The Role of Cadherin, β-Catenin, and AP-1 in Retinoid-regulated Carcinoma Cell Differentiation and Proliferation*

Received for publication, April 2, 2002, and in revised form, May 7, 2002
Published, JBC Papers in Press, May 8, 2002, DOI 10.1074/jbc.M203158200

Salimuddin Shah‡§, Michael J. Pishvaian‡§, Vijayasurian Easwaran‡§, Powell H. Brown¶, and Stephen W. Byers‡¶

From ‡The Lombardi Cancer Research Center and the Departments of Oncology and Cell Biology, Georgetown University School of Medicine, Washington, D. C. 20007 and the ¶Division of Medical Oncology, Department of Medicine, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284

Vitamin A derivatives (retinoids) are potent regulators of cell proliferation and differentiation. Retinoids inhibit the function of the oncogenic AP-1 and β-catenin/TCF pathways and also stabilize components of the adherens junction, a tumor suppressor complex. When treated with retinoic acid (RA), the breast cancer cell line, SKBR3, undergoes differentiation and reduction in cell proliferation. The present work demonstrates that in SKBR3 cells, which exhibit high AP-1 activity, RA-regulation of cadherin expression and function, but not changes in AP-1 (or β-catenin/TCF) signaling, is responsible for the epithelial differentiation. However, cadherin function and recruitment of β-catenin to the membrane is not required for RA to regulate DNA synthesis in these cells. RA also reduces the activity of an AP-1 and TCF-sensitive cyclin D1 reporter in SKBR3 cells in a manner that is independent of the TCF site. In contrast, in SW480 cells, which have high levels of β-catenin/TCF signaling, the activity and retinoid responsiveness of the cyclin D1 promoter was markedly inhibited by mutation of the TCF site. These data indicate that the remarkably broad effects of RA on the growth and differentiation of many different epithelial cancers may well be explained by the ability of RA to differentially regulate the activity of RAR/RXR, AP-1, and β-catenin/TCF pathways.

Retinoids are important regulators of cell proliferation and differentiation and can act as potent anti-tumor agents. For example, retinoic acid (RA) inhibits the formation of papillomas in the skin of mice and can prevent the transformation of mouse JB6 cells (1–3). Retinoids also inhibit the growth of several human cancers, including melanoma, as well as colon and prostate cancer (4–8). Retinoids are important regulators of cell proliferation and differentiation and can act as potent anti-tumor agents. For example, retinoic acid (RA) inhibits the formation of papillomas in the skin of mice and can prevent the transformation of mouse JB6 cells (1–3). Retinoids also inhibit the growth of several human cancers, including melanoma, as well as colon and prostate cancer (4–8).

† The abbreviations used are: RA, retinoic acid; TPA, 12-O-tetradecanoylphorbol-13-acetate; GFP, green fluorescent protein; FACS, fluorescence-activated cell sorter; RARE, retinoic acid responsive element; TCF, T cell factor; RAR, retinoic acid receptor; RXR, retinoid X receptor.

inhibit the growth of a number of breast cancer cell lines (10–12) and can reduce tumor incidence, average number of tumors, and average tumor burden in a rat breast cancer model (10). In some instances the effects of RA on cell growth have been attributed to the ability of RA to down-regulate AP-1 activity (1, 13, 14). However, it is not clear if the effects of retinoic acid on cell differentiation can be separated from its effects on cell growth. We and others have previously demonstrated that the effects of retinoids may involve modulation of adherens junction structure and function (11, 15–17).

The adherens junction is a molecular complex that is essential for initiating and maintaining strong cell-cell adhesion in epithelial cells (18). The basic components of the adherens junction include a trans-membrane cadherin molecule, the cytoplasmic catenins, and the actin cytoskeleton (19). Cadherins are calcium-dependent cell adhesion molecules that are involved in the organization of the developing embryo, and are essential for the maintenance of tissue integrity in the adult (20). In epithelial cells, loss of function or expression of E-cadherin is correlated with the progression of tumors to a more invasive phenotype (21). Moreover, expression of other cadherins can inhibit invasion and thus compensate for a loss of E-cadherin (22–24). Loss of cadherin function may also be mediated in other ways. For example, loss of α- or β-catenin protein expression can disrupt normal cell-cell adhesion (25, 26). Alternatively, cadherin function can be modulated by tyrosine kinase activity (27). For example, the v-src oncprotein phosphorylates tyrosine residues on β-catenin and cadherins and disrupts the adherens junction, resulting in a shift to a fibroblastoid phenotype that exhibits increased invasiveness (28, 29). Finally, loss of cadherin function may be mediated by activity of the transcription factor complex, AP-1. AP-1 is made up of the proto-oncogenes jun and fos, and its activity is associated with cell proliferation and neoplastic transformation (30). Activation of c-Jun in mammary epithelial cells resulted in a loss of epithelial polarity, a disruption of intercellular junctions and normal barrier function, and the formation of irregular multilayers. These morphological changes were accompanied by a reduction in the association between E-cadherin and β-catenin (17).

In the breast cancer cell line, SKBR3, RA reduces cell proliferation and induces a cell differentiation (15). We now show that modulation of AP-1 or β-catenin/TCF signaling is not involved in RA-induced cell differentiation. However, cadherin expression and function are necessary and sufficient to mediate the effects of RA on adhesion and differentiation but are not required for RA-mediated inhibition of cell proliferation.

EXPERIMENTAL PROCEDURES

Cell Lines and Treatments—SKBR3 and SW480 cells were obtained from ATCC and grown in Dulbecco’s modified Eagle’s medium (Invitrogen) plus 10% fetal bovine serum as described previously (31). Cells were treated with 1 μM 9-cis-RA or ethanol for 48 h. Several experi-
Subcellular Fractionation—Cells from confluent 10-cm dishes were isolated and subjected to sequential Dounce homogenization in a hypotonic solution (10 mM Tris, 0.2 mM MgCl₂, pH 7.5) (32). This procedure resulted in cell lysis and the isolation of sheets of plasma membrane rather than vesicles (32). The homogenate was spun first for 10 min at 10,000 × g to remove nuclei. The supernatant was then ultracentrifuged at 150,000 × g for 1 h. The supernatant, defined as the cytosolic fraction, was added to 4 volumes of ethanol, and the proteins were precipitated overnight. The proteins were then resolubilized by ultracentrifugation and solubilized in sample buffer (2% SDS, 60 mM Tris, pH 6.8, 10% glycerol). The pellet from the original ultracentrifugation was solubilized in a 1% Nonidet P-40 buffer (1% Nonidet P-40, 150 mM NaCl, 50 mM Tris, pH 8.0) for 30 min, then reclarified in a microcentrifuge for 15 min to remove insoluble material and cell debris. The resulting supernatant, the Nonidet P-40-soluble fraction, was then added to sample buffer.

Western Blotting—Bio-Rad DC Protein Assay kit was used to measure protein content in the samples. 25 μg of protein were separated on an 8% reducing polyacrylamide mini-gel (Novex), transferred onto nitrocellulose (Protran), and blocked overnight in 5% skim milk. The blot was then probed with an appropriate antibody followed by a secondary peroxidase-labeled antibody (Kirkegaard and Perry), and the bands were visualized by enhanced chemiluminescence (Amersham Biosciences). The blots were then stripped at 50 °C for 30 min (stripping solution: 62.5 mM Tris, pH 7.5, 2% SDS, 1.7% (v/v) β-mercaptoethanol), washed two times in phosphate-buffered saline, and blocked in 5% milk prior to reprobing.

Immunocytochemistry—Cells were grown on 12-mm coverslips. Most coverslips were fixed in 100% ice-cold methanol for 3 min at −20 °C. To retain green fluorescent protein staining, cells were fixed in 2% paraformaldehyde for 30 min followed by 0.5% Triton X-100 for 5 min. Coverslips were then blocked in 3% ovalbumin for 30 min. For double labeling, the cells were also treated with RA for 48 h prior to fixation. The cells were fixed and stained for β-catenin. RA dramatically increased the expression of β-catenin at sites of cell-cell contact in vector-treated cells (compare A to B). However, despite the high level of β-catenin expression there was no change in cell morphology at cell-cell contact sites (C). SKBR3 cells were transfected with vector (pcDNA3) or with wild type β-catenin, and Western blotting was performed using whole cell lysates (D).

FIG. 1. Transient overexpression of β-catenin is not sufficient to induce a morphologic change. SKBR3 cells were transfected with vector alone (A and B) or wild type β-catenin (C). In B, the cells were also treated with RA for 48 h prior to fixation. The cells were fixed and stained for β-catenin. RA dramatically increased the expression of β-catenin at sites of cell-cell contact in vector-treated cells (compare A to B). However, despite the high level of β-catenin expression there was no change in cell morphology at cell-cell contact sites (C). SKBR3 cells were transfected with vector (pcDNA3) or with wild type β-catenin, and Western blotting was performed using whole cell lysates (D).
trypsinized and sorted by FACS, isolating cells expressing high levels of GFP. Cells were replated on 12-mm coverslips, then treated with RA or vehicle for 48 h, fixed, and stained as described above. Luciferase Reporter Assays—Cells were seeded in 12-well plates at 1 × 10⁵ cells per well. Cells were transiently transfected using the calcium phosphate method (36). For luciferase assays, cells were transfected with 1 μg of either the wild type or mutant E-cadherin-luciferase construct, AP-1 luciferase construct, CD1-luciferase construct or with LEF-reporter pTOPFLASH/pFOPFLASH (35) along with 0.02 μg of pCMV-Renilla luciferase (Promega) (32). RA treatment was initiated 24 h posttransfection. Luciferase activity was monitored using the DUAL-luciferase Assay System (Promega). The experimental reporter activity was controlled for transfection efficiency by comparison with the constitutively expressed Renilla luciferase.

RESULTS

β-Catenin Overexpression Is Not Sufficient to Mimic the Effects of RA on Cell Morphology and Differentiation—RA has a profound effect on cell-cell adhesion and cell differentiation in a number of breast cancer cell lines, effects that are accompanied by an increase in membrane-associated β-catenin (10, 15). We also showed that RA treatment stabilizes β-catenin protein levels without affecting the steady state levels of its mRNA. To determine whether overexpression of β-catenin is sufficient to mimic the effects of RA on cell morphology and differentiation, SKBR3 cells were transfected with wild type β-catenin and its S37A-stable mutant form. Immunocytochemistry was performed to detect β-catenin protein. Fig. 1 shows that the morphology of the cells transfected with β-catenin was similar to that of untreated control cells despite the high expression of β-catenin, most of which was detected in the cytoplasm. Similar results were obtained in cells transfected with S37A β-catenin (not shown). However, upon RA treatment a dramatic change in cell morphology was observed in untransfected and β-catenin-transfected cells. Following RA treatment most of β-catenin was translocated to the cell membrane. These results showed that increased levels of β-catenin alone are not responsible for RA-induced changes in cell morphology and differentiation. However, it is possible that translocation of β-catenin to the membrane is mandatory for RA-mediated effects on cell morphology. To test this we examined if cadherin-mediated translocation of β-catenin to the membrane could mimic the effects of RA on cell morphology.

Cadherin Expression and RA-induced Cell Differentiation—To test if exogenous expression of E-cadherin is sufficient...
FIG. 3. Exogenous expression of E-cadherin phenocopies the effects of RA on β-catenin expression and localization. SKBR3 cells were transfected with a human E-cadherin construct, hecD pCDNA3 (A and B). The cells were then stained for E-cadherin (A) or β-catenin (B). Arrows point to the same cells in corresponding pictures. E-cadherin expression resulted in an increase in the expression of β-catenin, and both E-cadherin and β-catenin were localized to sites of cell-cell contact (A and B). C, Western blotting was performed using Nonidet P-40 lysates from cells treated and untreated with RA. SKBR3 cells were transfected with the empty vector, pCDNA3, or with E-cadherin. After transfection, cells were treated with RA (1 μM) for 48 h. Transient expression of E-cadherin increased the β-catenin levels in the transfected cells.

FIG. 4. RA treatment increases endogenous cadherin expression in SKBR3 cells. Cells were fixed in methanol and microwaved. A pan-cadherin antibody was used to detect cadherin expression in RA-untreated (A) and RA-treated (B) cells. RA dramatically increases the expression of a cadherin at sites of cell-cell contact (B). C, cellular subfractionation confirms that RA increases cadherin expression in the membrane-associated pool.
to mimic RA-induced effects, SKBR3 cells were transfected with an E-cadherin construct, hecD pCDNA3, along with a green fluorescent protein expression vector (pEGFP), and green cells were separated by FACS, replated, and grown with or without RA for 48 h. As expected RA markedly affected cell morphology (Fig. 2C; compare with untreated control in Fig. 2B). Similar effects were observed in the cells transfected with wild type E-cadherin expression vector (Fig. 2D) but not in the cells overexpressing $\beta$-catenin (Fig. 2E). E-cadherin expression also increases the translocation of $\beta$-catenin protein to the membrane as revealed by immunocytochemistry (Fig. 3B). Western blot analysis of whole cell lysates of cells overexpressing full-length E-cadherin revealed an increased level of $\beta$-catenin protein (Fig. 3C). Note that the increase in $\beta$-catenin expression is less than that induced by RA because these experiments were performed as transient transfection assays. To determine whether RA can directly regulate cadherin expression, SKBR3 and MCF-7 cells were transfected with an E-cadherin-luciferase promoter construct (37) and Renilla luciferase as a transfection efficiency control. After transfection, cells were grown in the presence or absence of RA and harvested after 48 h to measure reporter activity. A significant increase in the E-cadherin reporter activity was detected in the presence of RA (Fig. 2A). These results indicate that RA can increase the activity of an E-cadherin promoter and that exogenous expression of E-cadherin is sufficient to mimic RA-induced changes in cell morphology and recruitment of $\beta$-catenin to the cell membrane. We next examined if RA influenced endogenous cadherin expression.

The rounded and poorly adhesive phenotype of untreated SKBR3 cells is consistent with diminished cadherin expression or function and we showed several years ago that these cells do not express E-cadherin (31). Consistent with this Pierceall et al. showed that these cells have a homozygous deletion of the
E-cadherin gene (38). Nevertheless the calcium dependence of RA-induced morphological changes, the recruitment of β-catenin to the membrane, as well as the effects of RA on cadherin promoter activity strongly point to a role for an endogenous cadherin in mediating the effects of RA on SKBR3 cells.

To test RA effects on endogenous cadherin expression in SKBR3 cells, we used a polyclonal pan-cadherin antibody raised against a region of the highly conserved N-cadherin C-terminal, to detect cadherin protein. This antibody recognizes most cadherins except notably E-cadherin (39, 40). As presented in Fig. 4, A and B, RA treatment dramatically increased cadherin levels at cell-cell contact sites. Western blot analysis showed a dramatic RA-induced increase in an immunoreactive band at ~120 kDa, consistent with the molecular mass of most type I and II cadherins (Fig. 4C). A variety of approaches have shown that the RA-induced cadherin in SKBR3 cells is not E-, N-, P-, or LI-cadherin, cadherin-6 or-11 (results not shown). However, our attempts to identify this cadherin by classical methods were unsuccessful. This suggests that the unidentified cadherin may be a novel cadherin that is regulated directly or indirectly by RA. Taken together our results indicate that RA increases endogenous cadherin expression.

Inhibition of AP-1 Activity Is Neither Necessary nor Sufficient to Mimic the Effects of RA on Cell Morphology—High AP-1 activity can affect differentiation and changes in cadherin and catenin expression (17). As it is well known that RA can inhibit AP-1 activity we next investigated the role of AP-1 in RA-mediated cell differentiation. To determine the role of AP-1 in RA-induced changes in SKBR3 cells, reporter assays were performed using an AP-1 luciferase reporter construct. A mutant construct with a three-nucleotide mutation in the AP-1 site was used as a negative control. We confirmed that RA reduced AP-1 reporter activity in SKBR3 cells in a dose-dependent manner (Fig. 5A). As a control dominant-negative c-jun, TAM-67 also significantly reduces AP-1 reporter activity (Fig. 5A). To determine whether AP-1 function can inhibit the effects of RA on cell morphology and β-catenin protein levels, SKBR3 cells were transfected with TAM-67 or with a c-jun expression vector (41). Neither TAM-67 nor c-jun altered β-catenin proteins levels in cell lysates (Fig. 5, B–D). Similarly TAM-67 could not induce any changes in cell morphology, β-catenin levels, or localization as detected by immunocytochemistry (Fig. 6, A and B). Likewise, overexpression of c-jun (to increase AP-1 activity) was also not sufficient to inhibit the function of RA in altering cell morphology or β-catenin expression (Fig. 6, C and D). These results indicate that AP-1 activity is not required for RA-induced morphological changes and differentiation in SKBR3 cells.

Physiological Calcium Levels Are Necessary for RA Treatment to Change Subcellular Distribution of β-Catenin—The mechanism whereby RA increases the membrane pool of β-catenin is most likely the result of increased cadherin expression. This study and others have shown that exogenous expression of cadherins can recruit cytoplasmic β-catenin to the membrane pool, an effect that depends upon the ability of the cadherin cytoplasmic tail to bind β-catenin (42, 43). We next wanted to test if RA treatment could reduce cytoplasmic levels of exogenously expressed β-catenin in SKBR3 cells. We performed three-pool subcellular fractionation to isolate cytosolic, an Nonidet P-40-soluble plasma membrane fraction and an Nonidet P-40-insoluble fraction. Fig. 7A shows that cytoplasmic levels of β-catenin are markedly elevated following transient transfection. RA-treatment significantly reduces the level of cytoplasmic β-catenin with a corresponding increase in membrane fraction. RA also decreased the cytoplasmic level of the degradation-resistant S37A mutant form of β-catenin (data not shown). If the ability of RA to reduce cytoplasmic β-catenin depended upon cadherin function we would expect that treatment of cells with low calcium medium should reverse these effects of RA. Fig. 7B shows that exposure of cells to low calcium medium prevents the RA-mediated decrease in the level of cytoplasmic β-catenin in transiently transfected cells. These results strongly suggest that the ability of RA to reduce cytoplasmic β-catenin depends on cadherin expression.

β-Catenin protein levels and its subcellular distribution are tightly regulated. β-Catenin is present in two pools: a membrane pool, required for cell-cell adhesion; and cytoplasmic/nuclear pool, responsible for β-catenin/TCF signaling. Several studies have shown that translocation of cytoplasmic β-catenin to the membrane can reduce β-catenin/TCF signaling (44–46). However, our results showed that RA-treatment inhibits β-catenin/TCF reporter activity even in the low calcium medium (Fig. 7C), which is consistent with our demonstration that RAR is able to bind directly to β-catenin and inhibit β-catenin/TCF signaling in the presence of RA (47). We next investigated whether the RA effects on cell proliferation can be separated from its effects on cell differentiation.

Cadherin Expression and Function Is Not Necessary for RA-mediated Effects on Cell Proliferation—RA has a well documented inhibitory effect on cell proliferation. We showed previously that DNA synthesis is markedly inhibited by RA in SKBR3 cells (15). E-Cadherin has been implicated as a tumor/invasion suppressor, although there is little evidence that it...
directly affects cell proliferation (48, 49). Thus we wanted to determine whether RA effects on DNA synthesis could be mimicked by exogenous expression of E-cadherin or β-catenin. Fig. 8A shows that in control cells after 48 h of RA treatment, DNA synthesis, as measured by tritiated thymidine uptake, was reduced by ~50%. E-Cadherin transfection alone did not significantly reduce DNA synthesis in this time period but, as expected, did exert a marked morphological transformation (see Fig. 4). Moreover, DNA synthesis in E-cadherin-transfected cells was still reduced by RA. In this experiment, FACSSorted E-cadherin-transfected cells were used. These experiments show that cadherin expression does not mediate the growth inhibitory effects of RA. However, it is possible that calcium-dependent adhesion is still required for RA to inhibit cell proliferation. To test this we grew cells in physiological (2 mM) but not low (50 μM) calcium. RA treatment reduced cytoplasmic β-catenin levels in 2 mM calcium but not 50 μM calcium (data not shown but see Ref. 15). These results show that RA-mediated inhibition of cell proliferation is independent of the cadherin/β-catenin and calcium-dependent adhesion pathways in SKBR3 cells. To further investigate RA-induced inhibition of cell proliferation we tested the effects of RA on cyclin D1 expression.

**Regulation of Cyclin D1 Promoter Activity by RA**—RA inhibits cell proliferation by influencing progression from G1 to S-phase, which in turn depends on changes in cyclin D1 expression (CD1). Cyclin D1 expression and the activity of CD1 reporters are under complex regulation and are markedly influenced by AP-1 and β-catenin/TCF signaling pathways (50). To test if RA inhibition of β-catenin signaling plays a role in the regulation of CD1 expression, SKBR3 cells were transfected with wild type or TCF-deleted CD1-luciferase constructs (50). In SKBR3 cells, RA treatment significantly inhibits the activity of both wild type as well as TCF-deleted CD1 reporter activity showing that, in these cells, CD1-reporter activity is not predominantly regulated by β-catenin/TCF signaling (Fig. 8C). This is not surprising because SKBR3 cells have high AP-1 and low β-catenin/TCF activity (47). Therefore, the effects of RA on cell proliferation and CD1 expression in SKBR3 cells are probably mediated by inhibition of AP-1 not β-catenin/TCF activity. Nevertheless, it is possible that, CD1 activity in cells, which have high β-catenin/TCF signaling, is predominantly regulated by this pathway. To test this we used APC (adenomatous polyposis coli) mutant SW480 colorectal...
cancer cells, which have very high β-catenin/TCF activity but low AP-1 activity. In SW480 cells RA significantly decreased wild type CD1 reporter activity but had no effect on the β-catenin/TCF CD1 reporter (Fig. 8C). These results indicate that, unlike SKBR3 cells, RA repression of CD1 activity in SW480 cells depends on β-catenin/TCF signaling.

Taken together, our data strongly suggest that RA-mediated decreases in CD1-activity and DNA synthesis in SKBR3 breast cancer cells are due to changes in AP-1 and/or β-catenin/TCF activity that directly inhibit cell proliferation (47). In contrast, the effects of RA on cell morphology and differentiation are independent of AP-1 or β-catenin/TCF signaling. Therefore, it can be concluded that RA differentially regulates β-catenin/cadherin and β-catenin/TCF and AP-1 pathways.

DISCUSSION

Retinoids are potent regulators of cell proliferation and cell differentiation. The actions of RA are mediated through its receptors (RAR and RXR), which upon ligand binding affect at least three important transcriptional activation pathways. RA-activated RAR/RXR heterodimers bind to the RARE-containing promoter elements of many genes to regulate transcription (51). RA-activated RARs can also inhibit the action of the Fos/Jun AP-1 complex thereby blocking the transcription of AP-1-regulated genes (52). Finally, we have demonstrated that RA-activated RAR can inhibit β-catenin/TCF transactivation by directly binding to β-catenin (47). The pleiotropic actions of RA are probably a result of the differential regulation of these three pathways. However, it is not clear which of these pathways accounts for the effects of RA on cell differentiation and proliferation and whether the RA effects on cell proliferation can be separated from the effects on cell differentiation. Using the breast cancer cell SKBR3 as a model system we now show that these actions of RA can be separated.

RA Effects on SKBR3 Cell Differentiation Are Not Regulated by AP-1 or β-Catenin/TCF Pathways but Do Require Calcium-
expression of E-cadherin should show similar effects to RA. In SKBR3 cells, very little β-catenin signaling is detected even though these cells do not express E-cadherin and levels of TCF family members are comparable with other epithelial cells. Overexpression of β-catenin can increase β-catenin signaling indicating that this pathway can be activated in these cells (47). These observations indicate that SKBR3 cells might have adjusted their degradation machinery to remove free β-catenin very efficiently from the cytoplasm (53). In response to RA, β-catenin protein levels at the membrane increased significantly although mRNA levels remained constant (15). The translocation of β-catenin to the membrane could be due to either RA-mediated inhibition of β-catenin degradation or to RA-mediated increase in cadherin/s expression. If RA effects were solely due to β-catenin stabilization, we would expect that overexpression of β-catenin could mimic the effects of RA on cell morphology and differentiation. On the other hand, if RA effects are due to increased cadherin expression then over expression of E-cadherin should show similar effects to RA. Our experiments show that overexpression of cadherin but not β-catenin can mimic RA effects on cell morphology and differentiation in SKBR3 cells. However, it is also possible that both inhibition of degradation machinery as well as increased cadherin expression are involved and are working synergistically in RA-induced changes.

We also showed that RA-induced changes in cell morphology and differentiation are calcium-dependent, which confirms the requirement for cadherin. Because these cells do not express E-cadherin another cadherin must be involved in this process. Several unsuccessful attempts were made to identify the RA-responsive cadherin in SKBR3 cells. It should be noted that vitamin D-activated vitamin D receptor can also increase E-cadherin expression and promoter activity in colorectal cancer cells (54).

Promoter analysis indicates that there are no retinoic acid responsive elements in the 1-kb promoter of E-cadherin. Nonetheless our reporter assays showed that a 783-bp E-cadherin promoter responded to RA treatment. This paradox can be explained if we assume that RA effects on cadherin expression may not be direct. In several breast cancer cell lines including SKBR3, AP-1 activity is very high and might negatively regulate cadherin expression. Because RA treatment can inhibit AP-1 activity, it is possible that RA inhibition of AP-1 activity in mediating the RA effects on SKBR3 cell proliferation and cyclin D1 expression. In other cells such as SW480 colorectal cancer cells that do have high levels of β-catenin/TCF signaling RA could inhibit cell proliferation by directly interfering with this pathway rather than AP-1. Thus, the remarkable broad effects of RA on the growth and differentiation of many different epithelial cancers may well be explained by its ability to differentially regulate the activity of these three important pathways. In addition to co-activating TCF-regulated genes β-catenin can augment the ability of ligand to activate RA, androgen, and vitamin D-responsive promoters (54, 55). Taken together with the recent discovery that wnt and RA signaling cooperate to regulate the expression of the RA-responsive gene Stra6, these data indicate that cross-regulation of wnt/β-catenin/TCF and steroid receptor pathways may well be important in a number of embryological and neoplastic settings (56).

Acknowledgments—We acknowledge the generous contribution of cyclin D1 reporters by Dr. Richard Pestell.

REFERENCES

1. Li, J. J., Dong, Z., Dawson, M. I., and Colburn, N. H. (1996) Cancer Res. 56, 483–489
2. Verma, A. K., Garcia, C. J., Ashendel, C. L., and Boutwell, R. K. (1983) Cancer Res. 43, 3045–3049
3. Verma, A. K., Slaga, T. J., Wertz, P. W., Mueller, G. C., and Boutwell, R. K. (1980) Cancer Res. 40, 2367–2371
4. Fliegel, S. E., Inman, D. R., Talwar, H. S., Fisher, G. J., Voorhees, J. J., and Varani, J. (1992) J. Cutan. Pathol. 19, 27–33
5. Reiman, Z. M., and Mackie, R. M. (1992) Clin. Exp. Metastasis 10, 61–67
6. Housein, N. M., Brattain, D. E., and McKnight, M. K. (1988) Cancer Lett. 40, 219–232
7. O’Dwyer, P. J., Ravikuma, T. S., McCabe, D. P., and Steele, G., Jr. (1987) J. Surg. Res. 43, 550–557
8. Blatt, S. E., Allegretto, E. A., Pike, J. W., and Weigel, N. L. (1997) Endocrinology 138, 1491–1497
9. Hong, W. K., and Iri, L. M. (1994) In The Retinoids: Biology, Chemistry, and Medicine (Sporn, M. B., Roberts, A. B., and Goodman, D. S., eds) pp. 597–630, Raven Press, New York
10. Anzano, M. A., Byers, S. W., Smith, J. M., Peer, C. W., Mullen, L. T., Brown,
Retinoid-regulated Carcinoma Cell Differentiation and Proliferation

C. C., Roberts, A. B., and Sporn, M. B. (1994) Cancer Res. 54, 4614–4617

11. Fitzgerald, P., Teng, M., Chandraratna, R. A., Heyman, R. A., and Allegretto, E.A. (1997) Cancer Res. 57, 2642–2650, 6292–6297

12. Sheikh, M. S., Shao, Z. M., Chen, J. C., Hussein, A., Jetten, A. M., and Vermeulen, S. J., Bruyneel, E. A., Van Roy, F. M., Mareel, M. M., and Bracke, M. E. (1995) Br. J. Cancer 72, 1447–1453

13. Allegretto, E. A. (1997) Cancer Res. 57, 2642–2650, 6292–6297

14. Tozeren, A., Sporn, M., and Byers, S. (1997) Cancer Res. 57, 2642–2650, 6292–6297

15. Byers, S., Pishvaian, M., Crockett, C., Peer, C., Tozeren, A., Sporn, M., Anzano, M., and Lechleider, R. (1996) Endocrinology 137, 3265–3273

16. Takeichi, M. (1993) J. Membr. Biol. 159, 159–164

17. Fontana, J. A. (1993) Mol. Carcinog. 5, 120, 108–119

18. Hamaguchi, M., Matsuyoshi, N., Ohnishi, Y., Gotoh, B., Takeichi, M., and Angel, P., and Karin, M. (1991) Science 250, 2211–2215

19. Kemler, R. (1993) J. Cell Biol. 120, 571–576

20. Hoogewijs, A., Fiers, W. C., and Van Roy, F. M. (1991) Mol. Endocrinol. 5, 1415–1418

21. Takeichi, M. (1992) J. Cell Biol. 119, 703–714

22. Takeichi, M. (1993) EMBO J. 12, 307–314

23. Angel, P., and Karin, M. (1991) J. Cell Biol. 117, 1119–1157

24. Sommers, C. L., Thompson, E. W., Torri, J. A., Kemler, R., Gelmann, E. P., and Byers, S. W. (1991) Cell Growth Differ. 2, 365–372

25. Easwaran, V., Vakaet, L., Jr., Mareel, M., Fiers, W., and Van Roy, F. M. (1991) Mol. Endocrinol. 5, 1453–1456

26. Van den Berghe, P., and Schon, T. (1996) J. Biol. Chem. 271, 24735–24738

27. Schon, T., and Van den Berghe, P. (1996) J. Biol. Chem. 271, 24735–24738

28. Stockinger, A., Eger, A., Wolf, J., Beug, H., and Foss, F. (1991) Cell 66, 107–119

29. Vleminckx, K., Vakaet, L., Jr., Mareel, M., Fiers, W., and Van Roy, F. M. (1991) Mol. Endocrinol. 5, 1415–1418

30. Takeichi, M. (1990) Annu. Rev. Biochem. 20, 394–404

31. Kolch, W., and Lutz, H. (1997) Cell 8, 24735–24738

32. Matsumoto, N., Fuhito, H., Kimura, K., and Nagai, Y. (1993) J. Membr. Biol. 159, 159–164

33. Allegretto, E. A. (1997) Cancer Res. 57, 2642–2650, 6292–6297

34. Takeichi, M. (1990) Annu. Rev. Biochem. 20, 394–404

35. Takeichi, M. (1993) J. Membr. Biol. 159, 159–164

36. Sambrook, J., Fritsch, E., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2 Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

37. Batlle, E., Sancho, E., Franci, C., Dominguez, D., Monfar, M., Baulida, J., and Garcia, D. H. (2000) Nat. Cell Biol. 2, 84–89

38. Pierceall, W. E., Woodward, A. S., Morrow, J. S., Rimm, D., and Fearon, E. R. (1995) Oncogene 11, 1319–1326

39. Hazan, R. B., Kang, L., Wheeley, B. P., and Borgen, P. I. (1997) Cell Adhes. Commun. 4, 399–411

40. Pishvaian, M. J., Feltes, C. M., Thompson, P., Russemakers, M. J., Schalken, J. A., and Byers, S. W. (1999) Cancer Res. 59, 947–952

41. Brown, P. H., Chen, T. K., and Birrer, M. J. (1994) Oncogene 9, 791–799

42. Sadot, E., Simcha, I., Shuitman, M., Ben, Z., and Geiger, B. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 15339–15344

43. Fugotto, P., Panayotova, N., Gluck, U., and Gumbiner, B. M. (1996) J. Cell Biol. 132, 1105–1114

44. Gottardi, C. J., Wong, E., and Gumbiner, B. M. (2001) J. Cell Biol. 153, 1049–1060

45. Sellin, J. H., Umar, S., Xiao, J., and Morris, A. P. (2001) Cancer Res. 61, 2899–2906

46. Shabani, T., and Schon, T. (1996) J. Biol. Chem. 271, 24735–24738

47. Takeichi, M. (1993) J. Cell Biol. 120, 571–576

48. Takeichi, M. (1993) Curr. Opin. Cell Biol. 5, 806–811

49. Angel, P., and Karin, M. (1991) Molecular Cloning: A Laboratory Manual, 2 Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

50. Houle, J. D., and Reier, P. J. (1989) Science 247, 525–538

51. Easwaran, V., Vakaet, L., Jr., Mareel, M., Fiers, W., and Van Roy, F. (1991) Cell 66, 107–119

52. Houle, J. D., and Reier, P. J. (1989) Neurosci. Lett. 103, 253–258

53. Mangelsdorf, D. J., and Evans, R. M. (1994) in The Retinoids: Biology, Chemistry, and Medicine (Sporn, M. B., Roberts, A. B., and Goodman, D. S., eds) pp. 319–350, Raven Press, New York

54. Zhou, X. F., Shen, X. Q., and Shemeshdini, L. (1999) Mol. Endocrinol. 13, 276–285

55. Spiegelman, V. S., Slaga, T. J., Pagano, M., Minamoto, T., Ronai, Z., and Fuchs, S. Y. (2000) Mol. Cell 5, 877–882

56. Palter, H. G., Gonzalez-Sancho, J. M., Espada, J., Bericiante, M. T., Puig, I., Baulida, J., Quintanilla, M., Cano, A., de Herreros, A. G., Lafarga, M., and Muno, A. (2001) J. Cell Biol. 154, 369–387

57. Trucita, C., Byers, S., and Gelmann, E. (2000) Cancer Res. 60, 4709–4713

58. Szeto, W., Jiang, W., Tice, D. A., Rubinfeld, B., Hollingshead, P. G., Fong, S. E., Dugger, D. L., Pham, T., Yansura, D. G., Wong, T. A., Grimaldi, J. C., Corpuz, R. T., Singh, J. S., Franzt, G. D., Devaux, B., Crowley, C. W., Schwall, R. H., Eberhard, D. A., Rastelli, L., Polakis, P., and Pennica, D. (2001) Cancer Res. 61, 4197–4205

59. Schulte, R., Rangarajan, P., Yang, N., Kliwier, S., Ransome, L. J., Belado, J., Verma, I., and Evans, R. M. (1991) Proc. Natl. Acad. Sci. 88, 6092–6096
The Role of Cadherin, β-Catenin, and AP-1 in Retinoid-regulated Carcinoma Cell Differentiation and Proliferation
Salimuddin Shah, Michael J. Pishvaian, Vijayasurian Easwaran, Powell H. Brown and Stephen W. Byers

*J. Biol. Chem.* 2002, 277:25313-25322.
doi: 10.1074/jbc.M203158200 originally published online May 8, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M203158200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 54 references, 25 of which can be accessed free at http://www.jbc.org/content/277/28/25313.full.html#ref-list-1