CrkII Associates with BCAR3 in Response to Endothelin-1 in Human Glomerular Mesangial Cells

VICTORIYA A. RUFANOVA AND ANDREY SOROKIN[1]

Department of Medicine, Division of Nephrology and Kidney Disease Center, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

Endothelin-1 (ET-1) effects in human glomerular mesangial cells (GMC) include proliferation, contraction, and extracellular matrix synthesis. Calcium-regulated nonreceptor, proline-rich tyrosine kinase 2 (Pyk2) is a critical mediator of ET-1 signaling in human glomerulae. Working in concert with Pyk2, adaptor protein CrkII and a recently discovered guanidine exchange factor for certain small GTPases BCAR3 can be involved in ET-1 signaling in the kidney. Signaling through CrkII and BCAR3 might be critical in some proliferative kidney pathologies. The current study was designed to determine the possibility of CrkII and BCAR3 interaction in response to ET-1 in human GMC and the role of Pyk2 in the association of these proteins. Using adenovirus-mediated transfer of genes encoding either green fluorescent protein (control) or dominant interfering Pyk2 construct, we demonstrated that CrkII and BCAR3 can be coprecipitated from unstimulated and ET-1 stimulated GMC; ET-1 treatment time-dependently increased CrkII/BCAR3 complex formation; and inhibition of endogenous Pyk2 autophosphorylation led to a significant decrease in CrkII/BCAR3 association both basal and stimulated. Exp Biol Med 231:752–756, 2006

Key words: BCAR3; CrkII; endothelin; human glomerular mesangial cells; Pyk2

Introduction

Endothelin-1 (ET-1) is a strong vasoconstrictor peptide participating in the development of different types of kidney pathology (1, 2). ET-1 stimulation leads to contraction, proliferation, and induction of profibrotic extracellular matrix synthesis in glomerular mesangial cells (GMC) (3–8), directly decreasing glomerular filtration rate and contributing to sclerotic lesion formation (9). There are two types of G-protein–coupled ET-1 receptors, ETA and ETB, enabling the peptide effects in GMC. ET-1 receptor activation evokes a wide variety of intracellular signaling events.

Calcium-regulated nonreceptor, proline-rich tyrosine kinase 2 (Pyk2) is a critical mediator of ET-1 signaling in human GMC. Pyk2 activation provides a link between G-protein–coupled receptors and the induction of tyrosine phosphorylation *via* mobilization of intracellular calcium (10–14). We and others demonstrated that in different cell types Pyk2 can play a crucial role in ET-1–mediated cytoskeleton transformation, including migration, contraction, and adhesion (10, 15–19). The focal adhesion-dependent p130Cas/Crk/Pyk2/c-Src–mediated pathway is selectively involved in ET-induced JNK MAP kinase activation in cardiomyocytes (20).

CrkII is a member of the family of adaptor-type signaling molecules that consist mostly of SH2 and SH3 domains. Other members of CrkII family of adaptor proteins are v-Crk, CrkI, CrkIII, and CrkL (21, 22). Crk-family proteins are involved in a variety of signaling cascades, such as those induced by growth factors receptors, integrin molecules, T-cell receptors, B-cell antigen receptors, and cytokines. However, the role of CrkII in ET-1 signaling still remains uncertain. It has been postulated that the primary function of Crk is to recruit cytoplasmic proteins to the vicinity of tyrosine kinases through SH2-phosphotyrosine interaction. Thus the output from Crk depends on the SH3-binding proteins, which include the tyrosine kinase Abl, the C3G guanine nucleotide exchange factor for Rap1, DOCK180 for Rac, and Sos for Ras. In addition, another guanidine exchange factor for certain G-proteins, BCAR3 (its murine homolog is known as AND-34), can be indirectly engaged in CrkII-dependent transduction *via* p130Cas scaffolding protein, which has been shown to interact with BCAR3 (23, 24). Hypothetically, the BCAR3 proline-rich site and phosphotyrosines can directly associate with CrkII SH3 and SH2 domains, respectively. Despite the potential

This study was supported by the National Institutes of Health research grants DK 41684 and HL 22563 to Dr. A. Sorokin and by Postdoctoral Fellowship from AHA 0525801Z to Dr. V. (Petrukhina) Rufanova.

[1] To whom correspondence should be addressed at Medical College of Wisconsin, Department of Medicine/Kidney Disease Center, 8701 Watertown Plank Rd., Milwaukee, WI 53226. E-mail: sorokin@mcw.edu

Received September 29, 2005.
Accepted November 23, 2005.

1535-3702/06/2316-0752$15.00

importance of BCAR3 in proliferation cascades, the role of BCAR3 in ET-1–induced signaling remains obscure.

The current study was designed to examine the possibility that CrkII and BCAR3 interact in response to ET-1 in human GMC and to investigate the role of Pyk2 in this association. We demonstrated that CrkII and BCAR3 can be coprecipitated from GMC both before and after stimulation with ET-1; ET-1 treatment increased CrkII/BCAR3 complex formation; and inhibition of endogenous Pyk2 autophosphorylation led to significant decrease in CrkII/BCAR3 association.

Materials and Methods

Materials. Purified human ET-1 was from Calbiochem (San Diego, CA). The enhanced chemiluminescence detection system was supplied by Amersham Pharmacia Biotech (Baie d'Urfé, Quebec, Canada). The BCA protein assay kit was from Pierce (Rockford, IL). All other reagents, unless otherwise indicated, were from Sigma (St. Louis, MO).

Antibodies. To perform immunoprecipitation we used mouse monoclonal anti-human Crk from BD Transduction Laboratories (Lexington, KY), and control mouse IgG antibodies from BD Biosciences Pharmigen. For Western blotting, rabbit polyclonal anti-Pyk2 and anti-Crk and mouse monoclonal anti-FLAG antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse monoclonal anti-actin antibody was from ICN Biochemicals (Costa Mesa, CA). Polyclonal phosphorylation state-specific anti-Pyk2 Tyr-402 antibody was from Biosource International (Camarillo, CA). Previously described anti-human BCAR3 rabbit serum (25) was a gift from Dr. Adam Lerner (Boston University School of Medicine, Boston, MA). The horseradish peroxidase–E conjugated goat anti-mouse and goat anti-rabbit immunoglobulins were from Bio-Rad (Hercules, CA).

Cell Culture. All materials for cell culturing were purchased from Invitrogen (Carlsbad, CA). Previously characterized SV40-transformed human GMC (26) was cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 10 m*M* HEPES, 2 m*M* glutamine, penicillin (100 U/ml), and streptomycin (100 μg/ml) in a 37°C humidified incubator with 5% CO_2.

Adenovirus-mediated Gene Transfer. The adenoviral vector (Ad), encoding the carboxyl terminus of Pyk2, termed CRNK for calcium-dependent tyrosine kinase-related non-kinase (AdCRNK), was constructed from the replication-deficient adenovirus type 5 with deletions in the *E1* and *E3* genes as previously described (16). GMC, starved for 24–48 hrs, were incubated either with adenovirus encoding Green Fluorescent protein (AdGFP) or AdCRNK at a multiplicity of infection of 40 plaque-forming units/cell for 1 hrs at 37°C with periodic shaking, followed by the addition of the medium supplemented with 0.5% serum. Forty-eight hours later, cells were prestarved in serum-free media for 3 hrs, stimulated with ET-1, and lysed for further analysis. Efficiency of gene transfer in GMC was 80%–100%, as determined by fluorescence microscopy visualization of AdGFP control.

Cell Lysis, Immunoprecipitation, and Immunoblot Analysis. After stimulation with ET-1 (100 n*M*) for a given period, the cells were lysed as described previously (27). Briefly, cells were washed twice with ice-cold tris-buffered saline (TBS) after the treatment. They were then lysed for 20 mins at 4°C in 800 μl of lysis buffer containing the following: 10 m*M* Tris HCl, pH 7.5, 50 m*M* NaCl, 5 m*M* EDTA, 1% Triton X-100, 30 m*M* sodium pyrophosphate, 1 m*M* phenylmethylsulfonyl fluoride, 0.2 m*M* sodium orthovanadate, 10 m*M* sodium fluoride, 10 μg/ml leupeptin, and 10 μg/ml aprotinin. Cell lysates were collected in microcentrifuge tubes and cleared by centrifugation at 14,000 *g* for 8 mins at 4°C. Equalized for protein content, the supernatants were then resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) for Western blot analysis (20–30 μg) or subjected to immunoprecipitation (700–800 μg).

Cells lysates were immunoprecipitated with either 2 μg antibodies against CrkII, or control mouse IgG for 1.5 hr at 4°C with constant rotation. Protein A Sepharose (40 μl; Roche, Basel, Switzerland) was added, and the lysates were incubated for an additional 1 hr. After washing the immunoprecipitates three times in the lysis buffer, 3× Laemmli sample buffer was added, and the samples were boiled for 5 mins, subjected to SDS-PAGE, and transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA). After transfer of proteins, the polyvinylidene difluoride membrane was blocked with TBS containing 0.1% Tween 20 (TBS-T) and 3% bovine serum albumin (United States Biochemical, Cleveland, OH) for phosphorylated tyrosine-specific primary antibody, or TBS-T and 5% nonfat dry milk for all others for 2 hrs at room temperature. Primary antibodies were added, and the membranes were incubated overnight at 4°C with constant rotation. The membranes were then washed three times with TBS-T before the addition of secondary antibodies. Corresponding goat anti-rabbit or goat anti-mouse IgG-horseradish peroxidase conjugates antibodies were diluted 1:3000 in TBS-T containing 5% nonfat dry milk, and incubated with the membrane for 1 hr at room temperature. The membranes were then washed three times with TBS-T before development of immunoreactive signal by enhanced chemiluminescence reagent.

Results

Cell lysates from AdGFP and AdCRNK transfected cells were stimulated for 1, 2, and 6 mins with 100 n*M* ET-1 and subjected to either direct SDS-PAGE followed by immunoblot (IB) analysis or immunoprecipitation (IP) with anti-Crk antibody and then IB. We observed coprecipitation CrkII with BCAR3 in GMC (Fig. 1, first panel, top). Interaction between these two proteins was significantly increased at 2 and 6 mins after ET-1 stimulation compared with the basal level.

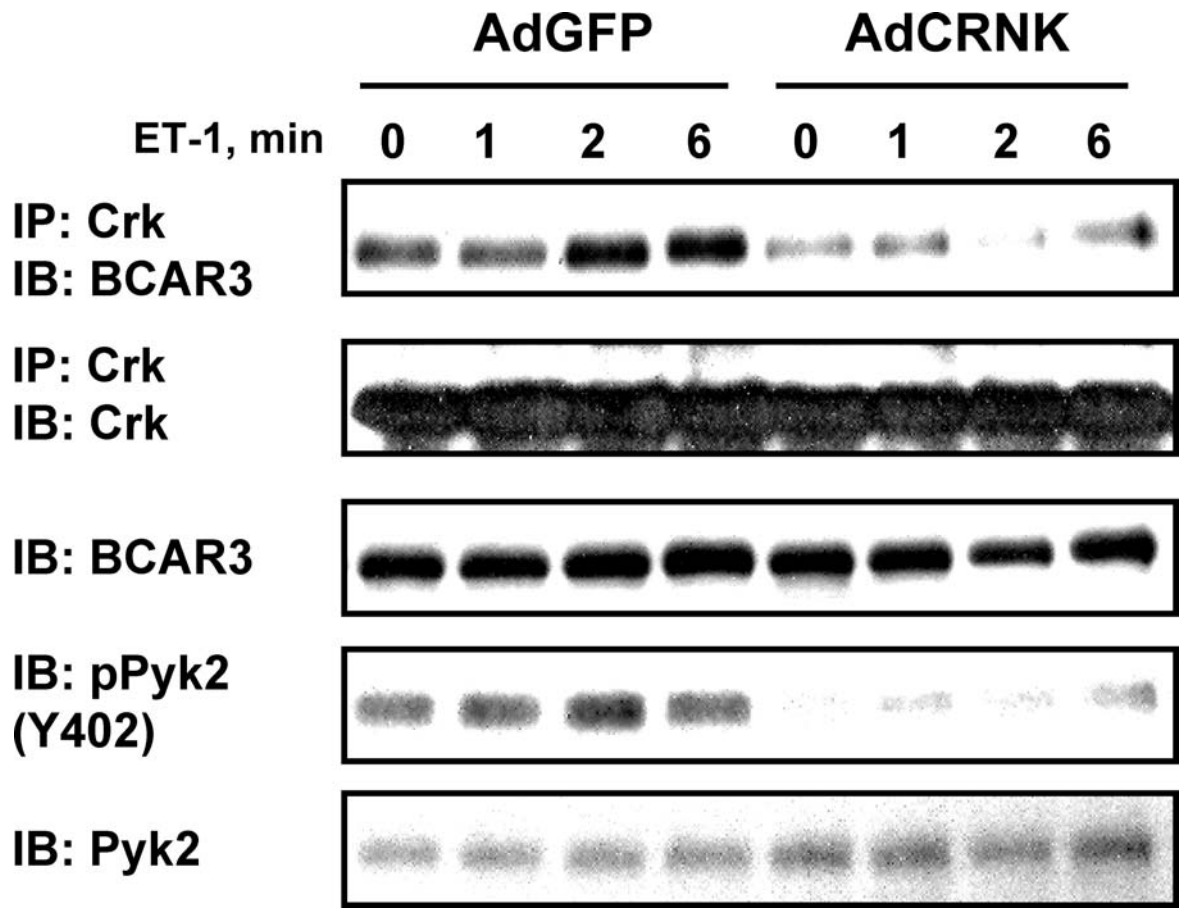

Figure 1. Adenovirus-mediated transfer of Pyk2 CRNK decreases CrkII association with BCAR3 in response to ET-1 in human glomerular mesangial cells. Lysates from AdGFP- and AdCRNK-infected human glomerular mesangial cells, quiescent or stimulated with endothelin-1 (100 nM) for indicated periods (min), were either subjected to immunoprecipitation (IP) with anti-Crk antibodies and immunoblotted (IB) either with anti-BCAR3 antibodies (first panel), or anti-Crk antibodies (second panel) or resolved by SDS-PAGE and IB with antiphospho-Pyk2 (fourth panel from the top) antibodies. Membrane from antiphospho-Pyk2 antibody blotting was stripped and reblotted with total Pyk2 antibody (fifth panel). Equal expression of BCAR3 was verified using IB of total cell lysates with anti BCAR3 antibodies (third panel from the top). Shown is representative of four experiments.

We used a Pyk2 CRNK–dominant negative construct to inhibit the endogenous Pyk2 autophosphorylation as seen by evaluating the phosphorylation of tyrosine-402. As demonstrated in Figure 1 (fourth and fifth panels, top), adenovirus-mediated CRNK expression caused significant reduction in Pyk2 autophosphorylation in response to ET-1 application without significant effect on total Pyk2 protein amount. In parallel, we observed noticeable diminishing of CrkII/BCAR3 complex formation in CRNK-expressing GMC with or without ET-1 treatment compared to GFP-expressing controls (Fig. 1, first panel). Equal amounts of BCAR3 were expressed in all experimental settings as detected by IB with anti-BCAR3 antibody (Fig. 1, third panel).

It has previously been shown that Pyk2 does not directly interact with CrkII adaptor protein (28), and we did not observe any coprecipitation of Pyk2 with CrkII in our experiments (data not shown). However, we cannot exclude the possibility that CRNK, *via* its proline rich sequences, can interfere with CrkII/BCAR3 assembly by directly binding to the SH3 domain of CrkII. To check this hypothesis, we conducted the next experiment.

Cell lysates from AdGFP- and AdCRNK-infected cells were stimulated for 2 and 6 mins with ET-1 and subjected to SDS-PAGE followed by IB or IP with anti-Crk antibody followed by SDS-PAGE and IB analysis. On ET-1 stimulation, CrkII was phosphorylated, as shown by the upward shift of CrkII band in total cell lysates IB (Fig. 2, second panel, top; 2 and 6 mins comparison to prestimulation level). Expression of CRNK did not affect CrkII activation in response to ET-1. Immunoprecipitation experiments resulted in the equal amount of CrkII protein collected from each time point (Fig. 2, second panel, top). No significant CRNK and CrkII association had been detected in IP samples, despite strong CRNK expression in GMC as revealed by IB with anti-FLAG antibody (Fig. 2, first panel).

Discussion

The glomerular mesangial cell is the target of both immune and nonimmune forms of injury in many diverse

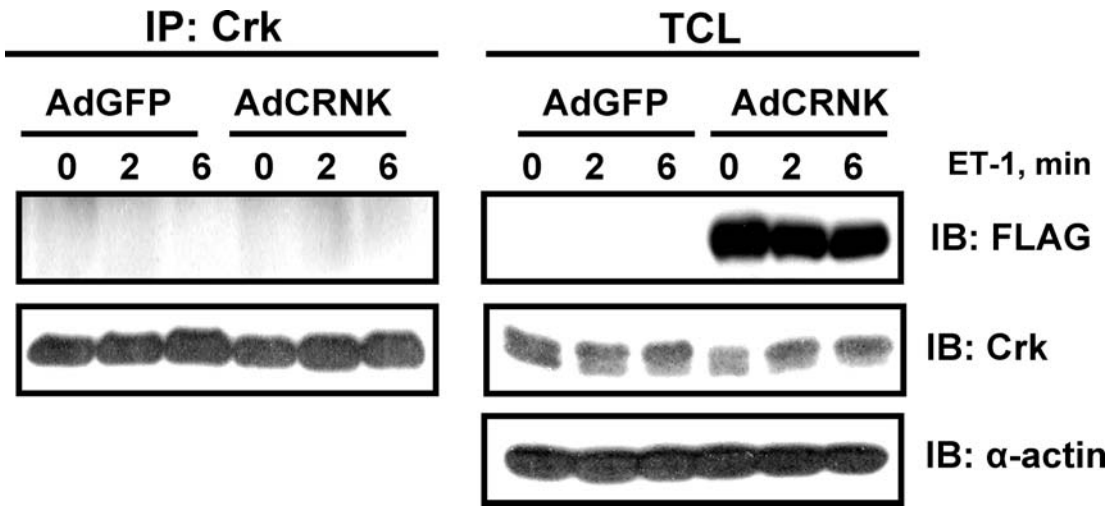

Figure 2. Pyk2 CRNK does not coprecipitate with CrkII in human glomerular mesangial cells. Total cell lysates (TCL) from AdGFP- and AdCRNK-infected human glomerular mesangial cells, quiescent or stimulated with endothelin-1 (100 n*M*) for indicated periods (min), were either subjected to immunoprecipitation (IP) with anti-Crk antibodies and immunoblotted (IB) either with anti-FLAG (first panel from the top) or anti-Crk antibodies (second panel) or resolved by SDS-PAGE and IB with anti-FLAG (first panel) or anti-Crk (second panel) antibodies. Equal loading was verified using IB of TCL with anti-α-actin antibodies (lower panel).

glomerular diseases. In response to injury, mesangial cells may undergo several scenarios, including contraction, proliferation, increased extracellular matrix synthesis, and apoptosis. These cellular responses may lead to progressive decrease in glomerular filtration rate and to glomerular scleralization. ET-1 is one of the significant para- and autocrine factors that regulate GMC homeostasis during development of kidney function abnormalities (1, 2). However, details of ET-1–induced cellular responses in glomerulae remain obscure. Pyk2 is an important non-receptor tyrosin kinase that mediates ET-1 signaling. Pyk2-mediated activation of intracellular cascades contributes to pathologic intracellular events in the kidney during hypertension and inflammatory disease, which are often accompanied by augmented GMC proliferation. Regulated by adhesion, inflammatory cytokines and ET-1, a BCAR3-involving complex, might also be important during mesangial proliferative kidney disease such as diabetic glomerulosclerosis or proliferative glomerulonephritis.

The present work is the first demonstration that CrkII interacts with BCAR3 and that ET-1 triggers the increase in CrkII/BCAR3 complex formation. We have also observed that activation of Pyk2 influenced CrkII/BCAR3 association without direct CRNK interaction with CrkII. Phosphorylation of Pyk2 and CrkII occurred independently of each other, because CRNK expression did not inhibit CrkII phosphorylation. Most likely, Pyk2 phosphorylation may play a role in ET-1–induced modifications of BCAR3 or CrkII translocation. Theoretically, SH2 and proline-rich domains of BCAR3 can participate in the protein-protein contact with phosphorylated tyrosines and SH3 domain of CrkII, respectively. In agreement with our speculation, the SH2 domain of BCAR3 was demonstrated to be important for activation of phosphatidylinositol 3-kinase (29). Also, our data suggest that an additional protein, presumably phosphorylated by Pyk2, might be required for the complex assembly. One of the possible candidates to link Pyk2 activation with BCAR3 and CrkII interaction might be the scaffolding protein p130Cas. In particular, p130Cas can be a substrate for Pyk2 and can form stable complex with CrkII (30). Furthermore, BCAR3 interacts *via* its C-terminal GEF domain and additional 70 amino acids with some unique sequence in p130Cas (23, 24). However, the exact mechanism of CrkII/BCAR3 complex assembling requires further experimental elucidation.

The hypothetical physiologic role of Pyk2-dependent CrkII/BCAR3 association may be the regulation of BCAR3 GEF activity for small GTPases, including Cdc42, Rac1, Ral, Rap1, and R-Ras (24, 31). In transmitting signals downstream to the cytoskeleton-dependent adhesion, contraction, proliferation, and migration, small G-proteins rotate between GDP- and GTP-bound forms. Activation of a particular small G-protein by BCAR3 may be a cell-specific event. Which particular GTP/GDP-binding molecule is employed, as a result of Pyk2-dependent CrkII/BCAR3 assemblance, needs to be determined. Alternatively, ET-1–induced Pyk2-dependent CrkII/BCAR3 cascade can be linked to the proliferation pathway involving activation of nuclear factor-κB (NFκB) (32). The NFκB family comprises ubiquitously expressed transcription factors that are known to be critical regulators of mammalian immune and inflammatory responses, proliferation, and more recently have been shown to associate with chemotherapy resistance in parallel with BCAR3. NfκB activation can provide a basis for the long-term ET-1 effects in kidney. However, the experimental examination of CrkII/BCAR3 complex physiologic importance is beyond the scope of our current study.

In summary, we have demonstrated that in human GMC ET-1, through Pyk2 kinase, induces association of adaptor protein CrkII with guanine nucleotide exchange factor BCAR3. The signaling complex described here may have short- and long-term pathophysiologic significance during development of proliferative renal diseases. Better understanding of postreceptor signaling through protein-protein interaction and tyrosine phosphorylation triggered by ET-1 can open beneficial approaches to intervention in the activation of selective pathways mediating mesangial cell contraction, hypertrophy, and proliferation.

We are grateful to Dr. Adam Lerner (Boston University School of Medicine, Boston, MA) for the generous gift of anti-BCAR3 rabbit serum.

1. Levin ER. Endothelins as cardiovascular peptides. Am J Nephrol 16: 246–251, 1996.
2. Sorokin A, Kohan DE. Physiology and pathology of endothelin-1 in renal mesangium. Am J Physiol Renal Physiol 285:F579–F589, 2003.
3. Badr KF, Murray JJ, Breyer MD, Takahashi K, Inagami T, Harris RC. Mesangial cell, glomerular and renal vascular responses to endothelin in the rat kidney. Elucidation of signal transduction pathways. J Clin Invest 83:336–342, 1989.
4. Gomez-Garre D, Ruiz-Ortega M, Ortego M, Largo R, Lopez-Armada MJ, Plaza JJ, Gonzalez E, Egido J. Effects and interactions of endothelin-1 and angiotensin II on matrix protein expression and synthesis and mesangial cell growth. Hypertension 27:885–892, 1996.
5. Herman WH, Emancipator SN, Rhoten RL, Simonson MS. Vascular and glomerular expression of endothelin-1 in normal human kidney. Am J Physiol 275:F8–F17, 1998.
6. Orth SR, Amann K, Gehlen F, Unger L, Wagner J, Raschack M, Ritz E. Adult human mesangial cells (HMCs) express endothelin-B-receptors which mediate endothelin-1-induced cell growth. J Cardiovasc Pharmacol 36:S232–S237, 2000.
7. Simonson MS, Wann S, Mene P, Dubyak GR, Kester M, Nakazato Y, Sedor JR, Dunn MJ. Endothelin stimulates phospholipase C, Na+/H+ exchange, c-fos expression, and mitogenesis in rat mesangial cells. J Clin Invest 83:708–712, 1989.
8. Takeda M, Breyer MD, Noland TD, Homma T, Hoover RL, Inagami T, Kon V. Endothelin-1 receptor antagonist: effects on endothelin- and cyclosporine-treated mesangial cells. Kidney Int 41:1713–1719, 1992.
9. Mene P, Simonson MS, Dunn MJ. Physiology of the mesangial cell. Physiol Rev 69:1347–1424, 1989.
10. Lev S, Moreno H, Martinez R, Canoll P, Peles E, Musacchio JM, Plowman GD, Rudy B, Schlessinger J. Protein tyrosine kinase PYK2 involved in Ca(2+)-induced regulation of ion channel and MAP kinase functions. Nature 376:737–745, 1995.
11. Herzog H, Nicholl J, Hort YJ, Sutherland GR, Shine J. Molecular cloning and assignment of FAK2, a novel human focal adhesion kinase, to 8p11.2-p22 by nonisotopic in situ hybridization. Genomics 32:484–486, 1996.
12. Earp HS, Huckle WR, Dawson TL, Li X, Graves LM, Dy R. Angiotensin II activates at least two tyrosine kinases in rat liver epithelial cells. Separation of the major calcium-regulated tyrosine kinase from p125FAK. J Biol Chem 270:28440–28447, 1995.
13. Avraham S, London R, Fu Y, Ota S, Hiregowdara D, Li J, Jiang S, Pasztor LM, White RA, Groopman JE, Avraham H. Identification and characterization of a novel related adhesion focal tyrosine kinase (RAFTK) from megakaryocytes and brain. J Biol Chem 270:27742–27751, 1995.
14. Sasaki H, Nagura K, Ishino M, Tobioka H, Kotani K, Sasaki T. Cloning and characterization of cell adhesion kinase beta, a novel protein-tyrosine kinase of the focal adhesion kinase subfamily. J Biol Chem 270:21206–21219, 1995.
15. Martinez MC, Randriamboavonjy V, Ohlmann P, Komas N, Duarte J, Schneider F, Stoclet JC, Andriantsitohaina R. Involvement of protein kinase C, tyrosine kinases, and Rho kinase in Ca(2+) handling of human small arteries. Am J Physiol Heart Circ Physiol 279:H1228–H1238, 2000.
16. Sorokin A, Kozlowski P, Graves L, Philip A. Protein-tyrosine kinase Pyk2 mediates endothelin-induced p38 MAPK activation in glomerular mesangial cells. J Biol Chem 276:21521–21528, 2001.
17. Tolloczko B, Tao FC, Zacour ME, Martin JG. Tyrosine kinase-dependent calcium signaling in airway smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 278:L1138–L1145, 2000.
18. Watts SW. 5-Hydroxytryptamine-induced potentiation of endothelin-1- and norepinephrine-induced contraction is mitogen-activated protein kinase pathway dependent. Hypertension 35:244–248, 2000.
19. Zubkov AY, Rollins KS, Parent AD, Zhang J, Bryan RM, Jr. Mechanism of endothelin-1-induced contraction in rabbit basilar artery. Stroke 31:526–533, 2000.
20. Kodama H, Fukuda K, Takahashi E, Tahara S, Tomita Y, Ieda M, Kimura K, Owada KM, Vuori K, Ogawa S. Selective involvement of p130Cas/Crk/Pyk2/c-Src in endothelin-1-induced JNK activation. Hypertension 1372–1379, 2003.
21. Prosser S, Sorokina E, Pratt P, Sorokin A. CrkIII: a novel and biologically distinct member of the Crk family of adaptor proteins. Oncogene 22:4799–4806, 2003.
22. Kiyokawa E, Mochizuki N, Kurata T, Matsuda M. Role of Crk oncogene product in physiologic signaling. Crit Rev Oncol 8:329–342, 1997.
23. Cai D, Clayton LK, Smolyar A, Lerner A. AND-34, a novel p130Cas-binding thymic stromal cell protein regulated by adhesion and inflammatory cytokines. J Immunol 163:2104–2112, 1999.
24. Gotoh T, Cai D, Tian X, Feig LA, Lerner A. p130Cas regulates the activity of AND-34, a novel Ral, Rap1, and R-Ras guanine nucleotide exchange factor. J Biol Chem 275:30118–30123, 2000.
25. Cai D, Iyer A, Felekkis KN, Near RI, Luo Z, Chernoff J, Albanese C, Pestell RG, Lerner A. AND-34/BCAR3, a GDP exchange factor whose overexpression confers antiestrogen resistance, activates Rac, PAK1, and the cyclin D1 promoter. Cancer Res 63:6802–6808, 2003.
26. Sraer JD, Delarue F, Hagege J, Feunteun J, Pinet F, Nguyen G, Rondeau E. Stable cell lines of T-SV40 immortalized human glomerular mesangial cells. Kidney Int 49:267–270, 1996.
27. Pratt PF, Bokemeyer D, Foschi M, Sorokin A, Dunn MJ. Alterations in subcellular localization of p38 MAPK potentiates endothelin-stimulated COX-2 expression in glomerular mesangial cells. J Biol Chem 278: 51928–51936, 2003.
28. Lee TN, Gold G, Workman R, Cook CA, Konrad RJ. Glucose stimulates the association of Crk with p130Cas in pancreatic beta cells. Pancreas 29:e100–e105, 2004.
29. Felekkis KN, Narsimhan RP, Near R, Castro AF, Zheng Y, Quilliam LA, Lerner A. AND-34 activates phosphatidylinositol 3-kinase and induces anti-estrogen resistance in a SH2 and GDP exchange factor-like domain-dependent manner. Mol Cancer Res 3:32–41, 2005.
30. Chodniewicz D, Klemke RL. Regulation of integrin-mediated cellular responses through assembly of CAS/Crk scaffold. Biochim Biophys Acta 1692:63–76, 2004.
31. Cai D, Felekkis KN, Near RI, O'Neill GM, van Seventer JM, Golemis EA, Lerner A. The GDP exchange factor AND-34 is expressed in B cells, associates with HEF1, and activates Cdc42. J Immunol 170:969–978, 2003.
32. Wu JT, Kral JG. The NF-kappaB/IkappaB signaling system: a molecular target in breast cancer therapy. J Surg Res 123:158–169, 2005.