

Treatment strategies for ovarian cancer focusing on prostaglandins

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DOI:10.31083/j.ejgo4205148

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Submitted: 23 June 2021 Revised: 26 July 2021 Accepted: 12 August 2021 Published: 15 October 2021

This work aims to researching the potential of anti-inflammatory therapy as an adjuvant in the antitumoral treatment for ovarian cancers. Drugs commonly used for dyslipidemia and diabetes mellitus and anti-inflammatory drugs have tumor-suppressing effects via anti-angiogenic and apoptosis-inducing actions. Proinflammatory prostaglandins (PGs) promote angiogenesis and suppress apoptosis. A therapeutic agent for dyslipidemia, Clofibrate acid increases carbonyl reductase 1 (CBR1), which inactivates PGs in the tumor, and exerts a tumor shrinkage effect. Oral hypoglycemic agents, ciglitazone and pioglitazone reduce PG synthase and cyclooxygenase (COX) 2 in tumors and shrink them. A selective COX-2 inhibitor, Meloxicam directly inhibits PG production and shrinks tumors. These facts remind us of the importance of drug repositioning. Considering that the safety has been established including side effects, these drugs have the potential to be used not only to treat ovarian cancer but also human solid cancers in general as a combination adjuvant drug with other anticancer agents or to be applied to tumor dormancy therapy with different properties from anticancer agents.

Keywords

Ovarian cancer; Prostaglandins; Clofibrate acid; Carbonyl reductase; Oral hypoglycemic agents

1. Introduction

The first-line treatments for advanced ovarian cancer are surgical cytoreduction and anticancer chemotherapy. However, ovarian cancer is often resistant to anticancer agents, and we often face difficulties in treatment. Angiogenesis is indispensable for tumor growth and regrowth of a metastatic lesion, and the role of various angiogenic factors has been attracting attention. Many researchers have reported the elucidated regulatory mechanisms of tumor angiogenesis and the strategies to suppress angiogenesis. It has been demonstrated that there are many angiogenic factors, among which vascular endothelial growth factor (VEGF) was first identified in 1989. Since VEGF has been shown to play an important role in the regulation of angiogenesis by many studies, a special focus has been placed on VEGF in the suppression of angiogenesis. A VEGF inhibitor, bevacizumab has shown to be effective not only for ovarian cancer but also various other cancers. We have confirmed the possibilities that the paracrine mechanism of VEGF and its receptors may have an action

on the stroma and vascular endothelium near the tumors in ovarian cancer and uterine cancer [1] and that expressions of VEGF-C and VEGF-D may play an important role in lymph node metastasis and intraperitoneal dissemination and is also a poor prognostic factor [2].

We have demonstrated that the reduced expression of carbonyl reductase 1 (CBR1), which converts prostaglandin (PG) E₂ to PGF_{2α}, is closely associated with cancer progression and retroperitoneal lymph node metastasis in human ovarian cancer [3]. PGE₂ is known to be involved in tumor metastasis and invasion capacity by inducing angiogenesis. In addition, we found that high levels of cyclooxygenase (COX)-2 expression and low levels of proliferator-activated receptor (PPAR) γ expression were involved in cancer initiation [4]. We also demonstrated that the expression regulatory mechanism of PPARγ and COX-2 mediated by PPARγ ligands and PG works in the ovarian cancer cells at the same time [4].

Based on these research results, we generated experimental models of ovarian cancer using subcutaneous cancer-bearing mice and carcinomatous peritonitis mice derived from multiple human ovarian cancer cells. Using these mouse models, we investigated the antitumor effects of clofibrate acid, which is a PPARα ligand targeting the enzymes involved in PGE₂ synthesis, ciglitazone and pioglitazone, which are PPARγ ligands, and a selective COX-2 inhibitor, meloxicam. Our results showed that all of these drugs exerted the same level of antitumor effect as cisplatin, which is one of the standard treatment drugs for ovarian cancer, when used as a single agent [5–7]. In the tumor to which any of these drugs was administered, angiogenesis was significantly suppressed, and apoptosis was significantly induced. Also, the PGE₂ levels in the serum and ascites were decreased in mice upon administration of each of these drugs. The mechanism of action of each drug is summarized in Fig. 1. Drugs commonly used for dyslipidemia and diabetes mellitus and anti-inflammatory drugs have tumor-suppressing effects via anti-angiogenic and apoptosis-inducing actions [8, 9]. This work aims to researching the potential of anti-inflammatory therapy as an adjuvant in the antitumoral treatment for ovarian cancers. We also discuss on the potential of the gene therapy for ovarian cancer by using CBR1 DNA.

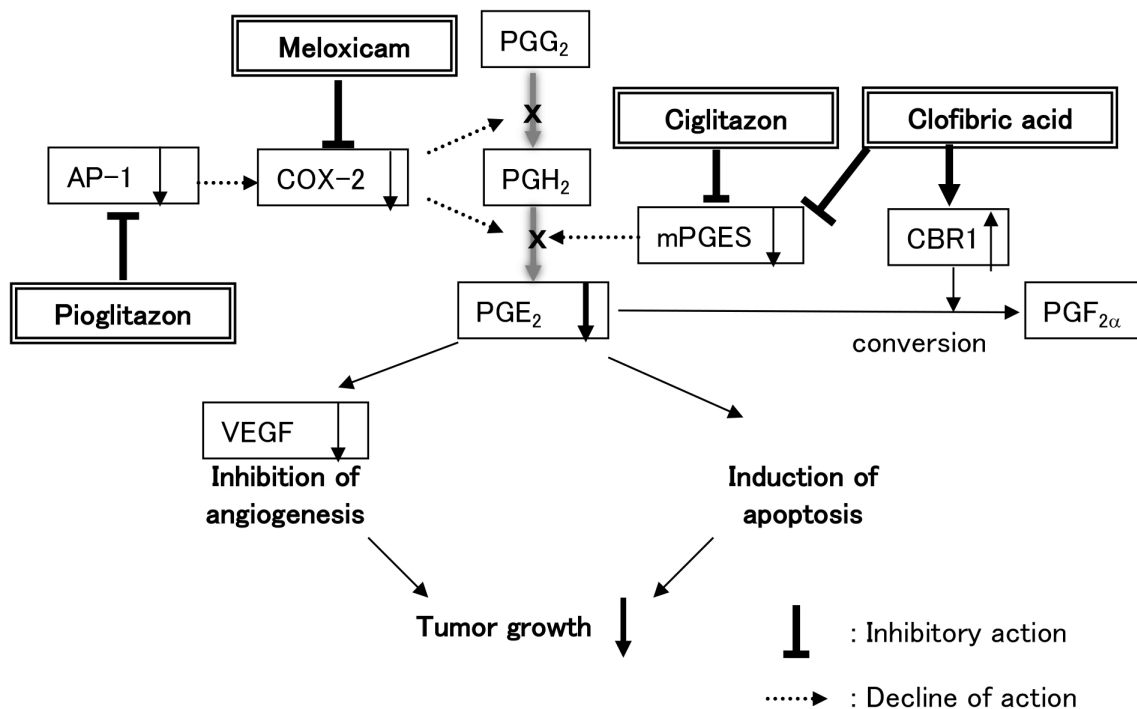


Fig. 1. The mechanism of action of PPAR α ligand, PPAR γ ligand and COX-2 inhibitor.

The data of this review article have been obtained by searching in PubMed and Web of Science until the end of December 2020. The search terms were “PPAR γ ligand”, “hypoglycemic agent”, “prostaglandin”, “anti-inflammatory effect” and “ovarian cancer” and we found 4 matched articles. The other search terms were “PPAR α ligand”, “prostaglandin”, “anti-inflammatory effect” and “ovarian cancer” and we found 4 matched articles and we found only 1 matched article.

2. Fibrate drug clobfibric acid (PPAR α ligand)

Clobfibric acid, the main component of fibrate drugs used as a treatment drug of dyslipidemia, is known as a PPAR α ligand. We reported for the first time that activation of PPAR α suppresses solid tumor growth *in vivo* [5]. We generated a cancer-bearing mouse model and a carcinomatous peritonitis mouse model using two types of human ovarian cancer cells. When we administered the PPAR α ligand clobfibric acid alone to these mice, the higher or equivalent levels of tumor shrinking effect and survival time prolongation as those of cisplatin, a key drug of ovarian cancer treatment, were observed [5]. CBR1 that was increased in the tumor due to clobfibric acid administration played the central role (Fig. 1). CBR1 is an enzyme present in the ovary and is known to metabolize carbonyl compounds in the presence of NADPH; however, in fact, it also has an important function to convert PGE $_2$ to PGF $_{2\alpha}$. We introduced CBR1 DNA into ovarian cancer cells, which reduced the PGE $_2$ activity to 20% [5]. PGE $_2$ is known to induce inflammation, and at the same time, to be involved in tumor progression by promoting angiogenesis and sup-

pressing apoptosis. Our gene transfection experiment also showed a significant reduction in VEGF expression as well as the PGE $_2$ activity. Furthermore, our result demonstrated that clobfibric acid has an effect of directly reducing mPGES (Fig. 1) [5]. We concluded that the antitumor effect of a PPAR α ligand, clobfibric acid in ovarian cancer is due to the decrease in the PGE $_2$ activity caused by the increase in CBR1 and decreased PG synthetase, which leads to suppression of angiogenesis and induction of apoptosis (Fig. 1).

Pozzi *et al.* [10] reported that administration of a PPAR α Wy14,643 showed a tumor-shrinking effect in human lung cancer. They stated that the tumor shrinkage was caused because angiogenesis did not occur due to the reduced expression of the gene encoding Cyp2c epoxygenase, which catalyzes arachidonic acid metabolism and has a stabilizing effect on vascular endothelium. These phenomena did not occur in PPAR α knockout mice, which proved that PPAR α activation by a ligand plays a major role in the antitumor effect. Moreover, Panigrahy *et al.* [11] reported that administration of a PPAR α ligand, fenofibrate suppressed tumor growth in all types of cancers tested. The mechanism of the antitumor effect was that fenofibrate suppressed angiogenesis by increasing thrombospondin-1, which has an anti-inflammatory effect. It has been confirmed that these antitumor effects were absent in PPAR α knockout mice. These results show that theoretically, activation of PPAR α by its specific ligands is effective in any solid tumors. The results of these three studies conducted at different research institutions unexpectedly demonstrated that PPAR α ligands exert the antitumor effect

by the same mechanism. In other words, it was suggested that inflammation, which is a commonplace event in tumor microenvironment, plays a central role in tumor growth. Inflammation leads to angiogenesis. Inflammatory cells around the tumor release not only angiogenic factors but also cytokines that nourish tumor cells, thereby playing an important role in promoting tumor growth. All these results revealed that the activation of PPAR α by its specific ligands has a function of removing "inflammation" around the tumor cells. We have found that the potential treatment for ovarian cancer with clofibric acid targeting PGE $_2$, and we are now headed to the next step.

3. Aiming for gene therapy using CBR1 DNA

3.1 What is CBR1?

CBR1, a protein present in the cytoplasm with a subunit with a molecular weight of 30,000, is an enzyme that reduces aldehydes and ketone bodies produced in the body to alcohol using NADPH as a coenzyme, and during the reaction, NADPH is converted to NADP $^+$. It also has an activity of reducing quinone to hydroquinone. The reduction of substances with carbonyl group is catalyzed not only by CBR1 but also by various enzymes, including short-chain dehydrogenase/reductase (SDR), aldo-keto reductase (AKR), medium-chain dehydrogenase/reductase (MDR, alcohol dehydrogenase), and quinone reductase. In other words, CBR1 is not a generic name for various enzymes that catalyze the reduction of carbonyl groups, but a name of one enzymatic protein. SDR, AKR, MDR, and ALDH each consists a superfamily of many molecular species, and CBR1 is a member of the SDR superfamily.

CBR1 was the first that was purified from the cytoplasm of the human brain cells, and its properties, such as its presence as a monomer, have been clarified. After that, an enzyme, which is present as tetramer and reduces carbonyl compounds, was purified from mitochondria of the lung and adipose tissue. The gene encoding cytoplasmic CBR1 is located on chromosome 21 (21q22.12). The superoxide dismutase (*SOD*) 1 gene and the gene related to Down syndrome are located in the proximity of the chromosome (21q22.12). CBR1 is the same enzyme as PG 9-ketoreductase. 15-hydroxyprostaglandin dehydrogenase inactivates PG, and in knockout mice of this gene, PGE $_2$ inactivation does not occur, thereby the ductus arteriosus does not close after birth and remains open.

In humans, CBR1 is expressed in many organs, of which the highest level of expression is seen in the brain, liver, and kidneys. The immunohistochemistry results have shown that CBR1 is expressed in nerve cells and glial cells in the brain, hepatocytes, and proximal tubules of the kidney at high levels, as well as in vascular endothelial cells, myocardium, anterior lobe of the pituitary gland, and gastric epithelial cells.

CBR1 reduces and detoxifies various low-molecular-weight compounds with a carbonyl group ketone group and aldehyde group. Since many drugs have a carbonyl

group, CBR1 may be considered as a drug-metabolizing enzyme. The substrate of CBR1 is quinones derived from benzo(a)anthracene and benzo(a)pyrene that are carcinogenic; however, since other enzymes are also involved in these detoxications, the significance of CBR1 in the detoxication of carcinogens is not clear. The lung carcinogen NNK contained in cigarette smoke is reduced and detoxified by microsomal and cytoplasmic CBR1 into 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol. These two enzymes show different selectivity to the optical isomers of NNK. These enzymes have CBR1 and prostaglandin 9-ketoreductase activities and reduce PGE $_2$ to PGF $_{2\alpha}$. CBR1 was reported to possibly catalyze a dehydrogenation reaction that produces 15-keto-PGE $_2$ from PGE $_2$. However, this reaction was revealed to be mediated by 15-hydroxyprostaglandin dehydrogenase. 15-ketoprostaglandins, including 15-keto-PGF $_{2\alpha}$ and 15-keto-PGD $_2$, have no activity as a PG.

3.2 Gene therapy with CBR1 DNA

Systemic administration of clofibric acid increases CBR1 in the tumor and exhibits an antitumor effect. However, it may also cause adverse effects such as rhabdomyolysis and weight loss. Therefore, we decided to figure out the way to have CBR1 taken up only into the tumor. The method of using a viral vector is not practical due to issues related to infection and the requirement of special equipment. We then focused on nanoparticles called dendrimers as a delivery tool for CBR1 DNA.

Prior to that, we performed an *in vivo* verification experiment to determine whether an increase in CBR1 in the tumor is directly linked to the suppression of tumor cell proliferation. CBR1 DNA was delivered to two types of ovarian cancer cells (serous adenocarcinoma cells, clear cell adenocarcinoma cells). The subcutaneously transplanted CBR1 DNA-introduced cells formed a small tumor, which showed no growth, while in the control group, tumors grew over time [12]. Tumors in the CBR1 DNA-introduced group exhibited extensive necrotic tissue and an increase in apoptotic cells due to the strong expression of caspase-8 and caspase-3 [12]. Conversely, injections of CBR1 siRNA into subcutaneous tumors of ovarian cancer significantly increased the growth rate compared to that of the control tumors [13]. Characteristically, the subcutaneous tumors grew penetrating into the intraperitoneal cavity, and the number of lung metastases significantly increased [13]. It was clearly shown that the infiltration and metastatic capacity of the tumor cells increased with the decrease in the CBR1 expression in the tumor cells [13].

We created ovarian cancer-related peritoneal carcinomatosis in nude mice and injected the CBR1 DNA dendrimer complex in the peritoneal cavity every 48 hours [14]. All mice in the dendrimer-alone control group died within 25 days, whereas all of the mice in the CBR1 DNA dendrimer complex injected group survived during the same 25-day period after the injection [14]. All these mice died about 25 days after

the CBR1 DNA dendrimer complex injection was discontinued [14]. The intraperitoneal observation revealed that the peritoneal foci were increasing in size in the dendrimer alone group, whereas the foci were smaller and fewer in the CBR1 DNA dendrimer complex group [14]. Neither weight loss or changes in feces were seen during CBR1 DNA dendrimer treatment, suggesting there was no toxicity. The results of the experiments suggest the potential of the future gene therapy with CBR1 DNA for treatment of ovarian cancer-related peritoneal carcinomatosis.

4. Oral hypoglycemic agents ciglitazone/pioglitazone (PPAR γ ligand) and selective COX-2 inhibitor meloxicam

PPAR γ expression was induced in the tumors in the mice to which ciglitazone was administered, but the COX-2 expression level did not change [6]. It was considered that ciglitazone decreases the mPGES level independently of COX-2 and lowers the PGE₂ level, which leads to suppressing angiogenesis and inducing apoptosis and thereby exerts antitumor effects [6].

Although administration of pioglitazone alone exerts an antitumor effect, the combination use with the PPAR α ligand clofibric acid markedly decreases the COX-2 expression regulator AP-1 expression in the tumor, resulting in lowering the COX-2 expression [7].

COX-2 expression was absent in the meloxicam-treated tumors in mice. Therefore, it was considered that meloxicam blocks the synthetic pathway of PGG₂ \rightarrow PGH₂ \rightarrow PGE₂, resulting in lowering the PGE₂ level, and thereby antitumor effect was achieved [6].

5. Conclusions

Although there are differences in the mechanisms, clofibrin acid, ciglitazone, pioglitazone, and meloxicam are all thought to exert the antitumor effects by inducing apoptosis, mainly by the suppression of angiogenesis through lowering the PGE₂ level. All these drugs are widely used in daily clinical practice; Clofibrin acid is the main component of fibrate drugs used for dyslipidemia treatment, ciglitazone and pioglitazone are oral hypoglycemic agents, and meloxicam is an oxicam non-steroidal anti-inflammatory drug. This is the first report that proves these four drugs have antitumor effects against ovarian cancer. Especially, it shows the possibility of an application of the gene therapy with CBR1 DNA for its action of increasing the CBR1 expression in the tumor, which is the main mechanism of the PPAR α ligand clofibrin acid.

The above are the research results using animal models of cancer-bearing and carcinomatous peritonitis related to ovarian cancer, which correspond to the late stage of cancer. Although new anticancer agents and molecular targeted drugs have been developed and new combination of anticancer agents have been tried out for advanced ovarian cancer, satisfactory treatment outcomes are yet to be ob-

tained. In this report, we demonstrate that drugs that are commonly used for dyslipidemia and diabetes and as anti-inflammatory drugs have tumor-suppressing effects via anti-angiogenic and apoptosis-inducing actions. Considering that the safety has been established including side effects, these drugs have the potential to be used not only to treat ovarian cancer but also human solid cancers in general as a combination adjuvant drug with other anticancer agents or to be applied to tumor dormancy therapy with different properties from anticancer agents.

Author contributions

TS wrote the manuscript. TS and YY analyzed the data. YY contributed in concept and design and supervised this work. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Acknowledgment

We would like to express our gratitude to all those who helped us during the writing of this manuscript and thanks to all the peer reviewers for their opinions and suggestions.

Funding

This work was supported by a Grant-in Aid for Cancer Research from the Ministry of Education, Science and Culture of Japan (Grant No.: 20K09589).

Conflict of interest

The authors declare no conflict of interest.

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