Mini Review

Anti-tumor effects of prostaglandin D_2 and its metabolites, 15-deoxy-$\Delta^{12, 14}$-PGJ_2, by peroxisome proliferator-activated receptor (PPAR) γ-dependent and -independent pathways.

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Hematopoietic prostaglandin (PG) D synthase (H-PGDS) is known as key enzyme in the production of PGD_2 and its J series metabolite, 15-deoxy-$\Delta^{12, 14}$-PGJ_2 (15d-PGJ_2), is thought to play an important role for anti-inflammatory effects. Anti-tumor effects of 15d-PGJ_2 has been shown to be involved in both peroxisome proliferator-activated receptor (PPAR) γ independent and dependent pathways. The independent pathway includes the repression of the telomerase reverse transcriptase (TERT) and cyclooxygenase (COX)-2 via the down-regulation of NFκB. The dependent pathway included repression of the vascular endothelial growth factor (VEGF) receptors and COX-2 with down-regulation of NFκB, followed by the activation of PPAR γ.

In this review, we focused on the role of 15d-PGJ_2 in anti-tumor effects including our evaluation in mouse bladder carcinoma model.

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Masaki Nakamura, Department of Environmental Microbiology, Kitasato University Graduate School of Medical Sciences, 1-15-1 Kitasato, Sagamihara City, Kanagawa, Japan, Phone: 042-778-8072, Fax: 042-778-9400, E-mail: hkita@kitasato-u.ac.jp

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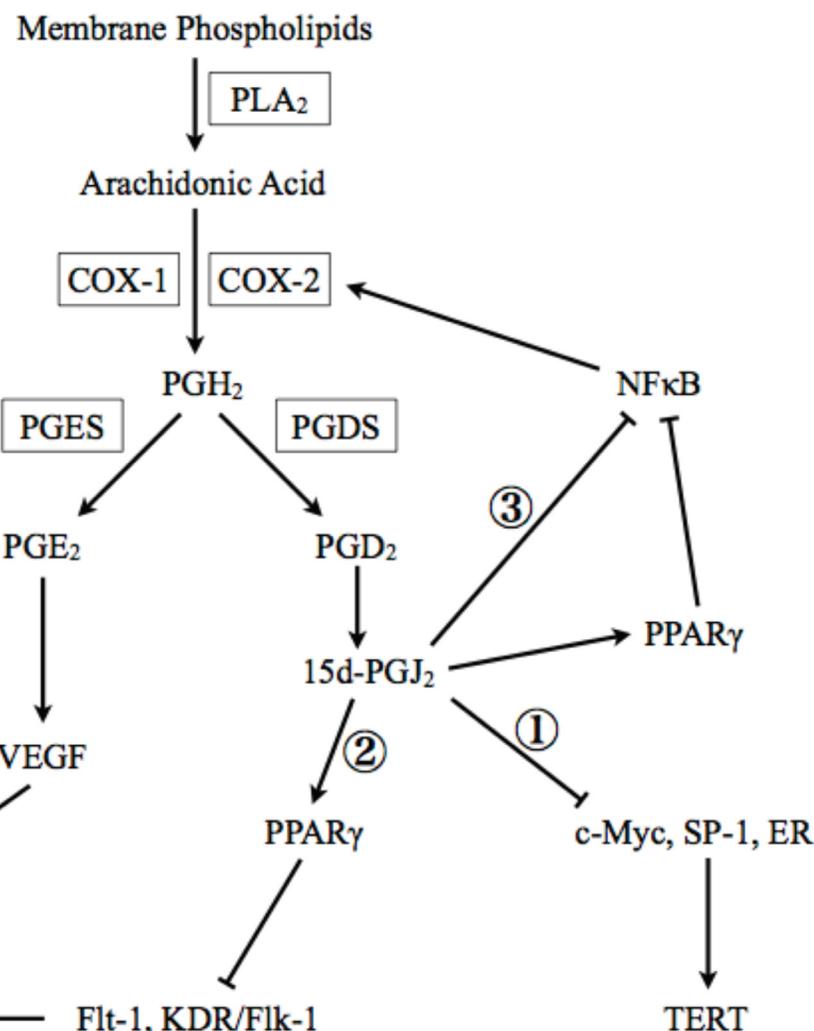## Prostaglandin D₂ and its metabolite, 15-deoxy-Δ¹²,¹⁴-PGJ₂

Prostaglandins (PGs) are autacoids synthesized from 20 carbon-containing polyunsaturated fatty acids, principally arachidonic acid (AA) generated from membrane phospholipids by phospholipase A_2 (PLA$_2$). Prostaglandin H_2 (PGH$_2$) is a common precursor of each PG and produced from AA by the cyclooxygenase-1 and -2 (COX-1, COX-2), then converted to the major active prostanoids such as PGD$_2$, PGE$_2$, PGF$_{2\alpha}$, PGI$_2$, and thromboxane A_2 (TXA$_2$), by PGD$_2$ synthase (PGDS), PGE$_2$ synthase (PGES), PGI$_2$ synthase (PGIS) and TXA$_2$ synthase (TXAS), respectively. Two kinds of PGDS, such as lipocalin (L-) and hematopoietic (H-) PGDS, are known. L-PGDS is a brain enzyme and its product, PGD$_2$, is the most potent somnogenic substance so far known and is involved in various physiological events, such as regulation of sleep and pain responses[1,2]. In contrast, H-PGDS is a splenetic enzyme and is expressed in antigen-presenting dendritic cells as well as in mast cells of various organs[3]. Its product PGD$_2$ and one of its metabolites, cyclopentenone 15-deoxy-Δ¹²,¹⁴-PGJ$_2$ (15d-PGJ$_2$), has been shown as a specific ligand of peroxisome proliferator-activated receptor γ (PPARγ)[4,5] and possess anti-neoplastic (anti-tumor) activity in human cancers of various organs[6,7,8,9,10,11] even no clinical application has carried out.

Fig. 1 A possible mechanism of 15d-PGJ₂ in anti-tumor effects.

Anti-tumor activity of 15d-PGJ$_2$ (Fig.1)

Repression of the TERT (Fig.1 ①)

Telomerase reverse transcriptase (TERT) is known as a major determinant of telomerase activity and to be up-regulated in various cancer cells[12]. 15d-PGJ$_2$ suppresses c-Myc mRNA expression, enhances Sp 1 protein degradation via the ubiquitin-proteasome pathway and inhibits estrogen receptor (ER) β phosphorylation at serine residues. A series of these event is followed by transcriptional repression on the TERT gene[13], and the induction of apoptosis in the colon cancer cells[14].

PPARγ dependent pathway (Fig.1 ②)

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated intracellular transcription factors, members of the nuclear hormone receptor superfamily[15]. PPAR-γ is the most extensively studied subtype of the PPARs and its role has been evaluated in important biological processes, including lipid biosynthesis, glucose metabolism, anti-inflammatory response and atherosclerosis[16,17]. It has been reported that PPARγ has been shown to be expressed in many cancers, including pancreas, breast, lung, thyroid, prostate, and bladder, and has been demonstrated to potentially play an important role in carcinogenesis[18,19]. 15d-PGJ$_2$, a ligand of PPARγ, have shown to inhibit tumor growth of the cells in various organs tumors such as stomach[6], colon[7], lung[8], breast[9], bone marrow[10] and bladder[11].

The activation of PPARγ upregulates the phosphatase and tension homolog (PTEN), which is tumor suppressor gene and modulates cellular proliferation. Thus the activated PPARγ exerts an anti-proliferative effect[20] and increases pro-apoptotic effect by positively regulation of Fas ligand expression[21]. Moreover, PPARγ activation by 15d-PGJ$_2$ inhibits the activities of the transcriptional factors AP-1, STAT and NFκB[22], and at least three important genes including the VEGF receptors 1 (Flt-1), 2 (Flk/KDR) and the urokinase plasminogen activator (uPA) during the angiogenic process[23].

PPARγ independent pathway (Fig.1 ③)

A large number of studies have reported the diverse effects of PPARγ agonists on tumor growth, progression, and metastasis. Nevertheless, emerging data indicate that some of these anti-tumor effects are not totally PPARγ-dependent, but rather are PPARγ-independent. PPARγ agonists 15d-PGJ$_2$ has been reported to inhibit prostate and bladder cancer cell growth in a PPARγ-independent fashion[19]. Sawano *et al.* showed that 15d-PGJ$_2$ inhibited IL-1β-induced COX-2 expression, followed by suppression of ERK and JNK pathways, and AP-1 in mesangial cells without activation of PPAR γ[24]. In addition, inhibition of COX-2 by COX-2 inhibitor and siRNA significantly reduced the production of VEGF in lung cancer cells[25]. Therefore, 15d-PGJ$_2$ played important role in suppression of angiogenesis, based on suppression of COX-2 without activation of PPARγ[26], resulting reduction of VEGF by down-regulation of PGE$_2$.

Bladder carcinoma and novel approach for the therapy

Bladder cancer is the second most common urological carcinoma, and its incidence is gradually incremental. The overall mortality from this disease has not decreased despite improvements in diagnostic and therapeutic modalities. Approximately 70% of bladder cancers are superficial, with the remaining 30% being muscle invasive and/or metastatic lesions at initial diagnosis[27, 28].

Non-muscle invasive cancers are primarily treated by transurethral resection, but approximately 50% of patients will develop disease recurrence within 5 years. Approximately 30% of these recurrent tumors have higher grade and more aggressive properties than the primary tumors. Intravesical administration of chemotherapeutic agents or BCG vaccine reduces the recurrence of the tumors. However, one third of patients will still experience disease recurrence. Surgery, chemotherapy, and radiation are the standard therapeutic options for an attempt to cure patients with cancers, particularly for muscle invasive lesions. Once disease progression or distant metastases are observed, current cancer therapies have been relatively inadequate for salvage or long-term survival. In addition, current anti-cancer therapeutic modalities come at a price because they are relatively nonselective and have detrimental effects on healthy tissues in the body. Novel approaches are desired for the treatment of bladder cancer.
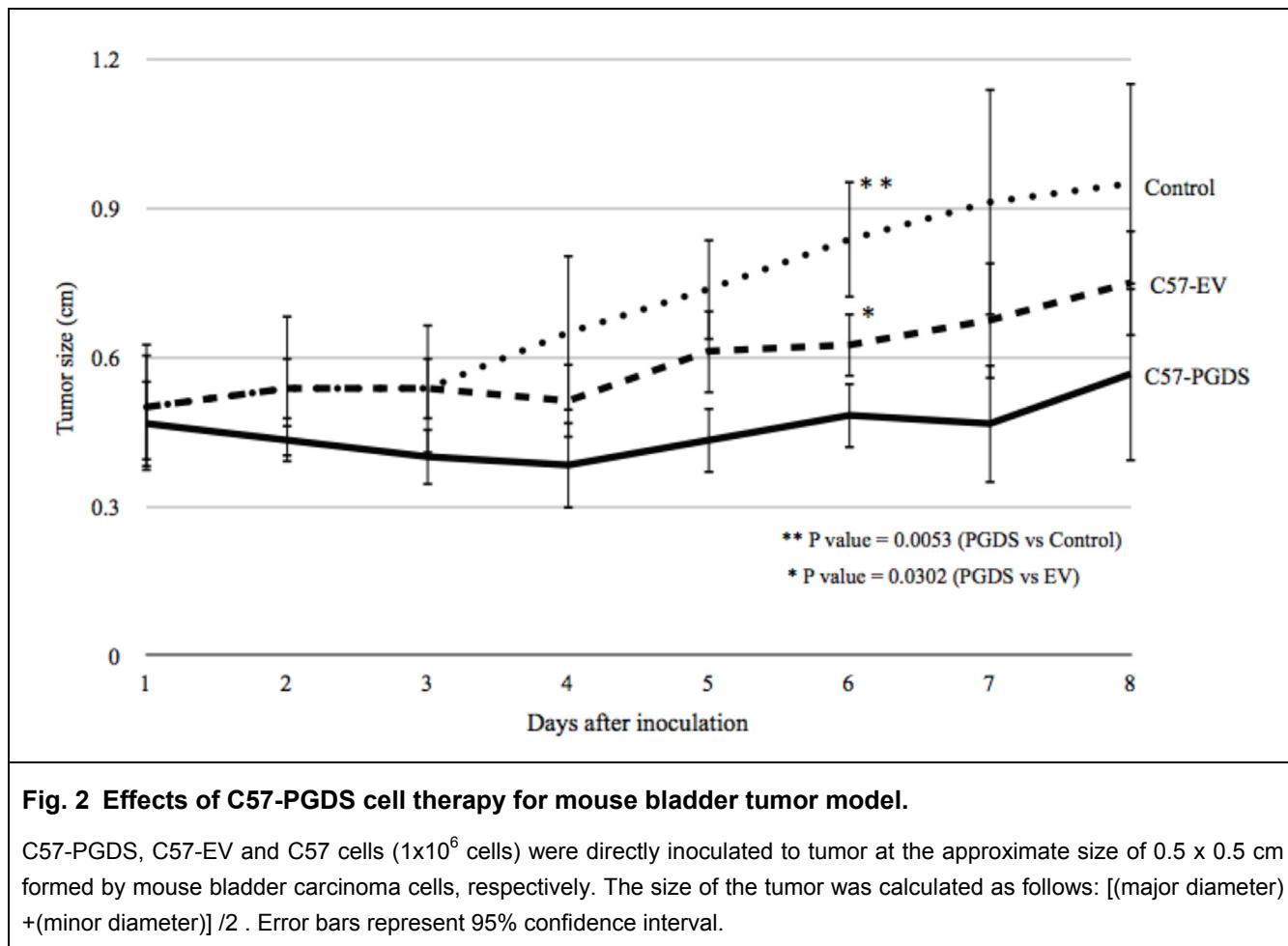
Anti-tumor effects of cell therapy using PGD$_2$ expressing cells in murine bladder carcinoma model

Cell therapy is thought to be an important tool to express the target gene in efficiently local area

without side effects. The risk of cell therapy such as propagation of the introduced cells might be avoided

by introduction of the suicide gene in the therapeutic cells.

Fig. 2 Effects of C57-PGDS cell therapy for mouse bladder tumor model.

C57-PGDS, C57-EV and C57 cells (1×10^6 cells) were directly inoculated to tumor at the approximate size of 0.5 x 0.5 cm formed by mouse bladder carcinoma cells, respectively. The size of the tumor was calculated as follows: [(major diameter) +(minor diameter)] /2 . Error bars represent 95% confidence interval.

Therapeutic cells using for the cell therapy, expressing PGD_2 were previously constructed by retrovirally introduced human H-PGDS cDNA into fibroblasts of C57BL/6J mice (C57), designated as C57-PGDS. The control cells expressing retrovirus vector only in C57 was designated as C57-EV. The amount of PGD_2 from C57-PGDS was estimated as approximate 400 pg/ml by ELISA. Down regulation of PGE_2 by overexpression of PGD_2 and conversion of PGD_2 to 15d-PGJ_2 in C57 and RBL-2H3 cells were confirmed by ELISA and thin-layer chromatography plate, respectively, as previously reported[29].

Murine bladder carcinoma model was made by transplanting MBT2 (mouse bladder tumor 2) cells into subcutaneous of C3H mouse. C57-PGDS and C57-EV were injected respectively into the tumor at approximate size of 0.5 cm in diameter. C57-PGDS cell therapy resulted in significantly reduction of the size of murine bladder carcinoma at 6 days after the cell inoculation in comparison with C57-EV or C57 inoculated mice (Fig.2). Histological samples were
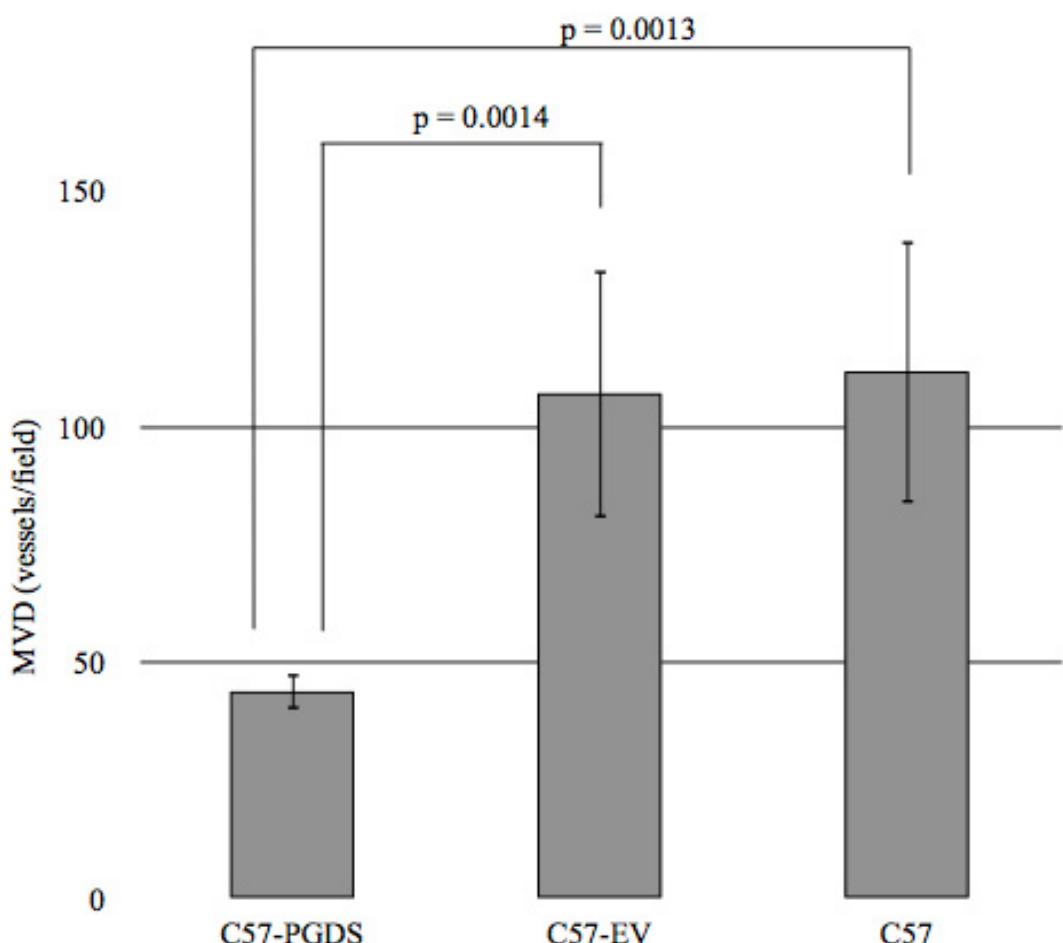
obtained at 7 days after the therapeutic cell inoculation and stained by hematoxylin-eosin (HE) followed by microvascular density (MVD) analysis. The result showed that the angiogenesis was significantly suppressed in mice treated with C57-PGDS (Fig. 3). In addition, suppression of COX-2 expression evaluated by real-time PCR was observed in the tumor inoculated with C57-PGDS cells (data not shown). On the other hand, no significant difference of transcriptional level of TERT was observed. These results suggest that cell therapy expressing PGD_2 significantly succeed to repress the volume of bladder tumor, followed by suppression of COX-2 transcription directly or indirectly via PPAR γ activation. The detail of the mechanism is under investigation.

Conclusion

Anti-tumor effects of 15d-PGJ_2 were evaluated including the repression of the telomerase reverse transcriptase (TERT), and inhibition of angiogenesis by suppression of COX-2 with down-regulation of

NFκB, with or without activation of the PPAR γ.

Cell therapy using PGD_2 expressing cells may

contribute to the development of specific therapy for carcinoma such as bladder carcinoma shown here.

Fig. 3

Microvascular density (MVD) analysis of the tumor treated with C57-PDGS, C57-EV and C57. Histological samples were stained by hematoxylin-eosin (HE), and the number of microvessels per one field was counted (MVD). Error bars represent 95% confidence interval (n = 5).

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