Antitumorigenic Effect of Wnt 7a and Fzd 9 in Non-small Cell Lung Cancer Cells Is Mediated through ERK-5-dependent Activation of Peroxisome Proliferator-activated Receptor γ*

Received for publication, May 1, 2006, and in revised form, July 10, 2006 Published, JBC Papers in Press, July 11, 2006, DOI 10.1074/jbc.M604145200

Robert A. Winn*§, Michelle Van Scoyk*, Mandy Hammond*, Karen Rodriguez§, Joseph T. Crossno, Jr.§, Lynn E. Heasley*, and Raphael A. Nemenoff§

From the*§Veterans Administration Medical Center, Denver, Colorado 80220 and the§Department of Medicine, University of Colorado Health Sciences Center, Denver, Colorado 80262

The Wnt pathway is critical for normal development, and mutation of specific components is seen in carcinomas of diverse origins. The role of this pathway in lung tumorigenesis has not been clearly established. Recent studies from our laboratory indicate that combined expression of the combination of Wnt 7a and Frizzled 9 (Fzd 9) in Non-small Cell Lung Cancer (NSCLC) cell lines inhibits transformed growth. We have also shown that increased expression of peroxisome proliferator-activated receptor γ (PPARγ) inhibits transformed growth of NSCLC and promotes epithelial differentiation of these cells. The goal of this study was to determine whether the effects of Wnt 7a/Fzd 9 were mediated through PPARγ. We found that Wnt 7a and Fzd 9 expression led to increased PPARγ activity. This effect was not mediated by altered expression of the protein. Wnt 7a and Fzd 9 expression resulted in activation of ERK5, which was required for PPARγ activation in NSCLC. SR 202, a known PPARγ inhibitor, blocked the increase in PPARγ activity and restored anchorage-independent growth in NSCLC expressing Wnt 7a and Fzd 9. SR 202 also reversed the increase in E-cadherin expression mediated by Wnt 7a and Fzd 9. These data suggest that ERK5-dependent activation of PPARγ represents a major effector pathway mediating the anti-tumorigenic effects of Wnt 7a and Fzd 9 in NSCLC.

Wnts are a family of secreted glycoproteins that serve as extracellular signaling molecules controlling diverse morphogenetic and developmental programs (1). Signaling is mediated by a family of distinct seven-membrane receptors known as Frizzled (Fzd)2 (2), and is further regulated by co-receptors LRP 5/6 (3). Aberrant Wnt signaling has been implicated in a variety of cancers (4, 5). Our laboratory has focused on the role of Wnt signaling in lung cancer. We have previously reported that the restoration of Wnt 7a and Fzd 9 signaling inhibited both cell proliferation and anchorage-independent growth, promoted cellular differentiation, and reversed the transformed phenotype in Non-small Cell Lung Cancer cells (NSCLC) (6). These findings unveil a novel tumor suppressor pathway in lung cancer and implicate Wnt 7a and Fzd 9 in the maintenance of epithelial cellular differentiation. The downstream effector pathways mediating these effects are not well understood.

Proliferator-activated receptor γ (PPARγ) is a member of the PPAR family of ligand-activated nuclear receptors implicated in a wide variety of biological functions (7). Three PPAR isoforms have been identified, α, γ, and β/δ, which all bind as heterodimers with the retinoic acid X receptor to specific regulatory elements in the promoter regions of their target genes. The role of PPARγ has been extensively studied in a variety of cancers including colon, breast, prostate, and lung (see Ref. 8 for review). Inactivating mutations in the PPARγ gene have been seen in colon cancers (9, 10), suggesting that PPARγ behaves as a tumor suppressor gene. Pharmacological activators of PPARγ inhibit growth of NSCLC cells and induce apoptosis (11, 12). In human lung tumors, decreased expression of PPARγ was correlated with poor prognosis (13). Our laboratories have demonstrated that overexpression of PPARγ in NSCLC inhibited transformed growth and metastasis of NSCLC and promoted epithelial differentiation (14, 15).

Because both Wnt 7a/Fzd 9 and PPARγ appeared to have similar tumor-suppressive effects on NSCLC, we postulated that they represented components of a common signaling pathway and that the cell surface genes Wnt 7a/Fzd 9 were likely upstream of the nuclear receptor gene PPARγ. The current study demonstrates that Wnt 7a/Fzd 9 signaling leads to the stimulation of PPARγ through activation of Extracellular Signal-Regulated Kinase 5 (ERK-5). Furthermore, pharmacological inhibition of PPARγ reversed the anti-tumorigenic effects of Wnt 7a/Fzd 9.

**EXPERIMENTAL PROCEDURES**

*Cell Culture and Retrovirus-mediated Gene Transfer—NSCLC lines of the adenocarcinoma (H2122) and squamous (H157) phenotype were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum at 37 °C in an humidified 5% CO₂.
Wnt 7a and Fzd 9 Activate PPARγ in NSCLC

FIGURE 1. Transfected Wnt 7a and Fzd 9 stimulate PPAR-RE activity and have a synergistic effect when co-transfected with wild-type PPARγ. H157 cells transfected with empty vector pcDNA3, Wnt 7a, Fzd 9, Wnt 7a and Fzd 9, wild-type PPARγ, or both Wnt 7a/Fzd 9 and wild-type PPARγ were transiently transfected with PPAR-RE and pCMV-β-galactosidase as described under “Experimental Procedures.” Similarly, H2122 cells were transfected with pcDNA3, Wnt 7a, Fzd 9, wild-type PPARγ, or both Fzd 9 and wild-type PPARγ. The cells were incubated for 72 h, and luciferase and β-galactosidase activities were measured. The data are presented as relative light units/millilunit of β-galactosidase activity and represent the mean of at least three independent experiments with the S.E. indicated. *, p < 0.05 versus pcDNA-3, Wnt7a alone, or Fzd 9 alone in H157 cells; **, p < 0.05 versus Wnt7a + Fzd 9 in H157 cells or Fzd 9 in H2122 cells.

incubator. Stable transfectants H157-LNCX-LPCX, H157-Wnt-7a/Fzd-9, H2122-LPCX, H2122-Fzd-9, and H157-LNCX, H157-PPARγ, H2122-LNCX, and H2122-PPARγ were prepared using retroviral-mediated gene transfer as previously described (6, 14). Wnt-7a cDNAs inserted into pcDNA3 encoding a C-terminal hemagglutinin epitope were kindly provided by Dr. Jan Kitajewski (Columbia University). The cDNA encoding mouse Frizzled 9 (mFzd-9) was provided by Dr. Terry Van Raay (Vanderbilt University School of Medicine).

Quantitative PCR—Total RNA was extracted from cultured cells with the RNeasy mini kit (Qiagen Inc., Valencia, CA). Aliquot of the RNA (10 μg) were converted to cDNA with Superscript II (Invitrogen) and random hexamers according to the manufacturer’s specifications. Primer sets for the quantitative PCR of human PPARγ and GAPDH are as follows: PPARγ (forward, 5′-CAT AAC ATT AGC TTT TGT TTT CC-3′; reverse, 5′-TCA GAG TGG CTG ATT GAA CTG-3′). GAPDH (forward, 5′-GCA CAA TAT GAC ATC AGG CAC GGA-3′; reverse, 5′-GTT GCT GCT GCT GAA AGA-3′). Aliquots (1 μl) of reverse transcription reactions were subjected to PCR using the following conditions: 95 °C x 10 min, followed by 95 °C x 30 s and 60 °C x 1 min for 40 cycles in 50-μl reactions with SYBR® Green Jumpstart Taq ReadyMix (Sigma). Initial real-time PCR amplification products were resolved by electrophoresis on 5% polyacrylamide gels to verify that the primer pairs amplified a single product of the predicted size. GAPDH mRNA levels were measured by quantitative PCR in the samples as a control gene. The real-time PCR data were analyzed with the Smart Cycler® software (version 1.2d) to calculate the threshold cycle values for the different samples and are presented as mRNA levels in arbitrary units.

Immunoblot Analysis—The following antibodies were used for immunoblotting: PPARγ, phospho-ERK-5, total ERK-5, phospho-p44/42 MAPK, and total p44/42 MAP kinase (Cell Signaling); E-cadherin and β-catenin (BD Transduction Laboratories); SMRT (Santa Cruz); and β-actin (Abcam). Cell extracts were prepared in MAP kinase lysis buffer (0.5% Triton X-100, 50 mM β-glycerophosphate, pH 7.2, 0.1 mM sodium vanadate, 2 mM MgCl2, 1 mM EGTA, 1 mM dithiothreitol, 2 μg/ml leupeptin, and 4 μg/ml aprotonin) as previously described (16). Aliquots of the different extracts were resolved by 10% SDS-PAGE and transferred to nitrocellulose. The filters were blocked in Tris-buffered saline (10 mM Tris-Cl, pH 7.4, 140 mM NaCl, containing 0.1% Tween 20 (TTBS) and 3% nonfat dry milk and then incubated with the same blocking solution containing the indicated antibodies at 1 μg/ml for 12–16 h. For immunoblotting of phospho-ERK-5, filters were blocked in TTBS containing 3% bovine serum albumin. The filters were extensively washed in TTBS, and bound antibodies were visualized with alkaline phosphatase-coupled secondary antibodies and LumiPhos reagent (Pierce, Rockford, IL) according to the manufacturer’s directions.

Soft Agar Colony Formation—For measurement of anchorage-independent cell growth, 5,000 cells were plated in triplic-
Wnt 7a and Fzd 9 Activate PPARγ in NSCLC

RESULTS

Wnt 7a and Fzd 9 Activate PPARγ in NSCLC Cell Lines—H157 and H2122 cells were transiently transfected with empty vector, Wnt 7a, and/or Fzd 9 or wild-type PPARγ along with a vector encoding three copies of a PPAR response element (PPAR-RE) linked to firefly luciferase. In H157 cells, which lack both Wnt 7a and Fzd 9 (6), simultaneous expression of both proteins led to a marked increase in PPAR-RE activity (Fig. 1, left panel). In H2122 cells, which express endogenous Wnt 7a, transient expression of Fzd 9 increased PPAR-RE activity 8-fold (right panel). Increases in PPARγ activity were also seen in both cell lines in response to expression of PPARγ. Co-expression of Wnt 7a and Fzd 9 with PPARγ resulted in a synergistic increase in PPARγ activity (Fig. 1). By contrast, co-expression of other Wnts (1, 2, 3, 4, 5a, 5b, and 6) and Fzd 9 failed to significantly increase PPAR-RE (data not shown). Similarly, co-expression of Wnt7a with Fzd 1, 2, 3, 4, 6, 7, or 8 failed to increase PPAR-RE activity (data not shown). We also examined activity of PPAR-RE in cells stably expressing Wnt 7a/Fzd 9 or PPARγ. As shown in Fig. 2A, similar increases were observed in these stable transfectants. Interestingly, stable expression of PPARγ failed to increase PPAR-RE activity in either cell line, confirming that this activity is due to PPARγ. To confirm the specificity of Wnt 7a/Fzd 9 on PPAR isoforms, we measured the activity of a transiently transfected PPARγ-specific promoter (PDRE) reporter (17). Expression of PPARγ, but not PPARδ, increased PDRE promoter activity (Fig. 2B), demonstrating the specificity of these PPAR-RE and PDRE luciferase reporters for PPARγ and
PPARγ, respectively. Expression of Wnt 7α/Fzd 9 in H157 cells or Fzd 9 in H2122 cells had no effect on PDRE activity (Fig. 2B). Taken together, these data indicate that expression of Wnt 7α/Fzd 9 results in increased PPARγ activity.

Neither PPARγ mRNA nor Protein Expression Is Induced in NSCLC Cells as a Result of Wnt 7α and Fzd 9 Expression—We sought to determine whether increased PPARγ activity in response to expression of Wnt 7α/Fzd 9 was mediated through increased expression of the protein. Our quantitative reverse transcription PCR experiments revealed that stable expression of Wnt 7α and Fzd 9 in either H157 or H2122 had no significant effect on PPARγ mRNA levels (Fig. 3A). This was confirmed by immunoblotting, where stable expression of Wnt 7α and Fzd 9 failed to cause a significant increase in the PPARγ expression (Fig. 3B). As a positive control, stable expression of wild-type PPARγ resulted in a marked increase in PPARγ protein and mRNA expression. These data indicate that Wnt 7α and Fzd 9 activation of PPARγ is not mediated through increased expression but by activation of PPARγ.

Wnt 7α and Fzd 9 Activation of PPARγ Is Mediated through Activation of ERK5—Previous studies have demonstrated that ERK5 is capable of mediating PPARγ transcriptional activation in endothelial cells (18). To test the hypothesis that ERK5 stimulates activation of PPARγ in NSCLC, we examined the effect of Wnt 7α and Fzd 9 expression in both H157 and H2122 cell lines on ERK5 phosphorylation by immunoblotting with a phospho-ERK5-specific antibody. Expression of Wnt 7α/Fzd 9 resulted in a 5- to 10-fold increase in pERK5 relative to the empty vector control cell lines (Fig. 4A). The levels of total ERK5 were not changed (Fig. 4A, lower panels). Under these conditions, phosphorylation of ERK5 was increased in both cell lines, indicating that Wnt 7α and Fzd 9 activation of PPARγ is mediated through activation of ERK5.
conditions we did not observe any significant increase in ERK1/2 activity, either by blotting with a phospho-ERK antibody (Fig. 4A) or by direct measurement of ERK1/2 kinase activity (data not shown).

We also examined the effects of pharmacological inhibitors of the ERK family of MAP kinases. PD 98059 (25 μM), a known MEK inhibitor, decreased expression of phospho-ERK1, 2, and 5 on immunoblot (Fig. 4A), consistent with what has been previously reported (19, 20). Exposure of either H157 or H2122 cells to this concentration of PD98059 had no effect on basal PPARγ activity in control cells transfected with empty vector but potently inhibited the increase seen in cells expressing Wnt7a/Fzd9 (Fig. 4B). Because these results do not rule out a role for ERK1/2 in regulation of PPARγ activity, we employed a second MEK inhibitor, U0126. This agent inhibited MEK1/2 but did not affect MEK5 activity at the concentration used in this study (10 μM) (Fig. 4, A and B) (21). The addition of U1026 to the various cell lines did not lead to a reduction of phospho-ERK5 protein expression or PPAR-RE reporter activity in either the H157 Wnt7a/Fzd9 or H2122 Fzd9 cell lines (Fig. 4, A and B). From these studies, we propose that increased PPARγ activity is mediated through activation of MEK5/ERK5. This finding is consistent with the lack of an effect of Wnt7a/Fzd9 on ERK1/2 activity (6).

To determine the role of ERK5 in activating PPARγ, we co-expressed dominant negative MEK5 an inhibitor of ERK5 with Wnt7a and Fzd9. Expression of dominant negative MEK5 had no effect on basal PPAR-RE activity but inhibited the activation stimulated by expression of Wnt7a and Fzd9 in both the H157 and H2122 cell lines. In H157 cells, PPARγ activity was decreased by ~60%, whereas in H2122 cells the decrease was >90% (Fig. 4C).

Wnt 7a and Fzd 9 Expression Results in Reduced Expression of the PPARγ Co-repressor SMRT—Past studies have shown that PPARγ activity is inhibited by the corepressors silencing mediator of retinoid and thyroid hormone receptor (SMRT) and NCoR (18, 22). Activation of PPARγ by ligands is associated with displacement of corepressors by coactivators, which is mediated through ubiquitination of corepressors, and subsequent degradation via the proteasome pathway. To determine whether this mechanism was involved in activation of PPARγ by Wnt7a/Fzd9, we examined expression of SMRT. Co-expression of Wnt7a and Fzd9 in NSCLC significantly reduced the protein expression of SMRT (Fig. 5).

A PPARγ Inhibitor SR 202 Blocks the Effects of Wnt 7a/Fzd 9 on Transformed Growth of NSCLC—The role of PPARγ in mediating the effects of Wnt7a/Fzd9 on transformed growth of NSCLC was assessed...
Wnt 7a and Fzd 9 Activate PPARγ in NSCLC

FIGURE 7. E-cadherin protein expression and promoter activity induced by Wnt 7a/Fzd 9 are reduced by the PPARγ antagonist SR202. A, extracts were prepared from the indicated transfectants using MAPK lysis buffer, and aliquots containing 100 μg of protein were resolved on 10% polyacrylamide SDS gels, transferred to nitrocellulose, and probed with an antibody to E-cadherin (125 kDa; BD Transduction Laboratories). The filters were stripped and re-immunoblotted for β-catenin (80 kDa; BD Transduction Laboratories) as a loading control. B, the indicated NSCLC cell lines were transiently transfected with the E-cadherin promoter construct, along with CMV-β-galactosidase to normalize for transfection efficiency. After an overnight incubation, cells were treated for 48 h with 20 μM SR 202 or vehicle. Extracts were prepared and promoter activity determined as luciferase units normalized to β-galactosidase. Results represent the mean of three independent experiments with the S.E. indicated. *, p < 0.05 versus empty vector; **, p < 0.05 versus Wnt7a and Fzd 9 in H157 cells or Fzd 9 in H2122 cells.

using a recently described pharmacological antagonist, SR202 (23, 24). To confirm the efficacy of this drug, H2122 cells, either stably expressing Fzd 9 or empty vector, were transiently transfected with the PPAR-RE-luciferase construct in the presence or absence of SR202 (20 μM). Exposure to SR202 completely prevented the increase in PPARγ activity seen with Fzd 9 expression (Fig. 6A), confirming our earlier conclusion that Wnt 7a and Fzd 9 selectively activate PPARγ. We next assessed the effects of this agent on colony formation in soft agar, a measure of transformed growth. Consistent with our earlier findings, expression of Fzd 9 in H2122 cells (6) strongly inhibited colony formation (Fig. 6B). Treatment with SR202 had no effect on colony formation in control cells transfected with empty vector. However, exposure to SR202 reversed the inhibition of colony formation seen with expression of Fzd 9 and resulted in ~50% of the number of colonies seen in control cells (Fig. 6, B and C). These data indicate that blocking PPARγ activation partially reverses the inhibitory effects of Wnt 7a/Fzd 9, suggesting that PPARγ is a major effector pathway downstream from Wnt 7a/Fzd 9.

E-cadherin Is Reduced in Cell Lines Expressing Wnt 7a and Fzd 9 by the PPARγ Inhibitor Compound SR 202—E-cadherin is a well known marker of epithelial differentiation (25, 26). We have previously shown that E-cadherin is induced by both Wnt 7a/Fzd 9 and PPARγ expression in NSCLC (6, 14, 15). We evaluated the effects of the PPARγ antagonist SR202 on NSCLC cell lines expressing Wnt 7a/Fzd 9. Expression of Wnt 7a/Fzd 9 resulted in a 5- to 10-fold increase in E-cadherin expression relative to the empty vector control cell lines, consistent with previous findings (Fig. 7A). The addition of SR202 to the cell lines expressing Wnt 7a/Fzd 9 resulted in a marked reduction of E-cadherin in both H157 and H2122 cells. Total β-catenin was used as a loading control (Fig. 7A, bottom panels).

We confirmed these findings by examining effects on the E-cadherin promoter (27). H157 and H2122 cells, either stably expressing Wnt 7a/Fzd 9 or empty vector, were transiently transfected with the E-cadherin-luciferase construct in the presence or absence of SR202. Wnt7a and Fzd 9 signaling stimulated E-cadherin promoter activity 5- to 10-fold, and SR202 inhibited this increase by >50% (Fig. 7B).

DISCUSSION

Deregulation of the Wnt pathway plays a key role in the development of various tumors (1, 5, 28). Whereas the canonical Wnt pathway has been demonstrated to be pro-tumorigenic, Wnt 7a is critical for the formation of normal epithelium in development and plays a significant role in maintaining a normal epithelial phenotype in the lungs of adults (29). Wnt 7a is frequently lost in NSCLC (6, 30), and its re-expression in the context of Fzd 9 has been shown to restore increased differentiation and decrease the transformed phenotype in cancer cell lines (6). In separate studies, we (14) and others have shown that thiazolidinediones, which are pharmacological activators of PPARγ, inhibit growth and promote increased differentiation in various types of tumors, including NSCLC (31, 32). We have also shown
that increased PPARγ activity, induced by overexpression of PPARγ, inhibited transformed growth and increased expression of epithelial markers in H2122 and H157 cells (15). In the current study we have demonstrated that these two pathways are linked. Specifically, expression of Wnt 7a/Fzd 9 leads to a marked increase in PPARγ activity. Furthermore, blocking PPARγ with a pharmacological inhibitor reverses both the inhibition of transformed growth and the induction of E-cadherin expression seen with Wnt 7a/Fzd 9, implying that activation of PPARγ is a major effector pathway in mediating the anti-tumorigenic effects of Wnt 7a/Fzd 9 in NSCLC.

Because Wnt 7a/Fzd 9 did not significantly increase expression of either PPARγ mRNA or protein, our data support a mechanism in which Wnt 7a and Fzd 9 activate PPARγ through a post-translational mechanism involving activation of ERK5. ERK5 is a member of the MAP kinase family that is activated by redox and hyperosmotic stress, growth factors, and pathways involving certain G-protein-coupled receptors (20, 33). ERK5 has a dual phosphorylation site like ERK1/2 but has a unique loop-12 structure and a large carboxyl-terminal, suggesting that its regulation and function may be different from that of ERK1/2 (34).

In endothelial cells, flow-mediated activation of ERK5 has been shown to lead to activation of PPARγ by direct binding to the hinge-helix region of PPARγ (18). This activation requires phosphorylation of ERK5 by the upstream activating kinase MEK5. Our data are consistent with this model, because expression of dominant negative MEK5 and pharmacological inhibition of MEK5 blocked the activation of PPARγ activity by Wnt 7a/Fzd 9. It has been demonstrated that ERK1/2 phosphorylates PPARγ on serine 82, which is in the AF1 region of the molecule (35, 36). This phosphorylation inhibits transcriptional activation by decreasing the affinity for ligand (37). Therefore, inhibition of ERK1/2 would not be expected to decrease PPARγ activity. The effects of ERK5 could be mediated by controlling the association of PPARγ with corepressors. Studies have shown that activation of MEK5/ERK5 caused displacement of silencing mediator of retinoid and thyroid hormone receptors (SMRT), a potent transcriptional co-repressor of PPARγ (38), resulting in PPARγ transcriptional activation (18). Similarly we have observed that Wnt 7a and Fzd 9 expression leads to reduced SMRT protein expression by immunoblotting (Fig. 5). This is an attractive model of activation of PPARγ, and in the absence of increases in PPARγ mRNA and protein expression could potentially account for PPARγ activation.

Wnt activation of PPARγ has been demonstrated in colon cancer cells (28). In these studies, pro-tumorigenic signaling through Wnts resulted in increased expression of PPARγ by a post-transcriptional effect. Overexpression of β-catenin also increased PPARγ, indicating that this effect was mediated through the canonical Wnt pathway (28). The biological effects of PPARγ in colon cancer remain controversial, with studies supporting both pro- and anti-tumorigenic roles. In contrast, studies in lung cancer, including data from our laboratory, support an anti-tumorigenic role for PPARγ. Furthermore, we have shown that Wnt 7a/Fzd 9 signaling is not mediated through the canonical Wnt pathway (6), indicating that the mechanisms of PPARγ induction are cell context specific.

Our studies using the pharmacological PPARγ inhibitor do not show a complete reversal of the effects of Wnt 7a/Fzd 9, and this may be due to other effector pathways that are independent of PPARγ. In this regard, we have previously demonstrated that Wnt 7a/Fzd 9 expression leads to activation of the JNK (c-Jun N-terminal kinase) pathway and increased expression of Sprouty-4 in NSCLC (6). It is likely that these pathways cooperate with activation of PPARγ in reversing the transformed phenotype. In summary, these data demonstrate a novel connection between Wnt signaling and activation of PPARγ in lung cancer. Our data as well as those of others raise the possibility that PPARγ activators may represent an effective treatment of patients with lung cancer. As with epidermal growth factor receptor inhibitors, these agents may be targeted to specific subpopulations of lung cancer cells that lack either Wnt 7a or Fzd 9. Our data would suggest that more specific pharmacological activators of ERK5 or PPARγ might have utility in the treatment of lung cancer.

**REFERENCES**

1. Polakis, P. (2000) *Genes Dev.* **14**, 1837–1851
2. Tamai, K., Svennum, M., Kato, Y., Spokony, R., Liu, C., Katsuyama, Y., Hans, F., Saint-Jeannet, J. P., and He, X. (2000) *Nature* **407**, 530–535
3. Gonzalez-Sancho, J. M., Brennan, K. R., Castelo-Soccio, L. A., and Brown, A. M. (2004) *Mol. Cell. Biol.* **24**, 4757–4768
4. Karim, R., Tse, G., Putti, T., Scolyer, R., and Lee, S. (2004) *Pathology* **36**, 120–128
5. Mazieres, J., He, B., You, L., Xu, Z., and Jablons, D. M. (2005) *Cancer Lett.* **222**, 1–10
6. Winn, R. A., Marek, L., Han, S. Y., Rodriguez, K., Rodriguez, N., Hammond, M., Van Scoy, M., Acosta, H., Mirus, I., Barry, N., Bren-Mattison, Y., Van Raay, T. J., Nemenoff, R. A., and Heasley, L. E. (2005) *J. Biol. Chem.* **280**, 19625–19634
7. Feige, J. N., Gelman, L., Michalik, L., Desvergne, B., and Wahl, W. (2006) *Prog. Lipid Res.* **45**, 120–159
8. Michalik, L., Desvergne, B., and Wahl, W. (2004) *Nat. Rev. Cancer* **4**, 61–70
9. Kinzler, K. W., and Vogelstein, B. (1996) *Cell* **87**, 159–170
10. Sarraf, P., Mueller, E., Smith, W. M., Wright, H. M., Kum, J. B., Aaltonen, L. A., de la Chapelle, A., Spiegelman, B. M., and Eng, C. (1999) *Mol. Cell.* **3**, 799–804
11. Chang, T. H., and Szabo, E. (2000) *Cancer Res.* **60**, 1129–1138
12. Tsoubouchi, Y., Sano, H., Kawahito, Y., Mukai, S., Yamada, R., Kohno, M., Inoue, K., Hia, T., and Kondo, M. (2000) *Biochem. Biophys. Res. Commun.* **270**, 400–405
13. Sasaki, H., Tanahashi, M., Yukiue, H., Mairiyama, S., Kobayashi, Y., Nakashima, Y., Kaji, M., Kiyriama, M., Fukai, I., Yamakawa, Y., and Fujii, Y. (2002) *Lung Cancer* **36**, 71–76
14. Wick, M., Hartueu, G., Dessev, C., Chan, D., Geraci, M. W., Winn, R. A., Heasley, L. E., and Nemenoff, R. A. (2002) *Mol. Pharmacol.* **62**, 1207–1214
15. Bren-Mattison, Y., Van Putten, V., Chan, D., Winn, R., Geraci, M. W., and Nemenoff, R. A. (2005) *Oncogene* **24**, 1412–1422
16. Winn, R. A., Brenness, R. M., Bemis, L., Franklin, W. A., Miller, Y. E., Cool, C., and Heasley, L. E. (2002) *Oncogene* **21**, 7497–7506
17. He, T. C., Chan, T. A., Vogelstein, B., and Kinzler, K. W. (1999) *Cell* **99**, 335–345
18. Akaie, M., Che, W., Marmarosh, N. L., Ohta, S., Osawa, M., Ding, B., Berk, B. C., Yan, C., and Abe, J. (2004) *Mol. Cell. Biol.* **24**, 8691–8704
19. Cameron, S. J., Malik, S., Akaie, M., Lerner-Marmarosh, N., Yan, C., Lee, J. D., Abe, J., and Yang, J. (2003) *J. Biol. Chem.* **278**, 18682–18688
20. Kamakura, S., Moriguchi, T., and Nishiida, E. (1999) *J. Biol. Chem.* **274**, 26563–26571
21. Mody, N., Leitch, J., Armstrong, C., Dixon, J., and Cohen, P. (2001) *FEBS Lett.* **502**, 21–24
22. Perissi, V., Aggarwal, A., Glass, C. K., Rose, D. W., and Rosenfeld, M. G. (2004) *Cell* **116**, 511–526
23. Rieusset, J., Touri, F., Michalik, L., Escher, P., Desvergne, B., Niesor, E., and
Wnt 7a and Fzd 9 Activate PPARγ in NSCLC

Wahl, W. (2002) Mol. Endocrinol. 16, 2628–2644
24. Santini, E., Fallahi, P., Ferrari, S. M., Masoni, A., Antonelli, A., and Ferrannini, E. (2004) Diabetes 53, Suppl. 3, S79–S83
25. Thiery, J. P. (2002) Nat. Rev. Cancer 2, 442–454
26. Thiery, J. P. (2003) Curr. Opin. Cell Biol. 15, 740–746
27. Haja, K. M., Chen, D. Y., and Fearon, E. R. (2002) Cancer Res. 62, 1613–1618
28. Jansson, E. A., Are, A., Greicius, G., Kuo, I. C., Kelly, D., Arulampalam, V., and Pettersson, S. (2005) Proc. Natl. Acad. Sci. U. S. A.
29. Kirikoshi, H., and Katoh, M. (2002) Int. J. Oncol. 21, 895–900
30. Calvo, R., West, J., Franklin, W., Erickson, P., Bemis, L., Li, E., Helfrich, B., Bunn, P., Roche, J., Brambilla, E., Rosell, R., Gemmill, R. M., and Drabkin, H. A. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 12776–12781
31. Demetri, G. D., Fletcher, C. D., Mueller, E., Sarraf, P., Naujoks, R., Campbell, N., Spiegelman, B. M., and Singer, S. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 3951–3956
32. Nemenoff, R. A., and Winn, R. A. (2005) Eur. J. Cancer 41, 2561–2568
33. Fukuhara, S., Marinissen, M. J., Chiariello, M., and Gutkind, J. S. (2000) J. Biol. Chem. 275, 21730–21736
34. Hayashi, M., and Lee, J. D. (2004) J. Mol. Med. 82, 800–808
35. Gelman, L., Michalik, L., Desvergne, B., and Wahli, W. (2005) Curr. Opin. Cell Biol. 17, 216–222
36. Hu, E., Kim, J. B., Sarraf, P., and Spiegelman, B. M. (1996) Science 274, 2100–2103
37. Shao, D., Rangwala, S. M., Bailey, S. T., Krakow, S. L., Reginato, M. J., and Lazar, M. A. (1998) Nature 396, 377–380
38. Yu, C., Markan, K., Temple, K. A., Deplewski, D., Brady, M. J., and Cohen, R. N. (2005) J. Biol. Chem. 280, 13600–13605