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Crosstalk Coregulation Mechanisms of G Protein-Coupled Receptors and Receptor Tyrosine Kinases

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Summary

G protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs) are transmembrane receptors that initiate intracellular signaling cascades in response to a diverse array of ligands. Recent studies have shown that signal transduction initiated by GPCRs and RTKs is not organized in distinct signaling cassettes where receptor activation leads to cell division and gene transcription in a linear manner. In fact, signal integration and diversification arises from a complex network involving crosscommunication between separate signaling units. Several different styles of crosstalk between GPCR- and RTK-initiated pathways exist, with GPCRs or components of GPCR-induced pathways being either upstream or downstream of RTKs. Activation of GPCRs sometimes results in a phenomenon known as "transactivation" of RTKs, which leads to the recruitment of scaffold proteins, such as Shc, Grb2, and Sos in addition to mitogen-activated protein kinase activation. In other cases, RTKs use different components of GPCR-mediated signaling, such as β-arrestin, G protein-receptor kinases, and regulator of G protein signaling to integrate signaling pathways. This chapter outlines some of the more common mechanisms used by both GPCRs and RTKs to initiate intracellular crosstalk, thereby creating a complex signaling network that is important to normal development.

Key Words: G protein-coupled receptor; growth factor receptor; crosstalk; transactivation; MAPK.

1. Introduction

Cells use a wide array of biochemical mechanisms to respond to extracellular signals, such as hormones, neurotransmitters, chemokines, odorants, and light. Three major classes of receptors on the surface of the cell detect these signals. The first class of receptor proteins is peripheral membrane proteins, which adhere only loosely to the biological membrane with which they are

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associated. These molecules do not span the lipid bilayer core of the membrane but attach indirectly, typically by binding to integral membrane proteins, or by interactions with the lipid polar head. Another major class of receptors is represented by intracellular receptors, such as those for steroid hormones. A third major class of receptors includes transmembrane proteins, which reside and operate typically within a cell's plasma membrane but also are found in the membranes of some subcellular compartments and organelles. Binding of a signaling molecule to the receptor on the extracellular domain helps transduce the signal through the transmembrane domain to the intracellular space of the cell. There are several types of transmembrane receptors including integrins, G proteins, and protein tyrosine kinases.

All G protein-coupled receptors (GPCRs) identified to date share a typical structural motif of seven membrane-spanning helices and are coupled with heterotrimeric G proteins. Agonist-stimulated GPCRs function as guanosine diphosphate (GDP)/guanosine triphosphate (GTP) exchange factors and promote the release of GDP and binding of GTP to the α-subunits. This process activates the G protein by dissociating GTP-bound Gα from the heterodimeric Gβγ subunit. Both GTP-Gα and Gβγ subunits interact with a variety of effector systems, such as adenylyl cyclase, phospholipase (PL) C isoforms, and ion channels, thereby modulating cellular signaling pathways through second messengers cyclic adenosine monophosphate (cAMP), protein kinase (PK) C, and Ca^{2+} and other intermediate molecules, such as phosphatidylinositol 3-kinase (PI3K), reactive oxygen species (ROS), Pyk2, and Src ***(1)***.

Receptor tyrosine kinases (RTKs) comprise another class of transmembrane proteins that span the membrane just once. Classically, RTKs are activated by ligands, such as growth factors and insulin. Upon ligand binding and receptor dimerization, the activated receptor acts as a tyrosine kinase, autophosphorylates itself on cytoplasmic tyrosine residues, and subsequently acts as a scaffold to assemble signaling partners. Classically these include Shc, Grb2, and Sos, which lead to Ras activation followed by an increase in mitogen-activated protein kinase (MAPK) activity ***(2,3)***.

Initially, it was thought that GPCRs and RTKs, along with their respective downstream effectors, represented distinct and linear signaling units that converged on downstream targets, such as the MAPKs. Recently, it has become clear that GPCR- and RTK-mediated signaling pathways are not mutually exclusive of one another and often function as partners, with G protein participation being either upstream or downstream of the RTKs, stimulating interactions at multiple levels between various molecules downstream of the receptors ***(4,5)***. For example, both pathways involve tyrosine phosphorylation of Shc and Ras activation upstream of MAPK activation ***(6–8)***. The involvement of common molecules initiates an integration of diverse stimuli through complex

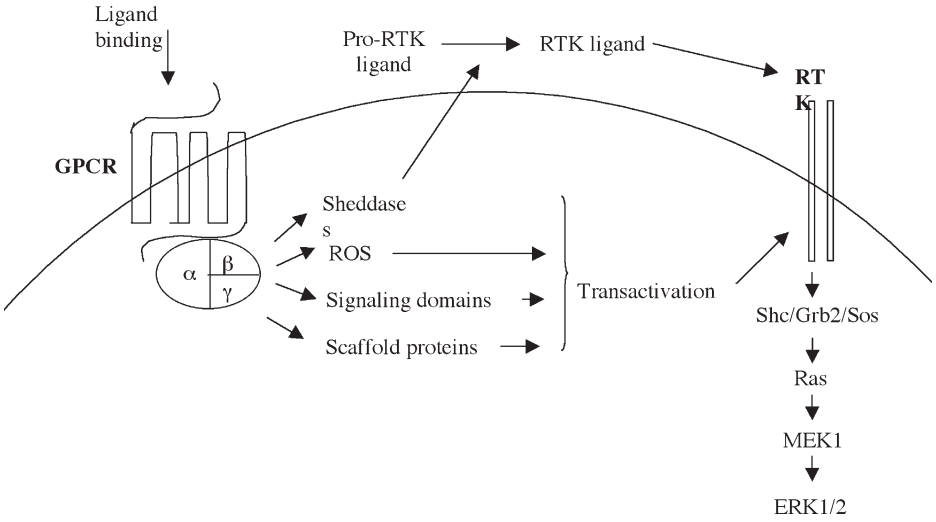

Fig. 1. Schematic showing G protein-coupled receptor–ligand-induced transactivation of receptor tyrosine kinase.

cross-communication and provides intricate control over regulatory mechanisms that affect cell proliferation, differentiation, growth, and survival. This chapter reviews the signaling pathways associated with crosstalk between GPCRs and RTKs that could be initiated by either GPCR or RTK ligands.

GPCRs initiate crosstalk in several different ways. In some cases, GPCRs can form homodimers and heterodimers in order to increase functional activity. Several such examples have been discovered, such as the heterodimerization of the γ-aminobutyric acid receptors, the homodimerization of the β2-adrenergic receptors, and the heterodimerization of the dopamine D2 and somatostatin SSTR5 receptor ***(9–11)***. In addition, treatment of cells with ligands for GPCRs results in tyrosine phosphorylation and subsequent activation of RTKs, by a phenomenon known as "transactivation" ***(12,13)***. In each case, increased dimerization of the RTKs leads to the recruitment of scaffold proteins, such as Shc, Grb2, and Sos, via their Src homology (SH)2 domains. Several GPCR agonists, such as angiotensin II (AngII), lysophosphatidic acid (LPA), bradykinin, and endothelin, transactivate RTKs such as the epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR).

In recent years, different concepts have emerged to explain mechanisms of transactivation as shown in **Fig 1**. Molecules such as PKC, Src, and ROS mediate RTK transactivation. In general, both calcium-dependent and -independent pathways leading to RTK transactivation have been suggested. One of the new concepts in transactivation mechanisms is that of GPCR ligands activating

"sheddases," proteases that cleave an RTK ligand molecule to its RTK-binding form. This active ligand in turn activates the RTK. Another mechanism of transactivation involves the creation of signaling domains by GPCR–ligand interaction, where there is a movement of RTKs to a specific subcellular location, leading to RTK–GPCR association and downstream signaling. Several adaptor/scaffold proteins such as Gab1, IRS-1, and GIT1, which serve as docking sites for multiprotein complexes at the RTK, also have been implicated as mediators of GPCR-ligand induced RTK transactivation, Activation of protein tyrosine phosphatases that "transinactivate" RTKs in response to GPCR activation also have been recently suggested as a mechanism of GPCR–RTK crosstalk.

In some cases, the RTK activation of downstream effector responses is sensitive to pertussis toxin, suggesting that G protein involvement is proximal to, and downstream of the RTKs. In this model, the RTKs use several different components of GPCR-mediated signaling, such as β-arrestin, regulator of G proteins (RGS), and G protein receptor kinases (GRKs). Studies by various groups have demonstrated two major models for G protein signaling downstream of RTKs. In the first scenario, activated RTKs have been shown to induce the activation of G proteins by dissociating the Gα subunit from the Gβγ subunit leading to downstream signaling (**Fig. 2A**) Alternatively, stimulation of an RTK by a ligand leads to a direct association between GPCRs and RTKs through scaffold proteins, such as RGS, leading to the use of G protein-associated molecules such as β-arrestin and Grk2, as shown in **Fig. 2B**. These data indicate the involvement of GPCRs both upstream and downstream of the RTK signal transduction. Outlined in **Headings 2** and **3** are a few common examples of crosstalk between GPCRs and RTKs. The novel crosstalk that may occur between two different RTKs also will be discussed.

2. GPCR/G Protein Ligand-Initiated Receptor Crosstalk

2.1. Angiotensin II

AngII, a multifunctional octapeptide of the renin–angiotensin system, influences the function of cardiovascular cells via intracellular signaling that is initiated at the AngII type 1 and type 2 receptors (AT_1R and AT_2R), which are GPCRs that have opposing effects on cell growth and other physiological functions ***(14,15)***. Crosstalk exists between AT_1R and AT_2R, and studies performed by Cui et al. demonstrate a role for SHP-1 tyrosine phosphatase in this cross talk that regulates survival of fetal vascular smooth muscle cells (VSMCs) ***(16)***. Activation of $G_{q/11}$ by AngII stimulates PLC to generate inositol (1,4,5)-triphosphate and diacyglycerol, thereby increasing intracellular Ca^{2+} levels and activation of PKC. Downstream effectors of AngII signaling include the following:

1. Extracellular signal-regulated kinase (ERK) 1/2, p38 MAPK, and JNK.
2. Tyrosine kinases, such as Src and Pyk2.

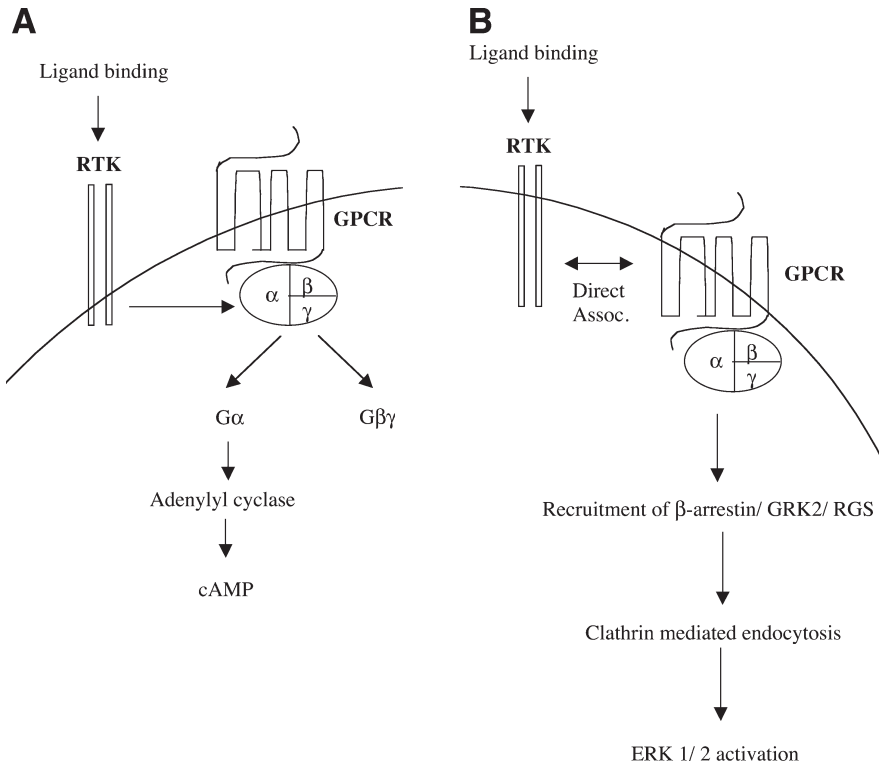

Fig. 2. Schematic showing receptor tyrosine kinase (RTK)–ligand-induced crosstalk with G protein-coupled receptors (GPCRs). (**A**) RTK–ligand-induced effect on G protein activation. (**B**) RTK–ligand-induced utilization of GPCR/G protein-regulating signaling components.

3. PI3K and PKB/Akt.
4. Janus-activating kinase (JAK) and signal transducers and activators of transcription (STATs).
5. RTKs, such as the EGFR and PDGFR ***(17–23)***.

2.1.1. EGFR Transactivation

AngII induces transactivation of the EGFR and, in turn, the EGFR serves as a scaffold for assembling signaling molecules, such as MAPKs and Akt that are important for downstream signaling, as well as the expression of the AT_1R signaling repertoire in VSMCs ***(20,24)***. Downstream, AngII-induced EGFR transactivation plays a role in inducing eukaryotic translation initiation factor 4E and 4E binding protein 1 phosphorylation, thereby playing a role in translational control and protein synthesis and this process upregulates proteins like the plasminogen activator inhibitor type 1 ***(25,26)***. AngII induces EGFR

transactivation by both Ca^{2+}-dependent and Ca^{2+}-independent processes ***(21,23,24,27,28)***. Three major mechanisms are involved in AngII-induced EGFR transactivation—an upstream tyrosine kinase, ROS, or through the use of metalloproteases that generate EGF-like ligands (sheddases in **Fig. 1**) In addition, recent studies from our laboratory indicate a novel mechanism by which glucose-dependent EGFR *N*-glycosylation and, hence, transactivation, modulates AngII signal transduction ***(29)***.

2.1.1.1. Non-RTKs

Two major non-RTKs have been shown to be involved in EGFR transactivation by AngII. Several studies done in VSMCs, cardiac myocytes, and rat anterior pituatory cells have shown that c-Src is necessary for the transactivation of the EGFR, and this in turn induces Ras/ERK activation downstream ***(12,24,30–32)***. In rat liver epithelial cells, Li et al. proposed an AngII-stimulated EGFR-dependent signaling pathway to Ras only when PKC activity was inhibited ***(33)***. Interestingly, in VSMCs, AngII-induced $p70^{rsk}$ activation is mediated via both the ERK and PI3K/Akt cascades that bifurcate at the point of EGFR-dependent Ras activation ***(34)***.

Another non-RTK, the proline-rich kinase 2 (PYK2)/cell adhesion kinase β also is induced by several GPCR agonists. Its role in the transmission of mitogenic signals via EGFR transactivation is somewhat controversial as shown in AngII-stimulated VSMCs, cardiac fibroblasts, and PC12 cells ***(27,35–37)***. Tyrosine phosphorylated Src is often found in association with the transactivated EGFR or with PYK2 on G_q-coupled receptor stimulation, suggesting activated Src to be the primary mediator of EGFR transactivation ***(35,36,38)***.

In addition to activating Src and PYK2, AngII induces the JAK/STAT signaling pathway, which has been implicated in ERK activation and subsequent cell growth in VSMCs, cardiac fibroblasts, and cardiomyocytes ***(39–41)***. Because JAK is involved in growth hormone-induced EGFR transactivation, the possibility of JAK-dependent EGFR transactivation by AngII also exists ***(42)***.

2.1.1.2. Reactive Oxygen Species

The generation of ROS, such as superoxide and hydrogen peroxide (H_2O_2) that act as intercellular and intracellular second messengers, is regulated by cytokines and growth factors, including AngII, in several cell types ***(43,44)***. AngII-induced transactivation of the EGFR is mediated, in part, through ROS derived from nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase, and this transactivation is strongly inhibited by antioxidants, such as, tiron, and *N*-acetylcysteine ***(28,45–47)***. Once produced, ROS activate several receptor- and non-RTKs, such as the JAK and Src families, PYK2, as well as the EGFR, stimulating the formation of the Shc–Grb2–Sos complex at the EGFR. This

complex subsequently activates Ras followed by the p38 MAPK and Akt/PKB pathways downstream of the EGFR ***(48,49)***. In addition, AngII promotes the movement of AT_1R to caveolae and lipid rafts leading to AT_1R–EGFR association in VSMCs through the tyrosine phosphatase SHP-2 ***(20,50)***. Depletion of membrane cholesterol by β-cyclodextrin disrupts caveolae structure and inhibits tyrosine phosphorylation of the EGFR and subsequent activation of PKB induced by AngII.

2.1.1.3. Metalloproteinase Cleavage of Heparin-Binding EGF

Prenzel et al. first showed that a chimeric RTK in rat fibroblasts, consisting of the EGFR ectodomain and the PDGFR transmembrane and intracellular domain, was transactivated with GPCR ligands, whereas the endogenous PDGFR was not, by the cleavage of proheparin-binding (pro-HB)-EGF to its active form HB-EGF by matrix metalloproteinases (MMPs; ***[51]***). Free HB-EGF subsequently binds to the EGFR, leading to EGFR transactivation. The role of MMPs in AngII-induced transactivation of the EGFR remains controversial; studies done in our laboratory on VSMCs did not show inhibition of EGFR transactivation with MMP inhibitors, whereas other studies have shown an inhibition by pharmacologically inhibiting the MMPs ***(22,23,52)***. Eguchi et al. suggest that MMP-dependent EGFR transactivation by AngII activates the ERK and p38 MAPK pathways, whereas JNK activation is regulated independent of EGFR transactivation ***(23)***.

Recent data suggests that different proteases (sheddases) may cleave pro-HB-EGF through PKC-dependent and PKC-independent mechanisms in response to different stimuli. Some data suggest that PKC mediates AngII-induced EGFR transactivation via activation of MMPs in response to GPCR agonists coupled to G_q ***(26,51,53–56)***. However, other studies, such as those done by Frank et al., showed that ROS transactivate EGF receptors through the release of HB-EGF by metalloproteases in VSMCs and that this transactivation is independent of PKC ***(57)***. In addition to the EGFR, the primary cognate HB-EGF receptor Erb1 has also shown to be transactivated by AngII in human prostate stromal cells, thereby promoting cell growth ***(58)***.

2.1.2. PDGFR Transactivation

Although PDGFR has two distinct receptor subtypes, rapid tyrosine phosphorylation of only the PDGFβ receptor by AngII has been reported ***(59–61)***. This transactivation induces association of the activated receptor with p66Shc, Grb2, and c-Src. In addition, PDGFR transactivation by AngII was not sensitive to BAPTA-AM, suggesting that this transactivation pathway was Ca^{2+}-independent ***(59)***. Like AngII-induced EGFR transactivation, PDGFR transactivation is redox-sensitive and is abrogated by *N*-acetylcysteine and

Tiron. Recently, the potential downstream signaling of the PDGFR to ERK 1/2 via AngII-mediated transactivation was proposed in mesangial cells ***(62)***. Additional studies by Conway et al. have shown that the activation of the MAPK pathway is dependent on both Src and complex formation of Grb2 with PI3K ***(63)***. New studies indicate that, like the EGF-family of ligands, a new ligand for the PDGFRα, PDGF-C, could be another growth factor that is released from the cell surface after limited proteolysis leading to transactivation of the PDGFR ***(64)***.

2.1.3. Insulin-Like Growth Factor 1 Receptor Activation

Another growth factor receptor that is transactivated by AngII is the insulin-like growth factor 1 receptor (IGF-1R) in VSMCs. IGF-1R becomes phosphorylated on its β-subunit and this in turn phosphorylates the adapter insulin receptor substrate-1 (IRS-1 ***[65]***). Transactivation of the IGF-1R has been shown to play a critical role in PI3K activation by AngII, but does not seem to be required for stimulation of the MAPK cascade ***(66)***. Touyz et al. demonstrated that AngII stimulates production of NADPH-inducible ROS partially through IGF-1R transactivation which leads to phosphorylation of p38 MAPK and ERK5, but not ERK 1/2 ***(49)***. Also, the role of insulin receptor substrate (IRS)-1-mediated signaling in response to AngII in VSMCs remains controversial as inhibition of insulin and IGF-1 signaling by AngII at the levels of IRS-1 and PI3K have been reported ***(67,68)***.

2.2. Lysophosphatidic Acid

LPA is an important component of serum that affects cell proliferation, survival, adhesion, and migration by transducing signaling through the Edg family of receptors that are coupled to Gi, Gq/11, and G12/13 proteins. LPA induces ERK 1/2 activation by mediator protein tyrosine kinases, such as Src, PYK2, and transactivated EGFR ***(13,69–73)***. LPA-induced EGFR tyrosine phosphorylation is weak but functionally significant in several cell lines tested ***(74)***. Inhibition of LPA induced EGFR transactivation suppressed tyrosine phosphorylation of adapter proteins Shc and Gab1, which in turn inhibited Shc-Grb2 and Gab1-SHP2 association that was necessary for ERK 1/2 activation. This indicates that LPA-induced transactivation is upstream of ERK 1/2 activation, *c-fos* induction and DNA synthesis ***(13,74,75)***.

Several studies have shown that LPA-mediated EGFR is dependent on calcium and ROS ***(76–80)***. In addition, LPA has been identified as a major serum factor for stimulating pro-HB-EGF ectodomain shedding via a Ras-Raf-MAPK/ERK pathway to transactivate the EGF receptor ***(81,82)***. Recently, LPA also has been shown to transactivate the HB-EGF receptors ErbB1 and ErbB4 via a Ca^{2+}-dependent pathway ***(83)***.

LPA receptors also interact with and transactivate the nerve growth factor receptor TrkA, stimulating translocation of the TrkA receptor to the nucleus and this regulates the ERK 1/2 pathway ***(84)***. LPA also mediates phosphorylation of the PDGFR-β in human bronchial epithelial cells via phospholipase D ***(85)***. In addition to transactivating these growth factor receptors, LPA induces phosphorylation of α_{1B}-adrenoreceptor phosphorylation through dissociated Gβγ subunits, EGFR transactivation, PI3K and PKC ***(86)***.

2.3. Endothelin

Endothelin (ET) isopeptides (ET-1, ET-2, and ET-3) are potent vasoconstrictors that bind specific ET (ET_A and ET_B) receptors coupled to G_q proteins. Similar to the angiotensin receptors, crosstalk between the two ET receptors has also been reported in rat mesenteric arteries ***(87)***. Activation of GPCRs by ET-1 phosphorylates the EGFR in a Ca^{2+}- and MMP-dependent manner, followed by an increased association of the phosphorylated EGFR with Shc and Grb2, subsequently leading to MAPK phosphorylation, $p70^{S6K}$ activation, *c-fos* induction, and cell proliferation ***(13,51,88,89)***. In addition, Hua et al. have shown that ET-1 activates ERK 1/2 in mesangial cells predominantly through a pathway involving EGFR transactivation and its attachment to caveolin, leading to compartmentalization of these signaling molecules ***(90)***. In a rat cardiac allograft model, Sihvola et al. demonstrated an increase in VSMC proliferation and migration via ET-1 induced PDGFR upregulation ***(91)***. ET-1 also signals through other GPCRs. ET-1 and norepinephrine signaling crosstalk through differential pathways regulating myocardial contractility, and this is mediated by Ca^{2+} transients, PKA, PKC, PKG, and phosphatases ***(92)***. PKC also plays a major role in ET-induced phosphorylation of the α_{1B}-adrenergic receptor ***(93,94)***.

2.4. Bradykinin

Bradykinin is an inflammatory mediator that exerts its biological effects through the activation of several bradykinin receptors. The B2 receptor (B2R) is capable of coupling to different classes of G proteins in a cell specific and time-dependent manner, resulting in simultaneous or consecutive initiation of different signaling chains that may crosstalk. Blaukat et al. have shown that bradykinin activates both $G\alpha_q$ and $G\alpha_i$ pathways simultaneously and cooperative signaling between these two activated G protein pathways is required for a synergistic stimulation of ERK 1/2 ***(95)***. Other studies have shown that the activated bradykinin receptor coupled to $G\alpha_q$ can activate $G\alpha_i$ and subsequently adenylate cyclase and cAMP. This activation leads to differential regulation of PLC preventing multiple stimulation of MAPK ***(96)***. Bradykinin modulates α_{1b}-adrenoreceptor phosphorylation in rat-1 fibroblasts ***(97)***. The B2R also has been shown to crosstalk with nucleotide receptors, such as P2Y, which are also coupled to G_q ***(98,99)***.

Schindelholz et al. report growth cone collapse of neuronal growth factor (NGF)-differentiated PC12 cells evoked by bradykinin, mediated by c-Src and paxillin, revealing a crosstalk between bradykinin and growth factor receptors, such as the NGF receptor ***(100)***. Bradykinin-induced transactivation of the KDR/Flk-1 (VEGF receptor 2) receptors associated with endothelial nitric oxide synthase production has also been shown in endothelial cells ***(101,102)***. Work done in several systems have shown that bradykinin induces transactivation of the EGFR via both PKC-dependent and PKC-independent mechanisms, which leads to phosphorylation of downstream molecules, such as ERK 1/2, AMP responsive element-binding protein (CREB), nuclear factor (NF)-κB, and E2F ***(103–105)***. EGFR transactivation by bradykinin also induces desensitization of EGFRs by a process associated with the loss of cell-surface EGFRs through clathrin-mediated endocytosis via β-arrestin and dynamin ***(104)***. Whether calcium and calmodulin are required for EGFR transactivation by bradykinin remains a matter of controversy ***(106–108)***. Finally, novel findings by Graness et al. show bradykinin-mediated "transinactivation" of EGFR by stimulation of a protein tyrosine phosphatase ***(109)***.

2.5. Sphinosine 1-Phosphate

Sphinosine 1-phosphate (S1P) is a bioactive lipid released by activated platelets that induces cell processes, such as migration and proliferation by binding the Edg family of GPCRs. S1P induces transactivation of the vascular EGFR (VEGFR) in human umbilical vein endothelial cells, followed by Src activation and phosphorylation of the adaptor protein CrkII, to induce membrane ruffling ***(110)***. In other studies, transactivation of the VEGFR by S1P is independent of ROS and is mediated by Ca^{2+} and Src, leading to the activation of the PI3K/Akt/endothelial nitric oxide synthase pathway ***(111)***. S1P also stimulates Akt phosphorylation via G_i-dependent PDGFRβ transactivation ***(112)***. Transactivation of EGFR by S1P has also been reported through a PKC-dependent pathway that results in the activation of the Ras–MEK–ERK pathway ***(113)***.

2.6. Thrombin

Thrombin is a procoagulant protease that signals through the protease-activated receptor family that are coupled to G proteins. Transactivation of the EGFR on thrombin stimulation has been shown in a number of systems through multiple mechanisms ***(114)***. Several groups also showed that thrombin transactivates the EGFR via HB-EGF, Src, and PYK2 followed by increased ERK 1/2 and p38 MAPK activation, leading to an increase in CREB activation DNA synthesis and interleukin 6 gene expression ***(115–119)***. In rat VSMCs, thrombin induces the release of basic FGF that results in FGF receptor transactivation-mediated cell proliferation ***(120)***. Thrombin also induces IGF-1R transactivation in rat VSMCs ***(121)***.

2.7. Adrenoreceptor Agonists

AngII stimulates the release of norepinephrine from the sympathetic nerves that is a ligand for the α_1-adrenergic receptor. In carotid injury models, Majesky et al. showed that α_1-adrenergic stimulation caused PDGF-A expression, suggesting crosstalk between AngII and PDGF signaling ***(122)***. Luttrell et al. also have demonstrated EGFR transactivation by G_i coupled-α-adrenergic receptors followed by tyrosine phosphorylation of the Shc adapter protein ***(12)***. In addition, PDGFRs reduce actions of α_{1B}-adrenergic receptors by phosphorylating the receptors and decreasing their association with their G proteins ***(93)***.

3. Growth Factor-Initiated Crosstalk Via G Proteins

3.1. Epidermal Growth Factor

Upon EGFR activation and autophosphorylation, numerous phosphotyrosines are generated that serve as docking sites for proteins, such as PLCγ, Shc, Gab1, and Grb2, which in turn activate downstream pathways. However, the EGFR also uses components involved in G protein signaling and bidirectionally interacts with GPCRs. EGF stimulation leads to increased association of $G\alpha_{12}$ with EGFR, which leads to the activation of PLCγ, ERK 1/2, and increased DNA synthesis ***(123–125)***. EGFR interaction with $G\alpha_i$ inhibits $G\alpha_i$. EGFR kinase phosphorylates and associates with $G\alpha_s$ leads to the activation of $G\alpha_s$ and in the heart this mechanism leads to increased cAMP accumulation via activation of adenylate cyclase ***(126–128)***.

Direct activation of EGFR also induces α_{1B}-adrenergic receptor phosphorylation by PKC via activation of PI3K ***(93)***. Also, Maudley et al. reported that the EGFR exists in a preformed complex with β2-adrenergic receptor ***(129,130)***. Transactivation of EGFR by GPCR agonists leads to the β-arrestin and Gβγ-mediated internalization of this complex, which is necessary for the activation of MAPK. However, EGF itself can stimulate the recruitment of β-arrestin to the EGFR, suggesting downstream interaction between the GPCR and EGFR pathways ***(130)***. EGF is also known to regulate other GPCR signaling component associations, such as that between GRK2 and PDEγ, thereby regulating MAPK activation and EGF-mediated phosphorylation of RGS increases GTPase activating protein activity ***(131,132)***.

3.2. Platelet-Derived Growth Factor

There is substantial evidence showing a requirement for G proteins in platelet-derived growth factor (PDGF)-stimulated pathways. Several studies have shown that activation of c-Src and ERK 1/2 downstream of PDGF stimulation is sensitive to pertussis toxin ***(63,133)***. In addition, Freedman et al. showed that GTPγS binding to $G\alpha_i$ increases on PDGF stimulation ***(134)***. PDGF induction of ROS also seems to require coupling of $G\alpha_{i1}$ and $G\alpha_{i2}$ to the PDGFR

(135). PDGF-induced cell migration requires the presence of EDG-1 a GPCR for S1P that activates Rac-dependent pathways *(136)*.

PDGFβ receptor signals through an endocytic pathway as well via GPCR-dependent machinery. The GRK2/β-arrestin complex constitutively associates with the PDGFR and is recruited via its association with the GPCR. On stimulation with PDGF, c-Src is recruited to the PDFGR–GPCR complex leading to β-arrestin-mediated signaling and ERK 1/2 activation *(134,137)*. RGS proteins, such as RGS**2**, that are GAPs involved in terminating GPCR signaling, are also recruited to the plasma membrane after PDGF stimulation, suggesting another component of GPCR signaling is involved in PDGFR signaling *(138)*.

3.3. Neuronal Growth Factor

NGF promotes the survival and differentiation of neurons and signals through its receptor TrkA, The TrkA receptor is constitutively bound to GRK2 and stimulation with NGF promotes binding of β-arrestin to this complex in a $G\alpha_{i/o}$-dependent manner. This initiates an integrative activation of the ERK 1/2 pathway via a process that involves β-arrestin 1 and clathrin-mediated endocytosis of the TrkA–GPCR/B-raf/MEK-1 signal complex. NGF also reduces cAMP levels in PC12 cells via a G protein-dependent mechanism *(139)*. Another level of GPCR crosstalk is with tyrosine kinase receptors through RGS proteins, where the RGS serves as a scaffold bridging together GPCRs and RTKs. Lou et al. were the first to show suppression of GPCR signaling by Trk, which is dependent on a PDZ domain in the RGS protein GIPC *(140)*.

3.4. Fibroblast Growth Factor

Fibroblast growth factors (FGFs) are members of a family of polypeptides synthesized by a variety of cell types that signal through one of four FGF receptors, i.e., FGFR1–4. Similar to other RTKs, FGFR stimulation with FGF results in receptor dimerization, phosphorylation, and activation of the Ras–Raf–MEK–MAPK pathway through either the Crk/FGFR substrate 2 (FRS2)/Grb2/Sos or Shc/Grb2/Sos complex. Fedorov et al. have shown that that $G_i\beta\gamma$ are involved in FGF-2 mediated activation of ERK 1/2 that promotes skeletal muscle differentiation *(141)*. Also, FGF-2 induces S1P-coupled G_i receptors by activating sphingosine kinase-1, the enzyme that converts sphingosine to S1P *(142)*. It has also been demonstrated that FGF-2 promotes dissociation of the $G_s\beta\gamma$ heterotrimer, leading to $G\alpha_s$ stimulation of adenylyl cyclase and $G\beta\gamma$ inhibition of NADPH oxidase *(143)*.

3.5. Vascular Endothelial Growth Factor

VEGF is a cytokine that is essential for angiogenesis and endothelial cell differentiation (vasculogenesis) during development *(144,145)*. VEGF regulates multiple biological functions through three major types of receptors—the

RTKs Flt1 (VEGFR-1), KDR/Flk1 (VEGFR-2), and Flt-4 (VEGFR-3), a nontyrosine kinase transmembrane protein Neuropilin-1 and heparan sulfate proteoglycans ***(146–151)***. Zeng et al. have demonstrated that VEGFR-2 (KDR) stimulates MAPK activation, migration, and proliferation via $G\alpha_q$ and $G\beta\gamma$ subunits ***(152,153)***. Also, KDR signaling is downregulated by VEGFR-1 (Flt-1)/G_i/$G\beta\gamma$-mediated activation of cdc42 and Rho, demonstrating opposing effects of the two VEGFRs ***(154)***.

3.6. Insulin and IGF

Insulin receptors have been shown to associate with and tyrosine phosphorylate Gi and Gs in several studies ***(155,156)***. Also, insulin phosphorylates the β2 adrenergic receptor (β2-AR), leading to increased Grb2/β2-AR interaction. Grb2 inturn binds PI3K and dynamin, and this leads to the internalization of β2-AR.

IGF-1 is a 12-kDa mitogenic and survival factor hormone peptide secreted by multiple cells that interacts with its own receptor, as well as the insulin receptor. IGF-1 preferentially interacts with and uses the G_i-dependent signaling pathway by promoting $G_i\beta\gamma$ dissociation to lower cAMP levels and activate ERK 1/2 and DNA synthesis in muscle cells and fibroblasts ***(157–159)***.

4. Growth Factor-Initiated RTK–RTK Crosstalk

Finally, EGFR and PDGFβ-R interact physically forming heterodimers and stimulation by EGF has been shown to increase the tyrosine phosphorylation of the PDGFβ-R leading to the recruitment of PI3K to the PDGFR ***(160,161)***. Bagowski et al. also provided evidence for the negative regulation of EGF-induced *c-jun* transcription by PDGF-mediated phosphorylation of the EGFR, demonstrating crosstalk between different members of the RTK family ***(162)***. Insulin receptors that are hormone-stimulated transactivate IGF-1 receptors ***(163)***. Recently, Roudabush et al. showed that ERK 1/2 activation downstream of IGF-1R stimulation is mediated by transactivation of the EGFR in Cos7 cells proposing an IGF-1R–EGFR crosstalk pathway based on metalloprotease-induced shedding of pro-HB-EGF ***(164)***.

5. Other Ligand-Induced Receptor Crosstalk

5.1. Integrins

Integrins, which are the primary link between extracellular matrix ligands and cytoskeletal structures, are a complex family of noncovalently associated heterodimeric transmembrane receptors composed of α and β subunits. They serve as both adhesive receptors and intracellular signaling mediators ***(165,166)***. In addition to transmitting signals from the extracellular matrix to the intracellular environment ("outside-in" signaling), integrins can be modified by agonists that bind nonintegrin cellular receptors like growth factor re-

ceptors. This concept of "inside-out" signaling in turn regulates integrin activation and function. In addition, it has been shown that integrin activation of growth factor receptors can occur even in the absence of the growth factor ***(167–169)***.

RTKs and growth factors interact spatially at multiple levels. At the plasma membrane, specific direct associations between integrins and RTKs, such as the PDGFR, EGFR, the insulin receptor, the IGF-1R and the VEGFR2, have been identified ***(170–172)***. Another level of interaction between growth factor receptors and integrins is at the level of plasma membrane lipid rafts as shown with PDGFR by Baron et al. ***(173,174)***. A third level of intersection between the growth factor and integrin pathways are at more downstream signaling molecules, such as focal adhesion kinase (FAK), and activation of a particular signaling cascade directly by integrins could lead to growth factor dimerization and phosphorylation/activation ultimately influencing MAPK activation ***(175,176)***.

In addition to interacting with growth factor receptors, integrins also interact with GPCRs, such as the LPA receptor 3. Studies by Sengupta et al. show that laminin-induced cell migration in ovarian cancer cells is mediated by LPA via PLA2 and PI3K, revealing a new mechanism of crosstalk between a β1 integrin receptor and a GPCR ***(177)***.

6. Conclusion

Signaling cascades often were considered to be discrete signaling cassettes that linked activation of a receptor to gene transcription and physiological function in a linear manner. Recent insights have broadened this view to encompass a complex network that allows multiple levels of crosstalk between the individual signaling units (stimulated by GPCR and RTK), leading to signal integration. This selective crosscommunication between different receptor classes generates common signals, including the stimulation of Ras GTPases and MAPKs, that control cell proliferation, differentiation, growth, and survival.

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