The inhibition of human mesangial cell proliferation by S-trans, trans-farnesylthiosalicylic acid

ARIF KHWAJA, CLAIRE C. SHARPE, MAZHAR NOOR, YOEL KLOOG, and BRUCE M. HENDRY

Department of Renal Medicine, GKT School of Medicine, King’s College London, London, United Kingdom; and Department of Neurobiochemistry, Tel Aviv University, Tel Aviv, Israel

The inhibition of human mesangial cell proliferation by S-trans, trans-farnesylthiosalicylic acid.

Background. Many of the proliferative cytokines implicated in human mesangial cell (HMC) proliferation signal through the superfamily of Ras GTPases. The Ras antagonist, S-trans, trans- farnesylthiosalicylic acid (FTS), was used to investigate the effects of the inhibition of Ras signaling on HMC proliferation.

Methods. Ras expression and membrane localization, MAPK, and Akt activation were analyzed by Western blotting. Ras activation was determined with a pull-down assay using the Ras-binding domain of Raf. HMC growth curves were assessed using the MTS assay of viable cell number, while DNA synthesis was measured with BrdU incorporation. Hoechst 33342 staining was used to determine apoptosis.

Results. FTS reduced the membrane localization of Ras in both serum and platelet-derived growth factor (PDGF). FTS (7.5–20 μmol/L) potently inhibited PDGF-induced HMC proliferation but had no effect on serum-induced proliferation. FTS (10–20 μmol/L) inhibited both Ras and phospho-MAPK activation by serum and PDGF. Furthermore, FTS (10–20 μmol/L) increased HMC apoptosis in the presence of PDGF but not in serum. Moreover, PDGF-stimulated activation of the survival protein Akt was inhibited by FTS. In contrast, serum-stimulated activation of Akt was unaffected by FTS.

Conclusion. FTS (5–20 μmol/L) inhibits PDGF-induced but not serum-induced HMC proliferation. FTS (10–20 μmol/L) also promotes HMC apoptosis in the presence of PDGF but not serum. These effects appear to be mediated by inhibitory effects on Ras-dependent signaling that occur as a result of the dislodgement of Ras from its membrane-anchorage sites by FTS. The selectivity of FTS toward PDGF-driven HMC proliferation suggests that FTS may be a valuable therapeutic in mesangio-proliferative renal disease.

Aberrant human mesangial cell (HMC) proliferation plays a critical role in the pathogenesis and progression of glomerular injury and glomerulosclerosis [1]. Indeed, HMC proliferation is a hallmark of both immune-mediated and non-immune–mediated glomerular injury [2]. In vivo studies have demonstrated that MC proliferation precedes and correlates with increased mesangial matrix production [3]. Furthermore, strategies to inhibit MC proliferation, such as complement depletion [4], and antibodies targeting platelet-derived growth factor (PDGF) [5] and basic fibroblast growth factor (bFGF) [6], have been shown to reduce mesangial matrix production and sclerosis. Elucidating the cellular processes that underlie HMC proliferation may lead to the development of more effective therapies for mesangio-proliferative disease.

A large number of cytokines, including PDGF, bFGF, angiotensin II, endothelin, interleukin-1 (IL-1), and IL-6 have been implicated in the regulation of HMC proliferation [7]. The intracellular signaling pathways of many of these mitogens converge upon the Ras superfamily of small monomeric GTPases [8], which act as molecular switches, in the transduction of extracellular proliferative signals from the plasma membrane to the cell nucleus. Within the Ras superfamily of proteins, Ras itself plays a key role in regulating cell proliferation, apoptosis, and differentiation [8]. Once activated, Ras cycles from an inactive, GDP-bound form to an active GTP-bound form. GTP-bound Ras is then able to activate a number of downstream effector cascades, of which the Ras-Raf-Mitogen activated protein kinase (MAPK) cascade is most strongly implicated in the control of cell proliferation [8, 9].

Ras exists as three closely related isoforms known as Harvey (Ha)-, Kirsten (Ki)-, and Neural (N)-Ras. Extracellular signals that activate Ras can be mediated through different types of receptors, such as receptor tyrosine kinases (e.g., PDGF, bFGF), cytokine receptors, and G protein–coupled receptors [10–12]. To transduce such growth signals, it is thought that Ras must be anchored to the inner surface of the plasma membrane [13]. To localize to the plasma membrane, Ras undergoes a series of post-translational modifications. The

Key words: farnesylthiosalicylic acid, Ras GTPases, mesangial, proliferation.

Received for publication October 1, 2004
and in revised form January 19, 2005
Accepted for publication February 28, 2005

© 2005 by the International Society of Nephrology

474
first step, known as prenylation, involves the binding of hydrophobic isoprenoid groups (farnesyl or geranylgeranyl residues) to the cysteine (C) in the carboxy-terminal CAAX motif (where A is an aliphatic acid and X is methionine or serine) [14]. Prenylation of Ras is followed by terminal amino acid (AAX) cleavage and methylation. Ha-Ras and N-Ras then undergo palmitoylation before translocating to the plasma membrane [15], while Ki-Ras has a polybasic-domain consisting of a string of six lysine residues that act as a membrane targeting sequence [16]. S-trans, trans-farnesylthiosalicylic acid (FTS) is a synthetic S-prenyl derivative of a rigid carboxylic acid that structurally resembles the carboxy-terminal farnesylcysteine group common to prenylated Ras proteins [17]. FTS appears to act as a functional Ras antagonist by dislodging Ras from specific membrane anchorage domains, thereby inhibiting Ras-dependent signaling [18]. FTS has been shown to inhibit the growth of Ha-Ras-transformed Rat-1 cells [19], and Ki-Ras transformed rodent fibroblasts in vitro [20]. As well as having potent antiproliferative effects in vitro, FTS has been shown to be effective in vivo, not only in models of cancer [21], but also in models of inflammatory disease, such as a rat model of cirrhosis [22], and in a genetic model of systemic lupus erythematosus (SLE) [23]. Recent work from our group has demonstrated that FTS can inhibit glomerular cell proliferation, proteinuria, and macrophage infiltration in the rat Thy 1.1 nephritis model of MC proliferation [24].

This work examines the role of Ras in HMC proliferation in vitro by using FTS to target Ras-dependent signaling.

METHODS

Materials
All reagents and antibodies were purchased from Sigma-Aldrich (Gillingham, UK) unless otherwise stated. Stock solutions of FTS were made up in chloroform. Prior to experimental use, FTS was dissolved in DMSO. U0126 was also made up in DMSO. Final DMSO concentrations were 0.2%, including vehicle-only control conditions.

Primary human mesangial cell culture
Primary culture HMC (Clonetics, Biowhitaker, Wokingham, UK) were maintained in RPMI 1640 (Invitrogen Limited, Inchinnan, UK) medium with 10% fetal calf serum (10% FCS). All experiments were carried out between passages 4 and 9.

Western blotting
For Western blotting experiments, HMC were grown to 80% confluence in 35- or 100-mm plates, in RPMI 1640 and 10% FCS. Cells were then serum-starved for 24 hours, and then treated overnight with FTS (0–20 μmol/L) in media containing either 10% FCS or PDGF-BB (200 ng/mL). Cells were lysed in cell-lysis buffer, and equal amounts of protein lysate [25] were immunoprecipitated with 15 μL of a rat anti-pan-Ras antibody/agarose conjugate (clone Y13-259/mL lysate (Merck, Nottingham, UK) followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western immunoblotting with a pan-Ras monoclonal antibody (Merck) and an antimouse immunoglobulin G (IgG) horseradish peroxidase conjugate (Merck).

Protein expression was detected by Amersham ECL chemiluminescence (Amersham Pharmacia Biotech, Little Chalfont, UK).

Total cell membrane and total cytosol fractions were obtained by suspending cell pellets in ice-cold hypotonic lysis buffer, and centrifuging at 320g for 10 minutes to clear debris, after which the supernatants were spun for 1 hour at 100,000g to separate membrane and cytosolic fractions. Aliquots of lysate were then resuspended in 2X cell lysis buffer and left on ice for 30 minutes. Equal volumes of lysate were then immunoprecipitated and Ras detected as outlined before.

To determine MAPK and Akt activation, cells were serum starved for 24 hours and then treated with FTS (5–20 μmol/L) or vehicle for 4 hours in serum-free media. The cells were then stimulated for 5 to 10 minutes with either PDGF (200 ng/mL) or 10% FCS. SDS-PAGE and Western blotting were performed as before using a Phospho-MAPK (Erk 1/2) or Phosph-Akt (Ser 473) antibody (New England Biolabs, Hitchin, UK). Separate immunoblots with equal amounts of cell lysate were probed with primary antibodies for total MAPK and total Akt (New England Biolabs).
triplicate into 96-well plates at a density of 5000 cell/well, which approximated to a confluence of 20% to 30%. FTS (5–20 μmol/L) was then added and cells were grown in either 10% FCS or 200 ng/mL of PDGF. Preliminary experiments showed HMCs to be responsive to PDGF, and this dose of PDGF gave optimal growth curves. Viable cell numbers were determined by the MTS assay (Cell titer 96; Promega, Southampton, UK), measuring absorbance at 490 nm.

DNA synthesis was assessed by Bromodeoxyuridine (BrdU) uptake. Cells were grown in 35-mm dishes up to a confluence of 90%, and serum starved for 24 hours prior to treatment with FTS in the presence of either PDGF (200 ng/mL) or 10% FCS. Six hours after the addition of FTS and growth factor, BrdU (10 μmol/L) was added to each dish for 16 hours. Dishes were then washed with phosphate-buffered saline (PBS), fixed (3 volume ethanol), incubated in 4M hydrochloric acid for 10 minutes, washed 3 times in 50 mmol/L glycine and 7 volume ethanol, and blocked in 5% goat serum/0.05% Tween/PBS for 15 minutes before being incubated overnight in anti-BrdU antibody (1 in 100 dilution) at 4°C. BrdU-positive cells were detected using a TRITC-labeled antimouse IgG antibody (Sigma), and cell nuclei were counterstained with Hoechst 33342 (Sigma) at a concentration of 5 μg/mL for 15 minutes. Cells were then visualized with a fluorescent microscope. BrdU-positive cells stain nuclei red, while all cell nuclei would stain blue. A total of at least 150 cells were counted from at least 5 different fields. Nuclei that appeared apoptotic on Hoechst staining were not counted.

Apoptosis

HMC were grown in 35-mm dishes and treated with FTS as before. Hoechst 33342 was added (5 μg/mL), and then incubated at 37°C for 15 minutes followed by propidium iodide (5 μmol/L) for 5 minutes. Cell nuclei were then visualized under fluorescent microscopy. Apoptotic nuclei were then identified as showing characteristic signs of chromatin condensation and fragmentation. Late apoptotic or necrotic cells stained red. A total of at least 150 cells were counted from at least 5 different fields.

Statistics

Statistical analysis was performed using analysis of variance (ANOVA) followed by Dunnett’s test of significance using Prism software (GraphPad, San Diego, CA, USA).

RESULTS

PDGF-stimulated but not serum-stimulated HMC growth is dependent on the Ras-Raf-MAPK pathway

The activation status of the Ras-Raf-MAPK pathway was assessed by determining the phosphorylation status of Erk 1 and Erk 2. UO126, a compound that inhibits the phosphorylation of Erk 1/2, was used to study the role of this pathway in HMC proliferation. As can be seen from Figure 1, UO126 (20 μmol/L) reduces the phosphorylation of Erk 1/2 both in PDGF and in serum. Figure 2 demonstrates that PDGF-stimulated growth curves are inhibited by UO126 in a dose-dependent manner. At 48 hours, UO126 (20 μmol/L) reduced cell number by 67.5%. In contrast, UO126 (10–20 μmol/L) had no effect on cell number in serum at 48 hours. While we did not perform cytotoxicity studies, higher doses of U0126 have been used in other cell models without evidence of cytotoxicity [26].

FTS reduces membrane-localized but not total cellular Ras

FTS had no effect on total cellular Ras expression in both PDGF and serum (data not shown). When assessing the membrane localization of Ras, 10 to 20 μL FTS caused a significant reduction in the expression of membrane-bound Ras, as shown in Figure 3. This effect was seen in both PDGF and serum.

FTS inhibits HMC proliferation in PDGF but not in serum

Figure 4 shows that FTS causes a marked dose-dependent reduction in cell number in the presence of PDGF. FTS 7.5 μmol/L reduces cell number by 13.6% at 24 hours, and 30.4% at 48 hours when compared to vehicle-treated cells. FTS 10 μmol/L reduces cell number by 70.7% at 24 hours, and by 89% at 48 hours when compared to vehicle-treated cells. At a dose of 20 μmol/L, FTS causes an even greater loss in cell number at 24 and 48 hours. In marked contrast to the effects in PDGF, serum-stimulated HMC growth curves were not affected by FTS at doses of up to FTS 20 μmol/L. Indeed, even when higher doses of FTS (50 μmol/L) were used in the presence of serum, there was still no effect on cell number at 48 hours (data not shown).

DNA synthesis was assessed by BrdU uptake, as shown in Figure 5. BrdU-positive cells were detected using an anti-BrdU antibody and a TRITC-labeled secondary antibody. Only nuclei that incorporated BrdU stain red when viewed with a fluorescent microscope. The total number of nuclei was counterstained with Hoechst33342, and these appeared blue under fluorescence. Cells of apoptotic morphology were not counted. Examples of BrdU-positive nuclei are shown in Figure 5A and C. The total number of nuclei in the same fields of view is shown in Figure 5B and D. As can be seen in Figure 6A, 5 to 20 μmol/L FTS causes a dose-dependent reduction in BrdU uptake in PDGF-stimulated cells. FTS 5 μmol/L reduces BrdU uptake by 72.8%, and 7.5 μmol/L reduces BrdU uptake by 83%. At doses of 10 to 20 μmol/L, FTS reduces BrdU uptake by over 95%. In marked contrast,
5 to 20 µmol/L FTS has no effect on BrdU uptake in FCS-stimulated cells (Fig. 6B).

**FTS induces HMC apoptosis in PDGF but not serum**

Figure 7 shows the effect of FTS on apoptosis in the presence of PDGF and serum. When quantifying the degree of apoptosis, it was apparent that FTS induced apoptosis in the presence of PDGF. Ten percent to 20% of cells had apoptotic features after treatment with 10 to 20 µmol/L of FTS. In marked contrast, FTS had no proapoptotic actions on HMC in the presence of serum, even at doses of up to 20 µmol/L.

**FTS inhibits Ras activation and Ras-dependent signaling**

In order to establish whether dislodging Ras from the plasma membrane had a functional effect on its signaling activity, the RBD pull-down assay was used to investigate the effects of FTS on Ras-GTP expression. As can be seen in Figure 8, there is minimal basal active Ras in serum-starved cells. Activation by PDGF for 5 minutes greatly increased the amount of Ras pulled down by the RBD-GST fusion protein. Furthermore, prior treatment of HMC with 10 to 20 µmol/L FTS caused marked attenuation of Ras activation by PDGF. Total cellular Ras remained unchanged. FTS treatment also inhibited serum-induced Ras activation.

The effect of FTS on signaling downstream of Ras was determined by examining the activation of phospho-Erk1/2. Figure 9 shows that 10 to 20 µmol/L FTS causes marked inhibition of PDGF-induced and serum-induced phosphorylation of Erk1/2. FTS had no effect on total cellular Erk1/2 expression, suggesting that the reduction in phospho-Erk1/2 is due to specific inhibition of phosphorylation, and not due to reductions in total cellular Erk1/2.

**Serum but not PDGF can activate AKT in the presence of FTS**

The effects of FTS on a second Ras effector cascade, the PI 3-kinase pathway, were also studied. Activation of this pathway results in the activation of the survival
Fig. 2. (A) The inhibitory effect U01026 on HMC growth curves in 200 ng/mL PDGF. Cell number was then estimated using the MTS assay by measuring the absorbance over 48 hours. Panel (B) shows that UO126 has no inhibitory effect on cell number in 10% FCS. The error bars are SD, N = 6. **P < 0.01 with respect to vehicle-treated cells.

Fig. 3. (A) Western blots for pan-Ras in cytosolic (C) and membrane (M) fractions of HMC treated for 24 hours with FTS. Cells were serum-starved for 24 hours prior to incubation with FTS (5–20 µmol/L) for 24 hours in the presence of 200 ng/mL PDGF or 10% FCS. Cells were then lysed and cytosolic and membrane fractions separated as described in methods. FTS reduced membrane-bound Ras in both growth factors. The ratio of M:C Ras expression is shown in (B). The error bars are SD, N = 6. * P < 0.05 with respect to vehicle-treated cells.
protein Akt. Figure 10 shows that there is minimal basal phospho-Akt expression, which is markedly up-regulated by PDGF. FTS 5 μmol/L has no effect on PDGF-induced activation of phospho-Akt, while 10 to 20 μmol/L FTS completely inhibits the response to PDGF. These changes in phospho-Akt expression do not appear to be a result of reductions in total Akt. In contrast, serum is able to activate Akt despite treatment with FTS (5–20 μmol/L).
DISCUSSION

Growth factor production, MC proliferation, and extracellular matrix production are key features of mesangioproliferative and crescentic glomerulonephritides. There is a considerable body of evidence to suggest that PDGF is a key mesangial mitogen. PDGF can stimulate MC proliferation in vitro [27], and plays a potent pro-proliferative role in the rat anti-Thy-1.1 nephritis model of mesangioproliferative disease [6]. In addition, there is increased expression of both PDGF and PDGF-β receptor mRNA in glomeruli from patients with mesangioproliferative diseases [28]. PDGF is also likely to have an important role in mediating vascular and interstitial injury in the kidney [29]. Ras GTPases act at a key convergent point in the PDGF-stimulated cell signaling pathways, suggesting that the targeting of Ras-signaling pathways may lead to the development of novel therapies for mesangioproliferative renal disease [30].

The inhibitory effect of UO126 on PDGF-stimulated growth curves suggests that the Ras-Raf-MAPK pathway plays a key role in PDGF-driven HMC proliferation. Activation of Ras ultimately results in the phosphorylation of Erk1/2, which then translocates into the nucleus and induces mitogenesis through the
transcription of growth-related genes such as c-jun and c-fos [10]. The lack of effect of UO126 on serum-stimulated growth curves suggests that non-Ras-Raf-MAPK signaling pathways are important in promoting serum-induced cell proliferation. G-protein–coupled receptor (GPCR) agonists, such as lysophosphatidic acid (LPA), are thought to account for much of the mitogenic activity of serum [12]. LPA may activate a number of signaling proteins that regulate cell transcription and proliferation, including PI 3-kinase [31], the Jun kinases (JNK), and possibly other members of the MAPK superfamily such as p38, Erk 5, Erk 6, and SAPK4 [12].

FTS is a small molecule that targets the Ras-Raf-MAPK signaling pathway. It dislodges activated, GTP-bound Ras from cell membrane anchorage domains [18]. Recent work suggests that galectin-1 acts as a Ras-binding protein, stabilizing the interactions between Ha-Ras (and to a lesser extent, Ki-Ras) and the plasma membrane [32]. It is this interaction between galectin-1 and Ras that is disrupted by FTS, though the precise mechanism of action of FTS remains to be elucidated. The dose-dependent inhibition in HMC of Ras membrane localization at concentrations by FTS (5–20 \( \mu \)mol/L) in both serum and PDGF has been seen in a variety of cell types [20, 33].

The results presented here show the exquisite sensitivity of PDGF-driven HMC proliferation to FTS. Furthermore, these effects of FTS on PDGF-stimulated HMC proliferation occur at doses that dislodge Ras from the plasma membrane, inhibit the activation of Ras, and down-regulate the activation of MAPK. These results...
suggest that FTS inhibits PDGF-stimulated HMC proliferation by suppressing the Ras-Raf-MAPK cascade. These observations are consistent with previous findings demonstrating that FTS can inhibit the activation of Ras [32], and also inhibit MAPK activation [34]. The remarkable sensitivity of PDGF-induced HMC proliferation to FTS contrasts strikingly with the lack of effect of much higher doses of FTS in serum. Even though FTS was still able to inhibit the membrane localization and activation of Ras in serum, it had no effect on serum-driven HMC proliferation. Similar effects have been observed in earlier work where FTS had no effect on serum-induced proliferation of Rat-1 cells, while potently inhibiting the proliferation of EGF- or FGF-stimulated proliferation [19]. Again, this suggests that serum can utilize signaling pathways other than the Ras-Raf-MAPK cascade. This hypothesis is consistent with our findings that both UO126 and FTS fail to inhibit serum-induced proliferation.

The effects of FTS on apoptosis appear to be partly mediated by modulation of Akt activity. Akt is a serine/threonine kinase that lies downstream of the PI 3-kinase, and provides a critical anti-apoptotic signal to the cell [35]. The proapoptotic effects of FTS in PDGF are associated with inhibition of the activation of the survival factor Akt. In contrast, FTS does not prevent serum-induced activation of Akt, and has no apoptotic effect in the presence of serum. The activation of Akt by serum in the presence of FTS suggests that mitogens, such as LPA, can activate the PI 3-kinase pathway in a Ras-independent manner. LPA acting through G-protein-coupled receptors has been shown to potently
activate the PI 3 kinase/Akt signaling pathway [36]. The inhibition of PDGF-induced activation of Akt in HMC by FTS suggests that direct activation of PI 3-kinase by PDGF requires some active Ras to be present in the cells. Thus, by inhibiting Ras activation, FTS prevents activation of PI 3-kinase. The proapoptotic effects of FTS have also been observed in melanoma cells [37], although interestingly, this effect was not dependent upon the inhibition of Akt activation.

These data suggest that FTS-induced apoptosis may be rescued by serum activation of Akt. It is therefore possible that FTS may induce apoptosis of normal quiescent HMCs in vivo. Clearly, further preclinical toxicology studies are required to address this issue. However, in an in vivo model of melanoma, FTS had no effect on murine glomerular structure, suggesting that the presence of growth factors or cell-cell interactions may protect mesangial cells from the proapoptotic effects of FTS in vivo [38].

Recent work from our group has shown that the in vitro selectivity of FTS toward PDGF-stimulated HMC proliferation has also been seen in rat mesangial cells (RMCs) both in vitro and in vivo in the rat model of Thy1.1 nephritis [24]. FTS markedly reduced glomerular cell proliferation and matrix expansion in this model. Interestingly, RMC proliferation was associated with increased glomerular expression of Ki-Ras and N-Ras, suggesting that increased expression of these Ras isoforms may be important in RMC proliferation in vivo. This increase in Ras expression was almost completely prevented by treatment with FTS, suggesting that the in vivo effects of FTS may be mediated by modulating Ras.

Fig. 9. Western blots for phospho-MAPK (Erk 1/2) and total MAPK (Erk 1/2) in stimulated HMC after overnight treatment with FTS and stimulation with PDGF and 10% FCS (A). As can be seen, FTS (10–20 µmol/L) inhibits the phosphorylation of Erk 1/2 by both serum and PDGF. Total Erk 1/2 remained unchanged. Panel (B) demonstrates the effect of FTS on relative Erk1/2 activation as compared to stimulated HMCs, as determined by densitometry. Error bars are SD,  \( N = 5 \). *** \( P < 0.001 \) with respect to vehicle-treated cells.
Fig. 10. (A) Western blots analysis for phospho-Akt in FTS-treated cells stimulated with PDGF 200 ng/mL and 10% FCS. FTS inhibits activation of phospho-Akt by PDGF but not by 10% FCS. Panel (B) shows the effect of FTS on relative Akt activation as determined by densitometry in HMCs stimulated by PDGF (upper graph) and 10% FCS (lower graph). Error bars are SD, N = 3. *P < 0.05 and ***P < 0.001 with respect to vehicle-treated cells.
expression. The current work in primary culture HMC suggests that FTS may have a specific effect on PDGF-driven mesangiproliferative human disease.

Targeting Ras with FTS appears to be a promising therapeutic development. A number of other strategies that can target Ras may also be of clinical value in proliferative renal disease [30]. These include the use of statins, which inhibit the prenylation of Ras and Rho GTPases, as well as newer compounds that target signals upstream or downstream of Ras. For example, a Raf-1 antagonist (BAY43-9006) is already undergoing clinical trials for the treatment of cancer [9], while a small-molecule tyrosine kinase inhibitor, currently being used as a novel treatment of chronic myeloid leukemia [39], has been shown to attenuate renal injury in the Thy 1.1 nephritis model [40].

CONCLUSION

PDGF-stimulated HMC proliferation is dependent on activation of the Ras-Raf-MAPK signaling pathway. FTS inhibits the membrane localization of Ras, thereby down-regulating Ras-dependent signaling pathways, inhibiting cell proliferation, and promoting apoptosis. The specificity of FTS to PDGF-driven MC proliferation both in vitro and in vivo suggests that it may be a promising new therapy for the treatment of mesangiproliferative renal disease.

ACKNOWLEDGMENTS

Ari Khwaja is a Medical Research Council Clinical Training Fellow. The Peck Medical Research Trust provided consumable funding. Thyesco Corporation (NJ) provided FTS.

Reprint requests to Professor B.M. Hendry, Renal Medicine, KCL, Bessemer Road, London, SE5 9PJ, United Kingdom.

E-mail: bruce.hendry@kcl.ac.uk

REFERENCES

16. HANCOCK JF, PATONER H, MARSHALL CJ: A polybasic domain or palmitoylation is required in addition to the CAAX motif to localize prenalted Ras to the plasma membrane. Cell 63:133–139, 1990