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Prostaglandin receptor EP2 is responsible for cyclooxygenase-2 induction by prostaglandin E_2 in mouse skin

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The EP2 prostanoid receptor is one of the four subtypes of receptors for prostaglandin E_2 (PGE_2). We previously reported that deletion of EP2 led to resistance to chemically induced mouse skin carcinogenesis, whereas overexpression of EP2 resulted in enhanced tumor development. The purpose of this study was to investigate the underlying molecular mechanisms. We found that EP2 knockout mice had reduced cyclooxygenase-2 (COX-2) expression after 12-*O*-tetradecanoylphorbol-13-acetate (TPA) treatment compared with wild-type (WT) mice. Further, primary keratinocytes from EP2 transgenic mice had increased COX-2 expression after either TPA or PGE_2 treatment and COX-2 expression was blocked by 10 μM SQ 22,536, an adenylate cyclase inhibitor. EP2 knockout mice had significantly decreased, whereas EP2 transgenic mice had significantly increased PGE_2 production in response to a single treatment of TPA. Cyclic AMP response element-binding protein (CREB) phosphorylation was elevated to a greater extent in keratinocytes from EP2 transgenic mice compared with those of WT mice following PGE_2 treatment. A protein kinase A (PKA) inhibitor reduced PGE_2-mediated CREB phosphorylation in keratinocytes from EP2 transgenic mice. Furthermore, we found that there was no CREB phosphorylation in EP2 knockout mice following PGE_2 treatment. PGE_2-induced DNA synthesis (cell proliferation) was significantly decreased in keratinocytes from EP2 knockout mice following pretreatment with 10 μM SQ 22,536. Taken together, EP2 activation of the PKA/CREB-signaling pathway is responsible for keratinocyte proliferation and our findings reveal a positive feedback loop between COX-2 and PGE_2 that is mediated by the EP2 receptor.

Introduction

There has been substantial interest in understanding the roles of COX in skin cancer (1,2). Non-steroidal anti-inflammatory drugs and cyclooxygenase-2 (COX-2) selective inhibitors have shown significant effects in reducing the incidence and multiplicity of skin tumors, suggesting that the COX prostaglandin products play an important role in the development of skin cancer (1,3).

Prostaglandin E_2 (PGE_2) is the major prostaglandin produced by COX enzymes in the skin (4) and is reported to increase cAMP levels in human and rodent skin (5). PGE_2 effects are mediated by seven transmembrane G-protein-coupled receptors, namely, EP1, EP2, EP3 and EP4. EP1 receptor-mediated signaling increases intracellular calcium levels. EP2 and EP4 receptor-mediated signaling increases cAMP levels via activation of adenylate cyclase (AC) whereas EP3 receptor-mediated signaling decreases cAMP levels (5). All four PGE_2 receptors were found to be present in normal human epidermis (6) and we have found that all four EP receptors are expressed in mouse epidermis (7). We have shown that deletion of the EP2, but not the EP3 receptor, for PGE_2 results in suppression of skin tumor development and is associated with decreased proliferation, angiogenesis, inflammation and cell survival (7). However, the signaling pathways and mechanisms by which the EP2 receptor regulates these processes are unknown. Based on previous studies, it was hypothesized in this study that EP2 signaling through protein kinase A (PKA) and cyclic AMP response element-binding protein (CREB) is responsible for the PGE_2 effects on proliferation.

The transcription factor CREB binds the cyclic AMP response element and activates transcription in response to a variety of extracellular signals including neurotransmitters, hormones, membrane depolarization and growth or neurotrophic factors (8). PKA stimulates phosphorylation of CREB at Serine 133, a key regulatory site controlling transcriptional activity (8). In cervical carcinomas, elevated PGE_2 can act in an autocrine/paracrine manner via cAMP-linked EP2/EP4 receptors to mediate an effect on target genes, including COX-2 (9). In human pulmonary artery smooth muscle cells, COX-2 induction by bradykinin is mediated by cyclic AMP response element through a novel autocrine loop involving endogenous PGE_2, EP2 and EP4 receptors (10). A similar positive feedback loop between COX-2 and PGE_2 may potentiate the progression of skin cancer. Thus, CREB may play an important role in the mechanistic basis of skin carcinogenesis.

It has been demonstrated that CREB plays an important role in promoting proliferation (8). Several cell cycle genes such as cyclin D1 and cyclin A are regulated by CREB via a functional cyclic AMP response element (11,12). Additionally, the involvement of CREB in the control of tumor metastasis was demonstrated in melanoma cells (13,14). A recent study showed that CREB controls hepatocellular carcinoma growth, supports angiogenesis and renders resistance to apoptosis (15). Also previous studies showed that genetic disruption of either COX-2 or EP2 receptor decreases the number and size of intestinal polyps in APCΔ716 mice (16). Tumor cell proliferation is significantly inhibited in adenomas of COX-2-deficient APCΔ716 mice (17). These findings indicate the potential link between COX-2 and tumor cell proliferation *in vivo* through EP2 activation. We have shown that EP2 knockout mice had significantly reduced cellular proliferation of skin keratinocytes *in vivo* and *in vitro* compared with that in wild-type (WT) mice (7). We also have shown that overexpression of the EP2 receptor increased 12-*O*-tetradecanoylphorbol-13-acetate-(TPA) and PGE_2-induced keratinocyte proliferation *in vivo* and *in vitro*, respectively, using BK5.EP2 transgenic mice (18). Thus, we have found that the EP2 receptor plays an important role in inducing cell proliferation in mouse skin. We hypothesized here that PKA signaling elicited by EP2 activation promotes cell proliferation and that this is a critical pathway in mouse skin carcinogenesis. Thus, we used primary skin keratinocytes from EP2 null, WT or EP2 transgenic mice to show that EP2 signaling through PKA and CREB is responsible for PGE_2 effects on proliferation and COX-2 induction.

Materials and methods

Materials

PGE_2 (Cayman Chemical Co., Ann Arbor, MI), SQ 22,536 (Sigma Chemical Co., St Louis, MO), [^{3}H]-methyl thymidine (79.20 Ci/mmol) (PerkinElmer Life Sciences, Boston, MA), PGE_2 immunoassay kit (NEN Life Sciences, Boston, MA), COX-2 antibody (Cayman Chemical Co.), CREB and phospho-CREB antibody (Cell Signaling, Beverly, MA) and β-actin antibody (Santa Cruz Bio Technology, Santa Cruz, CA), chemiluminescence detection system (ECL, PerkinElmer Life Sciences), BCA kit (Bio-Rad, Richmond, CA) were used.

Abbreviations: AC, adenylate cyclase; COX-2, cyclooxygenase-2; CREB, cyclic AMP response element-binding protein; EGFR, epidermal growth factor receptor; PGE_2, prostaglandin E_2; PKA, protein kinase A; SDS, sodium dodecyl sulphate; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; WT, wild-type.

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Animals

EP2 knockout mice on a 129 background were kindly provided by Dr Beverly Koller, University North Carolina, Chapel Hill. Genotyping of EP2 knockout mice was carried out as described previously (17). WT 129 mice, used as controls for EP2 knockout mice, were purchased from Taconic (Germantown, NY). BK5.EP2 transgenic mice on a FVB background were generated as we have described previously (18). Homozygous (+/+) EP2 transgenic mice were used for this study. FVB mice, used as WT controls for EP2 transgenic mice, were purchased from Harlan (Indianapolis, IN). All mice were maintained at Science Park and housed in an air-conditioned animal facility, which is Association for Assessment and Accreditation of Laboratory Animal Care accredited.

Northern blot analysis

Total RNA was extracted from whole skin of EP2 knockout and WT mice treated with 100 μM PGE_2 or 2.5 μg TPA in 200 μl acetone with Tri-reagent (Molecular Research Center, Cincinnati, OH) following the manufacturer's protocol. Ten μg of total RNA from each sample was denatured and separated on 1% agarose/6% formaldehyde gel, and then transferred to nylon membranes. A [^{32}P] dCTP-labeled cDNA probe for COX-2 was hybridized to the blots at 65°C for 2 h. The blots were then washed twice each for 15 min in 0.1% sodium dodecyl sulphate (SDS)/2× NaCl/sodium citrate solution (where 1× is 0.15 M NaCl/15 mM sodium citrate) at room temperature and once for 30 min with 0.1% SDS/0.1× NaCl/sodium citrate solution at 60°C and exposed to X-ray film at −80°C. The blots were stripped and reprobed with glyceraldehyde-3-phosphate dehydrogenase cDNA as a loading control.

Western blot analysis

For western analysis, total protein was isolated from epidermis or from primary keratinocytes and whole cell lysate was prepared with Triton-X 100 buffer and Radio Immuno Precipitation Assay buffer, respectively. Twenty-five to 50 μg of whole cell lysate of each sample was heated at 95°C for 10 min, denatured and fractionated by 10% SDS-polyacrylamide gel and electroblotted onto polyvinylidene difluoride membranes. The blot containing the transferred protein was blocked in blocking buffer for 1 h at room temperature followed by incubation with primary antibodies for COX-2 (1:500) or phospho-CREB (1:1000) or CREB (1:1000) in blocking buffer for 1.5 h to overnight at 4°C. This was followed by incubation with secondary antibody, antirabbit (1:2000) for 1 h and then washed three times with wash buffer and detected using a chemiluminescence kit. β-Actin was used as a loading control.

PGE_2 analysis

Epidermal PGE_2 levels were measured with an immunoassay kit (NEN Life Sciences). Three mice from EP2 knockout mice were dorsally shaved and topically treated with 2.5 μg TPA in 200 μl acetone. Six hours later, the mice were killed and immediately snap frozen in liquid nitrogen and stored at −80°C. For assay, a 1.5 cm^2 area of epidermis was chipped from the frozen skin into 2.5 ml of 0.05 M Tris buffer containing 50 μg/ml indomethacin (a COX inhibitor). Following the homogenization for 30 s at 4°C, homogenates were centrifuged at 12 000 r.p.m. for 10 min at 4°C and 50 μl aliquot was removed for determination of protein content, and the remainder was extracted according to slightly modified method of Chang *et al.* (19). For PGE_2 determination, supernatant was diluted to 15% and acidified water (pH 3–4) and was applied to a preconditioned C18 silica column (Alltech Associates, Deerfield, IL), the column was washed with 10 ml petroleum ether, and the prostaglandins were eluted with 10 ml methyl formate into a polypropylene tube. Following solvent evaporation, the prostaglandins were reconstituted in 1.0 ml buffer (supplied in the immunoassay kit) and PGE_2 levels were measured according to the supplier's instructions. Protein content was determined by the BCA method and calculation of PGE_2 concentrations (ng/μg protein) was based on a standard curve for each experiment.

Cell culture

Primary skin keratinocytes from newborn EP2 knockout or transgenic and corresponding WT mice were prepared as described by Yuspa and Harris (20). Briefly, 1- to 2-day-old pups were euthanized and washed in 75% ethanol. The skin was stripped off and floated on 0.25% trypsin overnight at 4°C. The epidermis was separated from the dermis and chopped in Waymouth's medium containing 1.2 mM calcium and 10% fetal bovine serum. The cells were filtered through a sterilized mesh and plated at 2×10^6 cells per dish for most purposes. Cells were incubated at 37°C with 5% CO_2 for 2 h in Waymouth's medium to allow them to attach to the plate. Cells were then washed with phosphate-buffered saline and grown in Keratinocyte Growth Medium (a serum-free medium containing 0.03 mM calcium, Cambrex, Walkers, MD) at 37°C with 5% CO_2 for experimental use and treated with 0.2% vehicle dimethyl sulfoxide or 10 μM PGE_2.

[^{3}H]-thymidine incorporation assay

Primary cultures of skin keratinocytes from WT and EP2 transgenic or knockout mice at ∼85–90% confluence in six-well plates were treated in triplicate with 10 μM PGE_2 for 20 h and pulsed with 1 μCi/ml [^{3}H]-thymidine, 2 h before harvest. The AC inhibitor SQ 22,536 (10 μM) was delivered 30 min prior to treatment with PGE_2. Cells were then washed twice with ice-cold phosphate-buffered saline and three times with ice-cold 10% trichloroacetic acid. Cells were lysed with 0.3 N NaOH, 1% SDS and the incorporated [^{3}H]-thymidine was counted in a scintillation counter and normalized to protein concentration. Protein concentration was determined with the BCA kit.

Statistical analysis

Data were shown as the mean ± standard deviation. Statistical differences between means were determined with one-way analysis of variance using SPSS10 (SPSS Mac V.10, SPSS, Chicago, IL).

Results

COX-2 expression is regulated by the EP2 receptor

PGE_2 has been shown to amplify its own production by inducing COX-2 expression in various cells (10,21). Our laboratory had previously observed that PGE_2 and dibutryl-cAMP, a cAMP analog, transcriptionally activate COX-2 expression in murine keratinocytes (22). These studies, in addition to our recent demonstration that EP2 activation contributes significantly to skin carcinogenesis (7), led us to hypothesize that the EP2 receptor may regulate COX-2 expression through a positive feedback loop.

To determine the effect of EP2 expression on COX-2 induction, WT and EP2 knockout mice were topically treated with 100 μM PGE_2, 2.5 μg TPA and/or a combination of these two treatments for 6 h. COX-2 expression was assessed at both the mRNA and the protein levels, as shown in Figure 1A and B. As has been described previously, TPA induced COX-2 in WT mice (23,24), but very little induction was observed in EP2 knockout mice. Additionally,

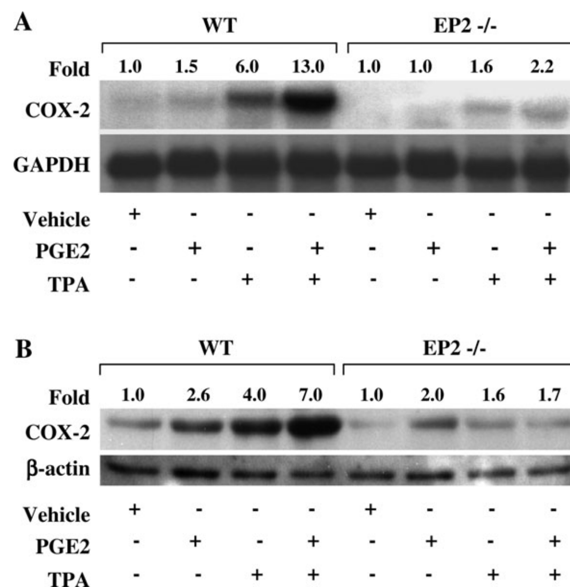

Fig. 1. Reduced COX-2 expression in EP2 receptor-deficient mice. (**A**) Northern blot analysis was performed to determine COX-2 mRNA expression in skin from WT (129 background) and EP2 knockout (EP2−/−) mice. Skins from WT and EP2 knockout mice were treated with vehicle (acetone) or 100 μM PGE_2 for 6 h, with or without TPA (2.5 μg/200 μl acetone). Glyceraldehyde-3-phosphate dehydrogenase cDNA was used as a loading control. The data are representative of at least two independent experiments. (**B**) Western blot of epidermal proteins from WT and EP2 knockout mice treated with vehicle (acetone) or 100 μM PGE_2 for 6 h with or without TPA (2.5 μg/200 μl acetone) visualized with antibody against COX-2 and β-actin. A set of representative data from two independent experiments is presented.

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treatment with PGE_2 alone upregulated COX-2 at least at the protein level. In WT mice, but not in EP2 knockout mice, the combination of TPA and PGE_2 synergistically enhanced COX-2 expression. To further evaluate this relationship, we compared COX-2 expression in WT and EP2 transgenic mice (Figure 2). TPA (Figure 2A) or PGE_2 (Figure 2B) induced COX-2 protein earlier, longer and to a greater extent, in cultures of primary skin keratinocytes from transgenic mice compared with WT mice. Consistent with these findings, we also saw that an AC inhibitor can block COX-2 expression in primary keratinocytes from EP2 transgenic mice (Figure 2C). These data suggest that COX-2 expression can be regulated by the EP2-signaling pathway.

PGE_2 production is regulated by EP2 signaling

TPA induction of COX-2 is reduced by treatment with indomethacin, a COX inhibitor, indicating that part of the mechanism by which TPA elevated COX-2 expression is through TPA-induced arachidonic acid release and metabolism in primary keratinocyte cultures (25). PGE_2 is the major PG synthesized by murine keratinocytes and is a comitogen for TPA-induced epidermal cell proliferation (26). Our recent studies showed that all four EP receptors are expressed in mouse epidermis (7). Furthermore, among the four receptors, the EP2 or the EP4 receptor has been implicated in breast (27,28) and skin (7). Thus, this suggests that the biological effects ascribed to PGE_2 (i.e. proliferation, apoptosis and angiogenesis) are EP2 dependent. This led us to hypothesize that EP2, among the EP receptors, may be crucial for driving TPA-treated mouse skin to produce PGE_2. As shown in Figure 3A, while TPA treatment significantly increased PGE_2 synthesis in WT mice, EP2 knockout mice had significantly reduced PGE_2 production following TPA treatment compared with their WT controls. Consistent with this observation, the EP2 transgenic mice produced twice as much PGE_2 than their counterparts (vehicle treated) and >2-fold more PGE_2 after TPA treatment (Figure 3B). The differences in the response to TPA between the WT mice (Figure 3A and B) is probably to be due to differences in the strains of mice used, i.e. FVB versus 129. Collectively, this study suggests that EP2 plays a critical role in TPA-induced PGE_2 production.

Deficiency of the EP2 receptor causes a reduction in cell proliferation

PGE_2 has been reported to regulate cell proliferation (29). We previously reported that EP2 knockout mice had significantly reduced keratinocyte proliferation following treatment with PGE_2 *in vitro* compared with that in WT mice (7). Therefore, we examined whether the proliferative effect of PGE_2 depends on its EP2 receptor and whether this involves the PKA/CREB pathway. To determine this, we performed [^{3}H]-thymidine incorporation assays using primary skin keratinocytes from EP2 knockout and/or transgenic and WT mice. [^{3}H]-thymidine incorporation assay reflects cellular DNA synthesis activity and is frequently used as a maker of cell proliferation. We found that cultures from EP2 transgenic mice showed a significantly increased ability to incorporate [^{3}H]-thymidine 20 h after 10 μM PGE_2 treatment compared with WT keratinocytes (Figure 4A), while EP2 knockout keratinocytes showed a significantly decreased DNA synthesis compared with controls (Figure 4B). As expected, PGE_2-induced DNA synthesis was significantly inhibited by pretreatment with 10 μM SQ 22,536, an AC inhibitor, in cultured keratinocytes

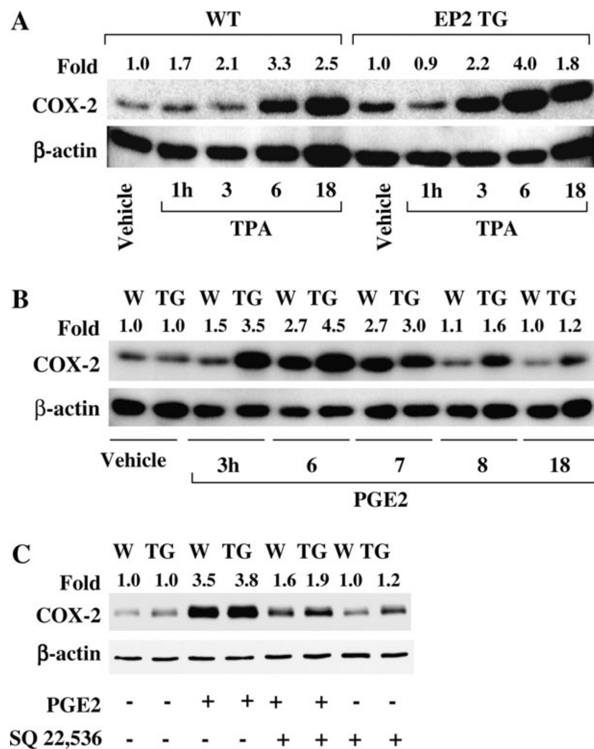

Fig. 2. Upregulation of COX-2 expression by overexpression of the EP2 receptor. (**A**) Western blot of whole cell lysate from WT (FVB) and EP2 transgenic mice (TG) treated with vehicle (acetone) or TPA (2.5 μg/200 μl acetone) for 1–18 h visualized with antibody against COX-2 and β-actin. (**B**) Western blot of whole cell lysate proteins from cultures of primary keratinocytes from WT and EP2 transgenic mice treated with vehicle dimethyl sulfoxide or 10 μM PGE_2 for 3–18 h visualized with antibody against COX-2 and β-actin. (**C**) Suppression of PGE_2-induced COX-2 expression by an AC inhibitor. Primary keratinocyte cultures from WT and EP2 transgenic mice at ∼85–90% confluence were treated with 10 μM SQ 22,536 for 30 min prior to 10 μM PGE_2 treatment for 6 h and blots were probed with antibodies against COX-2. Loading control was represented by actin. The data are representative of at least two independent experiments.

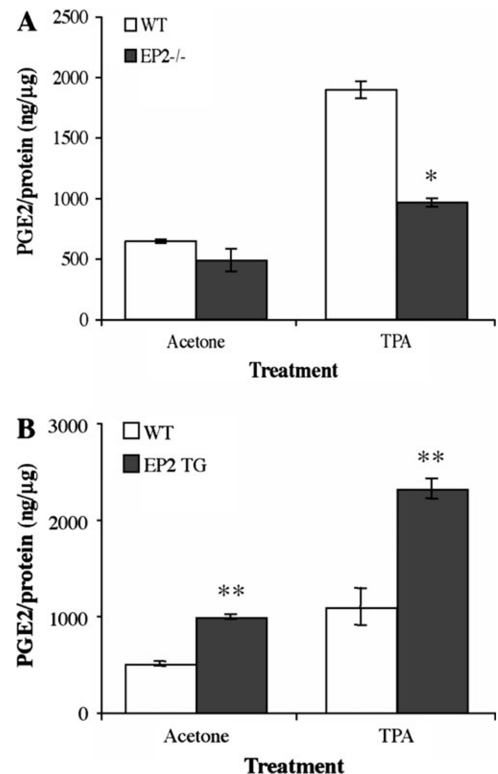

Fig. 3. PGE_2 levels in the skins of EP2 knockout, transgenic or WT mice with TPA treatment. Chipped epidermis from snap-frozen skins from EP2 knockout (**A**) or transgenic (**B**) and WT mice after dorsal TPA treatment (6 h) were used to measure PGE_2 using an immunoassay system. The data (calculated as ng PGE_2/μg protein) are representative of two independent experiments (three mice each per treatment group) and values are means ± standard deviations, $^*P < 0.05$, versus WT, $^{**}P < 0.01$, versus WT for each treatment group.

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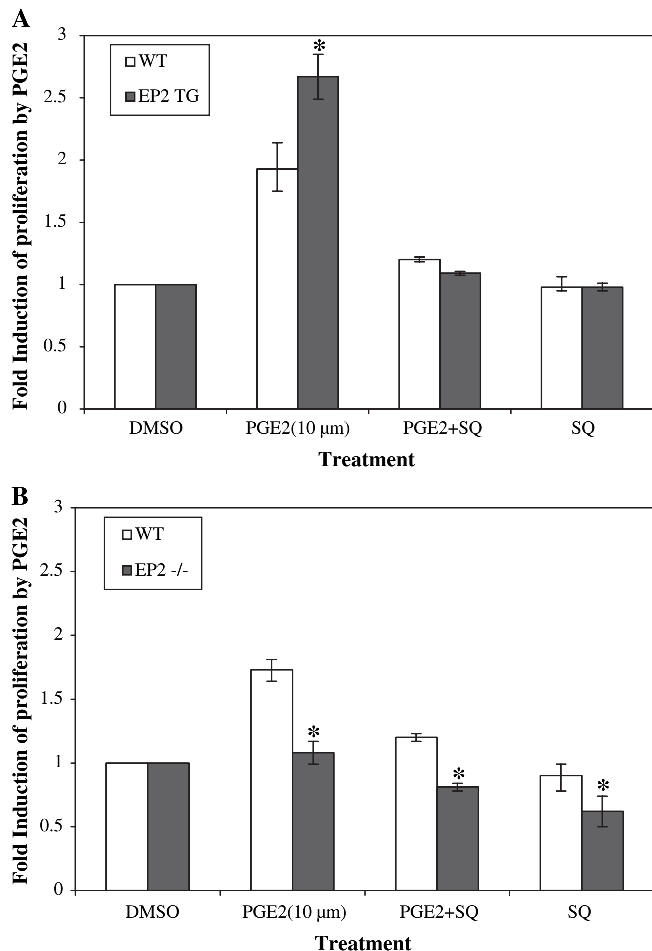

Fig. 4. Suppression of PGE_2-induced DNA synthesis by an AC inhibitor. Primary skin keratinocyte cultures from WT and EP2 transgenic or knockout mice at ~85–90% confluence were treated with 10 μM PGE_2 or 10 μM SQ 22,536 for 30 min prior to 10 μM PGE_2 treatment for 20 h and pulsed with 1 μCi/ml [^{3}H]-thymidine 2 h before harvest. The [^{3}H]-thymidine incorporated by cells was measured and normalized to protein concentration. Data are presented as fold induction of specific activity. A set of representative data from at least two independent experiments is presented and values are the means ± standard deviations, $^*P < 0.05$, versus WT for each treatment group.

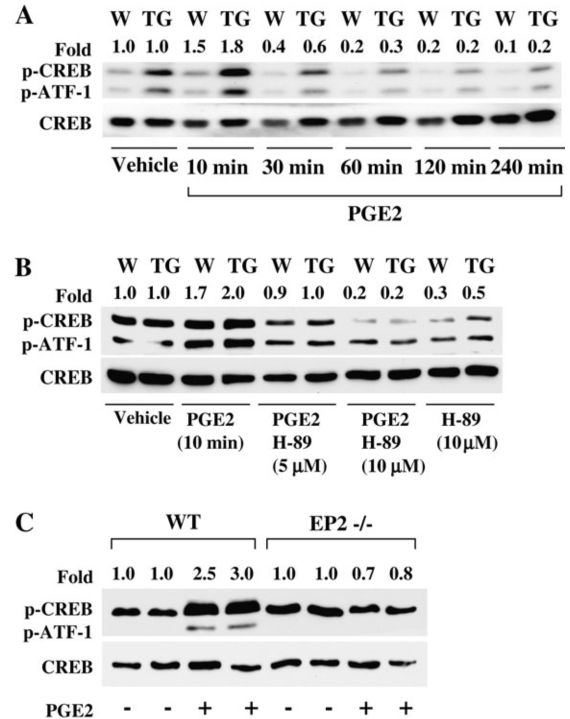

Fig. 5. PGE_2-induced CREB phosphorylation via the EP2 receptor. (**A**) Western blot of whole cell lysate proteins from cultures of primary keratinocytes from WT and EP2 transgenic mice treated with vehicle dimethyl sulfoxide or 10 μM PGE_2 for 10 min and up to 240 min visualized with antibodies against phosphorylated CREB and total CREB. (**B**) Western blot of whole cell lysate proteins from cultured primary keratinocytes from WT and EP2 transgenic mice treated with 5 or 10 μM PKA inhibitor (H-89) for 30 min before vehicle dimethyl sulfoxide or 10 μM PGE_2 for 10 min and immunostained with antibodies against CREB phosphorylation. (**C**) Western blot of whole cell lysate protein from EP2 knockout and WT mice treated with 100 μM PGE_2 for 30 min; the blot was immumostained with antibodies against phosphorylated CREB. Total CREB was used as a loading control. The data are representative of at least two independent experiments.

from WT as well as EP2 transgenic and knockout mice. This suggests that activation of the EP2 receptor can induce cAMP production and that cAMP-dependent PKA signaling may be a central pathway for the induction of cell proliferation in murine skin. The ability of the AC inhibitor to reduce proliferation in the EP2 knockout keratinocytes is probably to be due to the formation of cAMP from other receptors, possibly EP4.

EP2 regulates CREB phosphorylation via cAMP/PKA signaling

We previously reported that PGE_2 failed to induce cAMP production in the epidermis of EP2 knockout mice, whereas EP2 transgenic mice had significantly elevated cAMP after PGE_2 treatment compared with WT mice (7,18). This led us to hypothesize that activation of the EP2/cAMP/PKA-signaling pathway could lead to phosphorylation of CREB. We found that primary skin keratinocytes from EP2 transgenic mice have increased CREB phosphorylation compared with those of WT mice following PGE_2 treatment (Figure 5A). H-89 (a PKA inhibitor) significantly reduced PGE_2-induced CREB phosphorylation in primary skin keratinocytes from WT and EP2 transgenic mice (Figure 5B). In addition, we found that there was no CREB phosphorylation in EP2 knockout mice compared with those of WT mice following PGE_2 treatment *in vivo* (Figure 5C).

Based on recent studies in several carcinoma cell lines, increased EP2 receptor expression may induce PGE_2-mediated expression of the proangiogenic vascular endothelial growth factor through activation of the epidermal growth factor receptor (EGFR) and extracellular signal-regulated kinase 1/2-signaling pathways (30). However, in our *in vivo* and *in vitro* studies, we found no difference in the phosphorylation of EGFR, ERK1/2, Akt or c-src, in cultures of primary skin keratinocytes from WT and EP2 transgenic mice as well as WT and EP2 knockout mice (data not shown). Thus, we suggest that the major pathway by which the EP2 receptor regulates COX-2 expression and cell proliferation is via cAMP/PKA and not the EGFR/mitogen-activated protein kinase pathway.

Discussion

We recently reported that the EP2 receptor plays a significant role in the protumorigenic action of PGE_2 in skin tumor development using EP2 knockout and transgenic mice (7,31). We showed that deletion of the EP2, but not the EP3 receptor, for PGE_2 results in suppression of skin tumor development and is associated with decreased proliferation, angiogenesis, inflammation and increased cell survival (7). We also showed that overexpression of the EP2 receptor for PGE_2 results in enhancement of skin tumor development and is associated with increased proliferation, angiogenesis and inflammation (31). Thus, we hypothesized that downstream signaling from the EP2 receptor contributes significantly to the induction of skin tumor development. We propose a model (Figure 6) in which the EP2 receptor-mediated

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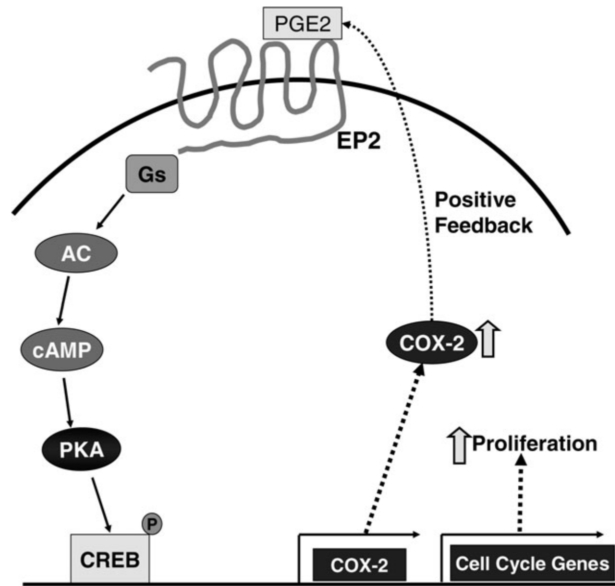

Fig. 6. Proposed positive feedback loop between COX-2- and PGE_2-mediated keratinocyte proliferation. cAMP/PKA/phospho-CREB-signaling pathway via the EP_2 receptor. PGE_2 derived from COX-2 binds to G-protein-coupled EP2 receptors, which in turn activates AC. Increased intracellular cAMP activates PKA, which can phosphorylate CREB on Serine 133, inducing the expression of COX-2. Prostaglandins produced by COX-2 can further activate the EP2 receptor, establishing a positive feedback loop.

phosphorylation of CREB on Serine 133 is primarily PKA-dependent and that EP2-mediated signaling pathways regulate COX-2 expression, and induces amplification of PGE_2 by elevating COX-2 expression, through a positive feedback loop. Here, we report that the PKA/phospho-CREB pathway is a central mechanism in mediating PGE_2 effects through EP2 in mouse skin keratinocytes. Previous studies using several carcinoma cell lines such as ovarian and endometrial adenocarcinomas, cell growth and cell invasion were associated with src-mediated EGFR transactivation by PGE_2 through EP2 or EP4 receptors (30,32). However, our findings suggest that only the classic PKA/phosphorylated CREB pathway was involved in inducing the expression of genes related to cell proliferation *in vivo* and *in vitro*. The difference between our results and other previous studies may depend on the differential expression and activation of the EP2 receptor in a number of tissues and cell types including skin (16,29), colon (32), breast (27,28,33) and prostate (30,34). In this study, we found that an AC inhibitor significantly blocks PGE_2-induced DNA synthesis. Furthermore, we found that an AC inhibitor also blocked PGE_2-induced COX-2 expression. Therefore, we suggest that the EP2-mediated signaling pathway is the major mechanism by which PGE_2 causes COX-2 expression and cell proliferation.

The EP1 and EP4 receptors also play an important role in colon carcinogenesis (35,36). Deletion of the EP1 and EP4 receptors resulted in inhibition of azoxymethane-induced colon cancer development (37,38). This suggests that the EP1 and EP4 receptors may also have a protumorigenic action in skin carcinogenesis. Recently, one group showed that the EP4 receptor can activate both the cAMP/PKA and the phosphatidylinositol-3-kinase pathways to induce phosphorylation of CREB in human embryonic kidney cells (39). In colon carcinoma cells, cell growth is associated with EP4 receptor activation of the phosphatidylinositol-3-kinase/extracellular signal-regulated kinase pathway (32). For this reason, we cannot rule out the possibility that the EP4 receptor also contributes to skin tumor development. However, EP4 mRNA levels are reduced by TPA treatment and are reduced in tumors from 7,12-dimethylbenz[*a*]anthracene/TPA protocol (data not shown), suggesting that it may not contribute significantly to tumor promotion. Further studies are needed to determine the roles of the EP1 and EP4 receptors in skin carcinogenesis and the underlying molecular mechanisms.

In summary, we have shown that EP2 signaling through PKA and CREB is responsible for the PGE_2 effects on cell proliferation in mouse skin and our findings reveal a positive feedback loop between COX-2 and PGE_2 mediated by the EP2 receptor.

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Conflict of Interest Statement: None declared.

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