Connective tissue growth factor [CTGF]/CCN2 stimulates mesangial cell migration through integrated dissolution of focal adhesion complexes and activation of cell polarization

J. K. CREAN,*1 F. FURLONG,† D. FINLAY,†,2 D. MITCHELL,* M. MURPHY,* B. CONWAY,† H. R. BRADY,* C. GODSON,* AND F. MARTIN†

Departments of *Medicine and Therapeutics and †Pharmacology, Conway Institute of Biomolecular and Biomedical Research, Mater Misericordiae Hospital, University College Dublin, Dublin, Republic of Ireland; and ‡Department of Renal Medicine, Queens University Belfast, Belfast, Northern Ireland, UK

To read the full text of this article, go to http://www.fasebj.org/cgi/doi/10.1096/fj.04-1546fje; doi: 10.1096/fj.04-1546fje

SPECIFIC AIMS

We have investigated the role of connective tissue growth factor [CTGF]/CCN2 in mesangial cell migration, with a particular emphasis on elucidating the mechanism through which treatment with CTGF leads to focal adhesion dissolution, actin disassembly and cell polarization.

PRINCIPAL FINDINGS

1. CTGF induces mesangial cell migration, disruption of focal adhesion assembly and concurrent actin disassembly

Scratching a confluent monolayer of fibroblast-like cells is a well-established model for cell migration and polarization, which we have used to investigate the mechanism of CTGF mediated directional migration. Addition of CTGF stimulated a >2-fold increase in the number of cells that migrated into the wound. To confirm that CTGF induced true migration and that closing of the wound was not just due to proliferation, the effect of CTGF on mesangial cell proliferation and ability of mesangial cells to migrate through collagen I coated transwell filters was determined. CTGF stimulated chemotaxis of mesangial cells compared with control cells while CTGF had no effect on thymidine incorporation. Primary human mesangial cells were also treated with CTGF and stained for F-actin, focal adhesion kinase and vinculin. CTGF induced actin cytoskeleton disassembly and loss of focal adhesions, as evidenced by the absence of staining for focal adhesion kinase and punctate vinculin in CTGF treated cells compared with control cells. During migration, this disassembly was often more evident in cells that had migrated into the wound where only cortical F-actin staining was apparent, compared with those that had not migrated.

2. CTGF-mediated focal adhesion disassembly involves dephosphorylation of focal adhesion kinase and paxillin through increased activity of protein tyrosine phosphatase SHP-2

We then investigated whether loss of focal adhesions resulted from altered phosphorylation status of components of the focal adhesion complex in response to treatment with CTGF. Dissolution of mesangial cell focal adhesions in cells treated CTGF was abrogated by pretreatment with tyrosine phosphatase inhibitor sodium orthovanadate (Fig. 1A). Western blot analysis subsequently showed that both focal adhesion kinase and paxillin are rapidly dephosphorylated in response to CTGF (Fig. 1B). Moreover, pretreatment with sodium orthovanadate inhibited mesangial cell migration (Fig. 1C) indicating that focal adhesion dissolution mediated by tyrosine dephosphorylation of focal adhesion kinase is necessary for mesangial cell chemotaxis. Given the role of protein tyrosine phosphatase SHP-2 in cell migration, we then measured its activity in response to CTGF. CTGF stimulated an almost 3-fold increase in SHP-2 activity compared with control (Fig. 1D).

1 Correspondence: Department of Medicine and Therapeutics, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland. E-mail: john.crean@ucd.ie

2 Current address: The Burnham Institute, 10901 North Torrey Pines Road, La Jolla, California 92037, USA
3. CTGF induces actin rearrangement in human mesangial cells via suppression of RhoA signaling

To probe the role of the Rho GTPase family in CTGF-induced actin cytoskeleton disassembly and mesangial cell migration, cells were treated with CTGF and lysates assayed for active RhoA, Rac 1, and Cdc42 over the period 0–24 h. CTGF induced a decrease in the activity of both RhoA and Rac 1 and a loss of RhoA from the cell membrane over the course of 24 h. Conversely, treatment with CTGF initially resulted in sustained activity of Cdc42 and over the time course of migration led to an increase in the activity of Cdc42.

4. CTGF induces phosphorylation of protein kinase C \( \zeta \) and GSK-3\( \beta \) and stimulates a polarized morphology

Increased activity of Cdc42 and the observation that inhibition of the protein kinase C pathway with broad-spectrum PKC inhibitor GF109203X inhibited CTGF-induced mesangial cell migration raised the possibility that CTGF may mediate cell migration via a mechanism similar to that involved in cell polarization. Consequently we examined the phosphorylation status of components of this pathway, namely PKC \( \zeta \) and GSK-3\( \beta \). Addition of CTGF to mesangial cells caused rapid and transient phosphorylation of protein kinase C \( \zeta \). There was no change in the phosphorylation state of other members of the PKC family. We then investigated whether the PKC \( \zeta \) pathway is required for CTGF induced mesangial cell migration. CTGF-induced mesangial cell migration was completely abolished by addition of the specific myristilated PKC \( \zeta \) pseudosubstrate peptide inhibitor.

PKC \( \zeta \) was most strongly expressed in a perinuclear fashion by mesangial cells treated with CTGF and it generally translocated to the leading edge of migrating cells. Neither sodium orthovanadate nor Toxin B inhibited phosphorylation of PKC \( \zeta \) indicating that this event is independent of both tyrosine dephosphorylation and Rho GTPases. Given structural similarities between CTGF-treated mesangial cells and polarized cells, and bearing in mind our previous observations that CTGF induced rapid phosphorylation of Akt/protein kinase B, we then investigated another component of the polarization complex and known PI3-kinase target, glycogen synthase kinase 3\( \beta \) (GSK-3\( \beta \)). GSK-3\( \beta \) was also rapidly phosphorylated in response to CTGF and there was a general increase in cytoplasmic staining in CTGF-treated cells.

Cells were also transiently transfected with expression vectors encoding either a constitutively active (wild-type) or a kinase inactive (dominant-negative) form of PKC \( \zeta \) and wounded. Overexpression of the constitutively active form of PKC \( \zeta \) and subsequent treatment with CTGF significantly increased the num-

![Figure 1](https://example.com/image1)

**Figure 1.** CTGF mediated focal adhesion disassembly involves dephosphorylation of focal adhesion kinase and paxillin through increased activity of protein tyrosine phosphatase SHP-2. Primary human mesangial cells were treated with CTGF and stained for focal adhesion kinase. Dissolution of mesangial cell focal adhesions in cells treated with CTGF was abrogated by pretreatment with tyrosine phosphatase inhibitor sodium orthovanadate (A). Primary human mesangial cells were treated with CTGF (25 ng/mL) and proteins separated by Western blot. Western blots were probed with antibodies to total focal adhesion kinase, phospho focal adhesion kinase (Tyr397), total paxillin and phospho paxillin (Tyr117). Western blot analysis illustrates that both focal adhesion kinase and paxillin are rapidly dephosphorylated in response to CTGF (B) while pretreatment with sodium orthovanadate inhibited mesangial cell migration (C). CTGF stimulated an almost 3-fold increase in SHP-2 activity compared with control as measured by phosphatase activity in SHP-2 immunoprecipitates.
ber of migrated cells compared with untreated ($P<0.01$). Cells expressing the dominant negative form of PKC $\zeta$ and subsequently wounded and treated with CTGF did not migrate into the wound. Cells transfected with the plasmid encoding the catalytically active PKC $\zeta$ construct demonstrated numerous membrane protrusions and spike formations while those expressing the dominant negative form did not. Primary cells stimulated with CTGF also demonstrated numerous membrane protrusions and filopodia throughout the time course of migration.

**CONCLUSIONS AND SIGNIFICANCE**

Our present studies indicate a mode of action for CTGF through integration of PKC $\zeta$ signaling and Rho GTPase family signaling leading to actin cytoskeleton disassembly, which is facilitative of increased mesangial cell migration. Dissolution of the focal adhesion complex in cells treated with CTGF may be a result of increased SHP-2 activity, which leads to tyrosine dephosphorylation of focal adhesion kinase and paxillin. Treatment of cells with CTGF results in decreased RhoA and Rac 1 activity and increased Cdc42 activity, which facilitates disassembly of the actin cytoskeleton and initiates microspike formation and cell protrusion respectively, resulting in increased cell motility.

F-actin disassembly have been attributed to high-glucose-activation of PKC $\zeta$, however the mechanism by which this occurs was unclear. Disruption of the actin cytoskeleton causes mesangial cells to lose their normal contractile responsiveness to vasoactive peptides. Lack of a mesangial cell contractile response in high glucose mimics hypocontractility observed in glomerular arteriolar smooth muscle cells in diabetes. Dynamic structural rearrangement of the actin cytoskeleton is fundamental for many cellular activities including adhesion and migration. Mesangial cells maintained in vitro have F-actin distributed as long stress fibers resembling tense cables. When subjected to mechanical stretch or indeed cultured for extended periods in high glucose, these fibers become shortened and show signs of gross disruption. This in turn is likely to mean impaired migratory ability. Cells cultured in high glucose exhibited partial disassembly of F-actin while F-actin content is significantly reduced compared with normal glucose exposure. Moreover, the fibrillar actin organization of mesangial cells is disrupted in long-term diabetes and in the STZ-induced diabetic rat. Together these findings suggest that high glucose alters actin assembly in both glomerular mesangial and epithelial cells in vitro and in vivo, thereby contributing to cellular dysfunction in early diabetes. Our data suggest that in addition to its role in mediating TGF$\beta$-induced fibronectin expression, CTGF may contribute significantly in mediating actin disassembly during the progression of diabetic nephropathy.

Here we demonstrate for the first time an integrated mechanism whereby CTGF regulates cell migration through facilitative actin cytoskeleton disassembly which is mediated by tyrosine dephosphorylation of focal adhesion kinase and paxillin, loss of Rho A activity, activation of cdc42, and phosphorylation of PKC $\zeta$ and GSK-3$\beta$. These changes indicate that initial stages of CTGF mediated mesangial cell motility are similar to those involved in the process of cell polarization.