Peroxisome proliferator-activated receptor and epithelial ovarian cancer

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Introduction

A nuclear receptor called peroxisome proliferator-activated receptor (PPAR) was discovered in 1990 [1]. To date, three PPAR subtypes, α, δ, and γ, have been identified. Each of them has been found to be expressed in different tissues [2]. PPARα is mainly distributed in the liver, kidney, heart, and skeletal muscle and plays a critical role in fatty acid metabolism. PPARγ is present in adipose tissue or in the small intestine and colon, and is involved in functions such as cell differentiation, lipid storage, and modulation of insulin action. PPARδ is extensively distributed in the body and has been reported to modulate intercerebral lipid metabolism, HDL metabolism, adipogenesis, and preadipocyte differentiation.

Each of these PPARs is activated by its specific ligand and regulates the expression of various genes by binding to the regulatory region of target genes. PPARs have received attention as potential targets for the treatment of lipid metabolism, hyperlipidemia, diabetes, and arteriosclerosis, although expression of PPARs is frequently seen in a variety of cancers. Therefore, not only studies on carcinogenic mechanisms but also on the development of new treatment strategies using PPARs as molecular targets have been underway. In this paper, the latest findings in this area are reviewed, focusing particularly on PPARα, PPARγ, and ovarian cancer and the possibility of their clinical applications for ovarian cancer treatment.

The role of PPARs in the ovary

PPARs have been shown to play a direct role in ovarian physiology since they were first discovered. PPARs were reported to have functions in regulating tissue remodeling during follicular growth, ovulation, and luteinization as well as in the expression and activation of proteinase-influencing angiogenesis [3-6]. In 2001, Komar *et al*. [7] identified the location of PPARs and their specific roles in follicular growth by in situ hybridization in normal rat ovarian tissue. PPAR was localized in granulosa cells, PPARα and PPARδ in the capsule and in the stroma [7]. PPARγ was found to be regulated by luteinizing hormone and to be highly expressed during follicular growth, but to decrease with ovulation [7]. Also, treatment with PPARγ ligand significantly increases progesterone and estrogen levels [7]. Elevated expression of PPARα and PPARδ are maintained despite estrous cycles, and so they were assumed to be associated with basic ovarian function. However, their exact mechanism of action remains unknown [7]. These results show the presence of PPARs in the ovary and that of PPAR-specific ligands certainly have some effect on physiological function in the ovary, but the history of investigation of the relationship between the ovary and PPARs is relatively short. Therefore, further studies to identify the various involvements of PPARs are expected.

PPARγ and ovarian cancer

Nicol *et al*. [8] reported in 2004 that the expression of PPARγ inhibited ovarian carcinogenesis induced by the carcinogen 7,12-dimethylbenz[a]anthracene (DMBA), and application of DMBA to PPARγ hetero-knockout mice and PPARγ wild-type mice increased the occurrence of ovarian cancer by more than 3-fold in the former mice compared with the latter, and the metastatic rate by 4.6-fold. Based on these results, they reported that PPARγ may be a regulator of ovarian carcinogenesis. In the same year (2004), Sakamoto *et al*. [9] found that the state of high cyclooxygenase (COX)-2 expression and low PPARγ expression in epithelial ovarian cells was strongly involved in ovarian carcinogenesis and that activation of PPARγ in ovarian cancer cells reduced the expression of COX-2 through the nuclear factor κB pathway. COX-2 is a known carcinogen in colon cancer and breast cancer. Our group identified that activating PPARγ with its specific ligand in ovarian cancer cells resulted in the loss of COX-2 expression induced by tumor necrosis factor-α, and reported that there was an inverse relationship between COX-2 expression and PPARγ [9]. Based on the findings of the two studies published in 2004, a specific ligand which can activate PPARγ was expected to be a promising drug candidate for the treatment of ovarian cancer as well as to inhibit ovarian carcinogenesis.

Since then, the relationship between the PPARγ ligand and ovarian cancer in vitro has been widely investigated and the following data have been published in succession: ciglitazone, a PPARγ ligand that induces cell cycle arrest and

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apoptosis in ovarian cancer cells, resulting in decreased cell proliferation [10], DIM-C-pPhtBu, another PPARγ ligand that induces PPARγ-dependent p21 expression in ovarian cancer cells leading to cell cycle arrest [11], and inhibition of cell proliferation can be achieved by inducing apoptosis as a result of reducing activation of non-PPARγ-dependent cyclin D1. We conducted an in vivo study in which we created ovarian carcinoma-bearing mice and cancerous peritonitis mice and directly administered a PPARγ ligand of ciglitazone. The study found administration of ciglitazone significantly reduced subcutaneous tumor growth and markedly prolonged the survival time of treated cancerous peritonitis mice [12]. There was a significant increase of PPAR in ciglitazone-treated subcutaneous tumors in mice, and induction of apoptosis was apparent and angiogenesis was suppressed. Moreover, interestingly, treatment with ciglitazone significantly reduced prostaglandin synthase (mPGES) activity in the tumors, although COX-2 expression in the tumors remained unchanged (Figure 1), suggesting that in vivo, depletion of prostaglandin (PG) E_2 in a COX-2-independent manner with ciglitazone can reduce tumor growth by inducing apoptosis and suppressing angiogenesis. Shigeto *et al*. [13] demonstrated that antitumor effects against ovarian cancer can be achieved with a similar mechanism using pioglitazone, the same PPAR ligand.

PPARα and ovarian cancer (including other types of cancer)

When the peroxisome proliferator-activated nuclear receptor was discovered in 1990, PPARα was the mediator responsible for the key mechanism [1]. It is not common for there to be an argument over whether the role of a molecule is beneficial or harmful, but in case of PPARα, this is subject to debate. Fibrate, a lipid-lowering drug is representative of peroxisome proliferators, is widely known to activate PPARα, enhance lipid metabolism, induce increased HDL cholesterol levels and to have a protective effect against arteriosclerosis, while it has been known for many years that rodents such as mice and rats chronically treated with peroxisome proliferators develop hepatocellular carcinoma [14]. Gonzalez *et al*. [15] created PPARα knockout mice and administered clofibrate or Wy-14,643, peroxisome proliferators. No liver tumors, peroxisome proliferation, or induction of a series of enzymes were observed, showing conclusively that PPARα plays a crucial role in peroxisome proliferator-induced liver carcinogenesis [15]. However, liver biopsy specimens obtained from human patients taking high doses of clofibrate for a few years showed no peroxisome proliferation [16]. Thus, even if there is a species difference in peroxisome proliferation, the fact that PPAR is involved in the mechanism of liver carcinogenesis (medication → peroxisome proliferation in the liver → liver carcinogenesis) is currently drawing attention in drug safety assessment during drug development [17]. PPARα being expressed in Sertoli cells in the testis is also known to be involved in reproductive toxicity caused by environmental chemicals [18].

Aside from whether PPARα is beneficial or not, it was first reported in 2006 that PPAR ligands decreased cancer cell proliferation in vitro [19], following which successive reports from 2007 to 2008 stating that activation of PPARα inhibited tumor growth were published [20-22]. These findings revealed the relationship between PPARα and antitumor activity.

Grau *et al*. [19] reported in 2006 that activation of PPARα by its ligand reduced transcriptional induction of COX-2 and vascular endothelial growth factor (VEGF) in colorectal-cancer cells. They concluded that this was due to the suppression of activator protein-1 (AP-1) gene induction involved in tumor development. The mechanism of tumor growth inhibition by PPARα was assumed to be that activated PPARα directly binds to the consensus DNA region and reduces AP-1 expression as well as inhibits transcriptional activity of c-Jun, although the expression of AP-1 is regulated by the c-Jun oncogene. This mechanism was supported by our follow-up study – we found that treatment with PPAR ligand reduced AP-1 expression and inhibited ovarian cancer growth in ovarian-cancer specimens in vivo [13].

In April 2007, we reported that activation of PPARα inhibited solid tumor growth in vivo, which was the first such report in the world [20]. We created tumor-bearing mouse models and cancerous peritonitis mouse models using two types of human ovarian-cancer cells. A single administration of clofibric acid, a PPARα ligand, produced a tumor response at least as effective as cisplatin (a key drug for ovarian cancer) and prolonged survival time in the animals. Carbonyl reductase, which was increased in the tumor by clofibric acid administration, had a pivotal role in the mechanism (Figure 1). Carbonyl reductase also exists in the ovary and metabolizes carbonyl compounds in the presence of NADPH on their surface [23], but it actually also has an important function in converting PGE_2 to $PGF_{2\alpha}$ [24]. Gene transfer of carbonyl reductase to ovarian cancer cells reduced the activation of PGE_2 by 20%. PGE_2 is known to be involved in tumor development by enhancing angiogenesis and inhibiting apoptosis as well as in eliciting inflammation [25, 26]. Our transfection study showed a significant decrease in VEGF expression with the decline of PGE_2 activity. Moreover, clofibric acid was found to have a direct reducing effect on mPGES (Figure 1). Thus, we concluded the mechanism of the antitumor effect against ovarian cancer of clofibric acid, a PPAR ligand, as follows: treatment with clofibric acid increased carbonyl reductase and decreased mPGES, which resulted in reduced PGE_2 activity followed by suppression of angiogenesis and induction of apoptosis (Figure 1).

Pozzi *et al*. [21] reported in June 2007 that administration of a PPARα ligand of Wy14,643 produced a tumor response in human lung cancer. They note the reason that angiogenesis did not occur to be the result of reduced expression of Cyp2c epoxygenase genes which catalyze arachidonic acid metabolism or synthesize epoxyeicosatrienoic acid having a stabilizing action on vascular endothelium. These findings were not observed in PPARα knockout mice, show-

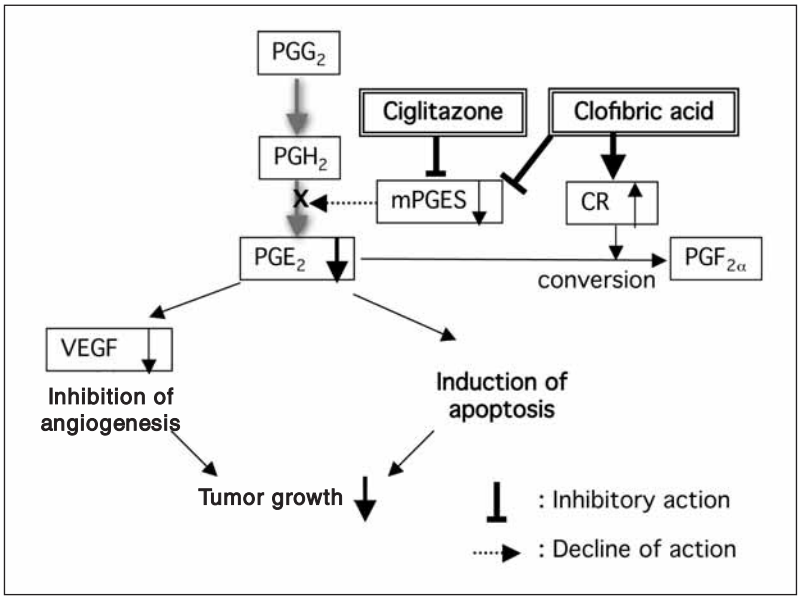

Figure 1. — Possible mechanisms of antitumor effect by the specific ligands for PPARa and PPARg. PG; prostaglandin, mPGES; prostaglandin synthase, CR; carbonyl reductase, VEGF; vascular endothelial growth factor.

ing that activation of PPARα by its ligand played a leading role. Panigrahy *et al*. [22] reported in January 2008 that administration of a PPARα ligand of fenofibrate in mesenchymal tumors and various types of cancer inhibited tumor growth in all treated tumors. The mechanism of the antitumor effects of fenofibrate is suppression of angiogenesis by increasing the anti-inflammatory effects of thrombospondin-1. They confirmed that these antitumor effects were not observed in PPARα knockout mice. Consequently, it was shown that the activation of PPARα by its specific ligand theoretically had an antitumor effect against any type of solid tumor. The results of these three studies conducted in different facilities demonstrated the same mechanism of antitumor effect of PPARα ligands. That is, it was suggested that the surrounding microenvironment of tumors commonly seen in inflammation was responsible for tumor proliferation. Inflammation induces angiogenesis [25]. Inflammatory cells around the tumor conduct an essential role in promoting tumor growth by releasing angiogenic factors and cytokines which are tumor cell nutrients [21, 22]. The activation of PPARα by its specific ligand was revealed to reduce "inflammation" around tumor cells. Our report served as a basis for the development of a potential treatment for ovarian cancer targeting PGE_2 using clofibric acid. It is recommended that future studies investigate antitumor effects against various types of cancers using different PPARα ligands.

PPARδ and ovarian cancer

At present, there are few studies investigating the relationship between PPARδ and malignant tumors. According to in vitro studies, the activation of PPARδ appears to inhibit tumor growth in breast cancer cells, lung cancer cells, and melanoma cells [27, 28], although a consensus has not been reached on its mechanism of action. Daikoku *et al*. [29] reported in 2007 that blocking PPARδ function by neutralizing activated PPARδ inhibited tumor growth of ovarian cancer in vivo. Aspirin, a NSAID and a COX-1 selective inhibitor, inhibited the growth and proliferation of ovarian cancer as well as reduced the function of PPARδ, suggesting inactivation of PPARδ is associated with the growth inhibition of ovarian cancer [29]. In our study, however, aspirin did not inhibit tumor growth of ovarian cancer [30]. Further studies on the relationship between PPARδ and malignant tumors may be necessary.

Future prospects

The basic treatment for ovarian cancer is cytoreductive surgery and postoperative chemotherapy. A characteristic of ovarian cancer is that even in patients with advanced ovarian cancer with residual lesions, the response rate is high since ovarian cancer is more sensitive to anticancer agents, but the recurrence rate is also high. For recurrent cancers, a single treatment with a variety of anticancer agents has been attempted, yet there is still no conclusive answer. The antiangiogenic agent bevacizumab (Avastin) is receiving attention as a molecular-targeted agent. However, the serious complications including intestinal perforation cannot be ignored. A molecular-targeted agent specific for the activation of PPARγ or PPARα discussed above would be a promising drug candidate from the aspect of side effects. The previously-reported PPARγ ligands ciglitazone and pioglitazone are oral hypoglycemic drugs, while PPARα ligands of clofibric acid and fenofibrate are drugs for hyperlipidemia. Both types of drugs are routinely used and their potential side effects are known. Therefore, it is desirable to promote clinical studies based on the fundamental accumulated data on these, in order to investigate antitumor effects by either of these drugs alone or with anticancer agents. Survival after

relapse is relatively long, particularly in patients with ovarian cancer. Although it is important to examine whether PPARγ ligands or PPARα ligands provide survival benefits, investigating their possible benefits as dormancy therapies would be very interesting.

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