THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 278, No. 52, Issue of December 26, pp. 52131–52138, 2003
Printed in U.S.A.

Microsomal Prostaglandin E_2 Synthase-1 Is Induced by Conditional Expression of RET/PTC in Thyroid PCCL3 Cells through the Activation of the MEK-ERK Pathway*

Received for publication, June 6, 2003, and in revised form, October 6, 2003
Published, JBC Papers in Press, October 10, 2003, DOI 10.1074/jbc.M306003200

Efisio Puxeddu‡§¶, Norisato Mitsutake‡¶‖, Jeffrey A. Knauf‡, Sonia Moretti§, Hei W. Kim, Karen A. Seta**, Diane Brockman‡‡, Leslie Myatt‡‡, David E. Millhorn**, and James A. Fagin‡§§**

*From the ‡Division of Endocrinology and Metabolism, the **Department of Genome Science, and the ‡‡Department of Obstetrics and Gynecology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267 and the §Dipartimento di Medicina Interna, University of Perugia, Perugia 06126, Italy*

***RET/PTC* rearrangements are believed to be tumor-initiating events in papillary thyroid carcinomas. We identified microsomal prostaglandin E_2 synthase-1 (mPGES-1) as a RET/PTC-inducible gene through subtraction hybridization cloning and expression profiling with custom microarrays. The inducible prostaglandin E_2 (PGE_2) biosynthetic enzymes cyclooxygenase-2 (COX-2) and mPGES-1 are up-regulated in many cancers. COX-2 is overexpressed in thyroid malignancies compared with benign nodules and normal thyroid tissues. Eicosanoids may promote tumorigenesis through effects on tumor cell growth, immune surveillance, and angiogenesis. Conditional RET/PTC1 or RET/PTC3 expression in PCCL3 thyroid cells markedly induced mPGES-1 and COX-2. PGE_2 was the principal prostanoid and up-regulated (by ~60-fold), whereas hydroxyeicosatetraenoic acid metabolites were decreased, consistent with shunting of prostanoid biosynthesis toward PGE_2 by coactivation of the two enzymes. RET/PTC activated mPGES-1 gene transcription. Based on experiments with kinase inhibitors, with PCCL3 cell lines with doxycycline-inducible expression of RET/PTC mutants with substitutions of critical tyrosine residues in the kinase domain, and lines with inducible expression of activated mutants of H-RAS and MEK1, RET/PTC was found to regulate mPGES-1 through Shc-RAS-MEK-ERK. These data show a direct relationship between activation of a tyrosine kinase receptor oncogene and regulation of PGE_2 biosynthesis. As enzymes involved in prostanoid biosynthesis can be targeted with pharmacological inhibitors, these findings may have therapeutic implications.**

The *RET* gene encodes a transmembrane tyrosine kinase receptor, expression and function of which are normally restricted to a subset of cells derived from the neural crest. Rearrangements of *RET* resulting in its constitutive activation are believed to play a causative role in the pathogenesis of a significant proportion of thyroid papillary carcinomas (PTC) (1). *RET* rearrangements have been found in 5–30% of PTC in the adult population. Notably, these rearrangements are more common in pediatric cases (2, 3), especially in individuals exposed to ionizing radiation during childhood (3–5). In thyroid follicular cells, RET activation occurs through chromosomal recombination resulting in illegitimate expression of a fusion protein consisting of the intracellular tyrosine kinase domain of RET coupled to an N-terminal fragment contributed by a heterologous gene (1). The promoter region of the partner gene drives constitutive expression of the chimeric gene. There are at least 10 different types of *RET/PTC*[1] that differ according to the 5′ partner gene involved in the rearrangement. *RET/PTC1* and *RET/PTC3* are the most common types, accounting for more than 90% of all the rearrangements. The fusion proteins generated by these chimeric genes dimerize in a ligand-independent manner (6, 7) and constitutively activate the tyrosine kinase function of RET, thus exerting their transforming potential. Several signaling pathways activated by RET/PTC have been identified. Among these, autophosphorylation of tyrosine residues Tyr-1015 and Tyr-1062 of full-length RET, which form docking sites for PLCγ and Shc, respectively (8–10), are required for its oncogenic function *in vitro*.

RET/PTC rearrangements are believed to be a very early step in thyroid cancer pathogenesis (11–17). Relatively little is known about the events that follow RET/PTC activation and that may be associated with PTC progression. Acute RET/PTC activation stimulates both DNA synthesis and apoptosis in thyroid cells, is associated with loss of thyroid-specific differentiation, and interferes with thyrotropin (TSH) receptor-mediated intracellular signaling at various levels (18). Taken together, these data suggest that acute expression of RET/PTC in normal follicular cells induces significant phenotypic changes oriented toward neoplastic transformation.

Epidemiological studies show decreased risk of several types of epithelial cancer in individuals regularly taking aspirin or

* This work was supported in part by National Institutes of Health Grant CA50706 (to J. A. F.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ Both authors contributed equally to this work.

‖ Supported in part by the Nakayama Foundation for Human Science and Sumitomo Life Social Welfare Services Foundation.

§§ To whom correspondence should be addressed: Division of Endocrinology and Metabolism, University of Cincinnati College of Medicine, P. O. Box 670547, Cincinnati, OH 45267-0547. Tel.: 513-558-4444; Fax: 513-558-8581; E-mail: james.fagin@uc.edu.

[1] The abbreviations used are: RET/PTC, RET/papillary thyroid carcinoma; PGE_2, prostaglandin E_2; AA, arachidonic acid; mPGES-1, microsomal prostaglandin E_2 synthase-1; COX, cyclooxygenase; MEK, mitogen-activated protein/extracellular signal-regulated kinase kinase; HETE, hydroxyeicosatetraenoic acid; PLCγ, phospholipase Cγ; TSH, thyroid-stimulating hormone; APC, adenomatous polyposis coli; rtTA, reverse tetracycline-dependent transactivator; LC-MS, liquid chromatography-mass spectrometry; HPLC, high performance liquid chromatography; ERK, extracellular signal-regulated kinase; SSH, subtractive suppression hybridization; PGJ, prostaglandin J; PGH, prostaglandin H; PGF, prostaglandin F; PGES, prostaglandin E synthase; cPGES, cytosolic prostaglandin E synthase; MAPK, mitogen-activated protein kinase; MAPEG, membrane-associated proteins in eicosanoid and glutathione metabolism; PI3K, phosphatidylinositol 3-kinase.

other nonsteroidal anti-inflammatory drugs (19, 20). This has been attributed to inhibition of prostaglandin production in tumor tissues, in particular prostaglandin E_2 (PGE_2) (21, 22). The synthesis of PGE_2 from arachidonic acid requires two enzymes acting sequentially. Cyclooxygenase (COX) catalyzes the synthesis of endoperoxide prostaglandin H_2 (PGH_2) from arachidonic acid (AA). There are two COX isoforms; COX-1 is constitutively expressed in most tissues, whereas COX-2 is normally not expressed but is induced by growth factors, cytokines, and certain oncogenes (reviewed in Ref. 23). The two COX isozymes play distinct physiological roles, although their function may overlap in certain settings (reviewed in Ref. 24). Thus, COX-1 is required for biosynthesis of thromboxane A_2 in platelets, which in its absence fail to undergo AA-induced aggregation. COX-1 is also required for onset of parturition via generation of $PGF_{2}\alpha$ (25, 26). By contrast COX-2 appears to play a more significant role in development, closure of the ductus arteriosus, and ovulation (24). COX-1 has a higher K_m for AA than COX-2. Thus, in the presence of low AA concentrations, COX-2 can operate when COX-1 is inactive (27), a mechanism that may account for the fact that prostanoids formed in the late phase (hours) after cell exposure to tumor promoters, cytokines, and growth factors are formed via COX-2 (reviewed in Ref. 24).

A variety of epithelial malignancies have increased expression of COX-2, including colorectal (28, 29) and thyroid (30–32) cancers. This appears to be important in disease pathogenesis, as genetic or biochemical disruption of COX-2 in APC Δ7–16 mice, a model of human familial adenomatous polyposis coli, results in a major reduction in the number and size of intestinal polyps (33, 34). Although most studies implicate COX-2, rather than COX-1, in colorectal carcinogenesis, a recent study indicates that deficiency of either enzyme reduces polyp burden in APC (−/+) mice (35).

Recently, three prostaglandin E synthase (PGES) isoforms involved in the terminal step of PGE_2 synthesis, the conversion of PGH_2 to PGE_2, have been identified. Based on ectopic overexpression of various COX and PGES isozymes, a hierarchy of functional interactions between them has been established (reviewed in Ref. 36). Cytosolic (cPGES) is expressed constitutively and has been proposed to exhibit preferential functional coupling with COX-1 (37). Microsomal PGES-1 (mPGES-1) is induced by proinflammatory stimuli and increases during the period when COX-2-dependent PGE_2 generation is ongoing (38, 39). mPGES-2, the most recently identified form of PGH-PGE isomerase, does not show homology with mPGES-1 (40), and has been proposed to couple functionally to both COX-1 and COX-2 (41). mPGES-1 is overexpressed in the majority of colorectal adenomas and carcinomas, suggesting that this may contribute to the increased amounts of PGE_2 in these tumors.

Relatively little is known about pathogenetic mechanisms that may account for mPGES-1 expression in cancer tissues. Here we report that activation of the RET/PTC oncoprotein is associated with a rapid and vigorous induction of mPGES-1 and COX-2 expression and stimulation of PGE_2 biosynthesis in thyroid cells. RET/PTC effects on mPGES-1 mRNA require coupling to Shc, likely via Tyr-1062, and activation of MEK-ERK. As RET/PTC is believed to be an early event in thyroid cell transformation, these data suggest that the PGE_2 biosynthetic machinery may be recruited into action shortly after tumor initiation, and may play a significant role in tumor progression.

EXPERIMENTAL PROCEDURES

Cell Lines and Reagents—PCCL3 cells, a clonal rat thyroid line requiring TSH for growth, were maintained in H4 complete medium consisting of Coon's medium/F12 high zinc supplemented with 5% fetal bovine serum, 0.3 mg/ml L-glutamine, 1 mIU/ml TSH, 10 μg/ml insulin, 5 μg/ml apo-transferrin, 10 nM hydrocortisone, and penicillin/streptomycin. H3 complete medium was identical to H4 complete medium but without addition of TSH. The following lines were derived from PCCL3 cells to obtain doxycycline-inducible expression of RET/PTC isoforms with or without the indicated mutations, as described elsewhere (18): rtTA-7 (stably expressing only the doxycycline-inducible transactivator rtTA), rtTA-3 (empty vector transfected rtTA-7 cells), PTC3-5 (doxycycline-inducible RET/PTC3), PTC1–31 (doxycycline-inducible RET/PTC1), PTC2-PDZ (doxycycline-inducible RET/PTC2 mutant unable to associate with Shc, in which the 22 most C-terminal amino acids, including Tyr-1062, were replaced with the PDZ domain of Enigma to ensure appropriate membrane localization) and $PTC3^{Y541F}$ (doxycycline-inducible RET/PTC3 tyrosine substituted unable to associate with PLCγ). In addition, we used clonal PCCL3 cell lines with doxycycline-inducible expression of $H\text{-}Ras^{V12}$ (Ras-25 cells) and $MEK1^{S217E/S221E}$ (MEK1–55 cells) (42, 43). PD98059, U0126, LY294002, wortmannin, and SB203580 were purchased from Calbiochem (San Diego, CA).

Construction of Subtractive Suppression Hybridization (SSH) Library and Custom Microarrays—We developed a SSH library to obtain an unbiased representation of the gene expression profile resulting shortly after activation of RET/PTC.

Briefly, PTC3-5 cells at 90–95% confluence were incubated with H3 medium for 3 days and harvested 12 h after treatment with/without 1 μg/ml doxycycline. Total RNA was isolated from cells using TRIreagent (Molecular Research Center, Inc., Cincinnati, OH), and poly(A) RNA using an Oligotex Direct mRNA minikit (Qiagen Inc., Valencia, CA) according to the protocols from the respective manufacturers. SSH cDNA libraries were constructed using PCR-Select cDNA subtraction kit (Clontech, Palo Alto, CA). Double-stranded cDNA was generated and subjected to digestion with RsaI, following which the doxycycline-treated sample (Tester) was split into two pools. Each pool was ligated with a different adaptor (N1 or N2R). Each ligated double-stranded cDNA pool was then denatured and rehybridized with excess denatured cDNA from cells that had not been treated with doxycycline (Driver). The hybridized pools were mixed, and a second round of hybridization was performed with an excess amount of denatured Driver ([+] library). All procedures were then repeated after switching the samples such that the cDNA from doxycycline-treated cells was the Driver and that from untreated cells was the Tester ([−] library). In each library, the hybridized sequences that contained both adaptors (N1 and N2R) represented the differentially expressed sequences in the Tester. Thus, the [+] library and [−] library contained RET/PTC3-up-regulated and -down-regulated genes, respectively. The hybridized molecules from each pool were subjected to PCR to amplify the specific sequences that contained both adaptors (N1 and N2R). The amplified fragments were then ligated into the pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA), and ligated clones introduced into DH10B *Escherichia coli* cells by electroporation. Single clones were isolated and the libraries titers determined. A number of clones corresponding to 10% of the estimated titer of each library were sequenced using the M13 reverse primer (MWG Biotech, Inc. High Point, NC). The identity of each clone was obtained by blasting its sequence against the NCBI GenBank™ data base using the "Standard Nucleotide-Nucleotide Blast" access.

To confirm RET/PTC-dependent expression, we developed custom arrays for hybridization. Inserts were amplified by PCR using primers for the adaptor sequences, and the products precipitated with isopropyl alcohol and resuspended in 3× SSC. PCR products (final concentration = 0.2–1 μg/μl) were then spotted onto poly-L-lysine-coated slides with an OmniGrid Robot (GeneMachines, San Carlos, CA). Slides were processed using the succinic anhydride method (44) and stored at room temperature in a desiccator cabinet until hybridization. Probes for the cDNA microarrays were generated using 40 μg of total RNA from cells treated with or without doxycycline for 12 h in a standard reverse transcriptase reaction in which some of the dTTP was replaced with either 50 μM Cy3-labeled dUTP or 75 μM Cy5-labeled dUTP (Amersham Biosciences). Dyes were switched so that, in half of the experiments, the doxycycline-treated sample was labeled with Cy3, and, in the other half, it was labeled with Cy5. Probes were cleaned using a QIAquick nucleotide removal kit (Qiagen Inc.) and hybridized to the array overnight at 58 °C in a buffer containing 0.57 μg/μl COT-1 DNA, 0.57 μg/μl $(dA)_{40-60}$, 0.23 μg/μl yeast tRNA, 3.5× SSC, and 0.3% SDS. Slides were serially washed, dried by centrifugation at room temperature and scanned immediately. Four independent experiments were performed.

Slides were scanned with a GenePix 4000A scanner (Axon Instruments, Inc., Foster City, CA) at 532 nm for Cy3 and 635 nm for Cy5. Exogenous plant genes from *Arabidopsis thaliana* (SpotReport 1–3, Stratagene, La Jolla, CA) were included on the array and spiked into

the Cy3 and Cy5 probe labeling reactions in equal amounts. Photo multiplier tube voltages for each wavelength were adjusted until the Cy3:Cy5 ratios for these spots averaged 1:1. This process eliminates biases resulting from scanning and from uneven dye incorporation in the labeling reactions. The images were analyzed using GenePix version 2.0 software. The background-subtracted median ratio value was calculated for each spot, and replicate spots on each slide were averaged.

Northern Blotting—Northern blotting was performed using total RNA isolated from cells treated with/without 1 μg/ml doxycycline for the indicated times as described (33, 34), followed by hybridization with [^{32}P]dCTP-labeled probes. The following cDNAs were used: a 430-bp fragment corresponding to 30–460 bp of rat mPGES-1 cDNA (NCBI GenBank™, NM_021583), a 783-bp fragment corresponding to 319–1101 bp of putative rat mPGES-2 cDNA (Ensembl, ENSRNOG00000014050), 438 bp of fragment of putative rat cPGES cDNA obtained using the following primers: (5′-ATGCAGCCTGCTTCTGCAAA-3′ and 5′-ATCTGCTCCGTCTACTTCTG-3′), which were designed with mouse cPGES cDNA (NCBI GenBank™, AY281130) and UCSC BLAT Search Rat Genome (genome.ucsc.edu/cgi-bin/hgBlat), a 691-bp fragment corresponding to 171–861 bp of rat COX-1 cDNA (NCBI GenBank™, S67721) and a 530-bp fragment corresponding to 1358–1887 bp of rat COX-2 cDNA (NCBI GenBank™, S67722), full-length human RET/PTC3, and full-length rat cyclophilin cDNA.

Western Blotting—Cells were lysed in cell lysis buffer containing 20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 150 mM NaCl, 0.5% Triton-X, and proteinase inhibitor mixture (Sigma). After measurement of protein concentration using Coomassie Plus Protein Assay Reagent Kit (PIERCE), 50 μg of each sample was separated by 4–15% SDS-PAGE and blotted onto nitrocellulose membrane. The following primary antibodies were used: anti-COX-1 (murine) polyclonal antibody (Cayman Chemical), anti-COX-2 (murine) polyclonal antibody (Cayman Chemical), mPGES-1 polyclonal antibody (Cayman Chemical), mPGES-2 polyclonal antibody (Cayman Chemical), and anti-p23 monoclonal antibody (Affinity Bioreagents). The antigen-antibody complexes were visualized using horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG antibody (Santa Cruz) and enhanced chemiluminescence (ECL) system (Amersham Biosciences).

HPLC and LC-MS Analysis of Arachidonic Acid Metabolites—After a 72-h incubation with H3 medium, PTC3-5 cells were treated with or without 1 μg/ml doxycycline for 24 h to induce RET/PTC3, and then labeled with [^{3}H]arachidonic acid (AA) (2 μCi for T75 flask) (PerkinElmer). After a 24-h incubation, the cells were washed with PBS and changed to fresh H3 medium without [^{3}H]AA. After another 48 h, conditioned media was acidified with formic acid (8.6 M, 50 μl for 10 ml of medium) and stored in siliconized tubes at −20 °C until assayed. HPLC analysis was performed as previously described (45). Briefly, the acidified medium was loaded onto a prewashed Sep-Pak C_{18} column and the column washed with distilled water and then with petroleum ether. AA metabolites were eluted with 5 ml of methyl formate and dried using oxygen-free nitrogen. The AA metabolites were then dissolved in 1 ml of solvent 1 (30:70 (v/v) acetonitrile:triethylamine formate ($4 \times 10-2$ M, pH 3.15)) and filtered using a 0.45 μM solvent-resistant filter (Millipore Millex). One milliliter of sample was then injected onto a reverse-phase HPLC column (Fatty Acid Analysis Column, Waters Corp., Milford, MA). The AA metabolites were eluted with a flow rate of 1 ml/min, and transition time between the following solvents was 1 min: 25 min with solvent 1, 25 min with solvent 2 (50:50 (v/v) acetonitrile:triethylamine formate ($4 \times 10-2$ M, pH 3.15)), and 8 min with solvent 3 (acetonitrile). The retention times of AA, PGE_2, and hydroxyeicosatetraenoic acids (HETEs) were determined using [^{14}C]AA, [^{3}H]PGE_2, and a mixture of unlabeled HETEs (5, 8, 11, 12, and 15), respectively.

As a complementary approach to identify prostanoids in condition medium of RET/PTC-expressing cells, AA metabolites eluted from the Sep-Pak C_{18} column were analyzed at the UC Mass Spectrometry Core Facility (www.chembus.uc.edu/massnew/maintbl.asp) using LC-MS (Waters QTOF II) in positive ion mode. The Sep-Pak C_{18} eluates were separated on a reverse phase C18 column (in house, 10 cm × 75 μm, inner diameter) using water-acetonitrile with 0.1% acetic acid as mobile phase at a flow rate of 7.0 μl/min. The mobile phase gradient started at 30% buffer A (95:5 water:acetonitrile with 0.1% acetic acid), increased to 100% buffer B (5:95 water:acetonitrile with 0.1% acetic acid) over 45 min, and held at 100% buffer B for 10 min. Detection was made after splitting flow (250 nl/min) at a spray voltage of +3.0 kV, maintaining the orifice voltage at 45 V. Selective ion scans at m/z 371, 353, 355, and 335 were used to detect 6=keto-$PGF_1\alpha$, PGE_2, $PGF_2\alpha$, and PGJ_2, respectively (46).

PGE_2 Assay—PTC3-5 and rtTA7 cells were grown to almost 100% confluence in 15- or 6-cm dishes. After a 72-h incubation with H3 medium, cells were treated with or without 1 μg/ml doxycycline for 24 or 48 h in the presence or absence of the indicated COX or mPGES-1 inhibitors: 100 μM aspirin, 10–1000 nM NS-398 (Calbiochem), and 10 μM MK-886 (Calbiochem). Twenty-four hours before harvesting the media, cells were changed to H3 media without addition of serum. Aliquots of the conditioned medium were collected and stored at −80 °C until assayed. Assays were performed on 1:10 dilutions of the conditioned media using the Prostaglandin E_2 EIA Kit, Monoclonal (Cayman Chemical Co., Ann Arbor, MI) as recommended by the manufacturer. PGE_2 levels were expressed as picograms/dish.

Assays for $PGF_2\alpha$, 6-Keto-$PGF_1\alpha$, and 15-Deoxy-$\Delta^{12,14}$-PGJ_2—PTC3-5 cells were grown to almost 100% confluence in 6-cm dishes. After a 72-h incubation with H3 medium, cells were treated with or without 1 μg/ml doxycycline for 24 or 48 h. Twenty-four hours before harvesting, cells were changed to H3 media without serum. Conditioned medium were collected and stored at −80 °C until assayed. The respective prostaglandin assays were measured by competitive immunoassay with alkaline phosphatase activity as the readout using the respective kits from Assay Designs, Inc. (Ann Arbor, MI), according to their recommended protocols.

Regulation of mPGES Promoter Activity—A pGL2 mPGES promoter vector consisting of a 510-bp fragment spanning −538 to −28 of the putative human mPGES promoter cloned into the pGL2-luciferase vector as well as a promoterless pGL2 control were kindly provided by Dr. Terry J. Smith (UCLA, Los Angeles, CA) (47). PTC3-5 cells were co-transfected with 800 ng of the appropriate pGL2 luciferase vector and 80 ng of pRL-TK vector (Promega; containing HSV-tk promoter and the *Renilla* luciferase gene) using 2 μl of LipofectAMINE 2000 (Invitrogen) in each well of 24-well plate. Five hours after transfection, medium was replaced by H3 medium with or without doxycycline. Cell lysates were prepared 48 h after transfection, and luciferase activities measured with the Dual-Luciferase Reporter Assay System (Promega) according to the protocol from the manufacturer.

RESULTS

Identification of mPGES-1 as a RET/PTC-inducible Gene Product in SSH Libraries—The sequence data obtained from the SSH libraries revealed that the [+] library contained 250 and the [−] library 114 clones corresponding to sequences in the NCBI data base. A comprehensive analysis of this experiment will be described elsewhere. A number of sequences in the libraries were present in multiple copies, *e.g.* two copies of mPGES-1 in the [+] library. As a first step toward verifying regulation of the target genes amplified from the SSH libraries, PCR products derived from each clone in the libraries were spotted onto glass slides and evaluated by cDNA microarray analysis. As expected RET/PTC3 was strongly up-regulated in doxycycline-treated PTC3-5 cells, as was mPGES-1 (data not shown). Because of its potential biological significance, the latter was selected for further study.

As shown in Fig. 1*A*, mPGES-1 mRNA levels were markedly induced in PTC3-5 cells 12 h after incubation with doxycycline, whereas this treatment had no effect on gene expression in rtTA-7 cells (PCCL3 cells stably expressing the tetracycline-dependent transactivator protein but not containing the tetO-RET/PTC3 construct). mPGES-1 abundance was induced both by RET/PTC1 or RET/PTC3, and was apparent as early as 6 h after doxycycline addition (Fig. 1*B*).

RET/PTC Induces COX-2 and mPGES-1 mRNA and Protein—RET/PTC strongly induced both COX-2 and mPGES-1 mRNA (Fig. 2*A*) and protein (Fig. 2*B*). There was a marginal increase in cPGES mRNA at 72 and 96 h, which was of a far lesser magnitude than that of mPGES-1 and not reflected in a concomitant increase in cPGES protein. COX-1 and mPGES-2 mRNA and protein were stably expressed and not changed by RET/PTC (Fig. 2, *A* and *B*).

RET/PTC Induction of Prostaglandin E_2 Biosynthesis—To obtain a profile of AA metabolites altered by acute activation of RET/PTC expression, PTC3-5 cells were metabolically labeled with ^{3}[H]AA and incubated with or without doxycycline as described under "Experimental Procedures," and the condi-

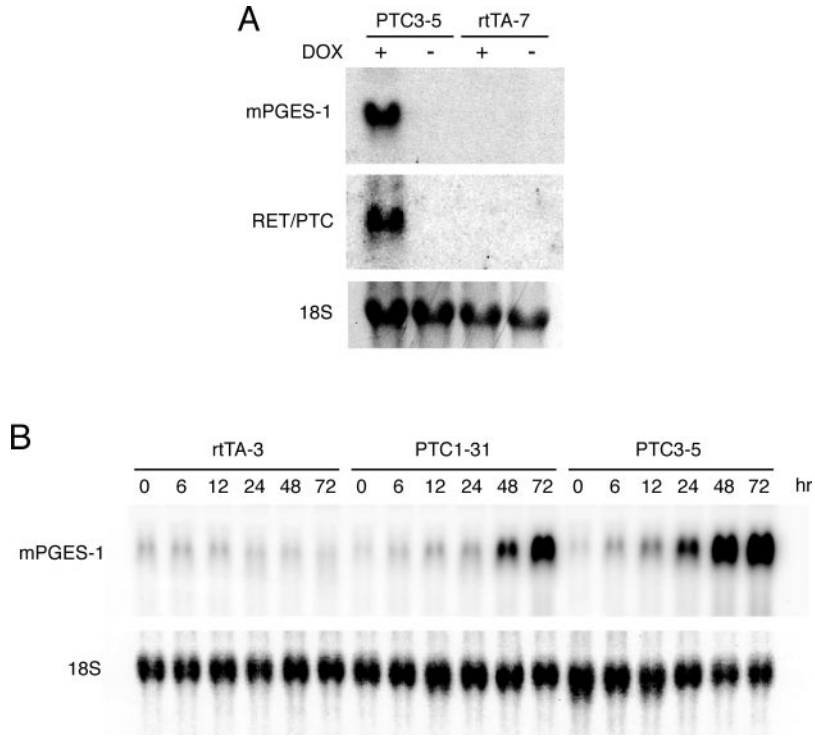

FIG. 1. **RET/PTC induces mPGES-1 mRNA.** The indicated cell lines were cultured until almost confluent and then preincubated with H3 medium (without TSH) for 3 days before addition of doxycycline (*DOX*). *A*, after the indicated preincubation, rtTA-7 (stably expressing only the doxycycline-dependent transactivator rtTA) or PTC3-5 (doxycycline-inducible RET/PTC3) cells were incubated with or without 1 μg/ml doxycycline for 12 h. Northern blotting was performed using the indicated probes. *B*, Northern blot of the indicated cells incubated with H3 medium containing 1 μg/ml doxycycline for the indicated times. Membrane was hybridized with the mPGES-1 cDNA probe. Ethidium bromide staining of 18 S ribosomal RNA was used as a loading control in all blots.

tioned media analyzed by HPLC. As shown in Fig. 3, there was a single peak exhibiting a significant increase after doxycycline-induced RET/PTC3 expression, and this corresponded to PGE_2 as determined by the retention time of ^{3}[H] PGE_2 (data not shown). Interestingly, RET/PTC-expressing cells also exhibited a decrease in two broad peaks with a retention time corresponding to that of HETE metabolites (Fig. 3). This is consistent with the co-activation of COX-2 and mPGES resulting in both increased PGH_2 biosynthesis, followed by preferential shunting toward PGE_2 generation. The robust increase in PGE_2 in conditioned media of doxycycline-treated PTC3-5 cells was completely abrogated by incubation with the pan-COX inhibitor aspirin (Fig. 4*A*). The COX-2/mPGES-1 antagonist NS-398 (48) also evoked a potent concentration-dependent inhibition of PGE_2 secretion (Fig. 4*B*). Maximal inhibition was achieved with 1 μM NS-398, which is within the range for COX-2 inhibition but lower than the IC50 for mPGES-1 (20 μM) (48). mPGES-1 is a member of the membrane-associated proteins in the eicosanoid and glutathione metabolism (MAPEG) family, which also includes 5-lipoxygenase-activating protein and leukotriene C_4 synthase. MK-886 inhibits the activity of MAPEG family members, including mPGES-1, but not COX-2 (39). MK-886 inhibited RET/PTC-induced PGE_2 production by ~50% at 10 μM, the highest concentration tolerated by these cells (Fig. 4*B*).

As an additional approach to identify the AA metabolites produced by RET/PTC-expressing PCCL3 cells, we analyzed 72-h conditioned medium of doxycycline-treated PTC3-5 cells by LC-MS. Selective ion scans at *m/z* 371, 353, 355, and 335 were used to detect 6-keto-$PGF_1\alpha$, PGE_2, $PGF_2\alpha$, and PGJ_2, respectively. Only selective ion scan at 353 *m/z* detected a significant metabolite peak, which had an identical retention time as the PGE_2 standard. We found no evidence for 6-keto $PGF_1\alpha$, $PGF_2\alpha$, or PGJ_2. PGD_2 also has a *m/z* of 353, but would be predicted to have a different retention time on this column (although this was not tested), and as shown below was not induced by RET/PTC as determined by enzyme immunoassay of its dehydration product.

We also checked the levels of other major prostaglandins, including $PGF_2\alpha$, 6-keto-PGF_{1a}, which is produced by nonenzymatic hydration of PGI_2, and 15-deoxy-$\Delta^{12,14}$-PGJ_2, which is one of the ultimate dehydration products of PGD_2. The levels of these molecules were quite low and not increased after RET/PTC expression (Fig. 4*C*). Thus, analysis of the major significant PG synthase products (other than thromboxane A_2, which was not tested by enzyme immunoassay) confirmed that PGE_2 was selectively increased by RET/PTC.

RET/PTC Induction of COX-2 mRNA Does Not Require Autocrine PGE_2 Production—As PGE_2 itself induces COX-2 expression in certain cell types (49–51), we tested the hypothesis that an early increase of this prostaglandin may be responsible at least in part for the COX-2 increase. PTC3-5 cells were treated with doxycycline to activate RET/PTC3 expression in the presence or absence of NS-398. Pretreatment with the COX-2/mPGES-1 inhibitor at a concentration shown to completely blunt PGE_2 biosynthesis failed to abrogate the RET/PTC3 induction of COX-2 mRNA (Fig. 5*A*). Similarly, pretreatment with aspirin, a pan-COX inhibitor, was without effect (data not shown). Furthermore, PCCL3 cells treated with PGE_2 did not show any induction of COX-2 or mPGES-1 in these cells (Fig. 5*B*).

RET/PTC Induces mPGES-1 Transcriptional Activity—To determine whether RET/PTC increases mPGES transcription, PTC3-5 cells were transiently transfected with a reporter construct consisting of 510 bp (−538 to −28) of the human mPGES promoter, previously shown to mediate interleukin-1β transcriptional regulation of mPGES in human orbital fibroblasts (47). RET/PTC activation was associated with a greater than 2-fold induction in mPGES promoter activity (Fig. 6).

RET/PTC-induced mPGES-1 Expression Occurs via Shc-RAS-RAF-MEK—There is now compelling genetic evidence that RET/PTC-mediated thyroid cell transformation to papillary carcinomas takes place through RAS activation via B-RAF (52). To determine the signaling pathways required by RET/PTC to induce expression of mPGES-1 we used PCCL3 cells stably transfected with doxycycline-inducible expression vectors for either $PTC3^{Y541F}$ or PTC2-PDZ, which are unable to associate with PLCγ and Shc, respectively (18). As shown in

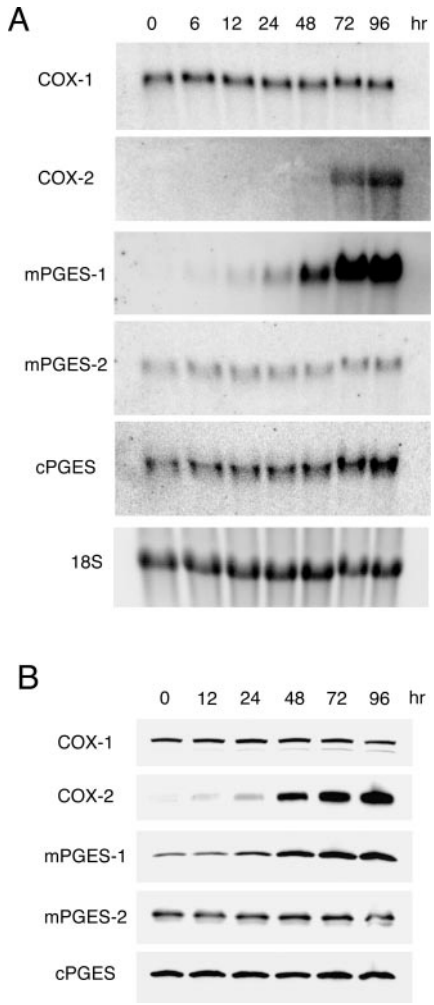

FIG. 2. **RET/PTC induces COX-2 and mPGES-1 mRNA and protein but not COX-1, mPGES-2, or cPGES.** PTC3-5 cells were cultured until almost confluent and then preincubated with H3 medium for 3 days before addition of doxycycline. After the preincubation, the cells were incubated with H3 medium containing 1 μg/ml doxycycline for the indicated times. *A*, Northern blots for COX-1, COX-2, mPGES-1, mPGES-2, and cPGES. Northern blotting was performed using the indicated probes. Ethidium bromide staining of 18 S ribosomal RNA was used as a loading control. *B*, Western blots for COX-1, COX-2, mPGES-1, mPGES-2, and cPGES. Equal amounts of protein (50 μg) from total cell lysates were separated by SDS-PAGE and blotted. Detection was performed using the indicated primary antibodies.

Fig. 7*A*, mPGES-1 mRNA was markedly increased by either RET/PTC3 or $PTC3^{Y541F}$ but not PTC2-PDZ. Whereas PI3 kinase inhibitors were without effect (Fig. 7*B*), pretreatment with the MEK inhibitors PD98059 or U0126 dramatically blunted RET/PTC-induced mPGES-1 expression (Fig. 7, *C* and *D*). Pretreatment with the p38 MAPK inhibitor SB203580 also failed to prevent RET/PTC-induced expression of mPGES-1 mRNA (data not shown). To further substantiate the involvement of Ras and MEK as intermediates in the stimulation mPGES-1 mRNA abundance, we used PCCL3 cells stably transfected with doxycycline-inducible expression vectors for either $H\text{-}Ras^{V12}$ (Ras-25 cells) or $MEK1^{S217E/S221E}$ (MEK1–55 cells), coding for constitutively active forms of these signaling proteins (42, 43). As shown in Fig. 7*E*, doxycycline-induced expression of $H\text{-}Ras^{V12}$ and $MEK1^{S217E/S221E}$ induced mPGES-1 mRNA levels, consistent with involvement of this pathway in control of its expression.

DISCUSSION

Eicosanoids are thought to play a significant role in survival, growth and metabolic support of tumor cells (53). Prominent among them are the prostaglandins, of which PGE_2 is the most abundant in nature. PGE_2 biosynthesis from arachidonic acid is controlled by two rate-limiting enzymatic reactions (54). The first step is catalyzed by COX, which transforms arachidonic acid into the unstable PGH_2. The second step is catalyzed by PGES, which converts PGH_2 into PGE_2. The inducible forms of these enzymes, COX-2 (22, 25, 45–49) and more recently mPGES-1 (28), have been linked to cancer, whereas COX-1 and cPGES are constitutive expressed in most tissues and involved in housekeeping functions. mPGES-2 was identified very recently, and is reported to process substrate arising from either COX isozyme, although its role in physiology and in disease pathogenesis is unknown (41). The genetic (33–35) and pharmacological (19, 20, 55–57) evidence implicating PGE_2 and its biosynthetic enzymes in cancer pathogenesis is now quite compelling.

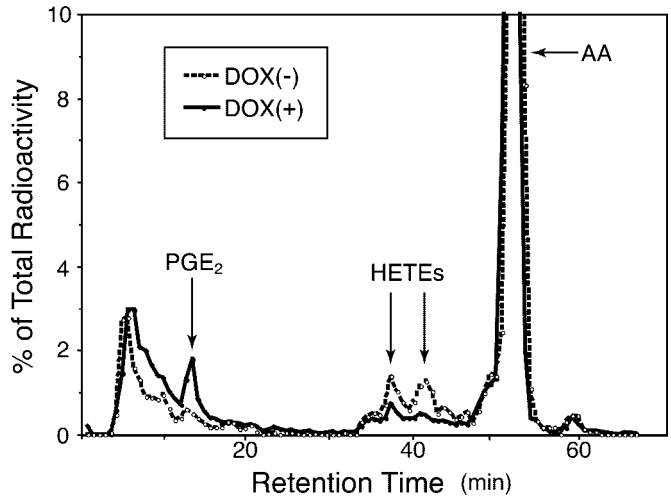

FIG. 3. **HPLC analysis of conditioned medium after induction of RET/PTC.** HPLC analysis of conditioned media from PTC3-5 cells metabolically labeled with [^{3}H]AA and treated in the presence (*solid line*) or absence (*dashed line*) of doxycycline (*DOX*) as indicated under "Experimental Procedures." A representative radiochromatogram is shown. The indicated AA metabolites were identified based on retention times as compared with standards.

Despite the ubiquitous dysregulation of gene expression of the PGE_2 biosynthetic enzymes in solid tumors, the molecular mechanisms involved in each tumor type remain to be determined. Here we demonstrate a causal relationship between well documented tumor-initiating genetic events in thyroid cells, namely the RET/PTC rearrangements, and induction of expression of mPGES-1 and COX-2. Regulation of mPGES-1 has been primarily explored in response to cytokines. Following an intravenous lipopolysaccharide *in vivo* challenge, mPGES-1 mRNA was induced in multiple tissues (39), and this is also seen in the forepaw during adjuvant-induced arthritis (39, 58). Interleukin-1β and -1α induce mPGES-1 through activation of both p38 MAPK as well as ERK in human orbital fibroblasts (47). The discovery that mPGES-1 is also up-regulated in certain cancers is relatively recent (28, 59–61), and the mechanisms accounting for this largely unknown. Our observation that mPGES-1 was one of the most highly expressed gene products 12 h after RET/PTC induction in an expression profiling experiment led us to explore this in greater detail. PCCL3 cells express almost no detectable mPGES-1 under basal, unstimulated conditions (*i.e.* in the absence of TSH). Activation of either RET/PTC1 or RET/PTC3 resulted in robust induction of mPGES-1 mRNA abundance. This was caused at least in part by increased transcriptional activity. There is now strong evidence pointing to requirement of signaling via Shc-RAS-B-RAF for thyroid cell transformation by RET/PTC *in vitro* (62, 63)

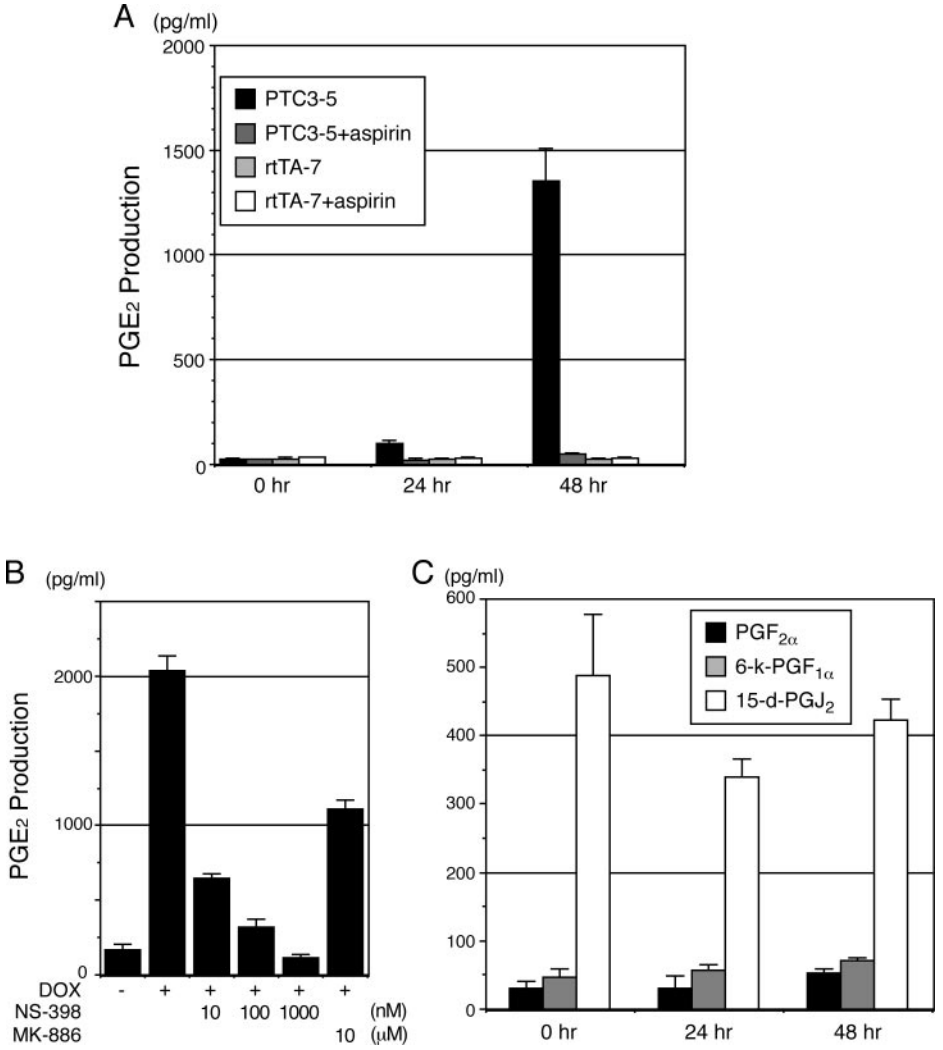

FIG. 4. **Effect of RET/PTC on secretion of specific prostaglandins into conditioned media as determined by enzyme immunoassay.** *A*, effects of doxycycline-induced RET/PTC expression on immunoassayable PGE_2 secretion into conditioned media. PTC3-5 or rtTA-7 cells were incubated in 15-cm dishes with H3 medium containing 1 μg/ml doxycycline with or without 100 μM aspirin for the indicated times. The medium was collected and subjected to PGE_2 immunoassay. PGE_2 levels are shown as picograms/ml. Data are from a single experiment that was replicated twice. *B*, inhibition of RET/PTC-induced PGE_2 secretion by COX-2 or mPGES-1 antagonists. PTC3-5 cells were incubated in 6-cm dishes with H3 medium containing 1 μg/ml doxycycline (*DOX*) and the indicated concentration of NS-398 or MK-886 for 48 h. The medium was collected and subjected to PGE_2 immunoassay. PGE_2 levels are shown as picograms/ml. Data are the average of a single experiment performed in triplicate. Similar results were obtained in two additional experiments. *C*, RET/PTC effects on other major prostaglandins. PTC3-5 cells were incubated in 6-cm dishes with 2 ml of H3 medium containing 1 μg/ml doxycycline for the indicated times. The medium was collected and assayed for $PGF_{2}\alpha$, 6-keto-$PGF_{1}\alpha$, or 15-deoxy-$\Delta^{12,\ 14}$-PGJ_2 by enzyme immunoassay. Data are the average of two experiments performed in triplicate.

and *in vivo* (52, 64). Constitutive activation of RET kinase results in autophosphorylation of Y1062 in the C terminus of the receptor, which couples to Shc-RAS, and drives transformation by RET in NIH3T3 cells (62). Similarly, RET/PTC-mediated dedifferentiation of thyroid cells requires engagement of this pathway (63). In human papillary thyroid carcinomas, mutations of *RET/PTC*, *RAS*, or *B-RAF* were found in 66% of cases, but in this series there was no single tumor with a clonal mutation in more than one of these oncogenes (52). The lack of concordance for these mutations provides strong genetic evidence for the centrality of this pathway in thyroid cell transformation to PTC, a premise that was recently confirmed (64). It was therefore of interest to determine the pathways downstream of RET/PTC required to induce mPGES-1 expression. Several lines of evidence point again to the requirement for Shc-RAS-MAPK signaling for this effect. 1) Expression of PTC2-PDZ, which lacks Y1062 and is unable to couple to Shc, failed to activate mPGES-1 expression, whereas $PTC3^{Y541F}$, which associates normally to Shc but not to PLCγ, did so normally. 2) RET/PTC induction of mPGES-1 was blocked by pretreatment with MEK inhibitors. 3) mPGES-1 was induced by expression of constitutively active mutants of H-RAS and MEK1.

Higher levels of COX-2 expression are found in human thyroid cancers compared with benign nodules and adjacent normal thyroid tissues (31, 32). Mechanisms accounting for this are unknown. TSH regulates arachidonic acid release from membrane phospholipids and increases COX-2 activity and PGE_2 production in thyroid FRTL5 cells (65). To our knowledge, there are no data on the potential role of receptor tyrosine kinases on COX-2 regulation in thyrocytes. In other cell types, such as colorectal cancer cells, activation of HER-2 or HER-3 receptors induces COX-2 transcription (66). In thyroid PCCL3 cells, however, RET/PTC activation induced COX-2 mRNA abundance with a markedly delayed time course, which is not consistent with transcriptional regulation, although this was not formally tested. Because in certain cell types PGE_2 itself can induce or potentiate COX-2 gene expression (49, 51, 67), we tested this premise by pretreating cells with COX inhibitors prior to activation of RET/PTC, yet this did not impair the up-regulation of COX-2 mRNA. Neither did treatment of PCCL3 cells with PGE_2. Thus, a burst of PGE_2 production mediated by the sharp early rise in mPGES-1 (perhaps acting in concert with COX-1) cannot account for the activation of COX-2 mRNA in these cells. In addition to being subject to transcriptional control, COX-2 gene expression is also regulated by mRNA stabilization. Conditional activation of $K\text{-}Ras^{V12}$ in rat intestinal epithelial cells is associated with marked induction of COX-2 gene expression, acting via MEK-ERK to induce gene transcription, and through PI3K/Akt/PKB to stabilize the transcript (68). By contrast, in alveolar macrophages PI3K negatively regulates mRNA stability (69). Pretreatment of PCCL3 cells with the PI3K inhibitors LY294002 or wortmannin inhibited COX-2 mRNA accumulation 72 h

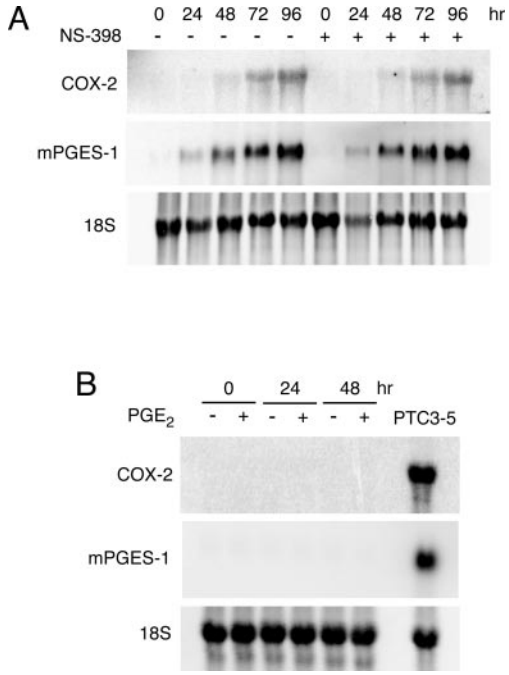

FIG. 5. **RET/PTC-induction of COX-2 mRNA does not require PGE_2.** *A*, PTC3-5 cells were preincubated with H3 for 3 days and then with H3 medium containing 1 μg/ml doxycycline with or without 10 μM of NS-398 for the stated times. *B*, PCCL3 cells were preincubated with H3 for 3 days and incubated with H3 medium with or without 10 μM PGE_2 for the indicated times. PTC3-5 cells treated with doxycycline for 96 h was used as a positive control. Northern blotting was performed using the indicated probes. Ethidium bromide staining of 18 S ribosomal RNA was used as a loading control.

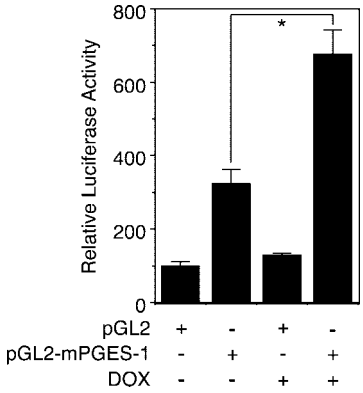

FIG. 6. **RET/PTC induces mPGES-1 gene transcription.** PTC3-5 cells were grown to ~80% confluence in 24-well plate with H4 medium. Cells were then cotransfected with 800 ng of either pGL2 (−538 to −28) mPGES-1 promoter vector or a promoterless pGL2 and 80 ng of pRL-TK as indicated under "Experimental Procedures." Five hours after transfection medium was replaced by H3 medium with or without doxycycline (*DOX*). Cell lysates were prepared 48 h after transfection, and luciferase activities measured with the Dual-Luciferase Reporter Assay System (Promega) according to the protocol from the manufacturer. Data are expressed as relative luciferase activities. Each *bar* indicates the mean and the standard deviation of four assay wells. Data are from a representative experiment that was replicated twice. *, $p < 0.05$

after RET/PTC induction, consistent with interference with mRNA stabilization.[2]

There is evidence that COX-1 preferentially couples functionally with cPGES (37), whereas COX-2 does so with mPGES-1 (38), based on cotransfection experiments in HEK293 cells. Accordingly, Han *et al.* (70) observed that mismatched expression of COX-2 and cPGES does not result in

[2] N. Mitsutake, unpublished data.

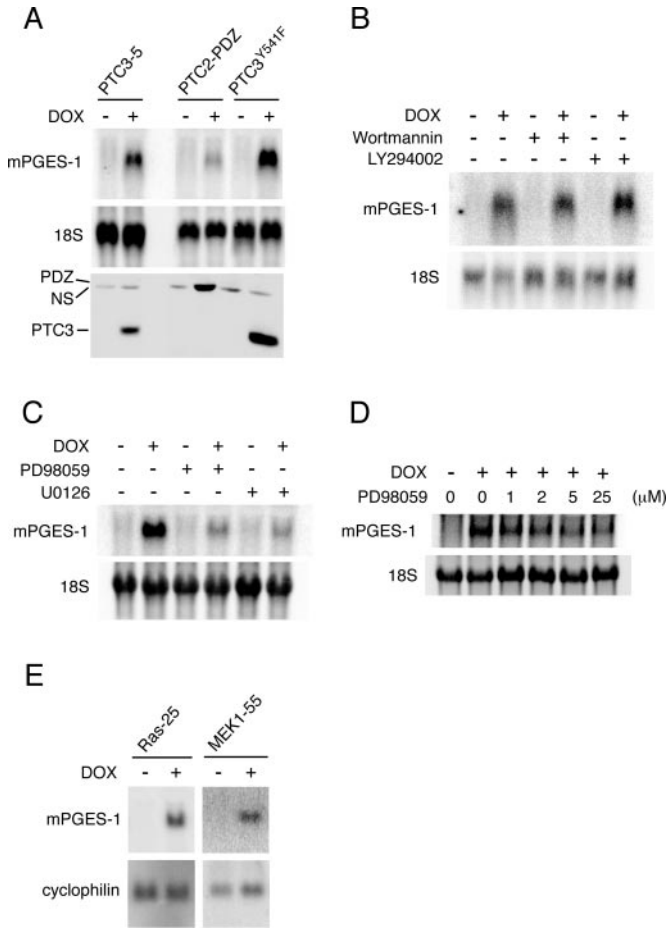

FIG. 7. **RET/PTC-induced mPGES-1 mRNA requires MEK-ERK activation.** All cells were grown in 10-cm dishes until almost confluent and then preincubated with H3 medium for 3 days. *A*, the indicated cell line was incubated with or without 1 μg/ml doxycycline (*DOX*) for 24 h. Northern blotting was performed using mPGES-1 cDNA (*upper lane*). Ethidium bromide staining of 18 S ribosomal RNA was used as a loading control (*middle lane*). Western blot for RET/PTC3 or the indicated mutants is shown (*lower lane*). *NS*, nonspecific bands. *B–D*, PTC3-5 cells were pretreated with or without the indicated inhibitors of PI3K (*B*, 300 nM wortmannin or 25 μM LY294002) or MEK (*C*, 25 μM PD98059 or 10 μM U0126; *D*, the indicated concentrations of PD98059) for 30 min prior to addition of 1 μg/ml doxycycline or vehicle for 24 h. Northern membranes were probed with mPGES-1 cDNA (*upper lanes*). Ethidium bromide staining of 18 S ribosomal RNA was used as a loading control (*lower lanes*). *E*, Ras-25 (PCCL3 cells with inducible expression of H-Ras^{V12}) or MEK1–55 (PCCL3 cells with inducible expression of $MEK1^{S217E/S221E}$) cells were incubated with or without 1 μg/ml doxycycline for 24 h. Northern blotting was performed with mPGES-1 (*upper lanes*) or cyclophilin (*lower lanes*) cDNA.

efficient PGE_2 production in KAT-50 thyroid cells, whereas following transfection of mPGES-1 PGE_2 biosynthesis is markedly increased. Thus, the ability of RET/PTC to activate expression of both COX-2 and mPGES-1 may explain the very robust activation of PGE_2 biosynthesis observed. Coactivation of mPGES-1 may also account for the preferential secretion of PGE_2, at the expense of other prostanoid metabolites, through shunting of PGH_2 toward this terminal step in PGE_2 biosynthesis. However, despite the evidence for preferential functional coupling of COX-2 and mPGES-1, it should be pointed out that in certain cell types both COX isoforms can funnel substrate to both cPGES and mPGES-1. Moreover, this proposed functional coupling cannot be explained based on different subcellular distribution, because both COX isoforms colocalize to the inner surface of the endoplasmic reticulum and nuclear envelope, and there is no evidence for direct binding between individual COX isoforms and PGE synthases (24).

If these findings are recapitulated in thyroid papillary thyroid carcinomas harboring *RET/PTC*, *RAS*, or *B-RAF* mutations (52, 64), it will be of considerable interest to understand how paracrine PGE_2 production might affect tumor development and progression. Because of the recognized function of PGE_2 in tumor angiogenesis and immunomodulation, these observations raise the possibility that pharmacological inhibition of this pathway may hold therapeutic promise in these cancers.

REFERENCES

1. Santoro, M., Melillo, R. M., Carlomagno, F., Fusco, A., and Vecchio, G. (2002) *Ann. N. Y. Acad. Sci.* **963,** 116–121
2. Bongarzone, I., Fugazzola, L., Vigneri, P., Mariani, L., Mondellini, P., Pacini, F., Basolo, F., Pinchera, A., Pilotti, S., and Pierotti, M. A. (1996) *J. Clin. Endocrinol. Metab.* **81,** 2006–2009
3. Nikiforov, Y. E., Rowland, J. M., Bove, K. E., Monforte-Munoz, H., and Fagin, J. A. (1997) *Cancer Res.* **57,** 1690–1694
4. Fugazzola, L., Pilotti, S., Pinchera, A., Vorontsova, T. V., Mondellini, P., Bongarzone, I., Greco, A., Astakhova, L., Butti, M. G., and Demidchik, E. P. (1995) *Cancer Res.* **55,** 5617–5620
5. Klugbauer, S., Lengfelder, E., Demidchik, E. P., and Rabes, H. M. (1995) *Oncogene* **11,** 2459–2467
6. Durick, K., Yao, V. J., Borrello, M. G., Bongarzone, I., Pierotti, M. A., and Taylor, S. S. (1995) *J. Biol. Chem.* **270,** 24642–24645
7. Tong, Q., Xing, S., and Jhiang, S. M. (1997) *J. Biol. Chem.* **272,** 9043–9047
8. Borrello, M. G., Alberti, L., Arighi, E., Bongarzone, I., Battistini, C., Bardelli, A., Pasini, B., Piutti, C., Rizzetti, M. G., Mondellini, P., Radice, M. T., and Pierotti, M. A. (1996) *Mol. Cell. Biol.* **16,** 2151–2163
9. Arighi, E., Alberti, L., Torriti, F., Ghizzoni, S., Rizzetti, M. G., Pelicci, G., Pasini, B., Bongarzone, I., Piutti, C., Pierotti, M. A., and Borrello, M. G. (1997) *Oncogene* **14,** 773–782
10. Durick, K., Gill, G. N., and Taylor, S. S. (1998) *Mol. Cell. Biol.* **18,** 2298–2308
11. Jhiang, S. M., Sagartz, J. E., Tong, Q., Parker-Thornburg, J., Capen, C. C., Cho, J. Y., Xing, S., and Ledent, C. (1996) *Endocrinology* **137,** 375–378
12. Powell, D. J. J., Russell, J., Nibu, K., Li, G., Rhee, E., Liao, M., Goldstein, M., Keane, W. M., Santoro, M., Fusco, A., and Rothstein, J. L. (1998) *Cancer Res.* **58,** 5523–5528
13. Viglietto, G., Chiappetta, G., Martinez-Tello, F. J., Fukunaga, F. H., Tallini, G., Rigopoulou, D., Visconti, R., Mastro, A., Santoro, M., and Fusco, A. (1995) *Oncogene* **11,** 1207–1210
14. Sugg, S. L., Ezzat, S., Rosen, I. B., Freeman, J. L., and Asa, S. L. (1998) *J. Clin. Endocrinol. Metab.* **83,** 4116–4122
15. Ito, T., Seyama, T., Iwamoto, K. S., Hayashi, T., Mizuno, T., Tsuyama, N., Dohi, K., Nakamura, N., and Akiyama, M. (1993) *Cancer Res.* **53,** 2940–2943
16. Nikiforov, Y. E., Koshoffer, A., Nikiforova, M., Stringer, J., and Fagin, J. A. (1999) *Oncogene* **18,** 6330–6334
17. Nikiforova, M. N., Stringer, J. R., Blough, R., Medvedovic, M., Fagin, J. A., and Nikiforov, Y. E. (2000) *Science* **290,** 138–141
18. Wang, J., Knauf, J. A., Basu, S., Puxeddu, E., Kuroda, H., Santoro, M., Fusco, A., and Fagin, J. A. (2003) *Mol. Endocrinol.* **17,** 1425–1436
19. Baron, J. A., Cole, B. F., Sandler, R. S., Haile, R. W., Ahnen, D., Bresalier, R., McKeown-Eyssen, G., Summers, R. W., Rothstein, R., Burke, C. A., Snover, D. C., Church, T. R., Allen, J. I., Beach, M., Beck, G. J., Bond, J. H., Byers, T., Greenberg, E. R., Mandel, J. S., Marcon, N., Mott, L. A., Pearson, L., Saibil, F., and van Stolk, R. U. (2003) *N. Engl. J. Med.* **348,** 891–899
20. Sandler, R. S., Halabi, S., Baron, J. A., Budinger, S., Paskett, E., Keresztes, R., Petrelli, N., Pipas, J. M., Karp, D. D., Loprinzi, C. L., Steinbach, G., and Schilsky, R. (2003) *N. Engl. J. Med.* **348,** 883–890
21. Chen, X., Li, N., Wang, S., Hong, J., Fang, M., Yousselfson, J., Yang, P., Newman, R. A., Lubet, R. A., and Yang, C. S. (2002) *Carcinogenesis* **23,** 2095–2102
22. Zweifel, B. S., Davis, T. W., Ornberg, R. L., and Masferrer, J. L. (2002) *Cancer Res.* **62,** 6706–6711
23. Smith, W. L., DeWitt, D. L., and Garavito, R. M. (2000) *Annu. Rev. Biochem.* **69,** 145–182
24. Smith, W. L., and Langenbach, R. (2001) *J. Clin. Invest.* **107,** 1491–1495
25. Langenbach, R., Morham, S. G., Tiano, H. F., Loftin, C. D., Ghanayem, B. I., Chulada, P. C., Mahler, J. F., Lee, C. A., Goulding, E. H., Kluckman, K. D., and. (1995) *Cell* **83,** 483–492
26. Gross, G. A., Imamura, T., Luedke, C., Vogt, S. K., Olson, L. M., Nelson, D. M., Sadovsky, Y., and Muglia, L. J. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 11875–11879
27. Shitashige, M., Morita, I., and Murota, S. (1998) *Biochim. Biophys. Acta* **1389,** 57–66
28. Yoshimatsu, K., Golijanin, D., Paty, P. B., Soslow, R. A., Jakobsson, P. J., DeLellis, R. A., Subbaramaiah, K., and Dannenberg, A. J. (2001) *Clin. Cancer Res.* **7,** 3971–3976
29. Eberhart, C. E., Coffey, R. J., Radhika, A., Giardiello, F. M., Ferrenbach, S., and DuBois, R. N. (1994) *Gastroenterology* **107,** 1183–1188
30. Nose, F., Ichikawa, T., Fujiwara, M., and Okayasu, I. (2002) *Am. J. Clin. Pathol.* **117,** 546–551
31. Cornetta, A. J., Russell, J. P., Cunnane, M., Keane, W. M., and Rothstein, J. L. (2002) *Laryngoscope* **112,** 238–242
32. Specht, M. C., Tucker, O. N., Hocever, M., Gonzalez, D., Teng, L., and Fahey, T. J., III (2002) *J. Clin. Endocrinol. Metab.* **87,** 358–363
33. Oshima, M., Murai, N., Kargman, S., Arguello, M., Luk, P., Kwong, E., Taketo, M. M., and Evans, J. F. (2001) *Cancer Res.* **61,** 1733–1740
34. Oshima, M., Dinchuk, J. E., Kargman, S. L., Oshima, H., Hancock, B., Kwong, E., Trzaskos, J. M., Evans, J. F., and Taketo, M. M. (1996) *Cell* **87,** 803–809
35. Chulada, P. C., Thompson, M. B., Mahler, J. F., Doyle, C. M., Gaul, B. W., Lee, C., Tiano, H. F., Morham, S. G., Smithies, O., and Langenbach, R. (2000) *Cancer Res.* **60,** 4705–4708
36. Fitzpatrick, F. A., and Soberman, R. (2001) *J. Clin. Invest.* **107,** 1347–1351
37. Tanioka, T., Nakatani, Y., Semmyo, N., Murakami, M., and Kudo, I. (2000) *J. Biol. Chem.* **275,** 32775–32782
38. Murakami, M., Naraba, H., Tanioka, T., Semmyo, N., Nakatani, Y., Kojima, F., Ikeda, T., Fueki, M., Ueno, A., Oh, S., and Kudo, I. (2000) *J. Biol. Chem.* **275,** 32783–32792
39. Mancini, J. A., Blood, K., Guay, J., Gordon, R., Claveau, D., Chan, C. C., and Riendeau, D. (2001) *J. Biol. Chem.* **276,** 4469–4475
40. Tanikawa, N., Ohmiya, Y., Ohkubo, H., Hashimoto, K., Kangawa, K., Kojima, M., Ito, S., and Watanabe, K. (2002) *Biochem. Biophys. Res. Commun.* **291,** 884–889
41. Murakami, M., Nakashima, K., Kamei, D., Masuda, S., Ishikawa, Y., Ishii, T., Ohmiya, Y., Watanabe, K., and Kudo, I. (2003) *J. Biol. Chem.* **278,** 37937–37947
42. Saavedra, H. I., Knauf, J. A., Shirokawa, J. M., Wang, J., Ouyang, B., Elisei, R., Stambrook, P. J., and Fagin, J. A. (2000) *Oncogene* **19,** 3948–3954
43. Shirokawa, J. M., Elisei, R., Knauf, J. A., Hara, T., Wang, J., Saavedra, H. I., and Fagin, J. A. (2000) *Mol. Endocrinol.* **14,** 1725–1738
44. Schena, M., Shalon, D., Davis, R. W., and Brown, P. O. (1995) *Science* **270,** 467–470
45. Rose, M. P., Myatt, L., and Elder, M. G. (1990) *Prostaglandins Leukotrienes Essent. Fatty Acids* **39,** 303–309
46. Nithipatikom, K., Laabs, N. D., Isbell, M. A., and Campbell, W. B. (2003) *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **785,** 135–145
47. Han, R., Tsui, S., and Smith, T. J. (2002) *J. Biol. Chem.* **277,** 16355–16364
48. Thoren, S., and Jakobsson, P. J. (2000) *Eur. J. Biochem.* **267,** 6428–6434
49. Murakami, M., Kuwata, H., Amakasu, Y., Shimbara, S., Nakatani, Y., Atsumi, G., and Kudo, I. (1997) *J. Biol. Chem.* **272,** 19891–19897
50. Faour, W. H., He, Y., He, Q. W., de Ladurantaye, M., Quintero, M., Mancini, A., and Di Battista, J. A. (2001) *J. Biol. Chem.* **276,** 31720–31731
51. Fournier, T., Fadok, V., and Henson, P. M. (1997) *J. Biol. Chem.* **272,** 31065–31072
52. Kimura, E. T., Nikiforova, M. N., Zhu, Z., Knauf, J. A., Nikiforov, Y. E., and Fagin, J. A. (2003) *Cancer Res.* **63,** 1454–1457
53. Koki, A., Khan, N. K., Woerner, B. M., Dannenberg, A. J., Olson, L., Seibert, K., Edwards, D., Hardy, M., Isakson, P., and Masferrer, J. L. (2002) *Adv. Exp. Med. Biol.* **507,** 177–184
54. Murakami, M., Nakatani, Y., Atsumi, G., Inoue, K., and Kudo, I. (1997) *Crit. Rev. Immunol.* **17,** 225–283
55. Grubbs, C. J., Lubet, R. A., Koki, A. T., Leahy, K. M., Masferrer, J. L., Steele, V. E., Kelloff, G. J., Hill, D. L., and Seibert, K. (2000) *Cancer Res.* **60,** 5599–5602
56. Harris, R. E., Alshafie, G. A., Abou-Issa, H., and Seibert, K. (2000) *Cancer Res.* **60,** 2101–2103
57. Reddy, B. S., Hirose, Y., Lubet, R., Steele, V., Kelloff, G., Paulson, S., Seibert, K., and Rao, C. V. (2000) *Cancer Res.* **60,** 293–297
58. Claveau, D., Sirinyan, M., Guay, J., Gordon, R., Chan, C. C., Bureau, Y., Riendeau, D., and Mancini, J. A. (2003) *J. Immunol.* **170,** 4738–4744
59. Yoshimatsu, K., Altorki, N. K., Golijanin, D., Zhang, F., Jakobsson, P. J., Dannenberg, A. J., and Subbaramaiah, K. (2001) *Clin. Cancer Res.* **7,** 2669–2674
60. Kamei, D., Murakami, M., Nakatani, Y., Ishikawa, Y., Ishii, T., and Kudo, I. (2003) *J. Biol. Chem.* **278,** 19396–19405
61. Jabbour, H. N., Milne, S. A., Williams, A. R., Anderson, R. A., and Boddy, S. C. (2001) *Br. J. Cancer* **85,** 1023–1031
62. Asai, N., Murakami, H., Iwashita, T., and Takahashi, M. (1996) *J. Biol. Chem.* **271,** 17644–17649
63. Knauf, J. A., Kuroda, H., Basu, S., Kimura, E. T., and Fagin, J. A. (2003) *Oncogene* **22,** 4406–4412
64. Soares, P., Trovisco, V., Rocha, A. S., Lima, J., Castro, P., Preto, A., Maximo, V., Botelho, T., Seruca, R., and Sobrinho-Simoes, M. (2003) *Oncogene* **22,** 4578–4580
65. Tahara, K., Grollman, E. F., Saji, M., and Kohn, L. D. (1991) *J. Biol. Chem.* **266,** 440–448
66. Vadlamudi, R., Mandal, M., Adam, L., Steinbach, G., Mendelsohn, J., and Kumar, R. (1999) *Oncogene* **18,** 305–314
67. Tjandrawinata, R. R., and Hughes-Fulford, M. (1997) *Adv. Exp. Med. Biol.* **407,** 163–170
68. Sheng, H., Shao, J., and DuBois, R. N. (2001) *Cancer Res.* **61,** 2670–2675
69. Monick, M. M., Robeff, P. K., Butler, N. S., Flaherty, D. M., Carter, A. B., Peterson, M. W., and Hunninghake, G. W. (2002) *J. Biol. Chem.* **277,** 32992–33000
70. Han, R., and Smith, T. J. (2002) *J. Biol. Chem.* **277,** 36897–36903

Microsomal Prostaglandin E_2 Synthase-1 Is Induced by Conditional Expression of RET/PTC in Thyroid PCCL3 Cells through the Activation of the MEK-ERK Pathway

Efisio Puxeddu, Norisato Mitsutake, Jeffrey A. Knauf, Sonia Moretti, Hei W. Kim, Karen A. Seta, Diane Brockman, Leslie Myatt, David E. Millhorn and James A. Fagin

J. Biol. Chem. 2003, 278:52131-52138.
doi: 10.1074/jbc.M306003200 originally published online October 10, 2003
