Journal of Traditional and Complementary Medicine 14 (2024) 19–25

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Zhoushi Qi Ling decoction inhibits the progression of castration-resistant prostate cancer in vivo by regulating macrophage infiltration via IL6-STAT3 signaling

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ARTICLE INFO

Article history:
Received 15 December 2022
Received in revised form
9 May 2023
Accepted 30 May 2023
Available online 7 June 2023

Keywords:
Prostate cancer
Castration-resistant prostate cancer
CRPC
IL6/STAT3
Macrophage infiltration

ABSTRACT

Background and aim: Prostate cancer is a leading malignant tumor in men, associated with a high rate of mortality. Androgen deprivation therapy is commonly used to treat prostate cancer, which contributes to the progression of castration-resistant prostate cancer (CRPC). The current therapy has a low survival rate in patients with CRPC. Our study aims to develop a novel effective approach for CRPC treatment and improve survival benefits.

Experimental procedure: CRPC cell line PC-3-Luc expressing luciferase and the CRPC cell line PC-3-IL6-Luc stably overexpressing IL-6 were used to establish the xenograft tumor mouse model. The tumor was monitored weekly using Bioluminescence imaging. Infiltrated macrophages were quantified by fluorescence-activated cell sorting using flow cytometry. IL6 mRNA level was determined using quantitative real-time PCR. The protein levels of total STAT3 and phosphorylated STAT3 were determined using Western blot.

Results and conclusion: Zhoushi Qi Ling decoction (ZQD) treatment significantly reduced PC3 the xenograft tumor progression and the number of infiltrated macrophages when compared with saline treatment. IL6 mRNA level was remarkedly suppressed by ZQD treatment. Notably, the protein level of phosphorylated STAT3 was significantly decreased in PC3 the xenograft tumor treated with ZQD compared to saline treatment. Our findings demonstrated that ZQD treatment significantly reduced the progression of prostate cancer, evidenced by the reduced population of infiltrated macrophages and the inhibition of the IL6/STAT3 pathway.

1. Introduction

Prostate cancer is the most common malignant tumor in men, associating with a high rate of mortality worldwide.[1] Although androgen therapy is the standard treatment for prostate cancer, most cases will progress to androgen-independent prostate cancer, also known as castration-resistant prostate cancer (CRPC).[2] However, most patients with prostate cancer patients progress to CRPC.[3,4] Mechanistically, the amplification and hypersensitivity of androgen receptor, androgen receptor mutant, androgen-independent receptor activation, etc., could contribute to CRPC.[5] Docetaxel is the first-line chemotherapeutic treatment for prostate cancer.[6,7] However, the poor survival outcome in patients with CRPC limits the application of docetaxel.[8,9] Therefore, gaining a better understanding of mechanisms biologically and

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Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.
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https://doi.org/10.1016/j.jtcme.2023.05.005

pathophysiologically of CRPC and developing an effective agent for prostate cancer treatment and management is urgently needed.

Zhou's "Qi Ling Recipe" is a traditional Chinese medicine prescription developed by the renowned Chinese medicine doctor Zhou Zhiheng for the treatment of advanced prostate cancer. It has the potential to slow the progression of CRPC. A previous study has shown that Zhoushi Qi Ling decoction (ZQD) induces prostate tumor cell apoptosis in patients with PC and who received androgen deprivation therapy.[10,11] In relation to the inhibitory effect on CRPC, it has been reported that IL6-STAT3 plays an important role in the progression of PC.[12] However, the role of ZQD in macrophage infiltration remain unclear. The mechanistic action of the "Qi Ling Recipe" needs to be further investigated, which limited the application of ZQD in clinical.

Therefore, in this study, we aim to investigate the role of Zhoushi Qi Ling decoction in the progression of CRPC using a CRPC cell line derived xenograft tumor mice model. This research will provide experimental evidence of ZQD for the treatment of prostate cancer, as well as the superiority of Chinese medicine in anti-tumor treatment.

2. Methods

2.1. ZQD treatment

The formula of ZQD was described as follows: 9 g of Herba Leonuri, 12 g of Radix Codonopsis, 15 g of Radix Rehmanniae, 30 g of Rabdosia rubescens Hara, 15 g of Astragalus mongholicus Bunge, 9 g of Rhizoma Curcumae Longae, and 9 g of Radix Glycyrrh. The decoction was made using the 10-function automatic decoction machine YJD20-GL (20000 cc). ZQD was boiled, and the resulting liquid extract of 200 mL was packed into two vacuum-sealed containers (100 mL in each container). According to the clinical dosage of the traditional Chinese medicine prescription, the equivalent dose of the rat was calculated, and the decoction of 10 times the equivalent dose of "Qi Ling prescription" was given by gavage daily at a concentration of 10 mL/kg body weight. After PC3-Luc or PC3-IL6-Luc tumors transplanted, the drug was administered daily till the end of the experiment. The control group was given 0.9% saline intragastrically.

2.2. Tumor xenografts

Male 8-week-old severe combined immunodeficient (SCID) mice underwent anesthesia (ether inhalation). After the longitudinal incision of the abdomen, opened the peritoneum. The root of the seminal vesicle on the dorsal side of the bladder root was the prostate tissue. The testicular led on both sides of the bladder, both testicles were removed. Two types of cells, the CRPC cell line PC-3-Luc expressing luciferase and the CRPC cell line PC-3-IL6-Luc stably overexpressing IL-6, were mixed with Matrigel (Biocoat, US) at a ratio of 1:1 and placed on ice. 20 μl of total amount of cells 1×10^6 of tumor cells were injected in the prostate of each mouse. The tip of an insulin syringe was used to gently pierce the prostatic capsule. Mice were put it back to the original cage. Saline (0.9%, intragastrically, daily), ZQD (10 mL/kg body weight, intragastrically, daily) or docetaxel (RP56976, Selleck, 5 mg/kg body weight, twice per week, i.p. injection) was given to mice after tumor transplantation according to divided groups. BLI was performed weekly to detect tumor burden. At day 35 tumor tissue were collected for further analysis.

2.3. Cell culture

Human prostate cancer cell lines PC3 were cultured with RPMI-1640 or DMEM medium supplemented with 10% fetal bovine serum, 1% penicillin and streptomycin (Gibco, Grand Island, NY). Cultured in the incubator (5% CO_2, 37 °C).

2.4. Bioluminescence imaging

The mice were weighed every three days and their tumors were measured by bioluminescence imaging weekly.[13] D-luciferin (150 mg/kg body weight, Thermo Fisher) was injected into the abdominal cavity, and the mice were anesthetized by isoflurane and imaged with the PerkinElmer IVIS Lumina XR in vivo imaging system. The track of PC3-Luc or PC3-IL6-Luc the xenograft growth was kept. At the end of experiment, the mice were euthanized, and the tumor was removed and stored in −80 °C for further analysis.

2.5. PC3-IL6-Luc cell line

PC3 cells were transfected with Lentiviral luciferase plasmid (LPP-Hluc-Lv201-100, GeneCopoeia, Guangzhou, China) or the commercial recombinant plasmid of IL6 and the non-targeting control plasmid according to the manufacturer's protocol.

2.6. Western blot

60 g of total proteins were loaded and analyzed with the antibodies listed. GAPDH was used as an internal control. The ECL the ImageMaster II scanner was then used to visualize protein bands. The antibodies included anti-GAPDH (1:5000, CST), anti-Cleaved Caspase 3 (1:2000, CST), anti-Stat3 (1:2000, CST), anti-p-Stat3 (1:1000, CST), and anti-Ki67 (1:1000, Abcam). The blots were analyzed with ImageQuant TL v2003.03 software (GE Healthcare). The results were expressed as a fold-change of target protein to GAPDH.

2.7. Immunohistochemistry

Tumor tissues were fixed in 10% formalin and embedded in paraffin. Tissue then was sectioned and incubated with Ki67 (1:2000, Abcam) and p-STAT3 (1:2000, CST) antibody.

2.8. ELISA

After homogenizing the tissues in ice-cold cell lysis buffer, they were then centrifuged at 1500 g for 15 min. The supernatants were collected and the levels of IL-6 were determined using corresponding ELISA kit (ab178013, Abcam).

2.9. QPCR

Total RNA was extracted from xenograft tumors using TRIzol reagent (Invitrogen, Waltham, MA USA). Following, the RNA was reversely transcribed to cDNA using the PrimeScript reagent kit (Takara, Dalian, China). Then, the real-time PCR was performed using ABI Quant Studio 7 Flex Real-time PCR system (Applied Biosystems, USA). ΔΔCt method was used to calculate the relative expression of target genes expression. The primer sequences were listed as follows:

IL6:
(forward) ACTCACCTCTTCAGAACGAATTG,
(reverse) CCATCTTTGGAAGGTTCAGGTTG.
GAPDH:
(forward) GGAGCGAGATCCCTCCAAAAT,
(reverse) GGCTGTTGTCATACTTCTCATGG.

2.10. Statistical analysis

The significant difference was analyzed using Student's t-test, two way-ANOVA in GraphPad prism 7. Error bar represents mean ± SD. It was considered statistically significant when $p < 0.05$.

3. Results

3.1. ZQD inhibits the progression of PC3 prostate cancer in vivo

To determine the effect of ZQD on the progression of PC3 prostate cancer, we first compared the efficacy of ZQD and docetaxel, a standard therapy drug for prostate cancer, in sensitive cell line PC3 xenograft tumor model. The results revealed that ZQD showed more effective on PC3 tumor in vivo (Fig. S1), which suggested the utilization potentiality in clinical treatment for prostate cancer. Further, we measured tumor burden by bioluminescence imaging weekly. We found that tumor burden was significantly reduced by the treatment with ZQD throughout 35 days when compared with saline-treated (Fig. 1A, $P < 0.001$). In parallel, tumor weight was significantly decreased by the treatment with ZQD compared to the saline-treated group (Fig. 1B, $P < 0.001$). Next, we evaluated the survival rate. As shown in Fig. 1C, ZQD improved survival from 0 to 50% at 60 days after PC3 prostate tumor injection (Fig. 1C, $P < 0.001$). Overall, these data suggest that ZQD could inhibit the growth and progression of PC3 prostate cancer.

3.2. ZQD decreases the proliferation but promotes apoptosis of PC3 cells in vivo

To further determine the effect of ZQD on the proliferation of PC3 prostate cancer cells in the xenograft tumors, we measured PC3 cancer cells with cell proliferation biomarker Ki67 and cell apoptosis executioner cleaved caspase 3. As shown in Fig. 2A and B, the number of Ki67 positive PC cells was significantly reduced by ZQD treatment ($P < 0.001$). In contrast, the cleaved caspase 3 protein expression level was significantly increased in ZQD treated group (Fig. 2C and D). These results suggest that ZQD inhibits PC3 proliferation and promotes PC3 cell apoptosis in vivo.

3.3. ZQD regulates IL6-STAT3 signaling in PC3 the xenograft tumor

The signal transducer and activator of transcription 3 (STAT3) plays a critical role in responding to cell growth and apoptosis. STAT3 can be activated by phosphorylation in response to cytokines such as IL5 and IL6. In our study, we measured total and phosphorylated STAT3 in PC the xenograft tumor using Western blot and IHC. It was found that ZQD significantly reduced pSTAT3 protein level when compared with the saline-treated group (Fig. 3A, B, and 3C, $P < 0.01$). Similarly, mRNA and protein level of IL6 was remarkedly lower in ZQD treated group than the saline-treated group (Fig. 3D and E, $P < 0.01$). These results reveal that ZQD inhibits PC growth and promotes PC apoptosis by inhibiting the phosphorylation of STAT3.

3.4. ZQD regulates immune microenvironment of PC3 the xenograft tumor

To determine whether ZQD could affect the immune microenvironment in PC3 the xenograft tumor, we measured macrophage infiltration in PC3 the xenograft tumors using FACS. As shown in Fig. 4A, ZQD treatment significantly decreased CD11b + F4/80+ subpopulation ($P < 0.05$, Fig. 4A and B), indicating that ZQD effectively inhibited macrophage infiltration in PC the xenograft tumor.

3.5. ZQD shows no effects on PC3-IL6-Luc the xenograft tumor

To determine the effect of ZQD on the STAT3/LI6 pathway, we inject IL6 overexpressed PC3 tumor into the mice. Tumor burden revealed that no significance was observed between the saline and ZQD treated group (Fig. 5A). In consistent with tumor burden results, tumor weights at day 35 showed no difference between the saline and ZQD group (Fig. 5B). Similarly, the survival rate in ZQD treated group was reduced to the level of saline group (Fig. 5C).

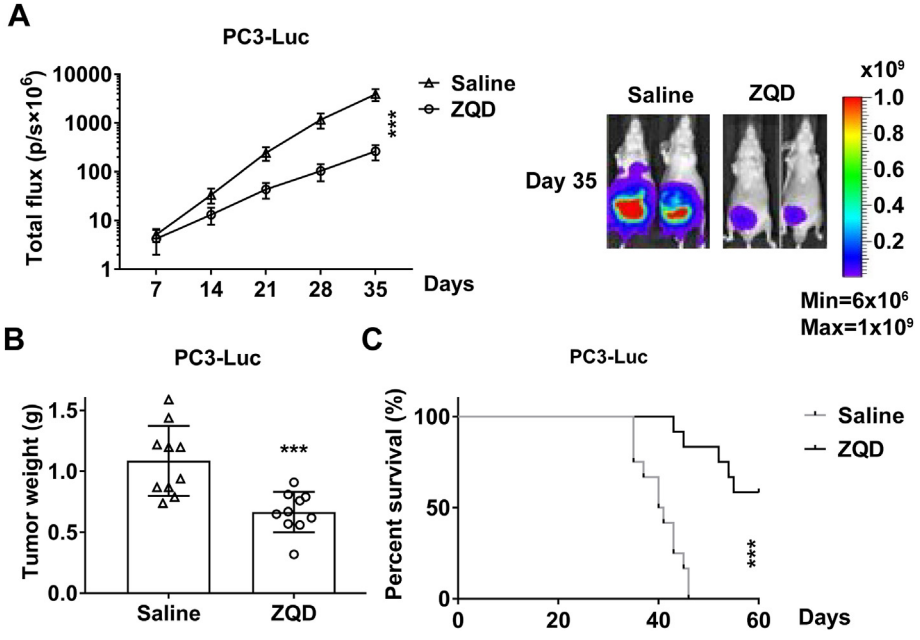

Fig. 1. ZQD inhibits the progression of PC3 prostate cancer *in vivo*. PC3-Luc tumor bearing mice were treated with saline or ZQD. (A) The tumor burden was revealed by bioluminescence imaging weekly. The significant difference of was analyzed using two way-ANOVA. (B) Tumor were removed and tumor weight at day 35 were weighted. 10 mice per group. The significant difference of was analyzed using *t*-test. (C) The percentage of survival mice in the two groups. 12 mice per group for survival assay. ***$p < 0.001$.

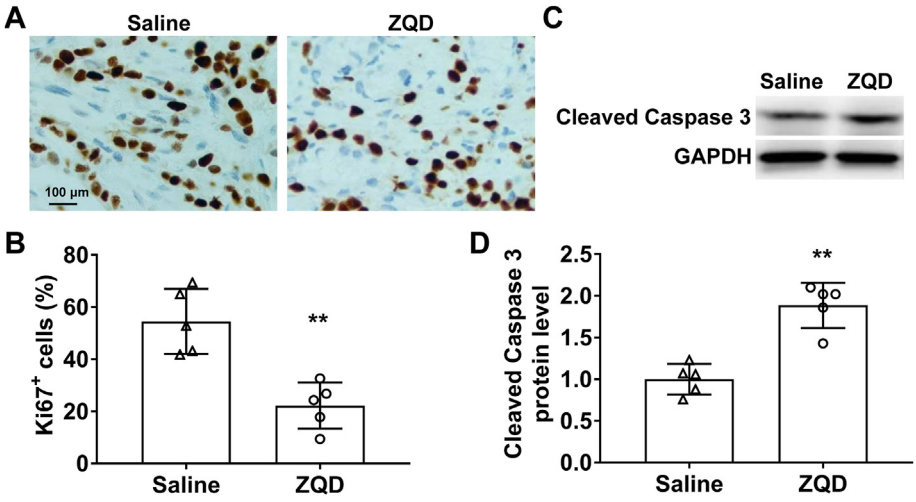

Fig. 2. ZQD decreased the proliferation but promoted apoptosis of PC3 cancer cells *in vivo*. PC3-Luc tumor bearing mice were treated with saline or ZQD. (A) Tumors were removed at day 35, then Ki67 protein level was analyzed by IHC. The representative images of Ki67 staining by IHC were shown. (B) The percentage of Ki67^{+} tumor cells in xenograft tumors were analyzed. (C) Tumors were removed at day 35, then cleaved Caspase3 protein level was analyzed by Western blot. The representative images of cleaved Caspase3 were shown. (D) Relative protein level of cleaved Caspase 3 was analyzed. n = 5. The significant difference of was analyzed using *t*-test. **p < 0.01.

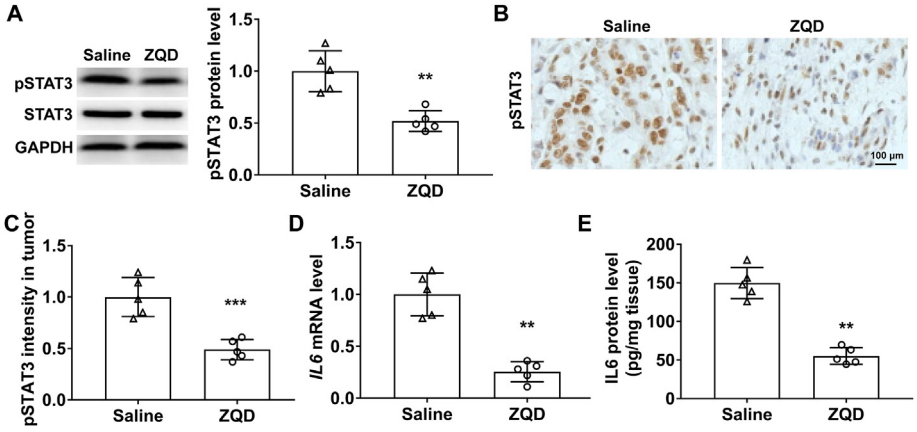

Fig. 3. ZQD modulated IL6-STAT3 signaling in PC3 xenograft tumor. PC3-Luc tumor bearing mice were treated with saline or ZQD. (A) Tumors were removed at day 35, then pSTAT3 and STAT3 protein level were analyzed by Western blot. And the representative images were shown. (B–C) Protein level of pSTAT3 in tumor were analyzed by IHC, and the representative images were shown. (D–E) Tumors were removed at day 35, then mRNA (D) and protein (E) level of human IL6 in PC3 xenograft tumors were analyzed by RT-qPCR and ELISA, respectively. n = 5. The significant difference of was analyzed using *t*-test. **p < 0.01.

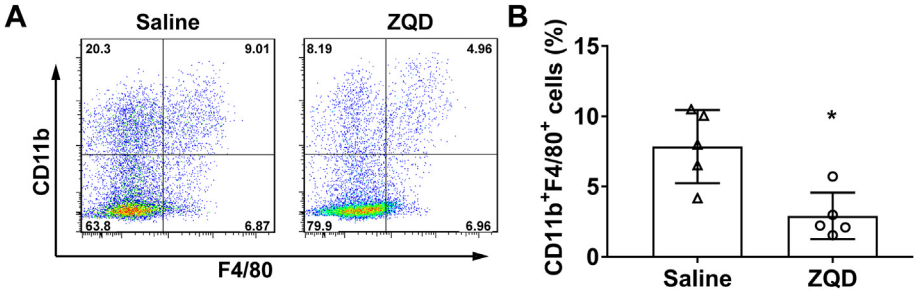

Fig. 4. ZQD modulated immune microenvironment of PC3 xenograft tumor. PC3-Luc tumor bearing mice were treated with saline or ZQD. (A) Tumors were removed at day 35, then infiltrated macrophages in tumor (CD11b^{+}F4/80^{+}) were analyzed by FACS. The representative images were shown. (B) The statistical results of (A). n = 5. The significant difference of was analyzed using *t*-test. *p < 0.05.

These results suggest that overexpression of IL6 overwhelmed the inhibitive effect of ZQD on PC the xenograft tumor. Furthermore, we measured infiltered macrophages using FACS. We found that the infiltrated macrophages CD11b + F4/80+ showed no significant change between the saline and ZQD-treated group (Fig. 6A and B). Taken together, after overexpression of IL6, the inhibitory effects of

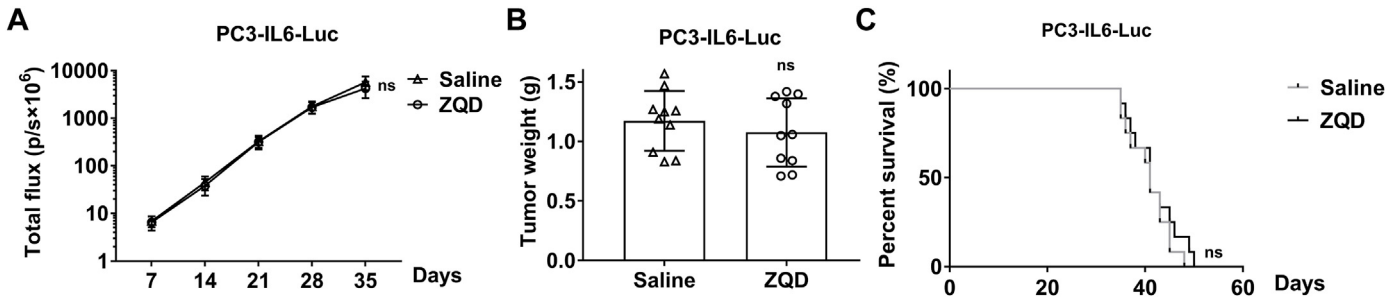

Fig. 5. The effect of ZQD on PC3-IL6-Luc xenograft tumors. PC3-IL6-Luc tumor bearing mice were treated with saline or ZQD. (A) The tumor burden was revealed by bioluminescence imaging weekly. The significant difference of was analyzed using two way-ANOVA. (B) Tumor were removed at day 35 weighted. 10 mice per group. The significant difference of was analyzed using *t*-test. (C) The percentage of survival mice in the two group. 12 mice per group for survival assay. ns, not significant.

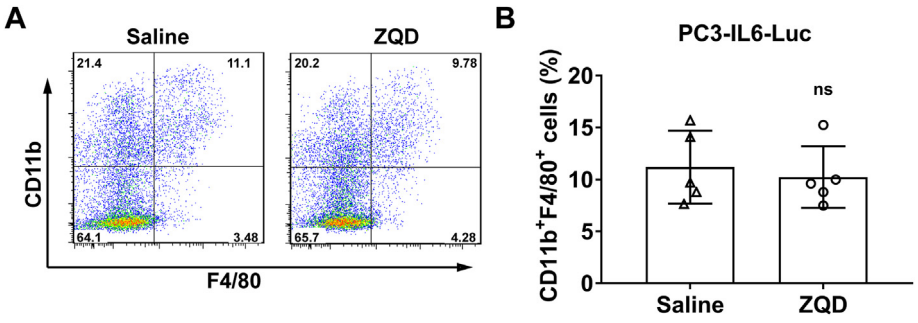

Fig. 6. ZQD had no effect on the immune microenvironment of PC3-IL6-Luc xenograft tumor. PC3-IL6-Luc tumor bearing mice were treated with saline or ZQD. (A) Tumors were removed at day 35, then infiltrated macrophages in tumor ($CD11b^+F4/80^+$) were analyzed by FACS. The representative images were shown. (B) The statistical results of (A). n = 5. The significant difference of was analyzed using *t*-test. ns, not significant.

ZQD on tumor progression and macrophage infiltration disappeared, indicating that ZQD may regulate the tumor immune microenvironment by regulating the IL6-STAT3 signaling pathway.

4. Discussion

Prostate cancer accounts for 3–5% of all cancer-related deaths in men, considering the second most common malignancy (after lung cancer) in men worldwide.[1] The common therapy to androgen deprivation therapy increased the likelihood of progression to CRPC, which has been identified as advanced prostate cancer. However, the mechanism of the progression of CRPC remains unclear. The discovery and development of an effective and safe pharmacological agent for the treatment of CRPC is urgently needed. Our study, for the first time, reported the effect of ZQD on macrophage infiltration in an in vivo xenograft tumor model. A xenograft mice model was also used to validate the regulation of ZQD macrophages that we found in in vitro experiments. Moreover, we investigated the effect of ZQD on PC3-IL6 transplanted tumors using PC3 cell lines overexpressing IL6. We found that overexpressing IL6 in PC3 derived tumors were insensitive to ZQD, validating that ZQD exerts regulatory effect on macrophages through IL6.

PC3 cell line derived the xenograft model is a well-established androgen-independent model to assess tumor growth.[14] In our study, we injected PC3 expressing luciferase into the prostatic capsule to create PC3 in the xenograft mouse model. Tumors were imaged weekly. It was found that treatment with ZQD effectively reduced tumor burden and significantly increased survival rate at 35 days after PC3 tumor transplant when compared with saline treatment. The efficacy of ZQD was confirmed by the reduction of proliferation biomarker Ki67 and elevated apoptosis executioner cleaved caspase 3 in the xenograft tumor tissue with the treatment of ZQD vs. saline. Docetaxel, the first-line chemotherapy for CRPC, has been demonstrated poor survival outcomes.[15] A 1006 patients clinical study showed no significant change in survival when administrated with docetaxel weekly. However, patients survival were extended from 17.2 months to 19.2 months when docetaxel was administrated every three weeks.[16] Mitoxantrone, a type II topoisomerase inhibitor, has been commonly used to manage CRPC. In the clinical study of 1006 patients were treated with prednisone twice a day and were randomly assigned to receive docetaxel or mitoxantrone every three weeks.[9] Results showed that treatment with docetaxel significantly improved survival and quality of life in patients with CRPC when compared with mitoxantrone treatment.[9] Notably, there was no significant difference in survival after 3 years between weekly and every three weeks treatment.[16] A novel treatment or docetaxel-based combination is still needed to overcome the poor survival benefits of current therapeutical agents.

Macrophage infiltration plays an important role in the tumor immune microenvironment and contributes to the tumor invasion, migration, and metastasis.[17–19] Macrophages secret cytokines and growth faction, promoting the alteration of tumor microenvironment.[20] The role of macrophage infiltration in tumor microenvironment inflammation is still unclear. It has been demonstrated that macrophage infiltration is associated with the progression of prostate cancer and could serve as a prognostic marker of prostate cancer.[21] It was found that a total number of tumor-associated macrophages significantly reduced after radical prostatectomy using quantitative immunohistochemistry.[21] In line with the previous study, we showed that infiltrated macrophages were significantly reduced by the treatment with ZQD when compared to saline treatment. These results revealed that ZQD effectively regulates tumor microenvironment by targeting infiltrated tumor-associated

macrophages.

STAT3 is a family member of signal transducers and activators of transcription (STAT), regulating gene transcription. Studies have shown that STAT3 phosphorylation plays an important role in promoting cell proliferation and inhibiting cell apoptosis in prostate cancer.[22,23] It has been reported that upregulation of chemokines such as CCL2 and CCL4 activates STAT3 and enhances infiltration macrophages during tumorigenesis.[23–25] The IL6/STAT3 pathway in regulating macrophage infiltration remains unclear. It was found that elevated IL6 level is contributing to the progression of prostate cancer and the reduction of survival.[26] In vitro study has shown that STAT3 was activated by phosphorylation in PC cells when treated with IL6.[27] In the current study, we found that IL6 mRNA level was significantly reduced by the treatment with ZQD. In line with IL6 results, the phosphorylated STAT3 protein level was significantly reduced by ZQD. To further investigate the role of ZQD in regulating IL6/STAT3 pathway, we established a PC3-Luc cell line overexpressing IL6 and developed PC3-IL6-Luc in the xenograft tumor mouse model. Results showed that overexpression of IL6 abolished the anti-tumor effects of ZQD. The inhibitive effect of ZQD on infiltrated macrophages was abolished in IL6 overexpression PC3 cells in the xenograft tumor. These results further confirmed that ZQD effectively regulated the immune environment by suppressing IL6/STAT3 pathway, thereby inhibiting prostate cancer tumor progression and macrophage infiltration.

There are a few limitations of this study. First, although it is not the first report on the regulation of IL6-STAT3 signaling by ZQD,[10,11] the mechanisms of inhibition of the IL6/STAT3 pathway by ZQD need further study in vitro. Lack of studies on normal mice to verify the effect of ZQD on macrophages as well as other immune cells. Further studies are also needed on the effect of ZQD on the immune microenvironment of prostate cancer. Moreover, the role of the IL6/STAT3 in promoting prostate tumor progression and enhancing macrophage recruitment needs to be included in further study. In addition, in the current study, we only compared ZQD with a first-line standard pharmacological agent docetaxel. In future research, standard treatments such as, abiraterone acetate and enzalutamide.

5. Conclusion

In conclusion, the presented study demonstrated that ZQD, a traditional Chinese decoction could effectively inhibit the progression of CRPC. ZQD treatment significantly reduced the population of infiltrated macrophages in PC3 the xenograft tumor. Additionally, our data showed that ZQD exerts anti-tumor effects via inhibiting the activation of the IL6/STAT3 pathway. Overall, our data provided evidence that ZQD could serve as a therapeutic approach for CRPC treatment.

Funding

The study was supported by Shanghai Municipal Health Commission special subject of Chinese traditional medicine research (2020JQ002); General Program of National Natural Science Foundation of China (82174199, 82274281); Shanghai Science and Technology Commission Shanghai Natural Science Foundation Project (19ZR1458200); National TCM clinical research base dragon medicine scholars (nursery plan) of LONGHUA Hospital Shanghai University of Traditional Chinese Medicine (LYTD-56); The third batch of young Chinese name training program of LongHua Hospital Shanghai University of Traditional Chinese Medicine (RC-2017-01-14).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2023.05.005.

References

1. Rawla P. Epidemiology of prostate cancer. *World J Oncol. Apr*. 2019;10(2):63–89. https://doi.org/10.14740/wjon1191.
2. Heidenreich A, Porres D. Prostate cancer: treatment sequencing for CRPC–what do we know? *Nat Rev Urol. Apr*. 2014;11(4):189–190. https://doi.org/10.1038/nrurol.2014.36.
3. Mizokami A, Namiki M. Reconsideration of progression to CRPC during androgen deprivation therapy. *J Steroid Biochem Mol Biol. Jan*. 2015;145:164–171. https://doi.org/10.1016/j.jsbmb.2014.03.015.
4. Merseburger AS, Russel C, Belz H, et al. [Early- vs. late-onset treatment using abiraterone acetate plus prednisone in chemo-naive, asymptomatic or mildly symptomatic patients with metastatic CRPC after androgen deprivation therapy]. *Aktuelle Urol*. Dec 2020;51(6):562–571. https://doi.org/10.1055/a-1121-7593. Fruhe vs. spatere Therapie mit Abirateron plus Prednison bei chemotherapie-naiven, asymptomatischen bis mild symptomatischen Patienten mit metastasiertem CRPC nach Androgendeprivation.
5. Chandrasekar T, Yang JC, Gao AC, Evans CP. Mechanisms of resistance in castration-resistant prostate cancer (CRPC). *Transl Androl Urol. Jun*. 2015;4(3):365–380. https://doi.org/10.3978/j.issn.2223-4683.2015.05.02.
6. Nagao K, Matsuyama H. [Docetaxel chemotherapy against CRPC]. *Nihon Rinsho. May 20* 2016;74(Suppl 3):619–623.
7. Meulenbeld HJ, van Werkhoven ED, Coenen JL, et al. Randomised phase II/III study of docetaxel with or without risedronate in patients with metastatic castration resistant prostate cancer (CRPC), The Netherlands prostate study (NePro). *Eur J Cancer. Nov*. 2012;48(16):2993–3000. https://doi.org/10.1016/j.ejca.2012.05.014.
8. Phillips R. Prostate cancer: dasatinib fails to improve on docetaxel for metastatic CRPC. *Nat Rev Urol. Jan*. 2014;11(1):5. https://doi.org/10.1038/nrurol.2013.283.
9. Tannock IF, de Wit R, Berry WR, et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N Engl J Med*. Oct 7 2004;351(15):1502–1512. https://doi.org/10.1056/NEJMoa040720.
10. Cao H, Wang D, Gao R, Chen L, Feng Y, Gao D. Zhoushi Qiling decoction induces apoptosis of human prostate cancer cells via miR-143/Bcl-2 axis. *Aging (Albany N Y)*. Jun 25 2021;13(13):17202–17210. https://doi.org/10.18632/aging.203171.
11. Cao H, Wang D, Gao R, Feng Y, Chen L. Qi Ling decreases paclitaxel resistance in the human prostate cancer by reversing tumor-associated macrophages function. *Aging (Albany N Y)*. Feb 22 2022;14(4):1812–1821. https://doi.org/10.18632/aging.203904.
12. Dai H, Wang C, Yu Z, et al. MiR-17 regulates prostate cancer cell proliferation and apoptosis through inhibiting JAK-STAT3 signaling pathway. *Cancer Biother Radiopharm. Apr*. 2018;33(3):103–109. https://doi.org/10.1089/cbr.2017.2386.
13. Huang R, Wang S, Wang N, et al. CCL5 derived from tumor-associated macrophages promotes prostate cancer stem cells and metastasis via activating β-catenin/STAT3 signaling. *Cell Death Dis*. 2020/04/16 2020;11(4):234. https://doi.org/10.1038/s41419-020-2435-y.
14. Wu X, Gong S, Roy-Burman P, Lee P, Culig Z. Current mouse and cell models in prostate cancer research. *Endocr Relat Cancer*. Aug 2013;20(4):R155–R170. https://doi.org/10.1530/ERC-12-0285.
15. Galsky MD, Vogelzang NJ. Docetaxel-based combination therapy for castration-resistant prostate cancer. *Ann Oncol. Nov*. 2010;21(11):2135–2144. https://doi.org/10.1093/annonc/mdq050.
16. Berthold DR, Pond GR, Soban F, de Wit R, Eisenberger M, Tannock IF. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer: updated survival in the TAX 327 study. *J Clin Oncol*. 2008;26(2):242–245. https://doi.org/10.1200/JCO.2007.12.4008.
17. Squadrito ML, De Palma M. Macrophage regulation of tumor angiogenesis: implications for cancer therapy. *Mol Aspect Med*. Apr 2011;32(2):123–145. https://doi.org/10.1016/j.mam.2011.04.005.
18. Zhang F, Lu W, Dong Z. Tumor-infiltrating macrophages are involved in suppressing growth and metastasis of human prostate cancer cells by INF-beta gene therapy in nude mice. *Clin Cancer Res. Sep*. 2002;8(9):2942–2951.
19. Andersen LB, Norgaard M, Rasmussen M, et al. Immune cell analyses of the tumor microenvironment in prostate cancer highlight infiltrating regulatory T cells and macrophages as adverse prognostic factors. *J Pathol*. Jul 13 2021. https://doi.org/10.1002/path.5757.
20. Leek RD, Harris AL, Lewis CE. Cytokine networks in solid human tumors: regulation of angiogenesis. *J Leukoc Biol*. Oct 1994;56(4):423–435. https://

doi.org/10.1002/jlb.56.4.423.
21. Shimura S, Yang G, Ebara S, Wheeler TM, Frolov A, Thompson TC. Reduced infiltration of tumor-associated macrophages in human prostate cancer: association with cancer progression. *Cancer Res*. Oct 15 2000;60(20):5857–5861.
22. Mora LB, Buettner R, Seigne J, et al. Constitutive activation of Stat3 in human prostate tumors and cell lines: direct inhibition of Stat3 signaling induces apoptosis of prostate cancer cells. *Cancer Res. Nov 15*. 2002;62(22):6659–6666.
23. Barton BE, Karras JG, Murphy TF, Barton A, Huang HF. Signal transducer and activator of transcription 3 (STAT3) activation in prostate cancer: direct STAT3 inhibition induces apoptosis in prostate cancer lines. *Mol Cancer Ther. Jan*. 2004;3(1):11–20.
24. Fang LY, Izumi K, Lai KP, et al. Infiltrating macrophages promote prostate tumorigenesis via modulating androgen receptor-mediated CCL4-STAT3 signaling. *Cancer Res. Sep 15*. 2013;73(18):5633–5646. https://doi.org/10.1158/0008-5472.CAN-12-3228.
25. Izumi K, Fang LY, Mizokami A, et al. Targeting the androgen receptor with siRNA promotes prostate cancer metastasis through enhanced macrophage recruitment via CCL2/CCR2-induced STAT3 activation. *EMBO Mol Med. Sep*. 2013;5(9):1383–1401. https://doi.org/10.1002/emmm.201202367.
26. Nakashima J, Tachibana M, Horiguchi Y, et al. Serum interleukin 6 as a prognostic factor in patients with prostate cancer. *Clin Cancer Res. Jul*. 2000;6(7):2702–2706.
27. Ueda T, Bruchovsky N, Sadar MD. Activation of the androgen receptor N-terminal domain by interleukin-6 via MAPK and STAT3 signal transduction pathways. *J Biol Chem. Mar 1*. 2002;277(9):7076–7085. https://doi.org/10.1074/jbc.M108255200.