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PERSPECTIVE

Scaffolds Direct Src-Specific Signaling in Response to Angiotensin II: New Roles for Cas and GIT1

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Angiotensin II (AngII) is the key product of the renin-angiotensin system and plays an important pathophysiological role in cardiovascular disease, including atherosclerosis, hypertension and cardiac hypertrophy. The importance of AngII in cardiovascular disease has been demonstrated in several clinical trials in which angiotensin-converting enzyme inhibitors and angiotensin receptor blockers proved to be beneficial in congestive heart failure, stroke and coronary artery disease (Pfeffer et al., 1992; Yusuf et al., 2000a,b). At the cellular level, AngII acts as a pro-inflammatory mediator by stimulating production of other growth factors and vasoconstrictors, transactivates several growth factor receptors, and influences cell functions such as contraction, cell growth, apoptosis, differentiation, gene expression, and cell migration (Yin et al., 2003).

AngII exerts its effects on various cell types, including fibroblasts, mesangial cells, macrophages, and vascular smooth muscle cells (VSMCs), by binding high-affinity angiotensin receptors. To date most of the physiological actions of AngII have been ascribed to activation of the AngII type 1 receptor (AT1R). Binding of AngII to the AT1R initiates several complex interacting pathways, including signaling through phospholipid metabolizing enzymes, such as phospholipase C (PLC), phospholipase D, and phospholipase A2, and receptor tyrosine kinases, such as the EGF, platelet-derived growth factor, and insulin-like growth factor receptors (Fig. 1). Ligand binding to the AT1R also induces the activation of nonreceptor tyrosine kinases, such as c-Src, Janus family kinases, focal adhesion kinase (FAK), and Pyk2.

The Src tyrosine kinase family, specifically c-Src, Fyn, and Yes, has been shown to be activated very rapidly by the AT1R (Sadoshima and Izumo, 1996; Ishida et al., 1998a; Tsygankova et al., 1998). Src family kinases are 52- to 62-kDa proteins composed of functional domains, including the SH4 domain that contains signals for lipid modification and membrane targeting, a catalytic domain, SH2 and SH3 protein binding domains, and a C-terminal negative regulatory domain (Thomas and Brugge, 1997). The Src kinase family consists of nine members, the best characterized of which is the ubiquitously expressed 60-kDa c-Src. Src kinase activity in the cell is dependent on a change in the phosphorylation of two tyrosines. For c-Src, autophosphorylation of Tyr419 activates the kinase whereas phosphorylation of Tyr527 inactivates it (Brown and Cooper, 1996). Intracellularly, Src kinases have been found in different subcellular locations, such as caveolae, focal adhesions, and endosomes.

The study by Kyaw et al. (2004) in this issue demonstrates that AngII stimulates c-Src and increases phosphorylation of its downstream target Cas, yet Src and Cas are differentially involved in AngII-stimulated migration of VSMCs via specific activation of ERK 1/2 and JNK (Fig. 1). The two novel findings of the present study are that Src and Cas colocalize with vinculin in focal adhesions after AngII stimulation and that Src and Cas differentially regulate migration (Fig. 1). Cas functions as a scaffold for a pathway involving Cas-Crk-Rac-JNK. These findings are of particular interest in the context of recent data from our laboratory reporting the novel role of GIT1 in Src-dependent signaling (Haendeler et al., 2003; Yin et al., 2004) and new data on JNK-mediated paxillin phosphorylation as a key aspect of cell migration (Huang and Schaller, 2004). The study by Kyaw et al. (2004), along with our GIT1 data, demonstrates a major contribution of Src in differentially regulating ERK1/2 and JNK pathways by phosphorylating the scaffold proteins GIT1 and Cas, respectively. The results suggest that scaffolds play an important role in segregating and defining the specificity of AngII-mediated signaling pathways.

ABBREVIATIONS: AngII, angiotensin II; VSMC, vascular smooth muscle cell; AT1R, angiotensin II type 1 receptor; PLC, phospholipase C; EGF, epidermal growth factor; FAK, focal adhesion kinase; JNK, c-Jun NH_2-terminal kinase; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; ROS, reactive oxygen species; GIT1, G protein-coupled receptor kinase-interacting protein-1.

Earlier work from our laboratory establishing that Src kinases are activated very early in AngII-induced signal transduction (Ishida et al., 1998a) is confirmed in the current work by Kyaw et al. (2004). It is now clear that Src activity is central to AngII-mediated effects on the cell cytoskeleton. Our lab showed that tyrosine phosphorylation of several focal adhesion proteins such as Cas, paxillin, and tensin was significantly reduced in rat VSMCs that had been retrovirally transduced with kinase inactive c-Src and this correlated with a reduced rate of cell spreading (Ishida et al., 1999). Others have demonstrated that Src phosphorylates FAK as well as other focal adhesion proteins such as paxillin and Pyk2 (Turner et al., 1995; Nishio et al., 1999). The roles of FAK and Src in regulating signaling pathways seem to be closely intertwined. After FAK activation by autophosphorylation at Tyr 397, it binds Src to form a stable complex. This in turn leads to the docking of paxillin and p130cas. Independent data showing p130cas and paxillin tyrosine phosphorylation by Src and interaction with Src (Sabri et al., 1998; Rocic et al., 2001) are consistent with the current data of Kyaw et al. (2004).

One of the most novel findings in the article by Kyaw et al. (2004) is the colocalization of Src and Cas with vinculin at focal adhesions in response to AngII. Although Src has been shown to be associated with these molecules in immunoprecipitates, this is the first report in which immunofluorescence was used to visualize the translocation of Src to focal adhesions from the cytoplasm (Siu et al., 2003; Kyaw et al., 2004). In addition, kinase activity of Src and Cas phosphorylation were necessary events for their translocation to the focal adhesions. This suggests a very clear mechanism for AngII-mediated changes at the focal adhesion that play an important role in migration.

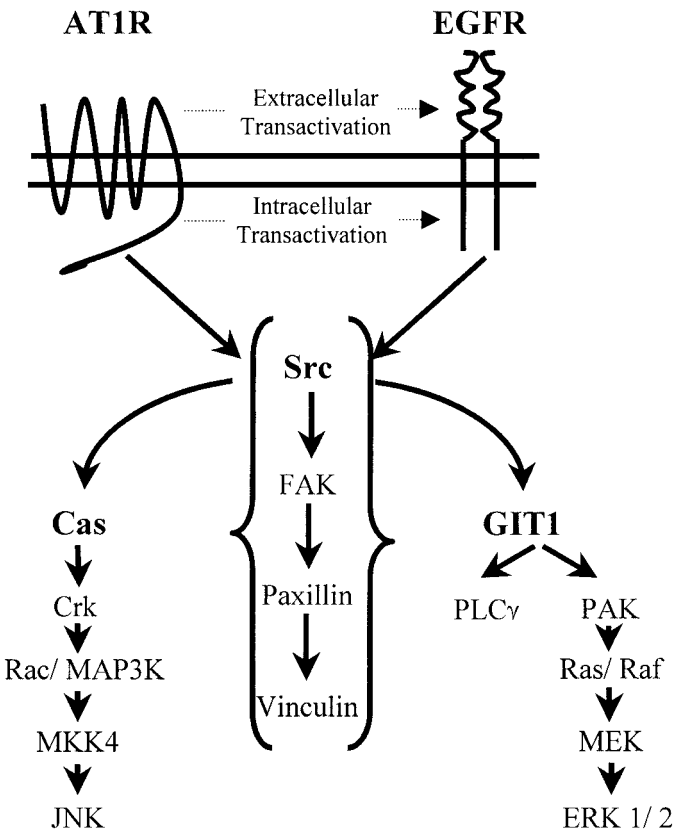

Fig. 1.

In VSMCs, at least three mitogen-activated protein kinases (MAPKs), p38, JNK, and ERK1/2, are activated in response to AngII. AngII activates JNK in VSMCs via several reported signaling mediators including Rac, ROS, Shc-PI3K. p-21 activated kinase or PYK2 (Schmitz et al., 1998, 2001; Frank et al., 2001; Yoshizumi et al., 2001; Sundberg et al., 2003). Src-mediated JNK phosphorylation, however, has not been described previously in AngII-mediated signaling in VSMCs and represents another novel finding in the work by Kyaw et al. (2004).

Several reports have also implicated a pivotal role for Src in cytoskeletal turnover and agonist- stimulated reorganization. The current findings by Kyaw et al. (2004) open several avenues to study mechanisms involved in Src-mediated migration of VSMCs in response to AngII. Their results along with the recent report that JNK phosphorylation of paxillin is involved in cell migration may help elucidate a mechanism for Src- and JNK-mediated migration in response to AngII (Huang and Schaller, 2004). It has been proposed previously that protein complexes containing Fak, Src, and Cas recruit the small adapter protein Crk to promote JNK activation and cell proliferation (Oktay et al., 1999). In addition, the expression of a Cas mutant lacking the substrate-binding domain is also inhibitory for JNK activation (Dolfi et al., 1998). Data from Klemke et al. (198) indicate that the Cas-Crk-Rac1 pathway is important for cell migration, suggesting Crk and Rac1 as potential downstream mediators for cell migration. In addition, reactive oxygen species (ROS), derived from NADP(H) oxidases, activate JNK in a Src- and Cas-dependent manner in VSMCs in response to AngII (Yoshizumi et al., 2000), suggesting that ROS may be upstream mediators in the pathway of JNK activation described by Kyaw et al. (2004).

Src also plays an essential role in AngII-mediated ERK1/2 activation. Kyaw et al. (2004) demonstrate this finding by showing a down-regulation in ERK1/2 phosphorylation in VSMCs that were stably transfected with kinase inactive Src (KI Src). Activation of ERK1/2 by AngII via a Src-dependent mechanism in VSMC was initially shown using Src −/− cells and by overexpressing dominant-negative Src (Ishida et al., 1998b). Src mediates ERK1/2 activation through the Ras-Raf-MEK pathway either directly or indirectly via the extracellular/intracellular transactivation of the EGF or platelet-derived growth factor receptors (Eguchi et al., 1998; Weng and Shukla, 2002) (Fig. 1). Intracellular transactivation is thought to involve AngII-mediated increases in calcium and ROS. These lead to the activation of Pyk2 (Lev et al., 1995) and Src (Eguchi et al., 1998, 1999; Weng and Shukla, 2002), leading to an interaction of Src with the EGFR and transactivation. Extracellular transactivation is mediated by matrix metalloproteinase cleavage of heparin-binding EGF, which then binds to the ligand-binding domain of EGFR (Saito et al., 2002; Hao et al., 2003). Both pathways promote recruitment of adaptor proteins Grb2 and Shc (Eguchi et al., 1999; Bokemeyer et al., 2000; Saito et al., 2002; Hao et al., 2003; Sayeski and Ali, 2003), followed by the translocation of Sos to the complex. Sos activates Ras, MEK1, and ERK1/2 (Eguchi et al., 1998). The importance of EGFR transactivation by AngII in regulating migration of VSMCs has been described previously by Saito et al. (2002). The work of Kyaw et al.

(2004) does not indicate whether transactivation of the EGF receptor is upstream of the ERK 1/2 and JNK activation that is important for cell migration in their system.

PLCγ is phosphorylated in response to AngII (Marrero et al., 1994) and is one of the downstream targets of Src-mediated signaling in VSMCs. This was demonstrated in experiments of Marrero et al. (1995) in which electroporation of Src antibodies into VSMC eliminated AngII-induced tyrosine phosphorylation of PLCγ and reduced inositol 1,4,5-triphosphate production, proving a direct involvement of Src in AngII-mediated PLCγ phosphorylation and activation. Subsequent studies by Schmitz et al. (1997) confirmed that AngII stimulates tyrosine phosphorylation of PLCγ by Src, and hypothesized that a linker protein mediates the interaction between these molecules. Our lab has identified this linker protein as the scaffold protein G protein-coupled receptor kinase-interacting protein-1 (GIT1) (Haendeler et al., 2003). In a recently published article, Haendeler et al. (2003) demonstrated that GIT1 is a substrate of c-Src that undergoes tyrosine phosphorylation in response to AngII. In addition, GIT1 constitutively associates with PLCγ. Deleting a novel SpaII homology domain present in GIT1 inhibited phosphorylation and activation of PLCγ, indicating a novel role for GIT1 as a mediator of c-Src activated PLCγ function. More recently, we found that GIT1 also associates with MEK1 in VSMCs and human embryonic kidney 293 cells after AngII stimulation. GIT1 acts as a scaffold for MEK1-ERK 1/2 activation and promotes activity probably by insulating MEK1-ERK 1/2 from phosphatases and assembling upstream activators such as Raf1. GIT1 thus serves as a scaffold protein to facilitate c-Src-dependent activation of MEK1-ERK 1/2 in response to AT1R activation (Yin et al., 2004).

Because the scaffold protein GIT1 identified in our lab is a substrate of Src, it is likely that this protein would modulate functions in cellular locations where Src is activated, including the cytoplasm and focal adhesions. To this end, data from our lab demonstrate that GIT1 modulates PLCγ and MEK1-ERK1/2 activation in the cytoplasm in response to AngII (Haendeler et al., 2003; Yin et al., 2004). In addition, GIT1 is recruited to focal adhesions in thrombin-stimulated endothelial cells in a Src -dependent manner (Shikata et al., 2003; G. P. van Nieuw Amerongen, G. Yin, R. J. Hoefen, M. Osawa, J. Haendeler, A. J. Ridley, K. Fujiwara, V. W. M. van Hinsbergh, and B. C. Berk, submitted), where it modulates focal adhesion assembly by promoting the turnover of focal adhesions, consistent with a potential increase in migration.

In summary, in their current work, Kyaw et al. (2004) have addressed the differential role of Src in activating either the ERK 1/2 or the JNK MAPK pathway. A key aspect of MAPK signaling is the generation of specificity and amplification. Specificity can be provided in part by scaffold proteins that help organize different components of MAPK pathways into modules. This study by Kyaw et al. (2004), along with work on GIT1 from our lab and others, suggests that Src, FAK, and paxillin may be common mediators of AngII signaling that play a central role in regulating two divergent MAPK pathways that are important in AngII-mediated cellular migration. One pathway involves Cas-mediated regulation of Crk-Rac-JNK and the other involves GIT1-mediated regulation of the p-21-activated kinase-Ras-ERK pathway. This concept suggests that the temporal and spatial differences in phosphorylation of different scaffold proteins by c-Src and subsequent protein-protein interaction define the activation of specific MAP kinase pathways in AngII-mediated signaling.

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