Abstract: “Lipid mediators” represent a class of bioactive lipids that are produced locally through specific biosynthetic pathways in response to extracellular stimuli. They are exported extracellularly, bind to their cognate G protein-coupled receptors (GPCRs) to transmit signals to target cells, and are then sequestered rapidly through specific enzymatic or non-enzymatic processes. Because of these properties, lipid mediators can be regarded as local hormones or autacoids. Unlike proteins, whose information can be readily obtained from the genome, we cannot directly read out the information of lipids from the genome since they are not genome-encoded. However, we can indirectly follow up the dynamics and functions of lipid mediators by manipulating the genes encoding a particular set of proteins that are essential for their biosynthesis (enzymes), transport (transporters), and signal transduction (receptors). Lipid mediators are involved in many physiological processes, and their dysregulations have been often linked to various diseases such as inflammation, infertility, atherosclerosis, ischemia, metabolic syndrome, and cancer. In this article, I will give an overview of the basic knowledge of various lipid mediators, and then provide an example of how research using mice, gene-manipulated for a lipid mediator-biosynthetic enzyme, contributes to life science and clinical applications.

Key words: eicosanoid, knockout mouse, lipid mediator, lysophospholipid, prostaglandin
among others. In this article, I will provide an overview of the classification and roles of various lipid mediators, such as eicosanoids, lysophospholipids, sphingolipids, and endocannabinoids. In the latter part, I will focus on a particular enzyme that produces the most intensively studied lipid mediator, PGE₂. On the bases of cell biological and clinical studies, together with recent analyses of mice with manipulation of its gene, PGE₂ synthase (PGES) now attracts much attention as a potential target for a new class of anti-inflammatory, analgesic and anti-cancer drugs which are currently being developed.

### Lipid Mediators

Lipid mediators can be historically and structurally grouped into three categories. **Class 1** includes well-known arachidonic acid (AA)-derived eicosanoids including prostaglandins (PGs), leukotrienes (LTs) and their relatives [4, 56]. **Class 2** includes lysophospholipids or their derivatives such as platelet-activating factor (PAF), lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P), which possess either glycerol or sphingosine backbone [9, 21, 78]. Endocannabinoids can also be categorized in this class, since the hallmark endocannabinoid, 2-arachidonoyl-glycerol, has a glycerol backbone and since endocannabinoid receptors cluster in close proximity to lysophospholipid receptors on the phylogenetic tree [11]. **Class 3** represents newly identified anti-inflammatory lipid mediators derived from ω-3 polyunsaturated fatty acids (PUFAs), such as resolvins [derived from eicosapentaenoic acid (EPA)] and protectins [derived from docosahexaenoic acid (DHA)] [2], whose biosynthetic enzymes and receptors are not yet fully understood. In addition, given the recent findings of a few unique GPCRs that recognize medium- to long-chain fatty acids [75], it is conceivable that some nutrient lipids abundantly present in the circulation or tissues could exhibit lipid mediator-like actions and be regarded as another class of lipid mediators.

**Class 1: eicosanoids**

It was about half a century ago when the first (or classical) class of lipid mediators, eicosanoids (PGs and LTs), which are produced from AA via the cyclooxygenase (COX) and lipoxygenase (LOX) pathways, respec-

![Image of lipid mediators](image)

![Diagram of lipid mediator pathways](diagram)

In 1982, Samuelsson and Bergstrom (Sweden) who had discovered PGs and LTs, and Vane (UK) who had found that aspirin exerts its anti-inflammatory action through inhibiting COX, were awarded the Nobel Prize. Thousands of studies involving biochemical, pharmacological and gene knockout strategies have delineated the regulatory actions of individual PGs, LTs and other related eicosanoids in a wide variety of physiological and pathological systems, and many compounds that can block the eicosanoid pathway are currently in clinical use. The major eicosanoid pathways are illustrated in Fig. 1.

So far, five major bioactive prostanoids that act on their cognate GPCRs, namely PGE₂, PGD₂, PGF₂α, PGI₂ and thromboxane A₂ (TXA₂), are known. They are produced through three sequential enzymatic steps: release of AA from membrane glycerophospholipids by various phospholipase A₂ (PLA₂) subtypes, oxygenated conversion of AA to PGG₂ and then to PGH₂ by either constitutive COX-1 or inducible COX-2, and finally isomerization of PGH₂ to individual bioactive prostanoids by specific terminal PG synthases [56]. On the basis of analyses of mice gene-manipulated for the biosynthetic enzymes and receptors, together with pharmacological studies using enzyme inhibitors and receptor agonists/antagonists, many physiological and pathological functions have been assigned to each prostanoid. For instance, sleep, allergy and adiposity are regulated by PGD₂, parturition and fibrosis by PGF₂α, anti-thrombosis and arthritis by PGI₂, and thrombosis and atherosclerosis by TXA₂. PGE₂ is the most pleiotropic prostanoid that is produced by a variety of cell types and controls many pathological events, such as inflammation, fever, pain, and cancer, through its receptors EP1 to EP4 [76]. Aspirin and related non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit COX enzymes and thereby shut off the synthesis of all prostanoids, are among the most commonly used drugs worldwide. However, since one prostanoid often counteracts another, blocking a specific prostanoid pathway, rather than multiple pathways altogether, would provide a more efficacious strategy for drug development. For instance, PGD₂, a major prostanoid produced by mast cells, exerts its pro-allergic action through the two receptors DP1 and DP2 [46], whereas PGE₂ produced from stromal cells
exerts an anti-allergic action through EP3 [36]. Accordingly, application of NSAIDs, which block both the pro-allergic PGD\(_2\) and anti-allergic PGE\(_2\) pathways, has no therapeutic effect on allergic asthma, or even exacerbates the disease, likely because the precursor AA shunts into the biosynthetic arm of LTs, which are potently pro-allergic. The development of a novel anti-asthmatic drug which specifically ablates the PGD\(_2\) pathway without affecting the PGE\(_2\) pathway is desirable.

Bioactive LTs, including LTB\(_4\) and cysteinyl LTs (cys-LTs; which include LTC\(_4\), LTD\(_4\), and LTE\(_4\)), represent critical lipid mediators of asthma. As in the case of PGs, LTs are produced through three sequential reactions: AA release by PLA\(_2\), metabolism of AA to LTA\(_4\) by 5-LOX in concert with 5-LOX activating protein (FLAP), and conversion of this unstable intermediate to LTD\(_4\) and LTC\(_4\) by LTD\(_4\) hydrolase and LTC\(_4\) synthase, respectively [4]. LTC\(_4\), which is synthesized by conjugation of LTA\(_4\) with glutathione by perinuclear LTC\(_4\) synthase, is exported extracellularly by an ABC transporter, and rapidly metabolized into LTD\(_4\) and then into LTE\(_4\). The actions of cys-LTs are mediated by their receptors CysLT1 (an LTD\(_4\) receptor) and CysLT2 (an LTC\(_4\) receptor). Accordingly, mice deficient in 5-LOX, FLAP, or LTC\(_4\) synthase are all highly resistant to asthmatic challenge [14, 31], and an antagonist of CysLT1 is now clinically

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**Figure 1.** Lipid mediator pathways. PLA\(_2\) hydrolyzes membrane phospholipids to liberate PUFAs (e.g., arachidonic acid, EPA, and DHA) and lyso-phospholipids (e.g., LPC). Of approximately 30 members of the PLA\(_2\) family, cytosolic Ca\(^{2+}\)-dependent PLA\(_2\) (iPLA\(_2\)) is responsible for the release of arachidonic acid in a wide variety of cells. Some Ca\(^{2+}\)-independent PLA\(_2\) (sPLA\(_2\)) enzymes can also participate in the release of PUFAs and LPC in cell type- and stimulus-specific manners. In the COX pathway, arachidonic acid is converted to PGH\(_2\) by COX-1 or COX-2 and then to bioactive prostanoids (PGD\(_2\), PGE\(_2\), PGF\(_{2\alpha}\), PGI\(_2\), and TXA\(_2\)) by various terminal PG synthases: hematopoietic and lipocalin-type PGD synthases (H- and L-PGDS), cytosolic and membrane-bound PGE synthases (cPGES, mPGES-1 and mPGES-2), PGI synthase, PGH synthase and TX synthase. In the 5-LOX pathway, arachidonic acid is converted to LTA\(_4\) by 5-LOX and FLAP and then to bioactive LTs by LT\(_A\) hydrolase (LTA\(_4\)H) or LTC\(_4\) synthase (LTC\(_4\)S) (5-LOX pathway). Arachidonic acid, EPA, and DHA are converted by 5-LOX and 12-LOX to the anti-inflammatory lipid mediators lipoxin A\(_4\) (LXA\(_4\)), resolving E\(_1\) (RevE\(_1\)) and protectin D\(_1\) (PD\(_1\)). LPC is converted to PAF by LPCAT2 or to LPA by autotaxin. Individual lipid mediators thus produced act on their cognate GPCRs on the target cell membrane.
used as an anti-asthmatic drug worldwide. In addition, it has been recently shown that CysLT1 and CysLT2 can form a heterodimer, that GPR17 counteracts the CysLT1 signaling, and that P2Y12, a purinergic GPCR, is important for the biological action of LTE_{4}, the most stable and abundant cys-LT in biological fluid [43, 62]. The first LTB_{4} receptor, BLT1, is a fundamental T_{H1} and T_{H2} immune regulator which acts through recruitment of T lymphocytes [80]. BLT2, originally regarded as a second, low-affinity LTB_{4} receptor, is now considered to be a high-affinity (and probably physiological) receptor for 12(S)-hydroxyheptadecatrienoic acid (12-HHT), a by-product of the COX-1/TXA\(_{2}\) synthesize pathway [58].

**Class 2: lysophospholipids and their derivatives**

The first recognized lysophospholipid-type lipid mediator was PAF. Biosynthesis of PAF involves production of 1-O-alkyl-lysophosphatidylcholine (LPC) by PLA_{2}, followed by its acetylation by LPC acyltransferase (LPCAT2) [70]. Analyses of PAF receptor (PAFR)-deficient mice have revealed important roles for this particular lipid mediator in inflammation and allergy [21, 29, 54]. Since PAFR also recognizes PAF-like oxidized phospholipids [71], some of the implied biological actions of PAF might actually be ascribed to some oxidized phospholipids. PAF undergoes rapid inactivation by plasma-type and intracellular PAF acetylhydrolases, which represent a special group of the PLA\(_{2}\) family [1].

LPA is produced extracellularly from LPC or other lysophospholipids by autotaxin (ATX; lysophospholipase D) and is degraded by a class of lipid phosphatases [85]. Currently, at least six LPA receptors have been identified (named LPA\(_{1}\) to LPA\(_{6}\)) [9]. From studies of knockout mice and hereditary diseases associated with these LPA receptors, it is now clear that LPA is involved in various physiological processes such as brain development, embryo implantation and hair growth, as well as pathological conditions such as neuropathic pain and pulmonary fibrosis [63, 77]. Biosynthetic pathways for lysophospholipid-type mediators are illustrated in Fig. 1. Accumulating evidence suggests that other lysophospholipids also act as lipid mediators. Specifically, LPC, lysophosphatidylserine and lysophosphatidylinositol act on GPR119, GPR34 and GPR55, respectively, whose pathophysiological roles await future studies [44]. LPC also exhibits immune-regulatory actions through G2A (probably indirectly), a GPCR belonging to the proton-sensing GPCR family [23].

SIP, a bioactive lysophospholipid structurally similar to LPA, has a sphingosine (instead of glycerol) backbone and exerts its actions through SIP\(_{1}\) to SIP\(_{3}\) receptors, which share homology with the LPA receptors LPA\(_{1}\) to LPA\(_{3}\) [78]. SIP also regulates a number of biological processes such as developmental vascular integrity, anaphylaxis, cancer, and lymphocyte egress from the lymphoid organs [78]. FYT720 is a powerful immune suppressant that blocks lymphocyte egress by down-regulating SIP\(_{1}\) [45]. SIP levels are tightly controlled through synthesis with sphingosine kinases and inactivation by SIP phosphatase and SIP lyase [5]. A recent study using zebrafish has identified a multipass trans-membrane protein, Sipn2, which plays a role in SIP export from the cell [28]. SIP stimulates the induction of COX-2, while ceramide-1-phosphate, another sphingolipid mediator, is important for PLA\(_{2}\) activation at the Golgi membrane, delineating the functional crosstalk between sphingolipid and eicosanoid pathways [38].

*N*-Acylethanolamines (NAEs) are endocannabinoids that transmit signals through the cannabinoid receptors (central CB\(_{1}\) and peripheral CB\(_{2}\)) and probably through other less understood mechanisms [11]. *N*-Arachidonoyl-ethanolamine (anandamide) is a representative NAE that exerts anti-nociceptive and anti-inflammatory effects through cannabinoid receptors. Development of inhibitors for the NAE-degrading enzymes, fatty acid amide hydrolase and NAE-hydrolyzing acid amidase, has unveiled the functions of endocannabinoids, which are otherwise promptly degraded in vivo, in various biological processes [11]. *N*-Palmitoyl-ethanolamine, an NAE that is abundantly present in the body, is gaining attention as an important analgesic, anti-inflammatory and neuroprotective mediator, and *N*-palmitoyl-ethanolamine itself, its analogs, or agents that inhibit its degradation may lead to the development of new therapeutic strategies for the treatment of pathological conditions [65]. *N*-Oleoyl-ethanolamine, another NAE, is produced in the gut after feeding and acts on the hypothalamus as an anorexigenic mediator [37]. 2-Arachidonyl-glycerol, a more potent cannabinoid receptor
agonist than NAEs, is produced by diacylglycerol lipase α, and is now considered to be a major CB1 ligand that regulates the retrograde suppression of neurotransmission at central synapses [79].

Class 3: ω-3 PUFA derivatives

Recent advances in lipidomic techniques have allowed the identification of new class of lipid mediators derived from ω-3 PUFA (e.g., EPA and DHA) [2]. Pioneered by Serhan and Bazan, these ω-3 lipid mediators such as resolvins, protectins and maresins, as well as lipoxins derived from ω-6 AA, have recently attracted considerable attention as critical mediators of the resolution of inflammation [6, 68]. Biosynthesis of these anti-inflammatory lipid mediators from PUFAs involves 12/15-LOX and other enzymes acting upstream and downstream of 12/15-LOX that have not yet been fully identified. Also, although several GPCRs have been proposed to act as receptors for the anti-inflammatory lipid mediators, such as ALX for lipoxin A4, Chemr23 for resolvin E1, and ALX and GPR32 for resolvin D1 [3, 34], their in vivo relevance awaits future studies using knockout mice of these receptor candidates.

**PGE2 Synthase**

Here, I highlight the pathophysiological roles played by one of the best-studied lipid mediators, i.e., PGE2, by focusing on mPGES-1, an enzyme that specifically converts the intermediate prostanoid PGH2 into PGE2 [51]. The reasons why I focus on PGE2 here are that it is one of the most pleiotropic lipid mediators, that its pathological and physiological roles, regulation of its biosynthesis, and signal transduction via its receptors have been well documented on the basis of numerous cell biological, pharmacological and genetic approaches, and that it represents a good example of how analyses of lipid mediators, through integration of the pathways from biosynthetic enzymes to receptors, are linked to basic biology and drug development.

Clinically, NSAIDs (COX inhibitors) are very effective at treating patients with inflammation, fever and pain. In addition, the regular use of NSAIDs has been shown in clinical trials to markedly reduce the relative risk of developing colorectal cancer by up to 40–50% [81]. As evidenced by gene targeting of the four PGE2 receptors EP1 to EP4 [76], it is evident that PGE2 is a major prostanoid that mediates various pathological events, and many of the pharmacological effects of NSAIDs can be attributed to the inhibition of PGE2 biosynthesis. However, since PGE2 is also involved in several homeostatic processes such as gastrointestinal mucosal protection, renal blood flow, airway homeostasis, and female reproduction, their adverse effects including gastric ulcer and renal damage often limit their long-term use. In view of the concurrent concept that pathogenic PGE2 is produced by inducible COX-2, while homeostatic PGE2 is produced by constitutive COX-1, a novel class of NSAIDs that selectively inhibits COX-2 in marked preference to COX-1 has been developed over the past decade. Even though COX-2-specific inhibitors exhibit less gastrointestinal toxicity than traditional NSAIDs that inhibit both COX-1 and COX-2, they still have a serious adverse effect, namely cardiovascular toxicity [33]. This is likely because specific inhibition of COX-2 affects the balance between platelet-derived pro-thrombotic TXA2 and endothelium-derived anti-thrombotic PGI2, leading to an increase in the risk of thrombosis due to altered vascular tone. Theoretically, a specific blockade of the PGE2-biosynthetic pathway, more specifically a step of PGES, could decrease the pathogenic PGE2 production while sparing other prostanoids, and this is indeed the reason why microsomal PGES (mPGES-1), a major and inducible subtype of PGES enzymes, has attracted much attention as a novel drug target. In support of this, a number of cell studies have demonstrated that mPGES-1 is induced by pro-inflammatory stimuli, couples with COX-2 in preference to COX-1, and is responsible for a large fraction of PGE2 synthesis. Gene targeting of mPGES-1 (Ptges<sup>−/−</sup>) have provided unequivocal evidence that mPGES-1 does play a role in pain, fever, inflammation and cancer [50]. Using research results from Ptges<sup>−/−</sup> mice, I will next give an overview of the functions of mPGES-1-driven PGE2 in pathophysiology. I will also describe recent efforts toward the development of a small molecule mPGES-1 inhibitor and note the concerns on whether such an mPGES-1-targeted strategy can avoid the adverse effects associated with NSAIDs.
**Inflammation**

Migration of macrophages after peritoneal injection of thioglycollate or pleural injection of carrageenan is strikingly reduced in Ptges$^{-/-}$ mice relative to replicate wild-type mice [27]. The formation of inflammatory granulation tissue and attendant angiogenesis in the dorsum induced by subcutaneous implantation of a cotton thread are significantly reduced in Ptges$^{-/-}$ mice as compared with wild-type mice [27]. This model, mPGES-1 deficiency is associated with reduced induction of vascular endothelial cell growth factor (VEGF) in the granulation tissue, implying that mPGES-1-derived PGE$_2$, in cooperation with VEGF, contributes to inflammation-associated angiogenesis and thereby to tissue remodeling.

In collagen-induced or collagen antibody-induced arthritis models (a mouse model of rheumatoid arthritis), Ptges$^{-/-}$ mice are protected from joint inflammation [27, 32, 82]. Similar phenotypes have been observed in mice lacking COX-2 [53] or the PGE receptor EP4 [18], thus revealing a metabolic flow of the COX-2/mPGES-1/EP4 pathway in the development of inflammatory arthritis. In addition to the synovial symptoms, defective generation of the humoral immune response is associated with reduced incidence and severity of arthritis in Ptges$^{-/-}$ mice [32], indicating that mPGES-1 also participates in adaptive immunity. In addition, mPGES-1-mediated PGE$_2$ production by osteoblasts plays a critical role in LPS-induced bone loss associated with inflammation [20]. Moreover, mPGES-1 deficiency is associated with impaired fracture healing in mouse models of skeletal disorders [90], indicating its role in bone metabolism. In experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis that is the most prevalent T$_{H1}$/T$_{H17}$-mediator autoimmune disorder of the CNS with neurological symptoms caused by inflammation and demyelination, Ptges$^{-/-}$ mice show less severe symptoms of EAE and lower production of IL-17 and IFN-$\gamma$ than wild-type mice [30]. PGE$_2$, acting on its receptor EP4 on T cells and dendritic cells, facilitates T$_{H1}$ cell differentiation and amplifies IL-23-mediated T$_{H17}$ cell expansion, and administration of an EP4-selective antagonist in vivo decreases accumulation of both T$_{H1}$ and T$_{H17}$ cells in regional lymph nodes and suppresses the disease progression in mice subjected to EAE or contact hypersensitivity [91].

**Pain, fever, and nerve injury**

Peripheral pain nociception, as assessed by the acetic acid writhing test, is markedly reduced in Ptges$^{-/-}$ mice [27]. This phenotype is particularly evident when the animals are primed with LPS, which induces simultaneous induction of COX-2 and mPGES-1. The basal pain response is also partially reduced in Ptges$^{-/-}$ mice [82], in which the COX-1-mPGES-1 coupling provides nociceptive PGE$_2$ to its receptor EP1 [74]. Ptges$^{-/-}$ mice are also refractory to mechanical allodynia or thermal hyperalgesia in a spinal nerve transection model, implying the role of mPGES-1-driven PGE$_2$ in neuropathic pain. Given that spinal inflammatory hyperalgesia is mediated by the EP2 subtype of PGE receptors [66], the spinal COX-2/mPGES-1/EP2 axis controls neuropathic pain in the CNS.

PGE$_2$ is a critical mediator of fever during infectious and inflammatory conditions, but not of circadian and psychological temperature regulation. LPS-induced febrile response is blunted in mice deficient in mPGES-1 as well as those deficient in COX-2 or the PGE receptor EP3 [13, 39, 86]. Cerebral vascular endothelial cells express COX-2 and mPGES-1 enabling pro-inflammatory cytokines to stimulate the synthesis of PGE$_2$, whose small size and lipophilic property allow it to pass across the blood-brain barrier into CNS neurons, where the PGE$_2$/EP3 signaling elicits G$_{i/o}$ activation in preoptic thermocenter neurons by decreasing preoptic GABA type A receptor expression [84].

mPGES-1 expression is induced in neurons, microglia, and endothelial cells in the cerebral cortex after transient focal ischemia. In Ptges$^{-/-}$ mice, postischemic PGE$_2$ production in the cortex is completely absent, and infarction, edema, apoptotic cell death, and caspase-3 activation in the cortex after ischemia are all reduced compared with those in wild-type mice [19]. Furthermore, the behavioral neurological dysfunctions observed after ischemia in wild-type mice are significantly ameliorated in Ptges$^{-/-}$ mice. Thus, mPGES-1-driven PGE$_2$ is a critical determinant of postischemic neurological dysfunctions.

**Cardiovascular disease**

The selective inhibition, knockout, or mutation of COX-2, or the deletion of the receptor for COX-2-de-
rived PGI$_2$, accelerates thrombogenesis and elevates blood pressure in mice, whereas these responses are attenuated by COX-1 knockdown, which mimics the beneficial effects of low-dose aspirin [92]. The deletion of mPGES-1 in mice reduces PGE$_2$ and increases PGI$_2$ in the circulation, has no effect on TXA$_2$ biosynthesis, and affects neither thrombogenesis nor blood pressure [8]. In mice with a low-density lipoprotein receptor knockout background, the deletion of mPGES-1 retards atherosclerosis development without an attendant impact on blood pressure [87], and protects against abdominal aortic aneurysm formation induced by angiotensin II [88]. These results suggest that inhibitors of mPGES-1 may retain their anti-inflammatory or anti-atherosclerotic efficacy by depressing PGE$_2$, while avoiding the adverse cardiovascular consequences associated with COX-2-mediated PGI$_2$ suppression. However, another study showed that the deletion of mPGES-1 leads to eccentric cardiac myocyte hypertrophy, left ventricular dilation, and impaired left ventricular contractile function after acute myocardial infarction [10], suggesting a cardio-protective role of mPGES-1-driven PGE$_2$.

Cancer

PGE$_2$ is widely recognized as a bioactive lipid metabolite with potent tumor promotion properties, and the in vivo roles of COX-2 [59] and EP receptors [52, 72] in tumor development have been established by studies using their knockout mice. Clinical studies have shown increased levels of COX-2 and mPGES-1 which are directly associated with increased PGE$_2$ production in a number of human cancers [49] (Fig. 2A). The functional role of mPGES-1 in cancer has also been supported by results from cell culture systems. For instance, co-transfection of COX-2 and mPGES-1 facilitates proliferation of HEK293 cells, which form large, well-vascularized tumors when injected into the flanks of nude mice [25]. In several cancer cell lines, mPGES-1 knockdown reduces cell growth, clonogenic capacity, motility, and matrix invasiveness [26], whereas mPGES-1 overexpression accelerates these events (Fig. 2B–D). Furthermore, mPGES-1-silenced lung carcinoma cell xenografts show decreased propagation and metastasis, with concomitant decreases in the density of microvascular networks, expression of pro-angiogenic vascular endothelial growth factor, and activity of matrix metalloproteinase-2 [26]. On the other hand, implantation of mPGES-1-overexpressed lung carcinoma cells results in increased lung metastasis (Fig. 2E).

Experimental observations made in cell culture studies, together with the well-recognized role of COX-2-dependent PGE$_2$ during tumor promotion, have led to several recent in vivo studies focused on the impact of mPGES-1 on tumorigenesis. Ptges$^{-/-}$ mice exhibit a significant reduction in the number and size of intestinal tumors generated on an Apc mutant background [55]. Interestingly, mPGES-1 deficiency is associated with a disorganized vascular pattern within primary adenomas, confirming a key role for PGE$_2$ in tumor angiogenesis. mPGES-1 deletion also results in both reduced size and numbers of pre-neoplastic aberrant crypt foci following treatment with the colon carcinogen azoxymethane [55]. Importantly, protection of the colonic mucosa is accompanied by a marked suppression of nuclear β-catenin translocation, a finding that confirms an earlier study in which PGE$_2$ stimulated colon cancer cell growth through the COX-2/mPGES-1/EP2/Axin/GSK3β/β-catenin axis [7]. Furthermore, lung carcinoma cells implanted into Ptges$^{-/-}$ mice show reduced tumor expansion and lung metastasis [26], suggesting the contribution of mPGES-1 in host stroma cells to tumor development. Finally, transgenic mice overexpressing both COX-2 and mPGES-1 are susceptible to gastric carcinogenesis, a process that is prevented by treatment of the animals with antibiotics [60], suggesting the influence of gut microbial flora and associated inflammation in the initiation of the tumor. The roles of mPGES-1-driven PGE$_2$ are summarized in Fig. 2F. Despite this evidence, one study has reported that Ptges$^{-/-}$ display accelerated, rather than decreased, intestinal tumorigenesis in Apc$^{Min/+}$ mice [12]. Although the reason for this discrepancy is unknown, it may be a reflection of the shunting of intermediate PGH$_2$ toward another pro-tumorigenic prostanoid pathway; for example, the potential contribution of the TXA$_2$/TP pathway to tumor progression has been reported in several cancers [48].

Metabolic syndrome

PGE$_2$ negatively regulates adipogenesis via its receptor EP4 [83]. mPGES-1 expression is down-regulated
Fig. 2. Tumorigenic role of mPGES-1, a PGE$_2$-biosynthetic enzyme. (A) Immunohistochemical staining of mPGES-1 in various human cancers. Human tumor sections were obtained by surgery at Toho University Ohmori Hospital (Tokyo, Japan) following approval from the ethical committee of the Faculty and receipt of informed consent from the patients. (B) Overexpression of mPGES-1 facilitates the migration of tumor cells. mPGES-1- or mock-transfected mouse lung carcinoma cells were seeded on Matrigel matrix (8 µm pore size) in the upper chamber, which was filled with serum-free medium, and placed on the lower chamber containing medium with 10% fetal calf serum. After culture for 16 h, the cells which had invaded the lower chamber were fixed and stained with crystal violet. More mPGES-1-transfected cells than mock-transfected cells were found in the lower chamber, indicating increased matrix invasiveness due to overexpression of mPGES-1. The cell migration was suppressed by the COX-2 inhibitor NS-398, suggesting the dependence of the migration on PGE$_2$. (C) Quantification of the cell migration assay shown in (B) (mean ± SD, n=4, *P<0.05). (D) Cell motility assay. mPGES-1- or mock-transfected mouse lung carcinoma cells were cultured in Petri dishes to near confluency, and then a portion of the cell monolayer was scratched off with a tip. After culture for 16 h, the cells were fixed with crystal violet. More mPGES-1-transfected cells than mock-transfected cells were found in the scratched area, suggesting that cell motility was increased by overexpression of mPGES-1. (E) Lung metastasis assay. mPGES-1-transfected and mock-transfected cells were injected intravenously into BALB/c mice. After 20 days, the mice were sacrificed and metastatic foci in the lung (arrowheads) were visualized by Bouin staining (left panel). The numbers of the lung metastatic foci were counted (right panel) (mean ± SD, n=4, *P<0.05). All procedures involving animals were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of Showa University, in accordance with Standards Relating to the Care and Management of Experimental Animals in Japan. (F) Summary of the roles of mPGES-1-driven PGE$_2$ in tumor development. PGE$_2$ produced by both cancer cell-associated and host stromal mPGES-1 contributes to tumor growth, migration, invasion, metastasis and angiogenesis. PGE$_2$ may also contribute to cellular transformation in the presence of other cancer-promoting factors.
in visceral adipose tissue of obese mice given a high-fat diet [16]. Mice with pancreatic β-cell-specific transgenic overexpression of both COX-2 and mPGES-1 show severe hyperglycemia [61]. In these mice, the relative number of β-cells to the total islet cell numbers was markedly reduced compared with wild-type mice, in accordance with a decreased proliferation rate in the islets. Thus, it is possible that increased PGE₂ signaling contributes to a reduction of pancreatic β-cell mass through inhibition of proliferation, thereby aggravating diabetes. Metabolic syndrome accompanies chronic, low-grade inflammation in the hypothalamic arcuate nucleus, and PGE₂ decreases feeding behaviors via its receptor, EP3 [67]. IL-1β induces anorexia in normal mice, whereas it fails to decrease food intake in Ptges⁻/⁻ mice [64]. Thus, the central mPGES-1/EP3 axis is involved in feeding control and is essential for immune anorexic behavior, and thus may constitute a potential therapeutic target.

Tissue homeostasis

Many of the above studies suggest mPGES-1 as a new drug target which would yield anti-inflammatory, antipyretic, analgesic and anti-tumor effects, while having little or no cardiovascular toxicity. Furthermore, while mice deficient in COX-2 or EP2 show defective female reproduction [17, 40] and mice deficient in both COX-1 and COX-2 or EP4 show neonatal lethality due to failure of ductus arteriosus closure [41, 57], mice deficient in mPGES-1 do not show any abnormality in these processes [35]. Hence, an mPGES-1 inhibitor may stand out as a better prospective tool than the currently used COX inhibitors for the management of pregnant women as well as premature infants with persistent ductus. However, accumulating lines of evidence suggest that the inhibition of mPGES-1 cannot fully avoid the side effects of classical NSAIDs.

First, Ptges⁻/⁻ mice develop progressive hypertension with an inappropriate increase in sodium balance when fed a high-salt diet [22], and also exhibit an impaired ability to excrete an acute water load [73]. These observations indicate that mPGES-1-driven PGE₂ is important for renal homeostasis. Second, PGE₂ is increased in inflammatory bowel diseases, including Crohn’s disease and ulcerative colitis, and traditional NSAIDs trigger or worsen the disease by inhibiting PGE₂ synthesis [69]. Ptges⁻/⁻ mice display exacerbated dextran sulfate-induced colitis compared with replicate wild-type mice, accompanied with increased fecal bleeding and diarrhea, decreased hematocrit scores, shortened colon (an indication of colon inflammation), splenomegaly, increased colonic ulceration, and elevated expression of pro-inflammatory cytokines [15]. An example of the worsened colorectal ulceration and stool bleeding in Ptges⁻/⁻ mice treated with 7% dextran sulfate is shown in Fig. 3A and 3B. These observations are in agreement with the fact that mice deficient in COX-2 or EP4 are more sensitive to dextran sulfate-induced colitis [24, 47], and imply the protective role of the COX-2/mPGES-1/EP4 axis in mucosal homeostasis and integrity. Third, in mouse models of allergic airway inflammation induced by a house dust mite, Ptges⁻/⁻ mice show marked increases in the numbers of vascular smooth muscle cells and the thickness of intrapulmonary vessels as well as modestly enhanced eosinophil infiltration compared with control mice [42]. This observation indicates that mPGES-1 and its product, PGE₂, possibly through EP3 [36], protect the pulmonary vasculature from remodeling during allergen-induced pulmonary inflammation, which agrees with the view that NSAIDs often exacerbate airway inflammation. Therefore, drugs targeting mPGES-1 may still have undesired side effects on the kidney, gut and lung. The pathological and physiological roles of mPGES-1 are summarized in Fig. 3C.

Drug discovery with mPGES-1 as a molecular target

Despite the above criticisms, selective drugs targeting mPGES-1 may be safe relative to COX-inhibiting NSAIDs, since much of the cardiovascular toxicity associated with COX-2 inhibition can potentially be circumvented. A compound that has been developed by Merck, MF63 [2-(6-chloro-1H-phenanthro[9,10-d]imidazol-2-yl)-isopthalonitrile], is a selective mPGES-1 inhibitor that potently inhibits human mPGES-1 (IC₅₀ of 1.3 nM), with a high degree (>1,000-fold) of selectivity over other prostanoid synthases [89]. MF63 retains NSAID-like efficacy at inhibiting LPS-induced pyresis, hyperalgesia, and iodoacetate-induced osteoarthritic pain in rodents. In addition, MF63 does not cause NSAID-like gastrointestinal toxic effects, such as mucosal ero-
Lipid signaling cascades are complex, often redundant and highly interconnected, and counter-regulatory in some cases. Nevertheless, enzymes and receptors involved in lipid mediator signaling have been and are still being targeted pharmacologically to alleviate the symptoms of various diseases. If the modulation of one lipid mediator pathway does not suffice therapeutically, a combination of targeting two or more pathways could be effective. At present, however, the tools that are available to follow the dynamics of individual lipid mediators and to monitor their precise modification and spatiotemporal localization in situ are still technically limited and thus hamper a more comprehensive description of these pathways and their crosstalk. Unlike proteins, the functions of which can largely be addressed individually, lipid actions are frequently masked by the large steady-state mass of the structural lipids in membranes, which make it difficult to detect the transient lipid signal. Further advances in this field and their integration into therapeutic use are likely to benefit from improved, time- and space-resolved lipidomics technology for monitoring individual lipid mediators within tissue niches. Clearly, more research is required to elucidate each of the many regulated pathways of bioactive lipids and define the regulatory mechanisms of their enzymes, the roles of the pathways in specific cell responses, and the mechanisms by which individual lipid mediators mediate their actions.

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