature of antigen delimits dendritic cells to secrete distinct cytokines for pathogen clearance. The dysregulation of this process can lead to a number of inflammatory diseases. The secretion of cytokines from dendritic cells is a tightly regulated process and the role of SNAREs in dendritic cell functioning can be exploited as novel targets of therapy in inflammatory diseases [46]. The role of SNAREs in dendritic cells both as facilitators of secretion and as useful tools to determine the pathways of secretion in opposing binding partners on vesicles and target membrane gives us an opportunity to explore these proteins as novel targets in inflammatory diseases.

Cytotoxic T lymphocytes (CTLs) and Natural killer cells (NK) selectively kills virally infected or cancer cells. Lytic granules are released at the contact area with target cells at the immunological synapse (IS). Maturation of lytic granules at the IS by SNARE mediations and exocytosis. Familial Hemophagocytic Lymphohystiocytosis is a disorder in which the immune system produces too many activated immune cells (lymphocytes) called T cells and SNARE mediated fusion events are deregulated [Refer **Table 2**]. It is reported that Syntaxin 11 and Syntaxin binding protein-2 are mutated and this deregulates lytic granule release during CTL activation and CTL mediated cytotoxicity and FHL pathophysiology [47].

The SNARE molecular machinery is involved in RBL-2H3 (Rat basophilic leukemia cell line) degranulation and probably depends on exocytosis process composed of Syntaxin 4, SNAP 23 and VAMP 8. V-SNARE which was originally localized in endosomal compartment functions in exocytosis and this possibility is due to the presence of VAMP8. It was also proposed that a secretory granule in mast cells is an intersection between exocytotic and endocytotic pathways and acts as a specialized compartment [48]. This process referred to as Endocytosis-Exocytosis coupling involves the incorporation of membrane during exocytosis. The endocytosis happens in mast cells after full collapse fusion, kiss-and run fusion, in which fusion pore just opens and closes without any significant membrane traffic. One can also observe multivesicular exocytosis, and bulk endocytosis that retrieves giant vesicles from the membrane [49]. The SNARE complexes are also reconstituted into individual SNARE proteins. This is made possible by the NSF ATPase and represents the energy consuming step of the SNARE cycle [50]-[52]. The kiss-and run exocytosis also known as “cavicapture” has been reported in chromoffin cells, and insulin-secreting pancreatic beta-cells and in mast cells. High calcium concentrations is known to shift the mechanism from kiss-and run to complete fusion. One SNARE complex is sufficient for the

bilayer fusion and three SNARE complexes are required to prevent the nascent fusion pore from reclosing, and the stability of fusion pore can be altered by accessory proteins like complexin and G$\beta\gamma$ [53].

Dendritic cell function can be better understood by identifying specific SNARE complexes and their regulators during inflammation. This could present potential therapeutic targets in a wide range of inflammatory diseases. The current strategy of using monoclonal antibodies and subsequent damages could be caused in cytokine production and blocking their activities. A number of antibody therapies in personalized medicine are being investigated. It was proposed that targeting SNARE proteins or other trafficking proteins and pathways which inhibit cytokine release could be an effective therapeutic approach in dendritic cells during inflammation [54].

2.5. Role of SNARE Protein in Control of Tumor Formation and as an Important Target for Cancer Therapy

Published studies using human endothelial cells have shown that syntaxin 6 (Stx6), a member of the target membrane-associated SNARE (t-SNARE) family protein is associated with VEGFR2/KDR and alpha5 beta1 integrin trafficking and angiogenesis [55].

SNARE protein may have important roles in cancer cell signaling, progression, invasion and onset of cancer through different mechanism. Mammalian Qb SNAREs are involved in vesicular transport with interaction with t-SNAREs (Vti1b). Vt1b forms complexes with Syntaxin 7(Qa), and Syntaxin 8 (Q_C). Vti1b is involved in Golgi-derived vesicle budding and is localized in the intracellular membrane with t-SNARE proteins. In Cancer Biology, lysosomes and mediated fusion of endosomes with lysosomes are emphasized through VAMP-8 and autophagy. Autophagosomes are trafficked to lysosomes and degraded by lysosomal hydrolases and autophagy is known to promote malignant cell survival, inflammation and tumorigenesis [56] [57].

In Glioblastoma which is most common and fatal type of primary brain tumor in adults, Stx1 over expression is detected, it is also expressed in small cell lung carcinoma and the most aggressive forms of colorectal cancer. the blockade of the SNARE protein Stx1 through three different approaches, including knock-down of Stx1a, expression of the H3TM dominant negative form of Stx1a and inhibition of Stx1 by treatment with BoNT/C1, consistently results in a dramatic decrease in glioblastoma tumor progression according to the study done in orthotopic mouse model. GBM *in vivo* experimental model study recommend that the possibility of using drugs [58] that targets Stx1 or other SNAREs [59]. Protein engineering of recombinant BoNT variants can he used to retarget BoNT SNARE-cleaving proteases into specific cell populations. Novel strategies have been devised in anticancer therapeutics to redirect BoNT protease into tumor cells by replacing neuronal binding domain with tumor targeting ligand. It is also possible to insert selected short peptide ligand into the loop region located between Light chain and Heavy chain in the BoNT molecule. This can trigger endocytosis in tumour cells rather than neurons and BoNT can translocate the SNARE protease into the cytoplasm. This will allow the cleavage of SNAREs and block the transportation of the signalling molecules which are known to cause tumorigenesis [60] [61].

In colorectal cancer, Netrin-1 receptor deleted in colorectal cancer forms a protein complex with Syntaxin-1 (Sytx1). This complex is Netrin-1 dependent both *in vitro* and *in vivo*, and requires specific Sytx1. Blockade of Sytx1 function by using various toxins, abolishes Netrin-1-dependent chemo-attraction of axons. Netrin-1 also provokes exocytosis at growing shafts in a Sytx1-dependent manner. Moreover, Sytx1/DCC complex associates with the v-SNARE in the spinal cord results in abnormal axonal direction phenotypes [62]. Silencing of SNARE protein NAPA sensitizes cancer cells to Cisplatin by inducing ERK 1/2 signaling, SINOVIOLIN ubiquitination and p53 accumulation. NAPA, an anti-apoptotic protein promotes degradation of tumor suppressor p53. The combination of Cisplatin and knockdown of NAPA represents a new strategy to eradicate p-53 sensitive cancer cells [63]. **Table 1** provides the summary of some of the studies carried out to understand the SNARE functions together with associated proteins.

Hemophagocytic Lymphohystiocytosis (HLH) [**Table 2**] with defects in cytotoxic activity and expansion of polyclonal CD-8 positive T cells and INF-gamma activated phagocytic macrophages, which infiltrates multiple organs and tissues including the nervous system cause neurological manifestations [86].

3. SNARE-Associated Proteins and Its Functional Regulation

Synaptotagmins and Other Ca^{2+} Sensors in Neuronal Cell

Synaptotagmin 1 is enriched in synaptic vesicles and is the major Ca^{2+} sensor particularly for ultrafast exocytosis.

Table 1. Different types of SNARE proteins that are important for trafficking molecules in various cancer cells during tumorigenesis and migration (modified after Ref. [56]).

The causative snare protein	Vesicular trafficking that are necessary for cancer cell formation and invasion	Ref.
1. Vamp-2	Alpha 5 beta 1 integrin trafficking, COS-7 cells.	[64]
2. Vamp-3	Integrin intracellular and cell surface distribution, PANC-1 pancreatic cancer cell, and alpha 5 beta 1 integrin trafficking in CHO cells.	[65] [66]
3. Vamp-7	ATP transportation in cancer cell during autophagy stimulation immunogenicity of cancer cell death or inflammation.	[67]
4. Vamp-8	Required for secretion of all platelet granules, Platelets contain α-granules, dense (δ-) granules, and a few lysosomes.	[68]
5. Syntaxin-8	Glioblastoma multiform (GBM): it is driven by aberrant signaling of growth factor receptors (EGFR). Unique tumor suppression mechanism involve the regulation of receptor trafficking.	[69]
6. Syntaxin-3 & -4	Alpha 5 beta 1 and alpha 3 beta 1 integrin trafficking, cervical adenocarcinoma, PANC-1 pancreatic adenocarcinoma.	[70]-[72]
7. Syntaxin-3, Vamp-7 & Vamp-8	Trafficking of serotonin mast cell line for triggering inflammation then cancer cell formation.	[73] [74]
8. Syntaxin-6 & Vamp-4	Cisplatin trafficking in ATP-11B mediated for ovarian cancer cell growth.	[75]
9. Syntaxin-6 & Vamp-3 Complex	Alpha 3 beta 1 integrin recycling in HeLa cell and DU145 cells.	[76] [77]
10. Syntaxin-6 complex with Vti1b	TNF-α trafficking for Upregulation of complex formation in activated macrophages.	[77]
11. Snap-25	VIP trafficking in neuroblastoma SH-SY5Y cells.	[79] [80]
12. Snap-23 & Syntaxin-4 complex	TNF-α in lipopolysaccharides (LPS)-induced macrophages.	[81]
13. Snap-23 & Vamp-3 complex	IL-1β induced IL-6 and TNF-α trafficking in human synovial sarcoma cell line.	[82]
14. Snap-23, Syntaxin-4 & Vamp-7	MMP-1 trafficking for proteolysis of ECM in MDA-MB 231-breast cancer cell and HT-1080 fibrosarcoma cell.	[83] [84]
15. Snap-23, Syntaxin-13 & Vamp-3	Beta 1 Integrin mediated Src and EGFR trafficking for invadopodia formation in breast cancer cell. MMP2 and MMP9 is also trafficked in human invasive fibrosarcoma HT-1080 cell line	[85]

Table 2. Mast cell vesicular trafficking and types of Familial Lymphohystiocytosis.

Associated secretory biogenesis and mast cell phenotype	Type of SNARE protein responsible for vesicular trafficking	Ref.
1. Familial Lymphohystiocytosis (FLH) type		
a. FLH1	Unknown	
b. FLH2	Defect in cytotoxic granule protein perforin/pore forming protein	[87]
c. FLH3	Defects in SNARE accessory protein Munc13-4	[88]
d. FLH4	Defects in t-SNARE syntaxin-11	[89]
e. FLH5	Defects in SNARE accessory protein syntaxin Binding Protein-2 (Munc18-2) that belonging to SM family of fusion accessory protein	[90] [91]
2. Hermansky-Pudlak syndrome (HPS)	Pulmonary fibrosis—endosomes to lysosomes related defects	[92] [93]

The presynaptic microinjection of synaptotagmin peptides or anti-synaptotagmin antibodies inhibits synaptic transmission. This protein contains two C2 domains (C2A and C2B), which are homologous to the regulatory C2 region of protein kinase C, both domains bind Ca^{2+} via their loops [94].

Ca^{2+} binding to the C2B domain is more vital for neurotransmitter release than Ca^{2+} binding to the C2A domain. Disturbing Ca^{2+} binding sites on the C2B domain has a significant effect on ultrafast exocytosis, whereas

disturbance of Ca^{2+} binding sites on the C2A domain has a small effect. Ca^{2+} bounding to the C2B domain of synaptotagmin1 stimulate interaction between two opposite membranes due to its highly positive electrostatic potential against negatively charged phospholipid head groups [95].

Asynchronous and spontaneous exocytosis lack synaptotagmin1, this indicates that asynchronous and spontaneous exocytosis are mediated by Ca^{2+} sensors other than synaptotagmin1. Attractive candidates for a slow Ca^{2+} sensor are the double C2domain (Doc2) proteins. Doc2 proteins are soluble proteins, which are enriched in synapses. Like synaptotagmins, these proteins contain C2 domains but have relatively high affinity for Ca^{2+}. Elimination of Doc2 in cultured hippocampal neurons results in normal aroused responses, but decreases spontaneous release rate by 50% [96].

Oncogene can control the potential expression of SNARE proteins as a consequence of numerous changes that are detected in human tumor cells through overexpression of Syntaxin 1, Syntaxin 2, Syntaxin 6, Syntaxin 7, and SNAP-25 [**Table 3**].

4. Conclusion

Obviously, numerous studies were done on membrane fusion as fusion process is mediated and controlled by various proteins such as SNAREs, Rap GTPases and SM proteins. SNARE proteins can be classified into three classes—Synaptobrevin (VAMP2), Syntaxin and Synaptosome associated proteins (SNAP) and based on their amino acid contents they grouped into Q-SNARE that contain Glutamine and R-SNARE that contain Arginine. Dendritic cells secrete distinct cytokines by a tightly regulated process by highly conserved trafficking protein families. SNAREs are known to act as facilitators of secretion by inherent specificity and opposing binding partners on vesicles and target membranes. This role of SNAREs in dendritic cell functioning may give an opportunity to explore these proteins as novel targets of therapy in inflammatory diseases. Specificity of membrane fusion is largely dependent on SNARE proteins, because different SNAREpins can catalyze distinct fusion events. Both SM proteins and tethering factors play an active role in SNAREpin formation and as a result contribute to the specificity of the fusion as well as to its speed and high fidelity. The tethering factors influence

Table 3. Lists of changes and alterations in SNARE regulatory protein detected in human tumor potentially activated by oncogene (modified after Ref. [56]).

Accessory protein for SNARE	Specific function of Accessary protein	Ref.
Complexins/synaphins	It is expressed in the brain. It is important for ultrafast exocytosis and facilitates spontaneous exocytosis of NTs. In addition, It can inhibits liposome or cell-cell fusion by clamping full zippering of the trans-SNARE complex.	[28] [97]-[99]
Munc13	It is necessary for ultrafast exocytosis of NTs. Munc13 removes the Munc18 capping from syntaxin, allow only one SNAP-25 molecule to bind syntaxin, whereby it prevents misfolding to the 2:1 complex.	[28] [100]-[102]
Snapin	It is identified as a SNAP25-binding protein and is expressed in both neuronal and non-neuronal cells. Snapin stabilizes the coupling between synaptotagmin1 and the SNARE complex during Ca^{2+} -triggered ultrafast exocytosis at central nerve terminals.	[103] [104]
Tomosyn	It is identified as a syntaxin-binding protein in rat brain cytosol. Tomosyn is a negative regulator of exocytosis because it's over expression causes a significant reduction in exocytosis and its deletion causes enhanced neurotransmitter release through increased synaptic vesicle priming.	[105] [106]
CAPS	CAPS was originally isolated from brain cytosol as a factor required for Ca^{2+}-triggered LDCV exocytosis. CAPS binds independently to each of the three SNARE proteins and markedly accelerates SNARE-dependent liposome fusion *in vitro* by promoting transSNARE complex assembly.	[107]
RIM	It is a binding partner for Rab3, a highly abundant protein in synaptic vesicles. RIM facilitate SNARE assembly by reversing autoinhibitory homodimerization of Munc13.	[108]
CAST	CAST directly or indirectly binds CAZ proteins to form a large molecular complex at the active zone. CAST also binds to RIM1 and, indirectly, to Munc13-1 to form a ternary complex. Deletion of CAST selectively affects the exocytosis of inhibitory synapses by reducing the size of the RRP.	[10]

SNAREp in assembly through stabilization of SNARE proteins or the entire SNARE complex, gathering of t-SNAREs on the target membranes, or activating the assembly process by interacting with SM proteins. The direct physical interactions of tethering factors with SNAREs and SM proteins mediate docking and fusion of vesicles. As normal function of SNARE protein is valuable for cell survival, the over expression of SNARE proteins can promote cancer cell formation. As a result, targeting SNARE protein in various types of cancer cell may present an opportunity to explore novel methods of therapy in various inflammatory diseases. Novel functions of SNARE proteins and SNARE-related proteins such as Synaptotagmin, Synaptophysin, have been implied in tumorigenesis. Analysis of the mechanisms by which these proteins contribute towards cancer malignance, through selective proteolysis of SNAREs and understanding the causative agents for the induction of chemo-resistance, will be rewarding in the future. It is hoped that, novel anti-cancer therapeutics will be developed using targeted delivery of a SNARE—inactivating proteases which could modulate cellular signaling cascades in the cancer cells.

Acknowledgements

We thank the Medical Biochemistry Department, School of Medicine, Addis Ababa University, Ethiopia for the Administrative support.

Competing Interests

Authors have declared that no competing interests exist.

References

[1] Ko, Y.J., Lee, M., Kang, K., Song, W.K. and Jun, Y. (2014) *In Vitro* Assay Using Engineered Yeast Vacuoles for Neuronal SNARE-Mediated Membrane Fusion. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 7677-7682. http://dx.doi.org/10.1073/pnas.1400036111

[2] Chen, Y.A., Scales, S.J. and Scheller, R.H. (2001) Sequential SNARE Assembly Underlies Priming and Triggering of Exocytosis. *Neuron*, **30**, 161-170. http://dx.doi.org/10.1016/S0896-6273(01)00270-7

[3] Söllner, T., Whiteheart, S.W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P. and Rothman, J.E. (1993) SNAP Receptors Implicated in Vesicle Targeting and Fusion. *Nature*, **362**, 318-324. http://dx.doi.org/10.1038/362318a0

[4] Kaiser, C.A. and Schekman, R. (1990) Distinct Sets of SEC Genes Govern Transport Vesicle Formation and Fusion Early in the Secretory Pathway. *Cell*, **61**, 723-733. http://dx.doi.org/10.1016/0092-8674(90)90483-U

[5] Bennett, M.K., Calakos, N. and Scheller, R.H. (1992) Syntaxin: A Synaptic Protein Implicated in Docking of Synaptic Vesicles at Presynaptic Active Zones. *Science*, **257**, 255-259. http://dx.doi.org/10.1126/science.1321498

[6] Trimble, W.S., Cowan, D.M. and Scheller, R.H. (1988) VAMP-1: A Synaptic Vesicle-Associated Integral Membrane Protein. *Proceedings of the National Academy of Sciences of the United States of America*, **85**, 4538-4542. http://dx.doi.org/10.1073/pnas.85.12.4538

[7] Oyler, G.A., Higgins, G.A., Hart, R.A., Battenberg, E., Billingsley, M., Bloom, F.E. and Wilson, M.C. (1989) The Identification of a Novel Synaptosomal-Associated Protein, SNAP-25, Differentially Expressed by Neuronal Subpopulations. *The Journal of Cell Biology*, **109**, 3039-3052. http://dx.doi.org/10.1083/jcb.109.6.3039

[8] Hong, W. and Lev, S. (2014) Tethering the Assembly of SNARE Complexes. *Trends in Cell Biology*, **24**, 35-43. http://dx.doi.org/10.1016/j.tcb.2013.09.006

[9] Kasai, H., Takahashi, N. and Tokumaru, H. (2012) Distinct Initial SNARE Configurations Underlying the Diversity of Exocytosis. *Physiological Reviews*, **92**, 1915-1964. http://dx.doi.org/10.1152/physrev.00007.2012

[10] Pieren, M., Schmidt, A. and Mayer, A. (2010) The SM Protein Vps33 and the t-SNARE Habc Domain Promote Fusion Pore Opening. *Nature Structural & Molecular Biology*, **17**, 710-717. http://dx.doi.org/10.1038/nsmb.1809

[11] Liu, W., Montana, V., Parpura, V. and Mohideen, U. (2011) Single-Molecule Measurements of Dissociation Rates and Energy Landscapes of Binary Trans Snare Complexes in Parallel versus Antiparallel Orientation. *Biophysical Journal*, **101**, 1854-1862. http://dx.doi.org/10.1016/j.bpj.2011.08.019

[12] Malsam, J. and Söllner, T.H. (2011) Organization of SNAREs within the Golgi Stack. *Cold Spring Harbor Perspectives in Biology*, **3**, a005249. http://dx.doi.org/10.1101/cshperspect.a005249

[13] Lee, J. and Littleton, J.T. (2015) Transmembrane Tethering of Synaptotagmin to Synaptic Vesicles Controls Multiple Modes of Neurotransmitter Release. *Proceedings of the National Academy of Sciences of the United States of America*,

112, 3793-3798. http://dx.doi.org/10.1073/pnas.1420312112

[14] Südhof, T.C. and Rothman, J.E. (2009) Membrane Fusion: Grappling with SNARE and SM Proteins. *Science*, **323**, 474-477. http://dx.doi.org/10.1126/science.1161748

[15] Lorentz, A., Baumann, A., Vitte, J. and Blank, U. (2014) The SNARE Machinery in Mast Cell Secretion. Deciphering New Molecular Mechanisms of Mast Cell Activation. *Frontiers in Immunology*, **4**, 100.

[16] Alpadi, K., Kulkarni, A., Comte, V., Reinhardt, M., Schmidt, A., Namjoshi, S., Mayer, A. and Peters, C. (2012) Sequential Analysis of Trans-SNARE Formation in Intracellular Membrane Fusion. *PLOS Biology*, **10**, e1001243. http://dx.doi.org/10.1371/journal.pbio.1001243

[17] Archbold, J.K., Whitten, A.E., Hu, S.H., Collins, B.M. and Martin, J.L. (2014) SNARE-ing the Structures of Sec1/Munc18 Proteins. *Current Opinion in Structural Biology*, **29**, 44-51. http://dx.doi.org/10.1016/j.sbi.2014.09.003

[18] Weninger, K., Bowen, M.E., Chu, S. and Brunger, A.T. (2003) Single-Molecule Studies of SNARE Complex Assembly Reveal Parallel and Antiparallel Configurations. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 14800-14805. http://dx.doi.org/10.1073/pnas.2036428100

[19] Takahashi, N., Hatakeyama, H., Okado, H., Noguchi, J., Ohno, M. and Kasai, H. (2010) SNARE Conformational Changes That Prepare Vesicles for Exocytosis. *Cell Metabolism*, **12**, 19-29. http://dx.doi.org/10.1016/j.cmet.2010.05.013

[20] Acuna, C., Guo, Q., Burré, J., Sharma, M., Sun, J. and Südhof, T.C. (2014) Microsecond Dissection of Neurotransmitter Release: SNARE-Complex Assembly Dictates Speed and Ca^{2+} Sensitivity. *Neuron*, **82**, 1088-1100. http://dx.doi.org/10.1016/j.neuron.2014.04.020

[21] Shen, J., Rathore, S.S., Khandan, L. and Rothman, J.E. (2010) SNARE Bundle and Syntaxin N-Peptide Constitute a Minimal Complement for Munc18-1 Activation of Membrane Fusion. *The Journal of Cell Biology*, **190**, 55-63. http://dx.doi.org/10.1083/jcb.201003148

[22] Zick, M., Orr, A., Schwartz, M.L., Merz, A.J. and Wickner, W.T. (2015) Sec17 Can Trigger Fusion of Trans-SNARE Paired Membranes without Sec18. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, E2290-E2297. http://dx.doi.org/10.1073/pnas.1506409112

[23] Peng, R. and Gallwitz, D. (2002) Sly1 Protein Bound to Golgi Syntaxin Sed5p Allows Assembly and Contributes to Specificity of SNARE Fusion Complexes. *The Journal of Cell Biology*, **157**, 645-655. http://dx.doi.org/10.1083/jcb.200202006

[24] Shah, N., Colbert, K.N., Enos, M.D., Herschlag, D. and Weis, W.I. (2015) Three αSNAP and 10 ATP Molecules Are Used in SNARE Complex Disassembly by N-Ethylmaleimide-Sensitive Factor (NSF). *Journal of Biological Chemistry*, **290**, 2175-2188. http://dx.doi.org/10.1074/jbc.M114.620849

[25] Rizo, J. and Xu, J. (2015) The Synaptic Vesicle Release Machinery. *Annual Review of Biophysics*, **44**, 339-367. http://dx.doi.org/10.1146/annurev-biophys-060414-034057

[26] Pérez-Vargas, J., Krey, T., Valansi, C., Avinoam, O., Haouz, A., Jamin, M., Raveh-Barak, H., Podbilewicz, B. and Rey, F.A. (2014) Structural Basis of Eukaryotic Cell-Cell Fusion. *Cell*, **157**, 407-419. http://dx.doi.org/10.1016/j.cell.2014.02.020

[27] Joshi, A., Garg, H., Ablan, S.D. and Freed, E.O. (2014) Evidence of a Role for Soluble N-Ethylmaleimide-Sensitive Factor Attachment Protein Receptor (SNARE) Machinery in HIV-1 Assembly and Release. *Journal of Biological Chemistry*, **289**, 29861-29871. http://dx.doi.org/10.1074/jbc.A111.241521

[28] Kiessling, V., Liang, B. and Tamm, L.K. (2015) Reconstituting SNARE-Mediated Membrane Fusion at the Single Liposome Level. *Methods in Cell Biology*, **128**, 339-363. http://dx.doi.org/10.1016/bs.mcb.2015.02.005

[29] de Paola, M., Bello, O.D. and Michaut, M.A. (2015) Cortical Granule Exocytosis Is Mediated by Alpha-SNAP and N-Ethilmaleimide Sensitive Factor in Mouse Oocytes. *PLoS ONE*, **10**, e0135679. http://dx.doi.org/10.1371/journal.pone.0135679

[30] Jahn, R. and Fasshauer, D. (2012) Molecular Machines Governing Exocytosis of Synaptic Vesicles. *Nature*, **490**, 201-207. http://dx.doi.org/10.1038/nature11320

[31] Rodkey, T.L., Liu, S., Barry, M. and McNew, J.A. (2008) Munc18a Scaffolds SNARE Assembly to Promote Membrane Fusion. *Molecular Biology of the Cell*, **19**, 5422-5434. http://dx.doi.org/10.1091/mbc.E08-05-0538

[32] Deák, F., Xu, Y., Chang, W.P., Dulubova, I., Khvotchev, M., Liu, X., Südhof, T.C. and Rizo, J. (2009) Munc18-1 Binding to the Neuronal SNARE Complex Controls Synaptic Vesicle Priming. *The Journal of Cell Biology*, **184**, 751-764. http://dx.doi.org/10.1083/jcb.200812026

[33] Südhof, T.C. and Rizo, J. (2011) Synaptic Vesicle Exocytosis. *Cold Spring Harbor Perspectives in Biology*, **3**, a005637. http://dx.doi.org/10.1101/cshperspect.a005637

[34] Kaeser, P.S. and Regehr, W.G. (2014) Molecular Mechanisms for Synchronous, Asynchronous, and Spontaneous

Neurotransmitter Release. *Annual Review of Physiology*, **76**, 333-363. http://dx.doi.org/10.1146/annurev-physiol-021113-170338

[35] Lalo, U., Palygin, O., Rasooli-Nejad, S., Andrew, J., Haydon, P.G. and Pankratov, Y. (2014) Exocytosis of ATP from Astrocytes Modulates Phasic and Tonic Inhibition in the Neocortex. *PLoS Biology*, **12**, e1001747. http://dx.doi.org/10.1371/journal.pbio.1001747

[36] Petrov, A.M., Yakovleva, A.A. and Zefirov, A.L. (2014) Role of Membrane Cholesterol in Spontaneous Exocytosis at Frog Neuromuscular Synapses: Reactive Oxygen Species-Calcium Interplay. *The Journal of Physiology*, **592**, 4995-5009. http://dx.doi.org/10.1113/jphysiol.2014.279695

[37] Jahn, R. (2004) Principles of Exocytosis and Membrane Fusion. *Annals of the New York Academy of Sciences*, **1014**, 170-178. http://dx.doi.org/10.1196/annals.1294.018

[38] Sedej, S., Klemen, M.S., Schlüter, O.M. and Rupnik, M.S. (2013) Rab3a Is Critical for Trapping Alpha-MSH Granules in the High Ca^{2+}-Affinity Pool by Preventing Constitutive Exocytosis. *PLoS ONE*, **8**, e78883. http://dx.doi.org/10.1371/journal.pone.0078883

[39] Chandra, S., Gallardo, G., Fernández-Chacón, R., Schlüter, O.M. and Südhof, T.C. (2005) α-Synuclein Cooperates with CSPα in Preventing Neurodegeneration. *Cell*, **123**, 383-396. http://dx.doi.org/10.1016/j.cell.2005.09.028

[40] Rizo, J. and Südhof, T.C. (2012) The Membrane Fusion Enigma: SNAREs, Sec1/Munc18 Proteins, and Their Accomplices-Guilty as Charged? *Annual Review of Cell and Developmental Biology*, **28**, 279-308. http://dx.doi.org/10.1146/annurev-cellbio-101011-155818

[41] Jackson, M.B. and Chapman, E.R. (2008) The Fusion Pores of Ca^{2+}-Triggered Exocytosis. *Nature Structural & Molecular Biology*, **15**, 684-689. http://dx.doi.org/10.1038/nsmb.1449

[42] Fang, Q. and Lindau, M. (2014) How Could SNARE Proteins Open a Fusion Pore? *Physiology*, **29**, 278-285. http://dx.doi.org/10.1152/physiol.00026.2013

[43] Jackson, M.B. (2011) Inferring Structures of Kinetic Intermediates in Ca^{2+}-Triggered Exocytosis. *Current Topics in Membranes*, **68**, 185-208. http://dx.doi.org/10.1016/B978-0-12-385891-7.00008-8

[44] Chang, C.W., Hui, E., Bai, J., Bruns, D., Chapman, E.R. and Jackson, M.B. (2015) A Structural Role for the Synaptobrevin 2 Transmembrane Domain in Dense-Core Vesicle Fusion Pores. *The Journal of Neuroscience*, **35**, 5772-5780. http://dx.doi.org/10.1523/JNEUROSCI.3983-14.2015

[45] Stow, J.L., Manderson, A.P. and Murray, R.Z. (2006) SNAREing Immunity: The Role of SNAREs in the Immune System. *Nature Reviews Immunology*, **6**, 919-929. http://dx.doi.org/10.1038/nri1980

[46] Collins, L.E., DeCourcey, J., Di Luca, M.S., Rochfort, K.D. and Loscher, C.E. (2015) An Emerging Role for SNARE Proteins in Dendritic Cell Function. *Frontiers in Immunology*, **6**, 133. http://dx.doi.org/10.3389/fimmu.2015.00133

[47] Giraudo, C., Spessott, W. and McCormick, M. (2013) SNARE Protein Requirements for Cytotoxic T Lymphocyte-Mediated Cell Killing (P1032). *The Journal of Immunology*, **190**, 65.17.

[48] Paumet, F., Le Mao, J., Martin, S., Galli, T., David, B., Blank, U. and Roa, M. (2000) Soluble NSF Attachment Protein Receptors (SNAREs) in RBL-2H3 Mast Cells: Functional Role of Syntaxin 4 in Exocytosis and Identification of a Vesicle-Associated Membrane Protein 8-Containing Secretory Compartment. *The Journal of Immunology*, **164**, 5850-5857. http://dx.doi.org/10.4049/jimmunol.164.11.5850

[49] Cabeza, J.M., Acosta, J. and Alés, E. (2013) Mechanisms of Granule Membrane Recapture Following Exocytosis in Intact Mast Cells. *Journal of Biological Chemistry*, **288**, 20293-20305. http://dx.doi.org/10.1074/jbc.M113.459065

[50] Shi, L., Shen, Q.T., Kiel, A., Wang, J., Wang, H.W., Melia, T.J., Rothman, J.E. and Pincet, F. (2012) SNARE Proteins: One to Fuse and Three to Keep the Nascent Fusion Pore Open. *Science*, **335**, 1355-1359. http://dx.doi.org/10.1126/science.1214984

[51] Wang, C.T., Lu, J.C., Bai, J., Chang, P.Y., Martin, T.F., Chapman, E.R. and Jackson, M.B. (2003) Different Domains of Synaptotagmin Control the Choice between Kiss-and-Run and Full Fusion. *Nature*, **424**, 943-947. http://dx.doi.org/10.1038/nature01857

[52] Wu, L.G., Hamid, E., Shin, W. and Chiang, H.C. (2014) Exocytosis and Endocytosis: Modes, Functions, and Coupling Mechanisms. *Annual Review of Physiology*, **76**, 301-331. http://dx.doi.org/10.1146/annurev-physiol-021113-170305

[53] Archer, D.A., Graham, M.E. and Burgoyne, R.D. (2002) Complexin Regulates the Closure of the Fusion Pore during Regulated Vesicle Exocytosis. *Journal of Biological Chemistry*, **277**, 18249-18252. http://dx.doi.org/10.1074/jbc.C200166200

[54] Vega-Ramos, J., Roquilly, A., Zhan, Y., Young, L.J., Mintern, J.D. and Villadangos, J.A. (2014) Inflammation Conditions Mature Dendritic Cells to Retain the Capacity to Present New Antigens but with Altered Cytokine Secretion Function. *The Journal of Immunology*, **193**, 3851-3859. http://dx.doi.org/10.4049/jimmunol.1303215

[55] Manickam, V., Tiwari, A., Jung, J.J., Bhattacharya, R., Goel, A., Mukhopadhyay, D. and Choudhury, A. (2011) Regu-

lation of Vascular Endothelial Growth Factor Receptor 2 Trafficking and Angiogenesis by Golgi Localized t-SNARE Syntaxin 6. *Blood*, **117**, 1425-1435. http://dx.doi.org/10.1182/blood-2010-06-291690

[56] Meng, J. and Wang, J. (2015) Role of SNARE Proteins in Tumourigenesis and Their Potential as Targets for Novel Anti-Cancer Therapeutics. *Biochimica et Biophysica Acta* (*BBA*)-*Reviews on Cancer*, **1856**, 1-12. http://dx.doi.org/10.1016/j.bbcan.2015.04.002

[57] Moreau, K. and Rubinsztein, D.C. (2012) The Plasma Membrane as a Control Center for Autophagy. *Autophagy*, **8**, 861-863. http://dx.doi.org/10.4161/auto.20060

[58] Arsenault, J., Ferrari, E., Niranjan, D., Cuijpers, S.A., Gu, C., Vallis, Y., O'Brien, J. and Davletov, B. (2013) Stapling of the Botulinum Type A Protease to Growth Factors and Neuropeptides Allows Selective Targeting of Neuroendocrine Cells. *Journal of Neurochemistry*, **126**, 223-233. http://dx.doi.org/10.1111/jnc.12284

[59] Ulloa, F., Gonzàlez-Juncà, A., Meffre, D., Barrecheguren, P.J., Martínez-Mármol, R., Pazos, I., Olivé, N., Cotrufo, T., Seoane, J. and Soriano, E. (2015) Blockade of the SNARE Protein Syntaxin 1 Inhibits Glioblastoma Tumor Growth. *PLoS ONE*, **10**, e0119707. http://dx.doi.org/10.1371/journal.pone.0119707

[60] Meng, J., Wang, J., Lawrence, G.W. and Dolly, J.O. (2013) Molecular Components Required for Resting and Stimulated Endocytosis of Botulinum Neurotoxins by Glutamatergic and Peptidergic Neurons. *The FASEB Journal*, **27**, 3167-3180. http://dx.doi.org/10.1096/fj.13-228973

[61] Ma, H., Meng, J., Wang, J., Hearty, S., Dolly, J.O. and O'Kennedy, R. (2014) Targeted Delivery of a SNARE Protease to Sensory Neurons Using a Single Chain Antibody (scFv) against the Extracellular Domain of P2X3 Inhibits the Release of a Pain Mediator. *Biochemical Journal*, **462**, 247-256. http://dx.doi.org/10.1042/BJ20131387

[62] Cotrufo, T., Pérez-Brangulí, F., Muhaisen, A., Ros, O., Andrés, R., Baeriswyl, T., Fuschini, G., Tarrago, T., Pascual, M., Ureña, J. and Blasi, J. (2011) A Signaling Mechanism Coupling Netrin-1/Deleted in Colorectal Cancer Chemoattraction to SNARE-Mediated Exocytosis in Axonal Growth Cones. *The Journal of Neuroscience*, **31**, 14463-14480. http://dx.doi.org/10.1523/JNEUROSCI.3018-11.2011

[63] Wu, Z.Z., Sun, N.K., Chien, K.Y. and Chao, C.C. (2011) Silencing of the SNARE Protein NAPA Sensitizes Cancer Cells to Cisplatin by Inducing ERK1/2 Signaling, Synoviolin Ubiquitination and p53 Accumulation. *Biochemical Pharmacology*, **82**, 1630-1640. http://dx.doi.org/10.1016/j.bcp.2011.08.018

[64] Hasan, N. and Hu, C. (2010) Vesicle-Associated Membrane Protein 2 Mediates Trafficking of $\alpha 5\beta 1$ Integrin to the Plasma Membrane. *Experimental Cell Research*, **316**, 12-23. http://dx.doi.org/10.1016/j.yexcr.2009.10.007

[65] Luftman, K., Hasan, N., Day, P., Hardee, D. and Hu, C. (2009) Silencing of VAMP3 Inhibits Cell Migration and Integrin-Mediated Adhesion. *Biochemical and Biophysical Research Communications*, **380**, 65-70. http://dx.doi.org/10.1016/j.bbrc.2009.01.036

[66] Tayeb, M.A., Skalski, M., Cha, M.C., Kean, M.J., Scaife, M. and Coppolino, M.G. (2005) Inhibition of SNARE-Mediated Membrane Traffic Impairs Cell Migration. *Experimental Cell Research*, **305**, 63-73. http://dx.doi.org/10.1016/j.yexcr.2004.12.004

[67] Fader, C.M., Aguilera, M.O. and Colombo, M.I. (2012) ATP Is Released from Autophagic Vesicles to the Extracellular Space in a VAMP7-Dependent Manner. *Autophagy*, **8**, 1741-1756. http://dx.doi.org/10.4161/auto.21858

[68] McConkey, D.J. (2009) Bortezomib Paradigm Shift in Myeloma. *Blood*, **114**, 931-932. http://dx.doi.org/10.1182/blood-2009-06-223230

[69] Ying, H., Zheng, H., Scott, K., Wiedemeyer, R., Yan, H., Lim, C., Huang, J., Dhakal, S., Ivanova, E., Xiao, Y. and Zhang, H. (2010) Mig-6 Controls EGFR Trafficking and Suppresses Gliomagenesis. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 6912-6917. http://dx.doi.org/10.1073/pnas.0914930107

[70] Day, P., Riggs, K.A., Hasan, N., Corbin, D., Humphrey, D. and Hu, C. (2011) Syntaxins 3 and 4 Mediate Vesicular Trafficking of $\alpha 5\beta 1$ and $\alpha 3\beta 1$ Integrins and Cancer Cell Migration. *International Journal of Oncology*, **39**, 863-871.

[71] Powelka, A.M., Sun, J., Li, J., Gao, M., Shaw, L.M., Sonnenberg, A. and Hsu, V.W. (2004) Stimulation-Dependent Recycling of Integrin $\beta 1$ Regulated by ARF6 and Rab11. *Traffic*, **5**, 20-36. http://dx.doi.org/10.1111/j.1600-0854.2004.00150.x

[72] Veale, K.J., Offenhäuser, C., Whittaker, S.P., Estrella, R.P. and Murray, R.Z. (2010) Recycling Endosome Membrane Incorporation into the Leading Edge Regulates Lamellipodia Formation and Macrophage Migration. *Traffic*, **11**, 1370-1379. http://dx.doi.org/10.1111/j.1600-0854.2010.01094.x

[73] Puri, N. and Roche, P.A. (2008) Mast Cells Possess Distinct Secretory Granule Subsets Whose Exocytosis Is Regulated by Different SNARE Isoforms. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 2580-2585. http://dx.doi.org/10.1073/pnas.0707854105

[74] Hibi, T., Hirashima, N. and Nakanishi, M. (2000) Rat Basophilic Leukemia Cells Express Syntaxin-3 and VAMP-7 in Granule Membranes. *Biochemical and Biophysical Research Communications*, **271**, 36-41. http://dx.doi.org/10.1006/bbrc.2000.2591

[75] Moreno-Smith, M., Halder, J.B., Meltzer, P.S., Gonda, T.A., Mangala, L.S., Rupaimoole, R., Lu, C., Nagaraja, A.S., Gharpure, K.M., Kang, Y. and Rodriguez-Aguayo, C. (2013) ATP11B Mediates Platinum Resistance in Ovarian Cancer. *The Journal of Clinical Investigation*, **123**, 2119-2130. http://dx.doi.org/10.1172/JCI65425

[76] Riggs, K.A., Hasan, N., Humphrey, D., Raleigh, C., Nevitt, C., Corbin, D. and Hu, C. (2012) Regulation of Integrin Endocytic Recycling and Chemotactic Cell Migration by Syntaxin 6 and VAMP3 Interaction. *Journal of Cell Science*, **125**, 3827-3839. http://dx.doi.org/10.1242/jcs.102566

[77] Tiwari, A., Jung, J.J., Inamdar, S.M., Brown, C.O., Goel, A. and Choudhury, A. (2011) Endothelial Cell Migration on Fibronectin Is Regulated by Syntaxin 6-Mediated $\alpha 5\beta 1$ Integrin Recycling. *Journal of Biological Chemistry*, **286**, 36749-36761. http://dx.doi.org/10.1074/jbc.M111.260828

[78] Murray, R.Z., Wylie, F.G., Khromykh, T., Hume, D.A. and Stow, J.L. (2005) Syntaxin 6 and Vti1b form a Novel SNARE Complex, Which Is Up-Regulated in Activated Macrophages to Facilitate Exocytosis of Tumor Necrosis Factor-α. *Journal of Biological Chemistry*, **280**, 10478-10483. http://dx.doi.org/10.1074/jbc.M414420200

[79] Purkiss, J.R., Friis, L.M., Doward, S. and Quinn, C.P. (2001) Clostridium Botulinum Neurotoxins Act with a Wide Range of Potencies on SH-SY5Y Human Neuroblastoma Cells. *Neurotoxicology*, **22**, 447-453. http://dx.doi.org/10.1016/S0161-813X(01)00042-0

[80] Héraud, C., Chevrier, L., Meunier, A.C., Muller, J.M. and Chadéneau, C. (2008) Vasoactive Intestinal Peptide-Induced Neuritogenesis in Neuroblastoma SH-SY5Y Cells Involves SNAP-25. *Neuropeptides*, **42**, 611-621. http://dx.doi.org/10.1016/j.npep.2008.05.005

[81] Pagan, J.K., Wylie, F.G., Joseph, S., Widberg, C., Bryant, N.J., James, D.E. and Stow, J.L. (2003) The t-SNARE Syntaxin 4 Is Regulated during Macrophage Activation to Function in Membrane Traffic and Cytokine Secretion. *Current Biology*, **13**, 156-160. http://dx.doi.org/10.1016/S0960-9822(03)00006-X

[82] Boddul, S.V., Meng, J., Dolly, J.O. and Wang, J. (2014) SNAP-23 and VAMP-3 Contribute to the Release of IL-6 and TNFα from a Human Synovial Sarcoma Cell Line. *FEBS Journal*, **281**, 750-765. http://dx.doi.org/10.1111/febs.12620

[83] Williams, K.C., McNeilly, R.E. and Coppolino, M.G. (2014) SNAP23, Syntaxin4, and Vesicle-Associated Membrane Protein 7 (VAMP7) Mediate Trafficking of Membrane Type 1-Matrix Metalloproteinase (MT1-MMP) during Invadopodium Formation and Tumor Cell Invasion. *Molecular Biology of the Cell*, **25**, 2061-2070. http://dx.doi.org/10.1091/mbc.E13-10-0582

[84] Miyata, T., Ohnishi, H., Suzuki, J., Yoshikumi, Y., Ohno, H., Mashima, H., Yasuda, H., Ishijima, T., Osawa, H., Satoh, K. and Sunada, K. (2004) Involvement of Syntaxin 4 in the Transport of Membrane-Type 1 Matrix Metalloproteinase to the Plasma Membrane in Human Gastric Epithelial Cells. *Biochemical and Biophysical Research Communications*, **323**, 118-124. http://dx.doi.org/10.1016/j.bbrc.2004.08.064

[85] Williams, K.C. and Coppolino, M.G. (2014) SNARE-Dependent Interaction of Src, EGFR and $\beta 1$ Integrin Regulates Invadopodia Formation and Tumor Cell Invasion. *Journal of Cell Science*, **127**, 1712-1725. http://dx.doi.org/10.1242/jcs.134734

[86] Schmid, J.P., Côte, M., Ménager, M.M., Burgess, A., Nehme, N., Ménasché, G., Fischer, A. and De Saint Basile, G. (2010) Inherited Defects in Lymphocyte Cytotoxic Activity. *Immunological Reviews*, **235**, 10-23. http://dx.doi.org/10.1111/j.0105-2896.2010.00890.x

[87] Stepp, S.E., Dufourcq-Lagelouse, R., Le Deist, F., Bhawan, S., Certain, S., Mathew, P.A., Henter, J.I., Bennett, M., Fischer, A., de Saint Basile, G. and Kumar, V. (1999) Perforin Gene Defects in Familial Hemophagocytic Lymphohistiocytosis. *Science*, **286**, 1957-1959. http://dx.doi.org/10.1126/science.286.5446.1957

[88] Feldmann, J., Callebaut, I., Raposo, G., Certain, S., Bacq, D., Dumont, C., Lambert, N., Ouachée-Chardin, M., Chedeville, G., Tamary, H. and Minard-Colin, V. (2003) Munc13-4 Is Essential for Cytolytic Granules Fusion and Is Mutated in a Form of Familial Hemophagocytic Lymphohistiocytosis (FHL3). *Cell*, **115**, 461-473. http://dx.doi.org/10.1016/S0092-8674(03)00855-9

[89] zur Stadt, U., Schmidt, S., Kasper, B., Beutel, K., Diler, A.S., Henter, J.I., Kabisch, H., Schneppenheim, R., Nürnberg, P., Janka, G. and Hennies, H.C. (2005) Linkage of Familial Hemophagocytic Lymphohistiocytosis (FHL) Type-4 to Chromosome 6q24 and Identification of Mutations in Syntaxin 11. *Human Molecular Genetics*, **14**, 827-834. http://dx.doi.org/10.1093/hmg/ddi076

[90] Côte, M., Ménager, M.M., Burgess, A., Mahlaoui, N., Picard, C., Schaffner, C., Al-Manjomi, F., Al-Harbi, M., Alangari, A., Le Deist, F. and Gennery, A.R. (2009) Munc18-2 Deficiency Causes Familial Hemophagocytic Lymphohistiocytosis Type 5 and Impairs Cytotoxic Granule Exocytosis in Patient NK Cells. *The Journal of Clinical Investigation*, **119**, 3765-3773. http://dx.doi.org/10.1172/JCI40732

[91] zur Stadt, U., Rohr, J., Seifert, W., Koch, F., Grieve, S., Pagel, J., Strauß, J., Kasper, B., Nürnberg, G., Becker, C. and Maul-Pavicic, A. (2009) Familial Hemophagocytic Lymphohistiocytosis Type 5 (FHL-5) Is Caused by Mutations in Munc18-2 and Impaired Binding to Syntaxin 11. *The American Journal of Human Genetics*, **85**, 482-492. http://dx.doi.org/10.1016/j.ajhg.2009.09.005

[92] Dell'Angelica, E.C., Ohno, H., Ooi, C.E., Rabinovich, E., Roche, K.W. and Bonifacino, J.S. (1997) A3: An Adaptor-Like Protein Complex with Ubiquitous Expression. *The EMBO Journal*, **16**, 917-928. http://dx.doi.org/10.1093/emboj/16.5.917

[93] Dell'Angelica, E.C., Ooi, C.E. and Bonifacino, J.S. (1997) *β*3A-Adaptin, a Subunit of the Adaptor-Like Complex AP-3. *Journal of Biological Chemistry*, **272**, 15078-15084. http://dx.doi.org/10.1074/jbc.272.24.15078

[94] Parisotto, D., Malsam, J., Scheutzow, A., Krause, J.M. and Söllner, T.H. (2012) SNAREpin Assembly by Munc18-1 Requires Previous Vesicle Docking by Synaptotagmin 1. *Journal of Biological Chemistry*, **287**, 31041-31049. http://dx.doi.org/10.1074/jbc.M112.386805

[95] Hobson, R.J., Liu, Q., Watanabe, S. and Jorgensen, E.M. (2011) Complexin Maintains Vesicles in the Primed State in *C. elegans*. *Current Biology*, **21**, 106-113. http://dx.doi.org/10.1016/j.cub.2010.12.015

[96] Lai, Y., Lou, X., Diao, J. and Shin, Y.K. (2015) Molecular Origins of Synaptotagmin 1 Activities on Vesicle Docking and Fusion Pore Opening. *Scientific Reports*, **5**, Article No.: 9267. http://dx.doi.org/10.1038/srep09267

[97] Zhang, Y., Diao, J., Colbert, K.N., Lai, Y., Pfuetzner, R.A., Padolina, M.S., Vivona, S., Ressl, S., Cipriano, D.J., Choi, U.B. and Shah, N. (2015) Munc18a Does Not Alter Fusion Rates Mediated by Neuronal SNAREs, Synaptotagmin, and Complexin. *Journal of Biological Chemistry*, **290**, 10518-10534. http://dx.doi.org/10.1074/jbc.M114.630772

[98] Falkowski, M.A., Thomas, D.D. and Groblewski, G.E. (2010) Complexin 2 Modulates Vesicle-Associated Membrane Protein (VAMP) 2-Regulated Zymogen Granule Exocytosis in Pancreatic Acini. *Journal of Biological Chemistry*, **285**, 35558-35566. http://dx.doi.org/10.1074/jbc.M110.146597

[99] Huntwork, S. and Littleton, J.T. (2007) A Complexin Fusion Clamp Regulates Spontaneous Neurotransmitter Release and Synaptic Growth. *Nature Neuroscience*, **10**, 1235-1237. http://dx.doi.org/10.1038/nn1980

[100] Shin, Y.K. (2013) Two Gigs of Munc18 in Membrane Fusion. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 14116-14117. http://dx.doi.org/10.1073/pnas.1313749110

[101] Henriette, K.O., Hofmann, K. and Brose, N. (2000) Definition of Munc13-Homology-Domains and Characterization of a Novel Ubiquitously Expressed Munc13 Isoform. *Biochemical Journal*, **349**, 247-253. http://dx.doi.org/10.1042/bj3490247

[102] Higashio, H., Nishimura, N., Ishizaki, H., Miyoshi, J., Orita, S., Sakane, A. and Sasaki, T. (2008) Doc2*α* and Munc13-4 Regulate Ca^{2+}-Dependent Secretory Lysosome Exocytosis in Mast Cells. *The Journal of Immunology*, **180**, 4774-4784. http://dx.doi.org/10.4049/jimmunol.180.7.4774

[103] Buxton, P., Zhang, X.M., Walsh, B., Sriratana, A., Schenberg, I., Manickam, E. and Tony, R.O. (2003) Identification and Characterization of Snapin as a Ubiquitously Expressed SNARE-Binding Protein That Interacts with SNAP23 in Non-Neuronal Cells. *Biochemical Journal*, **375**, 433-440. http://dx.doi.org/10.1042/bj20030427

[104] Pan, P.Y., Tian, J.H. and Sheng, Z.H. (2009) Snapin Facilitates the Synchronization of Synaptic Vesicle Fusion. *Neuron*, **61**, 412-424. http://dx.doi.org/10.1016/j.neuron.2008.12.029

[105] Yu, H., Rathore, S.S., Gulbranson, D.R. and Shen, J. (2014) The N-and C-Terminal Domains of Tomosyn Play Distinct Roles in Soluble N-Ethylmaleimide-Sensitive Factor Attachment Protein Receptor Binding and Fusion Regulation. *Journal of Biological Chemistry*, **289**, 25571-25580. http://dx.doi.org/10.1074/jbc.M114.591487

[106] Sakisaka, T., Yamamoto, Y., Mochida, S., Nakamura, M., Nishikawa, K., Ishizaki, H., Okamoto-Tanaka, M., Miyoshi, J., Fujiyoshi, Y., Manabe, T. and Takai, Y. (2008) Dual Inhibition of SNARE Complex Formation by Tomosyn Ensures Controlled Neurotransmitter Release. *The Journal of Cell Biology*, **183**, 323-337. http://dx.doi.org/10.1083/jcb.200805150

[107] Truong, C.Q., Nestvogel, D., Ratai, O., Schirra, C., Stevens, D.R., Brose, N., Rhee, J. and Rettig, J. (2014) Secretory Vesicle Priming by CAPS Is Independent of Its SNARE-Binding MUN Domain. *Cell Reports*, **9**, 902-909. http://dx.doi.org/10.1016/j.celrep.2014.09.050

[108] Inoue, E., Deguchi-Tawarada, M., Takao-Rikitsu, E., Inoue, M., Kitajima, I., Ohtsuka, T. and Takai, Y. (2006) ELKS, a Protein Structurally Related to the Active Zone Protein CAST, Is Involved in Ca^{2+}-Dependent Exocytosis from PC12 Cells. *Genes to Cells*, **11**, 659-672. http://dx.doi.org/10.1111/j.1365-2443.2006.00970.x

Abbreviations

SNARE: soluble NSF (N-ethylmaleimide-sensitive factor) accessory protein receptor;
SNAP: soluble NSF attachment protein;
GFP: green fluorescent protein;
NEM: *N*-ethylmaleimide;
NSF: NEM-sensitive factor;
PE-SA: phycoerythrin-streptavidin;
RBL-2H3: rat basophilic leukemia cell line;
SNAP23, 25, 29: synaptosome-associated protein of 23 kDa, 25kDa, 29 kDa;
t-SNARE: target-SNARE;
TI-VAMP: tetanus neurotoxin insensitive VAMP;
v-SNARE: vesicle-SNARE;
VAMP: vesicle-associated membrane protein;
BoNT: botulinum neurotoxin;
STX: syntaxin;
TGN: trans-Golgi network;
TM: trans-membrane domain;
Q-SNAREs: glutamate-SNARE;
R-SNAREs: Arginine SNARE;
SM: Sec1/Munc18-like proteins;
HIV: human immuno virus;
VIP: vasoactive intestinal peptide,
EGFR: epidermal growth factor receptor;
CAST: CAZ-associated structural protein;
RIM: Rab3-interacting molecule;
RRP: redially relasable protein;
ECM: extra cellular matrix;
MMP-1: type-1 matrix metalloproteinase.