

ADAMs as mediators of EGF receptor transactivation by G protein-coupled receptors

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Ohtsu, Haruhiko, Peter J. Dempsey, and Satoru Eguchi. ADAMs as mediators of EGF receptor transactivation by G protein-coupled receptors. *Am J Physiol Cell Physiol* 291: C1–C10, 2006; doi:10.1152/ajpcell.00620.2005.—A disintegrin and metalloprotease (ADAM) is a membrane-anchored metalloprotease implicated in the ectodomain shedding of cell surface proteins, including the ligands for epidermal growth factor (EGF) receptors (EGFR)/ErbB. It has been well documented that the transactivation of the EGFR plays critical roles for many cellular functions, such as proliferation and migration mediated through multiple G protein-coupled receptors (GPCRs). Recent accumulating evidence has suggested that ADAMs are the key metalloproteases activated by several GPCR agonists to produce a mature EGFR ligand leading to the EGFR transactivation. In this review, we describe the current knowledge on ADAMs implicated in mediating EGFR transactivation. The major focus of the review will be on the possible upstream mechanisms of ADAM activation by GPCRs as well as downstream signal transduction and the pathophysiological significances of ADAM-dependent EGFR transactivation.

ectodomain shedding; angiotensin II

THE EPIDERMAL GROWTH FACTOR (EGF)/ErbB family of type I receptor tyrosine kinases participate in various cellular functions, such as proliferation, migration, differentiation, and survival (44). The ErbB receptor family has four members, EGF receptor (EGFR)/ErbB1/HER1, ErbB2/Neu/HER2, ErbB3/HER3, and ErbB4/HER4. All members have a common extracellular ligand-binding region, a single membrane-spanning region, and a cytoplasmic protein tyrosine kinase domain. A family of ligands, the EGF-related peptide growth factors, binds to the extracellular domain of ErbB receptors inducing the formation of homo- and heterodimer of the receptors. As a consequence, the intrinsic tyrosine kinase domain is activated, resulting in phosphorylation of specific tyrosine residues within the cytoplasmic tail of the receptor. These autophosphorylated residues serve as docking sites for a variety of signaling molecules, some of them being substrates of the receptor, whose recruitment leads to the activation of intracellular signaling pathways (44). The EGF ligand family consists of EGF, heparin binding EGF-like growth factor (HB-EGF), transforming growth factor- α (TGF- α), epiregulin, amphiregulin, epigen, β -cellulin (BTC), and four neuregulins (NRG-1, 2, 3, and 4), and each ligand displays overlapping but distinct binding affinities toward ErbB receptors (40, 44, 97). No direct high-affinity ligand for ErbB2 has been identified and prevailing evidence suggests that the primary function of ErbB2 is to act as a coreceptor. Indeed, ErbB2 is the preferred heterodimerization partner for all ErbB family members and, as such, plays

an important role in the potentiation and diversification of ErbB receptor signaling. This is best exemplified in the case of ErbB2-ErbB3 heterodimers where heterodimerization of kinase-impaired ErbB3 with ligandless ErbB2 produces a potent receptor signaling complex (15). Thus activation of ErbB homo- and heterodimers by the different ErbB ligands could create multiple combinations of distinct signal transduction events. Importantly, all EGF ligand family members are made as inactive transmembrane precursors that can undergo ectodomain proteolytic cleavage to release mature active growth factor (40, 44). However, until recently, there has been a paucity of information regarding regulation and the identity of the proteases that are critical to stimulate posttranslational proteolytic “ectodomain shedding” of the ErbB ligands.

Several G protein-coupled receptors (GPCRs) have been demonstrated to activate EGFR/ErbB (an event referred to as transactivation), even though the GPCR agonists do not directly interact with EGFR. Transactivation of EGFR by some GPCR agonists was originally reported in 1996 (16), and is now attributed to a wide variety of GPCR agonists, including thrombin, ANG II, endothelin-1 (ET-1), carbachol, and lysophosphatidic acid (LPA) (37). The EGFR transactivation by GPCRs appears to mediate several critical downstream signals and functions, such as ERK activation, *c-fos* induction, and cell proliferation (22, 37, 75). The mechanism of EGFR transactivation and its pathological significance are currently one of the major topics of signal transduction research and recently several interesting findings have been reported on possible components involved in the EGFR transactivation. First, EGFR transactivation by GPCRs appears to require second messengers directly and/or signal transduction pathways operated by second messengers, such as elevation of intracellular Ca^{2+} (23,

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119), activation of protein kinase C (PKC) (103) and generation of reactive oxygen species (ROS) (28, 29, 70). Second, cytosolic nonreceptor tyrosine kinase such as Src and PYK2 may be involved in the EGFR transactivation (3, 61). Third, a metalloprotease is required for the EGFR transactivation by several GPCR agonists (21, 79). A series of hydroxamate-based metalloprotease inhibitors, which inhibit a disintegrin and metalloprotease (ADAM) and/or matrix metalloprotease (MMP), appear to block EGFR transactivation induced by many GPCR agonists (7, 59, 79, 83). In the following section of this review, we will focus our discussion on the signal transduction of GPCR-induced EGFR ligand shedding and subsequent EGFR transactivation through ADAM family metalloproteases.

THE BIOLOGY OF ADAMs

To date, most studies have implicated an important role for the family of membrane-anchored disintegrin-metalloproteases, ADAMs, in the ectodomain shedding of ErbB ligand precursors to produce a mature ligand under physiological and pathological conditions (9, 18). ADAMs belong to the metzincin superfamily and 40 ADAM orthologues have been identified, from protozoans to mammals (see http://www.people.virginia.edu/~7Ejw7g/Table_of_the_ADAMs.html). In mammals, ADAM2, 7, 18, 20, 21, 29, and 30 are predominantly expressed in the testis and associated structures, whereas ADAM8, 9, 10, 11, 12, 15, 17, 19, 22, 23, 28, and 33 show more broad somatic distribution (87).

A prototypical ADAM consists of a series of conserved protein domains: an NH₂-terminal signal sequence, followed by a pro-domain, a metalloprotease domain, a disintegrin domain, a cysteine-rich region, an EGF-like domain, a transmembrane domain, and a cytoplasmic domain (Fig. 1). However, only one-half of the ADAMs contain a consensus (HEXXH) motif within the catalytic site of the metalloprotease domain. Several of these ADAMs have been shown to be catalytically active, which implies that other ADAMs that contain the HEXXH sequence should also possess catalytic activity (9). The pro-domain of catalytically active ADAMs is believed to function as an intramolecular chaperone. Once an ADAM is properly folded, the pro-domain keeps the enzyme inactive until it is removed by a furin-type pro-protein convertase or possibly by autocatalysis. This pro-domain removal is likely to occur during transit through the *trans*-Golgi network but may also occur during later stages of the secretory pathway. Similar to MMPs, the cysteine switch box in the pro-domain is proposed to keep the metalloprotease domain of

ADAMs in an inactive state (9, 87). However, one report (33) demonstrates that the cysteine switch is not essential for inhibition of the ADAM17 enzymatic activity but rather the entire pro-domain has an inhibitory function.

ADAMs AS SHEDDASES

Previous studies have reported (18, 46) that EGFR ligands such as amphiregulin, BTC, EGF, epiregulin, HB-EGF, neu-regulins, and TGF- α are cleaved by multiple ADAMs, including ADAM9, 10, 12, 15, 17, and 19. In addition to these EGFR ligands, many ADAMs have multiple substrates, and thereby appear to be involved in various signaling pathways and cellular functions (46, 87). For example, the membrane-anchored cytokine TNF- α and the chemokines CX3CL-1 and CXCL-16 are cleaved by ADAM17/TNF- α -converting enzyme and/or other ADAMs (46). Interestingly, ADAM17 can cleave several membrane receptors, including ErbB4, TNF receptor-I and -II, colony-stimulating factor-I receptor, hepatocyte growth factor receptor Met, and nerve growth factor receptor (see Refs. 46 and 87 for reviews). In most cases, shedding of receptors would be predicted to lead to the termination of signal transduction and generation of soluble decoy receptors. In other cases, ectodomain cleavage may provide the permissive conditions for regulated intramembrane processing and the generation of intracellular and nuclear signaling events, as first described for Notch by Wolfe and Kopan (110). A further variation on this scenario has been reported for an ErbB4 receptor isoform where ligand-induced ectodomain cleavage is not only required to facilitate the release and translocation to the nucleus of the ErbB intracellular domain but is also necessary for the generation of the appropriate signaling events through the released kinase domain (5, 69).

ADAM KNOCKOUT MICE

To investigate the physiological functions of ADAM family, knockout mice have been generated. *ADAM17*^{-/-} mice resemble mice lacking TGF- α or EGFR because they have multiple defects in the maturation and morphogenesis of epithelial structures, including a failure to undergo eyelid fusion. Furthermore, *ADAM17*^{-/-} cells are defective in TGF- α shedding (74). More recent studies have uncovered additional defects in *ADAM17*^{-/-} mice that might also result from lack of EGFR-ligand processing. These include defects in branching morphogenesis of the lung (116), thickened and misshapen heart valves that resemble those of mice lacking HB-EGF (49), and regulation of amphiregulin-dependent mammary gland mor-

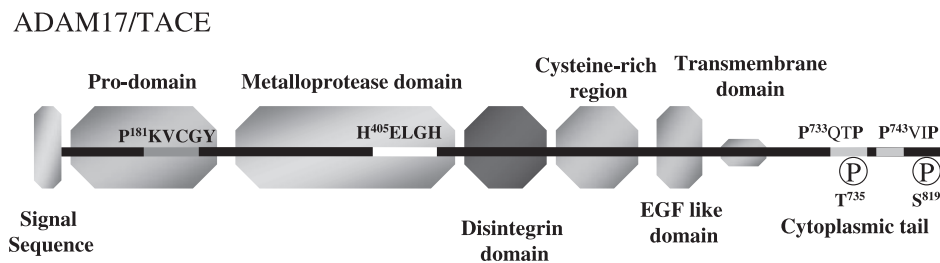


Fig. 1. Structure of a disintegrin and metalloprotease (ADAM). A prototypical ADAM, human ADAM17/TNF- α converting enzyme (TACE), structure is shown with consensus domains. PKVCGY, cysteine switch box motif, is located in pro-domain, and HEXXH (HEGLH), a catalytic-site consensus motif, is located in the metalloprotease domain. Thr⁷³⁵ and Ser⁸¹⁹ have been demonstrated as cytoplasmic phosphorylation (P) sites (24, 93). In addition, PXXP motifs are predicted to be associated with a SH3 domain of ADAM interacting proteins. EGF, epidermal growth factor.

phogenesis (94), further suggesting a role for ADAM17 in the ectodomain shedding of TGF- α , HB-EGF, and amphiregulin (see Ref. 9 for review). *ADAM10*^{-/-} mice die at *embryonic day 9.5* with malformed vessels in their yolk sacs, possibly due to defects in Notch signaling (42). In *ADAM12*^{-/-} mice, 30% of these animals did not survive and showed defects of brown adipose tissue (55). In *ADAM15*^{-/-} mice, the growth of implanted tumor cells was strongly inhibited, suggesting a role of ADAM15 in neovascularization (45). The heart valve defects observed in *ADAM19*^{-/-} mice indicate the involvement of ADAM19 in development of endocardial cushion. However, no evidence of pathological or developmental defects was observed in *ADAM8*^{-/-}, *ADAM9*^{-/-}, and *ADAM33*^{-/-} mice (9, 46).

ADAMs ARE REQUIRED FOR EGFR TRANSACTIVATION BY GPCRs

Several ADAM family members have been shown to mediate EGFR transactivation induced by GPCRs in various cells/tissues (Table 1). However, the specificity and regulation of ADAMs involved in GPCR-induced EGFR transactivation is complex and depends on GPCR agonists and cell types under investigation.

A cardiovascular hormone, ANG II, signals mainly through the angiotensin type I (AT₁) receptor (17, 35, 95, 102, 113). It has been demonstrated that ANG II via AT₁ induces EGFR transactivation through HB-EGF shedding in various cells (30, 100), such as vascular smooth muscle cells (VSMCs) (21). In three distinct cell types, ADAM17 appears to mediate HB-EGF shedding and subsequent EGFR transactivation induced by ANG II (64, 71, 86). Whereas ADAM17 is required for ANG II-induced TGF- α shedding and EGFR transactivation in the kidney (58), ADAM12 is responsible for ANG II-induced HB-EGF shedding in cardiac myocytes (7).

LPA is a bioactive phospholipid that binds a subfamily of GPCRs belonging to the LPA receptors (LPA₁₋₄) (4). LPA induces EGFR transactivation leading to ERK activation and cell growth (54). The roles of ADAM in mediating LPA-induced EGFR transactivation have been studied in several cancer cell lines. In kidney cancer cell lines, HB-EGF shedding

and subsequent EGFR transactivation induced by LPA is mediated through ADAM10 in ACHN cells, whereas ADAM17 is responsible for these events in CaKi2 cells and A498 cells (85). Similarly, in a squamous cell carcinoma cell line, SCC-9, ADAM17 mediates amphiregulin shedding and EGFR transactivation by LPA (36). By contrast, in bladder cancer cell lines, ADAM15 mediates LPA-induced shedding of TGF- α and amphiregulin in TccSup cells and in 5637 cells, respectively (85, 86).

Other GPCR agonists known to stimulate ADAM-dependent EGFR transactivation include phenylephrine, bombesin, platelet-activating factor, IL-8, and carbachol (7, 36, 59, 99, 112). Though numerous studies have demonstrated that ErbB ligand shedding and ErbB transactivation by various GPCRs is mediated by several ADAMs, including ADAM10, 12, 15, and 17, there is still no clear understanding about the specific requirements for individual ADAMs or ErbB ligands for these GPCR-induced ErbB transactivation events.

In addition, although the above reports strongly indicate a general requirement of ADAM in EGFR transactivation by GPCRs, there are a few exceptions demonstrating the involvement of MMPs in this process. In gonadotropic cells, gonadotropin-releasing hormone through its G_q-coupled gonadotropin-releasing hormone receptor transactivates EGFR via HB-EGF that appear to require MMP2 and MMP9 (81). In addition, MMP7 mediates HB-EGF shedding and EGFR transactivation in phenylephrine-stimulated arteries (39). These data are in agreement with the ability of some MMPs to cleave proHB-EGF to produce mature HB-EGF (96, 114), thus suggesting the possible participation of MMPs in GPCR-induced EGFR transactivation in some settings.

GPCR stimulation results in ADAM-dependent shedding of other proteins as well. Chemotactic GPCR agonists stimulated a metalloprotease-dependent IL-6 receptor shedding in neutrophils (62). In neuronal cells, stimulation of 5HT_{2B} receptors or α_{1D} receptors resulted in TNF- α shedding through ADAM17 (77). In astrocytoma cells, P2Y₂ receptors stimulated ADAM10/17-dependent shedding of amyloid precursor protein (14). Moreover, ADAM10 and 17 stimulation in particular cells generate the chemokine family of the GPCR ligands

Table 1. ADAM-dependent EGFR transactivation by GPCRs

Agonist	GPCR	ADAM Type	EGFR Ligand	EGFR	Cell, Tissue/Function	Reference No.
ANG II	AT ₁ *	17	HB-EGF	EGFR	ACHN tumor cell	86
	AT ₁	17	HB-EGF	EGFR	COS7 cell	64
	AT ₁ *	17†	TGF- α	EGFR	kidney/renal damage	58
LPA	LPA receptor ₁₋₄ *	10	HB-EGF	EGFR	ACHN tumor cell	86
		17	HB-EGF	EGFR	CaKi2, A498 kidney carcinoma/migration	85
		17	amphiregulin	EGFR	SCC cells/proliferation, migration	36
		15	TGF- α	EGFR/ErbB2	TccSup bladder carcinoma	85, 86
		15	amphiregulin	EGFR	TccSup and 5637 bladder carcinoma	85, 86
IL-8	CXCR 1 or 2*	10	HB-EGF amphiregulin	EGFR	KATO III gastric cancer cells	99
LTA	PAFR	10	HB-EGF	EGFR	epithelial cell/mucin production‡	59
Phenylephrine	α_1 AR*	12	HB-EGF	EGFR	cardiac myocyte/hypertrophy	7
Carbachol		17	amphiregulin	EGFR	SCC cells	36
Bombesin	BomR*	10	HB-EGF	EGFR	COS7 cell/prostate cancer cells	112

ADAM, a disintegrin and metalloprotease; AT₁, angiotensin type 1; HB, heparin binding; EGFR, epidermal growth factor (EGF) receptors; TGF- α , transforming growth factor- α ; GPCR, G protein-coupled receptor; CXCR, chemokine receptor; PAFR, platelet-activating factor receptor; ET-1, endothelin-1; LPA, lysophosphatidic acid; LTA, LPA transactivation; SCC, squamous cell carcinoma. *These receptors likely mediate the GPCR agonist function; †determined by a pharmacological inhibitor; ‡cells were stably transfected with mucin promoter.

(CX3CL1, CXCL-16) by shedding (1, 32, 34, 104). These data further indicate the important roles of ADAM in regulation of other GPCR-dependent functions.

MECHANISM OF ADAM FAMILY ACTIVATION BY GPCR

G Protein and Second Messengers

As mentioned above, multiple GPCRs are able to mediate ADAM-dependent EGFR transactivation, suggesting the involvement of two or more distinct heterotrimeric G proteins and their subunits in the ADAM activation. In this regard, requirement of G $\beta\gamma$ -subunits dissociated from activated G α_i was proposed to signal to c-Src, leading to HB-EGF shedding in COS7 cells stimulated by an α_{2A} -adrenergic receptor agonist (76). A Src inhibitor, PP1, blocked BTC shedding and EGFR transactivation stimulated by glucagon-like peptide 1 in pancreatic β -cells (12). By contrast, another Src inhibitor, PP2, did not affect TGF- α release but partially blocked EGFR transactivation induced by carbachol in colonic epithelial cells (63). However, the identities of the ADAM metalloprotease involved in these responses have not been determined. It should be noted that the aforementioned amphiregulin shedding and EGFR transactivation by LPA in SCC-9 mediated through ADAM17 was partially blocked by pertussis toxin (36). Pertussis toxin also inhibited LPA- or S1P-induced EGFR transactivation in MDA-MB-231 cells, which requires ADAM17-dependent HB-EGF generation (41). These data suggest that G $_i$ may be involved in the ADAM17 activation in response to particular GPCRs capable of coupling to G $_i$. Alternatively, requirements of PLC for HB-EGF shedding and ADAM17-dependent EGFR transactivation by the AT $_1$ receptor, which is mainly coupled to G $_q$, indicate the participation of G $_q$ for ADAM17 activation (64). In fact, overexpression of G $_q$ inhibitory mini-gene blocked HB-EGF shedding through the AT $_1$ receptor and no HB-EGF shedding was observed by an AT $_1$ mutant lacking G $_q$ coupling (64).

Little is known regarding the detailed upstream mechanisms involving G protein-derived second messengers and their effectors for ADAM activation by GPCR agonists. In many experimental systems, PKC activating phorbol esters stimulated ADAMs (46). In ADAM17 $^{-/-}$ mouse embryonic fibroblasts, shedding of TGF- α , amphiregulin, epiregulin, or HB-EGF induced by the phorbol ester, phorbol 12-myristate 13-acetate (PMA) was significantly decreased compared with wild-type cells (82), suggesting a critical role of PKC in ADAM17 activation. TGF- α and neuregulin shedding induced by PMA was also lost in ADAM17 $^{-/-}$ fibroblasts (65, 74). Neuregulin shedding by PMA was impaired in ADAM19 $^{-/-}$ fibroblasts (56), whereas PMA-stimulated HB-EGF shedding was reduced in ADAM12 $^{-/-}$ fibroblasts (55). In addition, PKC- δ has been shown to mediate HB-EGF shedding by PMA through its interaction with ADAM9 (48). Similarly, PKC-dependent metalloprotease activation involving Src has been proposed to mediate HB-EGF shedding and subsequent EGFR transactivation induced by gonadotropin-releasing hormone receptors and the AT $_1$ receptors in particular cell lines (91, 92). By contrast, LPA-induced HB-EGF shedding was independent of the PKC cascade but rather required an unidentified ADAM activated by the Ras/ERK cascade (105). On the basis of these findings, further investigations are necessary to determine the

role of PKC as an upstream candidate of the ADAM activation by GPCR agonists.

In addition to PKC, Ca $^{2+}$ and ROS are likely candidates to be involved in ADAM activation induced by GPCR agonists. In the human glioma cell line U251MG, CD44 cleavage by ADAM10 was stimulated through Ca $^{2+}$ influx and association between ADAM10 and calmodulin, whereas CD44 cleavage by ADAM17 was regulated through Rac activation via PKC (68). A Ca $^{2+}$ ionophore also specifically activated ADAM10 to mediate BTC shedding (84). Regarding ROS, a previous report (115) suggested that PMA activates ADAM17 through ROS generation in a monocytic cell line. H $_2$ O $_2$ -induced EGFR transactivation appears to be mediated by ADAM10 and 17 and to a lesser ADAM9 in COS7 cells, and by ADAM9, and 17 in NCI-H292 cells (26). Moreover, ROS mediated ADAM17-dependent TNF- α shedding activated by 5HT $_{2B}$ receptors and α_{1D} receptors (77). Ca $^{2+}$ may also signal to ROS to activate ADAMs. HB-EGF shedding through AT $_1$ receptor was mediated through ADAM17 activated through intracellular Ca $^{2+}$ elevation and ROS generation in COS7 cells and that Ca $^{2+}$ seems to exist upstream of ROS in the pathway (64).

ROS production may directly cause posttranslational modifications of ADAM that lead to its activation. As previously mentioned, a common feature of most metzincins including ADAMs and MMPs is that the pro-domain contains a regulatory cysteine switch box "PKVCGY". In the case of ADAM17, it has been proposed that ROS induces the oxidation of thiol group from the cysteine residue in the inhibitory motif of pro-domain, thereby interfering with its coordination with zinc in the catalytic domain to activate the latent form of the enzyme (115). However, recent studies (11, 33) have argued against the inhibitory role of the cysteine switch in ADAM17. Alternatively, because other cysteine residues (Cys 225 , Cys 600) within the ADAM17 catalytic domain and cysteine-rich domain appear to have regulatory function (60), it raises the possibility that these and possibly other residues within the ADAM ectodomain may be ROS targets. It is also possible that ROS may indirectly activate ADAM through modification of other accessory proteins or by activation of downstream protein kinases as described below. Taken together, second messengers and their direct downstream kinases, such as PKC, Ca $^{2+}$, and ROS, could be involved in ADAM activation induced by GPCRs.

Phosphorylation of ADAM

Although the detailed mechanism of ADAM activation by these signaling molecules has not been elucidated, there are some clues. First, phosphorylation of ADAM through a protein kinase activated by the second messengers may be involved in ADAM activation. Diaz-Rodriguez et al. (20) have shown that extracellular signal-regulated kinase (ERK) is associated with ADAM17 in response to PMA to phosphorylate ADAM17 at Thr 735 , which is partially required for TrkA receptor shedding in Chinese hamster ovary cells. The ERK-dependent phosphorylation of Thr 735 is also indispensable for maturation and inducible trafficking of ADAM17 to the cell surface (93). In addition, ERK-dependent ADAM17 Ser 819 phosphorylation but not any Thr or Tyr residues was reported in Chinese hamster ovary cells stimulated with fibroblast growth factor, although the functional role of this phosphorylation site re-

mains unknown (24). Similarly, p38MAPK seems to exist upstream of ADAMs, leading to HB-EGF shedding and EGFR transactivation induced by environmental stress, including ROS (26). However, MAPKs usually exist downstream of EGFR transactivation by GPCR agonists, implying the presence of additional Ser/Thr ADAM kinase(s) activated by GPCRs. Alternatively, it has been shown that PTPH1, a protein tyrosine phosphatase, can interact with ADAM17 through its PDZ domain and TNF- α shedding induced by PMA was inhibited by overexpression of PTPH1 (117), suggesting the possible involvement of tyrosine phosphorylation in the ADAM activation. To support this notion, c-Src has been implicated in metalloprotease-dependent EGFR transactivation by α_{2A} AR (76). In addition, Src, cAbl, and phosphorylation of the AT₁ at Tyr³¹⁹ are required for ANG II-induced EGFR transactivation (88, 89, 106). However, whether these signaling components exist upstream of ADAM activation by the AT₁ remains unclear.

Protein-Protein Interaction and Localization

Besides phosphorylation, ADAM activity could be regulated through direct or indirect protein-protein interactions. In this regard, the cytoplasmic domain of many ADAMs possesses several specific protein interaction domains such as PXXP motif to presumably interact with Src homology 3 (SH3) domain-containing proteins (Fig. 2). In fact, several direct ADAM interacting proteins have been identified and include kinases, adaptors, or substrates (87). PACSIN3 can interact with ADAM9, 10, 12, 15, and 19 (66). PACSIN3 associates with ADAM12 through its SH3 domain and is required for HB-EGF shedding induced by PMA and in part by ANG II in HT1080 cells. Eve-1 has several SH3 domains and proline-rich SH3 domain binding motifs. Eve-1 can be associated with ADAM9, 10, 15, and 17 through its SH3 domain and is required for HB-EGF shedding induced by PMA or ANG II (98). Other ADAM-interacting proteins include Grb2, phosphatidylinositol 3-kinase, p85 α , Src, endophilin I, SH3Pxl, and Fish (2, 87). These findings, together with ADAM cytoplasmic domain overexpression studies (31, 48), indicate a regulation of ADAM through cytoplasmic domain phosphorylation and/or protein-protein interaction. Surprisingly, the cytoplasmic domain of ADAM may not be essential for catalytic activity for ADAM17-dependent shedding of TGF- α or TNF- α shedding stimulated by FBS or PMA, respectively (24, 80). However, one may speculate that under more physiological

conditions, the ADAM cytoplasmic domain may have important regulatory functions. For example, the ADAM17 cytoplasmic domain may have a negative regulatory function linking regulated trafficking through the secretory pathway with functional catalytic activity (93).

Indeed, the trafficking and compartmentalization of substrate(s) and different components of the shedding machinery may provide temporal and spatial control for rapid induction of ectodomain shedding. The efficient and selective induction of substrate shedding by ADAMs leads to an interesting proposal of an integrated ADAM activation mechanism involving the substrates, ADAM metalloprotease activity and possibly other bridging/accessory protein(s), such as tetraspanins and integrins (67). Several integrins have been shown to interact with ADAMs via ADAM disintegrin domain (109). Similarly, tetraspanins are a family of widely expressed four-transmembrane-domain proteins that can form complexes with integrins implicated in signal transduction, compartmentalization, and trafficking (8). The direct association between the tetraspanin transmembrane protein CD9, HB-EGF and ADAM10 was induced in COS7 cells stimulated by bombesin (112). Also, integrins $\alpha_3\beta_1$ and $\alpha_6\beta_1$ can associate with CD9, ADAM10, and HB-EGF (67). These proteins may interact at lipid rafts (a microdomain of the local cholesterol-enriched plasma membrane) providing the spatial compartmentalization of the proteolytic machinery for regulated ADAM-dependent ectodomain shedding. In support of this theory, ADAM19-mediated ectodomain shedding is localized to the lipid rafts (108), and decreasing cellular cholesterol and disruption of the rafts increase the shedding activity of ADAM10 and ADAM17 (67). Importantly, the lipid raft may provide an environment for inducible association between a GPCR and the EGFR, such as between AT₁ and EGFR (72, 118). In fact, the interaction of AT₁ with caveolin-1 is essential for the trafficking of the AT₁ into the lipid raft and subsequent EGFR transactivation (118). Similarly, it has been hypothesized that β -arrestin-mediated endocytosis is involved in the metalloprotease-dependent EGFR transactivation by GPCRs at signaling microdomains such as clathrin coated pits (75). Therefore, it is likely that ADAM activation by GPCR and the resulting EGFR transactivation may require trafficking and compartmentalization of GPCR and ADAM providing temporal and spatial regulation necessary for the rapid and specific activation of the signaling events. Interestingly, several recent studies have reported that ADAM-dependent ectodomain shedding and the resultant re-

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ADAM10 -----KICSVHT-----PSSNPK
ADAM12 KRKTLIRLLFTNKKTTIEKLRRCVRFPSRPPRGFQPCQAHHLGHLGKGLMRKPPDSYPPKDNPR
ADAM15 -----GASYWYRARLHQRLCQLKGPCTQYRAAQSG-----PSERPQ
ADAM17 -----DKKLDKQYESLSLFFHPSNVEMLSMDSASVRIK-----PFPAPQ

ADAM10 -----LP-----PPKPLPGFLKRR--RPPQPI
ADAM12 RLLQCQNVDISRPLNGLNVPPQSTQRVLPPLHRAPRAPSVPARPLPAKPALR--QAQGTG
ADAM15 -----PPQRALLARGTKQASALSFPAPPSPRLPDPVSKRLQSQGPA
ADAM17 -----PGRLOPAPVIPSAPAAPKLDHQRMDTIQEDPST

ADAM10 QQPQRQRPRESYQMGHMRR-----
ADAM12 KPNPPQKPLPADPLARTTRLTHALARTPGQWETGLRLAPLRPAQYPHQVPRSTHTAYIK
ADAM15 KPPPRKPLPADPQGRCPGDLPGGAG-----IPPLVVP@RPAPPPPTVSSLYL-
ADAM17 DSHMDEDFEKDPFPNSSTAAKSFEDLTDHPVTRSEKAA@FKLQRQNRVDSKETEC-----

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Fig. 2. The cytoplasmic tails of ADAMs involved in EGF receptor (EGFR) transactivation by G protein-coupled receptors (GPCRs). The alignment of the human ADAMs was made using CLUSTALW software and the motifs were identified with Scansite (<http://scansite.mit.edu>). Predicted SH3 domains are underlined, with the central proline residue in bold-face text. The threonine residue in ADAM10, serine residue in ADAM15, and threonine (T) and serine (S) residues (in open italic text) in ADAM17 are predicted phosphorylation sites by PKC α / β / γ , PKC ζ , p38MAPK, and PKC α / β / γ / δ , respectively.

lease of soluble proteins, such as TNF receptor 1 (43) and the L1 adhesion molecule CD171 (38), may be regulated through exosome-like vesicles. Exosomes are small membrane-enclosed vesicles that correspond to the internal vesicles of endolysosome-related multivesicular bodies and are released from the cell via exocytic fusion with the plasma membrane (19). At present, the relationship between exosomal shedding and GPCR-mediated shedding events and particularly EGFR transactivation has not been explored. Taken together, GPCR-dependent signal transduction (G proteins, second messengers), ADAM kinases and interacting proteins together with specific membrane localization such as in lipid rafts might be involved in ADAM activation by GPCRs leading to EGFR ligand shedding and subsequent EGFR transactivation. The considered ADAM activation mechanisms by GPCRs are illustrated in Fig. 3.

Recently, Janes et al. (50) demonstrated that ADAM10 constitutively associates with the ephrin binding domain of the Eph receptor. ADAM17 activation leads to ectodomain shedding of the ligand only in *trans* with ADAM10 and the substrate being on the membranes of opposing cells. This is in contrast with other characterized ADAM-dependent shedding, which occurs in *cis* (ADAM and substrate interact within the same cells) (9). However, whether GPCR mediates the ADAM-dependent shedding in *trans* remain unclear.

DOWNSTREAM SIGNAL TRANSDUCTION AND FUNCTION OF ADAM-DEPENDENT EGFR ACTIVATION

GPCR-induced EGFR transactivation regulates various cellular functions such as proliferation, hypertrophy, and migration through its downstream signal transduction pathways (22, 27). These pathways include the Ras/Raf/MEK/ERK pathway and the phosphatidylinositol 3-kinase/Akt pathway that usually exist downstream of the EGFR (22, 37, 75, 90). Depending on the EGFR ligand produced by ADAM activity, distinct combinations of EGFR/ErbB receptor homo- and heterodimers could be formed and lead to unique downstream signaling. In

TccSup cells, LPA stimulated production of amphiregulin and TGF- α by ADAM15 and activated not only EGFR but also ErbB2/Neu, suggesting a heterodimer formation between EGFR and ErbB2 (86).

As expected, ADAM is critical in the activation of the Ras/ERK pathway by several GPCR agonists. It was reported that pharmacological metalloprotease inhibitors blocked EGFR transactivation and subsequent ERK activation induced by several GPCR agonists such as LPA, ANG II, and ET-1 (21, 79). Selective inhibition of ADAM isoform by small interfering RNA (siRNA) showed that ADAM17, but not ADAM12, is required for phosphorylation of Shc and ERK induced by LPA or carbachol in SCC-9 cells (36). Yan et al. (112) have also shown that overexpression of a dominant negative ADAM10 inhibited Ras and ERK activation induced by bombesin in COS7 cells. Akt/PKB, a serine/threonine kinase, has also been shown to exist downstream of EGFR transactivation induced by GPCRs. Indeed, ADAMs are involved in the Akt/PKB pathway activated by GPCRs. This is exemplified in SCC-9 cells, where phosphorylation of Akt/PKB induced by LPA and carbachol was inhibited by BB94 or siRNA created against ADAM17 (36).

In addition to ERK or Akt, other MAPKs, JNK, and p38MAPK, may exist downstream of ADAM activation depending on the cell type. Schafer et al. (85) have shown that BB94 inhibited JNK and p38MAPK phosphorylation induced by LPA in TccSup cells, whereas we have shown that BB94 and BB2116, a metalloprotease inhibitor, diminished activation of p38MAPK but not JNK induced by ANG II in VSMCs (21).

Through these downstream pathways, ADAM activation induced by GPCR agonists regulates several cellular functions. In regard to cell growth and hypertrophy, DNA synthesis of ACHN cells induced by ANG II was inhibited by dominant negative ADAM17, but not ADAM10, 12, or 15. (86). In cardiac myocytes, ADAM12 was proposed to mediate cardiac hypertrophy induced by phenylephrine, ANG II and ET-1.

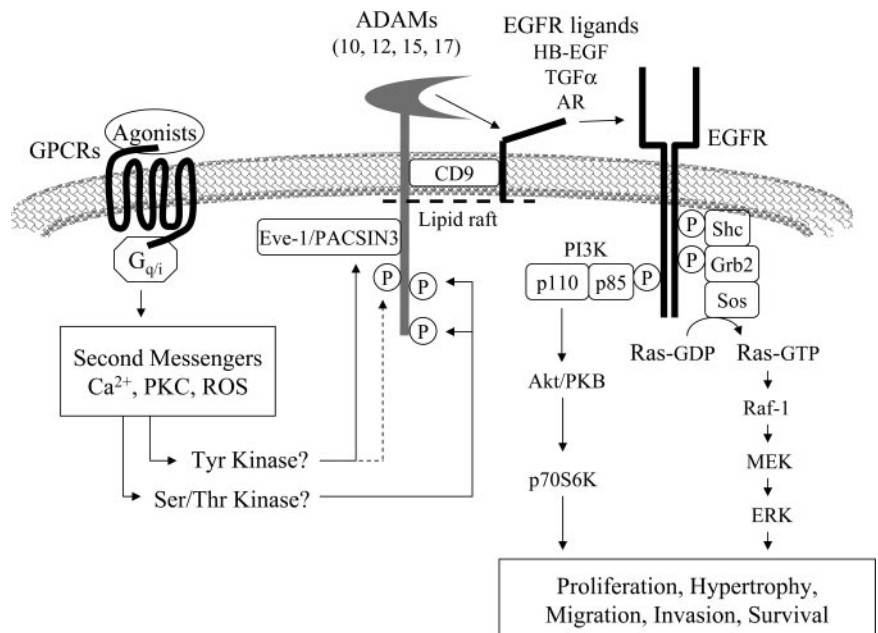


Fig. 3. Proposed signaling mechanism leading to ADAM-dependent EGFR transactivation by GPCR and its downstream significance. PI3K, phosphatidylinositol 3-kinase; ROS, reactive oxygen species; HB, heparin binding; AR, amphiregulin.

KB-R7785, a metalloprotease inhibitor that directly binds to ADAM12, inhibited protein synthesis in cardiac myocytes induced by these agonists (7). In addition, ADAM17 may be required for VSMC hypertrophy induced by ANGII (71).

Regarding cell migration, migration of MDA-MB-231 cells induced by S1P and that of SCC-9 cells induced by LPA were inhibited by siRNA for ADAM17 (36, 41). Furthermore, migration induced by LPA was inhibited by dominant negative ADAM17 in A498 kidney cells as well (85). In addition, pharmacological MEK inhibitor, PD-98059, or phosphatidylinositol 3-kinase inhibitor, LY-294002, also blocked cell migration induced by LPA or S1P in MDA-MB-231 cells (41), suggesting that ERK and Akt activation through ADAM17-dependent EGFR transactivation lead to cell migration. ADAMs may be involved in tumor cell invasion induced by GPCRs, because BB94 inhibited CaKi2 kidney carcinoma cell invasion induced by LPA (85). In addition to these cancer cells, ADAMs have been implicated in VSMC migration induced by ANGII (83). ADAMs may be involved in cell survival/prevention of apoptosis as well (86).

ADAMs MEDIATE HUMAN PATHOPHYSIOLOGY

ADAMs have been implicated in various human diseases, such as inflammatory diseases and cancer (46, 87). It is quite likely that GPCR-induced EGFR transactivation could be a key mechanism by which ADAMs contribute to these diseases. As mentioned above, many *in vitro* studies suggest a critical role of the GPCR/ADAM/ErbB transactivation pathway in cancer development, which is further supported by overexpression of ErbB ligands and/or receptors in various cancers. Borrel-Page et al. (10) have shown that TGF- α shedding possibly mediated through ADAM17 plays an important role in the progression of breast tumor *in vivo*, and ADAM17 is overexpressed in human breast cancers. In addition, ADAM12 is overexpressed in several carcinoma tissues and cell lines (47, 111). Peduto et al. (73) have demonstrated using ADAM9 loss-of-function and overexpression studies in mice an important role for ADAM9 in the pathogenesis of prostate carcinoma. However, further *in vivo* evidence to support this notion needs to be collected to link GPCR-dependent ADAM activation in cancer development.

Recent studies also suggest that ADAMs are involved in the development of cardiovascular diseases, inflammation, diabetes, lung fibrosis, asthma, and Alzheimer's disease. It has been demonstrated that HB-EGF shedding and subsequent EGFR transactivation by GPCR agonists mediated through ADAM12 plays an important role in cardiac hypertrophy (7) and ADAM17 may be required for VSMC hypertrophy induced by ANG II (71). Also, it has been shown that ANG II-induced EGFR transactivation via TGF- α production leads to renal deterioration (58). In TGF α ^{-/-} mice, ANG II did not induce renal damage and an ADAM17 inhibitor prevented renal damage induced by ANG II (58). ADAM17 may also play a role in diabetes and vascular inflammation (101). Insr^{+/-} mice are associated with deficiency of an endogenous inhibitor of ADAM17/TNF- α -converting enzyme, Timp3, leading to insulin resistance and vascular inflammation. Importantly, this phenotype could be reduced by an ADAM17 inhibitor (25) supporting the involvement of ADAM17 in the pathogenesis of type II diabetes and atherosclerosis.

Staphylococcus aureus lipoteichoic acid has been shown to induce a GPCR (platelet-activating factor receptor)-dependent EGFR transactivation through ADAM10, leading to mucin production (59), suggesting the contribution of this pathway in lung fibrosis. ADAM33 was identified as being significantly associated with asthma and bronchial hyperresponsiveness by using positional cloning to search for disease-causing gene (107). It has been also shown that ADAM8 is highly expressed in human eosinophils and mouse experimental asthma models by using microarray analysis (51, 52). However, there is no information available as to whether ADAM8 or 33 are involved in the GPCR-induced EGFR transactivation.

The cleavage of amyloid precursor protein shedding plays important role in Alzheimer's disease. It has been demonstrated that ADAM9, ADAM10, and ADAM17 can act as α -secretases in various cell lines (6, 13, 53, 57). In astrocytoma cells, GPCR stimulation leads to ADAM10/17-dependent shedding of amyloid precursor protein (14). Furthermore, it was reported that in ADAM10 transgenic mice, the formation of amyloid β -peptide is reduced and their deposition in plaques was prevented (78), suggesting a protective effect of ADAMs activated by GPCRs against Alzheimer's disease.

In conclusion, it is becoming clear that ADAMs are indispensable for ectodomain shedding of EGF family ligands required by various GPCRs to generate EGFR cross talk. Taken together with possible involvement of ADAM-dependent EGFR transactivation in mediating various human diseases, it will be important to further elucidate detailed activation/regulation mechanisms of ADAMs and pathophysiological significances of resultant signaling events. Such studies should help us to better understand molecular mechanism(s) of disease development and progression such as in cancer and cardiovascular remodeling, and provide interesting opportunities to develop novel treatments toward these diseases.

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