# Dihydroartemisinin Ameliorates Chronic Nonbacterial Prostatitis and Epithelial Cellular Inflammation by Blocking the E2F7/HIF1α Pathway

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**Research**

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# Abstract

**Objective:** Chronic nonbacterial prostatitis (CNP) has remained one of the most prevalent urological diseases, particularly in older men. Dihydroartemisinin (DHA) has been identified as a semi-synthetic derivative of artemisinin that exhibits broad protective effects. However, the role of DHA in inhibiting CNP inflammation and prostatic epithelial cell proliferation remains largely unknown.

**Materials and Methods:** CNP mice model was induced by carrageenan and Haemotoxylin Eosin (HE) ,immunofluorescence and immunochemistry staining were used to confirm CNP and E2F7 expression. Human prostatic epithelial cells (HPECs) and RWPE-1 was induced by lipopolysaccharide (LPS) to mimic CNP model *in vitro*. Real-time quantitative PCR and Western blot were used to detect proliferation and inflammatory genes expression. Cell proliferation was determined using MTT assay.

**Results:** DHA significantly alleviated the rough epithelium and inhibited multilamellar cell formation in the prostatic gland cavity and prostatic index induced by carrageenan. In addition, DHA decreased the expression of TNF-α and IL-6 inflammatory factors in prostatitis tissues and in LPS-induced epithelial cells. Upregulation of transcription factor E2F7, which expression was inhibited by DHA, was found in CNP tissues, human BPH tissues and LPS-induced epithelial cells inflammatory response. Mechanically, we found that depletion of E2F7 by shRNA inhibited epithelial cell proliferation and LPS-induced inflammation while DHA further enhance these effects. Furthermore, HIF1α was transcriptional regulated by E2F7 and involved in E2F7-inhibited CNP and cellular inflammatory response. Interestingly, we found that inhibition of HIF1α blocks E2F7-induced cell inflammatory response but does not obstruct E2F7-promoted cell growth.

**Conclusion:** The results revealed that DHA inhibits the CNP and inflammation by blocking the E2F7/HIF1α pathway. Our findings provide new evidence for the mechanism of DHA and its key role in CNP, which may provide an alternative solution for the prevention and treatment of CNP.


# 1. Introduction

Chronic nonbacterial prostatitis (CNP), characterized by pelvic discomfort, voiding and pain symptoms, and even sexual dysfunction, is a common urological disease mainly occurring in older men [1,2]. In clinical practice, patients with CNP account for more than 90% of those with chronic prostatitis [3]. Over the past few decades, several hypotheses have been proposed to explain the pathogenesis of CNP, including urothelial integrity and dysfunction, recessive infection, autoimmunity, endocrine imbalance, neuroplasticity, and psychosocial conditions [1,4]. Given the close relationship between CNP and male infertility, its prevention and treatment can enhance male reproductive function [5]. Although the specific mechanism and etiology of CNP remain unclear, recent studies have shown that inflammatory disorders play a key role in the pathogenesis of CNP[6].

Dihydroartemisinin (DHA), a semi-synthetic derivative of artemisinin, is a more water-soluble and effective antimalarial agent than artemisinin [7]. Studies found that DHA also inhibits cell proliferation, inflammation, angiogenesis, cell migration, etc. [8]. Our previous research found that dihydroartemisinin reduces the proliferation and inflammation of vascular smooth muscle cells induced by high glucose by inhibiting the miR-376b-3p/KLF15 pathway[9]. Ling et al. reported that DHA inhibits vascular endothelial growth factor-induced endothelial cell proliferation and migration through the p38 mitogen-activated protein kinase-independent pathway [10]. DHA can also target VEGFR2 by regulating the NF-kB pathway in endothelial cells, thereby inhibiting angiogenesis [11]. However, little is known regarding the underlying protective effects of DHA on prostatitis.

The E2F family of transcription factors has been known to regulate multiple genes responsible for cell proliferation, differentiation, apoptosis, and DNA damage response [12]. E2Fs can be classified as activators (E2F1, E2F2, and E2F3a) and inhibitors (E2F3b, E2F4, E2F5, E2F6, E2F7, and E2F8) based on their various functions, as well as typical (E2F1-6) and atypical (E2F7 and E2F8) on their structural characteristics [13]. Some studies reported that E2F7 plays an essential role in the regulation of cell cycle progression and is associated with several diseases, including lung adenocarcinoma, liver cancer, and head and neck cancer, that promote cell proliferation, inflammation, and metastasis [14–16]. One recent study reported that E2F7 promoted cell proliferation in glioblastoma [17]. However, the role of E2F7 in CNP remains unclear.

The present study utilized *in vivo* and *in vitro* analyses to examine the role of DHA in CNP. Accordingly, our results showed that DHA exerts anti-proliferative and anti-inflammatory effects by inhibiting the E2F7/HIF1α axis in CNP. The aforementioned findings provide new evidence for the mechanism of DHA in CNP treatment, thereby improving our understanding of phytochemicals.

## 2. Materials And Methods

# 2.1. Animal models

Experiments were conducted on 6–11 week-old male C57BL/6 mice. All mice were purchased from Beijing Vital River Laboratory Animal Technology Co.,Ltd. Accordingly, 50 mice were housed in the Hebei Province of Chinese Medicine/Affiliated Hospital of Hebei University of Chinese medicine animal facilities on a 12-hr light-dark cycle (7 a.m. to 7 p.m., light) in specific pathogen-free caging with free access to food and water in specific pathogen-free caging. These mice were randomly divided into five groups (n = 10): the con group, the carrageenan-induced CNP group, and the CNP with the DHA treatment group (1, 4, and 8 mg/kg/d). All mice were killed that were injected with intraperitoneal injection of pentobarbital (5%, 50mg/Kg) for anesthesia after 4 weeks, after which their prostates were immediately separated and weighed for prostate index analysis. The prostate index was calculated as follows: prostate index = prostate weight (mg)/bodyweight (g). Prostate tissues were then frozen for later use.

# 2.2. Clinical samples

Human benign prostatic hyperplasia (BPH) were collected from the Department of Urology, Hebei Province of Chinese Medicine from July 2013 to June 2020. All patients underwent radical nephrectomy for treatment. Normal prostate tissue specimens were also obtained from organ donors to act as controls. The research protocol has been approved by the Ethics Committee of Hebei Province of Chinese Medicine, and each patient's written consent has been obtained.

## 2.3. Cell culture and treatment

Human prostate epithelial cells RWPE-1 was purchased from Procell (Wuhan, China) and cultured in K-SFM mediates with supplemented with 0.05 mg/mL BPE and 5 ng/mL EGF and 1% P/S (PB180120). Primary cultures of human prostate epithelial cells (HPEC) established from prostatectomy specimens represent prostate progenitor cells or transit amplification cells. In short, the tissue was minced and digested with collagenase overnight. Inoculate the digested tissue into a petri dish coated with type I collagen and containing PFMR-4A medium supplemented with 10 ng/ml cholera toxin, 10 ng/ml epidermal growth factor, 40 μg/ml bovine pituitary extract, 4 μg/ml insulin, 1 μg/ml hydrocortisone, 100 μg/ml gentamicin, 0.1 mm phosphoethanolamine, 3 nm selenious acid, 2.3 μ m α-tocopherol, and 0.03 nm total reaction Formula retinoic acid. The cells grown in the primary culture were aliquoted and stored frozen in liquid nitrogen. The epithelial properties of these cells were confirmed by immunocytochemical staining of cytokeratin [18].

## 2.4. Western blotting

Proteins from RWPE-1 cells, HPECs, and prostate tissue were extracted using RIPA lysis buffer, after which they were separated through 8–10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane (Millipore). The membranes were then blocked with 5% milk solution in Tris-buffered saline for 2 h at 37°C and incubated overnight at 4°C with certain antibodies. Thereafter, the membranes were incubated with a 1:5000 dilution of anti-rabbit/horseradish peroxidase or anti-mouse/horseradish peroxidase (Santa Cruz Biotechnology) for 2 h and analyzed using the Chemiluminescence Plus Western Blot Analysis kit. All antibodies used were as follows: anti-E2F7 (1:1000; PAB12815), anti-VEGFR1 (1:1000; 13687-1-AP), anti-TNF-α (1:500, 17590-1-AP) anti-HIF1α (1:1000; 66730-1-Ig), and anti-β-actin (1:1000; 66009-1-Ig).

## 2.5. RNA isolation, reverse transcription, and real-time polymerase chain reaction

Total RNA from prostate tissues of CNP mice or RWPE-1 cells and HPECs was isolated using the RNAqueous kit (Ambion). RNA concentration and purity were evaluated using NanoDrop 2000 (Thermo Fisher). Reverse transcription was conducted using the M-MLV First Strand Kit (Life Technologies), whereas real-time polymerase chain reaction (PCR) was conducted using the Platinum SYBR Green quantitative PCR Super Mix UDG Kit (Invitrogen). Gene expression levels were determined using the comparative $2^{-\triangle\triangle Ct}$ method. All primers were listed in supplementary table 1.

## 2.6. MTS assay

A total of $1 \times 10^4$ cells were seeded into 96-well plates. After 24 h, cells received corresponding treatments according to the experimental design. The medium was then removed, and the cells were washed with PBS. Thereafter, 2 mg/mL MTS reagent was added to Hank's buffer and incubated for 1 h until dark blue crystals are observed in the cytoplasm under an optical microscope. The crystal was dissolved in DMSO, after which the absorbance was measured using a 490 nm thermal fluorescence scanning Ascent spectrometer, with the background-subtracted at 650 nm.

## 2.7. Colony formation assay

A total of 100 RWPE-1 cells and HPECs were seeded into six-well plates, cultured for 1 week, and then fixed with glacial acetic acid/methanol solution. Thereafter, the colony was stained with 0.5% crystal violet, and colony formation was analyzed under a microscope.

## 2.8. Morphometry and histology

Fresh CNP,prostate and BPH tissue are fixed in formalin. Conventional paraffin embeds the tissue. Tissue sections are 5-μm thick and stained with hematoxylin and eosin. The cross-sectional image was acquired with a Leica microscope (Leica DM6000B, Switzerland) and digitized with LAS V.4.4 (Leica).

## 2.9. Immunohistochemistry

The 4 μm indicated tissue section was decompressed and immersed in water for high-pressure repair. Use according to the instructions for immunohistochemistry (SP0041,Solarbio). Use primary anti-rabbit anti-human polyclonal antibody E2F7 (1:50, 24489-1-AP). Five media field microscopes were randomly selected to take pictures, and the percentage of brown particles in each field of view was quantitatively analyzed using IPP version 6.0 image analysis software.

## 2.10. Immunofluorescence staining

The 4 μm indicated tissue section was decompressed and washed with PBS. After preincubated with 10% normal goat serum (710 027, KPL), the cell smears were incubated with primary antibody anti-E2F7, anti-DMP1 at 37°C for 2 h. The secondary antibody was the fluorescence-labeled rabbit IgG antibody. Finally, the cell smears were incubated in DAPI for 10 min for nuclear counterstaining. Images were acquired using confocal microscopy (DM6000CFS, Leica) and digitized with LAS AF software.

## 2.11. Statistical analysis

Data were presented as mean ± SEM. Differences between two groups were analyzed using Student's *t* test, whereas correlations between two genes were analyzed using Spearman's conjunction analysis, with $p < 0.05$ indicating statistical significance.

# 3. Results

## 3.1 DHA alleviates carrageenan-induced CNP and prostate epithelial cells inflammatory response

Our previous research found that DHA reduces smooth muscle cell proliferation and inflammation induced by high glucose. However, it is unclear whether DHA can inhibit CNP. We first constructed a CNP mice model by used carrageenan, and then with different concentration of DHA treatment. Haemotoxylin& Eosin (H&E) staining results showed that carrageenan treatment significantly increased rough epithelium and non-monolayer cells in the gland cavity (Fig. 1A). However, DHA treatment markedly reduced these effect, especially when treating with a dose of 8 mg/kg (Fig. 1A). Next, we calculated the prostate index to observe the effect of DHA on carrageenan-induced CNP. As shown in Fig. 1B, carrageenan stimulation significantly increased in the prostatic index in CNP. Meanwhile, DHA dose-dependently decreased the carrageenan-induced CNP. In order to explore whether DHA inhibits CNP by reduced inflammation, we detected inflammatory factors in the CNP tissues. The results showed that that carrageenan treatment significantly increased TNF-α and IL-6 expression, whereas DHA decreased these factors level in a dose-dependent manner in CNP tissues (Fig. 1C and D). In order to verify whether DHA also exerts anti-inflammatory effects at the cellular level, cultured RWPE-1 and HPECs were treated with different concentrations of DHA after being stimulated by LPS. As showed in Fig. 1E and F, DHA significantly suppressed LPS-increased the expression of TNF-α and IL-6 in RWPE-1 and HPECs cells. Taken together, these results demonstrated that DHA play a role in reducing CNP and anti-inflammatory response in epithelial cells.

## 3.2 E2F7 participates in DHA-inhibited carrageenan-induced CNP and cell inflammatory response

To investigate how DHA depressed CNP and cellular inflammatory response, RT-qPCR was used to detected inflammatory genes expression in RWPE-1 cells that stimulated by LPS and with or without DHA treatment. The results showed that SMAD3, E2F7, RARB and ZNF730 were abnormal expression in PLS-treated RWPE-1, and these effects was reversed by DHA treatment (Fig. 2A). Next, we verify these genes expression in CNP tissues. As showed in Fig. 2B, only E2F7 was upregulated in carrageenan-induced CNP tissues and downreguated in DHA-treated CNP tissues. In order to examine whether E2F7 expression was elevated in clinical sample, we collected the BPH and normal prostate tissues from donors and detected E2F7 mRNA level by RT-qPCR. The results revealed that E2F7 mRNA expression significantly increased in BPH tissues than that in normal prostate tissues (Fig. 3C). Furthermore, immunofluorescence staining further confirmed this result (Fig. 3D and E). Besides, we detected E2F7 expression in epithelial cells with different concentration of DHA after LPS stimulation. Western blot and RT-qPCR showed that DHA inhibited LPS-induced E2F7 expression in dose dependent (Fig. 2F to H). Additionally, we measured E2F7 expression in carrageenan-induced CNP tissues by using immunochemistry staining. As showed in Fig. 2I, the expression of E2F7 markedly elevated in epithelial cell of CNP tissues while DHA decreased E2F7 expression in these cells in dose dependent. Taken together, these results suggested that E2F7 was

involved in carrageenan-induced CNP and DHA inhibited CNP and cell inflammation by downregulating E2F7 expression.

## 3.3 Downregulation of E2F7 inhibits prostatic epithelial cell proliferation and inflammation

To study the potential mechanism of E2F7 on CNP, we first designed two shRNAs specifically targeting E2F7. As shown in Fig. 3A, B, and C, both shE2F7-1# and shE2F7-2# vectors transfection significantly reduced E2F7 expression in transcription and protein level. Next, a colony formation assay was used to determine the proliferation effect of E2F7. Accordingly, our results showed that E2F7 knockout inhibited cell colony formation, whereas DHA treatment enhanced this effect in both RWPE-1 and HPECs cells (Fig. 3D). Besides, MTS assay showing the same results (Fig. 3E and F). These results demonstrated that DHA inhibits epithelial cell proliferation by decreasing E2F7 expression. Furthermore, RT-qPCR showed that E2F7 knockdown decreased TNF-α and IL-6 mRNA levels in RWPE-1 and HPECs cells (Fig. 3G and H). Together, these results indicated that E2F7 downregulation inhibited epithelial cell proliferation and inflammation, whereas DHA treatment enhanced this effect.

## 3.4 E2F7 regulates HIF1α expression and promotes cellular inflammation

Previous studies have already reported that E2F7 forms a transcriptional complex with hypoxia-inducible factor (HIF) 1 to stimulate downstream gene expression[19]. So we explored whether HIF1α was involved in E2F7-inhibited CNP. We first confirmed the HIF1α expression in transfected shE2F7-1# or shE2F7-2-transfected RWPE-1 and HPECs cells. RT-qPCR and western blot results showed that cell transfection with the E2F7-knockout vectors obviously decreased HIF1α mRNA and protein levels in RWPE-1 and HPECs cells (Fig. 4A, B, and C). Next, the Ensembl and Promo 3.0 website approaches were used to analysis potential binding sites on the 2-kb 5' promoter region of HIF1α, through which we found three potential binding elements within this region (Fig. 4D). ChIP analysis confirmed that E2F7 was bound predominantly to − 20 to − 100 nt of the HIF1α promoter (Fig. 4E), suggesting that E2F7 transcriptional regulated HIF1α expression. Previous studies have shown that HIF1α plays an essential regulatory role in cellular inflammation [20,21]. As a result, we examined HIF1α expression in E2F7-depleted epithelial cells after LPS treatment. RT-qPCR analysis revealed that LPS treatment significantly upregulated HIF1α expression. However, E2F7 knockout markedly decreased HIF1α expression regardless of LPS treatment or not (Fig. 4F). Furthermore, HIF1α expression in clinical sample or prostate tissues with CNP was determined using RT-PCR. The results showed that HIF1α expression significantly increased in BPH or CNP tissues (Fig. 4G and H), whereas DHA dose-dependently reversed its expression in CNP tissues(Fig. 4H). Clearly, HIF1α was transcriptional regulated by E2F7 and involved in E2F7-inhibited inflammation.

## 3.5 Inhibition of HIF1α blocks E2F7-induced cell inflammatory response but does not in E2F7-promoted cell growth

The aforementioned results showed that E2F7 increased HIF1α expression and enhanced cell inflammatory response; however, whether HIF1α affects the proliferation of E2F7-regulated cells remains unclear. Therefore, we constructed a recombinant plasmid expressing HIF1α. Using RT-PCR and western blotting, HIF1α overexpression was confirmed in RWPE-1 and HPECs cells. Figure 5A, B, and C shows that HIF1α overexpression promoted E2F7 expression. Next, RWPE-1 and HPECs cells were simultaneously transfected with E2F7 overexpression or shHIF1α vectors to examine HIF1α involvement in E2F7-regulated cell proliferation and inflammation. Figure 5D shows that E2F7 overexpression significantly increased, whereas shHIF1α transfection reduced the number of colonies but did not reverse the promotional effect of E2F7. The MTS assay obtained the same results (Fig. 5E). These findings suggested that HIF1α depletion did not affect the proliferative effects of E2F7 on cells. Interestingly, E2F7 overexpression increased TNF-α and IL-6 mRNA levels, whereas HIF1α knockout partially reversed this effect (Fig. 5F and G). Together, these results suggested that HIF1α mediated E2F7-regulated cell inflammatory response but not E2F7-promoted cell proliferation.

## 3.6 DHA inhibits the CNP and inflammation by blocking the E2F7/HIF1α pathway.

The aforementioned results showed that DHA was able to suppress E2F7 expression and reduce HIF1α levels. Therefore, we aimed to investigate whether E2F7/HIF1α participated in DHA-regulated cell proliferation and inflammation. As showed in Fig. 6A and B, knocked down of HIF1α did not inhibit E2F7 and VEGFR expression but reduced TNF-α protein levels. However, DHA treatment decreased E2F7, VEGFR, and HIF1α expression, as well as TNF-α protein levels. However, the expression of TNF-α was further reduced in both HIF1α-knockout and DHA-treated cells. Thereafter, RT-PCR was used to further examine TNF-α and IL-6 expression in RWPE-1 and HPECs cells after DHA treatment and shHIF1α transfection. Our results showed that DHA suppressed TNF-α and IL-6 mRNA levels, with HIF1α knockout enhancing this effect (Fig. 6C). Taken together, these results suggested that DHA inhibited cell proliferation and inflammation by disturbing the E2F7/HIF1α pathway (Fig. 6D).

## 4. Discussion

In this study, we investigated the anti-proliferation and anti-inflammation role of DHA in CNP. First, it was found that DHA inhibited carrageenan-induced CNP and prostate epithelial cells inflammatory response. Secondly, E2F7 was involved in carrageenan-induced CNP and DHA inhibited CNP and cell inflammation by downregulating E2F7 expression. Third, depletion of E2F7 reduced prostatic epithelial cell proliferation and inflammation. In addition, we found that HIF1α was transcriptional regulated by E2F7 and involved in E2F7-inhibited inflammation. Inhibition of HIF1α blocks E2F7-induced cell inflammatory response but

does not in E2F7-promoted cell growth. Our research results indicate that DHA inhibits CNP and inflammation by blocking the E2F7/HIF1α pathway, which may provide an alternative solution for the prevention and treatment of CNP.

A increasing evidence indicated that cell proliferation and inflammation play a key role in CNP [22]. However, the pathogenesis of CNP remains to be clarified. Inflammation leads to chronic prostatitis and therefore promotes cell proliferation. Considering that inflammation may cause chronic prostatitis, pro-inflammatory cytokines have been suspected to be closely related to chronic prostatitis [23]. Our research indicated that TNF-α and IL-6 levels were upregulated in CNP, whereas DHA treatment decreased the expression of both. CNP-induced inflammation has been identified through the presence of lymphocyte infiltration [24]. One study reported that epithelial cell proliferation in prostatitis and GHRH may promote prostatitis progression [25]. The present study showed that CNP promotes a significant proliferation of epithelial cells, which had been reversed with DHA. Although DHA inhibited cell proliferation and inflammation, the mechanisms through which DHA regulated this effect require further investigation.

DHA, a semi-synthetic derivative of artemisinin, is a more water-soluble and effective antimalarial agent than artemisinin [7]. One study reported that DHA could inhibit cell proliferation, inflammation, angiogenesis, and migration, among other processes. Moreover, Ling Guo et al. reported that DHA inhibited endothelial cell migration and reduced vascular endothelial growth through a p38 mitogen-activated protein kinase-independent pathway [10]. Our previous research showed that DHA inhibited cell proliferation and reduced inflammation by inhibiting the miR-376b-3p/KLF15 pathway in high glucose-induced VSMCs [9]. However, the role of DHA in carrageenan-induced CNP remains unknown. The present study showed that CNP-induced endothelial cell proliferation increased E2F7 expression. However, DHA was able to dose-dependently inhibit this effect. Additionally, our findings demonstrated that DHA increased HIF1α expression by directly targeting E2F7.

HIFs are transcription factors that react to alterations in obtainable oxygen at the cellular level. HIFs are heterodimeric complex proteins comprising an alpha (HIF1α, HIF2A, or HIF3A) and beta (HIF1B) subunits [26]. HIF1α is considered a major transcriptional regulator that responds to hypoxia and promotes glycolysis to regulate glucose metabolism. One study reported that HIF1α upregulates PAFAH1B2 protein and mRNA levels to regulate the transition between epithelial and mesenchymal phenotypes and promote migratory in PDAC cells [27]. HIF1α participates in multiple processes, such as tumorigenesis, angiogenesis, proliferation, metabolism, metastasis, differentiation, and response to radiation therapy [28]. However, its effects on CNP remains unknown.

# 5. Conclusion

In summary, LPS treatment upregulated E2F7 in RWPE-1 cells and HPECs, whereas DHA treatment reversed this effect. Furthermore, LPS exposure increased HIF1α in RWPE-1 cells and HPECs, which directly increased E2F7 expression. DHA exerts anti-proliferative and anti-inflammatory effects by

inhibiting the E2F7/HIF1α axis in CNP. Our findings provide new evidence for the mechanism of DHA and its key role in CNP, which may provide an alternative solution for the prevention and treatment of CNP. However, further research is required to determine its mechanism and elucidate indications for its clinical application.

# Abbreviations

CNP, Chronic nonbacterial prostatitis; DHA, Dihydroartemisinin; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor; IL-6, interleukin-6; ChIP, chromatin immunoprecipitation; qRT-PCR, quantitative polymerase chain reaction; FBS, fetal bovine serum.

# Declarations

### Conflicts of Interest

The authors declare that there are no conflicts of interests.


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### Author Contribution

Conception and design: Y.Z., Y.L. Development of methodology: Y.Z., J.W., J.H., J.F., and K.G. Acquisition of the data (provided animals, provided facilities and so on): F.D., W.C., and Y.Z. Analysis and interpretation of the data (for example, statistical analysis, biostatistics and computational analysis): J.W., J.H., and K.G. Writing, review and/or revision of the manuscript: Y.Z., J.W., K.G., and Y.L. Administrative, technical or material support (that is, reporting or organizing the data, constructing the databases): Y.Z., J.H., F.D., and W.C. Study supervision: K.G., W.C., and Y.L.


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### Ethics approval

The present study was authorized Ethics Committee of Hospital of Hebei University of Chinese Medicine. All animal studies were approved by the Institutional Animal Care and Use Committee of Hospital of Hebei University of Chinese Medicine (20090037) and were made to minimize suffering.

### Data Availability

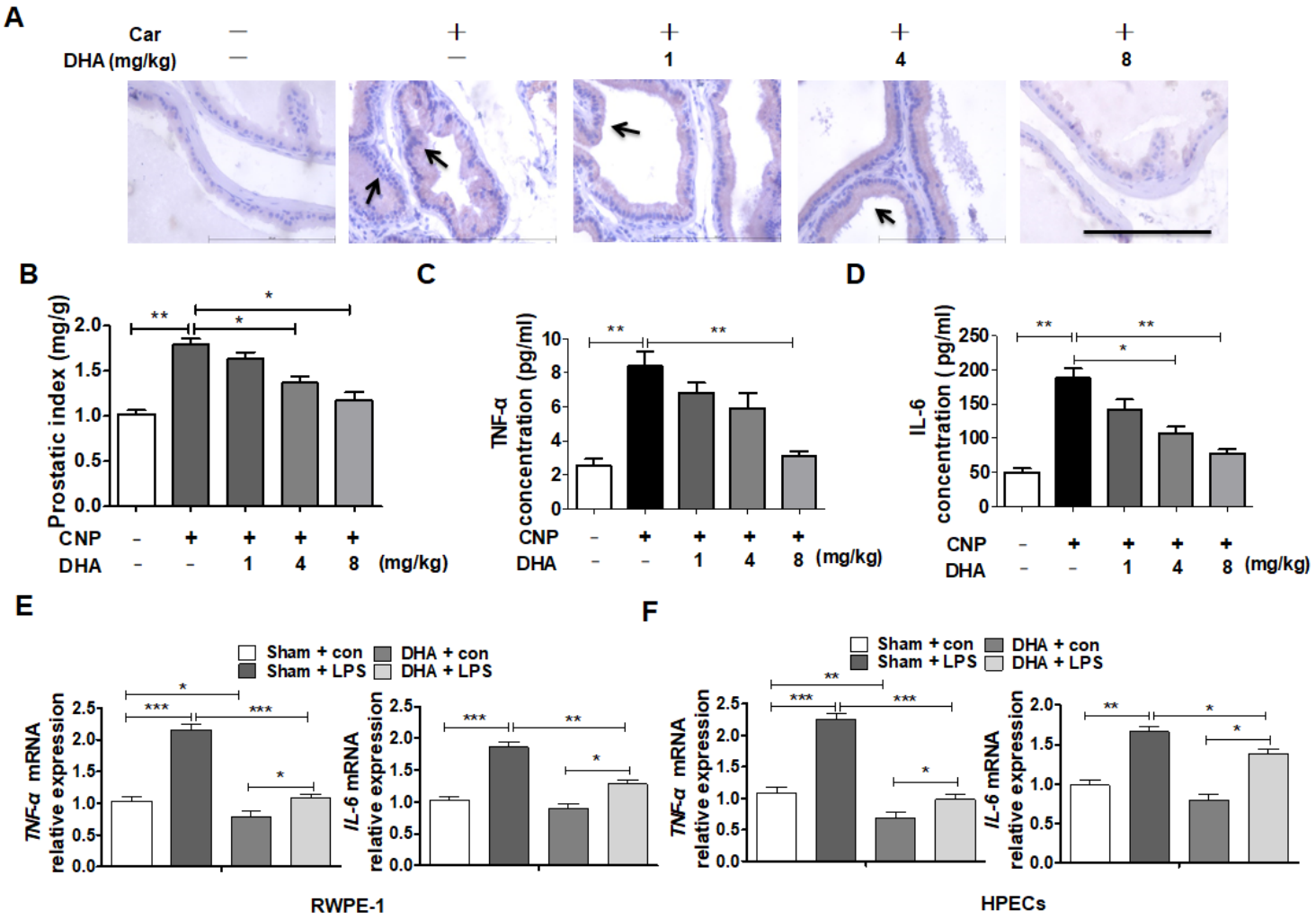
The data used to support the findings of this study are available from the corresponding author upon request.

# References


1. Zhang, L.*, et al.* MicroRNA expression profile in chronic nonbacterial prostatitis revealed by next-generation small RNA sequencing. *Asian J Androl* **21**, 351-359 (2019).
2. Polackwich, A.S. & Shoskes, D.A. Chronic prostatitis/chronic pelvic pain syndrome: a review of evaluation and therapy. *Prostate Cancer Prostatic Dis* **19**, 132-138 (2016).
3. Holt, J.D., Garrett, W.A., McCurry, T.K. & Teichman, J.M. Common Questions About Chronic Prostatitis. *Am Fam Physician* **93**, 290-296 (2016).
4. Breser, M.L., Salazar, F.C., Rivero, V.E. & Motrich, R.D. Immunological Mechanisms Underlying Chronic Pelvic Pain and Prostate Inflammation in Chronic Pelvic Pain Syndrome. *Front Immunol* **8**, 898 (2017).
5. Tantawy, S.A., Elgohary, H.M. & Kamel, D.M. Trans-perineal pumpkin seed oil phonophoresis as an adjunctive treatment for chronic nonbacterial prostatitis. *Res Rep Urol* **10**, 95-101 (2018).
6. Xiong, Y., Zhou, L., Qiu, X. & Miao, C. Anti-inflammatory and anti-hyperplastic effect of Bazhengsan in a male rat model of chronic nonbacterial prostatitis. *J Pharmacol Sci* **139**, 201-208 (2019).
7. Klayman, D.L. Qinghaosu (artemisinin): an antimalarial drug from China. *Science* **228**, 1049-1055 (1985).
8. Zhang, F.*, et al.* Dihydroartemisinin inhibits TCTP-dependent metastasis in gallbladder cancer. *J Exp Clin Cancer Res* **36**, 68 (2017).
9. Yang, B.*, et al.* Dihydroartemisinin alleviates high glucose-induced vascular smooth muscle cells proliferation and inflammation by depressing the miR-376b-3p/KLF15 pathway. *Biochemical and biophysical research communications* **530**, 574-580 (2020).
10. Guo, L.*, et al.* Dihydroartemisinin inhibits vascular endothelial growth factor-induced endothelial cell migration by a p38 mitogen-activated protein kinase-independent pathway. *Exp Ther Med* **8**, 1707-1712 (2014).
11. Dong, F.*, et al.* Dihydroartemisinin targets VEGFR2 via the NF-kappaB pathway in endothelial cells to inhibit angiogenesis. *Cancer Biol Ther* **15**, 1479-1488 (2014).
12. Xiang, S.*, et al.* E2F1 and E2F7 differentially regulate KPNA2 to promote the development of gallbladder cancer. *Oncogene* **38**, 1269-1281 (2019).
13. Crosby, M.E. & Almasan, A. Opposing roles of E2Fs in cell proliferation and death. *Cancer Biol Ther* **3**, 1208-1211 (2004).
14. Liang, R.*, et al.* SNHG6 functions as a competing endogenous RNA to regulate E2F7 expression by sponging miR-26a-5p in lung adenocarcinoma. *Biomed Pharmacother* **107**, 1434-1446 (2018).
15. Ma, Y.S.*, et al.* MicroRNA-302a/d inhibits the self-renewal capability and cell cycle entry of liver cancer stem cells by targeting the E2F7/AKT axis. *J Exp Clin Cancer Res* **37**, 252 (2018).

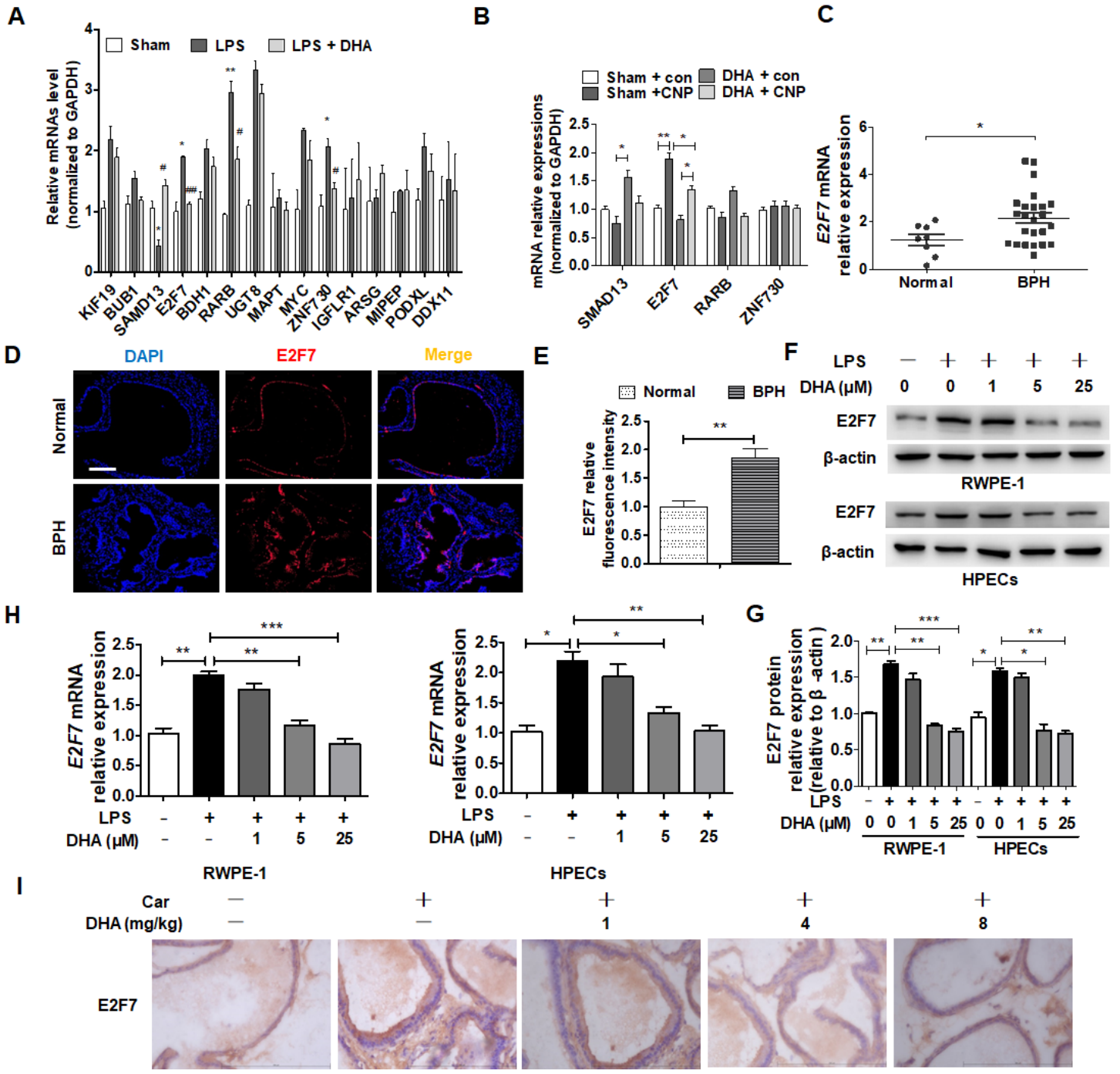
16. Saleh, A.D.*, et al.* Integrated Genomic and Functional microRNA Analysis Identifies miR-30-5p as a Tumor Suppressor and Potential Therapeutic Nanomedicine in Head and Neck Cancer. *Clin Cancer Res* **25**, 2860-2873 (2019).
17. Yang, R.*, et al.* E2F7-EZH2 axis regulates PTEN/AKT/mTOR signalling and glioblastoma progression. *Br J Cancer* (2020).
18. Sandhu, C., Peehl, D.M. & Slingerland, J. p16INK4A mediates cyclin dependent kinase 4 and 6 inhibition in senescent prostatic epithelial cells. *Cancer research* **60**, 2616-2622 (2000).
19. Weijts, B.G.*, et al.* E2F7 and E2F8 promote angiogenesis through transcriptional activation of VEGFA in cooperation with HIF1. *EMBO J* **31**, 3871-3884 (2012).
20. Neubert, P.*, et al.* HIF1A and NFAT5 coordinate Na(+)-boosted antibacterial defense via enhanced autophagy and autolysosomal targeting. *Autophagy* **15**, 1899-1916 (2019).
21. Rohwer, N.*, et al.* Non-canonical HIF-1 stabilization contributes to intestinal tumorigenesis. *Oncogene* **38**, 5670-5685 (2019).
22. Nadler, R.B.*, et al.* IL-1beta and TNF-alpha in prostatic secretions are indicators in the evaluation of men with chronic prostatitis. *J Urol* **164**, 214-218 (2000).
23. Wang, W., Chen, R. & Wang, J. Procyanidin B2 ameliorates carrageenan-induced chronic nonbacterial prostatitis in rats via anti-inflammatory and activation of the Nrf2 pathway. *Biochem Biophys Res Commun* **493**, 794-799 (2017).
24. Paulis, G. Inflammatory mechanisms and oxidative stress in prostatitis: the possible role of antioxidant therapy. *Res Rep Urol* **10**, 75-87 (2018).
25. Popovics, P., Schally, A.V., Salgueiro, L., Kovacs, K. & Rick, F.G. Antagonists of growth hormone-releasing hormone inhibit proliferation induced by inflammation in prostatic epithelial cells. *Proc Natl Acad Sci U S A* **114**, 1359-1364 (2017).
26. Semenza, G.L. Oxygen sensing, hypoxia-inducible factors, and disease pathophysiology. *Annu Rev Pathol* **9**, 47-71 (2014).
27. Ma, C.*, et al.* PAFAH1B2 is a HIF1a target gene and promotes metastasis in pancreatic cancer. *Biochem Biophys Res Commun* **501**, 654-660 (2018).
28. Yang, Y., Sun, M., Wang, L. & Jiao, B. HIFs, angiogenesis, and cancer. *J Cell Biochem* **114**, 967-974 (2013).

# Figures




## Figure 1

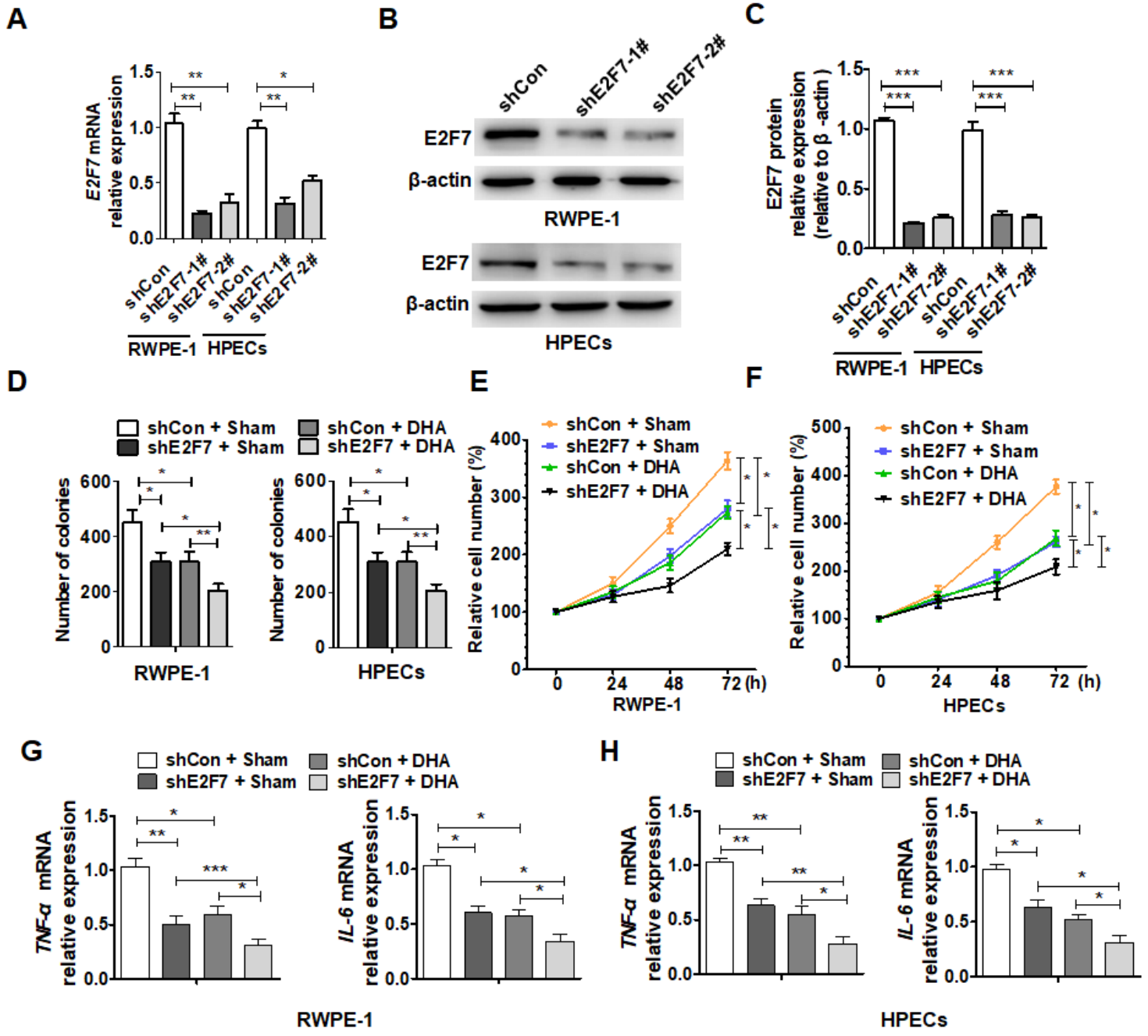
Dihydroartemisinin (DHA) inhibited carrageenan-induced chronic nonbacterial prostatitis (CNP) and prostate epithelial cells inflammatory response. (A and B) Mice model of carrageenan-induced CNP and then different DHA concentrations (1, 4, and 8 mg/kg/d) treated for 28 day. (A) Hematoxylin-eosin staining (HE) staining for morphological analysis of CNP tissues. (B) Analyze the index of the prostate. *p < 0.05, **p < 0.01 vs. the corresponding control. (C and D) RT-qPCR was used to determine TNF-α and IL-6 mRNA expression in CNP tissues. *p < 0.05, **p < 0.01 vs. the corresponding control. (E and F) Inflammatory response of RWPE-1 and HPECs cells were induce by LPS and then DHA (25 μM) was used to treatment for 24 h. RT-qPCR was used to examined TNF-α and IL-6 mRNA expression. *p < 0.05, **p < 0.01 ***p < 0.001 vs. the corresponding control.




## Figure 2

DHA suppressed inflammatory response by downregulating E2F7 expression. (A) RWPE-1 was treated with LPS or LPS + DHA for 24 h, and RT-qPCR was used to detected the candidate genes expression of inflammation. *p < 0.05 vs. Sham; #p < 0.05, ##p < 0.01 vs. LPS. (B) RT-qPCR was used to detected the SMAD13, E2F7, RARB and ZNF730 expression in tissues of CNP model with or without DHA treatment. *p < 0.05, **p < 0.01 vs. the corresponding control. (C) RT-qPCR detected the E2F7 mRNA expression in normal prostate or benign prostatic hyperplasia (BPH) tissues in clinical sample. *p < 0.05 vs. normal. (D and E) Immunofluorescence staining was used to detect E2F7 expression in normal prostate or BPH tissues in clinical sample. **p < 0.01 vs. normal. Scale Bar = 50 μm. (F-G) RWPE-1 cells and HPECs were

treated with indicated LPS and DHA, after which RT-qPCR and western blotting were used to analyze E2F7 protein (F and G) and mRNA (H) levels. *p < 0.05, **p < 0.01 ***p < 0.001 vs. corresponding control. (I) Immunohistochemical staining was used to detect the E2F7 expression level in CNP and normal prostate tissues. Scale Bar = 200 μm.




## Figure 3

E2F7 mediates DHA-inhibited epithelial cell proliferation and inflammation. (A–C) RWPE-1 cells and HPECs were transfected with shCon or shE2F7-1# or shE2F7-2# for 48 h, and then RT-qPCR and western blotting were used to analyze E2F7 mRNA (A) and protein (B and C) expression. *p < 0.05, **p < 0.01 vs. corresponding control. (D) RWPE-1 cells and HPECs were transfected with shE2F7 or shCon and then treated with DHA. Cell proliferation was then determined using a colony formation assay. *p < 0.05, **p <

0.01 vs. the corresponding control. (E and F) The MTS assay was used to determine proliferation of RWPE-1 and HPECs cells after treatment as (D). *p < 0.05 vs. the corresponding control. (G and H) RT-qPCR was used to determine TNF-α and IF-6 expression in RWPE-1 and HPECs cells after treatment as (D). *p < 0.05, **p < 0.01 vs. the corresponding control. All graph bars are expressed as mean ± standard error of the mean.

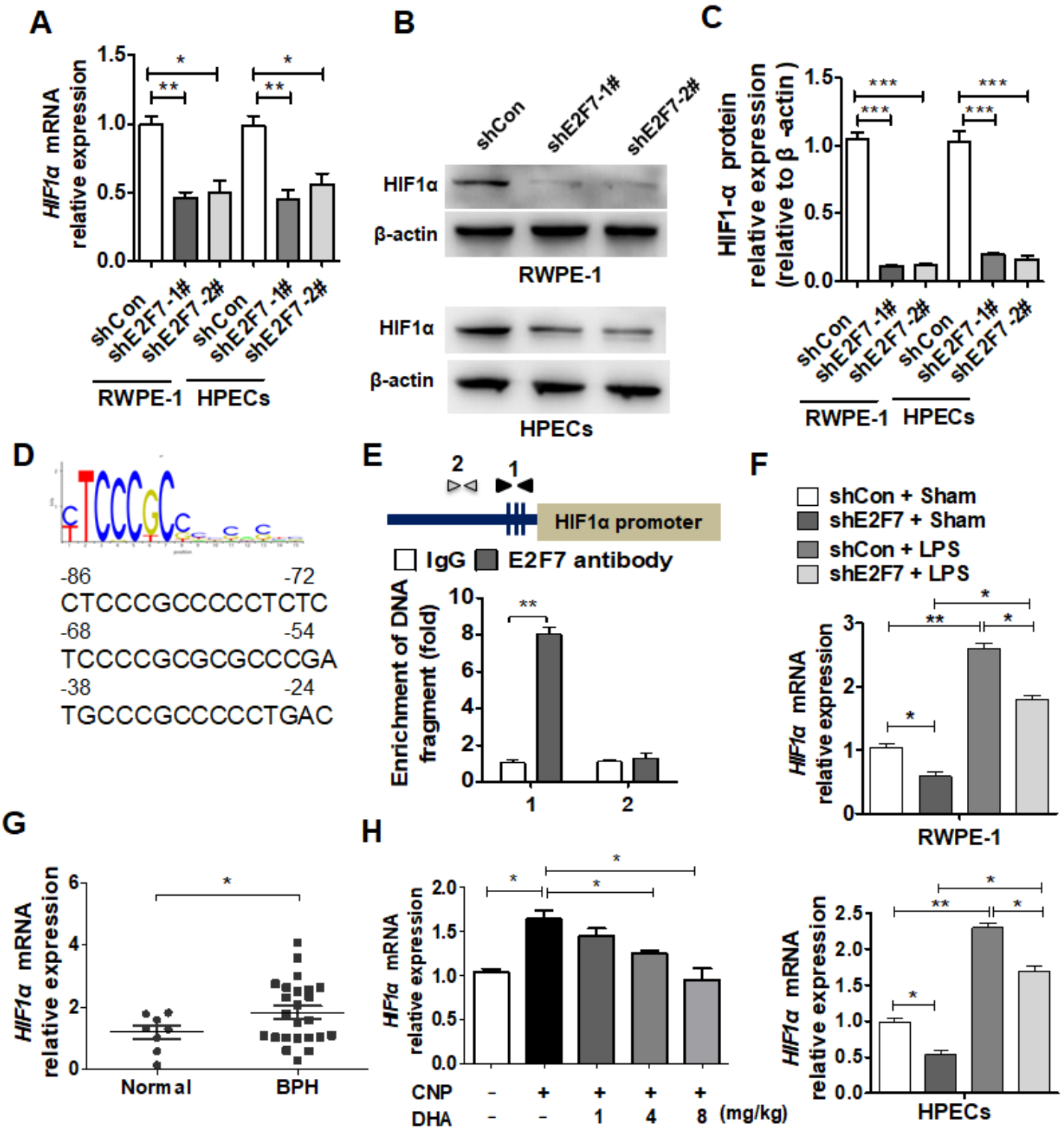



Figure 4

E2F7 promotes HIF1α expression and cell inflammation. (A and B) RWPE-1 and HPECs cells were transfected with shCon or shE2F7 for 48 h, and then RT-qPCR and western blotting were used to examine HIF1α mRNA (A) and protein (B and C) expression. *p < 0.05, **p < 0.01, ***p < 0.001 vs. shCon. (D) Three putative E2F7 binding elements within the 2-kb promoter region of HIF1α were identified. (E) ChIP-qPCR was used to determine E2F7 binding to the HIF1α promoter region in RWPE-1 cells. ** p < 0.01 vs. IgG. (F) RT-PCR was used to examine HIF1α expression in LPS-treated RWPE-1 and HPECs cells after transfection with shCon or shE2F7 vector. *p < 0.05, **p < 0.01 vs. the corresponding control. (G and H) RT-PCR was used to analyze HIF1α mRNA levels in normal prostate and BPH tissues (G) or CNP tissues with or without DHA treatment (H). *p < 0.05, vs. the corresponding control.

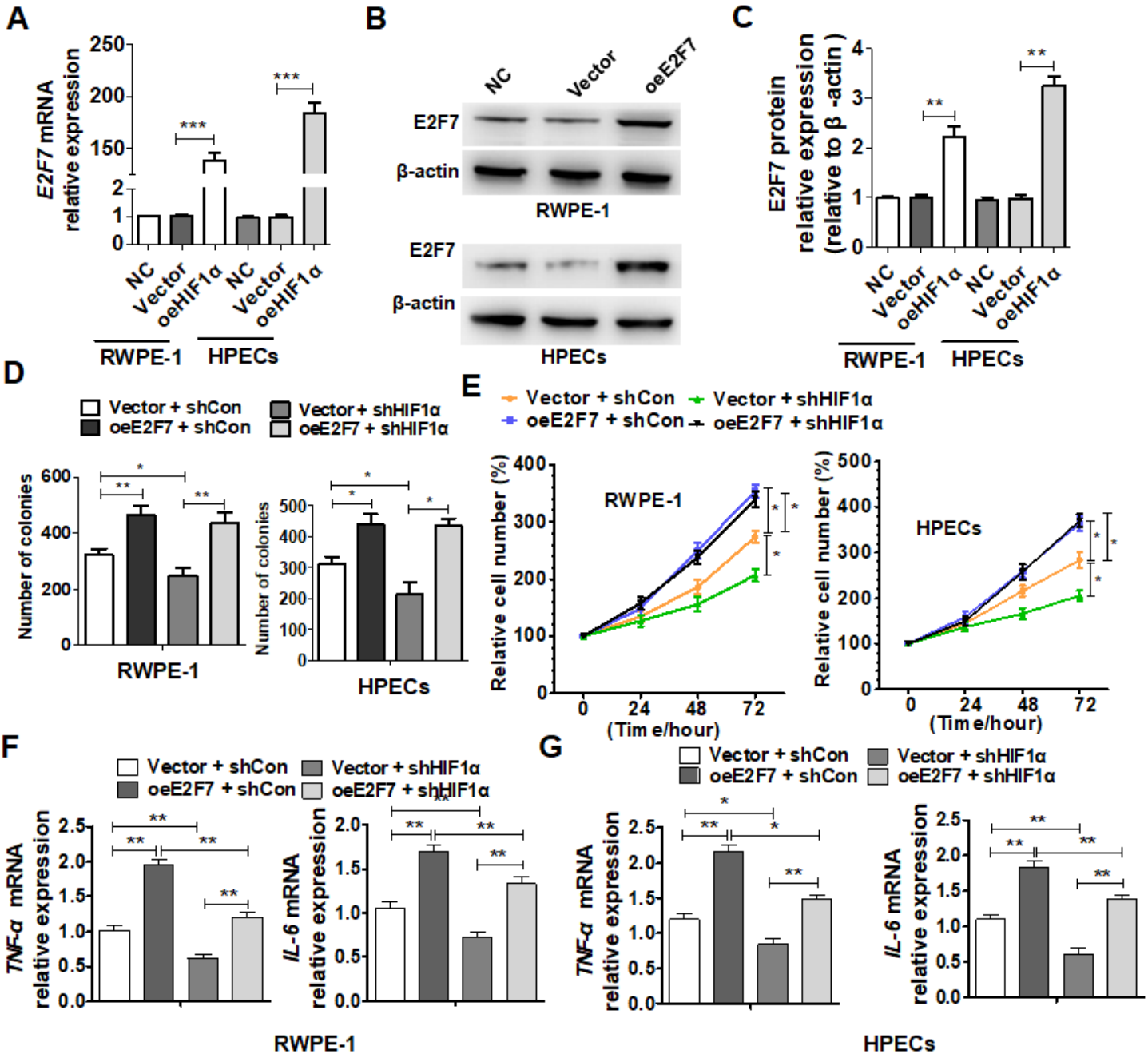



Figure 5

HIF1A mediates E2F7-regulated cellular inflammatory response but not on cell proliferation. (A–C) RWPE-1 and HPECs cells treated with or without DHA after indicated transfection, and then HIF1α mRNA (A) and protein (B and C) expression levels were examined. **p < 0.01, ***p < 0.001 vs. the corresponding control. (D) A colony formation assay was used to determine the RWPE-1 and HPECs cells proliferation after transfection of indicated vectors. *p < 0.05, **p < 0.01 vs. the corresponding control. (E) the MTS assay was used to determine the proliferation of RWPE-1 and HPECs cells after treatment as above. *p < 0.05 vs. the corresponding control. (F and G) RWPE-1 and HPECs cells were treated as in (D), and then RT-PCR was used to examine TNF-α and IL-6 mRNA levels. *p < 0.05, **p < 0.01 vs. the corresponding control.

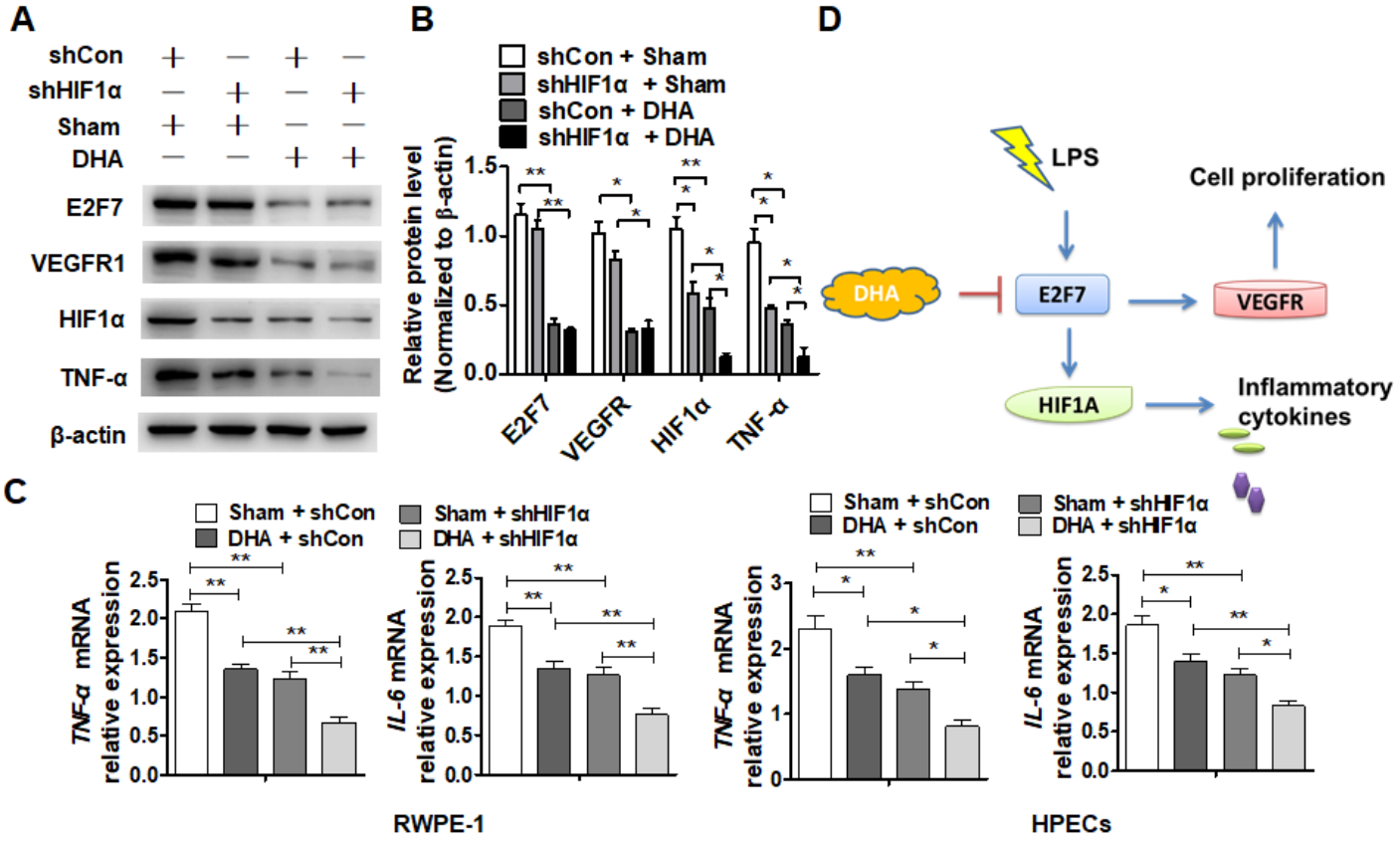



**Figure 6**

Inhibition of the E2F7/HIF1α axis reduces inflammation in carrageenan-induced CNP. (A) RWPE-1 cells were transfected with shHIF1α or their corresponding controls and then treated with or without DHA. Western blotting was used to analyze the indicated proteins. (B and C) TNF-α and IL-6 mRNA levels were examined in RWPE-1 cells and HPECs treated as in (A). *p < 0.05, **p < 0.01 vs. the corresponding control. (D) Proposed model for DHA regulated the process of CNP cell proliferation and inflammation.

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarytable1.docx