Am J Physiol Cell Physiol 291: C1–C10, 2006;
doi:10.1152/ajpcell.00620.2005.

Invited Review

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

Haruhiko Ohtsu,[1] Peter J. Dempsey,[2] and Satoru Eguchi[1]

[1]*Cardiovascular Research Center and Department of Physiology, Temple University School of Medicine, Philadelphia, Pennsylvania; and* [2]*Pacific Northwest Research Institute, and Department of Medicine, University of Washington, Seattle, Washington*

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 recetor 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,

Address for reprint requests and other correspondence: S. Eguchi, Cardiovascular Research Center, Temple Univ. School of Medicine, 3420 N. Broad St., Philadelphia, PA 19140 (e-mail: seguchi@temple.edu).

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 NH_2-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, neuregulins, 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-

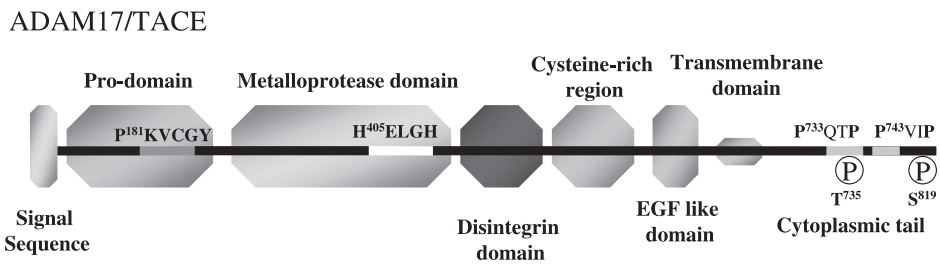

Fig. 1. Structure of a disintegrin and metalloprotease (ADAM). A prototypical ADAM, human ADAM17/TNF-α converting enzyme (TACE), structure is shown with consensus domains. PKVCGY, cysteine switch box motif, is located in pro-domain, and HEXXH (HEGLH), a catalytic-site consensus motif, is located in the metalloprotease domain. Thr^{735} and Ser^{819} 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 (AT_1) receptor (17, 35, 95, 102, 113). It has been demonstrated that ANG II via AT_1 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_{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 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_1AR*	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; AT_1, 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 Gβγ-subunits dissociated from activated $G\alpha_i$ 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-myristrate 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_2O_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 AT_1 at Tyr^{319} are required for ANG II-induced EGFR transactivation (88, 89, 106). However, whether these signaling components exist upstream of ADAM activation by the AT_1 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, phosphatidyl inositol 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 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 tetraspannins 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 AT_1 and EGFR (72, 118). In fact, the interaction of AT_1 with caveolin-1 is essential for the trafficking of the AT_1 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-

```
ADAM10  ---------------------------------KICSVHT----------------PSSNPK
ADAM12  KRKTLIRLLFTNKKTTIEKLRCVRPSRPPRGFQPCQAHLGHLGKGLMRKPPDSYPPKDNPR
ADAM15  -------------GASYWYRARLHQRLCQLKGPTCQYRAAQSG------------PSERPG
ADAM17  ---------DKKLDKQYESLSLFHPSNVEMLSSMDSASVRIIK------------PFPAPQ

ADAM10  ----------------------------LP----------PPKPLPGTLKRR--RPPQPI
ADAM12  RLLQCQNVDISRPLNGLNVPQPQSTQRVLPPLHRAPRAPSVPARPLPAKPALR--QAQGTC
ADAM15  --------------------PPQRALLARGTKQASALSFPAPPSRPLPPDPVSKRLQSQGPA
ADAM17  T----------------------------PGRLQPAPVIPSAPAAPKLDHQRMDTIQEDPST

ADAM10  QQPQRQRPRESYQMGHMRR----------------------------------------
ADAM12  KPNPPQKPLPADPLARTTRLTHALARTPGQWETGLRLAPLRPAPQYPHQVPRSTHTAYIK
ADAM15  KPPPPRKPLPADPQGRCPSGDLPGPGAG---------IPPLVVPSRPAPPPPTVSSLYL-
ADAM17  DSHMDEDGFEKDPFPNSSTAAKSFEDLTDHPVTRSEKAASFKLQRQNRVDSKETEC----
```

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 boldface 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 PKCα/β/γ, PKCζ, p38MAPK, and PKCα/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 bombsein 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.

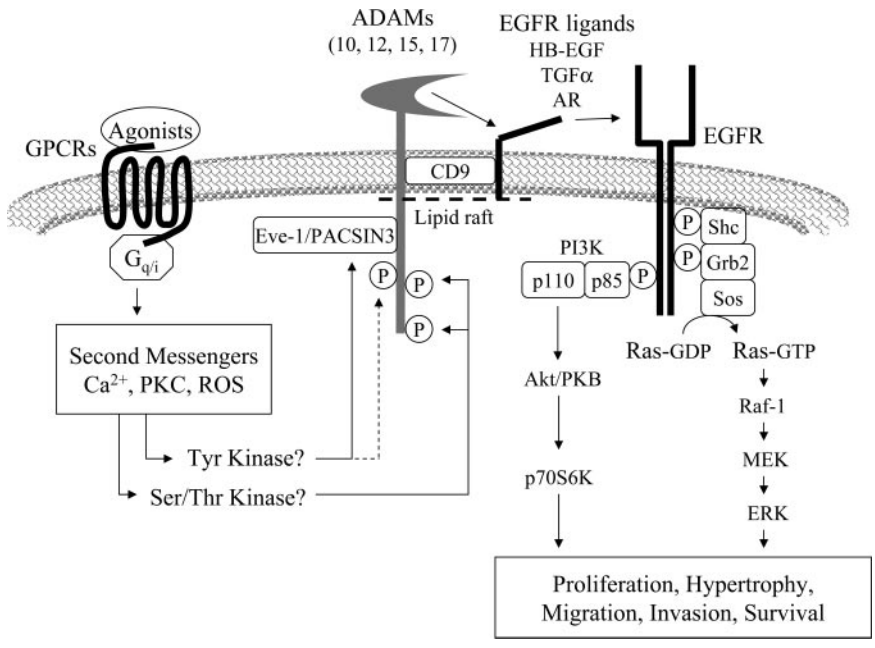

Fig. 3. Proposed signaling mechanism leading to ADAM-dependent EGFR transactivation by GPCR and its downstream significance. PI3K, phosphatidylinositol 3-kinase; ROS, reactive oxygen species; HB, heparin binding; AR, amphiregulin.

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-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 ANG II (71). Also, it has been shown that ANG II-induced EGFR transactivation via TGF-α production leads to renal deterioration (58). In $TGF\alpha^{-/-}$ 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.

ACKNOWLEDGMENTS

We thank Kunie Eguchi for excellent technical assistance.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grant HL-076770 (to S. Eguchi), National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-59778 and DK-63363 (both to P. J. Dempsey), a Crohn's and Colitis Foundation of America grant (to P. J. Dempsey), and funds from Tonohata Inc. and Kisyu Hosokawa Inc. (both to S. Eguchi).

REFERENCES

1. **Abel S, Hundhausen C, Mentlein R, Schulte A, Berkhout TA, Broadway N, Hartmann D, Sedlacek R, Dietrich S, Muetze B, Schuster B, Kallen KJ, Saftig P, Rose-John S, and Ludwig A.** The transmembrane CXC-chemokine ligand 16 is induced by IFN-γ and TNF-α and shed by the activity of the disintegrin-like metalloproteinase ADAM10. *J Immunol* 172: 6362–6372, 2004.
2. **Abram CL, Seals DF, Pass I, Salinsky D, Maurer L, Roth TM, and Courtneidge SA.** The adaptor protein fish associates with members of the ADAMs family and localizes to podosomes of Src-transformed cells. *J Biol Chem* 278: 16844–16851, 2003.
3. **Andreev J, Galisteo ML, Kranenburg O, Logan SK, Chiu ES, Okigaki M, Cary LA, Moolenaar WH, and Schlessinger J.** Src and Pyk2 mediate G-protein-coupled receptor activation of epidermal growth factor receptor (EGFR) but are not required for coupling to the mitogen-activated protein (MAP) kinase signaling cascade. *J Biol Chem* 276: 20130–20135, 2001.
4. **Anliker B and Chun J.** Cell surface receptors in lysophospholipid signaling. *Semin Cell Dev Biol* 15: 457–465, 2004.

5. **Arasada RR and Carpenter G.** Secretase-dependent tyrosine phosphorylation of Mdm2 by the ErbB-4 intracellular domain fragment. *J Biol Chem* 280: 30783–30787, 2005.
6. **Asai M, Hattori C, Szabo B, Sasagawa N, Maruyama K, Tanuma S, and Ishiura S.** Putative function of ADAM9, ADAM10, and ADAM17 as APP α-secretase. *Biochem Biophys Res Commun* 301: 231–235, 2003.
7. **Asakura M, Kitakaze M, Takashima S, Liao Y, Ishikura F, Yoshinaka T, Ohmoto H, Node K, Yoshino K, Ishiguro H, Asanuma H, Sanada S, Matsumura Y, Takeda H, Beppu S, Tada M, Hori M, and Higashiyama S.** Cardiac hypertrophy is inhibited by antagonism of ADAM12 processing of HB-EGF: metalloproteinase inhibitors as a new therapy. *Nat Med* 8: 35–40, 2002.
8. **Berditchevski F.** Complexes of tetraspanins with integrins: more than meets the eye. *J Cell Sci* 114: 4143–4151, 2001.
9. **Blobel CP.** ADAMs: key components in EGFR signalling and development. *Nat Rev Mol Cell Biol* 6: 32–43, 2005.
10. **Borrell-Pages M, Rojo F, Albanell J, Baselga J, and Arribas J.** TACE is required for the activation of the EGFR by TGF-α in tumors. *EMBO J* 22: 1114–1124, 2003.
11. **Buckley CA, Rouhani FN, Kaler M, Adamik B, Hawari FI, and Levine SJ.** Amino-terminal TACE prodomain attenuates TNFR2 cleavage independently of the cysteine switch. *Am J Physiol Lung Cell Mol Physiol* 288: L1132–L1138, 2005.
12. **Buteau J, Foisy S, Joly E, and Prentki M.** Glucagon-like peptide 1 induces pancreatic β-cell proliferation via transactivation of the epidermal growth factor receptor. *Diabetes* 52: 124–132, 2003.
13. **Buxbaum JD, Liu KN, Luo Y, Slack JL, Stocking KL, Peschon JJ, Johnson RS, Castner BJ, Cerretti DP, and Black RA.** Evidence that tumor necrosis factor α converting enzyme is involved in regulated α-secretase cleavage of the Alzheimer amyloid protein precursor. *J Biol Chem* 273: 27765–27767, 1998.
14. **Camden JM, Schrader AM, Camden RE, Gonzalez FA, Erb L, Seye CI, and Weisman GA.** P2Y2 nucleotide receptors enhance α-secretase-dependent amyloid precursor protein processing. *J Biol Chem* 280: 18696–18702, 2005.
15. **Citri A, Skaria KB, and Yarden Y.** The deaf and the dumb: the biology of ErbB-2 and ErbB-3. *Exp Cell Res* 284: 54–65, 2003.
16. **Daub H, Weiss FU, Wallasch C, and Ullrich A.** Role of transactivation of the EGF receptor in signalling by G-protein-coupled receptors. *Nature* 379: 557–560, 1996.
17. **De Gasparo M, Catt KJ, Inagami T, Wright JW, and Unger T.** International Union of Pharmacology. XXIII. The angiotensin II receptors. *Pharmacol Rev* 52: 415–472, 2000.
18. **Dempsey PJ, Garton K, and Raines EW.** Emerging roles of TACE as a key protease in ErbB ligand shedding. *Molec Inter* 2: 136–141, 2002.
19. **Denzer K, Kleijmeer MJ, Heijnen HF, Stoorvogel W, and Geuze HJ.** Exosome: from internal vesicle of the multivesicular body to intercellular signaling device. *J Cell Sci* 19: 3365–3374, 2000.
20. **Diaz-Rodriguez E, Montero JC, Esparis-Ogando A, Yuste L, and Pandiella A.** Extracellular signal-regulated kinase phosphorylates tumor necrosis factor α-converting enzyme at threonine 735: a potential role in regulated shedding. *Mol Biol Cell* 13: 2031–2044, 2002.
21. **Eguchi S, Dempsey PJ, Frank GD, Motley ED, and Inagami T.** Activation of MAPKs by angiotensin II in vascular smooth muscle cells. Metalloprotease-dependent EGF receptor activation is required for activation of ERK and p38 MAPK but not for JNK. *J Biol Chem* 276: 7957–7962, 2001.
22. **Eguchi S, Frank GD, Mifune M, and Inagami T.** Metalloprotease-dependent ErbB ligand shedding in mediating EGFR transactivation and vascular remodelling. *Biochem Soc Trans* 31: 1198–1202, 2003.
23. **Eguchi S, Numaguchi K, Iwasaki H, Matsumoto T, Yamakawa T, Utsunomiya H, Motley ED, Kawakatsu H, Owada KM, Hirata Y, Marumo F, and Inagami T.** Calcium-dependent epidermal growth factor receptor transactivation mediates the angiotensin II-induced mitogen-activated protein kinase activation in vascular smooth muscle cells. *J Biol Chem* 273: 8890–8896, 1998.
24. **Fan H, Turck CW, and Derynck R.** Characterization of growth factor-induced serine phosphorylation of tumor necrosis factor-alpha converting enzyme and of an alternatively translated polypeptide. *J Biol Chem* 278: 18617–18627, 2003.
25. **Federici M, Hribal ML, Menghini R, Kanno H, Marchetti V, Porzio O, Sunnarborg SW, Rizza S, Serino M, Cunsolo V, Lauro D, Mauriello A, Smookler DS, Sbraccia P, Sesti G, Lee DC, Khokha R, Accili D, and Lauro R.** Timp3 deficiency in insulin receptor-haploinsufficient mice promotes diabetes and vascular inflammation via increased TNF-α. *J Clin Invest* 115: 3494–3505, 2005.
26. **Fischer OM, Hart S, Gschwind A, Prenzel N, and Ullrich A.** Oxidative and osmotic stress signaling in tumor cells is mediated by ADAM proteases and heparin-binding epidermal growth factor. *Mol Cell Biol* 24: 5172–5183, 2004.
27. **Fischer OM, Hart S, Gschwind A, and Ullrich A.** EGFR signal transactivation in cancer cells. *Biochem Soc Trans* 31: 1203–1208, 2003.
28. **Frank GD and Eguchi S.** Activation of tyrosine kinases by reactive oxygen species in vascular smooth muscle cells: significance and involvement of EGF receptor transactivation by angiotensin II. *Antioxid Redox Signal* 5: 771–780, 2003.
29. **Frank GD, Mifune M, Inagami T, Ohba M, Sasaki T, Higashiyama S, Dempsey PJ, and Eguchi S.** Distinct mechanisms of receptor and nonreceptor tyrosine kinase activation by reactive oxygen species in vascular smooth muscle cells: role of metalloprotease and protein kinase C-δ. *Mol Cell Biol* 23: 1581–1589, 2003.
30. **Fujiyama S, Matsubara H, Nozawa Y, Maruyama K, Mori Y, Tsutsumi Y, Masaki H, Uchiyama Y, Koyama Y, Nose A, Iba O, Tateishi E, Ogata N, Jyo N, Higashiyama S, and Iwasaka T.** Angiotensin AT_1 and AT_2 receptors differentially regulate angiopoietin-2 and vascular endothelial growth factor expression and angiogenesis by modulating heparin binding-epidermal growth factor (EGF)-mediated EGF receptor transactivation. *Circ Res* 88: 22–29, 2001.
31. **Galliano MF, Huet C, Frygelius J, Polgren A, Wewer UM, and Engvall E.** Binding of ADAM12, a marker of skeletal muscle regeneration, to the muscle-specific actin-binding protein, α-actinin-2, is required for myoblast fusion. *J Biol Chem* 275: 13933–13939, 2000.
32. **Garton KJ, Gough PJ, Blobel CP, Murphy G, Greaves DR, Dempsey PJ, and Raines EW.** Tumor necrosis factor-α-converting enzyme (ADAM17) mediates the cleavage and shedding of fractalkine (CX3CL1). *J Biol Chem* 276: 37993–38001, 2001.
33. **Gonzales PE, Solomon A, Miller AB, Leesnitzer MA, Sagi I, and Milla ME.** Inhibition of the tumor necrosis factor-α-converting enzyme by its pro domain. *J Biol Chem* 279: 31638–31645, 2004.
34. **Gough PJ, Garton KJ, Wille PT, Rychlewski M, Dempsey PJ, and Raines EW.** A disintegrin and metalloproteinase 10-mediated cleavage and shedding regulates the cell surface expression of CXC chemokine ligand 16. *J Immunol* 172: 3678–3685, 2004.
35. **Griendling KK, Ushio-Fukai M, Lassegue B, and Alexander RW.** Angiotensin II signaling in vascular smooth muscle. New concepts. *Hypertension* 29: 366–373, 1997.
36. **Gschwind A, Hart S, Fischer OM, and Ullrich A.** TACE cleavage of proamphiregulin regulates GPCR-induced proliferation and motility of cancer cells. *EMBO J* 22: 2411–2421, 2003.
37. **Gschwind A, Zwick E, Prenzel N, Leserer M, and Ullrich A.** Cell communication networks: epidermal growth factor receptor transactivation as the paradigm for interreceptor signal transmission. *Oncogene* 20: 1594–1600, 2001.
38. **Gutwein P, Stoeck A, Riedle S, Gast D, Runz S, Condon TP, Marme A, Phong MC, Linderkamp O, Skorokhod A, and Altevogt P.** Cleavage of L1 in exosomes and apoptotic membrane vesicles released from ovarian carcinoma cells. *Clin Cancer Res* 11: 2492–2501, 2005.
39. **Hao L, Du M, Lopez-Campistrous A, and Fernandez-Patron C.** Agonist-induced activation of matrix metalloproteinase-7 promotes vasoconstriction through the epidermal growth factor-receptor pathway. *Circ Res* 94: 68–76, 2004.
40. **Harris RC, Chung E, and Coffey RJ.** EGF receptor ligands. *Exp Cell Res* 284: 2–13, 2003.
41. **Hart S, Fischer OM, Prenzel N, Zwick-Wallasch E, Schneider M, Hennighausen L, and Ullrich A.** GPCR-induced migration of breast carcinoma cells depends on both EGFR signal transactivation and EGFR-independent pathways. *Biol Chem* 386: 845–855, 2005.
42. **Hartmann D, de Strooper B, Serneels L, Craessaerts K, Herreman A, Annaert W, Umans L, Lubke T, Lena Illert A, von Figura K, and Saftig P.** The disintegrin/metalloprotease ADAM 10 is essential for Notch signalling but not for alpha-secretase activity in fibroblasts. *Hum Mol Genet* 11: 2615–2624, 2002.
43. **Hawari FI, Rouhani FN, Cui X, Yu ZX, Buckley C, Kaler M, and Levine SJ.** Release of full-length 55-kDa TNF receptor 1 in exosome-like vesicles: a mechanism for generation of soluble cytokine receptors. *Proc Natl Acad Sci USA* 101: 1297–1302, 2004.

44. **Holbro T and Hynes NE.** ErbB receptors: directing key signaling networks throughout life. *Annu Rev Pharmacol Toxicol* 44: 195–217, 2004.
45. **Horiuchi K, Weskamp G, Lum L, Hammes HP, Cai H, Brodie TA, Ludwig T, Chiusaroli R, Baron R, Preissner KT, Manova K, and Blobel CP.** Potential role for ADAM15 in pathological neovascularization in mice. *Mol Cell Biol* 23: 5614–5624, 2003.
46. **Huovila AP, Turner AJ, Pelto-Huikko M, Karkkainen I, and Ortiz RM.** Shedding light on ADAM metalloproteinases. *Trends Biochem Sci* 30: 413–422, 2005.
47. **Iba K, Albrechtsen R, Gilpin B, Frohlich C, Loechel F, Zolkiewska A, Ishiguro K, Kojima T, Liu W, Langford JK, Sanderson RD, Brakebusch C, Fassler R, and Wewer UM.** The cysteine-rich domain of human ADAM 12 supports cell adhesion through syndecans and triggers signaling events that lead to β_1 integrin-dependent cell spreading. *J Cell Biol* 149: 1143–1156, 2000.
48. **Izumi Y, Hirata M, Hasuwa H, Iwamoto R, Umata T, Miyado K, Tamai Y, Kurisaki T, Sehara-Fujisawa A, Ohno S, and Mekada E.** A metalloprotease-disintegrin, MDC9/meltrin-g/ADAM9 and PKCδ are involved in TPA-induced ectodomain shedding of membrane-ancored heparin-binding EGF-like growth factor. *EMBO J* 17: 7260–7272, 1998.
49. **Jackson LF, Qiu TH, Sunnarborg SW, Chang A, Zhang C, Patterson C, and Lee DC.** Defective valvulogenesis in HB-EGF and TACE-null mice is associated with aberrant BMP signaling. *EMBO J* 22: 2704–2716, 2003.
50. **Janes PW, Saha N, Barton WA, Kolev MV, Wimmer-Kleikamp SH, Nievergall E, Blobel CP, Himanen JP, Lackmann M, and Nikolov DB.** Adam meets Eph: an ADAM substrate recognition module acts as a molecular switch for ephrin cleavage in trans. *Cell* 123: 291–304, 2005.
51. **Johansson MW, Lye MH, Barthel SR, Duffy AK, Annis DS, and Mosher DF.** Eosinophils adhere to vascular cell adhesion molecule-1 via podosomes. *Am J Respir Cell Mol Biol* 31: 413–422, 2004.
52. **King NE, Zimmermann N, Pope SM, Fulkerson PC, Nikolaidis NM, Mishra A, Witte DP, and Rothenberg ME.** Expression and regulation of a disintegrin and metalloproteinase (ADAM) 8 in experimental asthma. *Am J Respir Cell Mol Biol* 31: 257–265, 2004.
53. **Koike H, Tomioka S, Sorimachi H, Saido TC, Maruyama K, Okuyama A, Fujisawa-Sehara A, Ohno S, Suzuki K, and Ishiura S.** Membrane-anchored metalloprotease MDC9 has an α-secretase activity responsible for processing the amyloid precursor protein. *Biochem J* 343: 371–375, 1999.
54. **Kranenburg O and Moolenaar WH.** Ras-MAP kinase signaling by lysophosphatidic acid and other G protein-coupled receptor agonists. *Oncogene* 20: 1540–1546, 2001.
55. **Kurisaki T, Masuda A, Sudo K, Sakagami J, Higashiyama S, Matsuda Y, Nagabukuro A, Tsuji A, Nabeshima Y, Asano M, Iwakura Y, and Sehara-Fujisawa A.** Phenotypic analysis of meltrin α (ADAM12)-deficient mice: involvement of meltrin α in adipogenesis and myogenesis. *Mol Cell Biol* 23: 55–61, 2003.
56. **Kurohara K, Komatsu K, Kurisaki T, Masuda A, Irie N, Asano M, Sudo K, Nabeshima Y, Iwakura Y, and Sehara-Fujisawa A.** Essential roles of meltrin β (ADAM19) in heart development. *Dev Biol* 267: 14–28, 2004.
57. **Lammich S, Kojro E, Postina R, Gilbert S, Pfeiffer R, Jasionowski M, Haass C, and Fahrenholz F.** Constitutive and regulated α-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. *Proc Natl Acad Sci USA* 96: 3922–3927, 1999.
58. **Lautrette A, Li S, Alili R, Sunnarborg SW, Burtin M, Lee DC, Friedlander G, and Terzi F.** Angiotensin II and EGF receptor cross-talk in chronic kidney diseases: a new therapeutic approach. *Nat Med* 11: 867–874, 2005.
59. **Lemjabbar H and Basbaum C.** Platelet-activating factor receptor and ADAM10 mediate responses to *Staphylococcus aureus* in epithelial cells. *Nat Med* 8: 41–46, 2002.
60. **Li X and Fan H.** Loss of ectodomain shedding due to mutations in the metalloprotease and cysteine-rich/disintegrin domains of the tumor necrosis factor-α-converting enzyme (TACE). *J Biol Chem* 279: 27365–27375, 2004.
61. **Luttrell LM, Della Rocca GJ, van Biesen T, Luttrell DK, and Lefkowitz RJ.** Gbetagamma subunits mediate Src-dependent phosphorylation of the epidermal growth factor receptor. A scaffold for G protein-coupled receptor-mediated Ras activation. *J Biol Chem* 272: 4637–4644, 1997.
62. **Marin V, Montero-Julian F, Gres S, Bongrand P, Farnarier C, and Kaplanski G.** Chemotactic agents induce IL-6Rα shedding from polymorphonuclear cells: involvement of a metalloproteinase of the TNF-α-converting enzyme (TACE) type. *Eur J Immunol* 32: 2965–2970, 2002.
63. **McCole DF, Keely SJ, Coffey RJ, and Barrett KE.** Transactivation of the epidermal growth factor receptor in colonic epithelial cells by carbachol requires extracellular release of transforming growth factor-α. *J Biol Chem* 277: 42603–42612, 2002.
64. **Mifune M, Ohtsu H, Suzuki H, Nakashima H, Brailoiu E, Dun NJ, Frank GD, Inagami T, Higashiyama S, Thomas WG, Eckhart AD, Dempsey PJ, and Eguchi S.** G protein coupling and second messenger generation are indispensable for metalloprotease-dependent, heparin-binding epidermal growth factor shedding through angiotensin II type-1 receptor. *J Biol Chem* 280: 26592–26599, 2005.
65. **Montero JC, Yuste L, Diaz-Rodriguez E, Esparis-Ogando A, and Pandiella A.** Differential shedding of transmembrane neuregulin isoforms by the tumor necrosis factor-alpha-converting enzyme. *Mol Cell Neurosci* 16: 631–648, 2000.
66. **Mori S, Tanaka M, Nanba D, Nishiwaki E, Ishiguro H, Higashiyama S, and Matsuura N.** PACSIN3 binds ADAM12/meltrin α and up-regulates ectodomain shedding of heparin-binding epidermal growth factor-like growth factor. *J Biol Chem* 278: 46029–46034, 2003.
67. **Moss ML and Bartsch JW.** Therapeutic benefits from targeting of ADAM family members. *Biochemistry* 43: 7227–7235, 2004.
68. **Nagano O, Murakami D, Hartmann D, De Strooper B, Saftig P, Iwatsubo T, Nakajima M, Shinohara M, and Saya H.** Cell-matrix interaction via CD44 is independently regulated by different metalloproteinases activated in response to extracellular Ca^{2+} influx and PKC activation. *J Cell Biol* 165: 893–902, 2004.
69. **Ni CY, Murphy MP, Golde TE, and Carpenter G.** γ-Secretase cleavage and nuclear localization of ErbB-4 receptor tyrosine kinase. *Science* 294: 2179–2181, 2001.
70. **Ohtsu H, Frank GD, Utsunomiya H, and Eguchi S.** Redox-dependent protein kinase regulation by angiotensin II: mechanistic insights and its pathophysiology. *Antioxid Redox Signal* 7: 1315–1326, 2005.
71. **Ohtsu H, Nakashima H, Suzuki H, Eguchi K, Higuchi S, Inagami T, Frank GD, and Eguchi S.** ADAM17/TACE mediates HB-EGF shedding and EGF receptor transactivation by angiotensin II in vascular smooth muscle cells (Abstract). *Hypertension* 46: 821, 2005.
72. **Olivares-Reyes JA, Shah BH, Hernandez-Aranda J, Garcia-Caballero A, Farshori MP, Garcia-Sainz JA, and Catt KJ.** Agonist-induced interactions between angiotensin AT1 and epidermal growth factor receptors. *Mol Pharmacol* 68: 356–364, 2005.
73. **Peduto L, Reuter VE, Shaffer DR, Scher HI, and Blobel CP.** Critical function for ADAM9 in mouse prostate cancer. *Cancer Res* 65: 9312–9319, 2005.
74. **Peschon JJ, Slack JL, Reddy P, Stocking KL, Sunnarborg SW, Lee DC, Russell WE, Castner BJ, Johnson RS, Fitzner JN, Boyce RW, Nelson N, Kozlosky CJ, Wolfson MF, Rauch CT, Cerretti DP, Paxton RJ, March CJ, and Black RA.** An essential role for ectodomain shedding in mammalian development. *Science* 282: 1281–1284, 1998.
75. **Pierce KL, Luttrell LM, and Lefkowitz RJ.** New mechanisms in heptahelical receptor signaling to mitogen activated protein kinase cascades. *Oncogene* 20: 1532–1539, 2001.
76. **Pierce KL, Tohgo A, Ahn S, Field ME, Luttrell LM, and Lefkowitz RJ.** Epidermal growth factor (EGF) receptor-dependent ERK activation by G protein-coupled receptors: a co-culture system for identifying intermediates upstream and downstream of heparin-binding EGF shedding. *J Biol Chem* 276: 23155–23160, 2001.
77. **Pietri M, Schneider B, Mouillet-Richard S, Ermonval M, Mutel V, Launay JM, and Kellermann O.** Reactive oxygen species-dependent TNF-α converting enzyme activation through stimulation of 5-HT2B and α1D autoreceptors in neuronal cells. *FASEB J* 19: 1078–1087, 2005.
78. **Postina R, Schroeder A, Dewachter I, Bohl J, Schmitt U, Kojro E, Prinzen C, Endres K, Hiemke C, Blessing M, Flamez P, Dequenne A, Godaux E, van Leuven F, and Fahrenholz F.** A disintegrin-metalloproteinase prevents amyloid plaque formation and hippocampal defects in an Alzheimer disease mouse model. *J Clin Invest* 113: 1456–1464, 2004.
79. **Prenzel N, Zwick E, Daub H, Leserer M, Abraham R, Wallasch C, and Ullrich A.** EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. *Nature* 402: 884–888, 1999.

80. **Reddy P, Slack JL, Davis R, Cerretti DP, Kozlosky CJ, Blanton RA, Shows D, Peschon JJ, and Black RA.** Functional analysis of the domain structure of tumor necrosis factor-α converting enzyme. *J Biol Chem* 275: 14608–14614, 2000.
81. **Roelle S, Grosse R, Aigner A, Krell HW, Czubayko F, and Gudermann T.** Matrix metalloproteinases 2 and 9 mediate epidermal growth factor receptor transactivation by gonadotropin-releasing hormone. *J Biol Chem* 278: 47307–47318, 2003.
82. **Sahin U, Weskamp G, Kelly K, Zhou HM, Higashiyama S, Peschon J, Hartmann D, Saftig P, and Blobel CP.** Distinct roles for ADAM10 and ADAM17 in ectodomain shedding of six EGFR ligands. *J Cell Biol* 164: 769–779, 2004.
83. **Saito S, Frank GD, Motley ED, Dempsey PJ, Utsunomiya H, Inagami T, and Eguchi S.** Metalloprotease inhibitor blocks angiotensin II-induced migration through inhibition of epidermal growth factor receptor transactivation. *Biochem Biophys Res Commun* 294: 1023–1029, 2002.
84. **Sanderson MP, Erickson SN, Gough PJ, Garton KJ, Wille PT, Raines EW, Dunbar AJ, and Dempsey PJ.** ADAM10 mediates ectodomain shedding of the betacellulin precursor activated by p-aminophenylmercuric acetate and extracellular calcium influx. *J Biol Chem* 280: 1826–1837, 2005.
85. **Schafer B, Gschwind A, and Ullrich A.** Multiple G-protein-coupled receptor signals converge on the epidermal growth factor receptor to promote migration and invasion. *Oncogene* 23: 991–999, 2004.
86. **Schafer B, Marg B, Gschwind A, and Ullrich A.** Distinct ADAM metalloproteinases regulate G protein-coupled receptor-induced cell proliferation and survival. *J Biol Chem* 279: 47929–47938, 2004.
87. **Seals DF and Courtneidge SA.** The ADAMs family of metalloproteases: multidomain proteins with multiple functions. *Genes Dev* 17: 7–30, 2003.
88. **Seshiah PN, Weber DS, Rocic P, Valppu L, Taniyama Y, and Griendling KK.** Angiotensin II stimulation of NAD(P)H oxidase activity: upstream mediators. *Circ Res* 91: 406–413, 2002.
89. **Seta K and Sadoshima J.** Phosphorylation of tyrosine 319 of the angiotensin II type 1 receptor mediates angiotensin II-induced transactivation of the epidermal growth factor receptor. *J Biol Chem* 278: 9019–9026, 2003.
90. **Shah BH and Catt KJ.** Matrix metalloproteinase-dependent EGF receptor activation in hypertension and left ventricular hypertrophy. *Trends Endocrinol Metab* 15: 241–243, 2004.
91. **Shah BH, Farshori MP, Jambusaria A, and Catt KJ.** Roles of Src and epidermal growth factor receptor transactivation in transient and sustained ERK1/2 responses to gonadotropin-releasing hormone receptor activation. *J Biol Chem* 278: 19118–19126, 2003.
92. **Shah BH, Yesilkaya A, Olivares-Reyes JA, Chen HD, Hunyady L, and Catt KJ.** Differential pathways of angiotensin II-induced extracellularly regulated kinase 1/2 phosphorylation in specific cell types: role of heparin-binding epidermal growth factor. *Mol Endocrinol* 18: 2035–2048, 2004.
93. **Soond SM, Everson B, Riches DW, and Murphy G.** ERK-mediated phosphorylation of Thr^{735} in TNFα-converting enzyme and its potential role in TACE protein trafficking. *J Cell Sci* 118: 2371–2380, 2005.
94. **Sternlicht MD, Sunnarborg SW, Kouros-Mehr H, Yu Y, Lee DC, and Werb Z.** Mammary ductal morphogenesis requires paracrine activation of stromal EGFR via ADAM17-dependent shedding of epithelial amphiregulin. *Development* 132: 3923–3933, 2005.
95. **Suzuki H, Motley ED, Frank GD, Utsunimiya H, and Eguchi S.** Recent progress in signal transduction research of the angiotensin II type-1 receptor: protein kinases, vascular dysfunction, and structural requirement. *Curr Med Chem* 3: 305–322, 2005.
96. **Suzuki M, Raab G, Moses MA, Fernandez CA, and Klagsbrun M.** Matrix metalloproteinase-3 releases active heparin-binding EGF-like growth factor by cleavage at a specific juxtamembrane site. *J Biol Chem* 272: 31730–31737, 1997.
97. **Sweeney C and Carraway KL 3rd.** Ligand discrimination by ErbB receptors: differential signaling through differential phosphorylation site usage. *Oncogene* 19: 5568–5573, 2000.
98. **Tanaka M, Nanba D, Mori S, Shiba F, Ishiguro H, Yoshino K, Matsuura N, and Higashiyama S.** ADAM binding protein Eve-1 is required for ectodomain shedding of epidermal growth factor receptor ligands. *J Biol Chem* 279: 41950–41959, 2004.
99. **Tanida S, Joh T, Itoh K, Kataoka H, Sasaki M, Ohara H, Nakazawa T, Nomura T, Kinugasa Y, Ohmoto H, Ishiguro H, Yoshino K, Higashiyama S, and Itoh M.** The mechanism of cleavage of EGFR ligands induced by inflammatory cytokines in gastric cancer cells. *Gastroenterology* 127: 559–569, 2004.
100. **Thomas WG, Brandenburger Y, Autelitano DJ, Pham T, Qian H, and Hannan RD.** Adenoviral-directed expression of the type 1A angiotensin receptor promotes cardiomyocyte hypertrophy via transactivation of the epidermal growth factor receptor. *Circ Res* 90: 135–142, 2002.
101. **Togashi N, Ura N, Higashiura K, Murakami H, and Shimamoto K.** Effect of TNF-α-converting enzyme inhibitor on insulin resistance in fructose-fed rats. *Hypertension* 39: 578–580, 2002.
102. **Touyz RM and Schiffrin EL.** Signal transduction mechanisms mediating the physiological and pathophysiological actions of angiotensin II in vascular smooth muscle cells. *Pharmacol Rev* 52: 639–672, 2000.
103. **Tsai W, Morielli AD, and Peralta EG.** The m1 muscarinic acetylcholine receptor transactivates the EGF receptor to modulate ion channel activity. *EMBO J* 16: 4597–4605, 1997.
104. **Tsou CL, Haskell CA, and Charo IF.** Tumor necrosis factor-α-converting enzyme mediates the inducible cleavage of fractalkine. *J Biol Chem* 276: 44622–44626, 2001.
105. **Umata T, Hirata M, Takahashi T, Ryu F, Shida S, Takahashi Y, Tsuneoka M, Miura Y, Masuda M, Horiguchi Y, and Mekada E.** A dual signaling cascade that regulates the ectodomain shedding of heparin-binding epidermal growth factor-like growth factor. *J Biol Chem* 276: 30475–30482, 2001.
106. **Ushio-Fukai M, Zuo L, Ikeda S, Tojo T, Patrushev NA, and Alexander RW.** cAbl tyrosine kinase mediates reactive oxygen species- and caveolin-dependent AT1 receptor signaling in vascular smooth muscle: role in vascular hypertrophy. *Circ Res* 97: 829–836, 2005.
107. **Van Eerdewegh P, Little RD, Dupuis J, Del Mastro RG, Falls K, Simon J, Torrey D, Pandit S, McKenny J, Braunschweiger K, Walsh A, Liu Z, Hayward B, Folz C, Manning SP, Bawa A, Saracino L, Thackston M, Benchekroun Y, Capparell N, Wang M, Adair R, Feng Y, Dubois J, FitzGerald MG, Huang H, Gibson R, Allen KM, Pedan A, Danzig MR, Umland SP, Egan RW, Cuss FM, Rorke S, Clough JB, Holloway JW, Holgate ST, and Keith TP.** Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature* 418: 426–430, 2002.
108. **Wakatsuki S, Kurisaki T, and Sehara-Fujisawa A.** Lipid rafts identified as locations of ectodomain shedding mediated by meltrin β/ADAM19. *J Neurochem* 89: 119–123, 2004.
109. **White JM.** ADAMs: modulators of cell-cell and cell-matrix interactions. *Curr Opin Cell Biol* 15: 598–606, 2003.
110. **Wolfe MS and Kopan R.** Intramembrane proteolysis: theme and variations. *Science* 305: 1119–1123, 2004.
111. **Wu E, Croucher PI, and McKie N.** Expression of members of the novel membrane linked metalloproteinase family ADAM in cells derived from a range of haematological malignancies. *Biochem Biophys Res Commun* 235: 437–442, 1997.
112. **Yan Y, Shirakabe K, and Werb Z.** The metalloprotease Kuzbanian (ADAM10) mediates the transactivation of EGF receptor by G protein-coupled receptors. *J Cell Biol* 158: 221–226, 2002.
113. **Yin G, Yan C, and Berk BC.** Angiotensin II signaling pathways mediated by tyrosine kinases. *Int J Biochem Cell Biol* 35: 780–783, 2003.
114. **Yu WH, Woessner JF Jr, McNeish JD, and Stamenkovic I.** CD44 anchors the assembly of matrilysin/MMP-7 with heparin-binding epidermal growth factor precursor and ErbB4 and regulates female reproductive organ remodeling. *Genes Dev* 16: 307–323, 2002.
115. **Zhang Z, Oliver P, Lancaster JJ, Schwarzenberger PO, Joshi MS, Cork J, and Kolls JK.** Reactive oxygen species mediate tumor necrosis factor α-converting, enzyme-dependent ectodomain shedding induced by phorbol myristate acetate. *FASEB J* 15: 303–305, 2001.
116. **Zhao J, Chen H, Peschon JJ, Shi W, Zhang Y, Frank SJ, and Warburton D.** Pulmonary hypoplasia in mice lacking tumor necrosis factor-α converting enzyme indicates an indispensable role for cell surface protein shedding during embryonic lung branching morphogenesis. *Dev Biol* 232: 204–218, 2001.
117. **Zheng Y, Schlondorff J, and Blobel CP.** Evidence for regulation of the tumor necrosis factor α-convertase (TACE) by protein-tyrosine phosphatase PTPH1. *J Biol Chem* 277: 42463–42470, 2002.
118. **Zuo L, Ushio-Fukai M, Ikeda S, Hilenski L, Patrushev N, and Alexander RW.** Caveolin-1 is essential for activation of Rac1 and NAD(P)H oxidase after angiotensin II type 1 receptor stimulation in vascular smooth muscle cells: role in redox signaling and vascular hypertrophy. *Arterioscler Thromb Vasc Biol* 25: 1824–1830, 2005.
119. **Zwick E, Daub H, Aoki N, Yamaguchi-Aoki Y, Tinhofer I, Maly K, and Ullrich A.** Critical role of calcium-dependent epidermal growth factor receptor transactivation in PC12 cell membrane depolarization and bradykinin signaling. *J Biol Chem* 272: 24767–24770, 1997.