Metformin mitigates PLCε gene expression and modulates the Notch1/Hes and androgen receptor signaling pathways in castration-resistant prostate cancer xenograft models

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Abbreviations: PDX, patient-derived xenograft; CRPC, castration-resistant prostate cancer; PLCε, phospholipase Cε; AR, androgen receptor; PSA, prostate-specific antigen; RT-qPCR, reverse transcription-quantitative PCR; ADT, androgen deprivation therapy

Key words: metformin, CRPC, patient-derived xenotransplantation, PLCε, Notch1/Hes signaling pathway, androgen receptor signaling pathway

Abstract. The present study aimed to establish a mouse model of patient-derived castration-resistant prostate cancer (CRPC) xenograft tumors, and to evaluate the effects of various doses of metformin on phospholipase Cε (PLCε) expression and the neurogenic locus notch homolog protein 1 (Notch1)/hairy and enhancer of split 1 and androgen receptor (AR) signaling pathways via western blotting and reverse transcription-quantitative PCR. Additionally, phorbol 12-myristate 13-acetate was used to activate PLC, and Jagged1 was used as a Notch activator to verify whether metformin could suppress CRPC development via the PLCε/Notch1/AR pathways. The results confirmed that metformin may serve critical roles in CRPC by significantly inhibiting the occurrence, growth and proliferation of CRPC tumors by decreasing PLCε/Notch1 expression and AR nucleation.

Introduction

Castration-resistant prostate cancer (CRPC), i.e., prostate cancer that responds poorly to anti-androgens, is characterized by strong local invasion, recurrence and distant metastasis, a low survival rate and a poor prognosis (1). It has been observed that metformin may decrease the risk of prostate cancer in patients with type 2 diabetes. The drug appears to be beneficial in delaying the development of castration resistance and improving overall survival time in patients with prostate cancer (2). However, there is still no definitive evidence. A meta-analysis conducted by Chen et al (3) demonstrated that there is no clear association between metformin and prostate cancer incidence in type 2 diabetes populations. Metformin may have potential protective effects on prostate cancer incidence in an Asian population with type 2 diabetes; however, no statistically significant difference and similar protective effects were found in a Western population with type 2 diabetes (3). Meanwhile, a clinical study confirmed that metformin improved the overall survival outcomes of patients with prostate cancer who were also diagnosed with type 2 diabetes (4). It should be noted that the retrospective studies introduced several interference factors. In vitro cell experiments revealed that metformin could inhibit the proliferation of prostate cancer cells, but this has not been demonstrated in any animal models. Ge et al (5) indicated that metformin inhibited the malignant biological behaviors of prostate cancer cells through alternative pathways between N-cadherin-expressing cells and N-cadherin-deficient cells. Yang and Wu (6) argued that the effect of metformin on the biological behavior of CRPC PC-3 cells may be activated by inhibiting the phospholipase Cε (PLCε) gene-mediated neurogenic locus notch homolog protein 1 (Notch1)/hairy and enhancer of split (Hes) and androgen receptor (AR) signaling pathways.

In the current study, a mouse model of patient-derived xenograft (PDX)-CRPC tumors was established to investigate the effects of metformin on CRPC. Furthermore, the mechanism related to PLCε gene expression and the Notch1/Hes and AR signaling pathways associated with the exogenous intervention of metformin or corresponding activators was elucidated upon.

Materials and methods

PDX-CRPC mouse model

Mice. Male mice with severe combined immunodeficiency NOD-NPG (age, 8 weeks; weight, 20±2 g; n=29) were purchased from Beijing Viton Lihua Experimental Animal Co., Ltd. (production license number: SCXK
The tissue sections (4 µm) were heated at 4˚C for 24 h immediately after surgical resection and then formaldehyde for histological analysis to determine the tumor stages.

Histological analysis. Part of the tumor was fixed in 4% formaldehyde for histological analysis to determine the tumor homology, compared with the patient section, and confirm the model. All tissues were fixed with 4% paraformaldehyde at 4˚C for 24 h immediately after surgical resection and then embedded in paraffin. The tissue sections (4 µm) were heated at 60˚C for 1 h then dewaxed and rehydrated by immersion in dimethylbenzene and an ethanol series, and hematoxylin and eosin (H&E) staining was performed at room temperature for 1-10 min. Stained sections were observed and images were captured using an optical microscope (Olympus).

Grouping method. Tumors under the renal capsule PDXs were surgically removed after tumor growth for 1 month, and the recurrence of tumor after several months was designated as the occurrence of CRPC. Based on the experimental requirements, to analyze the effects of Metformin, 20 CRPC mice (when the tumor exceeded 800 mm³) were grouped, with 5 mice in each group, as follows: High concentration group, 270 mg/kg/day; medium concentration group, 90 mg/kg/day; and low concentration group, 30 mg/kg/day (oral administration).

The control group was treated with 0.9% physiological saline. The expression of PSA, PLCε, Notch1/Hes and AR proteins was detected in the tumor tissues of each group, and the tumor size was recorded; the maximum tumor sizes observed in the study was 2,318 mm³. Metformin, PLC activator phorbole 12-myristate 13-acetate (PMA) and Notch activator Jagged1 were purchased from MilliporeSigma. An intraperitoneal injection of PMA (200 µg/kg) was administered to form the Metformin (90 mg/kg) + PMA group, while an intraperitoneal injection of Jagged1 (500 µg/kg) was administered to form the Metformin + Jagged1 group. The control group was treated with 0.9% physiological saline.

Immunohistochemistry (IHC). Paraffin-embedded sections were dewaxed and rehydrated in a graded alcohol series. Antigen retrieval was performed by boiling at 95˚C for 10 min in EDTA. Endogenous peroxidase quenching was performed using 3% hydrogen peroxide at room temperature for 30 min, and samples were blocked in 20% goat serum for 40 min at room temperature. Samples were incubated with anti-PSA primary antibody (1:100; catalog no. P07288; Beijing Solarbio Science & Technology Co., Ltd.) at 4˚C overnight, and incubation with HRP-conjugated sheep anti-rabbit secondary antibody (1:100; catalog no. SPA134; Beijing Solarbio Science & Technology Co., Ltd.) was performed at room temperature for 60 min. Sections were visualized using 2,2'-diaminobenzidine (MilliporeSigma) and counterstained with hematoxylin. Mouse IgG was used as the primary antibody for the negative control. The results were observed by fluorescent inverted/phase contrast microscope.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was isolated using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Total RNA (1 µg) was converted to cDNA using a GoScript™ Reverse Transcription System kit (Promega Corporation) according to the manufacturer's instructions. qPCR was performed using using the SYBR Green Step One Plus Real-Time PCR system (Santa Cruz Biotechnology, Inc.) according to the manufacturer's instructions. Complementary DNA was generated by reverse transcription, also using the Step One Plus Real-Time PCR system kit. PCR amplification conditions were set at 95˚C for 10 min, followed by 30 cycles at 95˚C for 15 sec, 60˚C for 30 sec and 72˚C for 15 sec. After the final cycle, the reaction was terminated. Results were quantified by the ΔΔCq method (8) and measured three times to take the average. Primers used in this study are as follows: PLCε forward,
5'-GGTTTCATCCAGGATCGAGC AGG-3' and reverse, 5'-ACAAAGATGGTCACGGTCTGCC-3'; Hes forward, 5'-ACGACACCGGAAAACCAAA-3' and reverse, 5'-CGGAGGTGCACTGTCAT-3'; Notch forward, 5'-CATCATCAATGGCTGCAAGGG-3' and reverse, 5'-CATTTTCACACGTGGCACC-3'; and GAPDH forward, 5'-ACCACAGTCCATGCCATCAC-3' and reverse, 5'-CCTGCTTCACCACCTTCTTGA-3'.

Western blotting. RIPA buffer (MilliporeSigma) was used for tumor tissue cell lysis, and the extraction and purification of intracellular protein. β-actin was used as the internal reference gene. BCA reagent (Thermo Fisher Scientific, Inc.) was used to quantify the protein concentration. A total of 30 µg protein were separated on an 8% gel using SDS-PAGE, and transferred to PVDF membranes. The membranes were blocked in 5% skimmed milk in 1X TBST at room temperature for 2 h and then incubated with rat anti-mouse Notch1, Hes, AR and β-actin primary antibodies (catalog nos. ab27526, ab71559; ab244058 and ab8226, respectively; dilution, 1:2,000; Abcam) overnight at 4˚C. After washing with 1X TBST three times, corresponding secondary antibody, HRP-conjugated anti-mouse IgG or Alexa Fluor 488-conjugated goat anti-rabbit IgG (catalog nos. ab131368 and ab150081, respectively; dilution, 1:500; Abcam) was added to the membrane and incubated at room temperature for 4 h. After washing again with TBST, SuperSignal® ECL kit (Pierce; Thermo Fisher Scientific, Inc.) was applied to develop the chemical signal. The Lab Works 4.5 gel imaging software (Invitrogen; Thermo Fisher Scientific, Inc.) was used to perform semi-quantitative analysis.

Nuclear separation. The tumor tissues were sonicated (4˚C; 20-25 kHz; 1 min), and then lysed on ice with a cytoplasmic lysis buffer (Enzo) for 15 min. Supernatant was collected after centrifugation (12,000 x g, 10 min, 4˚C) to obtain the cytoplasmic protein. Precipitate was re-suspended using a cytoplasmic lysis buffer without a protease inhibitor and washed 3 times with cytoplasmic lysis buffer, and then cleaved with a nuclear lysis buffer (Pierce Biotechnology, Inc.). The cleavage process was performed on a vortex oscillator at 4˚C for 30 min. Finally, the supernatant was collected as the nuclear protein fraction after centrifugation (12,000 x g, 10 min, 4˚C) for western blot analysis as aforementioned. Histone was used as a nuclear protein internal reference.

Statistical analysis. Data are expressed as the mean ± SD. Statistical analysis was performed using SPSS 20.0 statistical software (IBM Corp.). One-way ANOVA followed by Tukey's post hoc test, and unpaired Student's t-tests, were used to compare multiple groups or two groups, respectively. P<0.05 was used to indicate a statistically significant difference.

Results

PDX-CRPC mouse model. As depicted in Fig. 1A, surgical resection of the PDX tumor (pink) under the renal capsule of the mice delayed the tumor recurrence for a few months, which was characterized as CRPC. The growth of the tumor was monitored via real time imaging system in vivo (Fig. 1B). The tumor size measurements are presented in Fig. 1C. Mice were euthanized after the tumor size exceeded 800 mm³.
Histological analysis of the tumors indicated that the CRPC model was successfully established, as indicated by deeper HE staining, and stacked or inlaid cell clusters (Fig. 1D).

**Effects of metformin on CRPC growth.** Tumor sizes and weights were further observed in mice after 2 months of daily treatment with different doses of metformin (low, medium and high, and control) (8,9). As shown in Fig. 2, the results showed significant differences among tumor weights and sizes in a dose-dependent manner, with higher doses associated with lower tumor weight and size, indicating that metformin significantly inhibited tumor growth. Histological analysis (Fig. 2D) showed that metformin improved cell and nuclear morphology (H&E), inhibited tumor cell proliferation (Ki-67) and downregulated the proportion of PSA-positive cells. Overall these results indicated that an increase in metformin dose significantly inhibited prostate cancer proliferation.
Effect of metformin on PLCε expression. Western blotting and RT-qPCR analyses were next used to determine PLCε expression levels. The results indicated that the expression of PLCε was downregulated at the protein and mRNA levels by metformin in a dose-dependent manner (Fig. 3).

Effects of metformin on expression of Notch, Hes and AR proteins. It has been well documented that PLCε could affect the development of tumors by regulating the Notch signaling pathway and AR nucleation (6). In the present study, western blotting and RT-qPCR were used to analyze the expression levels of Notch and Hes at the protein and mRNA levels. As shown in Fig. 4A, Notch and Hes expression was downregulated at the protein and mRNA levels in the metformin (medium concentration)-treated group. Moreover, after nuclear separation, the nuclear AR expression was also significantly decreased in the metformin-treated group, indicating that metformin inhibited the nuclear relocation of AR (Fig. 4B).

Effects of metformin alone and in combination with PMA/Jagged1 on tumor growth. The effects of PMA/Jagged1 in combination with metformin on tumor growth were further investigated. Each group was monitored continuously for 45 days using a live animal imaging system. The results showed that tumor growth was significantly decreased in the mice treated with a combination of metformin and PLC activator PMA or Notch activator Jagged1, compared with that in the mice treated with metformin only (Fig. 5). This result could be attributed to PMA being able to increase PLC and Notch expression levels, and Jagged1 being able to increase Notch expression levels (Fig. 5A). Moreover, these two agents promoted AR expression (Fig. 5B).

Discussion

The multiple roles and mode of action of metformin in the treatment of various diseases are yet to be elucidated (9). Several studies suggested that metformin could affect the physiological function of prostate cancer cells by regulating the activity of adenosine 5'-monophosphate-activated protein kinase (10-12). It has been documented that metformin could directly regulate the AR, which is closely related to the occurrence and development of prostate cancer, and that it regulates the PI3K/Akt and MAPK signaling pathways, which are the downstream targets in several signaling pathways of insulin receptors. Thus, metformin may serve an important role in regulating insulin resistance and affect the function of prostate cancer cells (13). It was previously suggested that metformin significantly inhibited cell proliferation, invasion and apoptosis in the hormone-resistant prostate cancer PC3 cell line in time- and dose-dependent manners (14). Moreover, metformin targeted and inhibited PLCε gene expression and decrease the molecular expression of the AR and Notch1/Hes signaling pathways, which were closely associated with prostate cancer cell proliferation and invasion (6).

CRPC is recognized as the terminal stage of prostate cancer; it is characterized by distant metastasis and resistance to anti-androgen therapy, radiotherapy and chemotherapy. In a previous study, metformin reversed epithelial-mesenchymal transition in prostate cancer tissue to some extent and enhanced tumor cell sensitivity to neoadjuvant radiotherapy and chemotherapy (15).

There are two main prostate cancer cell lines for models of transplantation: PC3 and LNCaP. However, the probability of losing the three-dimensional structure of the tumor in long-term laboratory culture is high, which means that the heterogeneity of clinical prostate cancer would not
be captured, and ultimately, the important characteristics and diversity of prostate cancer would not be accurately summarized (16). The PDX model not only maintained the interaction between the micro-environmental components inside the tumor, but also accurately simulated the major characteristics of prostate cancer in patients, such as its hormone-dependent or non-dependent nature, and also induce the occurrence of CRPC through androgen ablation in mice (17). The present current study successfully established a mouse model of a PDX-CRPC transplanted tumor. Moreover, the PDX model of prostate cancer was improved by intermittent androgen supplementation and the selection of intrarenal transplantation sites. We consider that the PDX-CRPC transplanted tumor model has a high success rate, good stability and maintains the important characteristics of human prostate cancer, which guarantees the ability to use metformin treatment and information for the subsequent discussions on the molecular mechanisms involved. However, further investigations are required to determine how different metformin concentrations affect the human body. After the intervention with metformin for 2 months, the diameter of the tumors in the low and high concentration groups were significantly smaller than that in the control group, and the tumors in the high concentration group were smaller than those in the low concentration group (P<0.05). Meanwhile, mice in the control group developed multiple tumor metastases. This suggested that metformin intervention could inhibit in vivo growth and metastasis of prostate cancer in a dose-dependent manner.

PSA is a specific molecular target of prostate cancer tissue, and its positive expression is often associated with tumor malignancy, castration resistance and a poor clinical prognosis (18). IHC staining showed that expression of PSA was significantly higher in tumor tissues from the control group, compared with that in tumor tissues from the metformin-treated groups (P<0.05). Similarly, the tumors from mice treated with a high concentration of metformin exhibited lower expression levels of PSA compared with the tumors from mice treated with a low concentration of metformin. The results also indicated that the PDX-CRPC mouse model could express PSA, which further confirmed the similarity between the initial tumor of human origin and the PDX-CRPC tumor of the mouse. A previous study found that both the PC3 and LNCaP models lose their expression during passage to passage culture, which means that they do not fully reflect the important characteristics of human prostate cancer (19). In the present study, the expression level of PLCε mRNA in the control group was significantly higher than that in the metformin-treated groups (P<0.05), and the group with a high metformin concentration exhibited a low level of PLCε mRNA. This result suggested that metformin may regulate prostate cancer cell proliferation and invasion by inhibiting the expression of the PLCε gene. Additionally, previous studies reported that the Notch1/Hes and AR cell pathways played an important role in promoting the early onset, castration resistance and distant metastasis of prostate cancer (20,21). The present results showed that the expression levels of Notch1, Hes and AR proteins were lower in tumor tissues from the metformin-treated groups compared with that in tumor tissues

Figure 5. Effects of metformin only or a combination of metformin and PMA/Jagged1 on tumor growth. (A) Expression of PLCε and Notch in the metformin only group or the combination groups according to western blotting analysis. (B) Expression of AR protein in the metformin only group or the combination groups (x200 magnification). (C) Changes in recorded tumor volume in the metformin only group or the combination groups. **P<0.01 vs. metformin. (D) Images of tumors in the metformin only group or the combination groups. PLCε, phospholipase Cε; Notch, neurogenic locus notch homolog protein; PMA, phorbol 12-myristate 13-acetate; vol., volume.
from the control group (P<0.05). Moreover, the group treated with a higher dose of metformin exhibited a lower expression level of these proteins, suggesting that metformin may activate the Notch1/Hes and AR cell pathways to affect prostate cancer cell proliferation and invasion. Based on the aforementioned results and those of previous studies, further studies are required to investigate the potential role of the Notch1/Hes and AR cell pathways in prostate cancer.

Whitburn et al (22) indicated that the application of metformin in prostate cancer patients treated with androgen deprivation therapy (ADT) inhibited cancer cell proliferation and improve metabolic syndrome. However, as the number of cases in this study is limited, the randomized trial data provided is still insufficient. Similarly, Hankinson et al (15) reported that metformin treatment in patients with type 2 diabetes reversed metabolic conditions by decreasing the androgen levels, thereby leading to high levels of androgen stimulating prostate growth, proliferation and tumorigenesis. Although the antitumor properties of metformin are not obvious in the early stages of prostate cancer development, the drug could be effective in decreasing the mortality of patients with prostate cancer by significantly improving the parameters of metabolic syndrome after ADT, and it could exhibit a therapeutic effect on some patients with asymptomatic or mild metastatic CRPC (23,24). There is potential for metformin to be used as a monotherapy or an adjuvant agent in ADT, or in external therapy or other chemotherapies.

In conclusion, the present study reported that the PDX-CRPC mouse model of transplanted tumors enables investigation of the mechanism of prostate cancer occurrence and drug intervention. Moreover, metformin may have the potential to inhibit CRPC progression by activating the Notch1/Hes and AR signaling pathways, which could inhibit PLCe gene expression.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JP and QL conceived and designed the study. KX, JT and ZL analyzed and interpreted the data. JP, QL and KX drafted the manuscript. JT and ZL revised the manuscript. JP, QL and KX confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was ethically approved by the Institutional Review Board of Changshu Hospital Affiliated to Nanjing University of Chinese Medicine (Suzhou, China). Written informed consent was obtained from the patient prior to the start of the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Vlachostergios PJ, Puca L and Beltran H: Emerging Variants of Castration-Resistant Prostate Cancer. Curr Oncol Rep 19: 32, 2017.
2. Au Yeung SL and Schooling CM: Impact of glycemic traits, type 2 diabetes and metformin use on breast and prostate cancer risk: A Mendelian randomization study. BMJ Open Diabetes Res Care 7: e000872, 2019.
3. Chen CB, Eskin M, Earich DT, Majumdar SR and Johnson JA: Metformin, Asian ethnicity and risk of prostate cancer in type 2 diabetes: A systematic review and meta-analysis. BMC Cancer 18: 65, 2018.
4. Mayer MJ, Klotz LH and Venkateswaran V: The Effect of Metformin Use during Docetaxel Chemotherapy on Prostate Cancer Specific and Overall Survival of Diabetic Patients with Castration Resistant Prostate Cancer. J Urol 197: 1068-1075, 2017.
5. Ge R, Wang Z, Wu S, Zhuo Y, Osetov AG, Cai C, Zhong W, Wu CL and Ouami AF: Metformin represses cancer cells via alternate pathways in N-cadherin expressing vs. N-cadherin deficient cells. Oncotarget 6: 28973-28987, 2015.
6. Yang Y and Xu WX: Study on the influence of metformin on castration-resistant prostate cancer PC-3 cell line biological behavior by its inhibition on PLCe gene-mediated Notch1/Hes and androgen receptor signaling pathway. Eur Rev Med Pharmacol Sci 21: 1918-1923, 2017.
7. Abdel-Rahman O: Validation of American Joint Committee on Cancer eighth staging system among prostate cancer patients treated with radical prostatectomy. Ther Adv Urol 10: 35-42, 2017.
8. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(^ΔΔCT) method. Methods 25: 402-408, 2001.
9. Spiering MJ: The mystery of metformin. J Biol Chem 294: 6689-6691, 2019.
10. Akinuyeke T, Matsumura S, Wang X, Wu Y, Schalfer ED, Saxena A, Yan W, Logan SK and Li X: Metformin targets c-MYC oncogene to prevent prostate cancer. Carcinogenesis 34: 2823-2832, 2013.
11. Sarmento-Cabral A, L-López F, Gabete MD, Castaño JP and Luque RM: Metformin reduces prostate tumor growth, in a diet-dependent manner, by modulating multiple signaling pathways. Mol Cancer Res 15: 862-874, 2017.
12. Wu D, Hu D, Chen H, Shi G, Fetahu IS, Wu F, Rabidou K, Fang K, Tan L, Xu S, et al.: Glucose-regulated phosphorylation of TET2 by AMPK reveals a pathway linking diabetes to cancer. Nature 559: 637-641, 2018.
13. Delma MJ: Three May Be Better Than Two: A Proposal for metformin addition to PI3K/Akt inhibitor-antiandrogen combination in castration-resistant prostate cancer. Cureus 10: e3403, 2018.
14. Raffaele M, Pittalà V, Zingales V, Barbagallo I, Salerno L, Li Volti G, Romeo G, Carota G, Sorrenti V and Vanella L: Heme oxygenase-1 inhibition sensitizes human prostate cancer cells towards glucose deprivation and metformin-mediated cell death. Int J Mol Sci 20: 20, 2019.
15. Hankinson SJ, Fam M and Patel NN: A review for clinicians: Prostate cancer and the antineoplastic properties of metformin. Urol Oncol 35: 21-29, 2017.
16. Ranasinghe WK, Williams S, Ischia J, Wetherell D, Baldwin G, Shulkes A, Sengupta S, Bolton D and Patel O: Metformin may offer no protective effect in men undergoing external beam radiation therapy for prostate cancer. BJU Int 123 (Suppl 5): 36-42, 2019.
17. Lam HM, Nguyen HM, Labrecque MP, Brown LG, Coleman IM, Gulati R, Lakely B, Sondheim D, Chatterjee P, Marck BT, et al: Durable Response of enzalutamide-resistant prostate cancer to supraphysiological testosterone is associated with a multifaceted growth suppression and impaired DNA damage response transcriptomic program in patient-derived xenografts. Eur Urol 77: 144-155, 2020.

18. Lange T, Oh-Hohenhorst SJ, Joosse SA, Pantel K, Hahn O, Gosau T, Dyshlovoy SA, Wellbrock J, Feldhaus S, Maar H, et al: Development and characterization of a spontaneously metastatic patient-derived xenograft model of human prostate cancer. Sci Rep 8: 17535, 2018.

19. Zhao Y, Zeng X, Tang H, Ye D and Liu J: Combination of metformin and paclitaxel suppresses proliferation and induces apoptosis of human prostate cancer cells via oxidative stress and targeting the mitochondria-dependent pathway. Oncol Lett 17: 4277-4284, 2019.

20. Sun W, Li L, Du Z, Quan Z, Yuan M, Cheng H, Gao Y, Luo C and Wu X: Combination of phospholipase Cε knockdown with GANT61 sensitizes castration resistant prostate cancer cells to enzalutamide by suppressing the androgen receptor signaling pathway. Oncol Rep 41: 2689-2702, 2019.

21. Rice MA, Hsu EC, Aslan M, Ghoochani A, Su A and Stoyanova T: Loss of Notch1 activity inhibits prostate cancer growth and metastasis and sensitizes prostate cancer cells to antiandrogen therapies. Mol Cancer Ther 18: 1230-1242, 2019.

22. Whitburn J, Edwards CM and Sooriakumaran P: Metformin and Prostate cancer: A new role for an old drug. Curr Urol Rep 18: 46, 2017.

23. Zingales V, Distefano A, Raffaele M, Zanghi A, Barbagallo I and Vanella L: Metformin: A bridge between diabetes and prostate cancer. Front Oncol 7: 243, 2017.

24. Hong Y, Lee S and Won S: The preventive effect of metformin on progression of benign prostate hyperplasia: A nationwide population-based cohort study in Korea. PLoS One 14: e0219394, 2019.