Activation of Extracellular Signal-regulated Kinase 1/2 Inhibits Type I Collagen Expression by Human Skin Fibroblasts*

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* The abbreviations used are: ECM, extracellular matrix; MMP(s), matrix metalloproteinase(s); TNF-α, tumor necrosis factor-α; TGF-β, transforming growth factor-β; MAPK(s), mitogen-activated protein kinases; MEK, MAPK/ERK kinase; p38, extracellular signal-regulated kinase-1/2; ERK1/2, extracellular signal-regulated kinase-1/2; Jun N-terminal kinase (JNK), and p38 (11).

Here we show that activation of ERK1/2 signaling cascade as a potent negative regulatory pathway with respect to type I collagen expression in fibroblasts, suggesting that it mediates inhibition of collagen production in response to mitogenic stimulation and transformation.

Fibrillar type I collagen is an abundant component of the extracellular matrix (ECM) of various human connective tissues. Type I collagen is a heterotrimeric molecule consisting of two α1 chains and one α2 chain. The expression of proc1(I) and proc2(I) collagen genes is coordinately regulated during tissue development, growth, and repair resulting in their synthesis in a 2:1 ratio (1). The expression of type I collagen in fibroblasts is stimulated by transforming growth factor-β (TGF-β) (2), interleukin-4 (3), and connective tissue growth factor (4) and inhibited by epidermal growth factor (5), tumor necrosis factor-α (TNF-α) (2), interferon-γ (2), glucocorticoids (6), and tumor promoters (7, 8) and by contact with three-dimensional collagen (9). Excessive deposition of type I collagen is observed e.g. in fibrosis of skin, lungs, and liver (see Ref. 1).

TNF-α is a proinflammatory cytokine that inhibits the formation of ECM by suppressing the expression of type I collagen and by inducing the production of matrix metalloproteinases (MMPs) by fibroblasts (2, 10). Binding of TNF-α to its 55-kDa cell surface receptor activates neutral sphingomyelinase, which hydrolyzes cell membrane sphingomyelin to the lipid second messenger, ceramide. We have recently shown that synthetic cell-permeable C2-ceramide enhances fibroblast collagenase-1 (MMP-1) expression via coordinate activation of the following three distinct mitogen activated protein kinase (MAPK) pathways: extracellular signal-regulated kinase-1/2 (ERK1/2), c-Jun N-terminal kinase (JNK), and p38 (11).

Here we show that activation of ceramide signaling pathway potently inhibits the expression of type I collagen in human skin fibroblasts and that this involves coordinate activation of ERK1/2 and p38 MAPKs. In addition, we show that specific activation of ERK1/2 by adenovirus-mediated expression of constitutively active MEK1 results in potent suppression in type I and III collagen expression by normal skin fibroblasts. These results identify ERK1/2 as a potent negative regulatory pathway with respect to expression of type I collagen in fibroblasts, suggesting that it plays a role in the control of collagen deposition, e.g. in wound repair and tumor growth.
ATCC (Manassas, VA) and cultured in similar medium except with 10% CS. For experiments, fibroblasts were maintained at 18 h in culture medium supplemented with 0.5% FCS. C2-ceramide was added alone or in combination with TGF-β1, and the incubations were continued for 24 h. In experiments involving chemical signaling inhibitors, these were added 1 h prior to C2-ceramide. Cell viability was determined with trypan blue exclusion as described previously (8).

RNA Analysis—Total RNA was isolated from cells using the single-step method (12). Aliquots of total RNA were fractionated on gels, transferred to filters, and hybridized with cDNAs as described previously (11). The following cDNAs were used for hybridizations: a 0.7-kb human cDNA for procollagen II (procollagen II collagen)(13), a 1.2-kb human cDNA for procollagen III (procollagen III collagen)(14), a 0.7-kb human cDNA for procollagen I (procollagen I collagen)(15), a 2.0-kb human cDNA for collagenase-1 (MMP-1)(16), and a 1.3-kb rat cDNA for glyceraldehyde-3-phosphate dehydrogenase (GAPDH)(17). The [32P]cDNA-mRNA hybrids were visualized by autoradiography, quantitated by scanning densitometry, and corrected for the levels of GAPDH mRNA in the same samples.

Transient Transfections and Chloramphenicol Acetyltransferase (CAT) Assays—Confluent NIH-3T3 fibroblast cultures were transiently transfected with 2 μg of human procollagen II collagen promoter/CAT construct (18) kindly provided by Dr. F. Ramirez, Mount Sinai School of Medicine, New York, NY. Transfections were performed by the calcium phosphate/DNA co-precipitation method followed by a 2-min exposure to the calcium shock (11). The cells were incubated with C2-ceramide (50 μM) in DMEM and 1% CS for 40 h, and CAT activity was measured as described previously (11). The transfection efficiency was monitored by co-transfecting the cells with 2 μg of the Rous sarcoma virus β-galactosidase construct and correcting the CAT activities for β-galactosidase activity (11).

Determination of mRNA Stability—Confluent human skin fibroblasts in medium containing 1% FCS were incubated without or with C2-ceramide (50 μM) for 6 h, RNA polymerase II inhibitor DRB (60 μM) was added, and the cultures were harvested at 6-h intervals for RNA extraction and determination of procollagen I and procollagen II collagen and GAPDH mRNA abundance by Northern blot hybridizations.

Transduction of Fibroblasts with Recombinant Adenoviruses—Recombinant replication-deficient adenovirus RAdlacZ (RAd35), which harbors Escherichia coli β-galactosidase (lacZ) gene under the control of cytomegalovirus intermediate early promoter, and the empty adenovirus RAd66 were kindly provided by Dr. Gavin W. G. Wilkinson (University of Cardiff, Cardiff, Wales, United Kingdom) (19). Construction and characterization of recombinant adenoviruses harboring mutated, constitutively active MEK1 (RAdMEK1ca)(20), MKK3b (RAdMKK3bE)(21), and MKK6b (RAdMKK6bE)(21) genes driven by cytomegalovirus intermediate early promoter have been described previously. To determine the infection efficiency of normal human skin fibroblasts, cells in suspension were mixed with RAdlacZ at different multiplicities of infection, plated, incubated for 18 h in DMEM with 1% FCS, fixed, and stained for β-galactosidase activity (22, 23). In experiments, 5 × 10^5 cells in suspension were infected with RAd66, RAdlacZ, RAdMEK1ca, RAdMKK3bE, or RAdMKK6bE, at multiplicities of infection of 500 pfu/cell, which gives 100% transduction efficiency, plated, and incubated for 18 h. Culture medium with 10% FCS was replaced with one containing 1% FCS, the incubations continued for 24 h, and the cells were harvested for RNA extraction. In experiments involving PD98059, this was added to cultures at the time of infection.

Assay of MAPK Activation—Fibroblasts (5 × 10^5) were infected with RAdlacZ, RAdMEK1ca, RAdMKK3bE, or RAdMKK6bE, infected, and lysed in 100 μl of Laemmli sample buffer. The samples were sonicated, fractionated by 10% SDS-PAGE, and transferred to Hybond ECL membrane (Amer sham Pharmacia Biotech). Western blotting was performed with phosphospecific antibodies for ERK1/2 and p38 (New England Biolabs, Beverly, MA), in 1:1000 dilution, as described previously (11, 23). As loading controls, the same samples were analyzed by Western blotting using antibodies specific for total ERK1/2 and p38 (New England Biolabs). Binding of primary antibodies was detected with peroxidase-conjugated secondary antibodies and visualized by ECL.

Measurement of Fibroblast Collagen Synthesis—Cells were infected with adenovirus constructs RAd66, RAdMEK1ca, RAdMKK3bE, and RAdMKK6bE, as described above. C2-ceramide (50 μM) was added 1 h before or after TGF-β1, and the incubations were continued for 24 h. Culture medium with 10% FCS was replaced with one containing 1% FCS, the incubations continued for 24 h, and the cells were harvested for RNA extraction. In experiments involving PD98059, this was added to cultures at the time of incubation.

RESULTS

C2-ceramide Inhibits Type I and III Collagen Gene Expression by Human Skin Fibroblasts—We have recently shown that the TNF-α-generated lipid second messenger, C2-ceramide, induces expression of MMP-1 and MMP-3 in human skin fibroblasts (11). As TNF-α also potently suppresses the expression of type I collagen by dermal fibroblasts, we wanted to examine the role of the ceramide signaling pathway in this respect. Treatment of human skin fibroblasts with C2-ceramide for 24 h resulted in marked suppression of the expression of mRNAs for type I collagen α1 and α2 chains noted with concentrations of 25 μM, and maximal inhibition was observed at a 50 μM con-
Interestingly, induction of MMP-1 mRNA levels was also noted with C2-ceramide treatment with a concentration of 25 μM, and the maximal induction was obtained with 50 μM (Fig. 1A). Suppression of type I and III collagen mRNAs was first noted at 6 h of incubation and was nearly maximal at the 12-h time point (Fig. 1B). Interestingly, the inhibition of type I and III collagen mRNA expression by C2-ceramide was quite persistent, as it lasted at least 48 h (Fig. 1C).

Fig. 2. C2-ceramide reduces type I collagen mRNA stability in fibroblasts. A, confluent human skin fibroblasts in medium containing 1% FCS were treated with C2-ceramide (C2-cer; 50 μM) for 6 h or left untreated, RNA polymerase II inhibitor DRB (60 μM) was added, and the control (DRB) and C2-ceramide-treated cultures (C2-cer + DRB) were harvested at 6-h intervals for determination of proc1(I) and proc2(I) collagen and GAPDH mRNA abundance by Northern blot hybridizations. B, type I collagen mRNA levels in control (DRB) and C2-ceramide-treated cultures (C2-cer + DRB) were quantitated by densitometry, corrected for GAPDH mRNA levels, and are shown relative to the levels at the time of addition of DRB (100). Data from a representative experiment of two experiments with identical results are shown.

Fig. 3. Inhibition of type I collagen expression by C2-ceramide is mediated by ERK1/2, p38, and protein kinase C. A, human skin fibroblasts were incubated for 18 h in DMEM with 0.5% FCS, C2-ceramide (C2-cer; 50 μM) was added alone or in combination with MEK1,2 inhibitor PD98059 (40 μM) or p38 MAPK inhibitor SB203580 (40 μM), both added 1 h before C2-ceramide, and the incubations were continued for 24 h. B, human skin fibroblasts were incubated for 18 h in DMEM with 0.5% FCS. C2-ceramide (C2-cer; 50 μM) was added alone or in combination with protein kinase C inhibitors H7 (50 μM) staurosporine (stauro; 25 nM) and Ro-31–8220 (1 μM), all added 1 h before C2-ceramide, and the incubations were continued for 24 h. A and B, total RNA was isolated, and aliquots (15 μg) were analyzed for the expression of proc1(I) and proc2(I) collagen, MMP-1, and GAPDH mRNA with Northern blot hybridizations.

were added to cultures 1 h before or after C2-ceramide (Fig. 1C).

C2-ceramide Inhibits Proc2(I) Collagen Promoter Activity and Reduces Stability of Type I Collagen mRNAs—To determine whether the inhibitory effect of C2-ceramide on type I collagen expression takes place at the transcriptional level, we transiently transfected NIH-3T3 fibroblasts with the human proc2(I) collagen promoter/CAT construct −3766−21/V/CAT and subsequently treated the cells with C2-ceramide (50 μM) for 40 h. Assay of CAT activity revealed that C2-ceramide inhibited the activity of the proc2(I) collagen promoter by 55 ± 6% (mean ± S.E. of three independent transfections each performed in duplicate), as compared with the untreated control cells.

Next, we also examined whether the down-regulatory effect of C2-ceramide on type I collagen mRNA expression also involves reduced stability of the corresponding mRNAs. A 6-h pretreatment of human skin fibroblasts with C2-ceramide (50 μM) followed by co-treatment in the presence of the RNA polymerase II inhibitor DRB revealed that the decay rate of
Inhibition of Fibroblast Type I Collagen Production by C2- ceramide Is Mediated Coordinately by ERK1/2 and p38— We have recently observed that the maximal activation of fibroblast MMP-1 expression by C2-ceramide requires coordinate activation of ERK1/2, JNK, and p38 (11). To examine the roles of these MAPK pathways in the ceramide-elicited down-regulation of type I collagen expression, human skin fibroblasts were treated with C2-ceramide alone or in combination with the selective MEK1,2 inhibitor PD98059 and with a specific p38 MAPK inhibitor SB203580. As noted above, treatment with C2-ceramide (100 \( \mu \text{M} \)) resulted in nearly total inhibition of the expression of mRNA levels for proc1(I) and proc2(I) collagen in comparison with untreated control cells (Fig. 3A). Interestingly, treatment of cells with the combination of PD98059 and SB203580 markedly inhibited C2-ceramide-elicited down-regulation of proc1(I) and proc2(I) collagen mRNA abundance (Fig. 3A). PD98059 alone had a slight inhibitory effect, and SB203580 alone did not alter the effect of C2-ceramide on type I collagen mRNA levels (Fig. 3A). In comparison, the up-regulation of MMP-1 mRNA levels by C2-ceramide was potently inhibited (by 62%) by PD98059, as shown previously (11). Together, PD98059 and SB203580 entirely abrogated the induction of MMP-1 mRNA expression by C2-ceramide (Fig. 3A). Treatment with SB203580 or PD98059 had no effect on viability of the fibroblasts, as determined with trypan blue exclusion assay (not shown).

Inhibition of Type I Collagen Expression by C2-ceramide Is Dependent on Protein Kinase C Activity—Next, we further elucidated the signaling pathways mediating the inhibitory effect of the ceramide signaling pathway on type I collagen expression using chemical protein kinase C (PKC) inhibitors. Interestingly, the down-regulation of proc1(I) and proc2(I) collagen mRNA abundance by C2-ceramide was entirely abrogated by PKC inhibitors H7 (50 \( \mu \text{M} \)) and Ro-31–8220 (1 \( \mu \text{M} \)) and also potently by staurosporine (25 \( \mu \text{M} \)) (Fig. 3B). The induction of MMP-1 expression by C2-ceramide was also entirely inhibited by all three PKC inhibitors (Fig. 3B). Treatment of fibroblasts with PKC inhibitors H7 and Ro-31–8220 reduced the number of viable cells by 12%, whereas 24-h treatment with staurosporine resulted in reduced viability of nearly all cells, as determined with trypan blue exclusion assay (not shown).

Specific Activation of ERK1/2 Inhibits Type I and III Collagen Expression by Fibroblasts—To directly examine the role of ERK1/2 and p38 MAPK pathways in the regulation of the expression of the endogenous type I collagen genes, we used adenovirus-mediated gene delivery of constitutively active MEK1, MKK3b, and MKK6b to human skin fibroblasts. Transduction of cells with 500 pfu/cell of recombinant adenovirus RAdMEK1ca, harboring constitutively active MEK1, resulted in specific activation of endogenous ERK1/2, but not p38, as compared with control virus (RAd66)-infected cells (Fig. 4A). Interestingly, simultaneous treatment of RAdMEK1ca-infected fibroblasts with the MEK1/2 inhibitor PD98059 entirely inhibited activation of ERK1/2 (Fig. 4A). In parallel, transduction of cells with adenoviruses RAdMKK3bE and RAdMKK6bE bearing constitutively active MKK3b and MKK6b, respectively, resulted in specific activation of p38 and in reduction in the basal levels of phosphorylated ERK1/2 (Fig. 4A).

Interestingly, the activation of ERK1/2 by constitutively ac-
tive MEK1 resulted in marked reduction in the endogenous mRNA levels for proc(I) (85%), proc(II) (89%), and proc(III) (81%) collagen, as compared with cells transduced with control virus RAdlocZ (Fig. 4B). Inhibition of ERK1/2 activation with PD98059 almost completely restored the expression of type I and III collagen mRNAs in RAdMEK1ca-transduced fibroblasts (Fig. 4B). In contrast, adenovirus-mediated expression of constitutively active MKK3b slightly enhanced type I and III collagen mRNA abundance, and MKK6b alone had no marked effect (Fig. 4B).

Next, we also determined the effect of ERK1/2 and p38 activation on collagen production by dermal fibroblasts. For this, the cells were transduced with empty control adenovirus RAd66, or with RAdMEK1ca, RAdMKK3bE, and RAdMKK6bE, and subsequently labeled with 3H-labeled proline for 24 h in the presence of ascorbic acid and β-aminopropionitrile, followed by fractionation of equal aliquots of conditioned media by SDS-PAGE. As shown in Fig. 4C, expression of constitutively active MEK1 in dermal fibroblasts entirely inhibited their proc(I) and proc(II) collagen production, and this inhibition was potently abrogated by simultaneous incubation of cells with the MEK1,2 inhibitor PD98059. Expression of constitutively active MKK3b had no marked effect on type I collagen production, and MKK6b even slightly (1.8-fold) upregulated collagen production by fibroblasts.

DISCUSSION

MAPKs mediate extracellular signals, which regulate cell growth, differentiation, survival, and death (see Ref. 25). At present, the following three mammalian MAPK pathways have been characterized in detail: ERK1/2 pathway (Raf/MEK1,2/ERK1,2), which is activated by mitogenic signals, JNK/stress-activated protein kinase (SAPK) (MEK kinase 1–4/MKK4,7/JNK1–3), and p38 (MAPK kinase kinase/MKK3,6/p38α/β) pathways, which are activated by inflammatory cytokines and cellular stress (see Ref. 25). Phosphorylation of the conserved threonine and tyrosine residues of MAPKs by specific upstream dual-specificity kinases (MAPK kinases) results in activation and nuclear translocation of MAPKs, which in turn phosphorylate and activate nuclear protein kinases, e.g., MAPK-activated protein kinases 1,2, and -3 or transcription factors including Elk-1, c-Jun, and activating transcription factor-2 (see Ref. 25). It has been shown that activation of the ERK1/2 cascade by constitutively active Raf-1 or MEK1 results in transformation of fibroblasts (26, 27) and that the ERK1/2 pathway is activated in renal and breast carcinomas in vivo (28, 29). In addition, blocking the ERK1/2 pathway with specific MEK1,2 inhibitor reverses the transformed phenotype of colon carcinoma cells in vitro and arrests their growth in vivo (30). Recent observations also provide evidence that ERK1,2, JNK, and p38 regulate the proteolytic capacity of normal fibroblasts and malignant cells (9, 11, 23, 31–33).

In the present study, we show that activation of ceramide signaling pathway potently inhibits type I collagen gene expression in fibroblasts at the level of transcription and mRNA stability and that this effect requires PKC activity, as well as coordinate activation of ERK1/2 and p38 MAPKs. Furthermore, induction of MMP-1 mRNA expression by C2-ceramide was entirely abrogated by blocking both ERK1/2 and p38 MAPK pathways, as well as inhibition of PKC activity. Together with our previous observations (11), these results show that activation of the ceramide signaling pathway results in potent suppression of type I collagen expression and in induction of MMP-1 and MMP-3 expression in dermal fibroblasts, suggesting that in these cells ceramide pathway plays an important role in mediating the inhibitory effects of TNF-α on ECM deposition.

To examine the specific roles of ERK1/2 and p38 pathways in the regulation of type I collagen expression we utilized adenovirus-mediated gene delivery of the constitutively active ERK1/2 activator MEK1 and p38 activators MKK3b and MKK6b to normal human skin fibroblasts. The results of these experiments clearly identify the ERK1/2 cascade as a potent negative regulatory pathway with respect to type I and III collagen expression. In contrast, p38 appears to serve as a weak positive regulator of type I and type III collagen expression, possibly because of reduction in the basal levels of activated ERK1/2 in cells expressing constitutively active MKK3b or MKK6b. These observations are in accordance with previous studies showing that blocking the ERK1/2 pathway by dominant negative Raf-1 enhances α(1) collagen promoter activity in transiently transfected hepatic stellate cells (34) and that transformation of fibroblasts with oncovirus Ras, the upstream activator of Raf-1, results in suppression of type I collagen gene expression (35, 36). However, the effects of ERK1/2 activation on type I collagen expression appear to be cell-specific, as in human osteoblastic cells the ERK1/2 pathway mediates the stimulation of type I collagen gene expression by TGF-β and bone morphogenetic protein-2 (37) and hypergravity (38). In addition, blocking the ERK1/2 pathway with PD98059 in part inhibits the up-regulation of type I collagen gene expression by insulin-like growth factor-1 in hepatic stellate cells (39).

Controlled deposition of collagenous ECM is an important feature of normal tissue development, growth, and repair, whereas excessive collagen accumulation results in destruction of normal tissue architecture and function, as detected e.g. in lung fibrosis, liver cirrhosis, keloids, and hypertrophic scars (1). The results of this study show that activation of mitogen-responsive ERK1/2 MAPK is alone sufficient to inhibit type I and III collagen expression in normal human skin fibroblasts. Together with our recent observations showing that activation of ERK1/2 in human gingival (23) and dermal fibroblasts2 results in induction of MMP-1 and stromelysin-1 (MMP-3) expression and suppresses their decorin production (40), the results of the present study suggest that the ERK1/2 signaling pathway plays an important role in mediating the inhibitory signals on fibroblast ECM deposition, e.g. during wound repair and tumor growth.

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REFERENCES

1. Prockop, D. J., and Kivirikko, K. I. (1995) Annu. Rev. Biochem. 64, 403–434
2. Kähäri, V.-M., Chen, Y. Q., Su, M. W., Ramirez, F., and Uitto, J. (1990) J. Clin. Invest. 86, 1489–1495
3. Postlethwaite, A. E., Holness, M. A., Katai, H., and Raghov, R. (1992) J. Clin. Invest. 90, 1479–1485
4. Frazier, K., Williams, S., Kothapalli, D., Klapper, H., and Grotendorst, G. R. (1990) J. Invest. Dermatol. 107, 404–411
5. Laato, M., Kähäri, V.-M., Niinikoski, J., and Vuorio, E. (1987) Biochem. J. 247, 385–388
6. Hämäläinen, L., Oikarinen, J., and Kivirikko, K. I. (1985) J. Biol. Chem. 260, 720–725
7. Goldstein, R. H., Fine, A., Farszewski, L. J., Poliks, C., and Polgar, P. (1990) J. Biol. Chem. 265, 13623–13628
8. Westermark, J., Ilvanen, E., and Kähäri, V.-M. (1995) Biochem. J. 308, 995–999
9. Ravanti, L., Heino, J., López-Otin, C., and Kähäri, V.-M. (1999) J. Biol. Chem. 274, 2446–2455
10. Westermark, J., Hakkinen, L., Fiers, W., and Kähäri, V.-M. (1999) J. Invest. Dermatol. 105, 197–202
11. Reunanen, N., Reunanen, M., Foschi, J., Han, J., López-Otin, C., and Polgar, P. (1999) J. Invest. Dermatol. 123, 1362–13628
12. Westermark, J., Ilvanen, E., and Kähäri, V.-M. (1995) Biochem. J. 308, 995–999
13. Reunanen, N., Westermark, J., Hakkinen, L., Fiers, W., and Kähäri, V.-M. (1999) J. Invest. Dermatol. 105, 197–202
14. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
15. Sandberg, M., Makela, J. K., Multimaki, P., Vuorio, T., and Vuorio, E. (1989) Nucleic Acids Res. 16, 349
16. Makela, J. K., Vuorio, T., and Vuorio, E. (1990) Biochim. Biophys. Acta 1049, 171–176
17. Sandberg, M., Makela, J. K., Multimaki, P., Vuorio, T., and Vuorio, E. (1989)

2 N. Reunanen, M. Ahonen, M. Foschi, J. Han, C. López-Otin, and V.-M. Kähäri, submitted for publication.
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34639

Matrix 9, 82–91

16. Goldberg, G. I., Wilhelm, S. M., Kronberger, A., Bauer, E. A., Grant, G. A., and Eisen, A. Z. (1986) *J. Biol. Chem.* 261, 6690–6695

17. Fort, P., Marty, L., Piechaczyk, M., El Sahorey, S., Dani, C., Jeanteur, P., and Blanchard, J. M. (1985) *Nucleic Acids Res.* 13, 1431–1442

18. Boast, S., Su, M.-W., Ramirez, F., Sanchez, M., and Avvedimento, E. V. (1999) *J. Biol. Chem.* 265, 15351–15356

19. Wilkinson, G. W. G., and Akrigg, A. (1992) *Nucleic Acids Res.* 20, 2233–2239

20. Foschi, M., Chari, S., Dunn, M. J., and Sorokin, A. (1997) *EMBO J.* 16, 6439–6451

21. Wang, Y., Huang, S., Sah, V. P., Ross, J., Brown, J. H., Han, J., and Chien, K. R. (1998) *J. Biol. Chem.* 273, 2161–2168

22. Ahonen, M., Baker, A. H., and Kahari, V.-M. (1998) *Cancer Res.* 58, 2310–2315

23. Kawahara, S., Hokai, L., Larjava, H., Saarialho-Kere, U., Foschi, M., Han, J., and Kahari, V.-M. (1999) *J. Biol. Chem.* 274, 37292–37300

24. Cowley, S., Paterson, H., Kemp, P., and Marshall, C. J. (1994) *Cell* 77, 851–862

25. Mansour, S. J., Matten, W. T., Hermann, A. S., Candia, J. M., Rong, S., Fukasawa, K., Vande Woude, G. F., and Ahn, N. (1994) *Science* 265, 966–970

26. Oka, H., Chatani, Y., Hoshino, R., Ogawa, O., Kakehi, Y., Terachi, T., Okada, Y., Kawaichi, M., Kohno, M., and Yoshida, O. (1995) *Cancer Res.* 55, 4182–4187

27. Sivaraman, V. S., Wang, H., Nuove, G. J., and Malbon, C. C. (1997) *J. Clin. Invest.* 99, 1478–1483

28. Sebolt-Leopold, J. S., Dudley, D. T., Herrera, R., Van Beelvaere, K., Wiland, A., Gowan, R. C., Teche, H., Barrett, S. D., Bridges, A., Przybranowski, S., Leopold, W. R., and Slatier, A. R. (1999) *Nat. Med.* 5, 810–816

29. Slattery, J. M., Holmstrom, T., Ahonen, M., Eriksson, J. E., and Kahari, V.-M. (1999) *Matrix Biol.* 17, 547–557

30. Simon, C., Goepfert, H., and Boyd, D. (1998) *Cancer Res.* 58, 1135–1139

31. Johansson, N., Ala-aho, R., Uitto, V.-J., Grénman, R., Foschi, M., Han, J., and Kahari, V.-M. (2000) *J. Cell Sci.* 113, 227–235

32. Davis, B. H., Han, A., and Beno, D. A. (1996) *J. Biol. Chem.* 271, 11039–11042

33. Slatt, J. L., Parker, M. I., Robinson, V. R., and Bornstein, P. (1992) *J. Biol. Chem.* 12, 4714–4723

34. Andreu, T., Beckers, T., Thoenes, E., Hilgard, P., and von Melchn, H. (1998) *J. Biol. Chem.* 273, 13848–13854

35. Palay, S., and Goltzman, D. (1999) *Biochem. J.* 343, 21–27

36. Gebken, J., Lodder, B., Notbohm, H., Klein, H. H., Brinckmann, J., Muller, P. K., and Bittig, B. (1999) *J. Biochem.* 126, 676–682

37. Svegliati-Baroni, G., Ridolfi, F., Di Sario, A., Castini, A., Marucci, L., Gaggiotti, G., Orlandoni, P., Macarri, G., Perez, L., Benedetti, A., and Foll, P. (1999) *Hepatology* 29, 1743–1751

38. Laine, P., Reunanen, N., Ravanit, L., Foschi, M., Santra, M., Iozzo, R. V., and Kahari, V.-M. (2000) *Biochem. J.* 349, 19–25