Translational Repression of Human Matrix Metalloproteinases-13 by an Alternatively Spliced Form of T-cell-restricted Intracellular Antigen-related Protein (TIAR)*

Qing Yu‡, Steven J. Cok, Chenbo Zeng, and Aubrey R. Morrison§

From the Departments of Medicine and of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, Missouri 63110

Human matrix metalloproteinases-13 (HMMP13) shows a wide substrate specificity, and its expression is limited to pathological situations such as chronic inflammation and cancer. The coding sequence for HMMP13 is 86% identical to rat matrix metalloproteinases-13 (RMMP13); however, the regulation of HMMP13 and RMMP13 protein synthesis in renal mesangial cells is strikingly different. In human cells there is a discordance between HMMP13 mRNA levels and protein expression. Following IL-1β or TGF-β1 stimulation, HMMP13 mRNA levels increase significantly, whereas the protein expression is absent. This discordance is because of a species-dependent translational repression. In addition to the 3' untranslated region of the matrix metalloproteinases-13 (MMP13) gene, the differential expression of an alternatively spliced transcript of the RNA-binding protein TIAR in human cell cultures is also critical for this post-transcriptional regulation. Transient expression of the 17-amino acid insert of the alternatively spliced form of TIAR reverses the HMMP13 mRNA silencing observed in human and primate species. In addition, co-transfection of the alternatively spliced form of TIAR and HMMP13 into Rat2 cells suppresses HMMP13 protein expression. Thus, we report for the first time that a species-dependent TIAR isoform plays a major role in the post-transcriptional silencing for HMMP13.

Matrix metalloproteinases (MMPs) are proteolytic enzymes that function in extracellular matrix (ECM) remodeling (1). It is also clear that MMPs influence many cellular functions, such as migration, proliferation, apoptosis, and morphogenesis (2). Currently 20 distinct human MMPs have been described, of which 14 are secreted extracellular proteinases and 6 are membranes-type MMPs. MMP-13, together with MMP-1 and MMP-8, constitute the interstitial collagenase subfamily (3). HMMP13 was initially identified in fibrosing breast cancer (4). HMMP13 has a wide substrate specificity, and its expression is limited to pathological situations in which rapid and effective remodeling of collagenous ECM is required (5–7). High levels of expression of HMMP13 have been documented in certain cancers that are aggressive and invasive, and HMMP13 can serve as a prognostic marker of tumor progression in a specific subset of cancers (8).

Numerous studies have documented the transcriptional regulation of HMMP13 in several cell lines (9, 10). The HMMP13 gene can be either up-regulated or down-regulated by a number of cytokines, growth factors, and hormones. For example, HMMP13 expression was induced in chondrocytes by IL-1β and in human gingival fibroblasts by TGF-β. In contrast, IFN-γ inhibited HMMP13 expression in ras-transformed human keratinocytes (11). Recent work has also shown that the RMMP13 transcript is stabilized by cortisol (12) or alendronate (13) and destabilized by TGF-β in osteoblast cells (14). The change in message stability was mediated by adenine- and uridine-rich elements (AREs) in the 3' untranslated region (3'UTR) of the RMMP13 mRNA. However, our understanding of the mechanisms for regulation of HMMP13 expression is far from complete (15).

The 3'UTR of certain genes can specifically control the nuclear export, polyadenylation status, subcellular targeting, and rates of translation or degradation of transcripts (16). The AREs found in the 3'UTR of mRNA are one of the most prevalent and best studied elements (17). They play important roles in the post-transcriptional regulation of several genes involved in inflammation and tumorigenesis, including COX-2 (18), iNOS (19), and TNF-α (20). For example, in mice with a targeted disruption of the AREs from TNF-α mRNA, the animals developed chronic inflammatory arthritis and Crohn's-like inflammatory bowel disease due to increased TNF-α synthesis (21). In this study, we have shown that the HMMP13 gene undergoes a species-dependent translational silencing in human and primate cell cultures. This post-transcriptional regulation requires the participation of the 3'UTR of the transcripts and is affected by the expression of a peptide corresponding to the alternatively spliced form of TIAR, namely TIAR-α.

MATERIALS AND METHODS

Reagents—Unless indicated, all reagents used for biochemical methods were purchased from Sigma, VWR, or Fisher. Restriction enzymes, DNA Polymerase I large fragment (Klenow), DNase I, and alkaline phosphatase were all obtained from New England Biolabs Inc. The pcDNA3 vector, cell culture medium, and fetal bovine serum were from Invitrogen. Human recombinant IL-1β, TGF-β1, and TNF-α were all...
purchased from Roche Molecular Biochemicals.

Cell Cultures—Human mesangial cells were purchased from Clonetics (CC-2599) and grown in supplemented mesangial cell growth medium (MaGM BulletKit, CC-3146) that contained 5% fetal bovine serum with 50 μg/ml gentamicin and 0.05 μg/ml amphotericin B. Rat primary mesangial cell cultures were prepared from male Harlan Sprague-Dawley rats as previously described (22). Cells were grown in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 0.6% (v/v) insulin, 100 μg/ml streptomycin, 100 units/ml penicillin, 100 μg/ml amphotericin, 250 μg/ml amphotericin B, and 15 μM HEPEs. Where indicated, mesangial cells were stimulated with IL-1β (100 units/ml), TGF-β3 (5 ng/ml), or TNF-α (20 ng/ml). All experiments were performed with confluent cells and used at passages 3–8. HEK293 (CRL1573), COS-7 (CRL1651), and Rat2 (CRL1764) cells were all from American Type Culture Collection (ATCC) and grown at 37 °C in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. RT-PCR was performed using cDNA prepared from total RNA using reverse transcriptase (Qiagen, Inc.) using Oligo (dT) 15 primers.

RT-PCR and TaqMan Real-time PCR—To test the expression of MMP13 message, total RNA was isolated from cells using RNA STAT-60 reagent, DNase-treated, and reverse-transcribed by Omniscript reverse transcriptase (Qiagen, Inc.) using oligo (dT) 15 primers. The PCR amplifications were carried out using human MMP13 amplification primers, 5′-CCCAACCTTAAATCAGAAAGGC-3′ and 5′-CTTCTCCCAGGCGCTACTCT-3′, and rat MMP13 amplification primers, 5′-GGATGCTGACAACGGTACTACTG-3′ and 5′-AGGGTGGCTCCCTGTTCTC-3′. To quantify HMMP13 mRNA levels, TaqMan real-time PCR was performed using gene-specific primers and the double-stranded DNA-binding dye, SYBR green I. Fluorescence was detected using a ABI Prism 7500 sequence detection system (PE Biosystems). HMMP13 amplification primers were 5′-TGAACGTCGGACACTTCGGAACGGTTCACAATAAG-3′ and 5′-ACGATGGTTGATGGAGTTCCAGTTC-3′. Relative mRNA levels were calculated using the comparative Ct method.

Constructs—MMP13 expression vectors were made by cloning the RT-PCR-amplified human MMP13 coding region (HCR), human MMP13 3′-UTR (HUTR), and rat MMP13 3′-UTR (RUTR) into pcDNA3. Briefly, after first-strand synthesis, cDNAs encoding HCR, HUTR, and RUTR were generated by PCR using appropriate primers and then cloned into pcR2.1-TOPO-TA cloning vector (Invitrogen). HCR forward primer was 5′-ATCCAGATGATCATCAGGGGTC-3′ and reverse primer was 5′-CCTTAACTGACAAAGGCTG-3′. HUTR primers were 5′-CAATTTATCTTCTTGGTGTTAAGG-3′ and 5′-AATACCACTACATTCACTCTTGGC-3′. RUTR primers were 5′-GGAATATTTCTGTTTCACTAAG-3′ and 5′-GAACATGGTTTAGAACCAC-3′. HCR was subcloned from pcR2.1 into pcDNA3 using restriction enzyme EcoRI to get HMMP13-HCR. The HUTR were excised from pcR2.1 using BstXI (filled in with Klenow) and EcoRV, then ligated into HMMP13-HCR by using EcoRV and NotI (filled in with Klenow) to generate HMMP13-HFL. The RUTR were excised from pcR2.1 using BamHI (filled in with Klenow) and NotI and then inserted into HMMP13-HCR by using NotI and XhoI (filled in with Klenow) to obtain HMMP13-HCRU. The FLAG-TIARa and FLAG-TIARB constructs were generated by cloning the RT-PCR-amplified human TIAR (a) (1185 bp) or TIA-b (1134 bp) gene into pcDNA3/FLAG expression vector (Invitrogen). The forward primer was 5′-ACCATGATTGTCAGCAC-3′ and reverse primer was 5′-GTTGGATTTTCTGTTTTGCACTAAG-3′. The amplification primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were 5′-GGATGCT-3′ and 5′-GGATGATGTTGATGGAGTTCCAGTTC-3′. Primer pairs were tested to ensure a robust amplification signal of the expected size with no additional bands. The amount of HMMP13 message in each RNA sample was quantified and normalized to GAPDH content. Relative amounts of HMMP13 cDNA were calculated by the comparative Ct method.

Translational Repression of MMP13

FIG. 1. Differential regulation of MMP13 mRNA and protein expression in rat and human mesangial cells. Cells were treated with IL-1β (100 units/ml), TGF-β3 (5 ng/ml), or TNF-α (20 ng/ml) for 24 h. A, RT-PCR amplification of a 500-bp region of MMP13. IL-1β increased MMP13 mRNA levels. B, RT-PCR amplification of a 678-bp region of HMP13. IL-1β and TGF-β3 induced MMP13 mRNA expression. C, Western blot analysis of MMP13 expression. IL-1β increased MMP13 protein expression. p-amino-phenylmercuric acetate (APMA) treatment resulted in the cleavage of the latent form (60 kDa) of MMP13 to active form (48 kDa). D, Western blot analysis demonstrated that no MMP13 protein was expressed.

FIG. 2. DNA constructs utilized in this study. HMMP13-HCR contained only the coding region of HMMP13. HMMP13-HFL contained the full-length HMMP13 cDNA (coding region plus the 3′-UTR), and HMMP13-HCRU contained the coding region from HMMP13 plus the 3′-UTR from RMM13.

Translational Transcripts—Equimolar amounts of DNA were transiently transfected into cells using SuperFect transfection reagent (Qiagen Inc.) following the manufacturer’s protocol.

Western Blot Analysis—Culture medium from cells was concentrated by Centrifugus YM-30 centrifrons (Millipore Corp.), and equal amounts of proteins were assayed for MMP13 content by Western blotting. Monoclonal anti-HMMP13 (Chemicon International Inc.) and anti-HMMP13 (Oncogene) antibodies were used for Western blotting at a 1:500 dilution. Whole cell protein extracts were prepared from confluent cells as described previously (22). Western blots of whole cell extracts were probed with monoclonal anti-HuR (Santa Cruz Biotechnology), monoclonal anti-FLAG (Sigma), polyclonal anti-GFP (Clontech), or polyclonal anti-TIAR antibodies (Santa Cruz Biotechnology) at a 1:1000 dilution. Signal detection was carried out using enhanced chemiluminescence system (ECL, Amersham Biosciences). The monoclonal anti-HMMP13 and anti-RMMP13 antibodies were specific for human and rat MMP13, respectively. The monoclonal HuR and polyclonal TIAR antibodies reacted with both human and rat proteins. Bands corresponding to the MMP13 were quantitated on the Discovery Series densitometer, and the band intensities were measured using Quantity One software, both from Bio-Rad. The densitometer was calibrated against external standards and was linear to 2 AUFS. We also calibrated it against different amounts of expressed MMP-13 in serum-free cellular supernatants from HCR-transfected HEK293 cells; the response was linear over 10–100 μl of media, and we routinely used 50 μl for Western analysis.

RESULTS AND DISCUSSIONS

Differential Regulation of MMP13 mRNA and Protein Expression in Rat and Human Mesangial Cells—Previous studies in our laboratory have demonstrated that IL-1β induces inter-
stitial collagenase-3 (MMP13) gene expression and protein secretion in primary cultures of rat mesangial cells (23). To further investigate the regulation of MMP13 expression in renal mesangial cells, we used primary cultures of mesangial cells from both human and rat. Cultured mesangial cells were stimulated with IL-1β (100 units/ml), TGF-β1 (5 ng/ml), or TNF-α (20 ng/ml) for 24 or 48 h in serum-free medium. Total RNA was extracted and analyzed for MMP13 message by RT-PCR, and cell culture medium was concentrated and analyzed for MMP13 protein using Western blots. Rat mesangial cells exposed to IL-1β for 24 h showed increased MMP13 mRNA levels, whereas TGF-β1 and TNF-α had no effect (Fig. 1A). In human mesangial cells, IL-1β also increased MMP13 mRNA levels; however, TGF-β1 was a more effective ligand and TNF-α had no effect (Fig. 1B). Western blot analysis detected rat MMP13 (RMMP13) protein expression in media from IL-1β-treated rat mesangial cells (Fig. 1C). However, there was no HMMP13 protein detected in human mesangial cell media after 48 h of stimulation with IL-1β or TGF-β1 (Fig. 1D). We were also unable to detect HMMP13 protein expression in whole cell lysates, excluding the possibility that HMMP13 protein failed to be secreted (data not shown). Thus, the discordance between the increase in mRNA levels and the absence of HMMP13 protein expression following IL-1β or TGF-β1 treatment suggests that the HMMP13 gene is translationally silenced in human mesangial cells. Furthermore, the differential regulation of MMP13 in human and rat mesangial cells suggests a species-dependent mechanism for post-transcriptional regulation.

The 3′-UTR of MMP13 Decreased HMMP13 Protein Expression in HEK293 and COS-7 Cells but Not in Rat2 Cells—To determine whether the 3′-UTR was responsible for the differential post-transcriptional regulation of HMMP13 expression, we engineered a series of expression plasmids, shown in Fig. 2. HMMP13-HCR contains cDNA corresponding to the coding region of HMMP13, whereas HMMP13-HFL contains the full-length cDNA (coding region plus the 3′-UTR). HMMP13-HCR is the combination of the HMMP13 coding region and the 3′-UTR of RMMP13. HEK293, COS-7, and Rat2 cells were transiently transfected with equimolar amounts of these constructs. Human MMP13 protein expression was reduced by 70–80% in HEK293 cells transfected with HMMP13-HFL or -HCR when compared with cells transfected with HMMP13-HCR (Fig. 3, A and B). Similar results were also found in...
transiently transfected COS-7 cells (Fig. 3, C and D). In contrast, constructs expressed in Rat2 cells resulted in equivalent amounts of MMP13 protein expression regardless of the 3'-UTR present in the message (Fig. 3E). Interestingly, although only the latent form of HMMP13 was detected in transfected HEK293 and COS-7 cells, both latent and active forms were expressed in transfected Rat2 cells. These data suggest that the suppression of HMMP13 expression requires the 3'-UTR of MMP13, either human or rat, and occurs in human and primate cell lines but not in Rat2 cells.

Translational Silencing of HMMP13 Gene Expression by an Alternatively Spliced Form of RNA-binding Protein TIAR—Post-transcriptional regulation of certain genes has been shown to be dependent on AREs present in the 3'-UTR present in the message (Fig. 3E). Interestingly, only the latent form of HMMP13 was detected in transfected HEK293 cells and COS-7 cells, both latent and active forms were expressed in transfected Rat2 cells. These data suggest that the suppression of HMMP13 expression requires the 3'-UTR of MMP13, either human or rat, and occurs in human and primate cell lines but not in Rat2 cells.

Translational Silencing of HMMP13 Gene Expression by an Alternatively Spliced Form of RNA-binding Protein TIAR—Post-transcriptional regulation of certain genes has been shown to be dependent on AREs present in the 3'-UTR (24). Several proteins, including AUF1/hnRNPD, HuR, TIA1, and TIAR, are reported to bind AREs. Binding of these proteins to transcripts bearing AREs can have either a positive or negative effect on gene expression (25). To determine whether ARE binding proteins were differentially expressed, we performed Western blot analysis on the cell lines of interest, using anti-HuR and anti-TIAR antibodies. No significant differences in HuR protein expression were observed in human and rat mesangial cells (Fig. 4A). However, TIAR protein expression was different in human and primate cells compared with rat cells (Fig. 4B). Human and primate cells expressed two isoforms of TIAR, whereas rat cells expressed only one.

TIAR is a member of the RNA recognition motif (RRM) family of RNA binding proteins that has been shown to act as a translational silencer (26). This protein possesses three RRM domains that confer high affinity binding to AREs. The TIAR gene encodes two isoforms, TIAR-a and TIAR-b, that arise from alternative splicing of a common precursor transcript (27). TIAR-a differs from TIAR-b in that it contains an additional 51 nucleotides in the middle of RRM1 resulting in a 17-amino acid insertion (Fig. 4D), which has been suggested to play a role in RNA binding specificity (28).

Interestingly, translational silencing occurred only in the human or primate cell lines, the same cell lines that expressed TIAR-a. The possibility arises, therefore, that species-dependent translational silencing of the HMMP13 gene is because of the differential expression of TIAR-a in human and rat cell cultures. We reasoned that if the 17-amino acid insert of TIAR-a serves as a binding site required for the translational repression of HMMP13, then expression of the peptide QPD-SRRVNSSVGFSVLQ encoded by this 51-nucleotide insert may competitively inhibit this binding event and reverse mRNA silencing observed in human and primate cell lines. To test this possibility, we subcloned the 51-nucleotide cDNA into FLAG-tagged pcDNA3 vector to generate the FLAG peptide. HEK293 cells transfected with HMMP13-HCR, HMMP13-HFL, or

Fig. 5. Expression of the 17-amino acid insert of TIAR-a, but not the 11-amino acid insert of TIA1, inhibited the translational silencing of HMMP13 message. HEK293 cells transfected with HMMP13-HCR, HMMP13-HFL (HFL) or HMMP13-HCRU (HCRU) were co-transfected with either control vector (pcDNA3/FLAG), FLAG-TIAR peptide (A), or FLAG-TIA1 peptide (B) expression vector. The HMMP13 protein levels were quantitated and normalized to that of HCR-transfected cells. Data represent the mean ± S.E. from five independent experiments (*, p < 0.05 versus control).

Fig. 6. Expression of the GFP-TIAR peptide, but not the GFP-TIA1 peptide, inhibited the translational silencing of HMMP13 message. A, HEK293 cells transfected with HMMP13-HCR, HMMP13-HFL (HFL), or HMMP13-HCRU (HCRU) were co-transfected with either GFP-TIAR peptide or GFP-TIA1 peptide expression vector. The HMMP13 protein levels were quantitated and normalized to that of HCR-transfected cells. B, GFP protein expression was detected by Western blot analysis using polyclonal anti-GFP antibody. Data represent the mean ± S.E. from three independent experiments.
HMPM13-HCRU were co-transfected with pcDNA3/FLAG (as control) and FLAG peptide. Quantitative PCR demonstrated equivalent levels of HMPM13 mRNA for all experimental conditions, confirming that co-transfection of the plasmid encoding the 17-amino acid peptide had no effect on the steady state level of the message (data not shown). The inclusion of the 3'-UTR of MMP13 resulted in a 60–70% decrease in protein expression, and co-expression of the peptide blocked the reduction of HMPM13 protein expression because of the 3'-UTR, suggesting an interference of translational silencing conferred by TIAR-a (Fig. 5A).

TIAR-a is another RNA-binding protein reported to be a translational silencer similar to TIAR and has three RRM that can bind to mRNAs (29). TIAR-a can be expressed as two alternatively spliced forms, the difference due to an insert of a small (11-amino acid) peptide. In contrast to the TIAR peptide insert that is located in the middle of the first RRM, the TIA1 peptide insert (SSTTVVSTQRSQ) is located between RRM1 and RRM2 (30). We tested whether this peptide could also reverse the translational silencing of HMPM13. Co-transfection of the FLAG-TIA1 peptide had no effect on HMPM13 protein expression because of the 3'-UTR (rat) and the minimal but not significant effect with human 3'-UTR of MMP13 (Fig. 5B). In all of these experiments there was no difference between MPPM13 expression when co-transfected with pcDNA3, pcDNA3/FLAG, or pcDNA3/GFP vectors. The data were normalized to the HCR data because of the variability in intensity of Western blots between experiments. Within experiments all exposures were carried out on the same blot and film. This result suggests that interference of translational silencing of HMPM13 is specific for the TIAR peptide. To confirm that the FLAG peptides were expressed, we performed Western blot analysis for the FLAG protein. Unfortunately, we were unable to detect FLAG peptide expression by routine Western blotting. We believe this was because of technical problems generated by the small molecular mass (less than 3 kDa) of the FLAG peptides. To clearly document the expression of epitope-tagged peptide, we therefore engineered a GFP fusion protein (GFP-FLAG) whose expression was easily demonstrated by Western blotting (Fig. 6B). We repeated the transient transfection experiments using the GFP peptides and confirmed that, although the co-transfection of the GFP-TIAR peptide reversed the translational block of HMPM13 protein expression because of the 3'-UTR, the GFP-TIA1 peptide did not (Fig. 6A). Furthermore, the GFP peptides were in fact expressed at equivalent levels (Fig. 6B).

Expression of TIAR-a but Not TIAR-b Produces Translational Silencing on HMPM13 Protein Expression in Transfected Rat2 Cells—Rat2 cells express only the TIAR-b isoform, and the 3'-UTR-dependent suppression of HMPM13 expression did not occur in these cells (Fig. 4C). To determine whether TIAR-a could cause translational silencing of HMPM13 in transfected Rat2 cells, we co-expressed human TIAR-a or TIAR-b (as control) in HCR-, HFL-, or HCRU-transfected Rat2 cells and measured HMPM13 protein levels. FLAG-tagged TIAR-a and TIAR-b were expressed at similar levels in Rat2 cells (Fig. 7B). Co-expression of TIAR-a resulted in a significant decrease in HMPM13 protein expression when the 3'-UTR of HMPM13 was present, whereas TIAR-b had no significant effect (Fig. 7A). However, TIAR-b appeared to partially inhibit HMPM13 protein expression in Rat2 cells co-transfected with the HCRU construct (about 25%). The results of this experiment suggest that TIAR-a plays the major role in the 3'-UTR-dependent translational silencing in HMPM13 expression.

Thus we have shown, for the first time, species-dependent MMP13 gene silencing in human and primate cell cultures. This post-transcriptional regulation requires the participation of the 3'-UTR of the transcripts and is affected by the expression of a peptide corresponding to the alternatively spliced form of TIAR, namely TIAR-a. These results are consistent with the notion that TIAR-a binds to the 3'-UTR of HMPM13 and inhibits translation. Expression of the peptide competes for the RNA binding site or disrupts protein-protein interactions of TIAR-a. These experiments underscore the concept that alternatively spliced transcripts can regulate the diversity of gene expression. It may also provide a mechanism by which tumors derepress MMP13 expression that may affect their metastasis potential.

REFERENCES

1. Nagase, H., and Woessner, J. F., Jr. (1999) J. Biol. Chem. 274, 21491–21494
2. Vu, Th, and Werb, Z. (2000) Genes Dev. 14, 2123–2133
3. McCawley L.J., and Matrisian, L. M. (2000) Mol. Med. Today 6:149–156
4. Freije, M. J., Diez-Ita, I., Balbin, M., Sanchez, L. M., Blasco, R., Tolivi, J., and Lopez-Otin, C. (1995) J. Biol. Chem. 269, 17666–17673
5. Werb, Z. (1997) Cell 91, 439–442
6. Ste literal-Stevens on, W. G. (1999) J. Clin. Invest. 103, 1237–1241
7. Oh, J., Takahashi, K., Kondo, S., Mizoguchi, A., Adachi, E., Sasahara, R. M., Nishimura, T., Inamura, Y., Kitayama, H., Alexander, D. B., Ide, C., Horan, T. P., Arakawa, T., Yoshida, H., Nishikawa, S., Hob, Y., Seiki, M., Itohara, S., Takahashi, C., and Noda, M. (2001) Cell 107, 789–800
8. Brinckerhoff, C. E., Rutter, J. L., and Benbow, U. (2000) Clin. Cancer Res. 6, 4875–4880
9. Mengshol, J. A., Vincenti, M. P., and Brinckerhoff, C. E. (2001) Nucleic Acids Res. 29, 4361–4372
10. Ravanati, L., Hakkinen, L., Larjava, H., Saarialho-Kere, U., Foschi, M., Han, J., and Kahari, V. M. (1999) J. Biol. Chem. 274, 37292–37300
11. Ala-aho, R. F., Johansson, N. F., Grenman, R. F., Fusenig, N. E., Lopez-Otin, C. F., and Kahari, V. M. (2000) Oncogene 19, 248–257
12. Delany, A. M., Jeffrey, J. F., Rydziel, S., and Canalis, E. (1995) J. Biol. Chem. 270, 26607–26612
13. Varghese, S., and Canalis, E. (2000) J. Bone Miner. Res. 15, 2345–2351
14. Rydziel, S., Varghese, S., and Canalis, E. (1997) J. Cell. Physiol. 170, 145–152
15. Sternlicht, M. D., and Werb, Z. (2001) Annu. Rev. Cell Dev. Biol. 17, 463–516
16. Conne, B. F., Stutz, A. F., and Vassalli, J. D. (2000) Nat. Med. 6, 637–641
17. Mendell, J. T., and Dietz, H. C. (2001) Cell 107, 411–414
18. Dixon, D. A., Tolley, N. D., King, P. H., Nahers, L. B., McIntyre, T. M., Zimmerman, G. A., and Prescott, S. M. (2001) J. Clin. Invest. 108, 1657–1665
19. Rodríguez-Pascual, F., Hausding, M., Ihrig-Biedert, I., Furneaux, H., Levy, A. P., Forstermann, U., and Kleinert, H. (2000) J. Biol. Chem. 275, 26040–26049
20. Brewer, G. (2001) J. Exp. Med. 193, F1–F4
21. Kontoyiannis, D., Pasparakis, M., Pizarro, T. T., Cominelli, F., and Kollias, G. (1999) Immunity 10, 387–398
22. Guan, Z., Tetsuka, T., Baier, L. D., and Morrison, A. R. (1996) Am. J. Physiol. 270, F634–F641
23. Daphna-Iken, D., and Morrison, A. R. (1995) Am. J. Physiol. 269, F831–F837
24. Macdonald, P. (2001) Curr. Opin. Cell Biol. 13, 326–331
25. Chen, Cy, Gherzi, R. F., Ong, S. E, Chan, E. F., Rajimakers, R. F., Pruijn, G. J, Stoecklin, G. F., Moreni, C. F., Mann, M. F., and Karin, M. (2001) Cell 107:451–64
26. Kawakami, A., Tian, Q., Duan, X., Streuli, M., Schlossman, S. F., and Anderson, P. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 8681–8685
27. Le Guiner, C., Lejeune, F., Galiana, D., Kister, L., Breathnach, R., Stevenin, J., and Gatto-Konczak, F. (2001) J. Biol. Chem. 276, 40638–40646
28. Dember, L. M., Kim, N. D., Liu, K. Q., and Anderson, P. (1996) J. Biol. Chem. 271, 2783–2788
29. Piecyk, M., Wax, S., Beck, A. R., Pekarska, N., Gupta, M., Maritim, B., Chen, S., Gueydan, C., Kruys, V., Streuli, M., and Anderson, P. (2000) EMBO J. 19, 4154–4163
30. Beck, A. R., Medley, Q. G., O’Brien, S., Anderson, P., and Streuli, M. (1996) Nucleic Acids Res. 24, 3829–3835