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 EGF ligand leading to the EGFR transactivation. In this review, we describe the current knowledge on ADAMs implicated in mediating EGF 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
ADAM ACTIVATION BY GPCR

Invited Review

C2

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/~jw7g/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, and 19 are expressed in the testis and associated structures, whereas 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, neuropilins, 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-

ADAM17/TACE

![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, cytosine 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.](http://ajpcell.physiology.org/ by 10.1152/ajpcell.00334.2017)
phagogenesis (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 1 (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 (LPA receptor 1–4) (4). LPA is a bioactive phospholipid that binds a subfamily of GPCRs belonging to the LPA receptors (LPA receptor 1–4) (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 gonadotropin-releasing hormone through its G 12-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 3B Receptors or α1D 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

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**Table 1. ADAM-dependent EGFR transactivation by GPCRs**

<table>
<thead>
<tr>
<th>Agonist</th>
<th>GPCR Type</th>
<th>ADAM Type</th>
<th>EGFR Ligand</th>
<th>EGFR Cell, Tissue/Function</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANG II</td>
<td>AT1*</td>
<td>17</td>
<td>HB-EGF</td>
<td>EGFR ACHN tumor cell</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>AT1</td>
<td>17</td>
<td>HB-EGF</td>
<td>EGFR COS7 cell</td>
<td>64</td>
</tr>
<tr>
<td>LPA</td>
<td>LPA receptor1−4*</td>
<td>10</td>
<td>HB-EGF</td>
<td>EGFR CaK2, A498 kidney carcinoma/ migration</td>
<td>85</td>
</tr>
<tr>
<td>LTA</td>
<td>CXCR 1 or 2*</td>
<td>10</td>
<td>HB-EGF</td>
<td>EGFR SCC cells/proliferation, migration</td>
<td>86</td>
</tr>
<tr>
<td>IL-8</td>
<td>PAFR</td>
<td>10</td>
<td>HB-EGF</td>
<td>EGFR TccSup bladder carcinoma</td>
<td>85, 86</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>α1AR*</td>
<td>12</td>
<td>HB-EGF</td>
<td>EGFR cardiac myocyte/hypertrophy</td>
<td>7</td>
</tr>
<tr>
<td>Carbachol</td>
<td>BomR*</td>
<td>10</td>
<td>HB-EGF</td>
<td>EGFR SCC cells</td>
<td>36</td>
</tr>
<tr>
<td>Bombesin</td>
<td></td>
<td></td>
<td></td>
<td>EGFR COS7 cell/prostate cancer cells</td>
<td>112</td>
</tr>
</tbody>
</table>

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.
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 Gßγ-subunits dissociated from activated Goi was proposed to signal to c-Src, leading to HB-EGF shedding in COS7 cells stimulated by an α2A-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 SIP-induced transactivation in MDA-MB-231 cells, which requires ADAM17-dependent HB-EGF generation (41). These data suggest that Gi may be involved in the ADAM activation in response to particular GPCRs capable of coupling to Gi. Alternatively, requirements of PLC for HB-EGF shedding and ADAM17-dependent EGFR transactivation by the AT1 receptor, which is mainly coupled to Gi3, indicate the participation of Gi3 for ADAM17 activation (64). In fact, overexpression of Gi inhibitory mini-gene blocked HB-EGF shedding through the AT1 receptor and no HB-EGF shedding was observed by an AT1 mutant lacking Gi3 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 ADAM17-/- fibroblasts (56), whereas PMA-stimulated HB-EGF shedding was reduced in ADAM12-/- fibroblasts (55). In addition, PKCδ 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 AT1 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, Ca2+ 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 Ca2+ influx and association between ADAM10 and calmodulin, whereas CD44 cleavage by ADAM17 was regulated through Rac activation via PKC (68). A Ca2+ 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. H2O2-induced EGF 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 5HT2B receptors and α1D receptors (77). Ca2+ may also signal to ROS to activate ADAMS. HB-EGF shedding through AT1 receptor was mediated through ADAM17 activated through intracellular Ca2+ elevation and ROS generation in COS7 cells and that Ca2+ 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 (Cys225, Cys600) 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, Ca2+, 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 Thr735, which is partially required for TrkA receptor shedding in Chinese hamster ovary cells. The ERK-dependent phosphorylation of Thr735 is also dispensable for maturation and inducible trafficking of ADAM17 to the cell surface (93). In addition, ERK-dependent ADAM17 Ser819 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-
phosphatidylinositol 3-kinase, p85 (98). Other ADAM-interacting proteins include Grb2, phospho-AKD, ADAM9, 10, 15, and 17 through its SH3 domain and is SH3 domain binding motifs. Eve-1 can be associated with HT1080 cells. Eve-1 has several SH3 domains and proline-rich HB-EGF shedding induced by PMA and in part by ANG II in with ADAM12 through its SH3 domain and is required for with ADAM9, 10, 12, 15, and 19 (66). PACSIN3 associates kinases, adaptors, or substrates (87). PACSIN3 can interact ADAM interacting proteins have been identified and include several specific protein interaction domains such as PXXP motif to presumably interact with Src homology 3 (SH3) and with 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 α2AAR (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, p85α, Src, endophilin I, SH3pX1, 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 in the presence of 3 differed substrates 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 α3β1 and α2β1 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 cavin-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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**Fig. 2.** The cytoplasmic tails of ADAMS involved in EGFR 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 boldface. 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 PKCα/β, PKCδ, p38MAPK, and PKCα/β/γ, 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.
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-Pages 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 ANGII (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 pathophysiologically significant 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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ADAM ACTIVATION BY GPCR


