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Review

Crosstalk Between G Protein-Coupled Receptors and Tyrosine Kinase Signaling: Src Take Centre Stage

ANDRZEJ PTASZNIK* and ALAN M. GEWIRTZ

Department of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

Abstract. Although the role of G protein-coupled receptors in the regulation of metabolic, secretory and contractile responses is well established, they have only recently been recognized as important mediators of cellular growth and differentiation. G protein-coupled signaling pathways had been previously thought to be totally independent of the tyrosine kinase receptor pathway. It was previously believed that molecular switches responsible for growth factor tyrosine kinase receptor signaling and G protein-coupled signaling were divided into a distinct sets of protein families. Recent evidence has demonstrated, however, that G protein-coupled receptors can crosstalk to tyrosine kinase signaling. In the past few years several groups have found that G protein-coupled receptors utilize non-receptor tyrosine kinases, mostly that of Src family, and some adapter proteins, to regulate tyrosine kinase cascades in cells.

Key words: signal transduction; Src kinase; Shc protein; PI 3-kinase.

Receptors coupled to heterotrimeric GTP-binding proteins (G proteins) are the largest family of cell surface receptors in mammalian cells. These receptors are formed by a single polypeptide with an extracellular N-terminus, an intracellular C-terminus and 7 transmembrane domains. Several well-described groups of agonists, including hormones, neurotransmitters, chemokines and chemotactic agents, have been shown to bind to the N-terminus of their cognate receptor. When a receptor is stimulated with a ligand, its intracellular regions undergo conformational changes, allowing the receptor to interact noncovalently with G proteins[1]. This association, in turn, leads to conformational changes in the G proteins. Each receptor activates a specific class of trimeric G proteins, including Gi-α, Gq-α, G-β/γ and many others. G protein activity is tightly linked to the binding and hydrolysis of GTP. In the resting state, GDP is bound to the guanine nucleotide binding site on G-α. The binding of an agonist to its receptor promotes the release of GDP and its replacement with GTP. At the same time G-α undergoes a conformational transformation and dissociates from G-β/γ, leaving both in their biologically active state. The mechanism of utilizing trimeric G-proteins to transduce signals from an activated receptor into a cellular response is quite different from that of growth factor receptors which employ intrinsic tyrosine kinase activity. Nevertheless, it has been known for many years that G protein-coupled receptor dependent Ras and MAPK activation can be blocked with inhibitors of tyrosine phosphorylation, indicating that tyrosine phosphorylation plays an important role in this process. Since these receptors were not known to couple to previously defined pathways for tyrosine ki-

* Correnspondence to: Andrzej Ptasznik M.D., Ph.D., Department of Medicine, University of Pennsylvania (BRB-2, R-727), 421 Curie Blvd., Philadelphia, PA 19104, USA, fax: 215-573-7049, e-mail: andrzejp@mail.med.upenn.edu

nase/Ras activation, the means by which these receptors stimulate Ras and MAPK function has until recently been unclear. Several groups found that G protein--coupled receptors stimulate tyrosine phosphorylation cascade through association with Src family tyrosine kinases and the Shc adapter protein[12, 17, 18, 25]. These reports implicated Src/Shc signaling complexes as the go between that relay messages from the G protein--coupled receptors to the Ras and MAPK. Src and Shc--mediated integration between G protein-coupled signaling and growth factor receptor signaling occurs via obscure mechanisms involving phosphatidylinositol 3-kinase (PI3K) activity and perhaps other as yet unidentified proteins.

G Protein-Coupled Receptor Signaling via Src/Shc Complexes

The G protein-coupled N-formyl peptide chemoattractant receptor of leukocytes has been shown to mediate complex formation between a Src family tyrosine kinase and a tyrosine phosphorylated Shc adapter protein[18]. This is important because Shc is an adapter molecule that serves to link tyrosine kinase receptors to the Ras activation complex (i.e. Grb2 and Sos). Association between Src and Shc is sensitive to pertussis toxin (PTX), indicating that the signal is mediated by PTX-sensitive G proteins[18]. On the other hand, this event is not stimulated by elevations in intracellular Ca^{2+}, nor blocked by a low dose of staurosporine, suggesting that activation of protein kinase C is not required for Src/Shc interaction. This is of interest because the G protein-coupled receptor family has been classically linked to cell activation via phospholipase C, calcium and protein kinase C, but not via Src/Shc association[22]. Similarly, it has been recently shown in COS-7 cells that stimulation of G protein-coupled lysophosphatidic acid (LPA) receptor leads to an increase in c-Src autophosphorylation and the formation of a Src/Shc complex[12]. Coexpression of Csk in these cells, which inactivates Src family kinases by phoshorylating the regulatory c – terminal tyrosine residue, inhibited LPA stimulation of Shc tyrosine phosphorylation, Shc-Grb2 complex formation, and MAP kinase activation. Again, these events are pertussis toxin-sensitive, indicating that Src activity, which regulates mitogenic signaling cascade in this cellular system, is activated by PTX-sensitive G proteins. Consistent with this, activation of c-fos transcription by endothelin-1, which acts through a G protein-coupled receptor, is strongly inhibited by both Csk and a dominant negative mutant of c-Src[20]. This additionally indicates that non--receptor tyrosine kinases of the Src-family may play an important role in G protein-coupled receptor-mediated cell growth. Convergence of the G protein-coupled receptor and the growth factor receptor is further supported by observations that the epidermal growth factor receptor (EGF-R) became tyrosine phosphorylated after G protein activation[6]. LPA and β2A-adrenergic receptor stimulation induces tyrosine phosphorylation of the EGF receptor, and formation of a complex containing c-Src, Shc and EGF receptor[11]. Interestingly, the blockade of Src activity by expression of either Csk or dominant negative mutant of c-Src inhibits this complex assembly[11]. These results strongly indicate that Src plays an "upstream" role in growth factor receptor transactivation by G protein-coupled receptors. Thus, all available data support the concept that G protein--coupled receptors gain access to Ras and MAPK-dependent signaling pathways by regulating the activity of growth factor receptors through Src (see Fig. 1). However, little is still known about the precise biochemical mechanism whereby this is achieved.

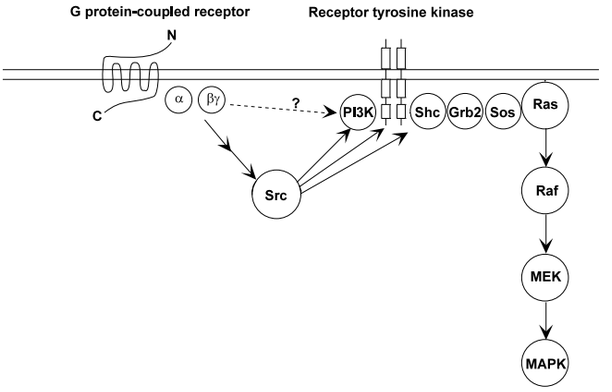

Fig. 1. G protein-coupled receptor-induced activation of the MAP kinase cascade via transactivated tyrosine kinase growth factor receptor. The interaction of G protein-coupled receptor with Src-related kinase, and subsequently with receptor tyrosine kinase, Shc adapter protein and PI3 kinase signaling complex, is shown schematically (details are described in the text)

G Protein-Coupled Receptor Signaling via PI3K

Associated with the Src-Shc complex formed during G protein-coupled receptor stimulation is PI3K an important signaling enzyme, whose activity may be regulated by this interaction[17, 18]. The lipid products of PI3K have been implicated in signaling pathways leading to cell growth[3, 16], differentiation[4, 13, 15, 16], apoptosis[7], chemotaxis[24] and secretion[2]. The mechanism by which PI3K is controlled by G protein-coupled receptors does

not appear to be a result of direct recruitment of PI3K to this receptor. Two mechanisms through which PI3K can be activated by these receptors have been described. One pathway involves the coupling of the src--related tyrosine kinases to the "classical" p85/p110 form of PI3K[17, 18]. The other pathway utilizes β/γ subunits of G proteins to activate a novel, "non-classical" form of PI3K[10, 21]. This form of PI3K has recently been cloned and shown to consist of unique p110γ catalitic subunit that lacks the p85 binding domain and, therefore, does not associate with the p85 subunit. An important question that has not yet been fully resolved is the contribution of the Src-regulated p85/p110 PI3K pathway versus that of the β/γ-regulated p110γ PI3K to the overall formation of phosphatidylinositol 3,4,5 triphosphate – PIP3, which is the main product of PI3K[24], in G protein-coupled receptor-stimulated cells. Previously, several studies provided indirect evidence that G protein-coupled receptors stimulate PIP3 formation mostly through the Src and the p85/p110 heterodimer and the tyrosine kinase-dependent signaling[9, 17]. Other studies suggested that G protein-coupled receptors may predominantly activate cell and PIP3 formation through p110γ, which is independent of tyrosine kinase[10, 21]. Recently, we have clearly shown, by transfecting cells with a p85 dominant negative mutant, that the p85/p110 PI3K is predominantly required for G protein-dependent cell activation, at least in epithelial cells[14]. This is of interest because p85/p110 PI3K is known to bind, through its SH2 domain on the p85 subunit, to a tyrosine phosphorylated motif on growth factor receptors. Thus, this isoform of PI3K forms a functional complex with the tyrosine kinase receptor, the Src non-receptor tyrosine kinase and the Shc adapter protein. As we already mentioned, Src protein plays an "upstream" role in assembling this signaling complex[11]. However, the role for PI3K in this process is more obscure. In contrast to inhibitors of Src activity, the PI3K inhibitors reportedly fail to prevent G protein--coupled receptor-mediated EGF receptor transactivation[5].

Nevertheless, they effectively block MAP kinase activation mediated by G protein-coupled receptors in COS-7 and CHO cells[8]. These inhibitors also prevented LPA-mediated activation of Shc[23] and Ras[8], as well as chemoattractant receptor-mediated activation of Shc (Ptasznik and Traynor-Kaplan, data unpublished), Thus, while p85/p110 PI3K activity is apparently involved in the mitogenic and chemoattractant signaling pathways triggered by G protein-coupled receptors, the PI3K-dependent intermediate step in this pathway is probably "downstream" from Src and receptor tyrosine kinase activation, and "upstream" from Shc tyrosine phosphorylation and Ras activation. A possible explanation of the role of PI3K in signaling upstream from Shc and Ras is that the product of PI3K activity, PIP3, has a high binding affinity for the SH2 domains of various proteins[19]. PIP3 recruits a subgroup of SH2-containing proteins to specific compartments of the cell that may be important for activation of these proteins. We therefore speculate that interactions between PIP3 and Shc-SH2 and Grb2-SH2 domain might have a regulatory effect on the Ras activation complex. Future studies directed at the mechanism by which G protein-coupled receptors couple to receptor tyrosine kinases should prove informative, as will further investigation of downstream signaling elements linked to this pathways.

Summary and Future Prospects

G protein-coupled signaling pathways synergize with other receptor pathways to enhance or inhibit signals elicited by these various receptors. Therefore, one of the most interesting challenges in this fields is to identify the signaling molecules that mediate crosstalk between these receptor pathways. These signaling molecules play a vital role in coordinating such cellular events as proliferation, differentiation, chemotaxis, apoptosis and secretion. Conversely, disintegration of these multiple regulatory pathways may lead to serious cell dysfunction. Thus, complete understanding of crosstalk signaling mechanisms may facilitate the design of new pharmacological agents for the treatment of diseases involving uncontrolled cell proliferation, abnormal cell differentiation, and premature or excessive apoptosis, such as atherosclerosis, tumorigenesis and inflammatory diseases.

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References

1. BOURNE H. R. (1997): How receptors talk to trimeric G proteins. Curr. Opin. Cell Biol., **9**, 134–142.
2. BROWN W. J., DE WALD D. B., EMR S. D., PLUTNER H. and BALCH W. E. (1995): Role for phosphatidylinositol 3-kinase in

the sorting and transport of newly synthetized lysosomal enzymes in mammalian cells. J. Cell. Biol., **130**, 781–796.
3. CANTLEY L. C., AUGER K. R., CARPENTER C., DUCKWORTH B., GRAZIANI A., KAPELLER R. and SOLTOFF S. (1991): Oncogenes and signal transduction. Cell, **64**, 281–302.
4. COOLICAN S. A., SAMUEL D. S., EWTON D. Z., MCWADE F. J. and FLORINI J. R. (1997): The mitogenic and myogenic actions of insulin-like growth factors utilize distinct signaling pathways. J. Biol. Chem., **272**, 6653–6662.
5. DAUB H., WALLASH C., LANKENAU A., HERRLICH A. and ULLRICH A. (1997): Signal characteristics of G protein-transactivated EGF receptor. EMBO J., **16**, 7032–7044.
6. DAUB H., WEISS F. U., WALLASCH C. and ULLRICH A. (1996): Role of transactivation of the EGF receptor in signalling by G protein-coupled receptors. Nature, **379**, 557–560.
7. FRANKE T. F., KAPLAN D. R. and CANTLEY L. C. (1997): PI3K: downstream AKTion blocks apoptosis. Cell, **88**, 435–437.
8. HAWES B. E., LUTTRELL L. M., VAN BIESEN T. and LEFKOWITZ R. J. (1996): Phosphatidylinositol 3-kinase is an early intermediate in the G beta/gamma mediated mitogen-activated protein kinase signaling pathway. J. Biol. Chem., **271**, 12133–12136.
9. KOWALSKI-CHAUVEL A., PRADAYROL L., VAYSSE N. and SEVA C. (1996): Gastrin stimulates tyrosine phosphorylation on insulin receptor substrate 1 and its association with Grb2 and the phosphatidylinositol 3-kinase. J. Biol. Chem., **271**, 26356–26361.
10. LOPEZ-ILASACA M., CRESPO P., PELLICI P. G., GUTKIND J. S. and WETZKER R. (1997): Linkage of G protein-coupled receptors to the MAPK signaling pathway through PI 3-kinase. Science, **275**, 394–397.
11. LUTTRELL L. M., DELLA ROCCA G. J., VAN BIESEN T., LUTTRELL D. K. and LEFKOWITZ R. J. (1997): G beta/gamma subunits mediate src-dependent phosphorylation of the epidermal growth factor receptor. J. Biol. Chem., **272**, 4637–4644.
12. LUTTRELL L. M., HAWES B. E., VAN BIESEN T., LUTTRELL D. K., LANSING T. J. and LEFKOWITZ R. J. (1996): Role of c-Src tyrosine kinase in G protein-coupled receptor and G beta/gamma subunit-mediated activation of mitogen-activated protein kinases. J. Biol Chem., **271**, 19443–19450.
13. MARCINKOWSKA E., WIEDLOCHA A. and RADZIKOWSKI C. (1998): Evidence that PI3-kinase and p70S6K protein are involved in differentiation of HL-60 cells induced by calcitriol. Anticancer Res., **18**, 3507–3514.
14. PAN Z. K., CHRISTIANSEN S. C., PTASZNIK A. and ZURAW B. L. (1999): Requirement of phosphatidyinositol 3-kinase activity for bradykinin stimulation of NF-kappa B activation in cultured human epithelial cells. J. Biol. Chem., **274**, 9918–9922.
15. PTASZNIK A. (1998): Letter to the Editor. Cancer Res., **58**, 4980.
16. PTASZNIK A., BEATTIE G. M., MALLY M. I., CIRULLI V., LOPEZ A. and HAYEK A. (1997): Phosphatidylinositol 3-kinase is a negative regulator of cellular differentiation. J. Cell Biol., **137**, 1127–1136.
17. PTASZNIK A., PROSSNITZ E. R., YOSHIKAWA D., SMRCA A., TRAYNOR-KAPLAN A. and BOKOCH G. M. (1996): A tyrosine kinase signaling pathway accounts for the majority of phosphatidylinositol 3,4,5-trisphosphate formation in chemoattractant-stimulated human neutrophils. J. Biol Chem., **271**, 25204–25207.
18. PTASZNIK A., TRAYNOR-KAPLAN A. and BOKOCH G. M. (1995): G protein-coupled chemoattractant receptors regulate Lyn tyrosine kinase-Shc adapter protein signaling complexes in human leukocytes. J. Biol. Chem., **270**, 19969–19973.
19. RAMEH L. E., CHEN C. S. and CANTLEY L. C. (1995): Phosphatidylinositol (3,4,5) P3 interacts with SH2 domain and modulates PI 3-kinase association with tyrosine-phosphorylated proteins. Cell, **83**, 821–830.
20. SIMONSON M. S., WANG Y. and HERMAN W. H. (1996): Nuclear signaling by endothelin-1 requires Src protein-tyrosine kinases. J. Biol. Chem., **271**, 77–82.
21. STOYANOW B., VOLINIA S., HANCK S., RUBIO T. et al. (1995): Cloning and characterization of a G protein-activated human phosphoinositide-3 kinase. Science, **269**, 690–693.
22. THELEN M. and WIRTHMUELLER U. (1994): Phospholipases and protein kinases during phagocyte activation. Curr. Opin. Immunol., **6**, 106–110.
23. TOUHARA K., HAWES B. E., VAN BIESEN T. and LEFKOWITZ R. J. (1995): G protein beta/gamma subunits stimulate ohosphorylation of Shc adapter protein. Proc. Natl. Acad. Sci. USA, **92**, 9284–9287.
24. TRAYNOR-KAPLAN A. E., HARRIS A. L., THOMPSON B. L., TAYLOR P. and SKLAR L. A. (1988): An inositol tetrakisphosphate--containing phospholipid in activated neutrophils. Nature, **334**, 353–362.
25. VAN BIESEN T., HAWES B. E., LUTTRELL D. K., KRUEGER K. M., TOUHARA K., PORFIRI E., SAKAUE M., LUTTRELL L. M. and LEFKOWITZ R. J. (1995): Receptor-tyrosine-kinase and G beta/gamma-mediated MAP kinase activation by a common signalling pathway. Nature, **376**, 781–784.

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