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Pathological Roles of Prostaglandin E2-specific E-type Prostanoid Receptors in Hormone-sensitive and Castration-resistant Prostate Cancer

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Abstract. *Background/Aim: Prostaglandin (PG) E_2 mediates malignant aggressiveness by binding to four specific E-type prostanoid receptors (EP1R – 4R). This study aimed to clarify the pathological significance of EPRs in hormone-sensitive prostate cancer (HSPC) and castration-resistant prostate cancer (CRPC). Materials and Methods: EP1R – 4R expression was examined in 102 HSPC and 27 CRPC specimens. The relationships between their expression and proliferation index (PI), apoptotic index (AI), and vascular endothelial growth factor (VEGF)-A expression were analyzed. Results: EP4R expression in CRPC was significantly higher compared to that in HSPC, even in advanced disease (T3/4, N1, and/or M1). EP4R expression was significantly correlated with PI, AI, and VEGF-A expression in CRPC. Such significant relationships were not detected between EP1R – 3R and CRPC. Conclusion: EP4R expression in CRPC was significantly higher than that in HSPC and was associated with cancer cell proliferation, apoptosis, and pro-angiogenetic potential.*

Prostaglandin (PG) E_2 plays an important role in mediating the carcinogenic process and malignant aggressiveness of many types of cancers (1, 2). Cyclooxygenase (COX)-2 has been established as the most important regulator of PGE_2 production in various pathologies, including cancers. Consequently, several studies have examined the pathological significance of the COX-2/PGE_2 pathway in cancer cells (3, 4). The downstream effects of the COX-2/PGE_2 pathway in various cancers are known to be mediated through the E-type prostanoid receptors (EPRs) (1, 4, 5). EPRs consist of four different members (EP1R – EP4R), and several studies have reported on their significant relationships with carcinogenesis, malignant aggressiveness, and the prognosis of various cancers (6-9). Furthermore, these previous reports have also shown that the pathological roles of EPRs in cancer tissues dependent on the type of cancer and associated pathological characteristics (6-10).

Prostate cancer (PC) is a major type of malignancy in males, and its prognosis is relatively improved with therapeutic interventions including hormonal therapy (10-12). Specifically, people with an advanced/metastatic form of PC, the elderly, and those with comorbidities are treated with hormonal therapy. However, most PC cells acquire resistance to this treatment over varying periods, triggering the gradual transition of hormone-sensitive prostate cancer (HSPC) cells into castration-resistant prostate cancer (CRPC) cells, which are androgen-independent (13). Although, the prognosis of patients with CRPC has improved with the development of new treatment strategies (11, 14, 15), radical cure for CRPC has not yet obtained. Therefore, a more detailed understanding of the molecular mechanisms underlying malignant aggressiveness of CRPC is essential to potentially improve patient outcomes.

Many investigators paid special attention to the pathological roles and clinical significance of EPRs in PC. For example, an *in vivo* study showed that EP2R plays a small role in the secretion of PGE_2-induced pro-angiogenic factor (vascular endothelial growth factor; VEGF) in LNCaP (androgen-irresponsive cell line), DU-145 and PC3 cells (androgen-responsive cell lines) (16). Moreover, another study has shown that EP4R is associated with the proliferation and invasion of PC-3 cells (17). On the other hand, in HSPC tissues, expression of EP1R, 2R, and 4R in cancer cells was reported to be significantly associated with

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Key Words: E-type prostanoid receptors EPRs), castration-resistant prostate cancer (CRPC), proliferation, apoptosis, vascular endothelial growth factor-A.

Table I. *Clinicopathological features of patients with hormone sensitive prostate cancer.*

Variables	n/%	Variables	n/%
Grade Group		N stage	
1	20/19.6	N0	78/76.5
2	18/17.6	N1	24/23.5
3	18/17.6	M stage	
4	22/21.6	M0	69/67.6
5	24/23.5	M1	33/32.4
Low (1-3)	56/54.9	Metastasis (N1 and/or M1)	
High (4+5)	46/45.1	Absence	66/64.7
T stage		Presence	36/35.3
T1	10/9.8	Advanced (T3/4, N1, and/or M1)	
T2	34/33.3	Absence	41/40.2
T3	39/38.2	Presence	61/59.8
T4	19/18.6		
Low (1+2)			
High (3+4)			

carcinogenesis and malignant potential (18). However, the pathological significance of EPR expression in PC, especially in CRPC, is not fully understood, despite the close association between PGE_2 and malignant aggressiveness of PC (19, 20).

The main aim of this study was to compare the expression of EP1R – EP4R between HSPC and CRPC tissue specimens. We also determined the relationship between EPR expression and cancer cell proliferation, apoptosis, and proangiogenic protein expression in CRPC. Collectively, our results demonstrate the pathological significance of EPRs and their potential as therapeutic targets for CRPC.

Materials and Methods

Specimens. In this study, we subjected 27 CRPC and 102 HSPC formalin-fixed, paraffin-embedded tissue specimens to various immunohistochemical analyses. The clinicopathological features of the corresponding patients are shown in Table I. Non-adenocarcinomas, such as neuroendocrine carcinoma or small cell carcinoma, were excluded from the study. The study design complied with the principles of the Declaration of Helsinki and its revisions and was approved by the Institutional Review Board of Nagasaki University Hospital (No. 16K15690). Written informed consent was obtained from all patients.

Immunohistochemistry. Immunohistochemical analyses of EPRs, Ki-67, cleaved caspase-3, and VEGF-A were performed according to previous reports (18, 21-23). Briefly, antigen retrieval of the tissue specimens was performed in 0.01 M sodium citrate buffer (pH 6.0) and then the specimens were immersed in 3% hydrogen peroxide. Primary antibodies against EPRs were obtained from the Cayman Chemical Corporation (Ann Arbor, MI, USA), while those for Ki-67, cleaved caspase 3, and VEGF-A were obtained from Dako Corp. (Glostrup, Denmark), R&D Systems, Inc. (Abingdon, UK), and Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA), respectively. Sections were incubated with primary antibodies at 4˚C overnight, treated with Dako EnVision+™ Peroxidase (Dako, Carpinteria, CA, USA), and then visualized using diaminobenzidine.

Estimation of immunoreactivity, proliferation index (PI), and apoptotic index (AI). Immunohistochemically stained cells were imaged and analyzed using a digital camera (Nikon DU100, Nikon Corp., Tokyo, Japan) and a computer-aided image analysis system (Win ROOF, version 5.0, MITANI Corp., Fukui, Japan). Immunoreactivity of EP1R – EP4R was expressed as the percentage of cancer cells exhibiting moderate to strong expression (18). The PI and AI were calculated as the percentage of cancer cells with nuclei positively stained for anti-Ki-67 antibody and cleaved caspase-3 antibody, respectively (21, 22). VEGF-A expression was semi-quantitatively analyzed using the immunoreactivity score, which is calculated by multiplying the staining intensity (grade 0=none, 1=weak, 2=moderate, and 3=strong) with the score for the percentage of positively stained cells (0, <1%; 1, 1%-25%; 2, 26%-50%; 3, 5175%; or 4, 76%-100%) (23).

Statistical analysis. All data are expressed as mean±standard deviation. Student's *t*-test was used to compare continuous variables. For correlation analyses, specimens were divided into two groups based on the grade: low [grade group (GG)1-3] and high (GG4 and 5). Likewise, the specimens were also divided into two groups on the basis of their T stage: low T stage (1 and 2) and high T stage (3 and 4). The N1 and M1 stages were used to classify the specimens as either metastatic or non-metastatic, while T3/4, N1, and/or M1 tumors were classified as advanced-stage cancer. Pearson's correlation was used to evaluate the relationship between continuous variables. Statistical significance was defined as $p<0.05$. All statistical analyses were performed using StatView for Windows (version 5.0, Abacus Concept, Inc., Berkeley, CA, USA).

Results

Expression of EPRs. Representative results of EP1R – 4R expression in CRPC tissues are shown in Figure 1A-D. We

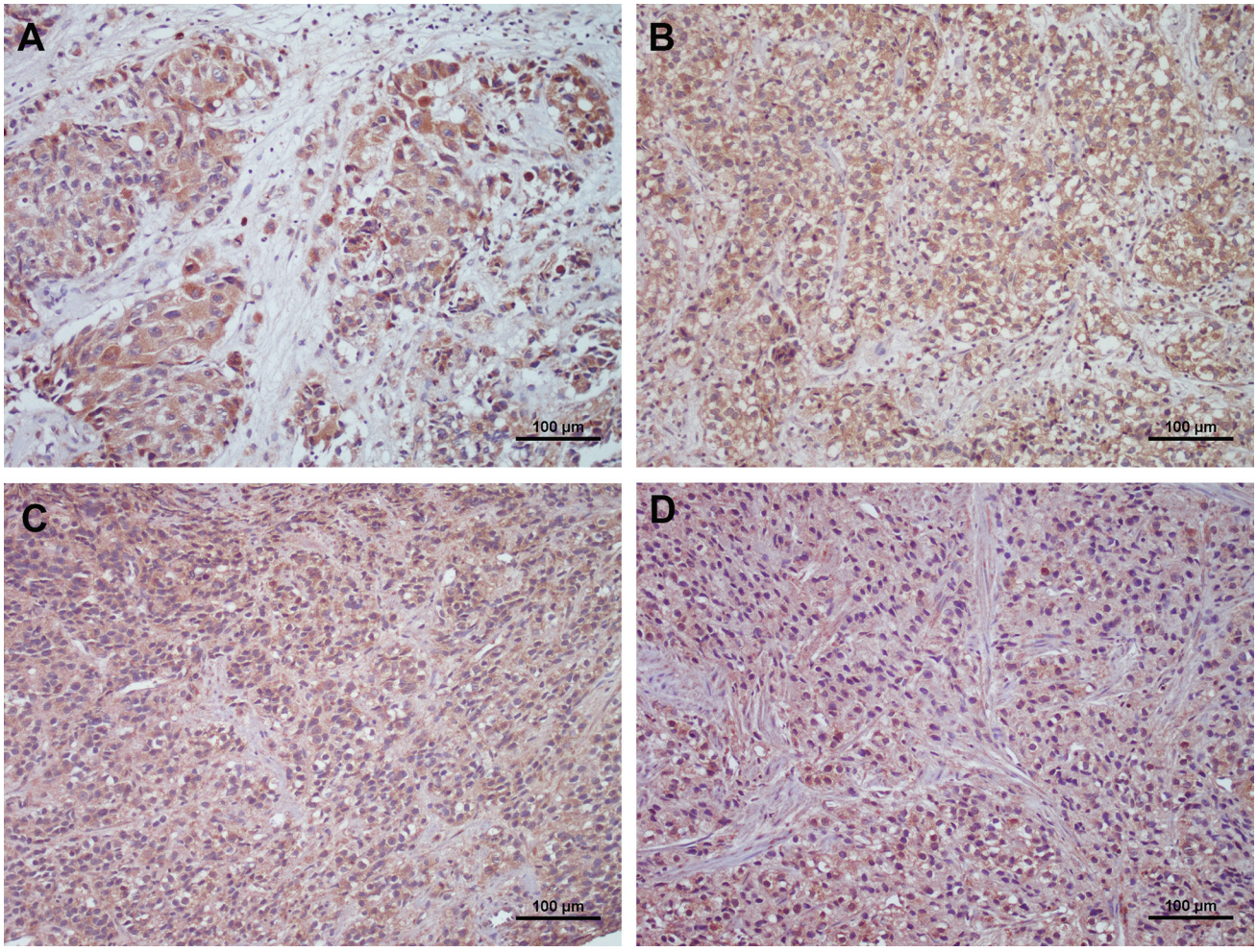

Figure 1. *Representative images of E-type prostanoid (EP)1-EP4 receptor expression in castration-resistant prostate cancer tissues (A-D). Each EP receptor was mainly detected in the cytoplasm of cancer cells.*

have previously reported their expression in HSPC tissue (18), and the pattern and distribution of the EPRs in CRPC were similar to those in HSPC. Briefly, immunohistochemical staining of each EPEs was detected mainly in the cancer cell cytoplasm. Finally, the percentage of cells positive for EP1R expression in CRPC was 40.1±7.0%, which is comparable to that in HSPC (37.6±14.4%; Figure 2A). Similarly, there was no significant difference in the expressions of EP2R and EP3R between the two types of cancers (Figure 2A). However, EP4R expression in CRPC tissues (18.9±5.6%) was significantly higher (p<0.001) than that in HSPC tissue specimens (10.3±5.0%; Figure 2A).

EP receptor expression in CRPC and low and high grade HSPC. A detailed analysis of the relationship between pathological features and EPR expression was performed. We found that the expression of EP2R and EP3R was not significantly different from those of low GG HSPC, high GG HSPC, and CRPC (Figure 2B). However, the proportion of cells positive for EP1R was significantly higher in high GG HSPC and CRPC than in low GG HSPC (p=0.019; Figure 2B). There was no significant difference in EP1R expression between high GG HSPC and CRPC groups. Although EP4R expression was similar between low GG HSPC and high GG HSPC, that in CRPC was remarkably higher than that in low and high GG HSPC (p<0.001; Figure 2B).

EP receptor expression in CRPC and low and high T stage HSPC. Expression of EP1R–4R in low T stage HSPC, high T stage HSPC, and CRPC is summarized in Figure 3A. Similar to the consequence of GG on EP receptor expression, the expression of EP1 receptors in high T stage HSPC was significantly higher than that in low T stage HSPC; however, there was no significant difference between CRPC and low

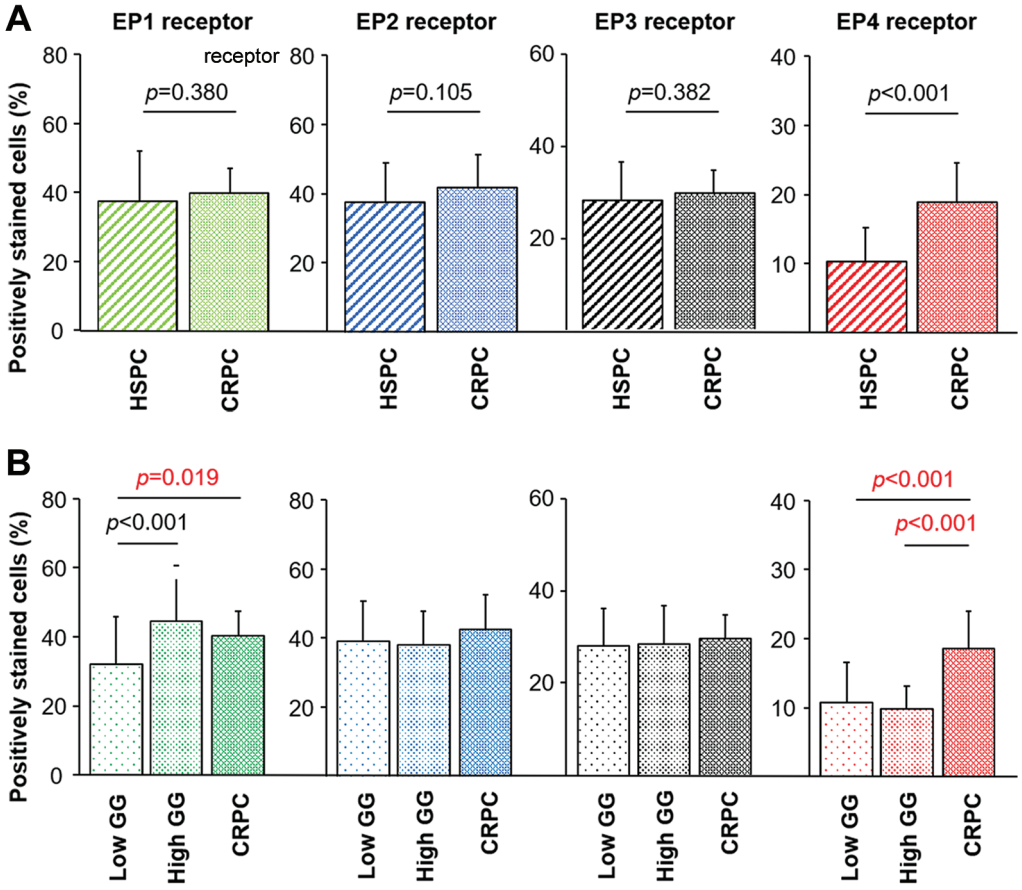

Figure 2. *Expression of E-type prostanoid receptors (EP1R-EP4R) in hormone-sensitive prostate cancer (HSPC) and castration-resistant prostate cancer (CRPC) (A). Expression of EP1R-EP4R in low grade group (GG) in HSPC, high grade group in HSPC, and castration-resistant prostate cancer (B). p-Values in red signify significant differences compared to CRPC values.*

T stage HSPC (p=0.052) or between CRPC and high T stage HSPC (p=0.902; Figure 3A). As shown in Figure 3B, similar relationships were also determined between EP1R expression and non-metastatic HSPC, metastatic HSPC, and CRPC. In contrast, EP4R expression in CRPC was remarkably higher (p<0.001) than that in low T stage HSPC and high T stage HSPC (Figure 3A), and similar differences were also detected between non-metastatic HSPC, metastatic HSPC, and CRPC (Figure 3B).

When similar analyses were performed among organ-confined HSPC (T1-2N0M0), advanced HSPC (T3-4, N1, and/or M1), and CRPC, EP4R expression levels in CRPC was significantly higher (p<0.001) than that in organ-confined HSPC and advanced HSPC, although there was no significant difference between organ-confined HSPC and advanced HSPC (Figure 3C). In addition to EP4R, EP1R expression in CRPC was higher than that in organ-confined HSPC (p=0.042), but not in advanced HSPC. The expression levels of EP2R and EP3R showed no significant difference between the different types of HSPC and CRPC (Figure 3A-C).

Correlations between EP receptor expression and malignant potential. In the case of HSPC, EP1R expression was positively correlated with PI and VEGF-A expression; a similar correlation was also seen for EP2R expression (Table II). In CRPC, the expression of EP1R and EP2R was not correlated with any of the cancer-related parameters, except for that between PI and EP2R (r=0.51, p=0.007; Figure 4A). As shown in Table II, EP4R expression was significantly associated with PI (r=–0.43, p=0.025; Figure 4B), AI (r=–0.42, p=0.030; Figure 4C), and VEGF-A expression (r=0.52, p=0.005; Figure 4D) in CRPC, although such significant correlations were not detected in HSPC. Furthermore, we noticed that the r values of these significant correlations in CRPC were remarkably higher (–0.42 to 0.52) than those in HSPC (0.21 to 0.36; Table II). In these analyses, EP3R expression was not significantly correlated with either HSPC or CRPC.

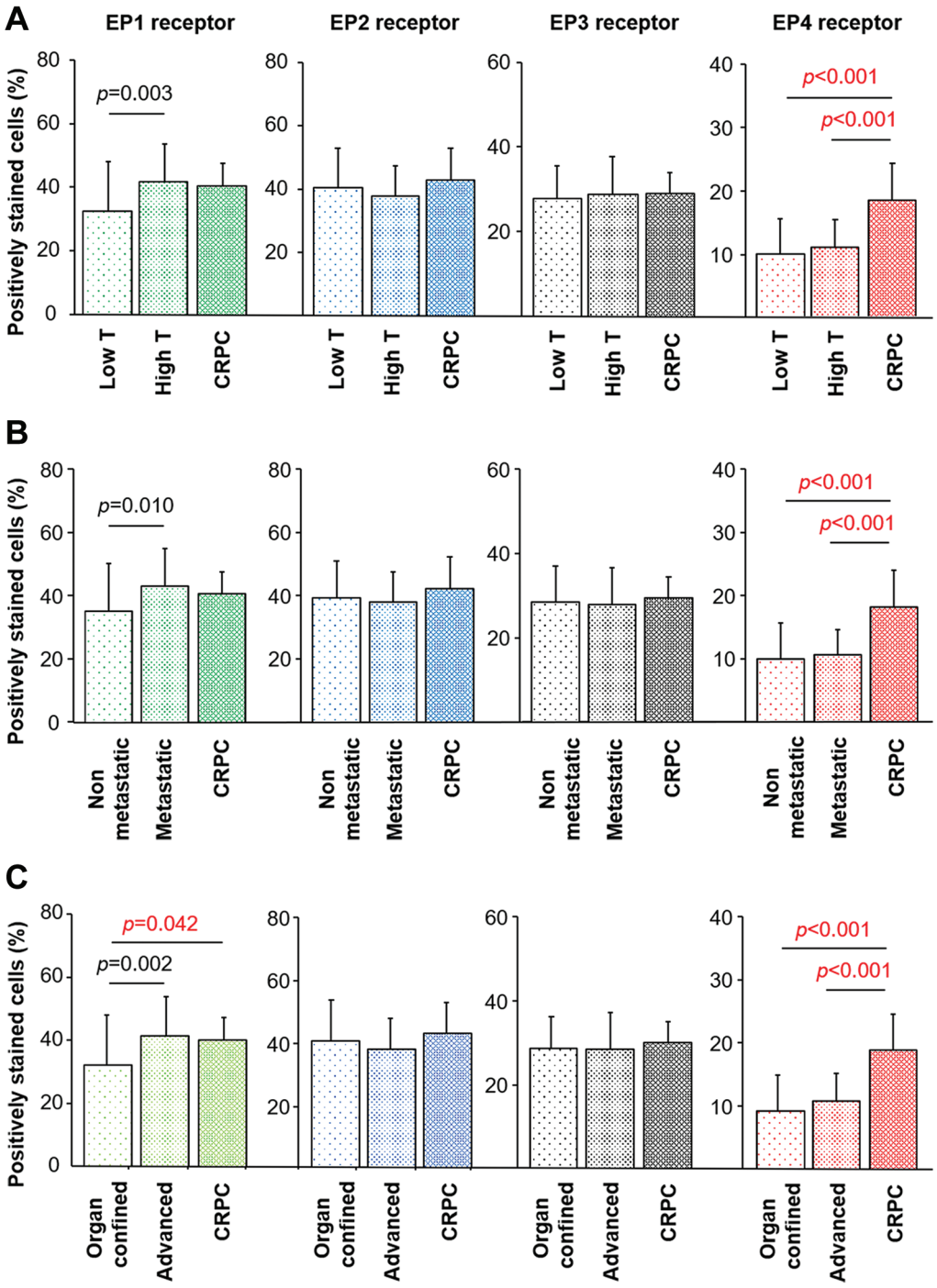

Figure 3. *Expression of E-type prostanoid receptors (EP1R-EP4R) in castration-resistant prostate cancer (CRPC) compared to that in hormone-sensitive prostate cancer (HSPC) according to different tumor (T) stages (A), status of metastasis (B), and tumor localization (C). p-Values in red signify significant differences compared to CRPC values.*

Discussion

In this study, we found that EP4R expression is higher in CRPC tissues than in HSPC tissues. We have previously reported that EP4R expression in HSPC cells is significantly higher than that in non-tumor gland cells (18). Based on these findings, we hypothesize that EP4R plays an important role in the acquisition of a malignant phenotype,

Table II. *Correlation between EP receptors and cancer-related factors.*

	EP1 receptor	EP2 receptor	EP3 receptor	EP4 receptor
For PI				
HSPC	0.36/<0.01	0.21/0.034	0.11/0.292	0.02/0.878
CRPC	**0.34/0.086**	**0.51/0.007**	**0.22/0.265**	**0.43/0.025**
For AI				
HSPC	– 0.07/0.481	0.04/0.710	–0.12/0.234	0.12/0.223
CRPC	**– 0.01/0.983**	**0.20/0.317**	**–0.19/0.351**	**–0.42/0.030**
For VEGF-A				
HSPC	0.26/0.008	0.25/0.012	0.06/0.557	0.11/0.281
CRPC	**0.32/0.100**	**0.17/0.409**	**0.08/0.692**	**0.52/0.005**

PI: Proliferation index; HSPC: hormone sensitive prostate cancer; CRPC: castration-resistant prostate cancer; AI: apoptotic index; VEGF: vascular endothelial growth factor; EP: E-type prostanoid. Data are presented as r/*p*-value. Italics indicate *p*-Value <0.05. Bold indicates data in CRPC.

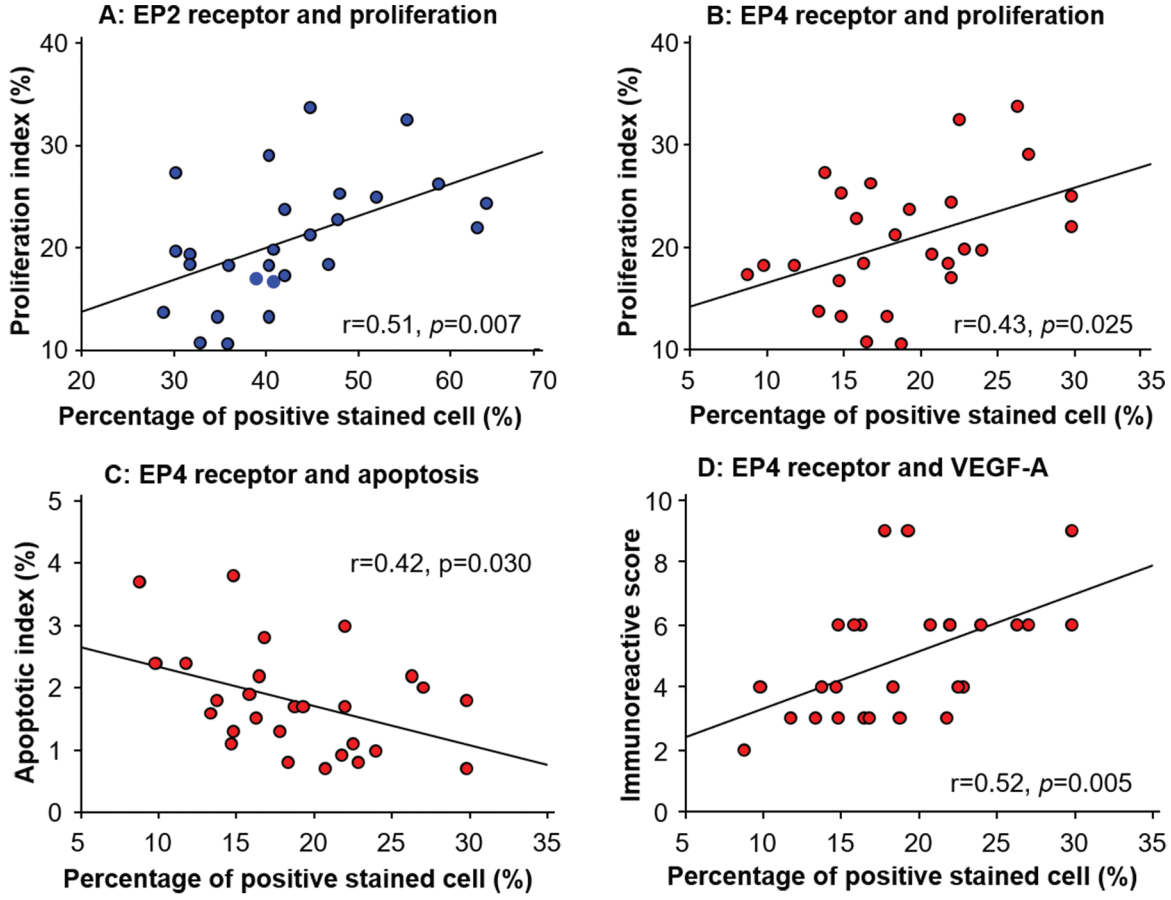

Figure 4. *Correlation between E-type prostanoid (EP) 2 receptor (EP2R) expression and proliferation index (PI) in castration-resistant prostate cancer (CRPC) (A). Correlation between EP4R expression and PI (B), apoptotic index (C), and vascular endothelial growth factor-A expression (D) in CRPC.*

carcinogenesis, and loss of androgen-dependency in HSPC and CRPC. We noticed that the pathological roles of EP4R expression were different between HSPC and CRPC, that is, EP4R expression was significantly associated with increased cancer cell proliferation and VEGF-A expression and a decrease in apoptosis in CRPC, but not in HSPC. In previous

in vitro studies involving the use of androgen-independent PC cell lines, EP4R has been shown to play an important role in mediating cell growth and development (17, 19). Moreover, EP4R has been associated with the inhibition of apoptosis in various malignancies (24, 25). In addition, EP4R has been reported to be closely associated with angiogenesis in PC, and the EP4R-related pathway may play a crucial role in VEGF-A secretion in androgen-independent PC cells (16, 26). Thus, these reports support our findings that EP4R expression is upregulated, and that this upregulation is significantly associated with the stimulation of cancer cell proliferation, angiogenic potential and suppression of apoptotic activity in CRPC.

In addition to EP4R, our results also show that EP1R expression in CRPC was significantly higher than that in organ-confined HSPC. Furthermore, EP2R expression was positively correlated with cancer cell proliferation in CRPC. Unfortunately, in this study, we could not determine the clinical significance of these findings. However, several reports have shown that PGE_2 plays an important role in various carcinogenic activities, such as epithelial-mesenchymal transition and the genesis of immunosuppressive microenvironments, which are closely related to the development of drug resistant CRPC (27, 28). Thus, it is possible that EP1R and EP2R play a significant role in mediating the malignant behavior of CRPC. Additionally, our results demonstrated the minimal pathological significance of EP3R in PC, including CRPC. However, an *in vitro* study has reported that EP3R mediates carcinogenesis, cancer cell growth, and castration resistance *via* the regulation of androgen receptor expression in PC (29). Thus, to obtain a better understanding of the pathological significance of each EPR, further *in vivo* and *in vitro* studies are necessary.

One of the major limitations of this study is the relatively small number of specimens, especially for CRPC. Therefore, the pathological significance and prognostic roles of EP4R in patients with CRPC should be confirmed in additional studies. We also did not examine the pathological mechanisms mediated by EPRs in CRPC. Several reports have shown that EP4R plays a crucial role in resistance to treatments, including hormonal therapy (30, 31). In addition, many investigators have suggested that inhibition of EP4R is a promising novel treatment strategy for various malignancies (5, 31, 32). Our results similarly emphasize the importance of studying the pathological roles of EP4R and its regulatory mechanisms at the molecular level in order to aid in the development of new therapeutic agents for CRPC. In recent years, the necessity of further preclinical and clinical trials to investigate the anti-cancer effects of EP4R inhibitors, particularly in combination with chemotherapy, endocrine therapy, or immune-based therapies has been suggested (31). Our results will be useful when planning future trial protocols for CRPC. Furthermore, the present study provides important information to elucidate the detailed pathological characteristics of CRPC and identify effective therapies for CRPC, which have been examined by other investigators (33-35).

Conclusion

The present study showed that EP4R expression in CRPC was significantly higher than that in HSPC, and EP4R expression was dependent on the grade and T, N, and M stage. In addition, EP4R expression was significantly correlated with an increase in cancer cell proliferation, VEGF-A expression and inhibition of apoptosis in CRPC, but not in HSPC. Thus, the pathological significance of EP4R in HSPC is distinct from that in CRPC. Additionally, EP1R and EP2R may play a significant role in the development of CRPC and cancer cell proliferation in CRPC. Finally, we suggest that the molecular basis of the pathological roles of EPRs, especially in EP4R, should be explored in further *in vivo* and *in vitro* studies. This will aid in identifying new treatment strategies for patients with CRPC.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

Study concept: Y Miyata. Study design: Y Miyata and HS. Immunohistochemical analyses: MM, Y Mukae, YN, T Matsuda, JH. Data collection: KM, T Mastuo, KO. Statistical analyses: Y Miyata, T Matsuo. Manuscript preparation: Y Mi, MM. Manuscript editing: SH. Manuscript review: HS. All Authors read and approved the final manuscript.

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References

1 Mizuno R, Kawada K and Sakai Y: Prostaglandin E2/EP signaling in the tumor microenvironment of colorectal cancer. Int J Mol Sci *20(24)*: 6254, 2019. PMID: 31835815. DOI: 10.3390/ijms20246254

2 Finetti F, Travelli C, Ercoli J, Colombo G, Buoso E and Trabalzini L: Prostaglandin E2 and cancer: insight into tumor progression and immunity. Biology (Basel) *9(12)*: 434, 2020. PMID: 33271839. DOI: 10.3390/biology9120434

3 Frejborg E, Salo T and Salem A: Role of cyclooxygenase-2 in head and neck tumorigenesis. Int J Mol Sci *21(23)*: 9246, 2020. PMID: 33287464. DOI: 10.3390/ijms21239246

4 Ye Y, Wang X, Jeschke U and von Schönfeldt V: COX-2-PGE_2-EPs in gynecological cancers. Arch Gynecol Obstet *301(6)*:

1365-1375, 2020. PMID: 32363546. DOI: 10.1007/s00404-020-05559-6

5 Karpisheh V, Joshi N, Zekiy AO, Beyzai B, Hojjat-Farsangi M, Namdar A, Edalati M and Jadidi-Niaragh F: EP4 receptor as a novel promising therapeutic target in colon cancer. Pathol Res Pract *216(12)*: 153247, 2020. PMID: 33190014. DOI: 10.1016/j.prp.2020.153247

6 Miyata Y, Kanda S, Nomata K, Eguchi J and Kanetake H: Expression of cyclooxygenase-2 and EP4 receptor in transitional cell carcinoma of the upper urinary tract. J Urol *173(1)*: 56-60, 2005. PMID: 15592025. DOI: 10.1097/01.ju.0000148272.77539.2d

7 Miyata Y, Ohba K, Kanda S, Nomata K, Eguchi J, Hayashi T and Kanetake H: Pathological function of prostaglandin E2 receptors in transitional cell carcinoma of the upper urinary tract. Virchows Arch *448(6)*: 822-829, 2006. PMID: 16609907. DOI: 10.1007/s00428-006-0198-1

8 Reader J, Harper AK, Legesse T, Staats PN, Goloubeva O, Rao GG, Fulton A and Roque DM: EP4 and class III β-tubulin expression in uterine smooth muscle tumors: implications for prognosis and treatment. Cancers (Basel) *11(10)*: 1590, 2019. PMID: 31635323. DOI: 10.3390/cancers11101590

9 Ye Y, Peng L, Vattai A, Deuster E, Kuhn C, Dannecker C, Mahner S, Jeschke U, von Schönfeldt V and Heidegger HH: Prostaglandin E2 receptor 3 (EP3) signaling promotes migration of cervical cancer *via* urokinase-type plasminogen activator receptor (uPAR). J Cancer Res Clin Oncol *146(9)*: 2189-2203, 2020. PMID: 32488496. DOI: 10.1007/s00432-020-03272-0

10 Ferini G and Pergolizzi S: A ten-year-long update on radiation proctitis among prostate cancer patients treated with curative external beam radiotherapy. In Vivo *35(3)*: 1379-1391, 2021. PMID: 33910815. DOI: 10.21873/invivo.12390

11 Grewal K, Grewal K and Tabbara IA: PARP inhibitors in prostate cancer. Anticancer Res *41(2)*: 551-556, 2021. PMID: 33517260. DOI: 10.21873/anticanres.14807

12 Mori K, Mostafaei H, Sari Motlagh R, Pradere B, Quhal F, Laukhtina E, Schuettfort VM, Kramer G, Abufaraj M, Karakiewicz PI, Kimura T, Egawa S and Shariat SF: Systemic therapies for metastatic hormone-sensitive prostate cancer: network meta-analysis. BJU Int, 2021. PMID: 34171173. DOI: 10.1111/bju.15507

13 Isaacs JT: The biology of hormone refractory prostate cancer. Why does it develop? Urol Clin North Am *26(2)*: 263-273, 1999. PMID: 10361549. DOI: 10.1016/s0094-0143(05)70066-5

14 Kim TJ, Lee YH and Koo KC: Current status and future perspectives of androgen receptor inhibition therapy for prostate cancer: a comprehensive review. Biomolecules *11(4)*: 492, 2021. PMID: 33805919. DOI: 10.3390/biom11040492

15 Lokeshwar SD, Klaassen Z and Saad F: Treatment and trials in non-metastatic castration-resistant prostate cancer. Nat Rev Urol *18(7)*: 433-442, 2021. PMID: 34002069. DOI: 10.1038/s41585-021-00470-4

16 Wang X and Klein RD: Prostaglandin E2 induces vascular endothelial growth factor secretion in prostate cancer cells through EP2 receptor-mediated cAMP pathway. Mol Carcinog *46(11)*: 912-923, 2007. PMID: 17427962. DOI: 10.1002/mc.20320

17 Xu S, Zhou W, Ge J and Zhang Z: Prostaglandin E2 receptor EP4 is involved in the cell growth and invasion of prostate cancer *via* the cAMP-PKA/PI3K-Akt signaling pathway. Mol Med Rep *17(3)*: 4702-4712, 2018. PMID: 29328471. DOI: 10.3892/mmr.2018.8415

18 Miyata Y, Kanda S, Maruta S, Matsuo T, Sakai H, Hayashi T and Kanetake H: Relationship between prostaglandin E2 receptors and clinicopathologic features in human prostate cancer tissue. Urology *68(6)*: 1360-1365, 2006. PMID: 17169665. DOI: 10.1016/j.urology.2006.09.035

19 Han IH, Kim JH, Jang KS and Ryu JS: Inflammatory mediators of prostate epithelial cells stimulated with Trichomonas vaginalis promote proliferative and invasive properties of prostate cancer cells. Prostate *79(10)*: 1133-1146, 2019. PMID: 31050003. DOI: 10.1002/pros.23826

20 Madrigal-Martínez A, Constâncio V, Lucio-Cazaña FJ and Fernández-Martínez AB: PROSTAGLANDIN E_2 stimulates cancer-related phenotypes in prostate cancer PC3 cells through cyclooxygenase-2. J Cell Physiol *234(5)*: 7548-7559, 2019. PMID: 30367494. DOI: 10.1002/jcp.27515

21 Watanabe S, Miyata Y, Kanda S, Iwata T, Hayashi T, Kanetake H and Sakai H: Expression of X-linked inhibitor of apoptosis protein in human prostate cancer specimens with and without neo-adjuvant hormonal therapy. J Cancer Res Clin Oncol *136(5)*: 787-793, 2010. PMID: 19946707. DOI: 10.1007/s00432-009-0718-x

22 Mitsunari K, Miyata Y, Asai A, Matsuo T, Shida Y, Hakariya T and Sakai H: Human antigen R is positively associated with malignant aggressiveness *via* upregulation of cell proliferation, migration, and vascular endothelial growth factors and cyclooxygenase-2 in prostate cancer. Transl Res *175*: 116-128, 2016. PMID: 27140699. DOI: 10.1016/j.trsl.2016.04.002

23 Asai A, Miyata Y, Matsuo T, Shida Y, Hakariya T, Ohba K and Sakai H: Changes in lymphangiogenesis and vascular endothelial growth factor expression by neo-adjuvant hormonal therapy in prostate cancer patients. Prostate *77(3)*: 255-262, 2017. PMID: 27527525. DOI: 10.1002/pros.23244

24 Li X, Yang B, Han G and Li W: The EP4 antagonist, L-161,982, induces apoptosis, cell cycle arrest, and inhibits prostaglandin E2-induced proliferation in oral squamous carcinoma Tca8113 cells. J Oral Pathol Med *46(10)*: 991-997, 2017. PMID: 28342204. DOI: 10.1111/jop.12572

25 Majchrzak-Celińska A, Misiorek JO, Kruhlenia N, Przybyl L, Kleszcz R, Rolle K and Krajka-Kuźniak V: COXIBs and 2,5-dimethylcelecoxib counteract the hyperactivated Wnt/β-catenin pathway and COX-2/PGE2/EP4 signaling in glioblastoma cells. BMC Cancer *21(1)*: 493, 2021. PMID: 33941107. DOI: 10.1186/s12885-021-08164-1

26 Jain S, Chakraborty G, Raja R, Kale S and Kundu GC: Prostaglandin E2 regulates tumor angiogenesis in prostate cancer. Cancer Res *68(19)*: 7750-7759, 2008. PMID: 18829529. DOI: 10.1158/0008-5472.CAN-07-6689

27 Tong D, Liu Q, Liu G, Xu J, Lan W, Jiang Y, Xiao H, Zhang D and Jiang J: Metformin inhibits castration-induced EMT in prostate cancer by repressing COX2/PGE2/STAT3 axis. Cancer Lett *389*: 23-32, 2017. PMID: 28043910. DOI: 10.1016/j.canlet.2016.12.031

28 Shackleton EG, Ali HY, Khan M, Pockley GA and McArdle SE: Novel combinatorial approaches to tackle the immunosuppressive microenvironment of prostate cancer. Cancers (Basel) *13(5)*: 1145, 2021. PMID: 33800156. DOI: 10.3390/cancers13051145

29 Kashiwagi E, Shiota M, Yokomizo A, Itsumi M, Inokuchi J, Uchiumi T and Naito S: Prostaglandin receptor EP3 mediates

growth inhibitory effect of aspirin through androgen receptor and contributes to castration resistance in prostate cancer cells. Endocr Relat Cancer *20(3)*: 431-441, 2013. PMID: 23493387. DOI: 10.1530/ERC-12-0344

30 Hiken JF, McDonald JI, Decker KF, Sanchez C, Hoog J, VanderKraats ND, Jung KL, Akinhanmi M, Rois LE, Ellis MJ and Edwards JR: Epigenetic activation of the prostaglandin receptor EP4 promotes resistance to endocrine therapy for breast cancer. Oncogene *36(16)*: 2319-2327, 2017. PMID: 27869171. DOI: 10.1038/onc.2016.397

31 Ching MM, Reader J and Fulton AM: Eicosanoids in cancer: Prostaglandin E_2 receptor 4 in cancer therapeutics and immunotherapy. Front Pharmacol *11*: 819, 2020. PMID: 32547404. DOI: 10.3389/fphar.2020.00819

32 De Paz Linares GA, Opperman RM, Majumder M and Lala PK: Prostaglandin E2 receptor 4 (EP4) as a therapeutic target to impede breast cancer-associated angiogenesis and lymphangiogenesis. Cancers (Basel) *13(5)*: 942, 2021. PMID: 33668160. DOI: 10.3390/cancers13050942

33 Holeckova K, Baluchova K, Hives M, Musak L, Kliment J Sr and Skerenova M: Germline mutations in DNA repair genes in patients with metastatic castration-resistant prostate cancer. In Vivo *34(4)*: 1773-1778, 2020. PMID: 32606146. DOI: 10.21873/invivo.11971

34 Ishii A, Shigemura K, Kitagawa K, Sung SY, Chen KC, Yi-Te C, Liu MC and Fujisawa M: Anti-tumor effect of Hedgehog signaling inhibitor, vismodegib, on castration-resistant prostate cancer. Anticancer Res *40(9)*: 5107-5114, 2020. PMID: 32878799. DOI: 10.21873/anticanres.14514

35 Iwamoto H, Kano H, Shimada T, Naito R, Makino T, Kadamoto S, Yaegashi H, Shigehara K, Izumi K, Kadonoa Y and Mizokami A: Effectiveness of vintage hormone therapy as alternative androgen deprivation therapy for non-metastatic castration-resistant prostate cancer. In Vivo *35(2)*: 1247-1252, 2021. PMID: 33622927. DOI: 10.21873/invivo.12375

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