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Endocytosis and membrane receptor internalization: implication of F-BAR protein Carom

Yanjie Xu[1,2], **Suxuan Liu**[2,6], **Jixiang Xia**[2], **Sam Stein**[2], **Cueto Ramon**[2], **Hang Xi**[2], **Luqiao Wang**[1,2], **Xinyu Xiong**[2], **Lixiao Zhang**[2], **Dingwen He**[7], **William Yang**[2], **Xianxian Zhao**[6], **Xiaoshu Cheng**[1], **Xiaofeng Yang**[2,3,4,5], and **Hong Wang**[2,3,4,5]

[1]Department of Cardiology, Second Affiliated Hospital of Nanchang University, Nan Chang, Jiang Xi, 330006, China

[2]Center for Metabolic Disease Research, Temple University School of Medicine, Philadelphia, PA, 19140

[3]Cardiovascular Research, Temple University School of Medicine, Philadelphia, PA, 19140

[4]Thrombosis Research, Temple University School of Medicine, Philadelphia, PA, 19140

[5]Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA, 19140

[6]Department of Cardiology, Changhai Hospital, Second Military Medical University, Shanghai, 200433, China

[7]Department of Orthopedics, Second Affiliated Hospital of Nanchang University, Nan Chang, Jiang Xi, 330006, China

Abstract

Endocytosis is a cellular process mostly responsible for membrane receptor internalization. Cell membrane receptors bind to their ligands and form a complex which can be internalized. We previously proposed that F-BAR protein initiates membrane curvature and mediates endocytosis via their binding partners. However, F-BAR protein partners involved in membrane receptor endocytosis and the regulatory mechanism remain unknown. In this study, we established a group of database mining strategies to explore mechanisms underlying receptor-related endocytosis. We identified 34 endocytic membrane receptors and 10 regulating proteins for vesicle formation in clathrin-dependent endocytosis (CDE), a major process of membrane receptor internalization. We found that F-BAR protein FCHSD2 (Carom) may facilitate endocytosis via 9 endocytic partners. Carom is highly expressed, along with highly expressed endocytic membrane receptors and partners, in endothelial cells and macrophages. We established 3 models of Carom-receptor complex and their intracellular trafficking based on protein-protein interaction and subcellular localization. We conclude that Carom may mediate receptor endocytosis and transport endocytic receptors to the cytoplasm for receptor signaling and lysosome/proteasome degradation, or to the nucleus for RNA processing, gene transcription and DNA repair.

Send correspondence to: Hong Wang, Centers for Metabolic Disease Research, Cardiovascular Research and Thrombosis Research, Temple University School of Medicine, MERB-1060, 3500 North Broad Street, Philadelphia, PA 19140, Tel: 1-215-707-5986, Fax: 1-215-707-5737, hongw@temple.edu.

Keywords

F-BAR proteins; Membrane receptor; Cellular trafficking; Nuclear translocation; Endocytosis

2. INTRODUCTION

Endocytosis is a cellular process by which molecules or substances are transported into the cell via cell membrane engulfment. Endocytosis is generally classified as phagocytosis and pinocytosis, which are distinguished by the size of the endocytic vesicles formed (Figure 1A & B) (1). Phagocytosis implies to the ingestion of large and solid particle (diameter 0.5–10μm) such as pathogens. Pinocytosis refers to internalization of various liquid via small endocytic vesicles and can be divided into four subtypes: macropinocytosis, clathrin-dependent, caveolae-dependent, and clathrin/caveolae-independent endocytosis based on clatherin or caveolae involvement (2). Pathogens or ligands induce endocytosis by binding to the cell membrane via receptor-dependent or -independent mechanisms, and then form phagosome or endocytic vesicle (Figure 1B & C). Endocytic vesicle may be coated with clathrin, caveolae or regulated by flotillin, Rho GTPase activating protein 26 (GRAF1), ADP-Ribosylation factor 6 (Arf6) and Ras homology family membrane A (RhoA). During phagocytosis, solid particle containing-phagosomes fuse with lysosomes (marked by lysosomal associated membrane protein (LAMP1)) and subjected to lysosomal degradation. In the process of pinocytosis, internalized vesicles are transported to early endosome (marked by Ras associated protein (Rab5)), which delivers the cargoes to three locations: 1) late endosome (marked by Ras associated protein (Rab7)) then lysosome for degradation, 2) recycling endosome (marked by Rab11) for signal transduction or receptor recycling to cell membrane, and 3) nucleus to regulate transcription factor and chromatin remolding (1–3).

Membrane receptors are responsible for transducing external signals into the cell by receiving extracellular molecules. It is suggested that some of the cell membrane receptors bind to their ligands and form a complex which can be internalized and translocated to the cytoplasm or nucleus for signaling or degradation mostly via clathrin-dependent endocytosis (CDE) mechanism (4). For example, receptor tyrosine kinases (RTKs) and G-protein coupled receptors (GPCRs) can be internalized via directly interacting with adaptor protein AP2, epsin or intersectin and CDE-mediated mechanism (5, 6) However, molecular mechanisms regulating CDE signaling are not fully elucidated.

We previously proposed that F-BAR (Fes/CIP4 homology-BAR) protein initiates membrane curvature and mediate endocytosis via its binding partners (7, 8). Beyond F-BAR domain which initiates endocytosis, most of the proteins in this superfamily contain other domains, such as SH3 (Src homology-3) and SH2 (Src homology-2), which can recruit adaptor proteins to form complexes. Such F-BAR protein complexes participate in multiple steps of endocytosis, ranging from the assembly of endocytic vesicles and their scissions, F-actin polymerization and nucleation, etc. As a novel member of F-BAR protein, FCHSD2 (Carom) displays such structures and contains a F-BAR domain and two SH3 domains. Although this protein's function hasn't been fully addressed, it is predicted as a critical

molecule in the process of CDE (Figure 2, Table 1). However, how Carom interacts with membrane receptor and facilitate receptor endocytosis is unknown.

In the past ten years, bioinformatics analysis has emerged as an important tool for functional interpretation of genomics and proteomics information (9–11). In this study, we established a group of database mining strategies and performed intensive literature searches to explore mechanisms underlying receptor-related endocytosis. We identified endocytic membrane receptors and potential regulating proteins for vesicle formation and investigated the relationship of F-BAR protein Carom with endocytic membrane receptors and endocytic partners. We established models of endocytosis and Carom-mediated membrane receptor internalization.

3. MATERIALS AND METHODS

3.1. Identification of regulating protein in CDE, F-BAR protein potential in membrane receptor endocytosis, and prediction of Carom-receptor complex signaling (PubMed)

We searched through PubMed literature to summarize 10 important regulating proteins that take part in vesicle formation in CDE and F-BAR protein potential in membrane receptor endocytosis (Figure 2B, Table 2). In order to identify the cell signaling connection to Carom-receptor complex, we summarized 3 types of Carom-receptor complexes, predicted their intracellular trafficking based on their subcellular localization and the nuclear localization signal (NLS) (Table 4), and predict the Function of Carom-receptor complexes based on receptor signaling reported in the literature (Table 5).

3.2. Identification of endocytic membrane receptor and Carom potential endocytic partners (Kegg database, Genecard database and PubMed)

Thirty-four cell membrane receptors were selected from 259 genes related to endocytosis identified from Kegg pathway database (hsa04144, http://www.genome.jp/dbget-bin/www.bget?hsa04144) (Table 1). The ligand and function of receptors were identified using Genecard database (http://ww.genecards.org). A total of 26 binding partners for Carom protein were identified from previous study, which were established via Affinity Capture-MS, Affinity Capture-RNA, Affinity Capture-Western, Reconstituted Complex and Two-hybrid technologies (12, 13). Nine of 26 Carom endocytic partner were selected based on literature review and their function (13–15) (Table 3). Functions and binding domains of Carom partners were defined based on information obtained from Genecard database. Binding receptor were identified from NCBI Gene database and their corresponding regulation on receptor were determined by PubMed search (16–18).

3.3. Cell expression profile of endocytic membrane receptor, Carom and Carom endocytic partner (Genevestigator database)

To investigate the expression profile of the endocytic membrane receptor, Carom and its endocytic partners in the cells, we used bioinformatics methods to gather extensive microarray information in the human primary cells. mRNA levels were obtained from microarray data available in the web site (https://www.genevestigator.com/gv/) and expressed as heat map (19) (Figure 3). The dark and light colour shading represents the

relatively high and low expression levels of the endocytosis receptor in the different human primary cells. The three darker shadings represent higher levels of expression.

3.4. Predicted interaction of endocytic membrane receptor with Carom and its endocytic partners. (String database, NCBI database)

To generate an overview of whether Carom and its 9 endocytic partners may relate to membrane receptor functionality. We carried out an analysis of their protein-protein interactions by using String database (19, 20) (Figure 4). Solid lines indicate known interaction deposited in NCBI Gene database which was established based on Affinity Capture-MS, Affinity Capture-RNA, Affinity Capture-Western, Reconstituted Complex and Two-hybrid experimental data. Dashed lines indicate computational-predicted interaction in String database based on analyzing genomic information ('genomic context'-methods) or from transferring associations/interactions between organisms ('interolog'-transfer).

3.5. Subcellular localization analysis of Carom endocytic partner and membrane receptor, and co-localization with Carom (Compartments database, cNLS Mapper)

Subcellular localization of Carom, its endocytic partner and membrane receptors were determined using Compartments database established by manually curated literature, high-throughput screens, automatic text mining, and sequence-based prediction methods (20) (Table 4). Numbers are indicated confidential levels. Protein nuclear import generally involves a NLS, or interaction with carrier proteins (21). Nuclear localization and nuclear export signals are identified by analyzing protein sequence in cNLS Mapper. We predicted Carom-receptor complex intracellular trafficking from cell membrane into cytoplasm based on the analysis of co-localization of endocytic membrane receptor and partner in plasma membrane and cytoplasm (CP)/cytoskeleton (CSK). We predicted Carom-receptor complex translocation into the nucleus (NC) based on the analysis of co-localization of endocytic membrane receptor and partner in PM, NC and NLS.

4. RESULT

4.1. Identification of 34 endocytic membrane receptors

We identified 34 membrane receptors from 259 endocytosis-related genes selected from Kegg pathway database and classified them into three groups (Table 1). 1) 8 G-protein coupled receptor (GPCR): Adrenoceptor β1-3 (ADRB1-3), chemokine C-C motif receptor 5 (CCR5), chemokine C-X-C motif 1-2/4 (CXCR1-2/4) and Coagulation factor II receptor (F2R), 2) 18 tyrosine kinase receptors (RTK): Colony stimulating factor 1 receptor (CSF1R), Epidermal growth factor receptor (EGFR), Erb-b2 receptor tyrosine kinase 2-4 (ERBB2-4), Fibroblast growth factor receptor 1-4 (FGFR1-4), Fms-related tyrosine kinase 1/Vascular endothelial growth factor receptor 1 (FLT1 or VEGFR1), Insulin-like growth factor 1-2 receptor (IGF1-2R), Kinase insert domain receptor/Vascular endothelial growth factor receptor 2 (KDR or VEGFR2) , Tyrosine-protein kinase met (MET), Neurotrophic tyrosine kinase receptor type 1 (NTRK1), Platelet-derived growth factor α receptor (PDGFRA), Transforming growth factor β receptor 1-2 (TGFBR1-2), and 3) 8 transmembrane receptor (TRM): Folate receptor 1-3 (FOLR1-3), Interleukin 2 receptor α, β, γ (IL2RA, B, G), Low density lipoprotein receptor (LDLR) and Transferrin receptor (TFRC). It is known that

endocytic membrane receptors can be internalized upon binding to its ligand and regulate various cellular functions including angiogenesis, proliferation, differentiation, and lipid/iron transport. The majority of these endocytic membrane receptors are internalized via CDE mechanism (22). Some of the receptors, such as EGFR, FGFR1-4, FLT1 (VEGFR1), IGF1R and TGFBR 1-2 can be internalized through both CDE and clathrin-independent endocytosis (CIE) (23). IL2R family appeared to be internalized by clathrin/caveolin-independent endocytosis (Indt) (Table 1) (24).

4.2. Key steps in vesicle formation in CDE and its regulating proteins

There are four steps during CDE process: 1) curvature initiation, 2) vesicle formation, 3) vesicle scission, and 4) un-coating vesicle (Figure 2A). A group of proteins are involved in regulating endocytic vesicle formation. At first, F-BAR protein binds to plasma membrane and initiates membrane curvature and clathrin-coated endocytic vesicle formation (25, 26). F-BAR protein also can recruit adaptor protein via its SH3 domain during vesicle formation. Clathrin are translocated to the site of adaptor-concentrated membrane from the cytosol via APs to form the clathrin-coated vesicle (2). The adaptor proteins link membrane cargo to clathrin and accessory proteins to form clathrin-coated endocytic vesicle. GTPase dynamin can then bind to the membrane and trigger vesicle scission and release upon GTP hydrolysis. Finally, ATPase heat shock cognate 70 (HSC70) binds to clathrin, disassociates clathrin, intersectin and dynamin from the vesicle and produces an un-coated endocytic vesicle containing the cargo molecules (27) (Figure 2B).

4.3. F-BAR protein participates in membrane receptor endocytosis

It is reported that F-BAR protein mediates membrane receptor endocytosis via initiating membrane curvature and endocytic vesicle formation in clathrin/caveolin-dependent endocytosis (Figure 1C) (8). F-BAR protein has 9 family members based on domain characterization. F-BAR protein contains one F-bar domain which binds to the cell membrane and other domains which interact with other molecules, such as Src homology-3 (SH3), Src homology-2 (SH2), protein kinase C-related kinase homology region 1 (HR1), F-BAR extension (FX), tyrosine kinase (Tyr-kinase), asparagine proline phenylalanine (NPF motif), μ-homology domain (μHD) and Rho GTPase-activating protein (RhoGAP) domains (Table 2). Six out of 9 F-BAR subfamily proteins are involved in membrane receptor endocytosis. CIP4 subfamily F-BAR proteins are required for EGFR internalization, trafficking and degradation (28). FCHO subfamily proteins are involved in LDLR endocytosis (29). PACSIN subfamily proteins participate in EGFR and transferrin/TFRC complex endocytosis (30). PSTPIP subfamily proteins can suppress transferrin/TFRC complex endocytosis (31). NOSTRIN subfamily protein is involved in assembling NOSTRIN-FGFR1-Rac1-Sos1 complex and regulating FGF signaling (28). FCHSDs subfamily proteins promote F-actin polymerization and membrane curvature which are key early events of endocytosis. However, endocytic receptors interacting with FCHSD protein have not been identified.

4.4. Identification of 9 Carom endocytic partners

The FCHSD2 gene encodes a protein termed as Carom, which is a novel membrane-associated protein with unknown function. Similar as most of the F-BAR family proteins,

Carom binds to cell membrane via N-terminal F-BAR domain association with membrane phospholipids and bridges the membrane with cytoskeleton. It interacts with proline-rich proteins, such as adaptor and signaling proteins, via its C-terminal SH3 domains to form a functional complex at cellular membranes. Based on their endocytosis-related function reported in the literature, we selected 9 proteins from 26 Carom partners we previously identified, and termed them as Carom endocytic partners (Table 3) (32). It was reported that these Carom endocytic partners played important role in regulating actin polymerization, endocytic vesicle formation and protein ubiquitination. The Carom endocytic partner proteins contain different domains, such as SH2, Pleckstrin homology (PH), Ubiquitin, CDC42-Rac interactive binding (CRIB), and WASP (Wiskott–Aldrich syndrome protein)-Homology 1/2 (WH1/2) domain, which can recruit proteins to organize signaling complexes at cellular membranes. Carom partner can bind to receptors. We found that DAPP1 bind to ERBB3 and that ITSN2/UBC/WAS/WASL bind to EGFR from experimental data generated via affinity capture-MS, affinity capture-RNA, affinity capture-western, reconstituted complex and two-hybrid technologies, and deposited in NCBI database at the gene/interaction branch. It is well documented that Carom endocytic partner ITSN1/UBC/UBD, VCP and WASL regulate EGFR ubiquitination, degradation and endocytosis process (8, 28, 29, 33, 34).

4.5. Endocytic membrane receptor, Carom and its endocytic partner are differently expressed in human primary cells

We anticipate that the expression of Carom, their corresponding partners and endocytic receptors is comparable in the cells where Carom-organized receptor endocytosis take place, and examined their relevant expression in 12 human body systems and 10 primary cells (Figure 3A). Gene expression levels (mRNA) were obtained from microarray database (https://www.genevestigator.com/gv/). We found that human body systems do not have distinguished patterns of Carom, endocytic membrane receptors and partners' expression. GPCR class endocytic membrane receptors had relative low levels of expression in most of the body system but highly expressed in immune system and in monocyte (MC), macrophage (Mϕ) and pancreatic islet cells. A few of RTK class receptors, such as EGFR, KDR (VEGFR2), MET, PDGFRA, appeared to be highly expressed in most of the body systems and in circulatory system cells, including cardiomyocyte (CMC), aortic vascular smooth muscle cell (VSMC), and aortic endothelial cell (EC). Cytokines and growth factor-related TRM class receptors had low levels of expression in most of body systems, but highly expressed in immune system (MC & Mϕ) and pancreatic islet cells. In contrast, TRM class receptors LDLR and TFRC, which transport lipid and iron into the cells, were highly expressed in all human body system and cells. Carom and its partners were expressed at medium levels in all body systems. In the circulatory system cells (Figure 3B), Carom was highly expressed in EC paralleled with highly expressed receptors (CXCR4, F2R, FLT1 (VEGFR1), KDR (VEGFR2), MET, IGF2R, LDLR and TFRC) and partners (GRASP, ITSN1-2, UBC, VCP and WASL). In LYM, Carom was highly expressed paralleled with highly expressed receptors (EGFR, ERBB3, MET, IGF1-2R, LDLR and TFRC) and partner (DAPP1, ITSN2 and WAS). In MC, highly expressed Carom was paralleled with receptors (CCR5, CXCR4, CSF1R, TGFBR1, IGF2R and FOLR3) and partners (DAPP1, ITSN2 and WAS). Mϕ had higher levels of Carom expression paralleled with large group of highly

expressed receptors (CCR5, CXCR4, CSF1R, FLT1 (VEGFR1), TGFBR1, IGF2R, FOLR3 and TFRC) and partners (DAPP1, ITSN1-2, UBC, UBD, WAS and WASL), which is comparable with that in EC.

4.6. Carom may directly or indirectly bind to endocytic membrane receptor through partner protein

To generate models of Carom-receptor complexes for receptor endocytosis and signaling, we analyzed the interaction of Carom with membrane receptor and partners using information from NCBI experimental database and computational String database (Figure 4). We proposed 3 Carom-receptor complex models: A) Carom-TGFBR1 complex, in which Carom binds directly to TGFBR1, B) Carom:partner-ERBBs complex, in which Carom indirectly interacts with ERBB2, ERBB3, ERBB4, EGFR via Carom partner DAPP1, ITSN1, ITSN2, WAS, and WASL, C) Carom:UBC-receptor complex in which Carom may bind to 25 of 34 membrane receptors (ADRB1, ADRB2, CXCR4, EGFR, ERBB3, ERBB4, FGFR1, FGFR2, FGFR3, FGFR4, FLT1 (VEGFR1), FOLTR1, F2R, IGF1R, IGF2R, IL2RB, IL2RG, KDR (VEGFR2), LDLR, MET, NTRK1, PDGFRA, TFRC, TGFBR1, TGFBR2) through partner UBC. In addition, Carom directly interacts with partner GRASP, UBD and VCP (model D, E and F) and this aids in the transduction of their signals.

4.7. Carom co-localization with endocytic membrane receptors and partners in different cellular micro-compartment

Interacting complexes are more likely to be presented within the same cellular compartment. We analyzed subcellular localization of Carom-receptor complex proteins identified in Figure 4 using Compartments database from manually curated literature, high-throughput screens, automatic text mining, and sequence-based prediction methods. We found that Carom is located in 4 major cell compartments, PM, NC, CP and CSK with the highest confidential level in the NC (confidential level 5) (Table 4A). Carom endocytic partners (DAPP1, ITSN1/2, UBC, WAS and WASL) are distributed in multiple compartments and mostly co-localized with Carom in 4 major cell compartments, with the exception of DAPP1 which is not located in the CSK. Except for UBC and WAS, all Carom-receptor complex related partner proteins contain NLS.

Carom-related membrane receptors are localized in multiple cell compartments (Table 4B). While looking at the 4 Carom-existent cell compartments, most of the membrane receptors can co-localize with Carom, except that ADRB1 was only located on the PM and CP, and FOLR1 is not sited in CP and CSK. Interestingly, FGFR3 is located in the PM, CSK and NC, but not in CP. We identified 13 receptors which can be potentially trans-localized to the nucleus because of the recognized NC localization and the detected NLS.

4.8. Carom-receptor complex intracellular trafficking and function

We analyzed intracellular trafficking and function of Carom-receptor complexes identified in Figure 4. Three models of Carom-receptor complexes are listed as, A) Carom-TGFBR1, B) Carom:partner-ERBBs, and C) Carom:UBC-receptor (Table 5). Based on their co-localization in the subcellular compartment and NLS (Table 4), we characterized 3 different Carom-receptor complex intracellular trafficking patterns: 1) only in PM, 2) PM to CP/CSK,

and 3) PM to NC. Among 13 potential nuclear trans-localized receptors identified (Table 4B), 9 receptors were found to interact with Carom via UBC which does not contain NLS (Table 4A). These 9 receptors were not justified as nuclear trans-localized receptors. The function of Carom-receptor complexes was determined based on signaling information related with its binding partner or receptor in literature.

As summarized in Table 5, the Carom-TGFBR1 complex can be transferred to CP and CSK via endocytosis for signaling transduction and lysosomal degradation (35). It can also be transported into the nucleus and participate in RNA processing (36). Carom:partner-ERBBs complex can be transferred to CP and CSK for signaling transduction and lysosomal degradation (37). Most of the Carom:partner-ERBBs complexes, except for Carom:DAPP1-ERBB3 and Carom:WAS-EGFR, can be transported into the nucleus to regulate transcription and DNA repair (38). The Carom:UBC-receptor complexes involve a large group of 24 receptors, including receptors for cytokine, growth factor, GPCR that are directed by UBC, which lacks NLS, and are subjected to ubiquitination and proteasome degradation (37, 39, 40). The Carom:UBC-FOLR1 complex may only stay in PM, because FOLR1 does not exist in CP and CSK.

5. DISCUSSION

Endocytotic trafficking of molecules is a highly regulated process involving multiple steps and molecules (Figure 1&2). In response to ligands stimulation, the BAR super family proteins can bind to cell membrane and bend to either positive or negative curvature. BAR proteins then recruit other adaptor proteins or accessory proteins to the deformed membrane to form endocytic vesicles. After endocytosis, cargos are destined to different subcellular organelles, including different endosome and lysosome.

Receptor trafficking is an important pathway for their signaling. The previous concept that receptor endocytosis would only contribute to its signal attenuation has already been challenged. Recent evidence demonstrated that receptor endocytosis and the following subcellular organelle redistribution regulate downstream signaling and gene regulation (41–44). As summarized in Table 1, at least 34 membrane receptors can be internalized via mostly CDE or CIE mediated endocytosis process, which contribute to their functions of regulating cell differentiation, proliferation, survival, angiogenesis, tumor transformation and immune regulation.

Recently, F-BAR proteins, a subfamily of BAR superfamily, have been identified as important coordinators that regulate endocytosis. In general, F-BAR proteins bind to the cell membrane via the association of F-BAR domain with membrane phospholipids. Through the SH3 domain, F-BAR proteins interact with WASP or GTPase dynamin to regulate the initiation and scission of the endocytic vesicle. We found out that at least 4 F-BAR protein subfamilies (CIP4, FCHO, PACSIN and NOSTRIN) are involved in the formation of endocytic vesicles and the assembly of endocytic complexes (Table 2) (7, 8). We listed 4 receptor endocytosis mechanisms, including CIP4 subfamily-related EGFR degradation, NOSTRIN subfamily-regulated FGFR signaling, FCHO2-regulated LDLR endocytosis and PACSIN3/PSTPIP1-regulated TFRC endocytosis. These findings presented fundamental

mechanisms for F-BAR protein-mediated receptor endocytosis. F-BAR protein-mediated receptor endocytosis, although less studied, may play critical roles in growth control, angiogenesis and lipid metabolism.

The FCHSD subfamily has two members, FCHSD1 and FCHSD2, each containing of one F-BAR domain and two SH3 domains (Table 2). The biological function of FCHSD subfamily proteins may be related to F-actin polymerization based on their direct interaction with WASP in E. Coli to promote WASP-Arp2/3-dependent F-actin polymerization (7). WASP is known to bind to Arp2/3 complex, via its C-terminal, to nucleate actin filaments, which then elongate at their free barbed ends to induce F-actin polymerization (45, 46). FCHSD2 (Carom) is a newly identified FCHSD subfamily member with unknown function. It is suggested that acute myeloid leukemia (AML) patients with high Carom expression have increased leukemia chemoresistance. We have previously proposed that Carom may regulate membrane curvature, promote F-actin polymerization and recruit adaptor proteins via its partner in the process of CDE (25). Carom related membrane receptor and endocytosis have not been studied. We hypothesized that Carom may regulate receptor endocytosis via its partner proteins and identified 9 Carom endocytic partners (Table 3). We found that Carom partners DAPP1 can bind to ERBB3 and that ITSN2/UBC/WAS/WASL can bind to EGFR. ITSN1/UBC/UBD/VCP bind to both Carom and EGFR leading to EGFR ubiquitination and degradation (47). We hypothesize that Carom regulate EGFR and other receptor internalization and signaling via interaction with its endocytic partners.

In the efforts to explore the functional connection of Carom and related receptor, we examined cell type expression profile of Carom, its endocytic binding partners and membrane receptors in human tissues and primary cells (Figure 3). High level Carom expression was found paralleled with some highly expressed endocytosis-related membrane receptors and Carom partners in aortic endothelial cell, lymphocytes, monocytes and macrophages, which usually display robust endocytosis phenomena. These results indicate that Carom and its partners regulate endocytosis-related endothelial function and myeloid cell related innate immune function.

We further analyzed the subcellular localization of these proteins to search potential signal partners in cell organelles in Carom-related membrane receptors endocytosis (Table 4). We found that Carom is located in all major subcellular domains, including NC, CSK, CP and PM, which is a typical pattern of trafficking signal molecules. The co-localization relationship of Carom with different endocytic binding partners and membrane receptors is dynamic. It appears that Carom co-localizes with all membrane receptors and endocytic binding partners at plasma membrane, suggesting the critical role of Carom in the initial step of receptor endocytosis on plasma membrane. In the NC, Carom is co-localized with all partners and most of the receptors in the NC, except for ADRB1 and IL2RB. We identified NLS in Carom and hypothesize that Carom can be translocated into the nucleus and is responsible for taking the Carom:partner-receptor complexes into the nucleus, because that NC-localized receptors (FGFR1/2/3/4, ERBB3, FOLR1, F2R, TFRC, TGFBR2) and partners (UBC and WAS) do not have identified NLS. We found that except for FGFR3 and FOLR1**,** Carom and most of receptors and partners are also located in the CP, suggesting that a proportion of Carom:partner-receptor complexes can be disassociated from membrane

structure and organelles, and released to the CP. The dynamic distribution of the component of Carom:partner-receptor complexes in various subcellular domain and organelles, including CSK, endosome (E), endoplasmic reticulum (ER), lysosome (Lys), mitochondrion (Mit) presented different intracellular trafficking pathways for Carom:partner-receptor complexes from CP to the NC.

Based on above findings, we presented three novel models for Carom-related receptor trafficking (Table 5 & Figure 5); Carom can **A)** directly bind to receptor (TGFBR1), **B)** indirectly binds to receptor through its partner (EGFR, ERBB and other yet characterized receptors) to initiate the formation of endocytic vesicle, and **C3)** facilitate membrane endocytosis through ubiquitination related proteins (UBC and UBD). Model C is likely responsible for endocytosis, ubiquitination and proteasome degradation of a large group of receptors. It is noticed that ubiquitin itself a sorting signal for membrane receptor endocytosis. There exist different sorting machineries that determine how receptors are selected by compartment specific ubiquitin-binding proteins and are delivered to cellular destination (48, 49). These three models suggest that Carom may play critical role in regulating intracellular trafficking and signaling of a large numbers of membrane receptors.

Transforming growth factor β (TGF-β) plays a critical role in embryogenesis and adult tissue development by regulating cell proliferation, differentiation, and migration (50). It is suggested that TGFBR1 plays an important regulatory role in TFG-β signaling via CDE to promote TGF-β-induced Smad activation and transcriptional regulation or Caveolae-dependent endocytosis to facilitate the degradation of TGF-β (35). We proposed a novel Carom-TGFBR1 complex for TGF-β signaling through database mining (Table 5). ERBBs family contains EGFR, ERBB2, ERBB3 and ERBB4. It is suggested that after ligand binding ERBBs are internalized mainly through CDE, which is followed by receptor activation or lysosomal degradation (51–53). Our data suggest that Carom can bind to ERBBs via its partners DAPP1, ITSN1/2, WAS and WASL, and such interaction complexes contribute to receptor endocytosis and control of signaling (Table 5). UBC gene encodes polyubiquitin-C protein, which is involved in the regulation of CDE and protein ubiquitination (54–56). It is reported. Through our analysis, we anticipate that Carom may mediate those 24 membrane receptor transport into cytoplasm and cytoskeleton via interacting with UBC for ubiquitination. As mentioned above, Carom may regulate receptor endocytosis through specific sorting machinery for individual receptor.

It is suggested that certain endocytic proteins translocate to the nucleus in response to extracellular signals which may affect gene transcription and chromatin remodeling machinery (57–59). The mechanism by which endocytic proteins enter the nucleus is based on NLS or interaction with carrier proteins (21). (Table 4). Carom and its endocytic partners (DAPP1, ITSN1-2 and WASL) were identified to have NLS through protein sequence analysis. Therefore, we propose that Carom-TGFBR1 and Carom:partner-ERBBs complexes may transport from the membrane to the nucleus to activate TGFBR1 and ERBB signaling for RNA processing, gene transcription or DNA repair (36, 60) (Table 5).

Traditional concept recognizes that the purpose of endocytosis of membrane receptors is to terminate receptor mediated signaling. However, it is now recognized that receptor

internalization, especially for RTK families, is highly regulated via various mechanism. For example, EGFR and FGFR employ different molecular mechanisms for nuclear translocation (60, 61). Unlike EGFR which displays NLS, FGFR is translocated into nucleus from early endosome (62). EGFR endocytosis is required for optimal activation of sub-populations of signal transducers (63). EGFR endocytosis and post-endocytic traffic display versatile pathways and such traffic can lead to different cellular behaviors, such as proliferation, survival, tumorigenesis and DNA repair (Figure 5). Different KDR (VEGFR2) trafficking pathways via different subcellular compartments effect different cellular behaviors, ranging from proliferation, migration, tubulogenesis and blood vessel formation (42, 64). The proposed 3 models in Figure 5 presented a simple network. Studies to define the subcellular localization of Carom:partner-receptor complexes in early and late endosome, lysosome or trans Golgi, nucleus should provide strong evidence and discover relevant molecular mechanisms.

A more complex regulatory network could be involved in regulating Carom-mediated receptor endocytosis and trafficking to the nucleus, especially considering the two SH3 domains which can associate with many other adaptor proteins. SH3 signaling may lead to phosphorylation of the receptor and adaptor proteins. The detailed mechanisms of surface receptors translocation to nucleus are largely unveiled. Whether the nuclear translocated receptors come from receptors embedded in endosomes are under debate and requires experimental clarification (61, 65).

The combinations of various bioinformatics tools employed in the study is very powerful for the identification of protein complexes involving complicated intracellular trafficking mechanism. While more and more online large databases become available, it is possible to develop model systems regulating important biological process and to predict molecular targets. The identified mechanistic model system can be important guidance for future experimental science and may lead to the discovery of novel mechanism for human disease and therapeutic targets.

6. CONCLUSIONS

In this study, we identified 34 endocytic membrane receptors and 9 Carom endocytic partners and established their expression profiles in human primary cells. We established 3 models of Carom-receptor complexes and their intracellular trafficking based on protein-protein interaction and subcellular localization. We propose that F-BAR protein Carom may mediate receptor endocytosis and transport endocytic receptors to the cytoplasm for receptor signaling and lysosome/proteasome degradation, or to the nucleus for RNA processing, gene transcription and DNA repair.

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Abbreviation

CIP4	CDC42-interacting protein 4
CRIB	CDC42-Rac interactive binding
CP	Cytoplasmic
CSK	cytoskeleton
ECM	Extracellular matrix
F-actin	Filamentous actin
F-BAR	Fes/CIP4 homology-Bin/Amphiphysin/Rvs
FCHO	FCH only
FCHSD	FCH and double SH3 domain proteins
FER	FES related
FX	F-BAR extension
GAS7	Growth arrest-specific 7
HR1	Protein kinase C-related kinase homology region 1
NOSTRIN	Nitric oxide synthase traffic inducer
N-WASP	Neural Wiskott-Aldrich syndrome protein
NC	Nucleus
PACSIN	Protein kinase C and casein kinase 2 substrates in neurons
PH	Pleckstrin homology
PM	plasma membrane
RhoGAP	Rho GTPase-activating protein
SH2	Src homology-2
SH3	Src homology-3
srGAP	Slit-Robo GTPase-activating protein
μHD	μ-homology domain
VCA	Verprolin, cofilin, acidic
WASP	Wiskott-Aldrich syndrome protein
WAVE	WASP family verproline-homologous protein
WH1/2	WASP-Homology 1/2

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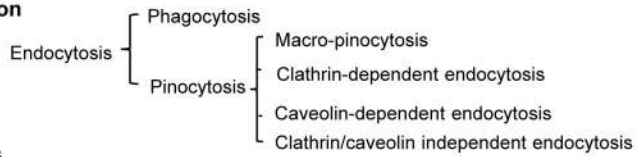
A. Endocytosis classification



B. Features of endocytosis

Endocytosis Class	Phagocytosis	Pinocytosis			
		Macro-pinocytosis	Clathrin-dependent endocytosis(CDE)	Caveolin-dependent endocytosis	Clathrin/caveolin independent endocytosis
Vesicle size	0.5-10μm	0.1-5μm	<120nm	<80nm	<50nm
Membrane domain localization	Lipid & non-lipid raft	Lipid & non-lipid raft	Non-lipid raft	Lipid raft	Lipid raft
Internalized Cargo	Pathogens	RTKs	RTKs	GPI-linked protein	GPI-linked protein
	Apoptotic remnants	Fluids	GPCR	TGFBRs	IL-2R
		Bacteria	TFR	IGF-1R	
			LDLR		
			Toxins		

C. Endocytosis process

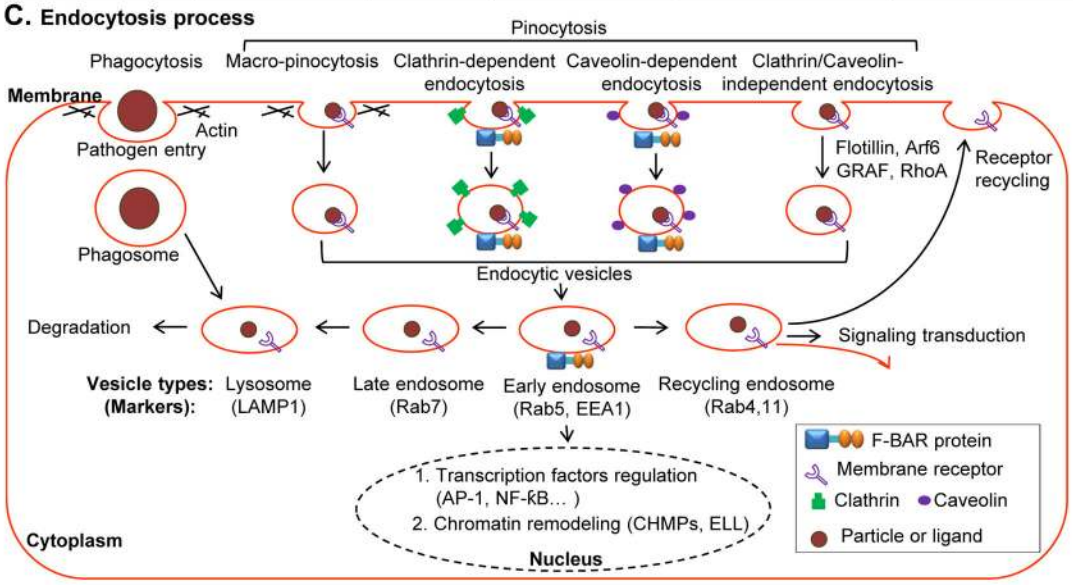
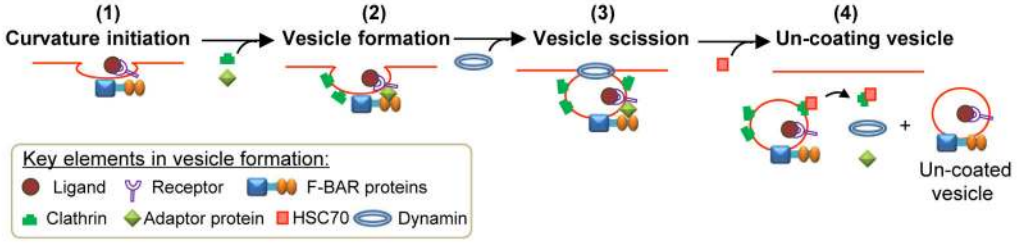


Figure 1. Overview of endocytosis

Endocytosis is a cellular process by which molecules or substances are transported into the cell via cell membrane engulfment. **A. Classification of endocytosis** Endocytosis is generally classified as phagocytosis and pinocytosis. Pinocytosis can be further divided into 4 subtypes; macropinocytosis, clathrin-dependent, caveolae-dependent, and clathrin/caveolae independent endocytosis based on clatherin or caveolae involvement. Most of the receptor-mediated endocytosis (REM) are processed via clathrin-dependent mechanism. **B. Features of endocytosis.** Features of endocytosis are summarized for the size of internalized particle, membrane domain localization and cargo content. **C. Schematic diagram of endocytosis process.** Pathogens and ligands induce endocytosis by binding to the cell membrane via receptor-dependent or -independent mechanism, and then form phagosome or endocytic vesicle which may be coated with clathrin/caveolae or regulated by flotillin, GRAF1, Arf6 and RhoA. Membrane–bounded F-BAR protein are linked to actin-associated proteins, can cause cell membrane curvature and facilitate clathrin-meditaed or caveolae-dependent endocytosis. During phagocytosis, cells bring in solid particles into phagosomes and then fuse with lysosomes (marked by LAMP1). During pinocytosis, internalized

vesicles are transported to early endosome (marked by Rab5). The early endosome can send the cargoes to three locations: 1) late endosome (marked by Rab7) then lysosome for degradation, 2) recycling endosome (marked by Rab11) for signal transduction or recycling to plasma membrane, and 3)nucleus for transcription factor regulation or chromatin remolding machinery. **Abbreviation:** RTK, receptor tyrosine kinase; GPCR, G protein-coupled receptor; TFR, transferrin receptor; LDLR, low-density lipoprotein receptor; GPI, glycosylphosphatidylinositol; TGF-βR, transforming growth factor-beta receptor; IGF-IR, insulin-like growth factor I receptor; IL-2RB, interleukin 2 receptor beta; Rab, Ras associated protein; EEA1, Early Endosome Antigene 1; LAMP1, Lysosomal associated membrane protein 1;GRAF1, Rho GTPase Activating Protein 26; Arf6, ADP-Ribosylation Factor 6; RhoA, Ras Homolog Family Member A;

A. Key steps in clathrin-dependent endocytosis (CDE):



B. Regulating proteins during vesicle formation in CDE

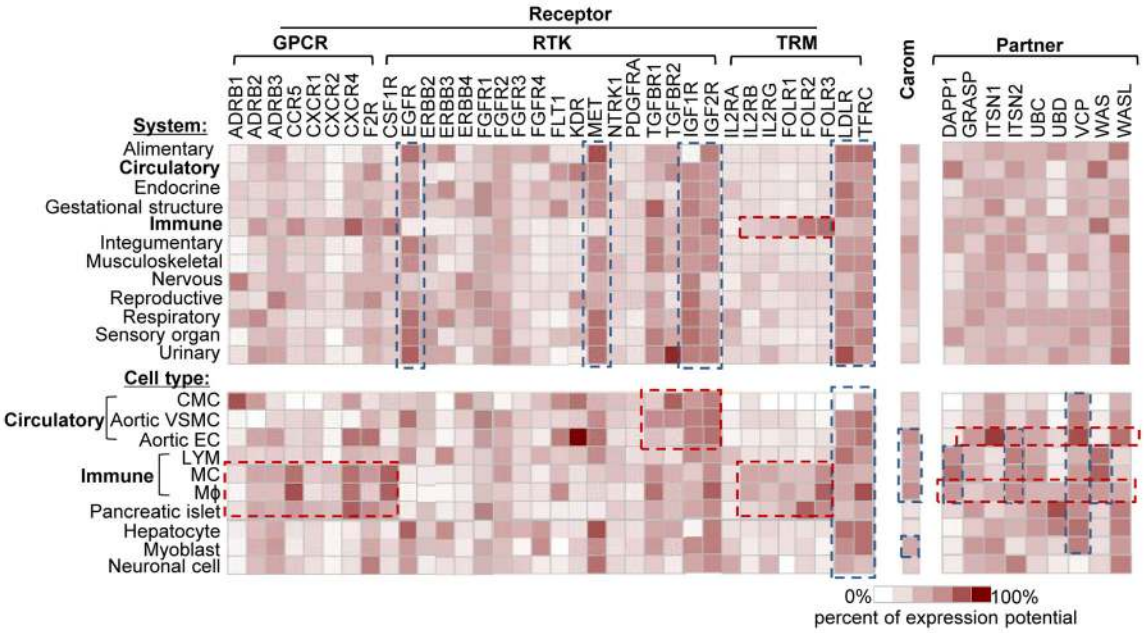
Proteins	Key steps in CDE				Function	PMID#
	Curvature initiation	Vesicle formation	Vesicle scission	Un-coating Vesicle		
1. F-BAR protein	√	√	√	√	Initialing membrane curvature and clathrin-coated endocytic vesicle formation	20448150
2. Clathrin		√	√		Composing three heavy and three light chains to form the clathrin triskelion	27627809
3. Intersectin		√	√		Linking various components of the clathrin machinery to form clathrin-coated endocytic vesicle	23986746
4. AP2		√	√		Linking membrane cargo to clathrin and accessory proteins	25788288
5. Epsin		√	√		A cargo-specific adaptor for mono-ubiquitylated receptors	1941647
6. CALM		√	√		Binding to AP2 and clathrin, and regulating vesicle size	27574975
7. Amphiphysin	√	√	√		Bending the membrane and recruiting dynamin to clathrin-coated endocytic vesicle	27093085
8. SNX9		√	√		Binding to AP2 and dynamin	25256216
9. Dynamin		√	√		Triggering vesicle scission upon GTP hydrolysis	25772449
10. HSC70				√	Triggering un-coating of endocytic vesicle	27478930

Adaptor protein

Figure 2. Key steps in clathrin-dependent endocytosis and its regulating proteins

A. Key steps in vesicle formation in clathrin-dependent endocytosis (CDE). There are four steps during vesicle formation: (1) curvature initiation, (2) vesicle formation, (3) vesicle scission, and (4) un-coating vesicle. . At first, F-BAR protein binds to plasma membrane and initiates membrane curvature. F-BAR protein can recruit adaptor protein via its SH3 domain during vesicle formation. Clathrin are recruited directly from the cytosol to the site of adaptor-concentrated membrane to help the formation of coated vesicle. GTPase dynamin can then bind to the membrane and cause vesicle constriction, scission, and release. HSC70 binds to Clathrin, disassociates Clathrin, Intersectin and Dynamin from the vesicle and produces an un-coated endocytic vesicle containing the cargo molecules. **B. Regulating proteins during vesicle formation in CDE.** A group of proteins are involved in endocytic vesicle formation. F-BAR protein initiates membrane curvature and clathrin-coated endocytic vesicle formation. Adaptor proteins (Intersectin, AP2, Epsin, CALM) links various components of the clathrin machinery to the membrane and helps the formation of adaptor-concentrated clathrin-coated vesicle. Dynamin triggers vesicle scission upon GTP hydrolysis. HSC70 triggers un-coating of endocytic vesicle. Abbreviation: AP2, adaptor protein 2; SNX9, sorting nexin 9; HSC70, ATPase heat shock cognate 70; CALM, clathrin assembly lymphoid myeloid leukaemia; (N-)WASP/WAVE, Wiskott-Aldrich Syndrome Like.

A. Endocytic membrane receptor, Carom, endocytic partner expression in human primary cells



B. Relationship of Carom, endocytic membrane receptor and partner expression in human circulatory and immune system cells

System	Cell type	Highly expressed endocytosis-related membrane receptor	Carom expression	Highly expressed Carom partners
Circulatory	CMC	ADAR1, KDR(VEGFR2),TGFBR2, IGF2R	Low	ITSN1, VCP, WASL
	Aortic VSMC	EGFR, ERBB4, MET,TGFBR1-2, IGF1-2R, LDLR TFRC	Low	ITSN1, UBC, VCP, WASL
	Aortic EC	CXCR4, F2R, FLT1 (VEGFR1), KDR(VEGFR2), MET, IGF2R, LDLR, TFRC	**High**	GRASP, ITSN1-2, UBC, VCP, WASL
Immune	LYM	EGFR, ERBB3, MET, IGF1-2R, LDLR, TFRC	**High**	DAPP1, ITSN2, WAS
	MC	CCR5, CXCR4, CSF1R, TGFBR1, IGF2R, FOLR3	**High**	DAPP1, ITSN2, WAS
	Mϕ	CCR5, CXCR4, CSF1R, FLT1(VEGFR1), TGFBR1, IGF2R, FOLR3, TFRC	**High**	DAPP1, ITSN1-2, UBC, UBD, WAS, WASL

Figure 3. Endocytic membrane receptor, Carom and Carom endocytic partner expression profile in human primary cells

A. Heat map of membrane receptor, Carom and Carom partner expression in human primary cells. mRNA levels are obtained from microarray data available in the web site (https://www.genevestigator.com/gv/) and expressed as heat map. Gradient bars indicate percent of expression potential. The dark and light color shadings represent relatively high and low expression levels, respectively. Dashed frames indicate body system and cells with relative high gene expression. **B. Relationship of Carom, endocytic membrane receptor and partner expression in human circulatory and immune system cells.** Noted that in aortic ECs, membrane receptor CXCR4, F2R, FLT1 (VEGFR1), KDR (VEGFR2), MET, IGF2R, LDLR and TFRC, and Carom endocytic partner GRASP, ITSN1-2, UBC, VCP and WASL are highly expressed. Carom is highly expressed in EC, LYM, MC, Mϕ and myoblast. Abbreviation: CMC, Cardiomyocyte; VSMC, vascular smooth muscle cell; EC, endothelial cell. LYM, lymphocyte; MC, Monocyte; Mϕ, Macrophage; GPCR, G-protein coupled receptor; RTK, Receptor tyrosine kinase; TRM, Transmembrane receptor; other refer to Table 1 and 3.

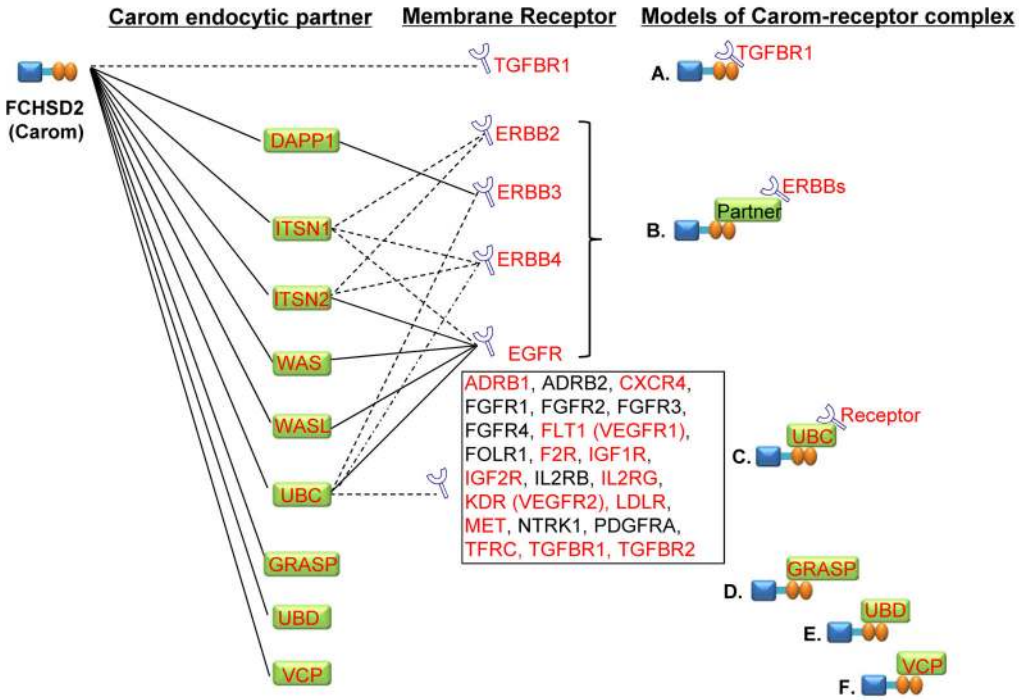


Figure 4. Models of Carom, endocytic partner and membrane receptor complexes

Interaction of membrane receptor with Carom and Carom partner were identified via NCBI and String databases. Solid lines indicate known interaction deposited in NCBI Gene database which was established from affinity capture-MS, affinity capture-RNA, affinity capture-western, reconstituted complex and two-hybrid experimental data. Dashed lines indicate computational-predicted interaction in String database based on analyzing genomic information ('genomic context'-methods) or from transferring associations/interactions between organisms ('interolog'-transfer). Letters in red indicate genes with comparable high level of expression paralleled with high levels Carom in human cells identified in Figure 3B. Noted that Carom may directly bind to TGBR1 (model A), indirectly interact with EGFR, ERBB2, ERBB3 and ERBB4 via Carom partner DAPP1, ITSN1, ITSN2, WAS, and WASL (model B), bind to receptors through the partner UBC (model C), and directly interact with partner GRASP, UBD and VCP (model D, E and F). Abbreviation: refer to Table 1 and 3.

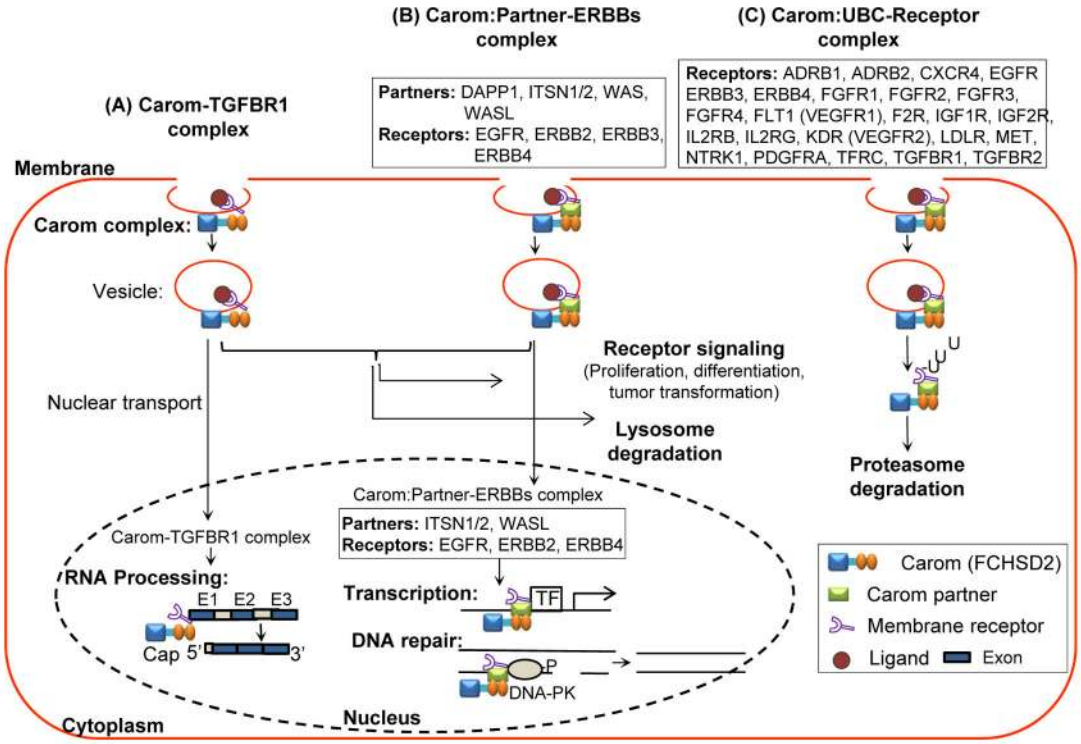


Figure 5. Hypothetic working model of Carom-mediated membrane receptor trafficking and endocytosis

Carom can form 3 types of receptor complexes and mediate membrane receptor trafficking and endocytosis. A) Carom-TGFBR1 complex can enter nucleus via nuclear localization signal and facilitate RNA processing. B) Carom:Partner-ERBBs complexes that Carom associate with receptor via its partner, such as Carom:DAPP1-ERBB3; Carom:ITSN1-ERBB2, RBB4, EGFR; Carom:ITSN2-ERBB2, ERBB4, EGFR; Carom:WAS-EGFR; Carom:WASL-EGFR (details in table 5). Carom:Partner-ERBBs complex (Carom:ITSN1-ERBB2, RBB4, EGFR; Carom:ITSN2-ERBB2, ERBB4, EGFR; Carom:WASL-EGFR) can be transported into nucleus, bind to transcriptional factor and promote transcription, or bind to damaged DNA to facilitate DNA repair via activating DNA-PK. Both Carom-TGFBR1 and Carom:Partner-ERBBs complexes can facilitate receptor signaling in the cytoplasm leading to proliferation, differentiation and tumor transformation, and can be degradated in lysosome. C) Carom:UBC-Receptor complexes may bind to its partner UBC which further bind to 21 membrane receptor and facilitate ubiquitination and proteasome degradation. Abbreviation: DNA-PK, DNA-protein kinase, others refer to Table 1 and 3.

Table 1
Classification of endocytic membrane receptor

We selected 34 cell surface receptors from 259 genes related to endocytosis identified from Kegg pathway database (hsa04144, http://www.genome.jp/dbget-bin/www.bget?hsa04144) and classified them into three groups: 1)G-protein coupled receptor, 2) Receptor tyrosine kinase, and 3) Transmembrane receptor. Ligand and function of receptors are identified using Genecard database (http://ww.genecards.org). Endocytosis type are defined by literature search. Noted that most of the receptor-mediated endocytosis generally occurs via CDE. Abbreviation: Leuc, leukocyte; PLT, platelets; CDE, Clathrin-dependent endocytosis; CIE, Clathrin-independent endocytosis; Indt, Clathrin/caveolin-independent endocytosis;

Gene (Symbol (Full name))	Ligand	Function	Endocytosis type
G-protein coupled receptor (GPCR)			
1. ADRB1 (Adrenoceptor β 1)	Epinephrine, norepinephrine	Mediate catecholamines action	CDE
2. ADRB2 (Adrenoceptor β 2)	Epinephrine, norepinephrine	Mediate catecholamines action	CDE
3. ADRB3 (Adrenoceptor β 3)	Norepinephrine	Mediate catecholamines action	CDE
4. CCR5 (Chemokine (C-C motif) receptor 5)	CCl3,CCl4,CCl5,CCl8,CCl13, CCl16	Leuc trafficking, angiogenesis, apoptosis	CDE
5. CXCR1 (Chemokine (C-X-C motif) receptor 1)	CXCl6,CXCl8	Leuc trafficking, angiogenesis, apoptosis	CDE
6. CXCR2 Chemokine (C-X-C motif) receptor 2)	CXCl1,CXCl2,CXCl3, CXCl5 CXCl6,CXCl7,CXCl8	Leuc trafficking, angiogenesis, apoptosis	CDE
7. CXCR4 (Chemokine (C-X-C motif) receptor 4)	CXCl14	Leuc trafficking, angiogenesis, apoptosis	CDE
8. F2R Coagulation factor II receptor)	Thrombin	PLT activation, vascular development	CDE
Receptor tyrosine kinase (RTK)			
9. CSF1R (Colony stimulating factor 1 receptor)	M-CSF,IL34	Macrophage regulator	CDE
10. EGFR (Epidermal growth factor receptor)	EGF	Proliferation, differentiation	CDE/CIE
11. ERBB2 (Erb-b2 receptor tyrosine kinase 2)	EGF	Proliferation, differentiation	CDE
12. ERBB3 (Erb-b2 receptor tyrosine kinase 3)	EGF	Proliferation, differentiation	CDE
13. ERBB4 (Erb-b2 receptor tyrosine kinase 4)	EGF	Proliferation, differentiation	CDE
14. FGFR1 (Fibroblast growth factor receptor 1)	FGF1,FGF2,FGF3,FGF6, FGF7	Proliferation, differentiation	CDE/CIE

Gene (Symbol (Full name))	Ligand	Function	Endocytosis type
15. FGFR2 (Fibroblast growth factor receptor 2)	FGF1,FGF4,FGF6,FGF7, FGF8	Proliferation, differentiation	CDE/CIE
16. FGFR3 (Fibroblast growth factor receptor 3)	FGF3,FGF4,FGF5,FGF6, FGF7	Proliferation, differentiation	CDE/CIE
17. FGFR4 (Fibroblast growth factor receptor 4)	FGF1,FGF3,FGF4,FGF5, FGF9	Proliferation, differentiation	CDE/CIE
18. FLT1 (Fms-related tyrosine kinase 1)/ VEGFR1 (Vascular endothelial growth factor receptor1)	VEGFA,VEGFB,PGF	Angiogenesis	CDE/CIE
19. IGF1R (Insulin-like growth factor 1 receptor)	IGF1,IGF2	Proliferation, differentiation	CDE/CIE
20. IGF2R (Insulin-like growth factor 2 receptor)	IGF2,Transferrin	Proliferation, differentiation	CDE
21. KDR (Kinase insert domain receptor)/ VEGFR2 (Vascular endothelial growth factor receptor2)	VEGFA,VEGFC	Proliferation, angiogenesis	CDE/CIE
22. MET (Tyrosine-protein kinase met)	HGF	Proliferation, angiogenesis	CDE
23. NTRK1 (Neurotrophic tyrosine kinase receptor type 1)	NGF	Differentiation	CDE
24. PDGFRA (Platelet-derived growth factor α receptor)	PDGFC	Proliferation, differentiation,	CDE
25. TGFBR1 (Transforming growth factor β receptor I)	TGF-β	Proliferation tumor transformation	CDE/CIE
26. TGFBR2 (Transforming growth factor β receptor I)	TGF-β	Proliferation, tumor transformation	CDE/CIE
Transmembrane receptor (TMR)			
27. FOLR1 (Folate receptor 1)	Folic acid	Transport folic acid	CDE
28. FOLR2 (Folate receptor 2)	Folic acid	Transport folic acid	CDE
29. FOLR3 (Folate receptor 3	Folic acid	Transport folic acid	CDE
30. IL2RA (Interleukin 2 receptor α)	IL2	Regulate immune system	Indt
31. IL2RB (Interleukin 2 receptor β)	IL2,IL15	Regulate immune system	Indt
32. IL2RG (Interleukin 2 receptor γ)	IL2,IL-4,IL15	Regulate immune system	Indt
33. LDLR (Low density lipoprotein receptor)	LDL, ApoB100, ApoE, IDL	Transport lipid	CDE

Gene (Symbol (Full name))	Ligand	Function	Endocytosis type
34. TFRC (Transferrin receptor)	Transferrin, HFE	Transport iron	CDE

Table 2

F-BAR proteins are involved in membrane receptor endocytosis

F-BAR protein have 9 family members, each protein contains one F-BAR domain and other domains such as SH3,SH2, WW, and RhoGAP. Most of the F-BAR protein are involved in endocytosis and play important roles in membrane receptor trafficking/signaling cited by PMID#. Symbols listed in the framed box indicate representative domains. Abbreviation: F-BAR, Fes/CIP4 homology-BAR; FX, F-BAR extension; HR1, Protein kinase C-related kinase homology region 1; NPF, Asparagine proline phenylalanine; RhoGAP, Rho GTPase-activating protein; SH2, Src homology-2; SH3, Src homology-3; μHD, μ-homology domain; CIP4, Cdc42-interacting protein 4; FBP17, Formin binding protein 17; Toca-1, TOCA homolog 1; FCHO1-2 , FCH domain only 1-2; srGAP1-3, SLIT-ROBO Rho GTPase activating protein 1-3; PSTPIP1- 2, Proline-serine-threonine phosphatase-interacting protein 1-2; FCHSD1-2, FCH and double SH3 domains 1-2; NOSTRIN, Nitric oxide synthase traffic inducer; GAS7, growth arrest specific 7; Others refer to Table 1.

F-BAR Proteins	Structure	Endocytosis	Roles in endocytosis	PMID#
1. CIP4 subfamily				
CIP4		✓	Required for EGFR trafficking /degradation	19632321
FBP17		✓	Required for EGFR internalization	19632321
Toca-1		✓	Required for EGFR trafficking from endosomes	19632321
2. FCHOs subfamily				
FCHO1		✓	Forming clathrin-coated vesicle	20448150
FCHO2		✓	Required for LDLR endocytosis	22323290
3. srGAPs subfamily				
srGAP1		N/A		
srGAP2		N/A		
srGAP3		N/A		
4. PACSINs subfamily				
PACSIN1		✓	Inhibiting endocytosis	11082044
PACSIN2		✓	Required for EGFR translocated to endosomes	23129763
PACSIN3		✓	Inhibiting transferrin/TFRC complex endocytosis	11082044
5. PSTPIPs subfamily				
PSTPIP1		✓	Suppressing transferrin/TFRC complex endocytosis	18480402
PSTPIP2		N/A		
6. FCHSDs subfamily				
FCHSD1		✓	Promoting F-actin polymerization and facilitate endocytosis	23437151
FCHSD2 (Carom)		✓	Stimulating F-actin polymerization and facilitate endocytosis	23437151
7. FES/FER subfamily				

F-BAR Proteins	Structure	Endocytosis	Roles in endocytosis	PMID#
FES		N/A		
FER		N/A		
8. NOSTRIN subfamily		✓	Assembling NOSTRIN-FGFR1-Rac1-Sos1 complex/regulate FGF signaling	22751148
9. GAS7 subfamily		N/A		

Table 3

Identification of F-BAR protein Carom endocytic partner involved in endocytosis

Nine Carom endocytic partners were selected based on literature review from 26 Carom partners we identified previously (Front Biosci (Landmark Ed), 2016; 21:856-72), which were established via Affinity Capture-MS, Affinity Capture-RNA, Affinity Capture-Western, Reconstituted Complex and Two-hybrid technologies. Functions and binding domains of Carom partners were defined based on information obtain from Genecard database. Binding receptor were identified from NCBI Gene database and its regulation on receptor were determined by PubMed search. All the Carom partners contain different domains such as SH2, PH, PDZ, EF and Ubiquitin which can recruit proteins to organize signaling complexes at cellular membranes. Noted that DAPP1 can bind to ERBB3, ITSN2/UBC/WAS/WASL bind to EGFR, and that ITSN1/UBC/UBD, VCP and WASL can regulate EGFR unbiquitilyation, degradation and endocytosis process. Abbreviation: CRIB, CDC42-Rac interactive binding; EF, EF hand; DH, DBL homology; AAA ATPase, ATPases associated with diverse cellular activities; PH, Pleckstrin homology; PDZ, PSD-95/Dlg-A/ZO-1; WH1/2, WASP-Homology 1/2; Others refer to Table 1. 1. Liu, S., et al., *Analysis for Carom complex, signaling and function by database mining.*

Carom endocytic partner Symbol (Full name)	Interaction identification approach	Function	Domains on partner	Binding Receptor	Receptor regulation
1. DAPP1 (B Lymphocyte Adapter Protein Bam32)	Two-hybrid	Regulates B-cell antigen receptor signaling	SH2,PH	ERBB3	N/A
2. GRASP (GRP1- Associated Scaffold Protein)	Two-hybrid	Regulate intracellular trafficking	PDZ	N/A	N/A
3. ITSN1 (Intersectin 1)	Two-hybrid	Regulate endocytic vesicle formation	EF,DH,PH,C2	N/A	EGFR ubiquitination
4. ITSN2 (Intersectin 2)	Two-hybrid	Regulate endocytic vesicle formation	EF,DH,PH,C2	EGFR	N/A
5. UBC (Ubiquitin C)	Affinity Capture-MS	Regulate protein ubiquitination	Ubiquitin	EGFR	EGFR ubiquitination
6. UBD (Ubiquitin D)	Affinity Capture-MS	Regulate protein ubiquitination	Ubiquitin	N/A	EGFR ubiquitination
7. VCP (Valosin Containing Protein)	Co- Immunoprecipitation Two-hybrid	Regulate vesicle trafficking	AAA ATPase	N/A	EGFR degradation
8. WAS (Wiskott-Aldrich Syndrome)	Two-hybrid	Regulate actin polymerization	WH1/2,PH,CRI B	EGFR	N/A
9. WASL (Wiskott-Aldrich Syndrome Like)	Two-hybrid	Regulate actin polymerization	WH1/2,PH,CRI B	EGFR	EGFR endocytosis

Table 4

Subcellular localization of Carom partner and membrane receptor, and co-localization with Carom (Compartments database/cNLS database)

Subcellular localization of Carom/partner and membrane receptors which can form 3 models Carom-receptor complex in Figure 4 were determined in Compartments database. **A. Subcellular localization and co-localization of Carom with endocytic partner. B. Subcellular localization of membrane receptor and co-localization with Carom**. Noted that most of the Carom partners and membrane receptors co-localize with Carom in PM, CP and NC. Some of Carom partner and membrane receptor contains NLS. 13 receptors can be potentially trans-localized to the nucleus because of the recognized NC localization and NLS, and labeled with numbers. Bolded words refer to molecules co-localized with Carom in subcellular sites as indicated in **A. Numbers** in parenthesis are confidence sores provided by Compartments database. Co-localization site with Carom are determined by present within the same cellular compartment and indicated by check marker (.). Nuclear localization signal are identified by using cNLS Mapper. Abbreviation: CP, cytoplasmic; CSK, cytoskeleton; E, endosome; ER, endoplasmic reticulum; EX, extracellular, Gol, golgi apparatus; Lys, lysosome; Mit, mitochondrion; NC, Nucleus; PM, plasma membrane, others refer to Table 2 and 3.

Table 4A. Subcellular localization of Carom and endocytic partner

Carom endocytic partner	Main subcellular locations (confidential levels)	Co-localization site with Carom				Nuclear localization signal
		NC	CSK	CP	PM	
Carom	**NC (5), CSK (2), CP (2), PM (2)**	√	√	√	√	√
DAPP1	**NC (2), CP (5), PM (5),**	√	√	N/A	√	√
ITSN1	**NC (2), CSK (2), CP (5), PM (5),** E (1)	√	√	√	√	√
ITSN2	**NC (2), CSK (5), CP (2), PM (2),** EX (5), Gol (1)	√	√	√	√	√
UBC	**NC (5), CSK (1), CP (5), PM (4),** E (4) EX (5),	√	√	√	√	N/A
WAS	**NC (4), CSK (5), CP (5), PM (2),** E (2), EX (5), Mit (1)	√	√	√	√	N/A
WASL	**NC (5), CSK (5), CP (5), PM (5),** E (1), EX (5), Gol (3), Mit (2)	√	√	√	√	√

Table 4B. Subcellular localization of endocytic membrane receptor

Membrane receptor	Main Subcellular locations (confidential levels)	Co-localization site with Carom				Nuclear localization signal
		NC	CSK	CP	PM	
ADRB1	**CP (1), PM (5),** E (5), ER (1), EX (2), Gol (1)	N/A	N/A	√	√	√
ADRB2	**NC (3), CSK (2), CP (1), PM (5),** E (5), ER (2), Lys (5)	√	√	√	√	N/A
1. CXCR4	**NC (2), CSK (2), CP (5), PM (5),** E (5), Lys (5)	√	√	√	√	√
2. EGFR	**NC (5), CSK (3), CP (5), PM (5),** E (5), ER (5), Gol (5),Lys (3),Mit (2)	√	√	√	√	√
3. ERBB2	**NC (5), CSK (2), CP (3), PM (5),** E (5), Lys (2)	√	√	√	√	√
ERBB3	**NC (2), CSK (3), CP (1), PM (5)**	√	√	√	√	N/A
4. ERBB4	**NC (5), CSK (1), CP (5), PM (5),** E (1), Lys (1),Mit (5)	√	√	√	√	√
FGFR1	**NC (5), CSK (2), CP (5), PM (5),** E (1), ER (1), Lys (1)	√	√	√	√	N/A
FGFR2	**NC (5), CSK (2), CP (1), PM (5),** E (1), ER (2), Gol (5), Lys (1)	√	√	√	√	N/A
FGFR3	**NC (3), CSK (2), PM (5),** ER (5), Gol (5), Lys (3)	√	√	N/A	√	N/A
FGFR4	**NC (5), CSK (2), CP (3), PM (5),** E (5), ER (5), Gol (5)	√	√	√	√	N/A
5. FLT1 (VEGFR1)	**NC (2), CSK (2), CP (2), PM (5),** E (5), ER (2), EX (2)	√	√	√	√	√
FOLR1	**NC (5), PM (5),** E (3), ER (4), EX (5),Gol (4)	√	N/A	N/A	√	N/A
F2R	**NC (1), CSK (2), CP (4), PM (5),** E (5), Gol (4)	√	√	√	√	N/A
6. IGF1R	**NC (2), CSK (2), CP (2), PM (5),** E (1), ER (1), EX (3), Mit (2)	√	√	√	√	√
7. IGF2R	**NC (4), CSK (1), CP (2), PM (5),** E (5), EX (5), Gol (5),Lys (4),Mit (1)	√	√	√	√	√
IL2RB	**CP (1), PM (5),** ER (1), EX (2), Gol (1)	N/A	N/A	√	√	N/A
IL2RG	**NC (1), CSK (1), CP (1), PM (5),** EX (3), Lys (1)	√	√	√	√	√
8. KDR (VEGFR2)	**NC (5), CSK (2), CP (2), PM (5),** E (5), ER (5), Gol (5), Lys (1),Mit (1)	√	√	√	√	√
9. LDLR	**NC (2), CSK (1), CP (2), PM (5),** E (5), ER (2), Gol (5), Lys (5),Mit (1)	√	√	√	√	√
10. MET	**NC (2), CSK (2), CP (3), PM (5),** ER (2)	√	√	√	√	√
11. NTRK1	**NC (5), CSK (5), CP (5), PM (5),** E (5), Gol (5), Lys (2),Mit (2)	√	√	√	√	√
12. PDGFRA	**NC (5), CSK (2), CP (3), PM (5),** EX (1)	√	√	√	√	√
TFRC	**NC (2), CSK (2), CP (3), PM (5),** E (5), ER (2), Lys (2),Mit (3)	√	√	√	√	N/A
13. TGFBR1	**NC (2), CSK (1), CP (1), PM (5),** E (2), ER (2)	√	√	√	√	√
TGFBR2	**NC (2), CSK (1), CP (5), PM (5),** Mit (2)	√	√	√	√	N/A

Table 5

Analysis of Carom:partner-receptor complex intracellular trafficking and function

Carom-receptor complex function are predicted based on its binding partner and receptor signaling reported in the literature (PMID#) referred. We characterized 3 different Carom-receptor complex intracellular trafficking patterns: 1) only in PM, 2) PM to CP/CSK and 3) PM to NC. In three Carom-receptor complex models, the Carom-TGFBR1 and Carom:partner-ERBBs complex can be transferred to CP and CSK via endocytosis for signaling transduction and lysosomal degradation. They can also be transported into the nucleus and participate in RNA processing, transcription regulation and DNA repair because of the recognized NC localization and the detected NLS, except for Carom:DAPP1-ERBB3 and Carom:WAS-EGFR complex. The Carom:UBC-receptor complexes involves a large group of 24 receptors for ubiquitination and proteasome degradation by UBC which lacks of NLS. Abbreviation: refer to Table 1, 3 and 4.

Carom-receptor complex models (Carom:Partner-receptor)	Predicted Carom complex trafficking				
	Only in PM	PM to CP		to NC	
		(Function)	PMID#	Function	PMID#
A. Carom-TGFBR1 complex		TGFBR1 signaling/ degradation	21295082	RNA processing	22473997
B. Carom:partner-ERBBs complex **Carom:**DAPP1-ERBB3		ERBB3 signaling/degradation	22436610	N/A	
Carom:ITSN1-ERBB2, ERBB4, EGFR		ERBB2/4, EGFR signaling/degradation	23472148	Transcriptional regulation/DNA repair	26719328
Carom:ITSN2-ERBB2, ERBB4, EGFR		ERBB2/4, EGFR signaling/degradation	23472148	Transcriptional regulation/DNA repair	20670598
Carom:WAS-EGFR		EGFR signaling/degradation	23472148	N/A	
Carom:WASL-EGFR		EGFR signaling/degradation	23472148	Transcriptional regulation/DNA repair	22127113
C. Carom:UBC-receptor complex **Carom:UBC**-ADRB1,ADRB2, CXCR4, EGFR, ERBB3, ERBB4, FGFR1, FGFR2, FGFR3, FGFR4, FOLTR1, FLT1 (VEGFR1), F2R, IGF1R, IGF2R,, IL2RB, IL2RG, KDR (VEGFR2), LDLR, MET, NTRK, PDGFRA, TFRC, TGFBR1, TGFBR2		Ubiquitination, degradation		N/A	