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Review

Cellular and molecular biology of prostacyclin synthase

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Abstract

Prostacyclin synthase (PGIS) cDNA comprises 1500 nucleotides coding for a 500 amino acid protein. It is a heme protein with spectral characteristics of cytochrome p450 (CYP). It does not possess the typical CYP mono-oxygenase activity but catalyzes the rearrangement of prostaglandin H_2 to form PGI_2. Analysis of its structure–function by molecular modeling and site-directed mutagenesis reveals a long substrate channel lined by hydrophobic residues. Cys-441 has been identified as the proximal axial ligand of heme. Tyr-430 is nitrated by peroxynitrite which results in reduced PGIS catalytic activity, suggesting that Tyr-430 is located close to the heme pocket. PGIS is constitutively expressed and may be upregulated by cytokines, reproductive hormones, and growth factors. It is physically colocalized with cyclooxygenases and phospholipases, and functionally coupled with these enzymes. PGIS coupling with COX-2 has been shown to play an important role in vascular protection, embryo development and implantation, and cancer growth.

Keywords: Prostacyclin synthesis; Cyclooxygenase-2; Cytochrome p450; Colon cancer; Embryo development

Prostacyclin (PGI_2) was discovered about three decades ago by Vane's group using the ingenious cascade tissue perfusion technique [1]. Its structure was deduced in the same year [2]. Data derived from these studies indicate that PGI_2 was a product of arachidonic acid (AA) metabolism. It was subsequently shown that it is converted from the prostaglandin endoperoxide, PGH_2, by an enzyme localized to the microsomal fractions of platelets, vascular endothelial cells, and vascular smooth muscle cells [3–6]. This PGI_2 synthetic enzyme, known now as PGI_2 synthase (PGIS), was proposed to be a cytochrome p450 (CYP) by Ullrich et al. [7] which was confirmed by DeWitt and Smith [8] when they purified PGIS from bovine aorta and characterized the enzyme as a hemoprotein with spectral properties consistent with CYP. PGIS is considered to be an atypical CYP as it does not possess any oxygenase activity. PGIS acts as an isomerase to rearrange PGH_2 to form PGI_2. The pioneering work reported by Ullrich's laboratory has shed light on the catalytic actions of PGIS and thromboxane synthase (TXAS). The reported work proposes that PGH_2 is bound to the active site of PGIS with its C-11 oxygen oriented to the heme iron of PGIS. This results in homolytic scission of the endoperoxide bond of PGH_2 to generate PGI_2 by a mechanism related to the peroxidase action of CYP [9]. By contrast, PGH_2 is bound to the active site of TXAS with its C-9 oxygen oriented to the heme iron resulting in the generation of TXA_2 [9]. The proposed mechanism provides plausible explanation for the conversion of the same substrate into two structurally and biologically distinct products by two enzymes sharing about 16% sequence homology. However, this proposal requires proof by direct structural analysis.

PGIS was purified in the early 1980s. The enzyme purified from bovine aorta by immunoaffinity chromatography was reported to be a hemoprotein with a

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molecular mass of ~52 kDa [8]. It exhibited a typical CYP spectrum when treated with carbon monoxide or sodium dithionite. It confirmed previous data that the enzyme was deactivated by PGH_2 and peroxides [10–12]. It was suggested that the enzyme is inactivated during catalysis by the production of oxidants that destroy the enzyme [12]. Interestingly, TXAS was not autoinactivated during catalysis.

PGIS cDNA was cloned from a bovine aorta by our group [13,14], bovine endothelial cells by Hara et al. [15], and human endothelial cells by Miyata et al. [16]. The sequence comparison shows identical bovine sequence between the two laboratories and a high degree of sequence homology among human, bovine, and murine sources. PGIS cDNA contains a 1500 bp open reading frame coding for a 500 amino acid protein with a molecular weight of 56 kDa. When compared with the sequence of other CYPs, it exhibits about 30% sequence identity to cholesterol 7-α-mono-oxygenase (CYP7) and only about 16% with TXAS. PGIS was considered to belong to a new CYP family and is now classified as CYP8 whereas TXAS is classified as CYP5.

There has been considerable progresses in the structure–function relationship and the genetic regulation of PGIS. This review highlights recent advances in the understanding of the cellular and molecular biology of PGIS.

Structure and function relationship

Hydropathy analysis of PGIS reveals a single hydrophobic region at the N-terminus, which was characterized by CD analysis and peptidoliposome reconstitution as having membrane anchoring properties and helical structural features [17]. The hydropathy plot of PGIS closely resembles those of other CYPs such as CYP2C1 and differs from TXAS which has an additional N-terminal hydrophobic region. Thus, PGIS like other CYP enzymes anchors to the membrane with a single N-terminal transmembrane domain [18]. The bulk of the enzyme is located at the cytosolic side of ER. Structural analysis by NMR spectroscopy has provided evidence that several hydrophobic residues (Val-224, Leu-217, and Leu-222) at the F/G loop of PGIS contact the ER membrane, thereby bringing the enzyme to be adjacent to the membrane [19]. It was further suggested that the F/G loop is involved in forming the opening of the substrate channel and at least two residues (Leu-214 and Pro-215) have been identified to interact with a substrate analog U46619 [20]. The substrate channel of PGIS is long and lined up with hydrophobic residues. By using molecular modeling coupled with site-directed mutagenesis, we have identified several residues (Ile-67, Val-76, Leu-384, Pro-355, Glu-360, and Asp-364) that line one side of the channel to be catalytically essential [21]. The analysis confirmed that Cys-441 is the proximal axial ligand of heme iron. Analysis by consensus sequence homology comparison revealed that PGIS possesses EXXR motif that is conserved in diverse CYP including mammalian and bacterial CYPs [22]. Site-directed mutagenesis has shown that Glu-347 and Arg-350 are critical for catalytic activity. Leu-112 which is corresponding to a Phe residue in BM_3 is situated at a crucial site at the catalytic center. Mutation of Leu-112 to Phe retains the activity. However, the activity is lost when this Leu residue is replaced with Asp or Gly [21]. Peroxynitrite has been shown to inhibit the catalytic activity of PGIS by nitration of Tyr-430 [23]. As this residue is not readily digested by pronase, it was suggested that Tyr-430 is embedded in the heme pocket. Nitration of this residue may interfere with the contact of substrate with the heme center. The catalytic center, heme environment, and substrate channel are only partially characterized. Full understanding of the catalytic property and reaction mechanism requires a resolution of the PGIS structure. X-ray crystallographic structure of PGIS has not been solved because of difficultly in obtaining sufficient quantities of functionally intact, soluble enzymes for crystallization and X-ray analysis. This problem appears to be partially resolved as methods for preparing large quantities of soluble PGIS have been developed [24]. It is anticipated that the structure of PGIS will be solved in the near future which will provide more precise information regarding the structure of heme environment and catalytic center as well as the substrate channel. Furthermore, it may shed light on the structural basis for lacking oxygenase activity in PGIS.

Inhibition of PGIS activity by peroxynitrite

Peroxynitrite is a reaction product of superoxide ion and NO [25]. Vascular cells such as endothelial cells and smooth muscle cells generate superoxide and NO when cells are stimulated by exogenous agonists resulting in the formation of peroxynitrite (PON). PON modifies protein functions by inducing nitration of tyrosine [26]. It is therefore of considerable interest to note that PON inhibits PGIS activity by nitration of tyrosine 430 [23,27]. As nitration of this tyrosine residue disrupts the catalytic activity, it has been postulated that this tyrosine residue is embedded in the heme region and is crucial for electron transfer. In an ex vivo experiment with bovine coronary artery strip, Zou et al. [28] have shown that PON impaired angiotensin-II-induced vasorelaxation which was accompanied by PGIS nitration and suppressed PGIS activity. It was further reported by Ullrich's group that the vasoconstrictive effect of PON-induced PGIS nitration occurs initially in vascular endothelial cells. They have shown that lipopolysaccharide treatment of endothelial cells induces PON

formation which inhibits PGIS activity, resulting in metabolic shunting to PGE_2 synthesis [29]. The physiological and pathophysiological implications of PGIS nitration by peroxynitrite remain to be evaluated by appropriate in vivo experiments.

PGIS transcriptional regulation

PGIS is constitutively expressed in vascular cells such as endothelial cells and smooth muscle cells. PGIS is encoded by a single gene copy located at the long arm of chromosome 20q13. Human PGIS gene spans 60 kb containing 10 exons [30,31]. The 3′-untranslated region contains multiple polyadenylation signals. Two transcripts of human PGIS, a major 6 kb and a minor 3.3 kb mRNA, have been shown in human cells. Human PGIS transcripts are longer than those of bovine, rat or mouse which has a transcript size of 2.7 kb. The biological meaning of longer polyadenylation signals in human PGIS is unclear. The 5′-flanking region of PGIS is TATA less and GC rich, consistent with structural features of housekeeping genes. Several potential transcription start sites are reported to be situated 46–54 bp upstream of the ATG translation codon [30]. Results from PGIS promoter analysis and gel shift assays have identified Sp1 binding to two Sp1 binding sites as being necessary for constitutive expression of PGIS in mouse smooth muscle cells [32]. There are several CCGCCAGCC repeats in the proximal region of the 5′-untranslated region which create several Sp1 binding sites. This region is highly polymorphic. Report from a study of Japanese population has identified polymerase of PGIS gene with various numbers of CCGCCAGCC repeats (3–7 repeats) among which 6 repeats is the most frequent genotype. Variation in the number of repeats alters the number of Sp1 binding sites. Functional assessment has shown a positive correlation between the number of repeats and thus Sp1 binding sites with promoter activity stimulated by interleukin 6 [33]. Interestingly, a case and control analysis shows a positive correlation between this polymorphism and hypertension. It is unclear whether similar polymorphisms exist in Caucasians and other ethnic groups. Nevertheless, this report suggests that the Sp1 sites are crucial for PGIS transcriptional activation by pro-inflammatory mediators. Results from this polymorphism study suggest that PGIS promoter activity is inducible by IL-6. This notion is supported by the report that PGIS mRNA levels are increased by tumor necrosis factor α [15]. The transcriptional mechanism for PGIS upregulation by pro-inflammatory cytokines requires further investigation.

PGIS has been shown to be overexpressed in rat intestinal epithelial cells transfected with Ras, suggesting that PGIS expression is induced via the Ras signaling pathway [34]. It is of interest to note that cytosolic phospholipase A_2 ($cPLA_2$) and COX-2 are also increased in Ras-transfected cells. PGI_2 generated via COX-2 and PGIS pathway has been implicated in colorectal cancer growth [35]. The mechanism by which PGI_2 promotes colorectal cancer growth is unclear. It has been postulated that PGI_2 acts through activation of PPARδ [35–37]. Stromal cells in colorectal cancer may contribute to PGI_2 production especially in cancer cells which do not produce PGI_2. A recent study has shown that fibroblasts from cancer mass produce PGI_2 whereas fibroblasts from adjacent normal tissues do not [38]. The reported data suggest that PGIS expression may be induced in fibroblasts by cancer and stromal cell-generated PGI_2 may contribute to cancer cell growth. Further studies are needed to confirm this.

PGIS and eicosanoid biosynthesis in endothelial cells

PGIS is expressed in vascular endothelial cells (EC) and smooth muscle cells as well as non-vascular cells including neurons [39]. Involvement of PGIS in endothelial cells (EC) eicosanoid has been more extensively investigated. PGIS in resting EC is colocalized with COX-1 [40]. It is also colocalized with COX-2 to nuclear envelope (NE) and endoplasmic reticulum (ER) in EC stimulated by PMA [40]. COX-2 in PMA-stimulated EC has been shown to be localized to caveolae by binding to caveolin-1 [41]. It has been reported that PGIS in EC stimulated by growth factors is localized to caveoli [42], suggesting that COX-2 and PGIS may also be colocalized to caveolae in mitogen-stimulated cells. As cytosolic PLA_2 is translocated to ER and NE in cytokine stimulated cells [43], it is increasingly accepted that $cPLA_2$ COX-1/COX-2 and PGIS are colocalized to NE and ER in stimulated cells and are functionally coupled to facilitate the transfer of intermediate metabolites generated from the upstream enzyme to the downstream enzyme. However, there is no clear evidence for a physical interaction among these enzymes. PGIS represents the ''terminal specific enzyme'' which takes in PGH_2 and converts it to PGI_2. The traditional view is that PGI_2 is released into the extracellular milieu where it acts on cells via PGI_2 specific receptors. However, there exists a possibility that PGI_2 may enter nucleus via NE and act on PPARδ. This interesting perspective is purely speculative at the present time. Further studies are needed to prove this possibility. The metabolic and functional properties of COX-2 and PGIS in caveoli are unknown. Caveolar COX-2 and PGIS may receive AA from soluble isoforms of PLA_2 which are localized to the plasma membrane. On the other hand, caveolae may simply serve as a site to sequester COX-2 and PGIS. The extent of PGI_2 synthesis is regulated by the levels of synthetic enzymes. For example, COX-2 overexpression in endothelial cells induced by cytokines, mitogens, and endotoxins is accompanied

by a several-fold increase in prostanoids notably PGI_2 and PGE_2 over the basal levels [15]. PGIS expression was reported to be stimulated by cytokines in bovine endothelial cells but was considered to be not inducible in human umbilical vein endothelial cells [44]. Thus, the endogenous regulation of PGI_2 and prostanoid synthesis of PGIS require further investigation.

To gain insight into the role of PGIS in endothelial cell prostanoid syntheses, we have employed adenoviral gene transfer technique to augment PGIS with or without COX-1 overexpression and determine the influence of the ratio of PGIS to COX-1 overexpression on prostanoid synthesis. Transduction of HUVEC with adenoviral PGIS construct (Ad-PGIS) (50 pfu/cell) results in a 6-fold increase in PGI_2 (6-keto-$PGF_{1\alpha}$) and, interestingly, a reduction in PGE_2 and $PGF_{2\alpha}$, suggesting shunting of PGH_2 entirely through the PGIS pathway. By contrast, transduction of Ad-COX-1 (50 pfu/cell) leads to a 2-fold increase in PGI_2, a >100-fold increase in PGE_2, and a 10-fold increase in $PGF_{2\alpha}$ [45]. These results indicate that HUVEC expresses a larger quantity of PGE synthase than PGIS and enrichment of PGIS expression causes a preferential shunt of PGH_2 via PGIS, despite a high capacity of PGES. When both PGIS and COX-1 are overexpressed, there is a great increase in PGI_2 but the extent of PGI_2 increase and the degree of suppression of other prostanoids depend on the ratio of Ad-PGIS/Ad-COX-1 viral titers. At a ratio of 10 pfu/cell of Ad-PGIS to 50 pfu/cell of Ad-COX-1, PGI_2 is increased by ~250-fold, PGE_2 by ~40-fold, and $PGF_{2\alpha}$ by ~6-fold but at a ratio of 50/50, the extent of PGI_2 increase is about the same as the 10/50 ratio but the $PGF_{2\alpha}$ and PGE_2 levels were no longer increased and remained close to baseline. Surprisingly, at a ratio of 100/50 (PGIS excess), PGI_2 is increased by ~100-fold, only half of the 50/50 ratio, while PGE_2 and $PGF_{2\alpha}$ remain close to the basal level. The reason for a decline of PGI_2 increase when PGIS is in excess of COX-1 is unclear. These results underscore the importance of relative enzyme levels in regulating PGI_2 production.

Data from these overexpression experiments provide several important insights into PGI_2 biosynthesis. First, the basal PGIS in endothelial cells is expressed at low levels when compared to PGES. Overexpression of PGIS either by gene transfer or through upregulation directs PGH_2 through the PGIS pathway thereby enhancing PGI_2 production. Second, PGI_2 synthesis is not determined simply by the level of PGIS but by an appropriate ratio of COX-1 to PGIS. An equal ratio appears to be optimal in PGI_2 synthesis. Third, the experimental data indicate that it is feasible to engineer the production of PGI_2 without concurrent increase in other prostanoids by transfer of bicistronic COX-1 and PGIS construct [45]. Lastly, as will be described in more detail in the next section, overexpression of PGIS with or without concurrent COX-1 overexpression is highly effective in controlling vascular diseases and ischemia-reperfusion tissue injury.

Control of vascular disease and tissue infarction by PGIS overexpression

Several studies have provided evidence for an important role of PGIS in vascular integrity. PGIS deficiency mice by gene targeting is associated with thickening of arterial wall, interstitial fibrosis with nephrosclerosis, and kidney infarction [46]. Patients with pulmonary hypertension have been reported to have decreased PGIS proteins in lung tissues [47]. In human atherosclerotic arterial diseases, endothelial dysfunction and deletion caused by oxidized low density lipoproteins and other insulting agents result in reduced expression of PGI_2 synthetic enzymes including PGIS. It is therefore logical to consider gene transfer for enhancing PGI_2 synthesis. There have been two strategies for enhancing PGI_2 by gene transfer: (1) PGIS and (2) bicistronic COX-1/PGIS.

PGIS overexpression in carotid arteries of experimental animals by viral or liposome-mediated transfer of PGIS cDNA has been shown to prevent intimal hyperplasia induced by carotid injury [48] which was reported to be associated with increased 6-keto-$PGF_{1\alpha}$ production [49]. Enhanced PGI_2 production in PGIS gene transfer is attributable to an increased COX-2 expression in vascular tissues following injury coupled with PGIS overexpression [50]. In vitro experiments suggest that PGIS gene transfer may play an important role in inhibiting smooth muscle cell proliferation [51]. Repeated injection of PGIS cDNA into rat skeletal muscle of a pulmonary hypertension rat model has been shown to produce an increased level of PGI_2 effective in controlling pulmonary hypertension and vascular remodeling [52].

Overexpression of PGIS alone may not achieve an optimal PGI_2 production as the extent of PGI_2 production is determined by an appropriate ratio of COX-1/COX-2 to PGIS. We, therefore, evaluated PGI_2 production by co-overexpression of COX-1 and PGIS using an adenoviral vector containing a bicistronic COX-1/PGIS construct. This construct contains human COX-1 and PGIS cDNAs driven separately by an identical human PGK (phosphoglyceryl kinase) promoter [53]. HUVEC transfected with this construct (Ad-COPI) have an equivalent increase in COX-1 and PGIS, and produce a large quantity of PGI_2 which is more than 200-fold over that produced by untransfected cells. Other eicosanoids are not significantly increased and remain at the basal level [45]. Direct infusion of Ad-COPI into the lateral ventricle of rat brain results in a selective increase in PGI_2 production in the ipsilateral cortex without an increase in other eicosanoids [53]. Several eicosanoids including TXA_2, leukotrienes B_4 and C4 (LTB_4 and

LTC_4) are recognized as important mediators of brain infarction following ischemia-reperfusion injury [53]. Direct infusion of Ad-COPI into the lateral ventricle for 72 h before ischemia-reperfusion injury results in a drastic change in eicosanoid production in the ipsilateral ischemic cortex. Ad-COPI induces a large increase in PGI_2 and suppression of other eicosanoids including leukotrienes when compared to Ad-null control [53]. Augmentation of PGI_2 is accompanied by a significant reduction in infarct volume induced by ischemia-reperfusion injury [53].

PGI_2 protects brain tissue from ischemia-reperfusion injury probably by multiple actions including protecting neurons from apoptosis via the PPARδ pathway. PGI_2 analogs have been shown to be a ligand of PPARδ [54] and PGI_2 has been implicated in protecting renal cell survival via PPARδ activation [55]. It is, therefore, possible that endogenously produced PGI_2 protects neurons from ischemia-reperfusion induced apoptosis by activating PPARδ. Gene transfer of bicistronic COX-1/PGIS has the potential for treatment of diverse clinical disorders including vascular diseases, pulmonary hypertension, and ischemia-reperfusion cerebral and cardiac injury.

PGIS expression in oviduct and embryo: role in embryo development

Oviduct (Fallopian tube) is pivotal for embryo development and transport to uterus. It is composed of a layer of epithelial cells lining the lumen, subepithelial smooth muscle cells and supportive tissues. The structure and function of oviduct resemble that of blood vessels. We, therefore, were interested in knowing whether oviduct produces PGI_2. Our results show that murine and human oviducts are capable of producing abundant PGI_2 [56,57]. Western blot analysis shows the expression of PGIS as well as COX-1 and COX-2 in oviduct tissues. Immunohistochemical analysis reveals that PGIS, COX-1, and COX-2 are detected in the epithelial cells, smooth muscle cells, and endothelial cells of intraductal blood vessels [57]. Similar to vascular tissues, COX-2 is the predominant contributor to PGI_2 production and is likely the major COX isoform that is coupled to PGIS in epithelial and smooth muscle cells [57]. PGI_2 production in oviducts is increased after mice have become pregnant [57]. Its level reaches maximal at day 2–3 post-coitus and declines to a level lower than the level of non-pregnant duct at day 4 post-coitus. The rise in PGI_2 production correlates with an increased expression of COX-2. Judging from a very high level of PGI_2 production at day 2–3 post-coitus, it is likely that PGIS is coordinately upregulated with COX-2. PGI_2 relaxes oviductal smooth muscle cells and its enhanced production within 3 days post-coitus may be crucial for maintaining ductal dilatation to allow for the transport of embryo to the uterus.

We have discovered that PGI_2 is also generated by embryo. Analysis of eicosanoid profile of AA-treated murine blastocysts reveals that PGI_2 is the major metabolite [58]. PGIS as well as COX-1 and COX-2 are detected in the lysates of blastocysts. Immunohistochemical analysis of PGIS, COX-1, and COX-2 at various stages of embryo development shows very little expression of these enzymes in the unfertilized egg or the fertilized egg. COX-2 and PGIS are detectable at 4-cell stage while COX-1 becomes detectable at 8-cell stage. At blastocyst stage, PGIS, COX-2, and COX-1 are expressed in the inner cell mass as well as trophectoderm, suggesting that both stem cells and the stromal trophoblasts express PGIS and COX enzymes, and are capable of PGI_2 production. To determine the role of PGI_2 in embryo development, we treated mouse embryos at different stages of development with PGI_2 analogs and analyzed embryo hatching which is used as an index of embryo development. PGI_2 enhanced complete embryo hatching [59]. To determine whether endogenous PGI_2 plays a role in embryo hatching, we treated mouse embryos with a selective COX-2 inhibitor. The results show that COX-2 inhibition resulted in reduced embryo hatching which was rescued by iloprost, a PGI_2 analog. Embryo hatching is a complex event which appears to depend on embryo cell numbers and break down of zona pallucida. The mechanism by which PGI_2 enhances embryo hatching is unknown at the present time. We suspect that it inhibits apoptosis of embryo cells. This hypothesis is being tested.

It is important to note that PGIS in conjunction with COX-2 is upregulated in oviduct and embryo following egg fertilization and embryo formation, resulting in synthesis of abundant PGI_2. PGI_2 may play a pivotal role in maintaining oviduct dilation to allow passage of developing embryo and ensuring a timely embryo development in the oviduct to permit hatching at a proper time when embryo reaches uterus. Both COX-2 and PGIS are likely to be coordinately upregulated during the early period of embryo formation.

PGIS has been shown to be involved in embryo implantation and decidualization [60]. It is coordinately upregulated with COX-2 with the generation of a high level of PGI_2. PGIS and COX-2 have been shown to be colocalized to maternal-embryo interface as well as in embryo trophoblasts [60]. It has been shown that PGI_2 exerts its action on embryo implantation by activating PPARδ [60]. Taken together, the results show that PGI_2 is a key player in embryo development, transport, and implantation. PGI_2 is produced by a well-coordinated program and is derived from the COX-2 and PGIS pathway. Both enzymes are probably upregulated at different stages of embryo growth and are expressed in maternal tissues including oviducts and uterus as well as embryonic stem cells and trophoblasts.

The coordinated expression of COX-2 and PGIS results in an augmented production of PGI_2 which is crucial for a timely development of embryo in the oviduct, transport of the developed embryo to the uterus, and implantation and decidualization of embryo. This coordinated program is regulated by temporal and spatial transcriptional activation of PGIS and COX-2. The transcriptional program of these genes is poorly understood at the present time. Further studies should provide valuable information regarding stem cell survival and proliferation.

Conclusion and perspective

PGIS is traditionally considered to be primarily expressed in vascular endothelial and smooth muscle cells and its product, PGI_2, is considered to be involved in controlling platelet and leukocyte reactivity, vascular interaction, and smooth muscle contractility via IP receptor coupled intracellular signaling pathway. Recent studies have shown that PGIS is expressed in non-vascular cells such as neurons, oviducts, embryonic cells, and cancer cells, and its functions have extended to neuroprotection, reproduction, and colon cancer growth. These novel actions of PGI_2 are thought to be mediated by activation of PPARδ.

PGIS is constitutively expressed in vascular and non-vascular cells. Analysis of the promoter of PGIS gene reveals housekeeping gene features. However, PGIS may be inducible possibly by diverse factors including cytokines, mitogenic growth factors, and reproductive hormones. Its upregulation is crucial in physiological functions and pathophysiological processes such as reproduction, neuroprotection, and cancer growth. The transcriptional mechanism for its upregulation is poorly understood and should be further investigated.

PGIS is colocalized with COX-1 and COX-2 to nuclear envelope and endoplasmic reticulum in resting cells. Following cell activation, $cPLA_2$ is translocated to the nuclear membrane and endoplasmic reticulum where it is functionally coupled with COX and PGIS. PGI_2 produced from PGIS is generally considered to be released into extracellular milieu. We postulate that the endogenously produced PGI_2 at the nuclear membrane enters nucleus, binds, and activates PPARδ to exert its cytoprotective action. This hypothesis is being tested in our laboratory.

PGIS membrane anchoring and substrate channel topology as well as substrate channel residues are partially resolved. On the other hand, the catalytic center and heme pocket of PGIS are poorly understood. The intriguing question as to why PGIS does not have mono-oxygenase activity remains unanswered. An essential step to understand the catalytic reaction of PGIS is to solve the PGIS structure. Progress is being made on this front. The availability of a large quantity of soluble, functionally intact PGIS will undoubtedly make it possible to crystallize PGIS for structural analysis.

PGIS deficiency plays a major role in diverse diseases including arterial intimal hyperplasia, pulmonary hypertension, and tissue infarction. Enhanced PGIS expression with or without a concurrent COX-1 overexpression has been shown to control these disorders and have potential for treating these diseases. We propose that concurrent overexpression of COX-1 and PGIS provides a superior approach to engineer a selective augmentation of PGI_2 production and maximalize the beneficial effect of endogenous PGI_2 on controlling tissue infarction and vascular diseases.

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