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Transcriptional Activation of *Cyclooxygenase-2* in Wnt-1-transformed Mouse Mammary Epithelial Cells[1]

Louise R. Howe, Kotha Subbaramaiah, Wen Jing Chung, Andrew J. Dannenberg, and Anthony M. C. Brown[2]

Strang Cancer Research Laboratory, The Rockefeller University, New York, New York 10021 [L. R. H., K. S., W. J. C., A. J. D., A. M. C. B.], and Departments of Cell Biology and Anatomy [L. R. H., A. M. C. B.] and Medicine [K. S., W. J. C., A. J. D.], Weill Medical College of Cornell University, New York, New York 10021

ABSTRACT

***Wnt-1* acts as a mammary oncogene when ectopically expressed in the mouse mammary gland. *APC* is a tumor suppressor gene, mutations in which cause intestinal tumorigenesis in humans and rodents. Both *Wnt-1* expression and *APC* mutation activate a common signaling pathway involving transcriptional activation mediated by β-catenin/Tcf complexes, but few targets relevant to carcinogenesis have yet been identified. Expression of the inducible prostaglandin synthase cyclooxygenase-2 appears critical for intestinal tumorigenesis resulting from *APC* mutation, suggesting that *cyclooxygenase-2* might be a transcriptional target for β-catenin/Tcf complexes. Here, we have investigated the effect of Wnt-1 on *cyclooxygenase-2* expression. *Wnt-1* expression in the mouse mammary epithelial cell lines RAC311 and C57MG induces stabilization of cytosolic β-catenin and morphological transformation. Expression of *Wnt-1* in these cells caused transcriptional up-regulation of the *cyclooxygenase-2* gene, resulting in increased levels of cyclooxygenase-2 mRNA and protein. Prostaglandin E_2 production was increased as a consequence of the elevated cyclooxygenase-2 activity and could be decreased by treatment with a selective cyclooxygenase-2 inhibitor. Cyclooxygenase-2 thus appears to be a common downstream target for *APC* mutation and *Wnt-1* expression. In view of the critical role of cyclooxygenase-2 in intestinal tumorigenesis, *cyclooxygenase-2* up-regulation in response to Wnt signaling may contribute to *Wnt*-induced mammary carcinogenesis.**

INTRODUCTION

Wnt-1 was originally identified as a mammary oncogene activated by proviral insertions of mouse mammary tumor virus (1–3). Ectopic expression of *Wnt-1* under the control of a mouse mammary tumor virus promoter leads to extensive mammary hyperplasia and subsequent generation of adenocarcinomas in mice (4). Cell culture experiments demonstrate that multiple *Wnt* gene family members including *Wnt-1* can cause partial cellular transformation of some epithelial and fibroblastic cell lines (5–12). Collectively, these data implicate *Wnt-1* as an oncogene when inappropriately expressed. Several *WNT* gene family members have been found to be overexpressed in a proportion of human breast cancers and may therefore contribute to carcinogenesis in humans (13–17).

The *Wnt-1* gene encodes a secreted protein that functions as an extracellular ligand capable of promoting mitogenesis (18–21). Wnt-1 appears to signal via a unique pathway, thought to be initiated by interaction of Wnt-1 with a member of the Frizzled family of seven-transmembrane receptors, leading to stabilization of a cytosolic pool of β-catenin (22). Accumulated β-catenin can translocate to the nucleus, interact with Tcf transcription factors, and thereby mediate transcriptional activation (11, 23–31).

β-Catenin/Tcf-mediated transcriptional activation has recently been implicated in human carcinogenesis. Elevated cytosolic β-catenin and transcriptionally active β-catenin/Tcf complexes have been detected in both colon carcinomas and melanomas (32–34). β-Catenin accumulation can occur as a consequence of mutation of either the *β-catenin* gene itself or the tumor suppressor gene *APC*, because wild-type APC protein contributes to β-catenin destabilization (32–36). Mutations in *APC* cause intestinal tumorigenesis in humans and mice. Although the molecular mechanism by which *APC* mutation induces tumorigenesis is unclear, many data implicate cyclooxygenase enzymes in this process (37). Cox-1[3] and Cox-2 are constitutively expressed and inducible isoforms of prostaglandin synthase, respectively (gene symbols, *Ptgs1* and *Ptgs2*; Ref. 38). *COX-2* expression has been detected in intestinal tumors of both mice and humans with *APC* mutations (39–41). Genetic ablation of the *Cox-2* gene or pharmacological inhibition of Cox-2 activity dramatically reduces the incidence of intestinal tumors in *Apc* mutant mice (42).

Thus, both *APC* mutation and ectopic *Wnt-1* expression can cause tumorigenesis, and this may be, at least in part, via a common signaling pathway involving β-catenin/Tcf complexes. Furthermore, Cox-2 appears critical for tumor formation resulting from *APC* mutation. Consequently, we reasoned that Cox-2 might also be a target for Wnt-1 signaling and might potentially contribute to *Wnt-1*-induced mammary tumorigenesis. We therefore tested the effect of *Wnt-1* expression on Cox-2 in mouse mammary epithelial cells. Here we show that *Wnt-1* expression in RAC311 and C57MG cells causes increased transcription of *Cox-2*, resulting in elevated Cox-2 protein levels. An increase in PGE_2 synthesis is also observed in *Wnt-1*-expressing cells, which can be reversed by treatment with a selective Cox-2 inhibitor. These data may be significant not only in terms of *Wnt*-mediated carcinogenesis in the mouse but also in relation to human cancers in which components of the Wnt signaling pathway are activated.

MATERIALS AND METHODS

Cell Culture. Two mouse mammary epithelial cell lines were used, C57MG (43) and RAC311, a clonal subline derived from RAC311c (44, 45). RAC311 cells were grown in DMEM (4.5 g/l D-glucose) containing 10% fetal bovine serum (Life Technologies, Inc.), 100 units/ml penicillin, and 100 μg/ml streptomycin. C57MG growth medium was supplemented with 10 μg/ml insulin (Sigma). Cells were infected with MV7 or MVWnt-1 retrovirus using helper-free virus stocks as described previously (21). Approximately 50–100 G418-resistant colonies were pooled to generate the pooled populations designated RAC/MV7, RAC/Wnt-1, C57/MV7, and C57/Wnt-1. A clonal subline of RAC/Wnt-1 was derived by limiting dilution, selected on the basis of highly transformed morphology, and designated RAC/Wnt-1 #9. For cell lysate and RNA preparation, cells were plated at 1×10^6 cells per 10-cm dish and grown until MV7-infected control cells were confluent (5 days for RAC/MV7; 4 days for C57/MV7). DFU was a generous gift of the Merck Frosst Center for Therapeutic Research (Quebec, Canada).

Cell Lysate Preparation and Analysis. For Wnt-1 protein analysis, ECM fractions were prepared after removing the cells from the dishes by incubation

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[2] To whom requests for reprints should be addressed, at Strang Cancer Research Laboratory, The Rockefeller University, Box 231, 1230 York Avenue, New York, NY 10021. Phone: (212) 734-0567, extension 232; Fax: (212) 472-9471; E-mail: amcbrown@mail.med.cornell.edu.

[3] The abbreviations used are: Cox, cyclooxygenase; PGE_2, prostaglandin E_2; DFU, 5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl-2(5*H*)-furanone; ECM, extracellular matrix; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

with Dulbecco's PBS (Life Technologies, Inc.) containing 2 mM EDTA. ECM remaining on the plates was solubilized in boiling Laemmli SDS sample buffer and stored at −20°C. For analysis of cytosolic β-catenin levels, lysates were prepared as described (46), and total protein was assayed using Bio-Rad Protein Assay reagent. For Cox-2 protein analysis, lysates were prepared essentially as described (47). Cells were washed twice with PBS and harvested in lysis buffer containing 150 mM NaCl, 100 mM Tris-Cl (pH 8.0), 1% Tween 20, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 50 mM diethyldithiocarbamic acid. After one cycle of freeze-thawing at −20°C, cells were sonicated (three times for 15 s each time) on ice, then debris was pelleted by centrifugation at 10,000 × *g* for 10 min at 4°C. Supernatants were stored at −80°C, and proteins were assayed using a Lowry-based protein assay kit (Sigma).

For Western analysis, samples were subjected to SDS-PAGE as follows: Wnt-1 ECM fractions, 10% gel; β-catenin samples, 8% gel, 5 μg of protein; Cox-2 lysates, 10% gel, 50 μg of protein. Proteins were transferred to polyvinylidene fluoride membrane (Immobilon; Millipore), blotted with anti-Wnt-1 antibody (MC123; Ref. 21), anti-β-catenin antibody (Transduction Laboratories; Ref. 46), or anti-Cox-2 antibody (715; Ref. 47), and developed with Amersham enhanced chemiluminescence reagents. The anti-Cox-2 antibody 715 was a rabbit polyclonal antibody, raised against the unique 18-amino acid sequence from the COOH-terminal region of human Cox-2, which does not react with Cox-1.

RNA Preparation and Northern Blotting. RNA was prepared from confluent cells using RNAzol B (Tel-Test, Inc.) according to the manufacturer's instructions. Twenty μg of RNA were subjected to electrophoresis in 1% agarose/formaldehyde/3-[*N*-morpholino]propanesulfonic acid gels and transferred to Zeta-Probe membrane (Bio-Rad). Radiolabeled random-primed probes were prepared using the Rediprime DNA labeling system (Amersham), and hybridization was undertaken at 65°C in 0.5 M Na_2HPO_4 (pH 7.2), 7% SDS, and 1 mM EDTA (48). Washes were performed in 40 mM Na_2HPO_4 (pH 7.2), 1% SDS at 65°C. Probes used were murine *Cox-2* (TIS-10; a gift from H. R. Herschman, University of California at Los Angeles, Los Angeles, CA), murine *Cox-1* (a gift from W. L. Smith, Michigan State University, East Lansing, MI), and murine GAPDH (obtained from A. Ashworth, Institute of Cancer Research, London, England). GAPDH was used to demonstrate equal loading of each lane.

Nuclear Run-Ons. Nuclei were prepared, and nuclear run-ons were performed as described (47).

Autoradiographic exposures of both Northern blots and nuclear run-ons were quantitated by analysis on a Macintosh computer using the public domain NIH Image program (developed at the United States NIH and available on the Internet at http://rsb.info.nih.gov/nih-image/). Values obtained were normalized to those obtained for GAPDH and 18S rRNA for Northern blots and nuclear run-ons, respectively.

PGE_2 Assays. Cells were plated in 12-well plates at 4×10^4 cells/well and grown to confluence. Culture medium was collected and assayed for PGE_2 by enzyme immunoassay (Cayman Co., Ann Arbor, MI). To assay PGE_2 production in the presence of excess arachidonic acid (AA "spiked"), cells were incubated with fresh medium containing 10 μM arachidonic acid for 30 min, and then this medium was harvested and assayed as above. For experiments assaying the effect of DFU on PGE_2 production, cells were plated in 6-cm dishes at 3×10^5 cells/dish. DFU was added in fresh medium 72 h after plating and readded at 96 h. PGE_2 production was assayed at 120 h after plating, at which time control cells were confluent.

RESULTS AND DISCUSSION

To examine the effect of Wnt-1 on *Cox-2* expression, we generated fresh cell populations expressing *Wnt-1* by infection of the mouse mammary epithelial cell lines C57MG and RAC311 with retrovirus encoding Wnt-1 (MVWnt-1) or control retrovirus (MV7). As observed previously (5, 6), both C57/Wnt-1 and RAC/Wnt-1 cells appeared morphologically transformed and grew to higher cell densities than control cells (C57/MV7 and RAC/MV7, respectively). An additional clonal subline, RAC/Wnt-1 #9, was generated from RAC/Wnt-1 by limiting dilution and selected because of its high degree of morphological transformation. Western blot analysis using an anti-Wnt-1 antibody revealed that RAC/Wnt-1 #9 produced more Wnt-1 protein than the pooled RAC/Wnt-1 population (Fig. 1*A*). Therefore, we included both RAC/Wnt-1 and RAC/Wnt-1 #9 in subsequent analyses. As demonstrated previously, expression of *Wnt-1* in C57MG led to accumulation of uncomplexed cytosolic β-catenin (Refs. 46 and 49; data not shown). In addition, cytosolic β-catenin was elevated in RAC311 cells expressing *Wnt-1* (Fig. 1*B*), and higher levels were detected in RAC/Wnt-1 #9 relative to RAC/Wnt-1, correlating with relative Wnt-1 protein production.

Cox-2 protein levels in control and *Wnt-1*-expressing cell lines were analyzed by Western blotting (Fig. 2). C57/MV7 exhibited a markedly higher basal amount of Cox-2 than RAC/MV7, in which Cox-2 protein was virtually undetectable. In both C57MG and RAC311 cell lines, however, expression of *Wnt-1* led to an increase in Cox-2 protein, and Cox-2 protein was more abundant in RAC/Wnt-1 #9 than in RAC/Wnt-1, correlating with *Wnt-1* expression levels. Analysis of Cox-2 RNA by Northern blotting demonstrated that

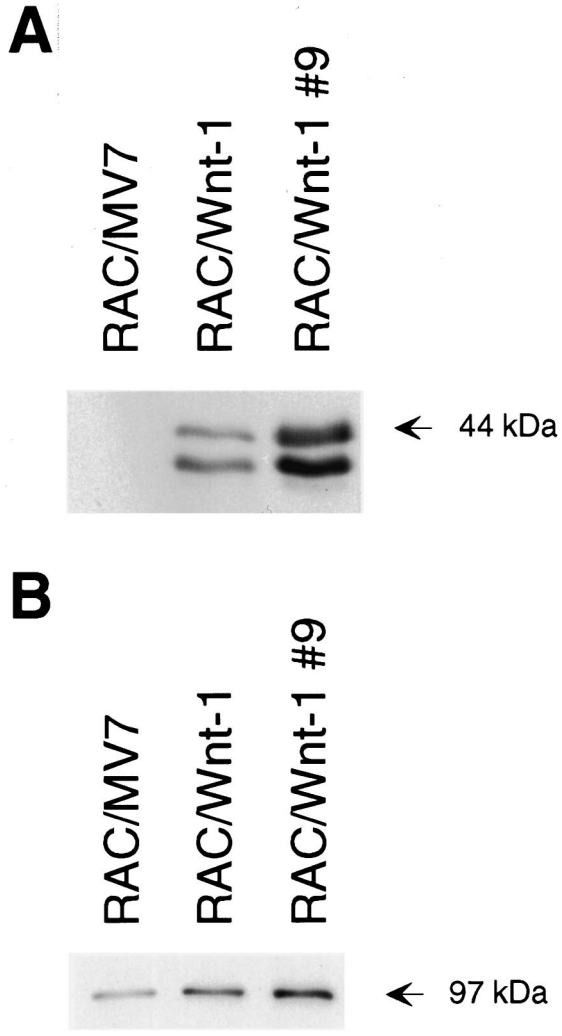

Fig. 1. Characterization of RAC311 cells expressing *Wnt-1*. RAC/MV7, RAC/Wnt-1, and RAC/Wnt-1 #9 cells were generated by retroviral infection as described in "Materials and Methods." Cells were analyzed by Western blotting for Wnt-1 protein and cytosolic β-catenin levels. *A*, *Wnt-1* expression. ECM fractions were prepared and assayed for Wnt-1 protein as described in "Materials and Methods." Anti-Wnt-1 antibody MC123 detected two bands of M_r 42,000 and M_r 44,000 in *Wnt-1*-expressing cells, as observed previously (71). These represent differentially glycosylated forms of Wnt-1 protein (20). No Wnt-1 protein was detected in ECM from control RAC/MV7 cells. *B*, cytosolic β-catenin. Cytosol fractions were prepared from cells and assayed for β-catenin as described in "Materials and Methods." The position of a M_r 97,000 molecular weight marker is shown.

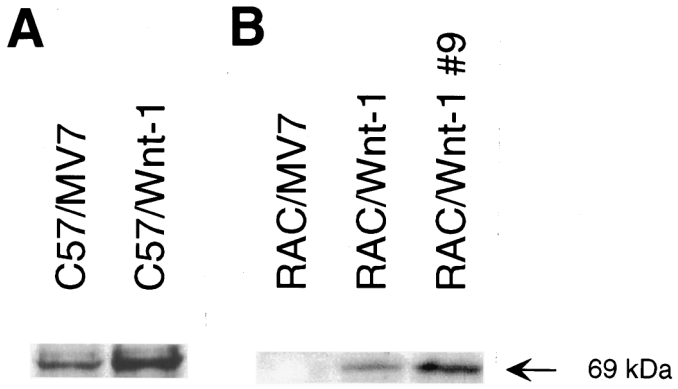

Fig. 2. Cox-2 protein is increased by *Wnt-1* expression. Lysates were prepared from C57MG-derived cells (*A*) and RAC311-derived cells (*B*). Fifty μg of lysate were analyzed by Western blotting for Cox-2 as described in "Materials and Methods," using rabbit polyclonal anti-Cox-2 antibody 715. Data shown in *A* and *B* are from separate experiments. The position of a M_r 69,000 molecular weight marker is shown.

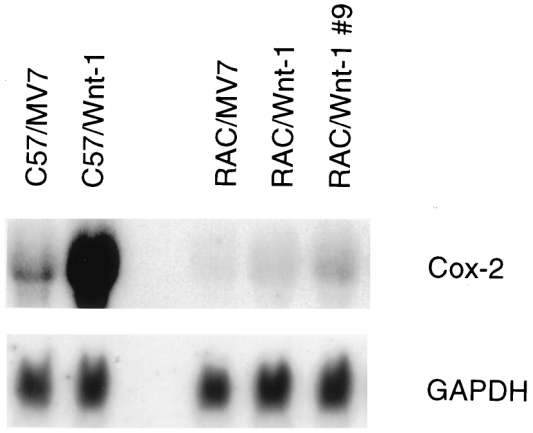

Fig. 3. Cox-2 mRNA is increased in cells expressing *Wnt-1*. Total RNA was prepared from cells, and 20 μg of each RNA sample were analyzed by Northern blotting as described in "Materials and Methods." The blot was probed sequentially with a murine *Cox-2* probe and a murine GAPDH probe. Cox-2 signals were quantitated using the program NIH Image and normalized to those obtained from GAPDH probing. Values obtained are expressed relative to the control MV7-infected cell line in each case. *C57/MV7*, 100%; *C57/Wnt-1*, 500%; *RAC/MV7*, 100%; *RAC/Wnt-1*, 174%; *RAC/Wnt-1 #9*, 327%.

Cox-2 mRNA levels closely reflected the changes observed in Cox-2 protein (Fig. 3), suggesting the effect of Wnt-1 on Cox-2 was likely to be due to transcriptional activation of the *Cox-2* gene. To test this directly, nuclear run-on assays were performed. These and subsequent assays were performed in the RAC311-derived cell lines in preference to C57MG-derived lines, because the latter tend to lose *Wnt-1* expression during continuous culture.[4] The rates of transcription from the *Cox-2* gene in RAC/Wnt-1 and RAC/Wnt-1 #9 were increased to 270 and 400%, respectively, relative to that in RAC/MV7 (Fig. 4), mirroring the differences observed in Cox-2 RNA and protein. Thus, expression of *Wnt-1* in RAC311, and most likely C57MG, causes transcriptional activation of the *Cox-2* gene.

Cox-2 is an inducible isoform of prostaglandin synthase (38). Thus, one predicted functional consequence of *Cox-2* up-regulation would be an increase in prostaglandin synthesis, of which PGE_2 is the predominant eicosanoid produced by most epithelial cells. We therefore assayed PGE_2 production in RAC311-derived cell lines. Spontaneous production of PGE_2 in RAC/Wnt-1 and RAC-Wnt/1 #9 was increased by 240 and 420%, respectively, over that in RAC/MV7 (Fig. 5, "spontaneous"). Spontaneous PGE_2 production was also measured in C57MG-derived lines and was increased ~100% in C57/Wnt-1 cells relative to C57/MV7 (data not shown). Given that arachidonic acid is the substrate from which cyclooxygenases synthesize prostaglandins, incubation of cells with excess arachidonic acid can increase PGE_2 production. In our experiments, treatment of the cells with arachidonic acid increased the absolute amounts of PGE_2 synthesis (Fig. 5, "AA spiked"), but PGE_2 production was still elevated in *Wnt-1*-expressing cells relative to RAC/MV7. This suggests that the measured spontaneous synthesis rates reflected relative levels of Cox-2 activity in the cells, rather than differential availability of arachidonic acid.

The observed increases in PGE_2 synthesis in *Wnt-1*-expressing cells could also be a consequence of changes in the level of Cox-1. Although *Cox-1* is constitutively and ubiquitously expressed, there have been reports of ligand-induced *Cox-1* up-regulation (50–53). Therefore, we addressed the involvement of Cox-1 using two assays: (*a*) we measured Cox-1 mRNA by Northern blotting and found little or no increase in Cox-1 mRNA in RAC/Wnt-1 and C57MG/Wnt-1, respectively, relative to control cells (Fig. 6); and (*b*) we tested the relative contribution of Cox-1 and Cox-2 to PGE_2 production in the RAC311-derived cell lines by using DFU, a selective Cox-2 inhibitor. DFU has at least a 1000-fold specificity for Cox-2 relative to Cox-1 in tissue culture cells (54). RAC/MV7, RAC/Wnt-1. and RAC/Wnt-1 #9 were treated with varying concentrations of DFU for 48 h, and culture supernatants were then assayed for PGE_2. A dose-dependent inhibition of PGE_2 production was observed, with 1 μM DFU being sufficient to reduce PGE_2 production to approximately the same basal level in all three cell lines (Fig. 7). Higher concentrations of DFU did not cause any additional inhibition of PGE_2 synthesis. The residual PGE_2 production observed in all cell lines after inhibition of Cox-2 with DFU is presumed to reflect Cox-1 activity. Because the amount of Cox-1-mediated PGE_2 synthesis is apparently constant in all three cell lines, we conclude that the enhanced production of PGE_2 in *Wnt-1*-transformed cells is attributable to increased Cox-2 activity, consistent with the observed differences in Cox-2 RNA and protein levels. Cell morphology was unaffected by treatment with DFU (data not shown). The failure of DFU to affect morphological transformation of *Wnt-1*-expressing cells suggests that elevated prostanoid pro-

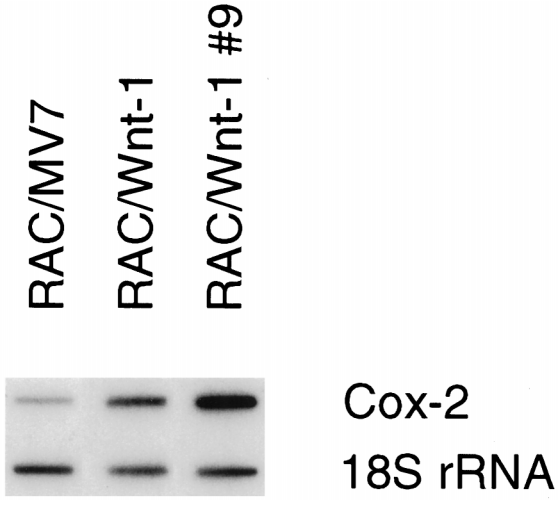

Fig. 4. *Cox-2* transcription is up-regulated in RAC311 cells expressing *Wnt-1*. Nuclei were prepared, and nuclear run-on assays were performed as described in "Materials and Methods." Labeled nascent transcripts were hybridized to 18S rRNA and *Cox-2* cDNAs, which were immobilized on nitrocellulose. Signals were quantitated using the program NIH Image, and Cox-2 was normalized to 18S rRNA. Values obtained are expressed relative to RAC/MV7. *RAC/MV7*, 100%; *RAC/Wnt-1*, 267%; *RAC/Wnt-1 #9*, 399%.

[4] A. M. C. Brown, unpublished observations.

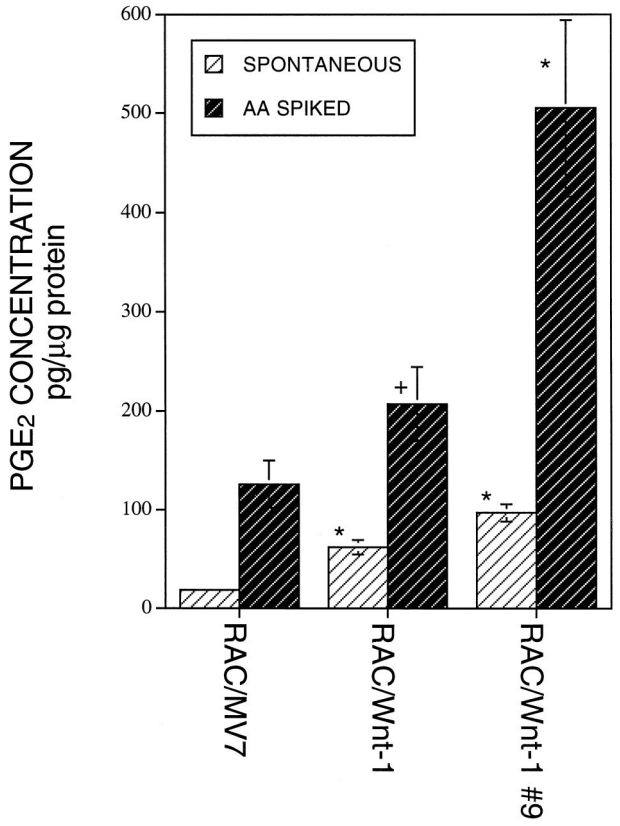

Fig. 5. PGE_2 production is increased by expression of *Wnt-1*. Cells were grown for 5 days after plating to achieve confluence, during which time no medium change was performed. Growth medium from the cells was harvested and assayed using enzyme immunoassay to measure spontaneous PGE_2 production (*SPONTANEOUS*, light hatching). Cells were incubated for an additional 30 min in fresh medium containing 10 μM sodium arachidonate. The medium was then collected and assayed for PGE_2 (*AA SPIKED*, dark hatching). Results were normalized to μg of protein obtained from the cells after harvesting of medium. Results shown are mean values of six replicates; *bars*, SD. PGE_2 production from both *Wnt-1*-expressing cell lines was significantly greater than that from RAC/MV7 control cells (*, $P < 0.001$; +, $P < 0.003$).

duction by Cox-2 is not necessary for maintenance of the transformed phenotype of these cells *in vitro*.

We have shown here that expression of *Wnt-1* in two mammary epithelial cell lines causes elevated expression and activity of Cox-2, via transcriptional activation, resulting in increased PGE_2 synthesis. The *Cox-2* gene was initially identified as an early response gene up-regulated in response to phorbol ester and serum and was subsequently found to be induced by multiple agents, particularly during inflammatory responses (38). A large body of evidence has accumulated implicating Cox-2 in intestinal carcinogenesis. *COX-2* expression is frequently detected in tumor tissue (39, 40, 55–58), and the incidence of intestinal tumorigenesis in both mice and humans can be reduced by pharmacological agents that inhibit Cox activity (41, 42, 59–64). A crucial role for *Cox-2* in tumorigenesis has been demonstrated by Oshima *et al.* (1996; Ref. 42), who found that intestinal polyposis in *Apc* mutant mice was markedly reduced by genetic ablation of *Cox-2*. However, *Cox-2* induction in response to Wnt proteins has not been demonstrated previously.

The mechanism by which Wnt-1 activates *Cox-2* transcription is unclear. Given that *COX-2* induction occurs in response to *Wnt-1* expression and *APC* mutation, both of which result in cytosolic β-catenin accumulation, our initial expectation was that the *Cox-2* promoter might be subject to direct regulation by β-catenin/Tcf complexes. The human *COX-2* promoter contains two potential Tcf-binding sites, although their overlap with the canonical TCF binding motif is only partial (ACTTTGATC and TCTTTGTAG compared with CCTTTGA/TA/TC; Ref. 27). One of these sites is not conserved in the murine *Cox-2* promoter, and the other lies outside the sequence presently reported for the mouse promoter. To investigate the mechanism of regulation, we have performed transient transfection assays using a human COX-2 promoter-luciferase reporter construct but thus far have been unable to detect increased reporter activity as a result of β-catenin overexpression. Thus, it is possible that *Cox-2* transcription may not be directly regulated by β-catenin/Tcf complexes but may be activated in *Wnt-1*-expressing cells by alternative transcription factors. We also cannot exclude the possibility that *Cox-2* induction is a more downstream or indirect consequence of Wnt signaling.

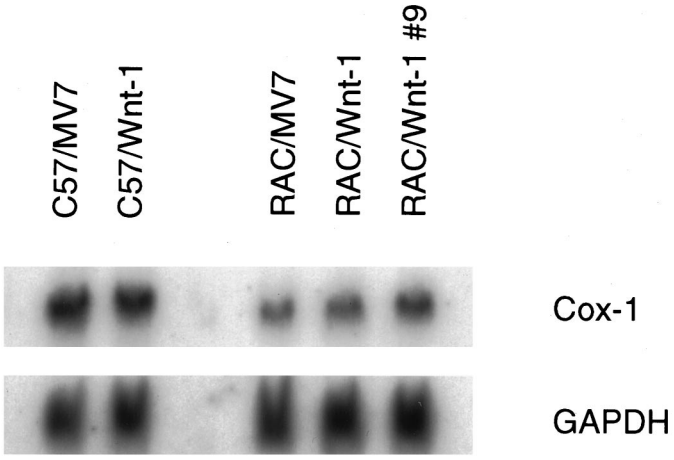

Fig. 6. Effect of *Wnt-1* expression on Cox-1 mRNA. RNA was prepared, and a Northern blot was generated as described in "Materials and Methods" using 20 μg of each RNA. The blot was probed sequentially with a murine *Cox-1* probe and a murine GAPDH probe. Cox-1 signals were quantitated using the program NIH Image and normalized to those obtained from GAPDH probing. Values obtained are expressed relative to the control MV7-infected cell line in each case. *C57/MV7*, 100%; *C57/Wnt-1*, 70%; *RAC/MV7*, 100%; *RAC/Wnt-1*, 118%; *RAC/Wnt-1 #9*, 163%.

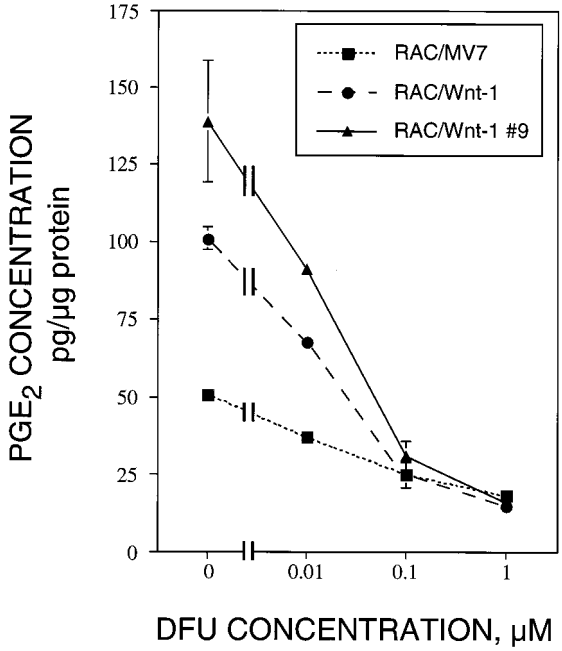

Fig. 7. Inhibition of PGE_2 production by a selective Cox-2 inhibitor DFU. Cells were treated with the indicated concentrations of DFU for 48 h. Culture medium was harvested and assayed for PGE_2 by enzyme immunoassay to determine spontaneous release of PGE_2. Results were normalized to μg of protein obtained from the cells after harvesting of the medium. Mean values of two replicates are shown; *bars*, spread.

Because *Wnt-1* is a mammary oncogene, our data suggest that *Cox-2* up-regulation might also contribute to mammary tumorigenesis. Consistent with this idea, *Cox-2* is expressed in ras- and virally transformed mammary cells, as well as in some human breast cancers and breast cancer cell lines (47, 65, 66). Interestingly, despite abundant evidence of the importance of Cox-2 during intestinal tumorigenesis, the precise mechanism by which Cox-2 contributes is unclear. Prostaglandin overproduction is likely to have multiple consequences. Prostaglandins can exert local immunosuppressive effects that could facilitate tumorigenesis (37, 67). Additionally changes in gene expression can occur because selected prostaglandins are ligands of the peroxisome proliferator-activated receptor γ (68). *Cox-2* induction in tumors may promote survival of cells otherwise destined to undergo apoptotic cell death; negative regulation of apoptosis by *Cox-2* overexpression has been demonstrated in intestinal epithelial cells (69). Recent data also demonstrate a role for Cox-2 in angiogenesis. Selective inhibition of Cox-2 reduces secretion of angiogenic factors from colon cancer cells, thereby suppressing *de novo* formation of endothelial tubules *in vitro* (70). Because of the pleiotropic consequences of *Cox-2* overexpression, it is difficult to predict what role Cox-2 might play in Wnt-1-induced mammary tumorigenesis. Transgenic mice that express *Wnt-1* ectopically in the mammary gland display extensive mammary hyperplasia at an early age and subsequently develop mammary adenocarcinomas stochastically after a latent period of several months (4). If Cox-2 contributes to Wnt-1-mediated tumorigenesis *in vivo*, it could do so either at the initial hyperplastic stage or by affecting progression to carcinoma. Our findings may also be pertinent to human breast cancer. Although expression of *WNT-1* itself in human mammary tissue has not been reported, several others members of the *WNT* gene family are overexpressed in human breast tumors relative to normal tissue (13–16). A number of Wnt proteins exhibit functional redundancy with Wnt-1, inducing stabilization of cytosolic β-catenin and morphological transformation of mammary cells (8, 10). Therefore, it is likely that some of the *WNT* genes overexpressed in human breast cancers may have transcriptional consequences similar to those of *Wnt-1*.

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