Oral administration of E-type prostanoid (EP) 1 receptor antagonist suppresses carcinogenesis and development of prostate cancer via upregulation of apoptosis in an animal model

Yasuyoshi Miyata (✉ yasu-myt@nagasaki-u.ac.jp)

Nagasaki University Graduate School of Biomedical Sciences

Masahito Masato

Nagasaki University Graduate School of Biomedical Sciences

Kensuke Mitsunari

Nagasaki University Graduate School of Biomedical Sciences

Akihiro Asai

Nagasaki University Graduate School of Biomedical Sciences

Yuichiro Nakamura

Nagasaki University Graduate School of Biomedical Sciences

Kyohei Araki

Nagasaki University Graduate School of Biomedical Sciences

Yuta Mukae

Nagasaki University Graduate School of Biomedical Sciences

Tsuyoshi Matsuda

Nagasaki University Graduate School of Biomedical Sciences

Junki Harada

Nagasaki University Graduate School of Biomedical Sciences

Tomohiro Matsuo

Nagasaki University Graduate School of Biomedical Sciences

Kojiro Ohba

Nagasaki University Graduate School of Biomedical Sciences

Hideki Sakai

Nagasaki University Graduate School of Biomedical Sciences

Hiroki Kurata

Nagasaki University Graduate School of Biomedical Sciences

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Abstract

Prostaglandin E2 plays important roles in carcinogenesis and malignant potential of prostate cancer (PC) by binding to its specific receptors, E-type prostanoid (EP) receptors. However, anti-carcinogenic effects of EP receptor antagonist are not clear. In this study, feed with or without EP1 receptor antagonist were given to a mouse model of prostate cancer. The mice were sacrificed at 10, 15, 30, and 52 weeks of age. Apoptosis was evaluated by immunohistochemical analysis using cleaved caspase-3. The incidence of cancer in the experimental group was significantly lower than that in the control group at 15, 30, and 52 weeks of age. The percentage of poorly differentiated PC cells was significantly lower in the experimental group than in the control group at 30 and 52 weeks of age. The survival period in the experimental group was significantly longer than that in the control group, and the percentage of apoptotic cells in the experimental group was significantly higher than that in the control group at 15, 30, and 52 weeks of age. Thus, an EP1 receptor antagonist delayed PC progression via the upregulation of apoptosis. We suggest that EP1 receptor may be a novel chemopreventive agent for the development of PC.

Introduction

Prostate cancer (PC) is the most common malignancy in men. Various treatments, such as surgery, hormonal therapy, chemotherapy, and radiotherapy, are performed for PC patients according to their clinicopathological features and background¹⁻³. In addition, the clinical usefulness of active surveillance has been reported in selected patients with favorable- and intermediate-risk PC^{3,4}. Conservative treatments, including active surveillance, can avoid adverse events, maintain quality of life, and save the patient from further medical treatments. Thus, information on suppression methods of malignant potential and tumor growth is important to discuss the treatment strategies aimed at both improving prognosis and maintaining quality of life in patients with PC.

Prostaglandin E2 (PGE2) is a strong mediator of various pathological conditions including cancers^{5,6}. Cyclooxygenase (COX)-2 plays crucial roles in the metabolism of arachidonic acid to PGE2. Therefore, COX-2 is well known to be positively associated with carcinogenesis, malignant aggressiveness, and poor outcomes in many types of malignancies⁷⁻⁹. However, pathological activities of PGE2 are not always dependent on COX-2 because various other factors besides COX-2 regulate PGE2 production^{10,11}. Briefly, although COX-2 inhibitors, including non-steroid anti-inflammatory drugs, are reported to act as tumor suppressors via regulation of PGE2 in many types of cancers^{12,13}, COX-2 inhibitors may inhibit some of the pathological activities of PGE2. On the other hand, we should know the facts that the binding of PGE2 to its specific receptor, E-prostanoid receptor (EP), is essential to exert pathophysiological functions of PGE2 [14]. The EP receptor family consists of four isoforms (EP1-4 receptor), and the interactions between PGE2 and EP receptors in malignancies vary depending on cell type and tumor-surrounding conditions^{5,7,15,16}.

Regarding the PGE2 / EP receptor axes in PC, many investigators have suggested that they play important pathological roles in malignant potential and tumor growth^{16–20}. However, the detailed pathological significance of each EP receptor in PC tissues is not fully understood. In addition, there is little information on the efficacy and safety of chemopreventive and treatment strategies using anti-EP receptor agents in PC by *in vivo* studies. In a previous study, we showed that EP1, EP2, and EP4 receptors play crucial roles in carcinogenesis in patients with hormone-sensitive PC¹⁷. In addition, EP1 receptor expression was shown to be positively associated with tumor grade and TNM stage¹⁷. Based on these results, we hypothesized that blocking of the EP1 receptor leads to suppression of carcinogenesis and tumor growth in PC *in vivo*. The main aim of this study was to test this hypothesis using a PC mouse model that showed close-to-human kinetics of tumor development. In addition, the influence of the EP1 receptor agonist on apoptosis in PC cells in the same mouse tissues was assessed.

Materials And Methods

Animals. The knock-in mouse adenocarcinoma prostate (KIMAP) model was used in this study. This model has previously been used to evaluate the pathological roles of cancer-related factors and anti-cancer effects of various foods in PC because pathological characteristics and tumor progression kinetics of PC in KIMAP are known to be similar to those in human PC^{21–23}. The detailed information on rearing environment, anesthesia, and welfare is described in our previous reports^{22,24}. All animal experiments were performed according to the Guidelines for Animal Experiments of Nagasaki University, and the protocol was approved by the Regulations of Animal Care and Use Committee of Nagasaki University. We confirmed that this study is reported in accordance with ARRIVE guidelines.

Food preparation. The EP1 receptor agonist was orally administered through a feed containing ONO-8713, which is a selective antagonist for the EP1 receptor (provided by ONO Pharmaceuticals, Osaka, Japan). In the experimental group, ONO-8713 was mixed with standard feed (AIN-76A, CLEA Japan, Inc., Tokyo, Japan) (final concentration of 1,00 ppm), according to a previous report²⁵. Feed with EP1 receptor antagonist was administered from 8 weeks of age, and we confirmed that the reduced amounts of feed were similar between the control and experimental groups every week.

Tissue collection and analyses. Mice were sacrificed and tissues were collected at 10, 15, 30, and 52 weeks of age. Hematoxylin and eosin (H&E) staining was performed on the collected tissues for histological examination. A schematic of the study protocol is shown in Figure 1. The apoptotic index (AI) was calculated by anti-cleaved caspase-3 (Asp 175) (R&D Systems, Minneapolis, MN) according to our previous report^{22,26}.

Statistical analyses.

All data were expressed as median and interquartile range (IQR). The Mann-Whitney U test was used to compare continuous variables. Kaplan-Meier survival curves and the log-rank test were performed for

survival analysis. A significance was defined as $p < 0.05$. All statistical analyses were performed by statistical package StatView for Windows (Version 5.0, Abacus Concept, Berkeley, CA).

Results

Changes of histological characteristics. PC cells were not detected in either group at 10 weeks of age. At 15 weeks of age, cancer cells were relatively rare in the experimental group (Fig. 2A); however, carcinogenic changes in the prostate glands were found in the control group (Fig. 2B). In fact, the median/IQR of the percentage of cancer cells in the experimental group (11.0/9.7–12.2%) was significantly lower ($p < 0.001$) than in the control group (50.7/49.4–51.6%). At 30 weeks of age, cancer tissues and normal prostate glands were mixed in the experimental group (Fig. 2C); however, PC tissues with glandular structures were found in the control group (Fig. 2D). Furthermore, at 52 weeks of age, the area of cancer tissues was increased in the experimental group, although glandular PC tissues and normal glands still existed (Fig. 2E). In contrast, in the control group, undifferentiated cancer cells clearly appeared at 52 weeks of age (Fig. 2F).

Frequency of cancer cells. The changes in the percentage of cancer cells in the experimental and control groups are shown in Figure. 3A. The frequencies of cancer cells in experimental group was significantly lower compared to control group at 15, 30 and 52 weeks of age. On the other hand, as shown in Fig. 3B, there were no significant differences in poorly differentiated PC cells between the groups at 10 and 15 weeks of age. However, the percentage of poorly differentiated PC cells was significantly lower ($p < 0.001$) in the experimental group (2.7/1.8–3.4% and 49.9/47.5–52.7%) than in the control group (19.6/19.2–22.1% and 98.4/97.3–100.0%) at 30 and 52 weeks of age, respectively (Fig. 3B). Thus, at 52 weeks of age, although almost all cancer cells were judged as poorly differentiated in the control group, the frequency of poorly differentiated PC cells in the experimental group was nearly half that of cancer cells.

Survival analyses and safety. In the control group, 2 of 15 mice (11.1%) died before 30 weeks of age, and 4 of 15 mice (26.7%) died from 31 to 52 weeks of age. In contrast, only one mouse (6.7%) died at 43 weeks of age in the experimental group. There was no injury, bite, or infection in any of the mice, including dead mice. Kaplan-Meier survival curves showed that the survival period in the experimental group was significantly longer than that in the control group (Fig. 4, $p = 0.043$). There was no significant difference in body weight or food intake between the control and experimental groups.

Change of frequency of apoptotic cells. As shown in Fig. 5, at 15 weeks of age, AI in the experimental group (2.8/2.5–3.3 %) was significantly higher ($p = 0.040$) than that in the control group (2.2/1.8–2.8). In addition, a similar significant difference was found at 30 and 52 weeks of age ($p = 0.040$ and 0.038, respectively; Fig. 5).

Discussion

The present study demonstrated that the EP 1 receptor antagonist delayed carcinogenesis and tumor growth in a PC animal model. Many investigators have suggested that COX-2 inhibitors are useful for the chemoprevention and treatment of malignancies in preclinical studies and clinical trials²⁷⁻²⁹. However, it should be noted that the addition of COX-2 inhibitor did not significantly affect the outcomes of randomized clinical trials of non-small cell lung cancer and colon cancer patients^{30,31}. In PC, several *in vivo* and *in vitro* studies showed that anti-cancer effects including improvement of prognosis of COX-2 inhibitors were limited³²⁻³⁵. Thus, the chemopreventive and anti-cancer effects of COX-2 inhibitors in PC are still controversial. On the other hand, there is the opinion that comprehensive regulation of PGE2 production by systematic administration of COX-2 inhibitors is speculated to lead to weakness of anti-cancer effects and increased risk of adverse events due to global prostanoid suppression³⁶. In fact, COX-2 inhibitors are known to upregulate the risk of various visceral disorders, such as gastrointestinal and cardiovascular toxicities³⁷⁻³⁹. In addition, other investigators have suggested that inhibition of the EP receptor pathway is a more effective approach for improving the anti-cancer effects compared to treatment strategies using COX-2 inhibitors⁴⁰. Based on these facts, we believe that more specific inhibition of PGE2 activity is necessary to improve the efficacy and safety of chemoprevention and treatment of PC patients.

Regarding expression pattern and pathological roles of EP receptors in PC, *in vitro* studies showed that EP2 and EP4 receptors were expressed in PC-3 cells and in PC-3, DU145, LNCaP, and PrEC cells, respectively⁴¹. Other *in vitro* studies have also shown that EP2 and EP4 receptors are mainly expressed in PC cell lines, and overexpression of EP2 and EP4 receptors and reduced EP3 expression were observed in PC tissues^{18,19}. Thus, these reports showed that the pathological significance of the EP1 receptor was minimal in PC. However, interestingly, inhibition of EP1 receptor signaling led to the suppression of proliferation in PC cell lines⁴². In addition, in an animal model, EP1 receptor-positive PC cells play a crucial role in cancer cell proliferation²⁰. Moreover, in human PC tissues, EP1 receptor expression is significantly associated with Gleason score, TNM stage, and cancer cell proliferation¹⁷. Although there was no general agreement on the pathological roles of the EP1 receptor in PC, we selected the EP1 receptor agonist according to the results obtained in PC cell lines, animal experiments, and human tissues.

The usefulness of treatment strategies by agonists of each EP receptor has been reported in various types of malignancies; for example, the EP1 and EP2 receptors for breast cancer^{43,44}, EP3 receptor for oral cancer⁴⁵, and EP4 receptor for lung cancer and breast cancer^{40,46,47}. On the other hand, regarding PC, the EP1 receptor antagonist (SC51322) showed anti-proliferative effects on cancer cells, whereas the EP2, EP3, and EP4 receptor antagonists did not⁴². Unfortunately, there is little information on the pro-apoptotic activity of EP1 receptor inhibitor in PC cells. However, oral intake of an EP1 antagonist was reported to have chemopreventive effects via stimulation of apoptosis without any side effects in a breast cancer animal model⁴³. These previous findings support our results on chemopreventive effects, stimulative function of apoptosis, and safety of EP1 antagonist.

A limitation of this study is that the chemopreventive effects of other EP receptor antagonists have not been investigated. In addition to the EP1 receptor, *in vitro* and animal experiments have shown that the EP4 receptor is a potential therapeutic target for PC⁴⁸. Furthermore, we previously reported that EP2 receptor- and EP3 receptor-expressing cancer stromal cells were positively associated with cancer cell progression and worse outcomes in patients with PC¹⁶. Thus, it is possible that EP2–EP4 agonists may have chemopreventive and anti-cancer effects in *in vivo* studies. In recent years, a combination therapy of anti-PD-L1 antibody and EP4 antagonist enhanced anti-tumor growth effects and prolonged survival in mice inoculated with murine lymphoma cells⁴⁹. Finally, we suggest further *in vivo* studies, including animal experiments, to discuss the usefulness, limitations, and safety of novel therapeutic strategies by inhibition of EP receptor pathways and of combined therapies with such treatments and other conventional therapies in PC.

Conclusion

Our *in vivo* study using KIMAP demonstrated that an EP1 receptor antagonist delayed carcinogenesis and prolonged survival periods. Induction of apoptosis was speculated to be associated with such chemopreventive effects. There was no toxicity, including pathological findings in the kidney and liver. Finally, we concluded that inhibition of the EP1 receptor pathway by an EP1 antagonist is a novel chemopreventive strategy for PC.

Declarations

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Author contributions

Y.Miyata, M.M., K.M., A.A., Y.N., K.A., Y. Mukae, T.Matsuda, J.H., T. Matsuo, K.O. and H.S. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Y. Miyata. Acquisition of data: M.M., K.M., A.A., Y.N., K.A., Y. Mukae, T.Matsuda, J.H., T. Matsuo, K.O. Analysis and interpretation of data: Y. Miyata, A.A., Y.N. Drafting of the manuscript: Y.Miyata, M.M. Statistical analysis: Y.Miyata. Study supervision: H.S.

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Competing interests

The authors declare that they have no conflict of interest

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Figures

Figure 1

Summary of the animal experiments. Mice were fed with or without EP1 receptor agonist at 8 weeks of age, and mice were sacrificed at 10, 15, 30, and 52 weeks of age.

Figure 2

Hematoxylin-eosin-stained tissues at 15, 30, and 52 weeks of age in experimental (A, C, and E, respectively) and control mice (B, D, F, respectively). Magnification x 200.

Figure 3

Percentage of cancer (A) and poorly differentiated cancer (B) at 10, 15, 30, and 52 weeks of age in control and experimental mice.

Figure 4

Kaplan-Meier survival curves in control and experimental mice.

Figure 5

The percentage of apoptotic cells in control and experimental mice at 15, 30, and 52 weeks of age.