Hydrogen Peroxide Stimulates Proliferation and Migration of Human Prostate Cancer Cells through Activation of Activator Protein-1 and Up-regulation of the Heparin Affin Regulatory Peptide Gene*

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It is becoming increasingly recognized that hydrogen peroxide (HP) plays a role in cell proliferation and migration. In the present study we found that exogenous HP significantly induced human prostate cancer LNCaP cell proliferation and migration. Heparin affin regulatory peptide (HARP) seems to be involved in the stimulatory effect of HP, because the latter had no effect on stably transfected LNCaP cells that did not express HARP. Moreover, HP significantly increased HARP mRNA and protein amounts in a concentration- and time-dependent manner. Curcumin and activator protein-1 (AP-1) decoy oligonucleotides abrogated both HP-induced HARP expression and LNCaP cell proliferation and migration. HP increased luciferase activity of the 5′-flanking region of the HARP gene introduced in a reporter gene vector, an effect that was abolished when one of the two putative AP-1 binding sites of the HARP promoter was mutated. The effect of HP seems to be due to the binding of Fra-1, JunD, and phospho-c-Jun to the HARP promoter. These results support the notion that HARP is important for human prostate cancer cell proliferation and migration, establish the role of AP-1 in the up-regulation of HARP expression by low concentrations of HP, and characterize the AP-1 dimers involved.

A growing body of evidence correlates the production of reactive oxygen species with many aspects of cellular functions (1). Reactive oxygen species include the superoxide, hydrogen peroxide (HP), and the hydroxyl radical. Of these, HP has the chemical stability required to establish significant steady-state concentrations in vivo (2, 3) and is small and uncharged, properties that allow it to freely diffuse across plasma membranes and make it an ideal candidate for a signaling molecule (4). Small quantities of HP are produced by all types of cells, and several signal transduction pathways in mammalian cells have been reported to be activated by HP, such as tyrosine kinases, mitogen-activated protein kinases, protein kinase C, the epidermal growth factor receptor, protein phosphatases, potassium channels, and the transcription factors activator protein-1 (AP-1), and nuclear factor κB (reviewed in ref. 1).

Heparin affin regulatory peptide (HARP), also called pleiotrophin or heparin-binding growth-associated molecule, is an 18-kDa secreted growth factor that displays high affinity for heparin. A growing body of evidence indicates that HARP is involved in cell proliferation, migration, and differentiation and plays a significant role in several cellular processes (5, 6). HARP seems to play a major role in physiological as well as tumor angiogenesis and is detected in various carcinomas, such as human breast and prostate cancer, neuroblastomas, gliomas, benign meningiomas, and small cell lung cancer and mammary tumors, exhibiting a proto-oncogene function (5–8).

HARP is highly conserved among human, rat, bovine, and mouse species, and its gene is expressed in a highly restricted temporal and spatial pattern during development, suggesting that HARP may be an important protein that potentially contributes to a number of different regulating systems (6). However, very little is known about the regulation of its expression. When the promoter region of HARP gene was first cloned in 1992, no binding sites for known general transcription factors in the immediate promoter region were detected, although two potential AP-1 sites were found in the distal 5′-promoter region (9). Very recently, HARP has been identified as a direct transcriptional target of HOXA5, a transcription factor with important roles in embryogenesis, hematopoiesis, and tumorigenesis (10).

The aim of the present study was to determine the effect of low concentrations of HP on human prostate cancer LNCaP cell proliferation and migration and to investigate whether HARP is involved in these effects. Our results argue for an HP-induced, AP-1-dependent transcriptional up-regulation of the human HARP gene, resulting in increased LNCaP cell proliferation and migration.

EXPERIMENTAL PROCEDURES

Cell Culture—The human prostate cancer epithelial cell line LNCaP (American Type Culture Collection) was grown routinely in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml gentamycin and 2.5 μg/ml amphotericin B. Cultures were maintained at 37 °C, 5% CO₂, and 100% humidity.

Generation of Human HARP Luciferase Reporter Constructs—Human genomic DNA was isolated from LNCaP cells using a genomic DNA mini kit (Geneaid Biotech Ltd., Taoyuan, Taiwan) according to the manufacturer’s instructions. To produce a construct that contains the region −1984 bp to +280 bp of the translational start site of the human HARP gene (GenBank™ accession number X65451), a fragment containing −2.3 kb of the human HARP 5′-flanking region was amplified by PCR using primers containing restriction sites (boldfaced) for MluI (5′-ATAAGCGGTGTCTCAGATCCGACAAGAACC-3′; sense) and BglII (5′-GCAAGATCGTTTTGAGTGGACCGTCCTCTC-3′; antisense). The fragment was ligated to a pGL3-basic firefly luc.
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briefly, LNCaP cells were harvested and resuspended at 10^5 cells per 0.1 ml in RPMI 1640 containing 0.5% bovine serum albumin. The bottom chamber of each transwell unit contained 0.6 ml of RPMI 1640 supplemented with 0.5% bovine serum albumin and 0.5% FBS. The plates were incubated for 20 h at 37 °C, and the filters were fixed with saline-buffered formalin and stained with 0.33% toluidine blue solution. The cells that migrated through the filter were quantified by counting the entire area of each filter by using a grid and an Optech microscope at a 20× magnification. For the experiments using ODNs, cells were pre-incubated with ODNs for 4 h.

Evaluation of DNA Binding Activity of AP-1 by Enzyme-linked Immunosorbent Assay (ELISA)—The DNA binding activity of AP-1 was quantified by ELISA using the TransAM™ AP-1 transcription factor family assay kit (Active Motif Europe), according to the manufacturer’s instructions. Briefly, nuclear extracts were prepared using a nuclear extract kit from Active Motif Europe and incubated in 96-well plates coated with immobilized oligonucleotide containing a consensus binding site for AP-1 (wild-type TPA response element). AP-1 binding to the target oligonucleotide was detected by incubation with primary antibodies specific for c-Fos, Fos-B, Fra-1, Fra-2, JunB, JunD, or the activated form of c-Jun, visualized with anti-IgG horseradish peroxidase conjugate and developing solution, and quantified at 450 nm with a reference wavelength of 655 nm. For the competition analyses, nuclear extracts were pre-incubated with the AP-1 like motifs of the HARP promoter and the respective mutated sequences or the wild type TPA response element.

Western Blot Analysis for HARP—The presence of HARP in the cell culture medium was investigated as described previously (8). The conditioned medium of the cells was incubated overnight with 100 μl of heparin-Sepharose (Amersham Biosciences) at 4 °C with continuous agitation. Bound proteins were eluted with 50 μl of Laemmli sample buffer under reducing conditions, fractionated by electrophoresis on 17.5% SDS-polyacrylamide gels, and transferred to Immobilon P membranes. Blocking was performed by incubating the polyvinylidene difluoride membranes with 3% bovine serum albumin in Tris-buffered saline (TBS) containing 0.05% Tween 20 (TBS-T). The membranes were then incubated with 45 ng/ml affinity purified anti-HARP antibody in TBS-T for 1 h at room temperature under continuous agitation and then with horseradish peroxidase-conjugated rabbit anti-goat IgG (Sigma) at a dilution of 1:7,500 in TBS-T for 1 h at room temperature under continuous agitation. Detection of HARP was performed by the ChemiLucent™ detection system kit (Chemicon International Inc.) according to the manufacturer’s instructions. The protein levels that corresponded to HARP were quantified using the ImagePC image analysis software (Scion Corporation, Frederick, MD). Results were normalized against total protein amounts.

Total RNA Isolation and Reverse Transcriptase-PCRs—Total RNA was extracted from LNCaP cells using the NucleoSpin® RNA II kit (Macherey-Nagel), according to the manufacturer’s instructions. Reverse transcriptase-PCRs were performed using the Access reverse transcription-PCR system (Promega) as described previously (8). Primers used for human HARP and β-actin were described previously (8). The reverse transcriptase-PCR products were subjected to electrophoresis on 2% agarose gels containing 0.5 μg/ml ethidium bromide and photographed using a digital camera. The ratio of electrophoretic band values of HARP versus β-actin represents the relative expression of HARP gene after different treatments of cells.

Statistical Analysis—The significance of variability between the results of each group and its corresponding control was determined by unpaired t test. Each experiment included triplicate measurements for
each condition tested unless otherwise indicated. All results are expressed as mean ± S.E. from at least three independent experiments.

RESULTS

Effect of HP on LNCaP Cell Proliferation and Migration—We have recently shown that HP increases angiogenesis in vivo (11) and human endothelial cell proliferation and migration in vitro (12). In the present work, we studied the effect of HP on proliferation and migration of human prostate cancer LNCaP cells. As shown in Fig. 1A, HP significantly increased the number of LNCaP cells in a concentration-dependent manner, with the maximum effect (70 ± 9% increase) observed at 5 μM. Concentrations of HP higher than 10 μM were toxic for the cells (data not shown). Similarly, HP significantly induced LNCaP cell migration (51 ± 14% increase) at the concentration of 5 μM (Fig. 1B).

HARP Expression Is Required for HP-induced LNCaP Proliferation and Migration—We have recently shown that endogenous HARP expression plays a key role in LNCaP cell proliferation and migration (8). To evaluate the role of HARP in HP-induced LNCaP cell proliferation and migration, we used LNCaP cells expressing either antisense HARP (AS-LNCaP) or the appropriate control vector (PC-LNCaP), as described previously (8). HP significantly increased PC-LNCaP cell proliferation and migration, similar to its effect on non-transfected LNCaP cells. In contrast, it had no effect on AS-LNCaP cell proliferation or migration (Fig. 1).

HP Increases the Expression and Secretion of HARP by LNCaP Cells—To determine whether HP affects HARP expression by LNCaP cells, we incubated the cells with HP and measured both protein and mRNA amounts of HARP at different time points after the addition of different concentrations of HP into the cell culture medium. HARP protein levels secreted into the culture medium of LNCaP cells were significantly increased in a concentration-dependent manner 24 h after the addition of HP. The effect was observed after the first 6 h after addition of HP into the cell culture medium and was maximal at 24 h (data not shown). Similarly, HARP mRNA levels were also increased 3 and 5 h after the addition of HP into the culture medium of LNCaP cells (Fig. 2).

Curcumin and AP-1 ODNs Abolish HP-induced HARP Expression and Secretion—AP-1 is generally considered to be a redox-regulated transcription factor complex. Because it has been suggested that HARP promoter may contain two putative AP-1 binding sites (6, 9), we investigated a possible role of AP-1 in the HP-induced HARP up-regulation. Curcumin, a c-Jun NH₂-terminal kinase (JNK)/AP-1 inhibitor (13), completely attenuated HP-induced HARP mRNA and protein up-regulation (Fig. 3) and LNCaP cell proliferation and migration (Fig. 4). In the same line, transfection of LNCaP cells with AP-1 ODNs prior to stimulation with HP resulted in marked attenuation of HP-induced mRNA and protein up-regulation (Fig. 3) as well as LNCaP cell proliferation and migration (Fig. 4). ODNs containing a mutated AP-1 consensus sequence had no effect on HP-induced protein up-regulation.
(Fig. 3) and did not attenuate HP-induced LNCaP cell proliferation and migration (Fig. 4). HP-stimulated proliferation, however, appeared reduced in the presence of ODNs containing mutated AP-1 consensus sequence (Fig. 4A). This result may be attributed to nonspecific effects of phosphorothioate oligonucleotides such as a nonspecific blockade of cell surface receptor activity or interference with other proteins, resulting in a non-sequence-specific inhibitory effect on cell proliferation as described previously (14).

**HP Induces the Transcriptional Activation of HARP Gene—**We examined the effects of HP on the human HARP promoter activity. For this reason, we made a plasmid construct containing the full-length promoter (2.3 kb) of the human HARP gene fused to a luciferase reporter gene (hHARPpro2.3-Luc; Fig. 5A) and used it to transfect LNCaP cells as described under “Experimental Procedures.” Reporter activity was increased in response to HP in LNCaP cells after the first 2 h following the addition of HP into the cell culture medium and was maximal at 6 h (Fig. 5B).

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**FIGURE 2.** Effect of HP on the expression and secretion of HARP by LNCaP cells. A, at the top is a photograph of a representative Western blot (from four independent experiments) used in analysis of the LNCaP culture medium for HARP. HARP protein amounts were quantified by densitometric analysis of the corresponding band in each lane. Results are expressed as mean ± S.E. of the percentage change of the amounts of HARP protein in cells treated with HP compared with that in the untreated cells. In the far left lane, 250 ng of human recombinant HARP were used as a positive marker. B, at the top is a photograph of a representative reverse transcriptase-PCR reaction (from six independent experiments) for HARP and β-actin mRNA in LNCaP cells. HARP mRNA amounts were quantified by densitometric analysis of the corresponding bands, and the ratio of HARP to actin mRNAs was calculated for each lane. Results are expressed as mean ± S.E. of the percentage change of HARP mRNA relative amounts in cells treated with HP compared with the untreated cells. **, p < 0.01.

**FIGURE 3.** Effect of AP-1 inhibition on HP-induced production and secretion of HARP by LNCaP cells. A, at the top is a photograph of a representative Western blot (from four independent experiments) used in analysis of the LNCaP culture medium for HARP. HARP protein amounts were quantified by densitometric analysis of the corresponding band in each lane. Results are expressed as mean ± S.E. of the percentage change of the amounts of HARP protein in treated cells compared with that in the untreated cells. B, at the top is a photograph of a representative reverse transcriptase-PCR reaction (from six independent experiments) for HARP and β-actin mRNA in LNCaP cells. HARP mRNA amounts were quantified by densitometric analysis of the corresponding bands, and the ratio of HARP to actin mRNAs was calculated for each lane. Results are expressed as mean ± S.E. of the percentage change of HARP mRNA relative amounts in treated cells compared with that in the untreated cells. **, p < 0.01.

**FIGURE 4.** Effect of AP-1 inhibition on HP-induced production and secretion of HARP by LNCaP cells. A, at the top is a photograph of a representative Western blot (from four independent experiments) used in analysis of the LNCaP culture medium for HARP. HARP protein amounts were quantified by densitometric analysis of the corresponding band in each lane. Results are expressed as mean ± S.E. of the percentage change of the amounts of HARP protein in treated cells compared with that in the untreated cells. B, at the top is a photograph of a representative reverse transcriptase-PCR reaction (from six independent experiments) for HARP and β-actin mRNA in LNCaP cells. HARP mRNA amounts were quantified by densitometric analysis of the corresponding bands, and the ratio of HARP to actin mRNAs was calculated for each lane. Results are expressed as mean ± S.E. of the percentage change of HARP mRNA relative amounts in treated cells compared with that in the untreated cells. **, p < 0.01.
We next evaluated the contribution of the two putative AP-1-like motifs of the HARP promoter to the increased transcription of HARP gene in HP-stimulated LNCaP cells. For this reason, we made constructs with point mutations in either one (AP-1 mutant-I and AP-1 mutant-II) or both (AP-1 double mutant) AP-1-like motifs, as shown in Fig. 6A. The reporter activities of both AP-1 mutant-I and AP-1 mutant-II, as well as AP-1 double mutant, were significantly lower in non-stimulated cells. Moreover, HP had no effect on the reporter activity (Fig. 6B), suggesting a pivotal role of these two motifs in both basal and HP-stimulated transcription of HARP gene in LNCaP cells.

**DISCUSSION**

HARP is an 18-kDa heparin binding growth factor that has a potent role in angiogenesis and tumor growth, although very little is known about the regulation of its expression (6). In the present work, we studied the mechanisms of regulation of HARP expression by HP that lead to increased prostate cancer cell proliferation and migration. To our knowledge, this is the first study that shows the involvement of AP-1 in the signaling mediated by low concentrations of HP, although the involvement of AP-1 in the apoptotic effects of increased concentrations of HP is well documented (15).

HP induced the transcriptional activation of HARP gene. A diagram of the reporter plasmid containing 2.3 kb of the human HARP 5'-flanking region (hHARPpro2.3-Luc). B, reporter activities of hHARPpro2.3-Luc in LNCaP cells are expressed as mean ± S.E. of relative luciferase units (RLU) per mg of total protein. Asterisks denote a statistically significant difference from the untreated cells (control) at the corresponding time points. *p < 0.05; **p < 0.01.

HP Induces the Binding Activity of Phospho-c-Jun, JunD, and Fra-1 on HARP Promoter—Having demonstrated that AP-1-like motifs are implicated in the up-regulation of HARP promoter activity upon stimulation with HP, we went on to determine the possible AP-1 subunits that constitute the active complex on HARP promoter. Therefore, a competitive ELISA was performed using soluble double-stranded oligonucleotides that corresponded to either the wild-type or the mutated AP-1 binding like motifs of the HARP promoter. Double-stranded oligonucleotides containing the wild-type TPA response element sequence were also used. When the AP-1 binding-like motifs of the HARP promoter were used, Fra-1, phospho-c-Jun and JunD binding was significantly decreased, whereas the binding of c-Fos, FosB, Fra-2, and JunB was not affected. Mutated AP-1 binding like motifs of the HARP promoter had no effect (Fig. 7B). When the TPA response element sequence was used, binding of all subunits was decreased up to 90% (data not shown).
support the stimulatory effect of either exogenously added or enzymatically produced HP on tumor growth and metastatic potential as well as on the mitotic and migratory rate of normal cells (12, 16, 17). The causative correlation of HP to the proliferation and migration of LNCaP cells is in line with and extends the notion that cell lines developed from LNCaP demonstrate increasing tumor and metastatic potential, in combination with a parallel increase in HP levels (18).

Using an antisense strategy, we have recently identified HARP as an important autocrine growth factor for human prostate cancer LNCaP cells (8). In the present study, using LNCaP cells expressing antisense...
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HARP, we sought to evaluate the possible role of HARP in HP-induced LNCaP cell proliferation and migration. HP stimulatory effects were abolished in AS-LNCaP cells, pointing at the necessity of HARP expression for HP-stimulated LNCaP cell proliferation and migration. Our hypothesis of an interventional role of HARP in HP effects was reinforced by the observation that HP resulted in increased HARP mRNA and protein levels in LNCaP cells.

To investigate a possible role of AP-1 in the biological phenomena triggered in LNCaP cells by low concentrations of HP, AP-1 activity was inhibited by using curcumin or AP-1 ODNs. The inhibition of HP-induced HARP expression and secretion, as well as LNCaP cell proliferation and migration, suggest a positive correlation between moderate HP concentration, AP-1 activity, HARP expression, and LNCaP cell functions. This notion is in line with previous studies that propose a proliferative and migratory effect of AP-1 complexes in different cell types (19–21).

The finding that HP treatment resulted in increased HARP mRNA and secreted protein levels, an effect that was reversed by AP-1 inhibition, indicated a probable transcriptional control mechanism. To corroborate this hypothesis, a reporter gene vector carrying the full-length promoter of HARP gene was constructed. The 5'-flanking nucleotide sequence of the human HARP gene promoter contains two putative AP-1-like binding sites (9), which seem to be involved in AP-1-mediated regulation of human HARP expression (this study). These two sites are located in the distal 5'-promoter region from −1737 to −1731 and −1406 to −1400, respectively. Such a cluster region for AP-1 responsive elements was also described for the human γ-glutamyltransferase (22), the human inducible nitric oxide synthase (23), the human γ-glutamylcysteine synthetase heavy subunit (24), and the mouse ornithine decarboxylase gene (25) promoters. In these cases, either one (22, 24) or both AP-1 binding sequences (23, 25) seemed to be required and/or functional. In the HARP gene promoter, AP-1-like binding sites were found to be similar in their ability to confer HP-mediated inducibility. Point mutation analyses revealed the requirement of both sites for the transcriptional up-regulation of HARP in HP-treated LNCaP cells. Furthermore, both AP-1 sites are implicated in the transcription of HARP at the basal non-induced state.

The binding of AP-1 to the corresponding sites of the HARP promoter most likely reflects the absolute requirement of a sequence containing the trinucleotide TGA, because a single mutation in the second base (G) was enough to inhibit the modulation of endogenous transcriptional regulation. Our data are in accordance with previous studies showing that oligonucleotides carrying similar sequences allowed the binding of AP-1 complexes, a phenomenon reversed when point mutations in the first triplet were introduced (25).

Various signal transduction pathways are linked to AP-1 in a manner controlled by upstream kinases, leading to both enhanced expression and/or interactions with other transcriptional regulators and resulting in AP-1 activity modulation (26–28). AP-1 proteins contain basic leucine zipper domains and act as a variety of homodimers or heterodimers composed of members of the Fos, Jun, and ATF families (29, 30). Jun proteins can form both homodimers and heterodimers, whereas Fos proteins form only heterodimers. In the present study, we demonstrate that low concentrations of HP are able to increase the binding activity of different Jun and Fos family members. Interestingly, the AP-1 complexes that bind to the corresponding sites of the HARP promoter consist of Fra-1, JunD, and c-Jun. Thus, three kinds of AP-1 complexes are most likely involved in the HP-stimulated HARP transcriptional activation, namely Fra-1-JunD, Fra-1-c-Jun, and c-Jun-JunD. These results come in line and extend the most recent notion that, in mouse fibroblasts, HARP expression is up-regulated by Jun (31). Interestingly, HARP expression is also regulated by Fra-1, a member of the AP-1 family that seems to regulate genes that are required for migration/invasion (21, 32, 33). HARP has been implicated in migration of several types of cells (reviewed in Ref. 6), including both endothelial (34, 35) and tumor cells (present study, 8, 36).

Three distinct mammalian mitogen-activated protein kinase modules, including JNK, extracellular signal-regulated kinase (ERK), and p38 mitogen-activated protein kinase, have been characterized as upstream kinases modifying AP-1 activity (37). The inhibition of HP-stimulated HARP expression by curcumin (a JNK inhibitor) suggests that the JNK signal transduction pathway may be involved. Moreover, preliminary data show the requirement of ERK activity for HP-induced HARP expression in human endothelial cells.3 Taken together, it would be tempting to speculate that AP-1-mediated HARP up-regulation is mediated by a coordinated activation of JNK and ERK signal transduction pathways.

3 C. Polytarchou, M. Hatziapostolou, and E. Papadimitriou, unpublished data.
In conclusion, under non-stress conditions HP exerts proliferative and migratory effects on LNCaP cells through up-regulation of HARP expression. The present study is the first to demonstrate the involvement of AP-1 response elements in the transcriptional regulation of the human HARP gene. Our data suggest that HARP gene is redox-sensitive and that HP-induced HARP expression is due to binding of specific AP-1 complexes to both of the AP-1 responsive sites of the promoter of HARP.

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REFERENCES

1. Dro¨ge, W. (2002) Physiol. Rev. 82, 47–95.
2. Chance, B., Sies, H., and Boveris, A. (1979) Physiol. Rev. 59, 527–605.
3. Cadenas, E., and Davies, K. J. (2000) Free Radic. Biol. Med. 29, 222–230.
4. Ohno, Y., and Gallin, J. I. (1985) J. Biol. Chem. 260, 8438–8446.
5. Kadomatsu, K., and Muramatsu, T. (2004) EMBO J. 23, 231–238.
6. Papadimitriou, E., Polykratis, A., Hatziapostolou, M., Parthymou, A., Polytarchou, C., and Mikelis, C. (2004) Curr. Cancer Drug Targets 4, 471–482.
7. Wu, H., Barusevicius, A., Babh, J., Klein-Szanto, A., Godwin, A., Elenitsas, R., Gelfand, J. M., Lessin, S., and Seykora, J. T. (2005) J. Cutan. Pathol. 32, 125–130.
8. Hatziapostolou, M., Delbe, J., Katsoris, P., Polytarchou, C., Courty, J., and Papadimitriou, E. (2005) Prostate 65, 151–158.
9. Li, Y. S., Hoffman, R. M., Le Beau, M. M., Espinosa, R., III, Jenkins, N. A., Gilbert, D. J., Copeland, N. G., and Deuel, T. F. (1992) J. Biol. Chem. 267, 26011–26016.
10. Chen, H., Rubin, E., Zhang, H., Chung, S., Jie, C. C., Garrett, E., Biswal, S., and Sukumar, S. (2005) J. Biol. Chem. 280, 19373–19380.
11. Polytarchou, C., and Papadimitriou, E. (2004) Free Radic. Res. 38, 501–508.
12. Polytarchou, C., and Papadimitriou, E. (2005) Eur. J. Pharmacol. 5, 31–38.
13. Kumar, A., Kingdon, E., and Norman, J. (2005) FASEB J. 19, 443–445.
14. Morishita, R., Tomita, N., Kaneda, Y., and Ogihara, T. (2004) Curr. Opin. Pharmacol. 4, 139–146.
15. Benhar, M., Engelberg, D., and Levitzki, A. (2002) EMBO Rep. 3, 420–425.
16. Stone, J. R., and Collins, T. (2002) Endothelium 9, 231–238.
17. Laurent, A., Nicco, C., Chereau, C., Goulvestre, C., Alexandre, J., Alves, A., Levy, E., Godlewski, F., Panis, Y., Soubrane, O., Weill, B., and Batteux, F. (2005) Cancer Res. 65, 948–956.
18. Lim, S. D., Sun, C., Lambeth, J. D., Marshall, F., Amin, M., Chung, L., Petros, J. A., and Arnold, R. S. (2005) Prostate 62, 200–207.
19. Oktyay, M., Wary, K. K., Dans, M., Birge, R. B., and Giancotti, F. G. (1999) J. Cell Biol. 145, 1461–1469.
20. Potapova, O., Gorospe, M., Rost, F., Dean, N. M., Gaarde, W. A., Mercola, D., and Holbrook, N. J. (2000) J. Biol. Chem. 275, 24767–24775.
21. Milde-Langsosch, K., Roder, H., Andritzký, B., Aslan, B., Hemminger, G., Brinkmann, A., Bamberger, C. M., Loning, T., and Bamberger, A. M. (2004) Breast Cancer Res. Treat. 86, 139–152.
22. Daubeuf, S., Duvill, A., Wellman-Rousseau, M., Diederich, M., and Visvikis, A. (2004) Biochem. Biophys. Res. Commun. 313, 300–307.
23. Marks-Konczelik, J., Chu, S. C., and Moss, J. (1998) J. Biol. Chem. 273, 22201–22208.
24. Mulcaly, R. T., Wartman, M. A., Bailey, H. H., and Gipp, J. J. (1997) J. Biol. Chem. 272, 7445–7454.
25. Bianchi, L., Tacchini, L., Matteucci, E., and Desiderio, M. A. (2002) Arch. Biochem. Biophys. 401, 115–123.
26. Deng, T., and Karin, M. (1994) Nature 371, 171–175.
27. Han, T. H., and Prywes, R. (1995) Mol. Cell. Biol. 15, 2907–2915.
28. Hill, C. S., Wynne, J., and Treisman, R. (1995) Cell 81, 1159–1170.
29. Pognonec, P., Boulukos, K. E., Aperlo, C., Fujimoto, M., Ariga, H., Nomoto, A., and Kato, H. (1997) Oncogene 14, 2091–2098.
30. Van Dam, H., and Castellazzi, M. (2001) Oncogene 20, 2453–2464.
31. Florin, I., Maas-Szabowski, N., Werner, S., Szabowski, A., and Angel, P. (2005) J. Cell Sci. 118, 1981–1989.
32. Belguike, K., Kersuel, N., Galtier, F., and Chalbos, M. (2005) Oncogene 24, 1434–1444.
33. Hong, S., Park, K. K., Magae, J., Ando, K., Lee, T. S., Kwon, T. K., Kwak, J. Y., Kim, C. H., and Chang, Y. C. (2005) J. Biol. Chem. 280, 25202–25209.
34. Papadimitriou, E., Polytarchou, C., Courty, J., Koolwijk, P., Heroult, M., and Katsoris, P. (2001) Biochem. Biophys. Res. Commun. 282, 306–313.
35. Polytarchou, A., Katsoris, P., Courty, J., and Papadimitriou, E. (2005) J. Biol. Chem. 280, 22454–22461.
36. Lu, K. V., Jong, K. A., Kim, G. Y., Singh, J., Dia, E. Q., Yoshimoto, K., Wang, M. Y., Cloughesy, T. F., Nelson, S. F., and Mischel, P. S. (2005) J. Biol. Chem. 280, 26953–26964.
37. Eferl, R., and Wagner, E. F. (2003) Nat. Rev. Cancer 3, 859–868.