Targeted Knockdown of EGR-1 Inhibits IL-8 Production and IL-8-mediated Invasion of Prostate Cancer Cells through Suppressing EGR-1/NF-κB Synergy*

Received for publication, May 3, 2009, and in revised form, October 8, 2009. Published, JBC Papers in Press, October 16, 2009, DOI 10.1074/jbc.M109.016246

Jiajia Ma, Zijia Ren, Yang Ma, Lu Xu, Ying Zhao, Chaogu Zheng, Yinghui Fang, Ting Xue, Baolin Sun, and Weihua Xiao

From the Hefei National Laboratory for Physical Sciences at Microscale and School of Life Sciences, University of Science and Technology of China, Hefei, Anhui 230027, China

IL-8 produced by prostate cancer cells may be responsible for the androgen-independent growth of advanced prostate cancers. Accumulating evidence from microarray analyses and animal genetic models highlights the central involvement of the transcription factor early growth response-1 (EGR-1) in prostate carcinoma progression. It is unknown, however, whether knockdown of EGR-1 inhibits IL-8 production and IL-8-mediated tumor metastasis. Here we show that EGR-1 knockdown by a specific shRNA-Egr1 inhibited gene transcription and production of IL-8 by the human prostate cancer cell line DU145. Conversely, enforced expression of EGR-1 in EGR-1-lacking PC3 prostate cancer cells markedly enhanced IL-8 transcription and secretion. By using wild type and a series of mutant IL-8 promoter luciferase constructs, we found that the NF-κB binding site is important for EGR-1 regulation of IL-8. Furthermore, silencing EGR-1 suppressed a synergistically functional interaction between EGR-1 and NF-κB. Consequently, knockdown of EGR-1 inhibited IL-8-mediated tumor colony formation and invasion. Thus, targeted knockdown of EGR-1 could be an effective therapeutic approach against prostate cancer.

Prostate cancer is currently the most prevalent noncutaneous cancer in men in the Western world and is the second leading cause of male death from cancer. There is considerable evidence from experimental models and studies conducted on patient samples to support a role for the pro-inflammatory chemokine interleukin 8 (IL-8)2 in the promotion of prostate cancer progression (1, 2). Several studies have now confirmed elevated expression of IL-8 and its associated receptors in prostate cancer (3–6), although these independent studies suggest markedly different distribution patterns for IL-8 and its receptors. By using immunohistochemistry staining, IL-8 expression was detected in glandular epithelial cells of prostate cancer tissue, with little or no IL-8 staining hypertrophy or normal prostate epithelium (7, 8). In contrast, Huang et al., reported that IL-8 was expressed solely by neuroendocrine rather than epithelial cells. Their analysis of benign and malignant prostate tissue cores confirmed an increased IL-8 expression that correlated with progressive disease (9). These studies suggested that there is a consistent trend of increased and concurrent expression of IL-8 and its two receptors in prostate cancer tissue; thus, indicating that prostate cancer cells are subject to a continuous autocrine/paracrine stimulus. In addition, several studies have reported the detection of increased IL-8 levels in the serum of patients with either localized or metastatic prostate cancer relative to control patients or patients with benign prostatic hypertrophy (10, 11). It is evident that future research providing a more comprehensive understanding of the transcriptional, translational, and post-translational signaling basis for IL-8-promoted cell motility and cell invasion will be required to identify viable and effective therapeutic strategies to attenuate the disease-progressing effects of IL-8.

EGR-1 expression results in either promotion or regression of cell proliferation, depending on the cellular context (12–14). Accumulating evidence indicates that EGR-1 plays a significant part in the development and progression of prostate cancer (15–21). There is a direct correlation between the level of EGR-1 expression and the prostate tumor Gleason grade (19). In addition, loss of the EGR-1 corepressor NAB2 enhances the EGR-1 levels in prostate cancer. Although there is no EGR-1 binding motif in the promoter of the interleukin 8 gene, EGR-1-associated IL-8 production was investigated in combination with three central transcription factors such as NF-κB, C/EBP, or AP-1 (22). The expression of EGR-1 can be induced by a range of stimuli, which in many cases overlap with those known to be capable of inducing NF-κB expression. NF-κB and EGR-1 have been shown to cooperatively stimulate the NF-κB promoter (23) despite a report that EGR-1 specifically represses NF-κB transcriptional activity in a regulatory way by preventing its interaction with promoter target elements (24). In addition, it has been reported that EGR-1 regulates proinflammatory cytokine gene expression by synergistic interaction with other transcription factors such as nuclear factors of activated T cells (NFAT) (25, 26). AP-1 is also positive downstream of
EGR-1 Knockdown Inhibits IL-8 in Prostate Cancer

EGR-1 in a cooperative way (27). It is unknown, however, whether knockdown of EGR-1 inhibits IL-8 production and IL-8-mediated tumor metastasis.

The proinflammatory chemokine IL-8 is undetectable in androgen-responsive prostate cancer cells (e.g. LNCaP and LAPC-4), but it is highly expressed in androgen-independent metastatic prostate cancer tissue (9) and cell lines such as DU145. Therefore, IL-8 is associated with chemoresistance, tumor growth, and angiogenesis in androgen-independent prostate carcinoma (28–31). In the present study, we used DU145/sh-EGR1 and DU145/sh-Control stable cell lines to evaluate the effect of EGR-1 knockdown on IL-8 transcription and production by human prostate cancer cells. Furthermore, it appears that EGR-1 functions cooperatively with NF-κB to stimulate IL-8 transcription and production. Our data demonstrate knockdown of EGR-1 inhibited tumor colony formation and invasion may be through the down-regulation of IL-8. Thus, targeted knockdown of EGR-1 could be an effective therapeutic approach against prostate cancer.

EXPERIMENTAL PROCEDURES

Reagent and Antibodies—All reagents and chemicals and affinity-purified anti-rabbit IgG and anti-mouse IgG coupled to horseradish peroxidase or fluorescent tags were purchased from Sigma. The following specific antibodies were used in this study: anti-NF-κB p65 and p50 (Upstate Biotech); anti-EGR-1, anti-actin (Santa Cruz Biotechnology), The IL-8 ELISA kit was purchased from R&D Systems. QCMTM 24-Well Cell Invasion Assay kit was purchased from Chemicon Corp.

shRNA Expression, siRNA Duplex, and Reporter Gene Constructs—The empty pU6 + 27, shRNA-control and shRNA-p65 vectors were purchased from Panomics Corp. (Fremont, CA). A 21-nucleotide sequence coding for amino acids 413–419 of human Egr-1 was selected as the targets for RNAi according to the manufacturer’s instructions. In all cases, the corresponding sequences were scrambled to generate a control vector. In initial studies, we used empty vector (pU6 + 27) and scrambled shRNA as controls. Double-strand siRNAs targeting NF-κB (p65) and scramble control were synthesized using the following sequences: siRNA-p65: forward 5’-GAU UGA GGA GAA ACG UAA AdTdT, reverse 5’-UUU ACG UUU CUC CUC AAU CdTdT; siRNA-scramble: forward 5’-AUG AAC GUG AAU UGC UCA AdTdT, Reverse 5’-UUG AGC AAU UCA CGU UCA UdTdT. siRNA duplexes were purchased from Invitrogen.

To construct the IL-8 promoter luciferase reporter, a 350-bp DNA fragment from the promoter region of the human IL-8 gene, including −323 to +27 nt upstream of the start ATG, was obtained by PCR using human genomic DNA as the template and primer pair IL-8-pf: 5’- CGA CCC GTC ACC TGC CAC TCT AGT ACT A-3’ and IL-8-pr: 5’- CGG CTC GAG AAG CTT GTG TGC TCT GCT G-3’, where the restriction enzyme sites for cloning are indicated by underlines. The PCR products were inserted into the pGL3-basic vector (Promega, Madison, MI) and designated as pGL3-IL-8-wt. The AP-1, CEBP, and NF-κB binding sites were each mutated using site-directed mutagenesis as indicated in Fig. 3A. All constructs were verified by sequencing. The Egr-1 expression plasmids containing a full-length cDNA coding for human EGR-1 were generous gifts from Dr. Jie Du, Baylor College of Medicine, Houston, TX.

Cell Culture and Transfection—Human prostate cancer cell lines, DU145 and PC3, were purchased from ATCC and cultured at 37 °C in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 2 mM L-glutamine. Lipofectamine-2000 (Invitrogen) was employed for transfection. The stably transfected cell lines were obtained after being selectively screened by G418 (800 µg/ml, Gibco) for 3–4 weeks.

RT-PCR and Real-time PCR—Total RNA was isolated by TRIzol (Invitrogen) according to the manufacturer’s instructions, and 1 µg was converted to cDNA using the Superscript III reverse transcriptase (Invitrogen). The following primer sets were used for RT-PCR and real-time PCR with Cyber-Green incorporation: hullL-8-RT: Re: TGG TGG CCG ATG GTG GTC CA, huLL-8-RT: Fw: AAG CTG GCC GTG GTC CTC TT; β-ActIN-RT: GTC ACA CTT CAT GAT GGA GTT GAA GG, β-ActIN-Fw: GAC TGT ACT GAC TAC CTG ATG AAT AT; Egr-1-RT: TGG GTG CCG CTG AGT AAA TG, Egr-1-Fw: CTG ACC GCA GAG TCT TTT CCT G.

Immunoblot Analyses—For preparing the whole cell extract, cells were harvested and lysed in the Nonidet P-40 cell lysis buffer containing 50 mM Tris-Cl, pH 7.4, 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, and 1 mM pepstatin (Sigma), 1 µg/ml leupeptin (Sigma), 1 µg/ml pepstatin (Sigma), and 1 µg/ml aprotinin (Sigma). Lysates were subjected to immunoblot analysis. Horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence detection (Pierce Chemical Co.) was used to detect specific immunoreactive proteins.

Immunoprecipitation—Cellular lysates were prepared using a radioimmune precipitation assay buffer (50 mM Tris-Cl, pH 7.4, 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 µg/ml leupeptin (Sigma), 1 µg/ml pepstatin (Sigma), and 1 µg/ml aprotinin (Sigma). Lysates were subjected to immunoprecipitation analysis. Horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence detection (Pierce Chemical Co.) was used to detect specific immunoreactive proteins.

Chromatin Immunoprecipitation Assay (ChIP)—Cells were seeded 24 h prior to fixation with 1% formaldehyde at 37 °C for 7 min. The cells were harvested in lysis buffer (50 mM Tris-Cl, pH 8.1, 10 mM EDTA, 1% SDS) and sonicated to shear the chromatin (~500 bp). The soluble fraction was collected by centrifugation and incubated with specific antibodies or control IgG at 4 °C overnight. The immune complexes were captured by incubation with protein A-agarose at 4 °C for 2 h, and the immunoprecipitated proteins were subjected to Western blotting with specific antibodies.
EGR-1 Knockdown Inhibits IL-8 in Prostate Cancer

FIGURE 1. The expression of IL-8 is correlated with the level of EGR-1 in prostate cancer cells. A, B, and C, whole cell lysates or total RNA were prepared from DU145 cells stably transfected with either the control vector or shRNA targeting EGR-1 (designated as DU145/sh-Con and DU145/sh-EGR-1, respectively). The expression of EGR-1 was monitored by Western blot (A), RT-PCR, and real-time PCR, whereas the level of IL-8 transcripts in the same sample was measured by RT-PCR (B) and real-time PCR (C). D, accumulated secretion of IL-8 was assayed by ELISA with the cell culture supernatants collected after a period of culture of 48 h. E and F, transcript levels of EGR-1 and IL-8 in PC3 cells that were either stably transfected with pcDNA3 control vector or EGR-1 expression plasmid (designated as PC3/pc-Con and PC3/pc-EGR1, respectively) were monitored by RT-PCR (E), whereas the accumulated production of IL-8 was assayed by ELISA (F).

RESULTS

Knockdown of EGR-1 Decreases Transcription and Secretion of IL-8 by Prostate Cancer Cells—To determine whether EGR-1 regulates transcription and secretion of IL-8 by prostate cancer cells, DU145 cells were transfected with shRNA-EGR1 plasmids or control plasmids to generate the DU145/sh-EGR-1 and DU145/sh-Con stable cell lines, respectively. The level of EGR-1 expression in both cell lines was analyzed by Western blotting, RT-PCR, and real-time PCR. As shown in Fig. 1A, a significant reduction of EGR-1 expression was observed, confirming the knockdown of Egr-1 in DU145/sh-EGR-1 stable prostate cancer cells. RT-PCR (Fig. 1B) and real-time PCR (Fig. 1C) showed that the level of transcripts of IL-8 was reduced in the DU145 cell with EGR1-knockdown compared with the control cells. Furthermore, IL-8 secretion by the above cell lines was also measured by ELISA. Knockdown of EGR-1 resulted in a significant decrease of IL-8 production (Fig. 1D).

Because PC3, another prostate cancer cell line, expresses little detectable EGR-1 mRNA, we used PC3 cells as a model system to directly assess the actual role of EGR-1 expression in the EGR-1-mediated IL-8 production by prostate cancer cells. The PC3 cells were transfected with plasmids containing either cDNA coding for full-length human EGR-1 wild type or empty vector pcDNA3.1. Compared with the control PC3 cells, the level of IL-8 transcription and secretion was significantly higher in the EGR1-overexpressing cells (Fig. 1, E and F). These results suggested that expression and secretion of IL-8 by prostate cancer cells are correlated with the level of EGR-1.

Importance of NF-κB Binding Site for EGR-1 Regulation of IL-8—Although there is no EGR-1 binding motif in the promoter of the interleukin 8 gene, EGR-1-associated IL-8 production may be regulated by other transcription factors such as...
NF-κB, C/EBP, or AP-1 because the IL-8 promoter contains these binding sites. We used wild type and a series of mutant IL-8 promoter luciferase constructs to assess whether EGR-1 affects activation of IL-8 promoter mediated by the above transcription factor binding sites. DU145/sh-Con and DU145/sh-EGR-1 cells were transiently transfected with the −323 bp IL-8 promoter construct and mutation constructs each containing AP-1 (−126 to −120), C/EBP (−94 to −81), or NF-κB site mutations (−80 to −69) (Fig. 2A) for 48 h and assayed for luciferase activity. As shown in Fig. 2B, compared with the control DU145 cells, knockdown of EGR-1 resulted in a 60% loss in IL-8 promoter luciferase activity of transfected cells with a wild-type construct, confirming that EGR-1 regulates IL-8 promoter activity in prostate cancer cells. When cells were transfected with an IL-8 promoter construct containing a single C/EBP site mutant (−94 to −81) or AP-1 site mutant (−126 to −120), IL-8 promoter activation was affected by knockdown of EGR-1. However, in the case of the transfected cells with the construct with a single mutation of the NF-κB site, there was a lower level of IL-8 promoter luciferase activity than that of the wild-type construct. In addition, no difference in transactivation of the IL-8 promoter containing an NF-κB binding site mutation between DU145/sh-EGR-1 and control cells was observed, demonstrating that EGR-1-regulating transactivation of IL-8 promoter is associated with NF-κB.

Furthermore, similar experiments were performed on EGR-1-overexpressing PC3 cells and their parent cells (Fig. 2C). Overexpression of EGR-1 in PC3 prostate cancer cells markedly enhanced IL-8 promoter activity, but did not affect activity of IL-8 promoter with a single NF-κB site mutation. These data indicate that the NF-κB site is the most important positive regulatory element and the target site for EGR-1 activation of IL-8 promoter transactivation in prostate cancer cells.

The EGR1-NF-κB Complex Binding to the IL-8 Promoter—We used ChIP to directly assess whether EGR-1 binds to the NF-κB binding site of IL-8 promoter. Stably transfected DU145/sh-con and DU145/sh-EGR-1 cells were harvested for the preparation of chromatin, which was immunoprecipitated with anti-EGR-1, -NF-κB p65, and -NF-κB p50 antibodies or normal IgG. Genomic fragments associated with immunoprecipitated DNA were amplified by PCR using specific primers flanking the NF-κB binding site within the IL-8 promoter (Fig. 3A, left panel). Not only did NF-κB bind to its specific binding site in the IL-8 promoter, but also the occupancy by EGR-1 of the NF-κB site was detected. Transfection with sh-Egr1 resulted in the decrease in such EGR-1 occupancy.
firming double knockdown of NF-κB p65 and EGR-1. IL-8 secretion by the same cells was measured by ELISA (Fig. 4C). Comparing the DU145 cells transfected with either sh-Egr1 or si-p65 alone, double knockdown of EGR-1 and NF-κB p65 resulted in a remarkable loss of IL-8 production. This suggested that EGR-1 functions cooperatively with NF-κB to stimulate IL-8 transcription and production. Silencing EGR-1 suppressed a synergistically functional interaction between EGR-1 and NF-κB.

**Knockdown of EGR-1 Inhibited IL-8-mediated Tumor Colony Formation and Invasion**—Because IL-8 has been reported to be involved in tumor colony formation and invasion, we investigated whether EGR-1 might further regulate IL-8-mediated tumor colony formation and invasion. Stably transfected DU145/sh-EGR-1 and DU145/sh-Con cells were transiently transfected with either siRNA-IL-8 or siRNA-Scramble. A human tumor colony-forming assay (Fig. 5, A and B) and in vitro chemoinvasion assay (Fig. 5C) were performed with the cells under different conditions. When the level of IL-8 in DU145 cells was reduced by transfection with siRNA-IL-8, both the tumor colony number and invasion activity of DU145 cells were significantly dropped off, confirming that IL-8 stimulates tumor colony formation and invasion of prostate cancer cells. In DU145 cells with knockdown of EGR-1, the number of tumor colonies and invasion of cells through the extracellular matrix were significantly decreased compared with the control DU145 cells (Fig. 5, A, B, and C, respectively). Silencing IL-8 also attenuated EGR-1 knockdown-caused inhibition of the tumor colony number and invasion activity on DU145 cells. The data indicated that knockdown of EGR-1 inhibited tumor colony formation and invasion that might be through down-regulation of IL-8.

Because other growth factors in addition to IL-8 may also participate in tumor invasion, we further examined the gene expression profile in EGR-1 knockdown prostate cells. In DU145/sh-EGR1 cells, the expression levels of EGF, PGDF-α, and IGF2 were significantly less than that in control DU145 cells (Fig. 5D). However, the expression of ICAM-1 and VEGF was not affected by EGR-1 knockdown. Therefore, besides IL-8, the role of other growth factors in tumor invasion needs to be further investigated.
DISCUSSION

The expression of IL-8 has been shown to correlate with the angiogenesis, tumorigenicity, and metastatic potential of prostate cancer cells. Inhibiting these pronounced effects of IL-8 signaling within the tumor microenvironment may have significant therapeutic potential in modulating disease progression (1, 31). This study revealed that EGR-1 knockdown resulted in significant reduction in the expression of IL-8 gene transcription and protein production. Small interfering RNA-mediated knockdown of EGR-1 in DU145 cells dramatically decreased IL-8-mediated tumor forming and invasion. Because the majority of clinical studies confirm overexpression of IL-8 in the most advanced stages of disease, this suggests that suppressing the effects of IL-8 may have important implications for the systemic treatment of aggressive and metastatic disease.

The present data, obtained from both knockdown and overexpression of EGR-1 experiments, demonstrate that EGR-1 suppresses transactivation of IL-8 via downregulation of the IL-8 promoter in an EGR-1-dependent manner. Interfering of EGR-1 expression repressed IL-8 induction as well as EGR-1 production. Although there is no EGR-1 binding motif in the promoter of the interleukin-8 gene, EGR-1-associated IL-8 production may be regulated by other transcription factors in an indirect way. The transcriptional regulation of the IL-8 gene has been analyzed extensively at the level of the IL-8 promoter. Cis-acting elements for several transcription factors have been identified within this regulatory region (22). The factors that bind to these motifs include C/EBP, AP-1, and NF-κB. By using wild type and a series of mutant IL-8 promoter luciferase constructs, we have found that the NF-κB binding site is an important positive regulatory element for EGR-1 regulation of IL-8 (Fig. 2). It is also worthy of note that deletion of the κB site seems to be too destructive to the promoter with or without EGR-1 knockdown. By using ChIP assays, we have found the decreased binding of EGR-1 to the IL-8 promoter after treatment with shRNA-Egr-1 (Fig. 3A). Such molecular interplay between EGR-1 and NF-κB in the regulation of IL-8 is dependent on p65. We further show that EGR-1 interacts with NF-κB forming an NF-κB/EGR-1 complex, which was consistent with Cogswell et al. (23) observations in T cell activation. Moreover, IL-8 transcription (mediated synergistically by the NF-κB and EGR-1 transcription factors) was significantly reduced by knockdown of EGR-1 in prostate carcinoma cells.

Taken together, these data provided the evidence that targeting knockdown of EGR-1 resulted in the reduction of IL-8 production and IL-8-mediated tumor colony formation and invasion of prostate cancer cells through suppression of a synergistically functional interaction between EGR-1 and NF-κB. Such a model could highlight the importance of transcriptional cross-talk in IL-8-mediated prostate cancer growth and progression. EGR-1 may be the therapeutic molecular target in androgen-independent prostate cancer cells by targeting IL-8 gene expression.

FIGURE 5. Knockdown of Egr-1 inhibited IL-8-mediated tumor colony formation and invasion. A and B, tumor colony formation was performed with DU145/si-con and DU145/si-Egr1 cells that were further transfected with siRNA silencing IL-8 or scramble RNA. The cells were plated in a 6-well plate (1 x 10^4 cells/well) and cultured for 14 days. Cell colonies were visualized under a microscope (A), and the number was counted and presented as number/cm^2 (B). C, for the chemoinvasion assay, the transfected cells were plated onto Matrigel-coated wells and cultured for 48 h. By the end, the cells in the lower chamber of the transwell were collected and measured by CyQUANT®EGR Dye (QCM™ 24-Well Cell Invasion Assay kit) per the manufacturer’s instruction. The data are presented as relative luminescence units and the mean of three samples in each experiment. D, gene expression profile of other growth factors and invasion proteins measured by RT-PCR.

EGR-1 Knockdown Inhibits IL-8 in Prostate Cancer
REFERENCES

1. Moore, B. B., Arenberg, D. A., Stoy, K., Morgan, T., Addison, C. L., Morris, S. B., Glass, M., Wilke, C., Xue, Y. Y., Sitterding, S., Kunkel, S. L., Burdick, M. D., and Strieter, R. M. (1999) *Am. J. Pathol.* **154**, 1503–1512

2. Xie, K. P. (2001) *Cytokine Growth Factor Rev.* **12**, 375–391

3. Waugh, D. J., Wilson, C., Seaton, A., and Maxwell, P. J. (2008) *Clin. Cancer Res.* **14**, 6735–6741

4. Uehara, H., Troncoso, P., Johnston, D., Bucana, C. D., Dinney, C., Dong, Z., Fidler, I. J., and Pettaway, C. A. (2005) *Prostate* **64**, 40–49

5. Ferrer, F. A., Miller, L. J., Andrawis, R. I., Kurtzman, S. H., Albertsen, P. C., Laudone, V. P., and Kreutzer, D. L. (1998) *Urology* **51**, 161–167

6. Murphy, C., McGurk, M., Pettigrew, J., Santinelli, A., Mazzucchelli, R., Johnston, P. G., Montironi, R., and Waugh, D. J. (2005) *Clin. Cancer Res.* **11**, 4117–4127

7. Huang, J., Yao, J. L., Zhang, L., Bourne, P. A., Quinn, A. M., di Sant'Agnese, P. A., and Reeder, J. E. (2005) *Am. J. Pathol.* **166**, 1807–1815

8. Veltri, R. W., Miller, M. C., Zhao, G., Ng, A., Marley, G., Jr., Vessella, R. L., and Ralph, D. (1999) *Urology* **53**, 139–147

9. Lehrer, S., Diamond, E. J., Mamkine, B., Stone, N. N., and Stock, R. G. (2004) *Technol. Cancer Res. Treat.* **3**, 411–411

10. Sukhatme, V. P., Cao, X. M., Chang, L. C., Tsai-Morris, C. H., Stamenkovich, D., Ferreira, P. C., Cohen, D. R., Edwards, S. A., Shows, T. B., Le Beau, M. M., and Adamson, E. D. (1988) *Cell* **53**, 37–43

11. Thiel, G., and Cibelli, G. (2002) *J. Cell. Physiol.* **193**, 287–292

12. Salah, Z., Maoz, M., Pizov, G., and Bar-Shavit, R. (2007) *Cancer Res.* **67**, 9835–9843

13. Xiao, D., Chinnappan, D., Pestell, R., Albanese, C., and Weber, H. C. (2005) *Cancer Res.* **65**, 9934–9942

14. Moris, G. R., Olivier, K. R., Mitchell, R. F., Jr, Jenkins, R. B., and Tindall, D. J. (2005) *Prostate* **63**, 198–207

15. Eid, M. A., Wilson, C., Seaton, A., and Maxwell, P. J. (2008) *Front. Biosci.* **13**, 4595–4604

16. Lentsch, A. B. (2006) *Future Oncol.* **13**, 4595–4604

17. Thiel, G., and Cibelli, G. (2002) *J. Cell. Physiol.* **193**, 287–292

18. Veltri, R. W., Miller, M. C., Zhao, G., Ng, A., Marley, G., Jr., Vessella, R. L., and Ralph, D. (1999) *Urology* **53**, 139–147

19. Ahmed, M. M., Chendil, D., Lele, S., Venkatasubbarao, K., Dey, S., Ritter, M., Rowland, R. G., and Mohiuddin, M. (2001) *Am. J. Clin. Oncol.* **24**, 500–505

20. Baran, V., Duss, S., Rhim, J., and Mercola, D. (2003) *Ann. N.Y. Acad. Sci.* **1002**, 197–216

21. Thigpen, A. E., Cala, K. M., Guileyardo, J. M., Molberg, K. H., McConnell, J. D., and Russell, D. W. (1996) *J. Urol.* **155**, 975–981

22. Moon, Y., Yang, H., and Lee, S. H. (2007) *Biochem. Biophys. Res. Commun.* **362**, 256–262

23. Cogswell, P. C., Mayo, M. W., and Baldwin, A. S., Jr. (1997) *J. Exp. Med.* **185**, 491–497

24. Chapman, N. R., and Perkins, N. D. (2000) *J. Biol. Chem.* **275**, 4719–4725

25. Decker, E. L., Skerka, C., and Zipfel, P. F. (1998) *J. Biol. Chem.* **273**, 26923–26930

26. Decker, E. L., Gehmann, N., Kampen, E., Eibel, H., Zipfel, P. F., and Skerka, C. (2003) *Nucleic Acids Res.* **31**, 911–921

27. Uzzo, R. G., Crispin, P. L., Golovine, K., Makhov, P., Horwitz, E. M., and Kolenko, V. M. (2006) *Carcinogenesis* **27**, 1890–1900

28. Araki, S., Omori, Y., Lym, D., Singh, R. K., Meinbach, D. M., Sandman, Y., Lokeshwar, V. B., and Lokeshwar, B. L. (2007) *Cancer Res.* **67**, 6854–6862

29. Seaton, A., Scullin, P., Maxwell, P. J., Wilson, C., Pettigrew, J., Gallagher, R., O’Sullivan, J. M., Johnston, P. G., and Waugh, D. J. (2008) *Carcinogenesis* **29**, 1148–1156

30. MacManus, C. F., Pettigrew, J., Seaton, A., Wilson, C., Maxwell, P. J., Berlingeri, S., Purcell, C., McGurk, M., Johnston, P. G., and Waugh, D. J. (2007) *Mol. Cancer Res.* **5**, 737–748

31. Inoue, K., Slaton, J. W., Eve, B. Y., Kim, S. J., Perrotte, P., Balbav, M. D., Yano, S., Bar-Eli, M., Radinsky, R., Pettaway, C. A., and Dinney, C. P. N. (2000) *Clin. Cancer Res.* **6**, 2104–2119

32. Peters, A. H., Kubicek, S., Mechtler, K., O’Sullivan, R. J., Derijck, A. A., Perez-Burgos, L., Kohlmaier, A., Opravil, S., Tachibana, M., Shinkai, Y., Martens, J. H., and Jenuwein, T. (2003) *Mol. Cell.* **12**, 1577–1589

33. Martens, J. H., O’Sullivan, R. J., Braunschweig, U., Opravil, S., Radolf, M., Steinlein, P., and Jenuwein, T. (2005) *EMBO J.* **24**, 800–812

EGR-1 Knockdown Inhibits IL-8 in Prostate Cancer