Prostaglandins and Other Lipid Mediators xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Prostaglandins and Other Lipid Mediators

journal homepage: www.elsevier.com/locate/prostaglandins

Review

Role of prostacyclin synthase in carcinogenesis

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ARTICLE INFO

Keywords:
Prostacyclin
Prostacyclin synthase
Carcinogenesis
Tumor
Peroxisome proliferator-activated receptor

ABSTRACT

Prostacyclin (PGI_2) synthase (PGIS) and microsomal prostaglandin (PG) E synthase-1 (PGES-1) functionally couple with inducible cyclooxygenase-2 (COX-2) as their upstream enzymes to produce PGI_2 and PGE_2, respectively. Non-steroidal anti-inflammatory drugs exert their pharmacological effects including antitumor effects by the inhibition of COX-2 and thereby suppress this PG biosynthesis. PGIS is abundantly expressed in vascular endothelial and smooth muscle cells and was shown to be critical for the regulation of platelet aggregation and vascular tone. In addition to its role in vascular regulation, PGIS was shown to be frequently down-regulated in several types of cancers, and the involvement of PGIS in carcinogenesis has been suggested. In this review, we summarize the current understanding of the roles of PGIS and PGIS-derived PGI_2 in carcinogenesis.

1. Introduction

Numerous studies of rodent cancer models and human cancers have shown that nonsteroidal anti-inflammatory drugs (NSAIDs) have anti-neoplastic properties [1,2]. A well-known effect of NSAIDs is their ability to inhibit the enzyme cyclooxygenase (COX) and thereby suppress prostaglandin (PG) synthesis. Of the two COX isozymes COX-1 and COX-2, COX-1 is expressed constitutively in most tissues and is generally responsible for the immediate production of PGs that control normal physiological functions. COX-2 is induced in response to mitogens, cytokines, and cellular transformation and mediates the delayed PG generation related to carcinogenesis [3,4]. High levels of the constitutive expression of COX-2 have been detected in various cancer cells and tissues. Moreover, pharmacological, cell biological and gene targeting studies investigating COX-2 have demonstrated that PGs produced through the COX-2-dependent pathway contribute to the progression of several types of cancer [2].

A COX metabolite, PGH_2, is converted to each PG species by species-specific PG terminal synthases. Among the PG terminal synthases, microsomal PGE synthase-1 (mPGES-1) is induced by various types of stimuli including mitogens as well as COX-2 [5,6]. Furthermore, studies employing cotransfection of mPGES-1 with either COX isozymes in HEK293 cells revealed that mPGES-1 is functionally coupled with COX-2 in marked preference to COX-1, although COX-1-mPGES-1 coupling can also occur under certain conditions [7]. It was reported that mPGES-1 is constitutively expressed in several cancers, most of which also express COX-2 constitutively [8]. Tumorigenic potential of mPGES-1 has been also shown by the observations that transfection of mPGES-1 in combination with COX-2, but not with COX-1, into HEK293 cells led to cellular transformation [8]. Moreover, other research groups and our group have used mPGES-1 knockout (KO) mice, finding that mPGES-1 plays a critical role in carcinogenesis [9–12].

However, not only mPGES-1 but also prostacyclin (PGI_2) synthase (PGIS) functionally couples with COX-2 [13]. In mPGES-1 KO mice, COX-2-derived PGH_2 is metabolically shunted into PGIS-mediated PGI_2 production [12]. We isolated PGIS cDNA and showed that PGIS belongs to cytochrome P450 and is expressed constitutively in vascular endothelial and smooth muscle cells [14–16]. PGIS is critical for the regulation of platelet aggregation and vascular tone [17–19]. Thus, in addition to its role in vascular regulation, the involvement of PGIS in carcinogenesis has been suggested. In this review, we summarize the current understanding of the roles of PGIS and PGIS-derived PGI_2 in carcinogenesis and discuss their possibility as novel therapeutic targets for cancer chemoprevention and chemotherapy.

2. Reduced expression of PGIS in cancerous tissues

As described above, PGIS is abundantly expressed in vascular cells,

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http://dx.doi.org/10.1016/j.prostaglandins.2017.05.001
Received 10 February 2017; Received in revised form 8 May 2017; Accepted 10 May 2017

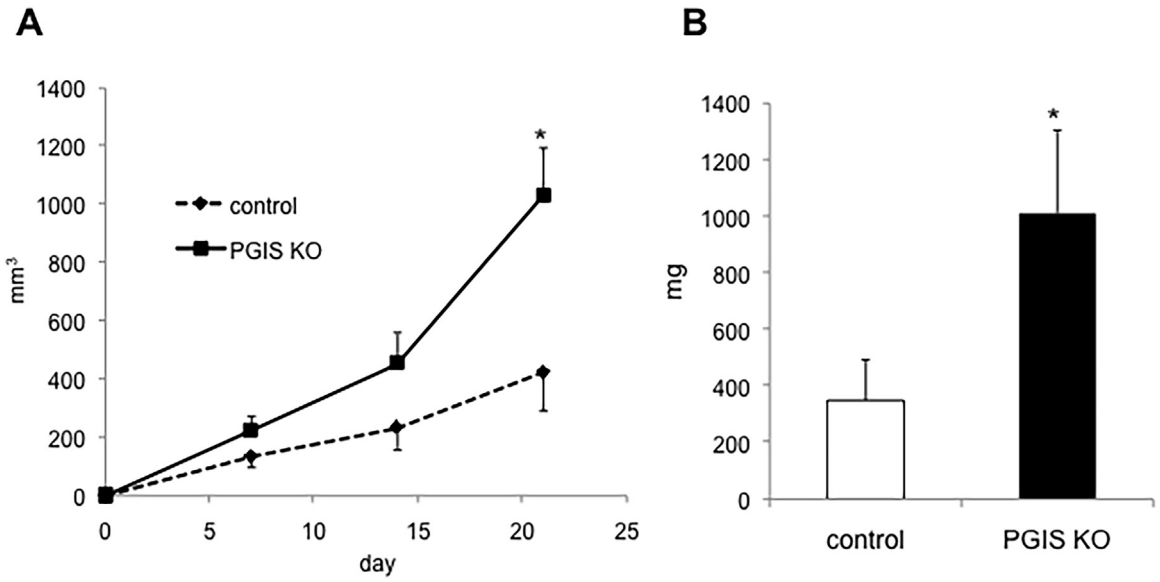

Fig. 1. Growth of colon-26 cells subcutaneously implanted into PGIS KO and control mice. A total of 1×10^6 cells were injected into the subcutaneous tissue of PGIS KO (n = 8) and littermate co;1;ntrol mice (n = 13). A: The size of the tumor was determined by direct measurement of the tumor dimensions on the indicated days. The volume was calculated according to the equation: $V = (L \times W^2) \times 0.5$, where V = volume, L = length and W = width. Results are means + S.E.M. $^*P < 0.05$ *vs.* control mice. B: On day 21 after implantation, the tumor tissues were dissected and weighed. Results are means + S.E.M. $^*P < 0.05$ *vs.* control mice.

but our *in situ* hybridization analysis revealed that this enzyme is expressed not only in vascular cells but also in cells such as lung parenchyma cells [16]. A tissue distribution study demonstrated that PGIS mRNA is particularly abundant in human and rat lung as well as in ovary, heart, skeletal muscle and prostate [15,16]. Although the PGIS expression level is high in normal lung tissues and PGI_2 is one of the most abundant PGs in those tissues, several investigators showed that PGIS is frequently down-regulated in human lung tumors. For instance, a very marked reduction of PGIS mRNA levels was observed in human lung tumor samples, relative to matched normal controls [20].

Comparative immunohistochemical analyses of nonsmall cell lung cancer (NSCLC) and normal human lung tissue reveled that, while the tumors were positive for the expressions of COX-2, mPGES-1 and thromboxane synthase (TXS), immunoreactive signals for PGIS protein were not observed [21]. A microarray gene analysis of NSCLCs also showed that PGIS is down-regulated but mPGES-1 is up-regulated in lung adenocarcinoma tissues compared to adjacent normal tissues [22]. As in NSCLC, the PGIS expression, in terms of mRNA and protein, were lower in head and neck squamous cell carcinoma (HNSCC) tumor samples than in non-tumoral mucosa, whereas, as expected, COX-2 expression was increased in HNSCC tumor samples [23]. These findings suggested that the down-regulation of PGIS might be associated with carcinogenesis in these tissues.

Stearman et al. investigated the expression pattern for PGIS in 108 different NSCLC biopsies, and they found that the expression of PGIS was primarily absent in tumor cells, but 13% of the lung tumors retained PGIS expression, although at very low levels [22]. They generated Kaplan-Meier survival curves to test the survival benefit of positive PGIS staining *versus* negative PGIS staining for all of the NSCLC samples, and they observed a significant correlation between positive PGIS staining and increased survival. The detection of PGIS immunostaining in tissue samples might have strong prognostic value in predicting patient survival.

The precise mechanisms responsible for the down-regulation of PGIS in cancer are still unclear, but several investigators have suggested the involvement of CpG methylation of PGIS promoter in cancerous cells. The 5′-flanking region of the human PGIS gene contains a CpG island region, and our transient transfection experiments using vascular endothelial cells demonstrated that the CpG island region possesses significant promoter activity [24]. Stearman et al. observed hypermethylation of CpG dinucleotides within the PGIS promoter CpG island region in several types of human lung cancer cell lines, and this hypermethylation was associated with a decreased expression of PGIS [25]. The down-regulation of PGIS expression by CpG methylation was reversed by 5-aza-dC, an analogue of deoxycytidine that cannot be methylated. Both a DNA methylation inhibitor, 5-aza-2-deoxycytidine, and a histone deacetylase inhibitor, trichostatin A, also increased PGIS mRNA expression in NSCLC cell lines [26]. Hypermethylation of the CpG dinucleotide in the PGIS promoter CpG island region was also observed in several human colon cancer cell lines and colorectal cancer tissues [27]. Figola et al. examined 15 paired normal and tumor colorectal cancer tissues, and they found that 10 samples showed partial or hypermethylation of the promoter and low relative PGIS expression (tumor tissues *vs.* their normal counterpart).

The PGIS promoter CpG island region contains a 9-bp variable number of tandem repeats (VNTR) sequence (5′-CCAGCCCCG-3′), including Sp1 and AP-2 transcription binding sites. Individual PGIS promoter alleles range from three to nine copies of 9-bp VNTR, and Iwai et al. demonstrated that the repeat polymorphism is associated with promoter activity [28]. A large case-control study of adenomatous and hyperplastic colon polyps by Poole et al. showed an increased risk for these polyps in individuals when both PGIS promoter alleles have a variable number of VNTR lengths less than six [29]. In addition, several single-nucleotide polymorphisms (SNPs) in PGIS gene have been identified, and some were shown to affect PGIS enzymatic activity or PGIS promoter activity [25,30]. An association between a certain type of the SNPs and the risk of cancers was also reported [31]. Genotypes of PGIS gene may be useful as a predictive factor for cancer.

3. Gene deletion of PGIS exacerbates carcinogenesis in mice

We established PGIS KO mice [19] and have investigated the effects of the gene deletion of PGIS on carcinogenesis in experimental animal models. As shown in Fig. 1, we first implanted colon-26 (colon cancer) cells subcutaneously into control and PGIS KO mice, and the development of solid tumors around the injected sites was evaluated over time. Colon-26 tumors grafted into PGIS KO mice grew faster than did those grafted into littermate control mice. On day 14, the tumor weight was increased in the PGIS KO mice. These results suggested that PGIS-derived PGI_2 produced in host microenvironments might suppress