Identification of a Novel Class of Retinoic Acid Receptor β-Selective Retinoid Antagonists and Their Inhibitory Effects on AP-1 Activity and Retinoic Acid-induced Apoptosis in Human Breast Cancer Cells*

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Four candidate retinoid antagonists (LE135, LE511, LE540, and LE550) were designed on the basis of the ligand superfamily concept and synthesized. Analysis of these related retinoids by transient transfection assay demonstrated that LE135, LE540, and LE550 are effective retinoic acid receptor (RAR) antagonists, whereas LE511 selectively induced RARβ transcriptionsal activity. Both LE135 and LE540 inhibited retinoic acid (RA)- induced transcriptionsal activation of RARβ, but not RARα, RARγ or retinoid X receptor α (RXRα), on a variety of RA response elements. The retinoid antagonists also inhibited all-trans-RA-induced transcriptional activation of RARα/RXRα heterodimers, although they did not show any effect on transactivation activity of RXR/RXR homodimers. In ZR-75-1 human breast cancer cells, cotreatment of LE135 and LE540 with all-trans-RA inhibited all-trans-RA-induced apoptosis of the cells, further demonstrating that RARβ plays a role in RA-induced apoptosis of breast cancer cells. We also evaluated the effect of these retinoids on AP-1 activity. Our data showed that LE135 and LE540 strongly repressed 12-O-tetradecanoylphorbol-13-acetate-induced AP-1 activity in the presence of RARα and RXRα. Interestingly, LE550 induced AP-1 activity when RARβ and RXRα were expressed in HeLa cells but not in breast cancer cells. These results demonstrate that LE135 and LE540 were a novel class of RARβ-selective antagonists and anti-AP-1 retinoids and should be useful tools for studying the role of retinoids and their receptors.

Retinoids, the natural and synthetic vitamin A derivatives, are known to regulate many biological processes, including growth, differentiation, and development (1–3). They are currently used in the treatment of epithelial cancer and promyelocytic leukemia and are being evaluated as preventive and therapeutic agents for a variety of other human cancers (4). One of the major drawbacks of retinoid therapy has been the wide range of undesirable side effects. Development of anticancer-specific retinoids with improved clinical value is largely dependent on the understanding of the mechanistic basis of the pleiotropic activities induced by retinoids and their receptors. The effects of retinoids are mainly mediated by two classes of nuclear retinoid receptors: the retinoic acid receptors (RARs)1 (5–9) and the retinoid X receptors (RXRs) (10–14), and both receptors are members of the steroid-thyroid hormone receptor superfamily and are encoded by three distinct genes, α, β, and γ (5–14). All-trans-retinoic acid (RA) acts as a ligand for RARs, while 9-cis-RA is a ligand for both RARs and RXRs. RARs and RXRs modulate the expression of their target genes by interacting as either homodimers or heterodimers with RA response elements (RAREs) (11, 14–17). A RARE (β-RARE) in the RARβ gene promoter mediates RA-induced RARβ gene expression in many different cell types (18, 19). Up-regulation of the RARβ gene by RA plays a critical role in amplifying the RA response and is required for RA-induced growth inhibition and apoptosis in human breast cancer (20) and lung cancer cells (21).

In addition to the regulation of RARE-containing genes, retinoid receptors can inhibit the effect of the tumor promoter TPA by repressing the transcripational activity of AP-1 (22). Inhibition of AP-1 activity by retinoid receptors may involve either direct protein-protein interaction between retinoid receptor and components of AP-1, such as c-Jun (23), or competition for a common coactivator CBP (24). Recent studies have suggested that different receptor conformational changes may account for gene regulation on RAREs and inhibition of AP-1 activity and that they can be dissociated as anti-AP-1-specific retinoids have been described (25–27). Interaction between membrane and nuclear receptor signaling pathways mediated by RAR/AP-1 interaction may represent an important mechanism underlying the potent antineoplastic effects of retinoids. Because many of the AP-1 responsive genes, such as collagenases and stromelysins, are involved in cancer cell proliferation and transformation (28), retinoids that specifically inhibit AP-1 activity may be therapeutically desirable because they may have reduced side effects associated with gene activation, but retain their anticancer activity. This is demonstrated by recent studies showing that retinoids that inhibit AP-1 activity but cannot induce transactivation of RARE-containing genes were able to inhibit TPA-induced transformation and the clonal growth of mouse epidermal JB6 cells (29). In addition, a group of anti-AP-1-specific retinoids inhibited the proliferation of lung and breast cancer cells but had impaired ability to induce differentiation of F-9 cells (25).

Each subtype of RARs has been implicated in the regulation of cancer development and the anticancer activities of retinoids. Translocation of the RARα gene is responsible for the development of acute promyelocytic leukemia (30), whereas RARγ may play a role in mediating growth inhibition and

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‡ The abbreviations used are: RAR, retinoic acid receptor; RA, retinoic acid; TPA, 12-O-tetradecanoylphorbol-13-acetate; RXR, retinoid X receptor; RARE, RA response element.
apoptosis by certain retinoids (31). Recently, evidence is emerging showing that RARβ may play a critical role in the regulation of cancer cell growth. RARβ is located in chromosome 3p24, a region that is often deleted or mutated in a variety of cancer (32), and RARβ was found to be integrated by hepatitis B virus in human liver cancer (33). It is not expressed in many different types of cancer cell lines (20, 34–38), and re-expression of RARβ in RARβ-negative cancer cells restored the ability of RA to induce growth inhibition and apoptosis (20). Despite these studies, further investigation is needed to dissect function of each retinoid receptor in cancer cells. Several approaches have been used often to determine specific function of each receptor subtype, including loss of function, such as knock-out and antisense technique, and gain-of-function, such as ectopic expression of a receptor subtype. RAR subtype-selective agonists and antagonists are being developed (39–43), and they have been widely used to study the function of each receptor. However, the degree of selectivity and receptor transactivation activity needs to be improved. In addition, development of more and effective RARβ-selective retinoids is important to further study RARβ function in cancer. Furthermore, receptor-selective retinoids allow dissociation of desired and undesired effects of retinoids and are also believed to be more specific and with less toxicity in cancer prevention and treatment.

To further study RARβ function in cancer cells, we analyzed a class of conformational restricted retinoids. Our data demonstrated that LE135, LE540, and LE550 inhibited all-trans-RA-induced transcriptional activation of RARβ but not RARα, RARγ, or RXRα on a number of RAREs, whereas LE511 selectively induced transactivation of RARβ. The RARβ-selective antagonist effect was further demonstrated by their ability to inhibit all-trans-RA-induced apoptosis of ZR-75-1 human breast cancer cells. Interestingly, the antagonists LE135 and LE540 also exert anti-AP-1 activity. They effectively repressed TPA-induced AP-1 activity in both HeLa and breast cancer cells when RARβ was expressed. In contrast, LE550 induced AP-1 activity in HeLa but not in breast cancer cells. Together our results demonstrate that a novel class of RARβ-selective retinoids with variable biological functions should represent useful tools for studying RARβ function.

**EXPERIMENTAL PROCEDURES**

**Retinoids—All-trans-RA** was obtained from Sigma. LE135 was prepared as described by Eyrolles et al. (44). LE511, LE540, and LE550 were prepared as described by (45). Ro 41-5253 was kindly provided by Dr. Michael Klaus (46).

**Cell Culture—**Monkey kidney CV-1 and HeLa cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, and ZR-75-1 breast cancer cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum.

**Plasmid Constructions—**The receptor expression plasmids pCECRARα, -RARγ, and -RXRα and the construction of the reporter plasmids βRARE-tk-CAT, TREpal-tk-CAT, CBP1-RARE-tk-CAT, and ApoaIRE-RARE-tk-CAT have been described previously (15, 16, 47, 48).

**Transient Transfection Assay—**CV-1 cells were seeded at 5.0 × 10⁵ cells/well in 24-well plates. A modified calcium phosphate precipitation procedure was used for transient transfection as described previously (20). 100 ng of reporter plasmid, 100 ng of β-galactosidase expression vector (pCH110, Amersham Pharmacia Biotech), and various amounts of receptor were mixed with carrier DNA (pBluescript, Stratagene) to 1,000 ng of total DNA/well. After 16–24 h, transfected cells were treated with or without 10⁻⁷ M all-trans-RA in the absence or presence of the indicated concentrations of retinoid antagonists. For anti-AP-1 assay, a reporter construct containing the collagenase promoter linked with the CAT gene, -73-Col-CAT (23), was used in HeLa and ZR-75-1 cells. After transfection, cells were grown in a medium containing 0.5% charcoal-treated fetal calf serum with retinoids and/or TPA (100 ng/ml). Transfection efficiency was normalized to β-galactosidase activity. The data shown are the means of three separate experiments.

**Apoptosis Assay—**Nuclear morphological change analysis and DNA fragmentation (TdT) assay were as described previously (20, 21). For nuclear morphological analysis, ZR-75-1 cells were treated with or without 10⁻⁷ M all-trans-RA in the absence or presence of 10⁻⁶ M LE135, LE540, or LE550 for 4 days, trypsinized, washed with phosphate-buffered saline, fixed with 3.7% paraformaldehyde, and stained with 50 μg/ml 4,6-diamino-2-phenylindole containing 100 μg/ml DNase-free RNase A to visualize the nuclei. Stained cells were examined by fluorescent microscopy. For DNA fragmentation (TdT) assay, ZR-75-1 cells were treated with or without 10⁻⁷ M all-trans-RA in the absence or presence of 10⁻⁶ M LE135, LE540, or LE550. After 3 days, cells were trypsinized, washed with phosphate-buffered saline, fixed in 1% formaldehyde in phosphate-buffered saline, washed with phosphate-buffered saline, resuspended in 70% ice-cold ethanol, and immediately stored at -20 °C overnight. Cells were then labeled with biotin-16-dUTP by terminal transferase and stained with avidin-fluorescein isothiocyanate (Roche Molecular Biochemicals). The labeled cells were analyzed using a FACSscater-Plus. Representative histograms are shown.

**RESULTS**

**Transactivation Activity of the Synthetic Retinoids—**Recently, we designed several retinoid antagonists, LE135, LE511, LE540, and LE550 (49, 50), based on the structure-activity relationship of retinoids (Fig. 1). Transcriptional activation of these retinoids on RARα, RARγ, or RXRα was determined by transient transfection assay using the reporter construct TREpal-tk-CAT (15) in CV-1 cells. As shown in Fig. 2, all-trans-RA strongly induced transcriptional activation of each RARs, whereas 9-cis-RA effectively promoted RXX homodimer activity. However, LE135, LE540, and LE550, at 10⁻⁷ M and 10⁻⁸ M, had very little effect on transcriptional activation of RARα, RARγ, or RXRα. LE135 and LE540 even showed inhibitory effect on basal RARβ activity in a dose-dependent manner. Interestingly, LE511, a LE135 analog with bulky acyclic alkyl group, showed a strong induction of RARβ transactivation activity. Activation of RARβ could be observed when 10⁻⁷ M LE511 was used, and it is about 65% of efficiency as compared with all-trans-RA. Together, these results demonstrate that LE135, LE540, and LE550 are ineffective on transcriptional activation of RARs and RXR, whereas LE511 is a RARβ-selective agonist.

**Antagonistic Effect of the Synthetic Retinoids on RA-induced RARβ Transcriptional Activation—**LE135 was previously shown to selectively bind to RARβ (44). The observations that LE135 could not induce RARβ transactivation activity and that it inhibited basal RARβ activity (Fig. 2) suggested that binding of RARβ by LE135 might repress its transactivation function. We therefore analyzed the effect of LE135 on all-trans-RA-induced transcriptional activation of RARβ on βRARE-tk-CAT reporter (19). For comparison, its effect on RARα or RARγ was analyzed. As shown in Fig. 3a, 10⁻⁷ M all-trans-RA-induced RARβ activity was strongly inhibited by LE135 in a concentration-dependent manner, with more than 70% inhibition when 10⁻⁶ M LE135 was used. For comparison, RARα-selective antagonist Ro 41-5253 did not show any effect on RARβ activity. When RARα was analyzed, LE135 did not
exhibit clear inhibitory effect on all-trans-RA-induced RARα activity, whereas Ro 41-5253 significantly repressed the reporter transcription. Both LE135 and Ro 41-5253 did not show any influence on all-trans-RA-induced RARγ activity. Similar results were obtained on another reporter, the TREpal-tk-CAT (Fig. 3b and data not shown). Because RARβ is likely to function as RARα/RXR heterodimer in cells, we determined whether LE135 could act as a RARβ/RXR heterodimer antagonist. As shown in Fig. 3b, LE135 exhibited a similar degree of inhibition on both all-trans-RA-induced RARβ and RARβ/RXR heterodimer activity (Fig. 3b), whereas it had no effect on 9-cis-RA-induced RXR homodimer activity. Similar results were obtained with LE540 (data not shown). Together, these data demonstrate that LE135 and LE540 are effective antagonist of RARβ and RARβ/RXR heterodimer.

To further analyze the antagonist effect of LE135 and its analogs, reporter constructs containing different RAREs, including βRARE, CRBP1-RARE and ApoAI-RARE were used. As shown in Fig. 3c, LE135, LE540, and LE550 inhibited all-trans-RA-induced RARβ transcriptional activity on these different RAREs. Similar degrees of inhibition (about 60–70%) were observed with the βRARE and the ApoAI-RARE, whereas a less degree of inhibition (50–60%) was obtained with the CRBP1-RARE. Interestingly, LE540 showed a more effective inhibition on all RAREs than its isomer LE550, consistent with their antagonist effect on HL-60 cell differentiation (45). These results demonstrate that LE135, LE540, and LE550 could inhibit transactivation of RARβ and that the antagonistic effect of these retinoids is response element independent.

Effect of the Retinoid Antagonists on RA-induced Growth Inhibition and Apoptosis in Human Breast Cancer Cells—We have previously demonstrated that expression of RARβ is required for all-trans-RA-induced apoptosis of human breast cancer cells (20). We then analyzed whether inhibition of RARβ activity by RARβ-selective antagonists could repress all-trans-RA activity in ZR-75-1 human breast cancer cells. ZR-75-1 cells underwent extensive apoptosis when they were treated with all-trans-RA as revealed by both morphological analysis (DAPI staining) (Fig. 4a) and DNA end-labeling assay (TdT) (Fig. 4b). However, all-trans-RA-induced apoptosis was strongly prevented when 10⁻⁷ M all-trans-RA was used together with 10⁻⁶ M of LE135, LE540, or LE550. Morphological analysis showed that all-trans-RA-induced apoptosis was reduced from about 40 to 20% by LE135, LE540, or LE550 (Fig. 4a). Similar results were obtained by TdT assay (Fig. 4b). These results are in agreement with previous observation made by ectopic expression of RARβ (20), and suggest that these RARβ-selective antagonists are useful tools for studying RARβ function.

Effect of Retinoid Antagonists on TPA-induced AP-1 Activity—Recently, it was reported that RA can inhibit AP-1 activity (22) and that receptor conformational change required for AP-1 inhibition is different from that required for receptor transcriptional activation and they can be dissociated (25–27). To determine whether LE135, LE540, and LE550 could also induce RAR conformational changes for inhibiting AP-1 activity, we investigated their effect on TPA-induced AP-1 activity. When a reporter containing the collagenase (23) linked with the CAT gene, -73Col-CAT (23), was transiently transfected into HeLa cells, treatment of the cells with TPA strongly induced the reporter activity, consistent with previous observations. Co-treatment of the cells with either all-trans-RA, LE135, LE540, or LE550 did not show a clear effect on TPA-induced reporter activity (Fig. 5a). However, when RARβ was cotransfected, the TPA-induced reporter transcription was slightly inhibited, which was further inhibited when cells were treated with all-trans-RA but not with LE135, LE540, and LE550. Interestingly, treatment with LE550 slightly enhanced reporter transcription. When RARβ and RXRα were cotransfected, however, LE135 and LE540 showed a strong inhibition of the TPA-induced reporter activity. The enhancing effect of LE550 was also increased. These data suggest that LE135 and LE540 could induce a conformational change of RARβ required for inhibiting AP-1 activity only when RARβ is heterodimerized with RXRα and that LE550 may induce another RARβ conformation that stimulates AP-1 transcriptional activity. To determine whether these retinoids could inhibit AP-1 activity in breast cancer cells, the -73Col-CAT was transiently transfected into ZR-75-1 cells (Fig. 5b). Treatment of the cells with TPA led to an increase of reporter gene transcription for about 7-fold. Both LE135 and LE540 inhibited the TPA-induced activity in a concentration-dependent manner when RARβ expression vector was cotransfected. These data demonstrate that LE135 and LE540 could also inhibit AP-1 activity in breast cancer cells. The fact that LE135 and LE540 could inhibit the TPA-induced reporter transcription without RXRα cotransfection is consistent with observation that RXRα is expressed in ZR-75-1 cells (20). Interestingly, we did not observe any induction of the reporter activity when cells were treated with LE550, suggesting that the AP-1-inducing effect of LE550 is cell-type specific.

DISCUSSION

Previous studies on receptor-ligand interaction have suggested requirements for being a potent retinoid antagonist: (i) strong binding to the receptor mediated by the hydrophobic
alkylated benzo group of a retinoid contributes to its binding affinity to RARs and (ii) inhibition of the conformational changes, such as proper folding of the helix 12 where the binding site for co-activators (AF2 domain) exists. This has established structure-activity relationships in both retinoid agonists and antagonists. We describe here a class of related synthetic retinoids, LE135, LE540, LE511, and LE550, which either specifically activate or inhibit RARβ transactivation function. In assaying for transactivation function of the retinoids on each subtype of RARs and RXRα, LE511 selectively activated RARβ (Fig. 2), suggesting that it is a RARβ-selective agonist. In contrast, other LE compounds, LE135, LE540, and LE550, did not display any activation function on the retinoid receptors tested (Fig. 2). Instead, LE135 and LE540 showed a strong inhibition of RARβ basal transactivation activity. When they were used together with all-trans-RA, LE135 or LE540 effectively inhibited all-trans-RA-induced RXRα/RARβ activity (Fig. 3). LE135 does not bind to RXRs and RARγ in receptor binding assay (45). It binds with higher affinity to RARβ \((K_i = 0.22 \mu M)\) than to RXRs \((K_i = 1.4 \mu M)\) (45). Thus, LE135 and its related analog LE540 act as RARβ-selective antagonists. These results suggest that the tetramethyltetrahydronaphthalenyl group as seen in LE135, LE540, and LE550 may be required for RARβ antagonist effect, because its replacement with effective mono-tertiary butylphenyl group as seen in LE511 resulted in loss of RARβ antagonism effect (Fig. 3). The smaller hydrophobic tert-butyl group of LE511 not only decreases the binding affinity to RARs but also may change the binding occupation in the ligand-RAR complex with less disturbance of the helix folding. This may explain the critical agonistic activity of LE511. The existence of another benzo (or naphtho) group impairs the transcriptional activating activity due to the different conformational change in the ligand-receptor complex. From the reported crystal structures of RXRα and RARγ (51, 52), proper folding of the helix 12 in the ligand-binding domain of RARs is critical for receptor activation. The benzo/naphtho group fused to the diazepine ring may disturb the proper folding of the helix to elicit the antagonistic activity. The bulkier naphtho group of LE540 is expected to be more effective than the benzo group of LE135 and that of LE550 with a different direction seems to affect weakly the conformation around the helix as it could not inhibit RARβ basal transactivation activity (Fig. 2).

The pleiotropic effects of retinoids are mainly mediated by RARs and RXRs. Both types of retinoid receptors are encoded by three distinct genes, \(\alpha\), \(\beta\), and \(\gamma\). The fact that these receptors display distinct patterns of expression during development and differentiation suggests that each of them may have specific function, which is being unraveled recently by a variety of technologies, such as homologous recombination, antisense, and ectopic expression of a receptor of interest. The complexity of retinoid responses also can be dissected with the use of both
receptor-selective agonists and antagonists, activating or interfering specifically or preferentially with one given receptor. We have previously reported (20) that all-trans-RA-induced apoptosis of human breast cancer cells requires RAR\(\beta\) expression. This was based on our observation that stable expression of RAR\(\beta\) in RAR\(\beta\)-negative cells induced apoptosis, whereas expression of RAR\(\beta\) antisense RNA in RAR\(\beta\)-positive cells abolished apoptotic effect of all-trans-RA (20). Here, we used RAR\(\beta\)-selective antagonists to study the involvement of RAR\(\beta\) in all-trans-RA-induced growth inhibition and apoptosis of ZR-75-1 human breast cancer cells. When RAR\(\beta\)-selective antagonists LE135, LE540, or LE550 was used together with all-trans-RA, the effect of all-trans-RA on apoptosis in ZR-75-1 cells was largely reduced (Fig. 4). This result further supports the role of RAR\(\beta\) in all-trans-RA-induced growth inhibition and apoptosis of human breast cancer cells. It also demonstrates that LE135 and its analogs are valuable tools for studying RAR\(\beta\) function.

In this study, we also show that RAR\(\beta\) antagonists could act as effective anti-AP-1 retinoids (Fig. 5). These retinoids, which did not show any transactivation function on RARs and RXRs, could repress AP-1 activity in the presence of RAR\(\beta\)/RXR heterodimer. Therefore, they are anti-AP-1 specific retinoids. Interestingly, they could not affect AP-1 activity in the presence of RAR\(\beta\) alone but required RXR for effective inhibition of AP-1 activity. This suggests that binding of retinoids to RAR\(\beta\) alone is not sufficient to induce a specific anti-AP-1 conformational change. Interestingly, LE550 could induce AP-1 activity in HeLa cells but not in ZR-75-1 cells (Fig. 5). The mechanism by which LE550 induces AP-1 activity is unclear. It is likely that LE550 induces a different conformational change of RAR\(\beta\). This is supported by our observation that LE540 and LE550 are regio-isoforms, in which the bulkier naphtho group of both compounds is arranged in different directions, which may be critical for AP-1 interaction (Fig. 5). Previous studies have demonstrated that AP-1 can either inhibit or stimulate nuclear receptor activity, depending on cell type, promoter, and nuclear receptor (53). Our observation that LE550 could induce AP-1 activity in HeLa cells suggests that the retinoid receptor could also stimulate AP-1 activity in response to appropriate ligand. The fact
that induction of AP-1 activity by LE550 was only observed in HeLa cells but not in ZR-75-1 cells implies that inhibition or induction of AP-1 activity by retinoid receptor is also cell-type specific. LE550 may induce RARβ in a conformation that allows a positive effect on AP-1 transcription, probably through transcriptional mediators specifically expressed in HeLa cells. Such a compound may be a valuable tool for studying mechanisms underlying AP-1/nuclear receptor interaction and for dissecting complexity of AP-1/nuclear receptor interaction.

REFERENCES
1. Gudas, L. J., Sporn, M. B., and Roberts, A. B. (1996) in The Retinoids (Sporn, M. B., Roberts, A. B., and Goodman, D. S., eds) pp. 443–520, Raven Press, New York
2. Lotan, R. (1981) Biochim. Biophys. Acta 665, 53–91
3. Roberts, A. B., and Sporn, M. B. (1984) in The Retinoids (Sporn, M. B., Roberts, A. B., and Goodman, D. S., eds) pp. 299–286, Academic Press, Orlando, FL
4. Lotan, R. (1987) Cancer Treat. Rep. 71, 493–515
5. Benbrook, D. (1998) Nature 333, 624–629
6. Brand, N., Petkovich, M., Krust, A., Chambron, P., de The, H., Marchio, A., Tiollais, P., and Dejean, A. (1988) Nature 332, 850–853
7. Giguere, V., Ong, E. S., Segui, P., and Evans, R. M. (1987) Nature 330, 624–629
8. Krust, A., Kastner, Ph., Petkovich, M., Zelent, A., and Chambron, P. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 5310–5314
9. Petkovich, M., Brand, N. J., Krust, A., and Chambron, P. (1987) Nature 330, 444–450
10. Hamada, K., Gleason, S. L., Levi, B. Z., Hirschfeld, S., Appella, E., and Ozato, K. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 8269–8273
11. Leid, M., Kastner, P., Lyons, R., Nakshatri, H., Saunders, M., Zacharewski, T., Chen, J. Y., Staub, A., Garnier, J. M., and Mader, S. (1992) Cell 68, 377–395
12. Mangelsdorf, D. J., Borgmeyer, U., Heyman, R. A., Zhou, J. Y., Ong, E. S., Ora, A. E., Kakizuka, A., and Evans, R. M. (1992) Genes Dev. 6, 329–344
13. Mangelsdorf, D. J., Ong, E. S., Dyck, J. A., and Evans, R. M. (1990) Nature 345, 274–279
14. Yu, V. C., Delaert, C., Andersen, B., Holloway, J. M., Devaray, O. V., Naar, A. M., Kim, S. Y., Boutin, J. M., Glass, C. K., and Rosenfeld, M. G. (1991) Cell 67, 1251–1266
15. Zhang, X.-k., Hoffmann, B., Tran, P. B., Graupner, G., and Pfahl, M. (1992) Nature 355, 441–446
16. Zhang, X.-k., Lehmann, J., Hoffmann, B., Dawson, M. I., Cameron, J., Graupner, G., Hermann, T., Tran, P., and Pfahl, M. (1992) Nature 358, 587–591
17. Zhang, X.-k., Zhang, X.-k., Salbert, G., Lee, M. O., and Pfahl, M. (1994) Mol. Cell. Biol. 14, 4311–4323
18. de The, H., del Mar Vivanco-Ruiz, M., Tiollais, P., Stunnenberg, H., and Dejean, A. (1990) Nature 343, 177–180
19. Hoffmann, B., Lehmann, J. M., Zhang, X.-k., Graupner, G., and Pfahl, M. (1990) Mol. Endocrinol. 4, 1734–1743
20. Liu, Y., Lee, M.-O., Wang, H.-G., Li, Y., Hashimoto, Y., Klaus, M., Reed, J. C., and Zhang, X.-k. (1996) Mol. Cell. Biol. 16, 1138–1149
21. Li, Y., Dawson, M. I., Agadir, A., Lee, M. O., Jong, L., Hobbs, P. D., and Zhang, X. K. (1998) Int. J. Cancer 78, 88–95
22. Pfahl, M. (1993) Endocr. Rev. 14, 651–658
24. Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S.-C., Heyman, R. A., Rose, D. W., Glass, C. K., and Rosenfeld, M. G. (1996) Cell 85, 403–414
25. Fanjul, A., Dawson, M. I., Hobbs, P. D., Jong, L., Cameron, J. F., Harlev, E., Graupner, G., Lu, X. P and Pfahl, M. (1994) Nature 372, 107–111
26. Chen, J. Y., Penco, S., Ostowski, J., Balaguer, P., Pons, M., Starrett, J. E., Reczek, P., Chambon, P., and Gronemeyer, H. (1995) EMBO J. 14, 1187–1197
27. Nagpal, S., Atchanikar, J., and Chandraratna, R. A. S. (1995) J. Biol. Chem. 270, 923–927
28. Angel, P., and Karin, M. (1991) Biochim. Biophys. Acta 1072, 129–157
29. Li, J. J., Dong, Z., Dawson, M. I., and Colburn, N. H. (1996) Cancer Res. 56, 483–489
30. Warrell, R. P., Jr., De The, H., Wang, Z.-Y., and Degos, L. (1993) N. Engl. J. Med. 329, 177–189
31. Fanjul, A. N., Bouterfa, H., Dawson, M., and Pfahl, M. (1996) Cancer Res. 56, 1571–1577
32. Kok, K., Osinga, J., Carritt, B., Davis, M. B., van der Hout, A. H., van der Veen, A. Y., Landsvater, R. M., de Leij, L. F. M. H., Berendsen, H. H., Fostmus, P. E., Poppema, S., and Buys, C. H. C. M. (1987) Nature 330, 578–581
33. Dejean, A., Bougueleret, L., Grzeschik, K.-H., and Tiollais, P. (1986) Nature 322, 70–72
34. Houle, B., Ledue, F., and Bradley, W. E. C. (1991) Exp. Cell Res. 195, 163–170
35. Gebert, J. F., Moghal, N., Frangioni, J. V., Sugarbaker, D. J., and Neel, B. G. (1991) Oncogene 6, 1859–1868
36. van der Lee, B. M., van den Brink, C. E., and van der Saag, P. T. (1993) Mol. Carcinogen. 8, 112–122
37. Nervi, C., Volleberg, T. M., George, M. D., Zelent, A., Chambon, P., and Jetten, A. M. (1991) Exp. Cell Res. 195, 163–170
38. Zhang, X.-k., Liu, Y., Lee, M.-O., and Pfahl, M. (1994) Cancer Res. 54, 5663–5669
39. Lehmann, J. M., Dawson, M. I., Hobbs, P. D., Husmann, M., and Pfahl, M. (1991) Cancer Res. 51, 4804–4809
40. Lehmann, J. M., Jong, L., Fanjul, A., Cameron, J. F., Liu, X. P., Haeftner, P., Dawson, M. I., and Pfahl, M. (1992) Science 256, 1944–1946
41. Jong, L., Lehmann, J. M., Hobbs, P. D., Harlev, E., Huffinan, J. C., Pfahl, M., and Dawson, M. I. (1993) J. Med. Chem. 36, 2605–2613
42. Delescluse, C., Cavey, M. T., Martin, B., Bernard, B. A., Reichert, U., Maignan, J., Darmon, M., and Shroot, B. (1991) Mol. Pharmacol. 40, 556–562
43. Bernard, B. A., Bernardon, J.-M., Deleschue, C., Martin, B., Lenoir, M.-C., Maignan, J., Charpenteir, B., Pilgrim, W. R., Reichert, U., and Shroot, B. (1992) Biochem. Biophys. Res. Commun. 186, 977–983
44. Eyrolles, L., Kagechika, H., Kawachi, E., Fukasawa, H., Iijima, T., Matsuushima, Y., Hashimoto, Y., and Shudo, K. (1994) J. Med. Chem. 37, 1508–1517
45. Umemiya, H., Fukasawa, H., Ebisawa, M., Eyrolles, L., Kawachi, E., Eisenmann, G., Gronemeyer, H., Hashimoto, Y., Shudo, K., and Kagechika, H. (1997) J. Med. Chem. 40, 4222–4234
46. Apfel, C., Bauer, F., Crettaz, M., Forni, L., Kamber, M., Kaufmann, F., Lemotte, P., Pirson, W., and Klaus, M. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7129–7133
47. Tran, P., Zhang, X.-k., Salbert, G., Hermann, T., Lehmann, J. M., and Pfahl, M. (1992) Mol. Cell. Biol. 12, 4666–4767
48. Lee, M.-O., Liu, Y., and Zhang, X.-k. (1995) Mol. Cell. Biol. 15, 4194–4207
49. Eyrolles, L., Kawachi, E., Matsuushima, Y., Nakajima, O., Kagechika, H., Hashimoto, Y., and Shudo, K. (1991) Med. Chem. Res. 1, 220–225
50. Bourguet, W., Ruff, M., Chambon, P., Gronemeyer, H., and Moras, D. (1995) Nature 375, 377–382
51. Renaud, J.-P., Rochel, N., Ruff, M., Vivat, V., Chambon, P., Gronemeyer, H., and Moras, D. (1995) Nature 378, 681–689
52. Shemshedini, L., Rudolf, K., Sassone-Corsi, P., Ponnorn, A., and Gronemeyer, H. (1991) EMBO J. 10, 3839–3849