Cytoplasmic Prostaglandin E₂ Synthase Is Dominantly Expressed in Cultured KAT-50 Thyrocytes, Cells That Express Constitutive Prostaglandin-endoperoxide H Synthase-2

BASIS FOR LOW PROSTAGLANDIN E₂ PRODUCTION*

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Rui Han and Terry J. Smith‡
From the Division of Molecular Medicine, Department of Medicine, Harbor-UCLA Medical Center, Torrance, California 90502 and the David Geffen School of Medicine at the University of California, Los Angeles, California 90095

The recent identification and cloning of two glutathione-dependent prostaglandin E₂ synthase (PGES) genes has yielded important insights into the terminal step of PGE₂ synthesis. These enzymes form efficient functional pairs with specific members of the prostaglandin-endoperoxide H synthase (PGHS) family. Microsomal PGES (mPGES) is inducible and works more efficiently with PGHS-2, the inflammatory cyclooxygenase, while the cytoplasmic isoform (cPGES) pairs functionally with PGHS-1, the cyclooxygenase that ordinarily exhibits constitutive expression. KAT-50, a well differentiated thyroid epithelial cell line, expresses high levels of PGHS-2 but surprisingly low levels of PGE₂ when compared with human orbital fibroblasts. Moreover, PGHS-1 protein cannot be detected in KAT-50. We report here that KAT-50 cells express high basal levels of cPGES but mPGES mRNA and protein are undetectable. Thus, KAT-50 cells express the inefficient PGHS-2/cPGES pair, and this results in modest PGE₂ production. The high levels of cPGES and the absence of mPGES expression result from dramatic differences in the activities of their respective gene promoters. When mPGES is expressed in KAT-50 by transiently transflecting the cells, PGE₂ production is up-regulated substantially. These observations indicate that naturally occurring cells can express a suboptimal profile of PGHS and PGES isoforms, resulting in diminished levels of PGE₂ generation.

The field of prostaglandin biology has benefited enormously from the recent identification and cloning of two prostaglandin-endoperoxide H synthase (EC 1.14.99.1, PGHS)¹ isoenzymes (1–6) and two forms of prostaglandin-E₂ synthases (EC 5.3.99.3, PGES) (7–9). The activities of PGHS involve the conversion of arachidonate, first into PGG₂ and subsequently into PGG₂ in two rate-limiting steps, catalyzed by discrete active sites on these enzymes (10). PGHS-1 is a constitutive enzyme involved in many “housekeeping” functions in most of the tissues and cell types thus far examined (1, 2). In contrast, PGHS-2, the inflammatory cyclooxygenase, is expressed at extremely low levels under basal conditions in most cell types but is highly inducible by cytokines and growth factors (3–6). The PGES enzymes are glutathione-dependent and involved in the conversion of PGG₂ to PGE₂. PGES localizes to the cytosol and is a constitutively expressed enzyme, identical to p23, a chaperone for the hsp90/glucocorticoid receptor complex (8). On the other hand, mPGES represents a microsomal protein, is inducible by cytokines, and glucocorticoid repressible (9). A number of different cell types have been examined and found to express both mPGES and cPGES (7–9).

A functional association linking PGHS-1 with cPGES and PGHS-2 with mPGES has been made on the basis of studies conducted in transfected cells overexpressing the enzymes (8, 9). The authors of those earlier studies concluded that these associations yield efficient enzyme pairs. These initial findings have been extended subsequently to human synovial fibroblasts where treatment with IL-1β or tumor necrosis factor-α up-regulated mPGES mRNA and protein in a manner that is coordinated with an induction of PGHS-2 (11). More recently, this relationship between PGHS-2 and mPGES has also been demonstrated in orbital fibroblasts (12), cells that produce particularly high levels of PGE₂ when provoked by inflammatory cytokines such as IL-1β, leukoregulin, and CD154 (13–15). In those cells, an overlap in the signal transduction pathways utilized by IL-1β to induce both enzymes was demonstrated (12). This signaling involved both p38 and ERK1/2 components of the mitogen-activated protein kinase pathways (12). Moreover, the induction by IL-1β of mPGES in orbital fibroblasts depends, at least in part, on the activity of PGHS-2. Glucocorticoids can block the up-regulation by IL-1β of both PGHS-2 and mPGES in synovial and orbital fibroblasts (11, 12). Because these fibroblasts are believed to play important roles in the pathogenesis of rheumatoid arthritis and Graves’ ophthalmopathy, respectively, it is tempting to implicate both PGHS-2 and mPGES in these disease processes.

KAT-50, an established line of human thyroid epithelial cells derived from non-neoplastic tissue, expresses high levels of constitutive PGHS-2 (16, 17). Moreover, basal generation of PGE₂ in these cells can be substantially diminished by treating them with PGHS-2 selective inhibitors, such as SC58125. In fact, several types of mammalian cells exhibit high levels of unstimulated PGHS-2 expression, including hepatic stellate cells (18), bronchial epithelium (19), pancreatic islet (20), and granulosa cells (21). While the function of unprovoked PGHS-2 is uncertain, this enzyme may serve housekeeping roles under...
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certain circumstances and therefore might replace the functions ordinarily ascribed to PGHS-1. In fact, PGHS-1 protein is undetectable in either un-stimulated or cytokine-activated KAT-50 cells. Moreover, most of the PGE2 production detected in KAT-50 cells derived from the action of PGHS-2 (16). Thus KAT-50 cells represent a growing list of cell types, both well differentiated and neoplastic, where PGHS-2 represents the dominant, constitutive cyclooxygenase. Of particular interest is the lack of up-regulation of PGHS-2 expression in KAT-50 cells by factors such as serum and IL-1β, agents that ordinarily enhance cyclooxygenase expression in most cell types. It was noted in the course of earlier studies that despite the high basal PGHS-2 levels, KAT-50 cells produce rather low levels of PGE2 (16). Even when treated with exogenous arachidonate, KAT-50 cultures were found to generate PGE2 levels substantially lower than those found in orbital fibroblasts when high PGHS-2 levels are induced. These observations suggested that some critical component(s) of the prostanoid synthetic machinery in KAT-50 might be suboptimally represented.

In this paper, we report that KAT-50 cells, despite expressing high levels of PGHS-2, produce very low levels of PGE2 compared with orbital fibroblasts. The basis for this diminished prostanoid generation relates to an absence of detectable mPGES expression. Instead, KAT-50 cells express high levels of cPGES mRNA and protein. This disparity results from dramatically different activities of the respective PGES gene promoters. Thus, in these cells, PGHS-2 and cPGES are predominately co-expressed, creating an inefficient enzyme pair. When mPGES expression was introduced by transiently transfecting KAT-50 cells, PGE2 production was enhanced substantially. These observations suggest that a collaboration of PGHS-2 and cPGES can occur naturally in some cells. This pairing results in relatively low levels of PGE2 generation.

EXPERIMENTAL PROCEDURES

Materials—cDNA encoding human mPGES and anti-human mPGES antibodies were kindly provided by Dr. Per-Johan Jakobsson (Karolinska Institute, Stockholm, Sweden). Professor I. Kudo (Showa University, Tokyo, Japan) kindly supplied cDNA encoding p23, which was a gift of Dr. David Toft (Mayo Clinic, Rochester, MN). Anti-PGHS-1 and -PGHS-2 monoclonal antibodies were purchased from Cayman Chemical Co. (Ann Arbor, MI). IL-1β was obtained from Bio-source International (Camarillo, CA), and dexamethasone (1,4-pregnadien-9-fluoro-16,21-triol-3,20-dione) was from Sigma. SC55125 was a generous gift from Searle & Co. (St. Louis, MO). Human PGHS-1 and PGHS-2 cDNA plasmids were gifts from Drs. Donald Young and Kerry O'Banion (University of Rochester, Rochester, NY). Dr. Stephen M. Prescott (University of Utah, Salt Lake City, UT) generously provided plasmids containing fragments of the human PGHS-2 promoter. Plasmid –1800pGL2 contained the sequence –1840/+123. pBR-TK (Promega, Madison, WI) was used as a transfection efficiency control, pGL2 Basic and pGL2 Promoter were also purchased from Promega. Human orbital connective tissues were obtained from surgical waste. The Institutional Review Board of Harbor-UCLA Medical Center has approved these activities. 

Cell Culture—KAT-50 cells were a generous gift from Dr. K. Aín (University of Kentucky, Lexington, KY) (22). They were maintained in a humidified, 5% CO2 atmosphere, and then the transfection complexes were allowed to form over 15 min at room temperature. Complexes were allowed to form over 15 min at room temperature. Complexes were allowed to form over 15 min at room temperature.
ther empty vector or mPGES/pcDNA3.1(−) (0.6 µg) was mixed with LipofectAMINE PLUS as described above. In control experiments conducted under identical conditions, cultures received the expression plasmid pIRE2-EGFP (CLONTECH) containing the coding region for green fluorescent protein (GFP) (0.6 µg). Transfection efficiency was determined by trypanosmization of the cells and manually counting those expressing GFP. The efficiency was used to normalize the data presented in Fig. 4A (inset) concerning PGE2 production in cells before and after transfection with mPGES.

PGE2 Analysis—PGE2 levels were determined as described previously (12) utilizing an enzyme immunoassay (Amersham Biosciences). Briefly, KAT-50 cells were incubated in 24-well plates covered with medium supplemented with 10% FBS. One day prior to experimental manipulation, culture wells were shifted to medium without FBS, and the following day, the test compounds indicated in the figure legends were added. Thirty min prior to monolayer harvest, medium was removed and replaced with 150 µl of PBS with the respective additives.

Following the incubation, the PBS was removed quantitatively and subjected to the assay for PGE2 following the manufacturer’s instructions. These studies were conducted with three separate wells per treatment group. Data are expressed as the mean ± S.D. of triplicate cultures from representative experiments.

RESULTS

KAT-50 Cells Generate Considerably Less PGE2 than Do Cytokine-activated Fibroblasts—KAT-50 cells express extraordinarily high levels of PGHS-2 under basal culture conditions. Despite high levels of enzyme, these cultures generate and release low levels of PGE2 (Fig. 1). Moreover, IL-1β (10 ng/ml) treatment fails to enhance the production of this prostanoid. In contrast, orbital fibroblasts, maintained under identical conditions, exhibit a dramatic increase in PGE2 synthesis in response to IL-1β, resulting from an induction of PGHS-2. A substantial fraction of the low basal PGE2 generation in KAT-50 cells can be attributed to constitutive PGHS-2 activity. This activity is susceptible to the PGHS-2-selective inhibitor, SC58125 (control, 123.8 ± 3.2 pg/ml; SC58125, 45.2 ± 2.4 pg/ml) (mean ± S.D., n = 3). The fractional inhibition is unchanged with IL-1β treatment. The extremely low levels of PGE2 production, when expressed on a “per cell” basis, were dramatically below those observed in orbital fibroblasts (Fig. 1B).

In contrast, the PGE2 production in untreated fibroblast cultures derives, in large part, from the activity of PGHS-1, which is expressed at relatively high levels in untreated and cytokine-exposed fibroblast cultures. SC58125 exerts modest effects on production of the prostanoid in fibroblasts under control conditions. In contrast, the vast majority of PGE2 synthesis in IL-1β-treated fibroblasts can be inhibited with PGHS-2-selective agents, including SC58125, consistent with earlier findings in these cells (12–15).

KAT-50 Cells Express High Levels of PGHS-2 and cPGES under Basal Culture Conditions—We next began to investigate the basis for why KAT-50 cells generate low levels of PGE2, despite high constitutive PGHS-2 expression. Confluent cultures of KAT-50 cells incubated in medium supplemented with low concentrations of FBS, or in serum-free medium, express high levels of PGHS-2 protein (Fig. 2A) and mRNA (Fig. 2B). PGHS-2 protein migrates as a 72-kDa band on Western analysis, while the transcript appears as a 5-kb band on Northern blot analysis. Basal levels of PGHS-2 are considerably higher in KAT-50 cells than those in orbital fibroblasts incubated under identical culture conditions. The impact of IL-1β on PGHS-2 expression also differs substantially in fibroblasts and KAT-50 cells. The cytokine fails to up-regulate PGHS-2 expression in KAT-50 cells. Levels of PGHS-2 are essentially unchanged with addition of IL-1β after 6 h. The same analysis reveals very different responses in fibroblasts. In those cells, IL-1β induces PGHS-2 mRNA and protein dramatically at 6 and 16 h, respectively, consistent with its actions in many other cell types (27).

Analysis of PGES isoform expression in KAT-50 cells reveals that cPGES is an abundant protein under basal culture conditions, and the levels of both mRNA and protein are invariant with regard to IL-1β treatment (Fig. 2, A and B). This was also the case in fibroblasts, although the levels of cPGES mRNA were considerably lower than those found in KAT-50. In contrast, mPGES mRNA and protein were undetectable in KAT-50 cells, regardless of whether the cultures had been treated with cytokine or were maintained under control conditions (Fig. 2). Fibroblast cultures, in contrast, exhibited a low level of basal expression, but the levels of mPGES mRNA and protein were enhanced substantially when cultures were treated with IL-1β for 6 and 16 h, respectively. Thus, the predominant synthase expressed in KAT-50 cells under control and IL-1β-treated conditions is cPGES. Moreover, the levels of cPGES mRNA and protein are invariant in these cells with regard to cytokine
protein expression in the absence or presence of IL-1. Confluent cultures of KAT-50 cells and orbital fibroblasts were incubated overnight in serum-free medium and then some received IL-1β (10 ng/ml) for 6 and 16 h, respectively. Monolayers were rinsed and proteins solubilized and subjected to Western blot analysis of the enzyme proteins using the procedures described under "Experimental Procedures." Proteins were subjected to electrophoresis and were then transferred to membranes. These were then analyzed using primary antibodies directed specifically against mPGES, cPGES, PGHS-2, and PGHS-1. The ECL detection system was used to generate specific signals. These were then analyzed using primary antibodies directed specifically against mPGES, cPGES, PGHS-2, and PGHS-1. The ECL detection system was used to generate specific signals.

**FIG. 2. Western and Northern blot analyses of PGHS and PGES expression in KAT-50 cells and orbital fibroblasts.** A, analysis of protein expression in the absence or presence of IL-1β. Confluent cultures of KAT-50 cells and orbital fibroblasts were incubated overnight in serum-free medium and then some received IL-1β (10 ng/ml) for 6 and 16 h, respectively. Monolayers were rinsed and proteins solubilized and subjected to Western blot analysis of the enzyme proteins using the procedures described under "Experimental Procedures." Proteins were subjected to electrophoresis and were then transferred to membranes. These were then analyzed using primary antibodies directed specifically against mPGES, cPGES, PGHS-2, and PGHS-1. The ECL detection system was used to generate specific signals. B, comparison of mRNA expression under control and IL-1β-treated conditions. Cells were allowed to proliferate to confluency in 100-mm diameter plates covered with medium supplemented with 10% FBS. The cultures were shifted to serum-less medium overnight and then some received IL-1β (10 ng/ml) for 6 h. Monolayers were rinsed, and RNA was extracted as described under "Experimental Procedures." Samples were subjected to electrophoresis and then transferred to membranes and subjected to hybridization with the relevant cDNA probes. Radioactive DNA/RNA hybrids were detected by exposing the membranes to X-Omat film at −70 °C. The signals were normalized by rehybridizing membranes with a cDNA probe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

treatment. Consistent with earlier studies, KAT-50 cells express high levels of PGHS-1 mRNA. PGHS-1 protein, in contrast, is undetectable under all culture conditions assessed. It would appear that the transcript for PGHS-1 is not translated in these cells. The molecular basis for this apparent blockade is not currently understood but KAT-50, unlike most cells, expresses a single functional cyclooxygenase isoform.

Absence of mPGES Promoter Activity in KAT-50 Cells under Basal and IL-1β-treated Culture Conditions Accounts for Undetectable mPGES in These Cells—We next determined whether differences in the respective promoter activities could account for the disparate levels of PGHS-2 and mPGES mRNA and protein expression observed in KAT-50 cells and orbital fibroblasts. Reporter genes linked to human promoter fragments for PGHS-2, cPGES, and mPGES were transiently transfected into both types of cells, and their activities were compared under basal and cytokine-treated conditions. With regard to PGHS-2 promoter activity, levels in untreated fibroblasts were at least 20-fold greater than the promoter-less reporter gene, and IL-1β (10 ng/ml) treatment for 3 h resulted in a 2-fold increase (Fig. 3). We have reported that the substantial impact of IL-1β on increasing the steady-state PGHS-2 mRNA levels in orbital fibroblasts is mediated primarily through the enhancement of transcript stability and not on PGHS-2 gene transcription (12). In untreated KAT-50 cells, the PGHS-2 promoter activity was twice that seen in control fibroblasts and IL-1β reduced the activity by 25%. With regard to mPGES promoter activity, fibroblasts not treated with the cytokine exhibited levels similar to those in cultures transfected with reporter genes lacking a promoter, and IL-1β treatment increased the activity levels by 3-fold. The mPGES promoter construct transfected in KAT-50 cells failed to exhibit any detectable activity, either in untreated cultures or in those treated with IL-1β. With regard to the cPGES (p23) promoter, its activity was low in orbital fibroblasts but at least 30-fold higher in KAT-50 cells (Fig. 3). IL-1β treatment failed to influence cPGES promoter activity in either cell type, consistent with the findings concerning the relative steady-state mRNA levels observed on Northern blot analysis (Fig. 2B). Thus, the undetectable mPGES expression found in KAT-50 cells can be directly attributed to an absence of mPGES promoter activity in these cells. In contrast, the promoter for cPGES exhibited extremely high levels of activity in KAT-50 cells.

KAT-50 Cells Transfected with mPGES cDNA and Expressing the Terminal Synthase Generate Higher Levels of PGE₂—We speculated that the limited capacity of KAT-50 cells to generate PGE₂ is a consequence of the “wrong” profile of prostanooid biosynthetic enzymes being expressed in those cells. We therefore next determined whether introducing the expression of mPGES to KAT-50 cells and thus creating the theoretically more effective PGHS/PGES pairing would enhance the production of PGE₂ in these cells. Cultures were transiently transfected with mPGES cDNA cloned into pcDNA3.1(−), and the resulting expression of that protein culminated in a substantial increase in PGE₂ synthesis in KAT-50 cells under basal culture conditions (Fig. 4A). The levels were up to 3-fold above those observed in cultures transfected with empty vector. Moreover, addition of graded concentration of exogenous arachidonate further enhanced the PGE₂ production. As Fig. 4B indicates, Western blots using anti-mPGES antibodies confirmed that the synthase protein was expressed in the transfected cultures. Potentially low transfection efficiency could result in a substantial underestimation of the impact mPGES expression might have on PGE₂ synthesis in KAT-50 cells. Therefore, we determined the efficiency by transfecting sister cultures with pcDNA GFP. Those studies revealed that the efficiency was 25.3% (range 19–29%, n = 3 experiments). Data presented in Fig. 4A (inset) represent the normalized PGE₂ generation in cultures transfected with mPGES expressed as a function of transfection efficiency. As the figure indicates, PGE₂ generation was enhanced more than 6.9-fold in cultures transfected with mPGES and treated with arachidonate (10 μM). Thus, introducing mPGES expression in KAT-50 results in
a substantial increase in PGE$_2$ production, presumably by allowing the functional coupling of the synthase with PGHS-2.

**DISCUSSION**

Thyrocytes express high basal levels of PGHS-2 in culture and in situ (16, 17). The cell line, KAT-50, retains the elevated PGHS-2 expression found in primary thyrocytes in vivo but the cells have lost their ability to express detectable PGHS-1 protein. These observations suggest that activities of PGHS-2 must, in some manner, suffice with regard to meeting the metabolic needs of the cell. Of note is the coincident expression in these cells of cPGES at relatively high levels but the absence of detectable mPGES mRNA or protein. This particular profile of enzyme expression results in considerably lower levels of PGE$_2$ generation in KAT-50 cells than those observed in orbital fibroblasts which express both PGHS-2 and mPGES following cytokine treatment (Fig. 2) (12). It is possible that the absence of an efficient pair of cyclooxygenase/PGES enzymes has resulted from adaptation to the high constitutive levels of PGHS-2, effectively protecting the cells from excess prostanoid concentrations. When the more efficient pair of enzymes is expressed, in this case by transfecting the cells with mPGES, PGE$_2$ synthesis is enhanced. This suggests strongly that the endogenously expressed PGHS-2 functions more efficiently when functionally coupled to mPGES. These results support the functional nature of the constitutively expressed PGHS-2 in KAT-50 cells. They indicate that abnormal compartmentalization of PGHS-2 protein has not rendered the enzyme inaccessible to critical pools of substrate. The current results indicate further that the disparity between mPGES and cPGES expression in KAT-50 cells is a consequence of vastly different levels of activity exhibited by the respective gene promoters. There exists no detectable activity of the mPGES promoter in these cells, in contrast to orbital fibroblasts where the promoter is active under both basal and IL-1β-treated conditions.

The functional coupling between specific PGES and PGHS isoforms that leads to highly efficient PGE$_2$ production was first demonstrated in HEK293 cells that had initially undergone stable transfections with PGHS-1 or PGHS-2 and then were transiently transfected with one of the PGES isoforms (8, 9). mPGES co-localized with PGHS-2 and exhibited a marked enzymatic preference for PGHS-2 when arachidonate was supplied from endogenous or exogenous sources (9). Likewise, cPGES and PGHS-1 formed a considerably more efficient pairing in these transfected cells (8). It would seem that KAT-50 cells represent a naturally occurring example of cells expressing a pair of enzymes not optimized for efficient PGE$_2$ production. Restoration of theoretically optimal fidelity between the cyclooxygenase and terminal prostaglandin synthase results in enhanced PGE$_2$ synthesis.

From the current studies, it would appear that IL-1β does not influence levels of cPGES expression in KAT-50 cells (Fig. 2). This finding is consistent with that in some cells types, such as HeLa, MKN45, HEK293, WI-38, CHO, and L929, but differs from the modest increase found in rat brain in vivo following treatment with lipopolysaccharide (8). In previous studies, we also failed to observe a change in cPGES levels in orbital fibroblasts treated with a variety of cytokines (12). mPGES was undetectable in KAT-50 by Western and Northern blot analysis, suggesting extremely low levels of expression of that enzyme. In contrast, the cells express high levels of cPGES. PGHS-1 constitutes the natural enzyme partner for cPGES (8). While KAT-50 cells express high levels of PGHS-1 mRNA, the enzyme protein is undetectable under all culture conditions assessed. It is possible that, as a component of adaptation to culture, these cells have lost their ability to translate the
mRNA. Further studies will be necessary to determine whether this transcript is competent to be translated.

An important role for PGE₂ in normal or pathological thyrocyte function has yet to be firmly established. High levels of PGHS-2 expression found in untreated KAT-50 cells and cultured primary thyrocytes suggest that this enzyme may predominate in prostaglandin production found in thyroid. This possibility is supported by the finding of PGHS-2 protein in situ in thin-sectioned thyroid tissue from apparently healthy glands (16). The frequent involvement of the thyroid in inflammatory processes suggests that inherent properties of thyrocytes might underlie disease susceptibility. Likely participants in the tissue remodeling integral to inflammatory diseases of the thyroid include the arachidonate synthetic pathways. Limited numbers of observations have thus far been made concerning the capacity of thyrocytes to generate eicosanoids or the physiological implications of their synthesis in thyroid tissue. In FRTL-5 cells, thyrotropin can regulate arachidonate release from membrane phospholipids, the activity of PGHS, and influence the formation of prostaglandins (28). These actions were enhanced by insulin-like growth factor-1. Thyroid-stimulating immunoglobulins from patients with Graves’ disease activate phospholipase A₂ in FRTL-5 cultures, promote the release of free arachidonate, and enhance 1,4,5-trisphosphate generation (29, 30). This action appears to be mediated through a different pathway from those effects that result in cAMP generation.

Another study has shown that proliferation of FRTL-5 cells is accelerated by stimulating IgGs from patients with Graves’ disease and that the inhibition of cyclooxygenase activity with indomethacin can block this effect of disease-specific immunoglobulins (31). The net impact of PGE₂ on many vascular beds often involves substantial vasodilatation (32). Because the thyroid is extremely vascular, local production of the prostanoid may represent a key step in the regulation of perfusion patterns in this organ. PGE₂ generated in thyroid tissue, regardless of the cell type generating it, can influence the immunity and condition the nature of inflammatory responses occurring in the gland. This prostanoid can bias the differentiation of naïve T lymphocytes from TH1 to the TH2 phenotype (33). Moreover, PGE₂ can influence B lymphocyte and mast cell development (34–36). Thus the profile of prostaglandin-generating enzymes expressed by thyrocytes can potentially influence the nature of immunocompetent cells infiltrating the gland in disease.

Our findings identify, for the first time, a cell type that expresses constitutive PGHS-2 as a single, dominant cyclooxygenase isoform and yet fails to express detectable mPGES under basal or IL-1β-treated conditions. These results suggest that development of therapeutic agents selectively targeting mPGES might not interrupt the full spectrum of PGHS-2-dependent prostaglandin production. Coupling of PGHS-2 with cPGES might be more widespread than is currently appreciated. It will be of considerable interest to examine whether other cells expressing high constitutive levels of PGHS-2 exhibit similar patterns of PGES. Of particular potential importance is the high level of constitutive PGHS-2 found in the pancreatic islet (37). The inhibitory action by PGE₂ on glucose-provoked insulin release suggests that the functional relationship between PGHS-2 and the PGE₂ synthases could determine, at least in part, levels of glucose tolerance.
The current observations are potentially important in helping to define the spectrum of relationships that exist between enzymes in the PGE₂ biosynthetic pathway. They imply that, despite forming a more efficient pairing with mPGES, PGHS-2 may sometimes be identified. Examination of neoplastic cells may prove especially interesting, since overexpression of PGHS-2 dominates the phenotype and apparently influences the clinical behavior of some tumors, including colorectal adenocarcinomas (38).

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