Prostaglandin E$_2$ Regulates the Nuclear Receptor NR4A2 in Colorectal Cancer*

Received for publication, July 18, 2005, and in revised form, October 24, 2005. Published, JBC Papers in Press, November 17, 2005, DOI 10.1074/jbc.M507152200

Vijaykumar R. Holla‡, Jason R. Mann†, Qiong Shi†, and Raymond N. DuBois§*†

From the Departments of ‡Medicine and †Cancer Biology, Vanderbilt University Medical Center and the §Vanderbilt-Ingram Cancer Center, Nashville, Tennessee 37232-6838

Many lines of research implicate cyclooxygenase 2-derived prostaglandins in tumor growth and metastasis. More specifically, we have shown that prostaglandin E$_2$ (PGE$_2$) promotes cell proliferation and invasion through transactivation of the epidermal growth factor receptor, initiates immune evasion through induction of decay accelerating factor, and transactivates peroxisome proliferator-activated receptor $\delta$, leading to increased polypl size and multiplicity. We continue to identify novel PGE$_2$ target genes in colorectal carcinoma cells and report here that an immediate early gene, nuclear receptor $\alpha_2$ (NR4A2, Nurr1), is induced by PGE$_2$ that in turn regulates cell death. Originally described as a critical dopaminergic neuron growth factor receptor, NR4A2 expression is rapidly but transiently induced by PGE$_2$ in a CAMP/protein kinase A-dependent manner. NR4A2 binds to the cognate NBRE response element and enhances transcription of a reporter construct in colorectal carcinoma cells. Furthermore, NR4A2 expression is elevated in Apc$^{-/-}$ mouse adenomas and its levels were further increased following PGE$_2$ treatment. Human colorectal cancers relative to matched normal mucosa showed increased NR4A2 expression. Although not previously described in epithelial tissues, NR4A2 protein localizes to proliferating crypts of Apc$^{-/-}$ mouse intestine. Finally, functional studies reveal that PGE$_2$-mediated protection from apoptosis is completely inhibited by a dominant-negative NR4A2 construct. Building on previous reports from our group on the peroxisome proliferator-activated receptor family of nuclear receptors, these most recent data suggest that NR4A2, a member of another family of nuclear receptors can stimulate progression of colorectal cancer downstream from cyclooxygenase 2-derived PGE$_2$.

Numerous reports demonstrate increased cyclooxygenase (COX)-2 at sites of inflammation (1, 2) and in a variety of human malignancies, including colorectal cancer (3–9). In addition, high COX-2 expression correlates with poor clinical outcomes, and inhibition of its activity by non-steroidal anti-inflammatory drugs reduces colorectal cancer risk (10–13). High levels of COX-2-derived prostaglandin E$_2$ (PGE$_2$) leads to reduced programmed cell death (14), compromised immune surveillance (15–17), and stimulation of cell migration, proliferation, and angiogenesis (18).

PGE$_2$, the most abundant prostaglandin in colorectal cancer (19), can be generated from arachidonic acid by either COX-1 or COX-2. PGE$_2$ signals by binding to four distinct G protein-coupled cell surface receptors (EP1–EP4): leading to increased calcium flux (EP1), increased (EP2 and -4) or decreased (EP3) cAMP levels all of which modulate downstream networks (20). Our laboratory is actively investigating precisely which genes are regulated by PGE$_2$ in colorectal cancer and during embryogenesis (21). We now show that expression and activity of NR4A2, a nuclear receptor superfamly member, are induced in colorectal carcinoma cells by PGE$_2$.

Transcription factors in the nuclear receptor superfamily regulate gene expression upon ligand binding (22, 23). Well known steroid receptors including progesterins, estrogens, androgens, glucocorticoids, and mineralocorticoids comprise the type I group. Conversely, receptors of thyroid hormone, all-trans-retinoic acid, 9-cis-retinoic acid, and vitamin D$_3$ (VDR) belong to the type II group. The orphan receptors comprise a third class for which physiologic ligands have yet to be identified. The type III group includes, among others, peroxisome proliferator-activated receptors and the NR4A family of receptors.

The NR4A family includes three members: Nur77 (NGIF-B/NR4A1), Nurr1 (NOT/NR4A2), and Nor-1 (MINOR/NR4A3). NR4A can trans-activate target genes through monomer binding of a consensus NBRE sequence (AAAGGTCA) or homodimer binding of the palindromic NurRE sequence (AAAT/G/A/C/T/CA) (24–26). NR4A1 and NR4A2 have also been shown to heterodimerize with 9-cis-retinoic acid receptor (RXR) through DR5 elements in mediating retinoid signaling (27, 28). Crystal structure and NMR data indicate that NR4A2 can function as a ligand-independent transcription factor because the putative ligand binding domain is occupied by several bulky hydrophobic side chains (29, 30).

Based on the expression pattern in the brain, NR4A family members have been strongly implicated in Parkinson disease (31), schizophrenia (32), manic depression (33), and Alzheimer disease (34). NR4A2 is important for dopaminergic neuron function via regulation of tyrosine hydroxylase expression (35). Nur77$^{-/-}$ mice lack mesencephalic dopaminergic neurons, which are known to degenerate in Parkinson disease (36, 37). Preliminary reports suggest a role for this family of receptors in rheumatoid arthritis and cancer through modulation of apoptosis (38). Other functions associated with NR4A2 include regulation of osteocalcin in osteoblasts (39, 40), aldosterone synthase in adrenal cortex (41), and aromatase in ovarian granulosa cells (42). With regard to aromatase, our results may help explain another mechanism by which PGE$_2$ regulates its expression in certain contexts.

We seek a more complete understanding of the role of prostaglandins and downstream targets in epithelial biology, developmental biology, and colorectal carcinogenesis. We have identified key genes that are regulated by PGE$_2$ in colorectal carcinoma cells. Here we present data suggesting that the nuclear receptor NR4A2 is regulated by PGE$_2$ in colorectal cancer. This is the first demonstration that prostaglandins...
regulate this family of transcription factors in neoplastic cells. Ultimately, this novel observation may shed light on the precise role of PGE₂ in colorectal carcinogenesis.

MATERIALS AND METHODS

Reagents—PGE₂ was obtained from Cayman Chemical (Ann Arbor, MI). LY294002 and H-89 were purchased from Calbiochem. Antibodies to NR4A were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) (catalog number SC-990) and R&D Systems (Minneapolis, MN) (catalog number AF2156). β-Actin antiserum was obtained from Sigma.

Cell Culture—LS-174T, HT-29, LoVo, HCT-15, HCT-116, and SW480 cells were purchased from the ATCC (Manassas, VA) and HCA-7 cells were a generous gift from Susan Kirkland. These cells were maintained in McCoy's 5A medium containing 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin in a 5% CO₂ atmosphere.

Northern Blotting—Total cellular RNA was isolated from cells by TRI Reagent (Molecular Research Center, Cincinnati, OH) following the manufacturer’s protocol. Five μg of total RNA was fractionated with a MOPS/formaldehyde-agarose gel and transferred to Hybond N1 membrane (Amersham Biosciences). Following UV cross-linking, the blots were pre-hybridized for 30 min at 42 °C in Hybriol I (Intergen Company, Purchase, NY), hybridized using 32P-labeled cDNA in the same buffer at 42 °C, and subjected to autoradiography. The 0.35-kb NR4A2 (NM_006186) probe was amplified by reverse transcripase-PCR using primers 5'-CAGAGGTTGCAATGCGTTCCG-3' (sense: 1746–1765) and 5'-TCAATTATGTGGCCCGTG-3' (antisense: 2089–2105).

Quantitative Real-time PCR—cDNA for each RNA sample was synthesized in 20-μl reactions using the SuperScript First Strand synthesis system for reverse transcripase-PCR (Invitrogen) following the manufacturer’s protocol. Primers for PCR were designed using Primer3 software. The real-time PCR contained iQ SYBR Green supermix (Bio-Rad), 50 ng of each primer, and 5 μl of 1/1000 diluted reverse transcriptase template in a 25-μl reaction volume. Amplification was carried out using the MyiQ Single Color Real-time PCR Detection system (Bio-Rad), with incubation times of 3 min at 95 °C, followed by 50 cycles of 95 °C for 10 s and 63 °C for 30 s. Specificity of the amplification was checked by melt–curve analysis. Relative levels of mRNA expression were calculated according to the ΔΔCt method (BMC Biotechnol). Individual expression values were normalized by comparison with β-actin mRNA expression. Oligonucleotide sequences were used: human NR4A2 forward, 5'-CGAATTCTGGAGGCGCAGGTT-TCTTTAACCAGC-3' and reverse, 5'-GGCGATTTAAGAATCTT-TGCCGCCCTACAGGTGCG-3'. Following amplification of the Nurr1-pCMX construct using Pfu turbo DNA polymerase (Stratagene), the PCR product was digested with DpnI enzyme and transformed into DH5α-competent cells. Each construct was sequence-verified before further evaluation. The C283G mutation disrupts Nurr1 binding to the NBRE (39).

Transient Transfection and Luciferase Assay—For luciferase assays, 1.3 × 10⁵ LS-174T cells were cultured in a 12-well plate 24 h before transfection. The transfection was carried out using Lipofectamine (Invitrogen) in serum-free media containing 100 ng of reporter plasmid and 5 ng of Renilla luciferase reporter plasmid pRL-SV40 as an internal control following the manufacturer’s protocol. This transfection mixture was added to cells and the plates were incubated at 37 °C for 24 h. Prostaglandins and other reagents were added after 24 h and incubated for an additional 24 h. Firefly and Renilla luciferase activities were measured using a dual luciferase assay kit (Promega, Madison, WI) and a Luminometer. Firefly luciferase values were normalized to Renilla values.

Immunohistochemistry—Adult ApoC⁻/⁻ mice were treated with PGE₂ or vehicle for 7 weeks, as previously described (43). Tissue sections from intestine were stained as follows. After CO₂ asphyxiation, the intestine was dissected, washed in phosphate-buffered saline, and fixed in 10% neutral buffered formalin. Paraffin sections (5 μm) were de waxed with xylene and rehydrated; the epitopes were revealed following treatment in a microwave oven. Once endogenous peroxidase activity was quenched, nonspecific immunoglobulins were blocked with normal goat serum (Vector Laboratories, Burlingame, CA) and samples were incubated overnight at 4 °C with goat monoclonal antibody against NR4A2 at a dilution of 1:25 (R&D Systems, Minneapolis, MN). Negative controls received no primary antibody. The Vectastain ABC peroxidase system (Vector Laboratories, Burlingame, CA) was used for immunodetection following the manufacturer’s instructions, and immunolocalization was visualized with the peroxidase substrate 3,3-diaminobenzidine. Samples were counterstained with hematoxylin and mounted. All results were verified by a blinded second independent observer.

Apoptosis Assay—HCT-116 cells (2.5 × 10⁵ cells/well of 6-well plate) were transfected with empty vector or a dominant-negative NR4A2 construct. 24 h after transfection the cells were washed with phosphate-buffered saline and replaced with (10%) or without serum containing different concentrations of PGE₂. FACS analysis was used to measure apoptotic cells using an Annexin V-FITC Apoptosis Detection Kit according to the manufacturer’s instructions (R&D System). Briefly, the cells were harvested, washed, and then incubated with annexin V-FITC and propidium iodide followed by FACS analysis. This method allows the identification of different apoptotic cell populations: early apoptotic cells (annexin V-FITC positive), late apoptotic cells (annexin V-FITC and propidium iodide positive), necrotic cells (propidium iodide positive), and viable cells (both negative).

Human Colorectal Tissue Samples—Human colorectal tumor specimens were obtained from surgical resections with Vanderbilt Internal Review Board approval. For each tumor sample, matched adjacent normal mucosa was collected for comparison. All samples were snap frozen and stored in liquid nitrogen until use. Tissue RNA was processed with TRI Reagent (Molecular Research Center, Cincinnati, OH) as described above.

Statistical Analysis—Each experiment was performed at least three times and data are expressed as the mean ± S.E. Statistical significance
was determined by paired Student’s t test. p values < 0.05 were considered statistically significant.

RESULTS

PGE2 Induces NR4A2 mRNA and Protein Expression in Colorectal Carcinoma Cells—To identify potential target genes for PGE2 in colorectal cancer, we performed microarray analysis on LS-174T cells treated with PGE2 (1 μM) at different time points. PGE2 appears to regulate several transcription factors, including an immediate early gene, NR4A2. In confirmation of the microarray results, Northern blot analysis revealed that PGE2 rapidly induces NR4A2 expression (Fig. 1A). Upon further investigation, PGE2 induces NR4A2 in other colon carcinoma cell lines as well, with the greatest response observed in LS-174T and LoVo cells (Fig. 1B). Because NR4A2 induction is most dramatic in LS-174T cells, subsequent experiments focused on this model system. LS-174T cells were originally isolated from a mucinous adenocarcinoma derived from a well differentiated goblet cell lineage.

Quantitative real-time PCR assays and immunoblotting was utilized to determine the kinetics of NR4A2 induction. NR4A2 mRNA induction is strong but transient, with its levels peaking at 1 h (Fig. 1C). At the protein level, NR4A2 expression peaks at 2 h (Fig. 1D) as expected from the temporal pattern seen with changes in RNA expression. PGE2 induction of NR4A2 is dose-dependent, and the effect is observed at very low levels of PGE2, indicating that a receptor mediated process is likely involved (Fig. 1, E and F).

PGE2-induced NR4A2 Activates NBRE—Like other nuclear receptors, NR4A family members activate target genes through direct interaction with specific promoter-derived cis-response elements. NR4A2 binds the consensus NBRE site (AAAGGTCA) as a monomer, homodimer, or heterodimer, which is found in the promoter region of genes that are regulated by this nuclear receptor. Thus, we determined whether PGE2-induced NR4A2 can bind and activate a 3xNBRE reporter gene construct using transient transfection assays. PGE2

FIGURE 1. PGE2 induces NR4A2 mRNA and protein expression in colorectal cancer cells. A, LS-174T cells were cultured in serum-free media for 48 h prior to PGE2 (1 μM) treatment. Total RNA was isolated following harvest of the cells at the indicated time points. Equal amounts of RNA were loaded and the levels of NR4A2 mRNA were determined by Northern blot. B, cells were cultured in serum-free conditions prior to PGE2 (1 μM) treatment for 4 h. Total RNA was isolated and 5 μg were reverse transcribed to cDNA. NR4A2 RNA levels were determined by quantitative real-time PCR. C, the Northern blot time course data were confirmed by real-time PCR as noted above. D, following the isolation of total cellular protein, equal amounts of protein were separated by SDS-PAGE and visualized with NR4A2 antibody. E, LS-174T cells were cultured in serum-free media for 48 h prior to PGE2 (0.05–10 μM) stimulation; cells were harvested after 4 h. The levels of NR4A2 mRNA were determined by real-time PCR analysis as noted above. F, following the isolation of total cellular protein, equal amounts of protein were separated by SDS-PAGE and visualized with NR4A2 antibody.

FIGURE 2. PGE2-induced NR4A2 activates NBRE. A, LS-174T cells were cultured in 12-well plates and transiently co-transfected with a 3xNBRE-Luc construct and internal standard controls. Transfected cells were treated with PGE2 (1 μM) for 24 h prior to measurement of firefly luciferase activity normalized to Renilla luciferase activity, as described under “Materials and Methods.” B, LS-174T cells were co-transfected with 3xNBRE-Luc construct and internal standard controls. Transfected cells were treated with PGE2 (0.1–10 μM) for 24 h prior to measurement of firefly luciferase activity normalized to Renilla luciferase activity. DMSO, dimethyl sulfoxide.
induction of NR4A2 increased luciferase expression by 5-fold via binding the NBRE sites (Fig. 2A). This experiment evaluated different concentrations of PGE2 and found that activation of NBRE mirrors the increases we observe in NR4A2 mRNA and protein levels (Fig. 2B).

PGE2 Regulates NR4A2 Expression and Activity in a cAMP/Protein Kinase A (PKA)-dependent Manner—Four G protein-coupled receptors mediate PGE2 action on target cells via distinct second messenger pathways (EP1–EP4). Upon ligand binding these receptors activate several downstream signaling cascades including the epidermal growth factor receptor, phosphatidylinositol 3’-kinase/Akt, Src, and PKA. To elucidate the specific mechanism by which PGE2 induces NR4A2 expression, we employed several inhibitors to identify which signaling pathways mediate PGE2 regulation of NR4A2. Induction of NR4A2 RNA and protein by PGE2 is completely blocked by H-89, a selective inhibitor of the cAMP-dependent protein kinase A, PKA (Fig. 3, A and B). These results were further corroborated at a functional level: inhibition of NR4A2 expression by treatment with H-89 also blocks NBRE activation (Fig. 3C). These findings are consistent with the hypothesis that PGE2 induces NR4A2 expression and activity in a cAMP/PKA-dependent manner.

PGE2 Can Block Apoptosis Through NR4A2—Cancer cells must evolve to survive in a hostile environment. These hardy clones can modulate gene expression by blocking pathways that promote programmed cell death. We have shown previously that 24-h serum starvation (24 h) of LS-174T cells consistently induces apoptosis by 4–5-fold (assessed by FACS analysis). Significantly, treatment with PGE2 can rescue cells from undergoing programmed cell death (43). However, the molecular basis for this observation has not been well elucidated. Here we provide the first data that NR4A2 is an important mediator of this “anti-apoptotic” effect. We transfected cells with the dominant-negative NR4A2 construct and then added increasing amounts of PGE2 following serum starvation. As shown in Fig. 4, A and B, cells transfected with dominant-negative NR4A2 were not protected from undergoing apoptosis following PGE2 treatment. These experiments were also replicated with multiple stable clones expressing empty vector or dominant-negative NR4A2 (data not shown).

To further analyze the molecular mechanism by which PGE2-induced NR4A2 protects cells from apoptosis, we analyzed whole cell lysates for caspase-3. Whereas PGE2 blocks caspase-3 cleavage upon serum starvation, introduction of dominant-negative NR4A2 abrogates this effect (Fig. 4C). A recent report (44) also indicated that NR4A2 is involved in cell transformation and apoptosis in cervical cancer cells (HeLa), where the authors employed the use of NR4A2 small interfering RNA to inhibit its expression.

NR4A2 Expression Is Increased in Colorectal Cancer—Increased expression of COX-2 has been associated with poor prognosis in several different types of malignancies, including colorectal cancer (45–47). PGE2 is the most abundant bioactive lipid in this setting (19). Because PGE2 was found to induce NR4A2 expression in cultured cells, we sought to determine whether COX-2 expression and NR4A2 expression correlate in vivo. Analysis of 15 week-old Apc-/- mouse adenos revealed elevated levels of NR4A2 mRNA and protein, whereas intestinal mucosa with a microscopically normal appearance exhibited little expression (Fig. 6A). To our knowledge, this is the first demonstration that NR4A2 is expressed in intestinal epithelia. Consistent with a role in tumorigenesis, NR4A2 is strongly expressed in the proliferative crypt compartment, where expression is localized to the same region as that of Ki67 (Fig. 5).

To further analyze the role of PGE2-induced NR4A2 in vivo, mice were treated with PGE2 of vehicle for 7 weeks and then intestinal tissues were evaluated for NR4A2 expression levels by immunohistochemistry and quantitative real-time PCR. Immunohistochemistry of tissue sections revealed increased NR4A2 expression in the intestine following PGE2 treatment (Fig. 6D). Furthermore, quanti-
tative PCR analysis indicates that PGE$_2$ treatment leads to increased NR4A2 transcript levels in colonic mucosa (Fig. 6C). These data support the hypothesis that NR4A2 is induced by PGE$_2$ in vivo.

Finally, to extend our in vivo studies and assess the clinical relevance of these observations, we examined NR4A2 levels in human colorectal carcinomas. Comparison of 16 paired human colon cancers and matched normal tissue demonstrates increased expression of NR4A2 in malignant tissue relative to normal colonic mucosa (Fig. 6B). These data support the hypothesis that NR4A2 is induced in colorectal cancer, a setting known to involve elevated levels of PGE$_2$.
A wide range of growth factors and cytokines are known to induce NR4A2 gene expression. These include vascular endothelial growth factor, basic fibroblast growth factor, parathyroid hormone, corticotrophin releasing hormone, tumor necrosis factor-α, and interleukin-1β. NR4A2 has been shown to regulate NR4A2 in inflamed synovial tissue as well. The cAMP/PKA signaling pathway is known to regulate NR4A2 expression in osteoblasts. The functional role of NR4A2 receptors in colon cancer has not been examined previously. In this report we demonstrate that PGE2 induces NR4A2 in colon cancer cells and plays a significant role in mediating the anti-apoptotic effects of PGE2.

Future studies will continue to yield greater insight into the effector genes that mediate tumorigenesis downstream of COX-2-derived PGE2. A better understanding of this process may reveal new strategies for the treatment and/or prevention of colorectal cancer. Evasion of apoptosis is a critical requirement for tumor progression. Building on previous reports from our group on the peroxisome proliferator-activated receptor family of nuclear receptors, these most recent data suggest a novel mechanism by which COX-2-derived PGE2 protects carcinoma cells from apoptosis.

Acknowledgments—We gratefully acknowledge Dingzhi Wang and Greg Buchanan for valuable contributions to the animal studies.

REFERENCES

1. Ristimaki, A. (2004) Novartis Found. Symp. 256, 215–221, 221–226, 259–269
2. Thun, M. J., Henley, S. J., and Gansler, T. (2004) Novartis Found. Symp. 256, 6–21, 22–28, 49–52, 266–269
3. Dannenberg, A. J., Lippman, S. M., Mann, J. R., Subbaramaiah, K., and DuBois, R. N. (2002) J. Clin. Oncol. 20, 254–266
4. Turini, M. E., and DuBois, R. N. (2002) Annu. Rev. Med. 53, 35–57
5. Sakamoto, A., Yokoyama, Y., Unemoto, M., Futagami, M., Sakamoto, T., Bing, X., and Mizunuma, H. (2004) Br. J. Cancer 91, 633–638
6. Denkert, C., Winzer, K. J., Muller, B. M., Weichert, W., Pest, S., Kohel, M., Kristiansen, G., Reles, A., Siegert, A., Guski, H., and Hauptmann, S. (2003) Cancer 97, 2978–2987
7. Dubinett, S. M. (2002) Cancer Res. 62, 6706–6711
8. Zweifel, B. S., Davis, T. W., Ornberg, R. L., and Masferrer, J. L. (2002) Cancer Res. 62, 6706–6711
9. Gupta, R. A., and DuBois, R. N. (2001) Nat. Rev. Cancer 1, 11–21
PGE2 Regulates NR4A2 in Colorectal Cancer

10. Zha, S., Yegnasubramanian, V., Nelson, W. G., Isaacs, W. B., and De Marzo, A. M. (2004) Cancer Lett. 215, 1–20
11. Thun, M. J., Namboodiri, M. M., and Heath, C. W., Jr. (1991) N. Engl. J. Med. 325, 1593–1596
12. Dannenberg, A. J., and Subbaramaiah, K. (2003) Cancer Cell 4, 431–436
13. Steinbach, G., Lynch, P. M., Phillips, R. K., Wallace, M. H., Hawk, E., Gordon, G. B., Wakabayashi, N., Saunders, B., Shen, Y., Fujimura, T., Su, L. K., and Levin, B. (2000) N. Engl. J. Med. 342, 1946–1952
14. Sheng, H., Shao, J., Morrow, J. D., Beauchamp, R. D., and DuBois, R. N. (1998) Cancer Res. 58, 362–366
15. Huang, M., Stolina, M., Sharma, S., Mao, J. T., Zhu, L., Miller, P. W., Wollman, J., Herschman, H., and Dubinett, S. M. (1998) Cancer Res. 58, 1208–1216
16. Holla, V. R., Wang, D., Brown, J. R., Mann, J. R., Katkuri, S., and Dubinett, S. M. (2005) J. Biol. Chem. 280, 476–483
17. Sharma, S., Stolina, M., Yang, S. C., Baratelli, F., Lin, J. F., Atianzar, K., Luo, J., Zhu, L., Lin, Y., Huang, M., Dohadwala, M., Batra, R. K., and Dubinett, S. M. (2003) Clin. Cancer Res. 9, 961–968
18. Rozic, J. G., Chakraborty, C., and Lala, P. K. (2001) Int. J. Cancer 93, 497–506
19. Rigas, B., Goldman, I. S., and Levine, L. (1993) J. Lab. Clin. Med. 122, 518–523
20. Breyer, R. M., Bagdassarian, C. K., Myers, S. A., and Breyer, M. D. (2001) Anto. Rev. Pharmacol. Toxicol. 41, 661–690
21. Cha, Y. I., Kim, S. H., Solnica-Krezel, L., and Dubois, R. N. (2005) Dev. Biol. 282, 274–283
22. Gronemeyer, H., and Laudet, V. (1995) Protein Profile 2, 1173–1308
23. Aranda, A., and Pascual, A. (2001) Physiol. Rev. 81, 1269–1304
24. Wilson, T. E., Fahner, T. J., Johnston, M., and Milbrandt, J. (1991) Science 252, 1296–1300
25. Giguere, V. (1999) Endocr. Rev. 20, 689–725
26. Castro, D. S., Hermanson, E., Joseph, B., Wallen, A., Aarnisalo, P., Heller, A., and Perlmann, T. (2001) J. Biol. Chem. 276, 43277–43284
27. Forman, B. M., Umesono, K., Chen, J., and Evans, R. M. (1995) Cell 81, 541–550
28. Perlmann, T., and Jansson, L. (1995) Genes Dev. 9, 769–782
29. Wang, Z., Benoit, G., Liu, J., Prasad, S., Aarnisalo, P., Liu, X., Xu, H., Walker, N. P., and Perlmann, T. (2003) Nature 423, 555–560
30. Codina, A., Benoit, G., Gooch, J. T., Neuhaus, D., Perlmann, T., and Schwabe, J. W. (2004) J. Biol. Chem. 279, 53338–53345
31. Le, W. D., Xu, P., Jankovic, J., Jiang, H., Appel, S. H., Smith, R. G., and Vassilatis, D. K. (2003) Nat. Genet. 33, 85–89
32. Chen, Y. H., Tsai, M. T., Shaw, C. K., and Chen, C. H. (2001) Am. J. Med. Genet. 105, 753–757
33. Buerenich, S., Carmine, A., Arvidsson, M., Xiang, F., Zhang, Z., Sydow, O., Jonsson, E. G., Sedvall, G. C., Leonard, S., Ross, R. G., Freedman, R., Chowdari, K. V., Nimgaonkar, V. L., Perlmann, T., Anvret, M., and Olson, L. (2000) Am. J. Med. Genet. 96, 808–813
34. Newman, S. J., Bond, B., Crook, B., Darker, J., Edge, C., and Maycock, P. R. (2000) Brain Res. 857, 131–140
35. Kim, K. S., Kim, C. H., Hwang, D. Y., Seo, H., Chung, S., Hong, S. J., Lim, J. K., Anderson, T., and Iacson, O. (2003) J. Neurochem. 85, 622–634
36. Zetterstrom, R. H., Solomin, L., Janson, L., Hoffner, B. J., Olson, L., and Perlmann, T. (1997) Science 276, 248–250
37. Wallen, A., Zetterstrom, R. H., Solomin, L., Arvidsson, M., Olson, L., and Perlmann, T. (1999) Exp. Cell Res. 253, 737–746
38. Wu, Q., Liu, S., Ye, X. F., Huang, Z. W., and Su, W. J. (2002) Carcinogenesis 23, 1583–1592
39. Lammi, J., Huppusen, J., and Aarnisalo, P. (2004) Mol. Endocrinol. 18, 1546–1557
40. Pirh, F. Q., Tang, A., Ozturk, I. C., Nervina, J. M., and Tetrakis, S. (2004) J. Biol. Chem. 279, 53167–53174
41. Bassett, M. H., Suzuki, T., Sasano, H., White, P. C., and Rainey, W. E. (2004) Mol. Endocrinol. 18, 279–290
42. Wu, Y., Ghosh, S., Nishi, Y., Yanase, T., Nawata, H., and Hu, Y. (2005) Endocrinology 146, 237–246
43. Wang, D., Wang, H., Shi, Q., Katkuri, S., Walhi, W., Desvergne, B., Das, S. K., Dey, S. K., and DuBois, R. N. (2004) Cancer Cell 6, 285–295
44. Ke, N., Claussen, G., Yu, D. H., Albers, A., Fan, W., Tan, P., Griffman, M., Hu, X., Defile, K., Ngoc, M., Meyhre, D., Brachat, A., Wong-Staal, F., and Li, Q. (2004) Cancer Res. 64, 8208–8212
45. Ladetto, M., Vallet, S., Trojan, A., Dell’Aquila, M., Monitillo, L., Rosato, R., Santo, L., Drandi, D., Bertola, A., Falco, P., Cavallo, F., Ricca, I., De Marco, F., Mantoan, B., Bode-Lesniewska, B., Pagliano, G., Frances, R., Rocc, A., Astolfi, M., Compagn, M., Mariani, S., Godio, L., Marino, L., Ruggeri, M., Ormed, P., Palumbo, A., and Boccardo, M. (2005) Blood 105, 4784–4791
46. Brown, R. J., and DuBois, R. N. (2005) J. Clin. Oncol. 23, 2840–2855
47. Raspollini, M. R., Amunni, G., Villanucci, A., Boddi, V., and Taddei, G. L. (2003) J. Gynecol. Cancer 43, 518–523
48. Gupta, R. A., Tejada, L. V., Tong, B. J., Das, S. K., Morrow, J. D., Dey, S. K., and DuBois, R. N. (2003) Cancer Res. 63, 906–911
49. Tsujii, M., and DuBois, R. N. (1997) Cell 81, 961–968
50. McEvoy, A. N., Murphy, E. A., Ponnio, T., Connelly, O. M., Bresnihan, B., FitzGerald, O., and Murphy, E. P. (2002) J. Immunol. 168, 2979–2987
51. Murphy, E. P., McEvoy, A., Connelly, O. M., Bresnihan, B., and FitzGerald, O. (2001) Arthritis Rheum. 44, 782–793
52. Tetradis, S., Bezougaia, O., and Tsingotjidou, A. (2001) Endocrinology 142, 663–670
53. Martinez-Gonzalez, J., and Badimon, L. (2005) Cardiovasc. Res. 65, 609–618