Cancer-associated fibroblasts exosomes promote prostate cancer metastasis through miR-500a-3p/FBXW7/HSF1 axis under hypoxic microenvironment

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Article

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Abstract

Metastasis is the main cause of deaths in prostate cancer (PCa). However, the mechanisms of PCa metastasis remain unknown. In this study, severe hypoxia was identified in primary lesions of metastasis PCa (mPCa). The exosomes secreted by cancer-associated fibroblasts (CAFs) significantly promoted PCa metastasis in vitro and in vivo. miRNA sequencing analysis and reverse transcription quantitative PCR (RT-qPCR) experiments indicated miR-500a-3p was significantly increased in CAFs exosomes under hypoxia. RT-qPCR, western blotting, and dual luciferase reporter assays were used to identify FBXW7 was a target of miR-500a-3p. Using immunohistochemistry, we found a decrease in FBXW7 expression with PCa progression, while there was an increase in HSF1 expression. After FXBW7 plasmid transfection, the metastasis ability was suppressed and HSF1 expression significantly decreased in PCa cells. These data indicated that CAFs exosomes promote PCa metastasis through miR-500a-3p/FBXW7/HSF1 axis under hypoxic microenvironment. Targeting hypoxia or exosomal miR-500a-3pmay provide a potential treatment strategy for the management of PCa.

Introduction

Prostate cancer (PCa) is the most common tumor affecting the male urinary and reproductive system, with over 14 million PCa new patients and 370,000 deaths worldwide in 2020. Most PCa-specific deaths occur due to the distant spread of the tumor. Despite the prognosis has improved with the use of second-generation antiandrogens abiraterone acetate and enzalutamide, the five-year survival rate for patients with mPCa has drastically dropped from nearly 100–31%[1]. Therefore, it is imperative to investigate the mechanisms of PCa metastasis and identify new therapeutic targets that can improve patient's prognosis and treatment outcomes.

The tumor microenvironment (TME) comprises the immediate surroundings of tumor, including not only epithelial cells but also mesenchymal cells, macrophages, and other types of cells, playing a crucial role in the process of tumor initiation, progression and metastasis[2]. Among these cells, cancer-associated fibroblasts (CAFs) are considered crucial in PCa[3, 4]. CAFs regulate tumor progression by synthesizing and remodeling the extracellular matrix (ECM) and releasing cytokines, thereby affecting angiogenesis, tumor mechanics, and therapeutic response[5]. Additionally, during PCa and other solid tumors' rapid growth, hypoxia in the microenvironment is commonly observed and usually associated with a poor prognosis. Current studies suggested that targeting hypoxia in the tumor microenvironment could be an effective approach to inhibit tumor progression[6]. However, most studies have focused on the effect of hypoxia on cancer cells, neglecting the potential impact of hypoxia on CAFs in the context of PCa.

Exosomes are small lipid bilayer vesicles, approximately 100nm in diameter, that transport a diverse array of RNA, proteins, lipids, and other functional components. These vesicles can directly fuse with the membrane of recipient cell, releasing their contents and modulating cellular functions[7]. In exosomes, non-coding RNAs (ncRNAs), especially microRNAs(miRNAs), are the primary active components. These ncRNAs regulate the expression of multiple genes in recipient cells, promoting tumor growth and

invasion, and affecting the microenvironment of distant metastatic sites[8]. Under the hypoxic microenvironment, anoxic tumor cells can drive their own progression through the interaction between exosomes and surrounding cells[9]. Exosomes derived from tumor cells have been reported to play a pivotal role in the progression of PCa, leading to the development of exosomal RNA-based PCa detection methodologies[10]. However, the specific role that exosomes derived from CAFs plays in PCa progression under hypoxic conditions remains an intriguing area that requires further investigation.

In this study, a more severe hypoxia was observed in primary tissues of mPCa. CAFs-secreted exosomes significantly enhanced the metastasis of prostate cancer under hypoxia. Moreover, CAFs-secreted exosomal miR-500a-3p was proved to promote cancer metastasis through targeting FBXW7/HSF1. Based on these findings, we hypothesize that CAFs-secreted exosomes under hypoxia conditions promote prostate cancer metastasis through miR-500a-3p/FBXW7/HSF1 signaling pathway.

Materials and methods

Isolation of CAFs and NFs

PCa tissues and corresponding adjacent normal tissues were procured from patients who were diagnosed with PCa before surgery through prostate biopsy. This research was approved by the Ethic committee of Beijing Friendship Hospital, and each participant provided written informed consent to take in this study. Briefly, the tissues were meticulously sectioned using sterile scissors, followed by digestion with a solution containing 0.1g of collagenase I and II (Sigma) and 0.1g of DNAse enzyme (Solarbio) in 50ml of phosphate-buffered saline for 2 hours. The digested tissues underwent filtration through a 100-mesh sieve (Falcon). Following centrifugation at 1200 revolutions per minute (rpm) for 10 minutes, the cells were cultured in dishes and fibroblasts could be observed after 3 days.

Exosomes isolation and identification

The isolation of CAFs-derived exosomes in this study was facilitated using a combination of the ultrafiltration method and an exosome extraction kit [11]. Briefly, when CAFs grow to 90% confluent, the medium was replaced with DMEM/F12 without FBS. Subsequently, the supernatant of CAFs was harvested and subjected to filtration through a 0.22μm filter to eliminate cells and cellular debris. The filtered supernatant was introduced into a 10kDa Molecular Weight Cut Off (MWCO) ultrafiltration filter (Millipore) and centrifuged in accordance with the provided instructions. After exosomes were suspended by PBS, exosome extraction kit (101Bio, P100) was used to purify the exosomes.

PKH67-labeled exosomes and cy3-labeled miR-500a-3p transfer

PKH67 Green Fluorescent Labelling Kit (Sigma—Aldrich) was employed for exosome visualization. PCa cells were treated with labeled exosomes for 16h, followed by documentation using a fluorescence microscope. Nuclei were stained with DAPI to facilitate accurate localization.

Additionally, to investigate the potential transfer of miR-500a-3p from CAFs to PCa cells via exosomes, we transfected cy3-miR-500a-3p into CAFs. Subsequently, we isolated and labeled the exosomes present in the supernatant with PKH67. PCa cells were then treated with these labeled exosomes for 16h, after which documentation was carried out using a fluorescence microscope.

Animal models

Six-week-old male BALB/c nude mice were obtained from the Charles River (Beijing, China). PC-3 cells were treated with CAFs exosomes for 48h, followed by injection into the mice via tail vein (1×10^6 cells in 100 μL PBS, with a group size of n=6). Subsequently, exosomes (30μg) were administered to the mice via tail vein every third day for two weeks. Eight weeks after injection, the mice were sacrificed. Metastatic tumor foci in the lungs were visualized through the fixation of lung tissues in 4% paraformaldehyde, followed by paraffin embedding. This study was approved by the Ethics Committee of Beijing Friendship Hospital, and animals were maintained following the institutional guidelines.

Bioinformatics analyses

The expression levels of miR-500a-3p in PCa and normal tissues were retrieved from The Cancer Genome Atlas (TCGA) database. Additionally, the expression levels of miR-500a-3p in the serum of patents with PCa were obtained from the Gene Expression Omnibus (GEO) dataset GSE112264. Furthermore, the expression levels of HSF1 in various PCa tissues were extracted from the UALCAN database[14].

Statistical analysis

All error bars in graphical data represent mean ±SD. All statistical analyses were performed using the SPSS 23 software (SPSS, Chicago) and GraphPad Prism 8 software. Comparisons between datasets were carried out using Student's t-test. *P 0.05; **P 0.01; ***P 0.001 were considered statistically significant.

All other methods and statistical approaches used in this study are described in the Supplementary methods.

Results

CAFs-CM promotes PCa progression under hypoxic condition

In previous studies, a characteristic hypoxic microenvironment has been identified in PCa tissues, potentially contributing to tumor development via multiple signaling pathways [15, 16]. Consequently, we detected the expression of HIF-1α, a member of the hypoxia-inducible factor, in 15 BPH, 15 organ-confined PCa, and 15 primary tumor tissues of mPCa. IHC analysis revealed minimal HIF-1α expression in BPH tissues, while significantly increased expression with PCa progression (Fig. 1A).

To explore the influence of stromal cells on PCa progression under hypoxic condition, we collected the supernatants from cultured WPMY-1 exposed to hypoxic and normoxic conditions. These supernatants were subsequently employed as conditioned media (CM) for treating PCa cells. Regrettably, treatment with WPMY-1 CM did not result in any significant alterations in the proliferation or metastatic potential of PCa cells (Supplementary Fig. 1A, B). Subsequently, primary CAFs and normal fibroblasts (NFs) were isolated from PCa tissues and adjacent normal tissues, respectively. Under white light microscopy, both CAFs and NFs displayed a spindle-shaped morphology, with CAFs exhibiting a more elongated appearance (Fig. 1B). Western blotting and immunofluorescence analysis revealed elevated levels of α-SMA expression within CAFs (Fig. 1C).

To investigate the effect of CAFs on PCa cells in hypoxic conditions, PC-3 and C4-2 cells were exposed to hypoxic and normoxic CM obtained from CAFs. PC-3 and C4-2 cells were slightly accelerated in their growth capability by hypoxic CM, but their apoptosis levels were not affected (Fig. 1E, Supplementary Fig. 2A). Additionally, scratch assays revealed an augmented migratory capacity of PC-3 cells following treatment with hypoxic CM derived from CAFs (Fig. 1F). In addition, Transwell invasion assays demonstrated that hypoxic CM promoted the invasive ability of PC-3 and C4-2 cells (Fig. 1G). Collectively, these findings indicated that hypoxia could promote PCa progression through the modulation of CAFs in TME.

CAFs-secreted exosomes under hypoxia promote PCa in vitro and in vivo.

Considering the significant regulatory influence of exosomes on tumor progression[8], we collected exosomes secreted by CAFs to investigate their impact on PCa cells under hypoxia and normoxia conditions. The exosomes released by CAFs were identified through the common exosomal markers (TSG101, Alix, CD63), nanoparticle tracking analysis (NTA) and transmission electron microscope (TEM). The results showed that exosomal markers were highly expressed in CAFs exosomes while being nearly absent in CAF cells (Fig. 2A). Furthermore, NTA revealed that these vesicles possessed a diameter of approximately 100nm, with TEM revealing their characteristic cup-shaped morphology (Fig. 2B, C). These results indicated that the isolated vesicles were exosomes.

To investigate whether PCa cells could internalize CAFs-derived exosomes, PC-3 and C4-2 cells were treated with exosomes labeled by PKH67 for 16 hours. Confocal microscope showed the successful internalization of exosomes by both PC-3 and C4-2 cells (Fig. 2D). Subsequently, we assessed the impact of CAFs-secreted exosomes on PCa cells. As anticipated, CAFs hypoxic exosomes significantly enhanced the migration and invasion capacities of PC-3 and C4-2 cells (Fig. 2E, F). However, only minor changes were observed in the proliferation and apoptosis of both PC-3 and C4-2 cells following treatment with CAFs hypoxic exosomes (Fig. 2G, Supply Fig. 2B). Notably, the promoting effect exerted by CAFs hypoxic exosomes on PCa cells could be counteracted by the exosome inhibitor GW4869 (Supplementary Fig. 3A, B). To assess the influence of CAFs hypoxic exosomes on PCa metastasis in vivo, we established a lung metastasis model by injecting PC-3 cells via the tail vein. As illustrated in the schematic (Fig. 2.H), PC-3 cells were treated with CAFs-derived exosomes for 48 hours prior to injection. Subsequently, exosomes

(40μg) were administered via tail vein injection every third day for two weeks after tumor cells injection. Tumor metastases were evaluated after eight weeks. We observed a steady increase in body weight in nude mice during the first six weeks after tumor injection, followed by a decline from the seventh week (Supplementary Fig. 4). Histological examination confirmed the presence of tumor metastases, with a higher incidence of lung metastases observed in the group of mice injected with CAFs hypoxic exosomes (Fig. 2I). Additionally, P504S staining confirmed the histological origin of PCa (Fig. 2J). Collectively, these results underscored the role of CAFs-derived exosomes in accelerating PCa metastasis under the hypoxic microenvironment.

miR-500a-3p is elevated in CAFs hypoxic exosomes.

Exosomes are known to contain various non-coding RNAs (ncRNAs), including miRNAs, lncRNAs, and circRNAs. MiRNAs derived from exosomes had been shown to form a new mode among cells communications in diseases[17]. To identify the differentially expressed miRNAs in normoxic and hypoxic exosomes secreted by CAFs, we conducted a miRNA sequencing analysis. The results revealed 14 upregulated and 19 downregulated miRNAs in CAFs hypoxic exosomes compared to their normoxic counterparts (Fig. 3A, B). Results of the target genes of different expressed miRNAs (DE-miRNAs) by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were shown in Fig. 3C, D, respectively. Subsequently, we further validated the expression levels of the top-ranked 13 miRNAs from the sequencing date in CAFs hypoxic and normoxic exosomes using RT-qPCR. The results confirmed that miR-500a-3p and miR-2682-3p were significantly upregulated, while miR-184 exhibited downregulation in CAF hypoxic exosomes, consistent with RNA sequencing assay findings (Fig. 3E). However, following treatment with CAFs hypoxic exosomes, only miR-500a-3p displayed the anticipated upregulation in PCa cells (Fig. 3F). Therefore, we embarked on exploring whether miR-500a-3p could affect the progression of PCa.

miR-500a-3p in CAFs exosomes promote PCa cells migration and invasion.

Firstly, we investigated whether miR-500a-3p derived from CAF exosomes could be taken by PCa cells. Cy3-labelled miR-500a-3p mimics were transfected into CAFs using siRNA-transfectmate (Genepharma). Subsequently, exosomes were isolated from the transfected CAFs and introduced into PC-3 and C4-2 cells. Under a fluorescence microscope, abundant cy3-miR-500a-3p could be observed within PC-3 and C4-2 cells (Fig. 4A). To visualize the spatial distribution of miR-500a-3p within CAFs- derived exosomes following transfection with cy3-miR-500a-3p, we co-labeled exosomes with PKH67. The results showed that the majority of labeled miR-500a-3p was effectively packaged within CAFs exosomes, which were subsequently taken by PCa cells (Fig. 4B).

To investigate the impact of miR-500a-3p on prostate cancer, miR-500a-3p mimics and inhibitor were transfected into PC-3 and C4-2 cells. The results demonstrated that miR-500a-3p mimics promoted the migration and invasion capabilities of PC-3 and C4-2 cells, while the miR-500a-3p inhibitor exhibited the opposite effect (Fig. 4C, D). However, miR-500a-3p did not influence the proliferation of PCa cells (Supplementary Fig. 5). Additionally, we found elevated miR-500a-3p expression in the serum exosomes

of PCa patients with metastasis (Fig. 4E). Furthermore, an analysis of the TCGA database revealed a significant increase in miR-500a-3p expression in PCa tissues compared with normal tissues (Fig. 4F). Similarly, data from the GEO database indicated elevated miR-500a-3p levels in the serum of PCa patients (Fig. 4G). Collectively, these findings indicated that miR-500a-3p promoted the migration and invasion of PCa cells and might serve as a potential marker for mPCa diagnosis and prognosis.

miR-500a-3p promoted PCa progression through targeting FBXW7.

To investigate the mechanisms underlying the regulation of PCa progression by miR-500a-3p, we utilized several miRNA databases, including miRDB, TargetScan, miRWalk, mirDIP, and RNA22, to identify potential target mRNAs of miR-500a-3p. The analysis revealed 63 potential target mRNAs of miR-500a-3p (Fig. 5A). Among these candidates, we focused on 11 mRNAs with target scores exceeding 90 in the miRDB database (Supplementary Fig. 6). One among these mRNAs, FBXW7, is a recognized tumor suppressor gene validated by previous studies[18, 19]. Moreover, TCGA data indicated a decreased FBXW7 expression levels in PCa tissues (Fig. 5B), suggesting that FBXW7 could probably serve as a potential marker for PCa. Subsequently, we assessed the expression of FBXW7 in PCa cells upon treatment with CAFs-derived exosomes. Both RT-qPCR and western blot analysis demonstrated a significant decrease in FBXW7 expression following CAFs hypoxic exosomes treatment (Fig. 5C, D). Additionally, the transfection of miR-500a-3p mimics into PC-3 and C4-2 cells resulted in a significant reduction of FBXW7 mRNA and protein expression (Fig. 5E, F). To further verify the direct interaction between miR-500a-3p and the FBXW7 mRNA, we constructed two FBXW7 3’ untranslated regions (3’UTR) luciferase reporter constructs – one containing the conserved seed sequence and the other carrying a mutated (MUT) version of the seed sequence with nine nucleotides exchanged (Fig. 5G). When miR-500a-3p mimics and the wild-type (WT) FBXW7 3’UTR reporters were transfected into 293T cells, there was a noticeable decrease in luminescence, while not observed in case of the mutant reporters (Fig. 5H). These findings indicated the inhibitory role of exosomal miR-500a-3p on FBXW7 expression in PCa cells.

FBXW7 inhibit the progression of tumor by regulating the HSF1 expression in PCa cells

To explore the relationship between FBXW7 and PCa, we conducted IHC to examine the FBXW7 expression in BPH, organ-confined PCa, and primary lesions of mPCa. FBXW7 was distributed in both the nucleus and cytoplasm, with notably higher expression in the cytoplasm. Notably, as PCa progression, FBXW7 expression was found gradually decreased (Fig. 6A), indicating a negative correlation between FBXW7 levels and PCa progression.

To investigate the functional role of FBXW7 in PCa, we overexpressed FBXW7 in PC-3 and C4-2 cells using a pcDNA3.1(+) plasmid. RT-qPCR and western blotting confirmed a significant increase in FBXW7 expression after plasmid transfection (Supplementary Fig. 7A, B). The overexpression of FBXW7 triggered increased cell death in PC-3 cells and inhibited the growth of C4-2 cells (Supplementary Fig. 7C). Additionally, FBXW7 overexpression significantly suppressed PCa cells proliferation, migration, and

invasion (Fig. 5B, C). Furthermore, the overexpression of FBXW7 effectively counteracted the stimulatory effects of miR-500a-3p on PCa cells (Supplementary Fig. 7D).

Previous studies have revealed that FBXW7 exerts its inhibitory effects on cancer progression by ubiquitinating multiple oncogenes, including c-Myc, cyclin E and Mcl-1[20]. Among these oncogenes, HSF1 and MAP7D1 expression were negatively correlated with FBXW7 in PCa tissues (Supplementary Fig. 7E). Furthermore, it has been demonstrated that abundance of HSF1 expressed in PCa cells nuclear and HSF1 inhibitor robustly inhibited cancer progression[21]. Therefore, we investigated whether FBXW7 inhibited PCa progression through the HSF1 pathway. IHC analysis demonstrated that HSF1 mainly localized in the nucleus and limited expression in the cytoplasm, and the HSF1 expression escalates with PCa progression, indicating a negative correlation between FBXW7 and HSF1 (Fig. 6D). Furthermore, FBXW7 overexpression resulted in significantly decreased HSF1 expression (Fig. 6E), indicating that FBXW7 negatively regulate the expression of HSF1 in PCa cells. In conclusion, our findings suggest that exosomes derived from CAFs under hypoxic conditions promote PCa progression through the miR-500a-3p/FBXW7/HSF1 signaling axis. FBXW7 acts as a tumor suppressor through repressing HSF1 expression, and its downregulation mediated by miR-500a-3p contributes to the progression of PCa.

Discussion

In solid tumors, including PCa, the rapid and uncontrolled growth of tumors creates a hypoxic microenvironment[22]. Numerous studies have demonstrated that hypoxia in the tumor microenvironment promotes cancers progression through various mechanisms, such as modulation of tumor cells metabolism, promoting tumor cell heterogeneity and plasticity, and the augmentation of tumor aggressiveness [23, 24]. In this study, we observed that hypoxia was more severe in PCa tissues compared with BPH, and even more severe in primary lesions of mPCa. This observation raised the possibility that hypoxia could serve as a potential marker for indicating the severity of the tumor. Subsequently, we identified CM and exosomes derived from CAFs under hypoxia condition could significantly promoted the migration and invasion ability of PCa cells. Additionally, miR-500a-3p in CAFs exosomes was proved to promote PCa cells migration and invasion through miRNA sequencing and RT-qPCR. Furthermore, FBXW7 was identified as a direct target of miR-500a-3p, influencing the metastasis of PCa by regulating HSF1 expression.

CAFs, as a crucial component of the tumor microenvironment, plays a critical role in PCa progression[25]. Shan et al. reported that CAFs-derived exosomes carrying miR-423-5p inhibit GREM2 via TGF-β to enhance PCa cell resistance to chemotherapy[26]. Conversely, Jia et al. revealed that CAFs with elevated FOXF2 expression can repress tumor growth and metastasis by improving antitumor immunity[27]. In our current study, we uncovered that the hypoxic TME stimulates CAFs to release exosomes rich in miR-500a-3p, which subsequently influence PCa phenotypes by targeting specific mRNA. Notably, miR-500a-3p predominantly enhances the capability of migration and invasion ability of PCa cells, while having minimal effect on their proliferation. Additionally, our investigation revealed an upregulation of miR-500a-3p expression in serum exosomes derived from metastasis PCa patients in comparison to those from

patients with localized PCa and BPH, indicating that miR-500a-3p could potentially serve as a marker for cancer progression, especially for metastasis.

miR-500a-3p has been investigated in several cancers. Some researchers identified miR-500a-3p as a proponent of cancer progression, contributing to the growth and advancement of tumors[28, 29]. However, a contrasting perspective was presented by liu et al., who reported that miR-500a-3p could inhibit cancer cell proliferation and aerobic glycolysis via targeting CDK6 [30]. In our current study, we identified FBXW7 as a direct target of miR-500a-3p in PCa cells through bioinformatics analysis, dual-luciferase assay and western blotting. Previous studies had revealed that FBXW7 was an important cancer suppressor gene through controlling proteasome-mediated degradation of oncoproteins, such as cyclin E, c-Myc, Mcl-1, mTOR, Jun, Notch and AURKA[20]. The expression of FBXW7 was regulated by multiple mechanisms and several miRNAs had been identified suppressing FBXW7 expression, including miR-25-3p, miR-32, miR-92b, miR-96, miR-155-3p, and others [18]. Consistent with our study, Lin et al. reported that exosomal miR-500a-3p promoted drug resistance and stemness of cancer cells by inhibiting FBXW7 expression in gastric cancer[31]. Our IHC results provided further evidence, demonstrating a marked decrease in FBXW7 expression as PCa progressed, underscoring the suppressive role of FBXW7 in PCa.

As a member of the F-box protein family, FBXW7 plays a critical role in oncogenesis by mediating phosphorylation-dependent ubiquitination and proteasome degradation of oncoproteins[18]. HSF1 is the most intensively studied member of the family of heat shock factors and its activity downregulation could be beneficial to treat cancer[32]. Dong et al. reported a significant increase in nuclear HSF1 expression in PCa cells, particularly in neuroendocrine prostate cancer, and demonstrated that HSF1 inhibitors could attenuate PCa progression [21]. Besides, Kourtis et al. identified FBXW7 modulated the ability of metastasis in melanoma through ubiquitinating the HSF1 in cancer cell nuclear[33]. Interestingly, we observed that overexpression of FBXW7 led to a reduction in HSF1 expression, confirming the negative correlation between FBXW7 and HSF1 in PCa cell. Moreover, elevated HSF1 expression was associated with higher Gleason scores and was notably prominent in mPCa[34], suggesting that HSF1 might play a promoting role during PCa progression. However, the specific pathway through which HSF1 regulates cancer progression and whether FBXW7 regulates HSF1 expression through ubiquitination were beyond the scope of this article, highlighting the need for further in-depth research.

Nevertheless, there are some limitations in our study. Primarily, our study focused only the different miRNAs between CAFs hypoxic and normoxic exosomes. However, there might be additional ncRNA, such as circRNAs and lncRNAs, or proteins in CAFs exosomes affecting PCa progression. More ncRNA detection and liquid chromatography-mass spectrometry may provide a more comprehensive understanding of the specific molecular mechanisms affecting the progression of PCa in CAFs exosomes. Additionally, we were unable to assess the expression of HIF-1α, FBXW7, HSF1 in metastatic lesions of PCa owing to the difficulties for tissues collection. Lastly, further investigation of the specific mechanisms by which FBXW7 affects HSF1 expression is not included in this article. Whether FBXW7

regulates HSF1 expression in PCa through ubiquitination or other posttranslational modifications remains unclear.

In summary, our research has unveiled a novel insight into the role of the hypoxic microenvironment in promoting PCa metastasis through inducing CAFs to release exosomal miR-500a-3p. These exosomes are absorbed by PCa cells, and their cargo miR-500a-3p stimulates PCa progression by regulating FBXW7/HSF1 signaling pathway (Fig. 7). We hope that these findings will contribute to the improvement of PCa treatment and management.

Declarations

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Data availability statement

All data generated and analyzed during this study are available on request.

Authorship contribution statement

Zhanliang Liu: Formal analysis, Investigation, Validation, Writing- original draft. Zhemin Lin: Formal analysis, Visualization, Data curation. Mingxin Jiang and Guangyi Zhu: Resources. Tianyu Xiong and Fang cao: Visualization. YN Niu and Cui Yun: Supervision, Funding acquisition, Writing – review & editing.

Declaration of competing interest

All authors have declared that they have no conflict of interest.

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Figures

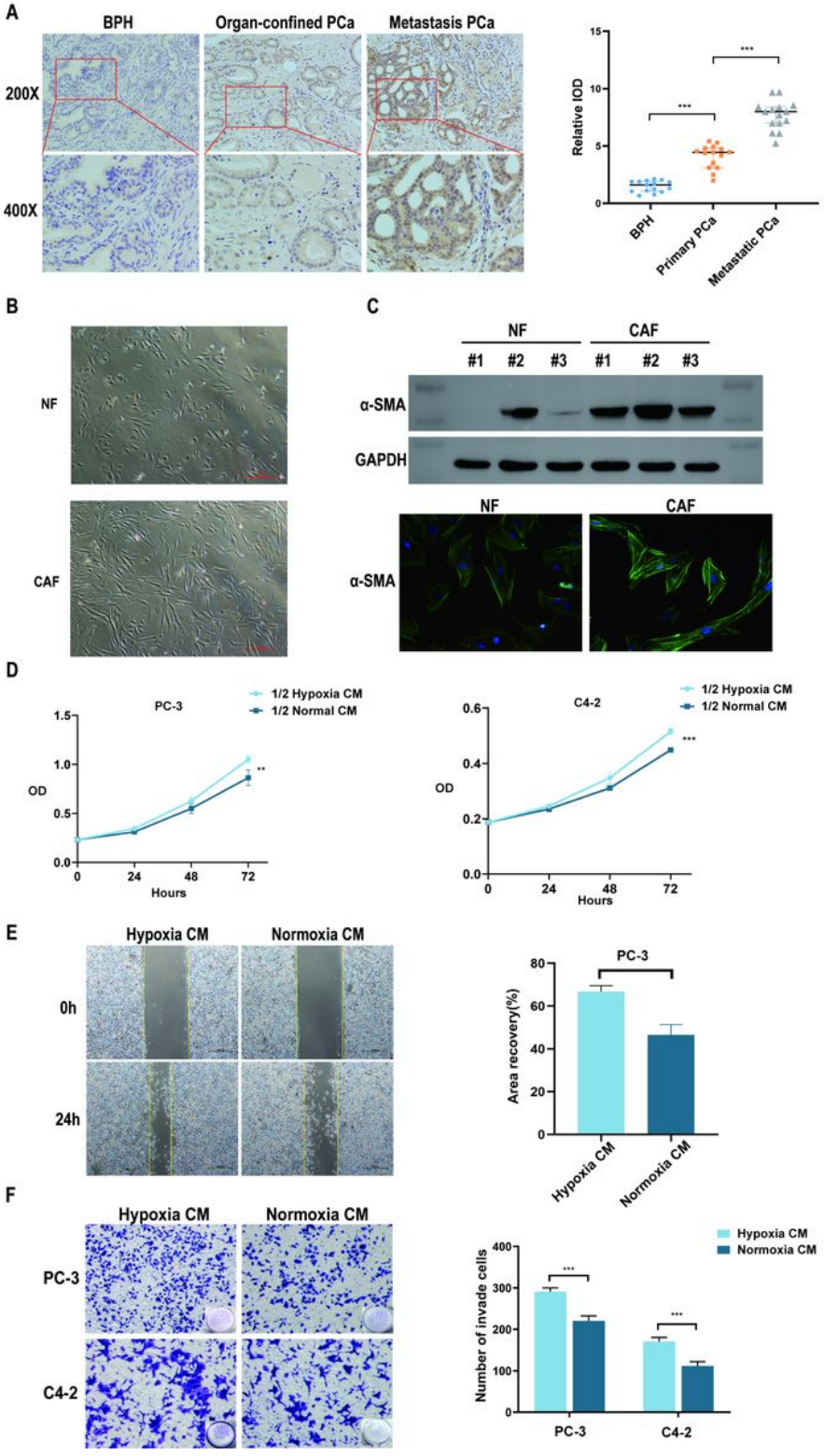

Figure 1

Influence of CAF-CM on the progression of PCa cells under hypoxic microenvironment. (A) Representative images of IHC staining for HIF-1α in both BPH and PCa tissues with or without metastasis (left). Quantitative evaluation of IHC scores is presented (right). (B) Visual depiction of the cellular morphology of NFs and CAFs under light microscopy. (C) Western blotting and immunofluorescence analysis for α-SMA in NFs and CAFs. Patient samples are represented as #1, #2, and #3. (D) CCK8 assay, (E) scratching

assay, and (F) invasion assay was performed to detect the proliferation, migration, and invasion ability of PCa cells after CAF-CM treatment. **P 0.01, ***P 0.001.

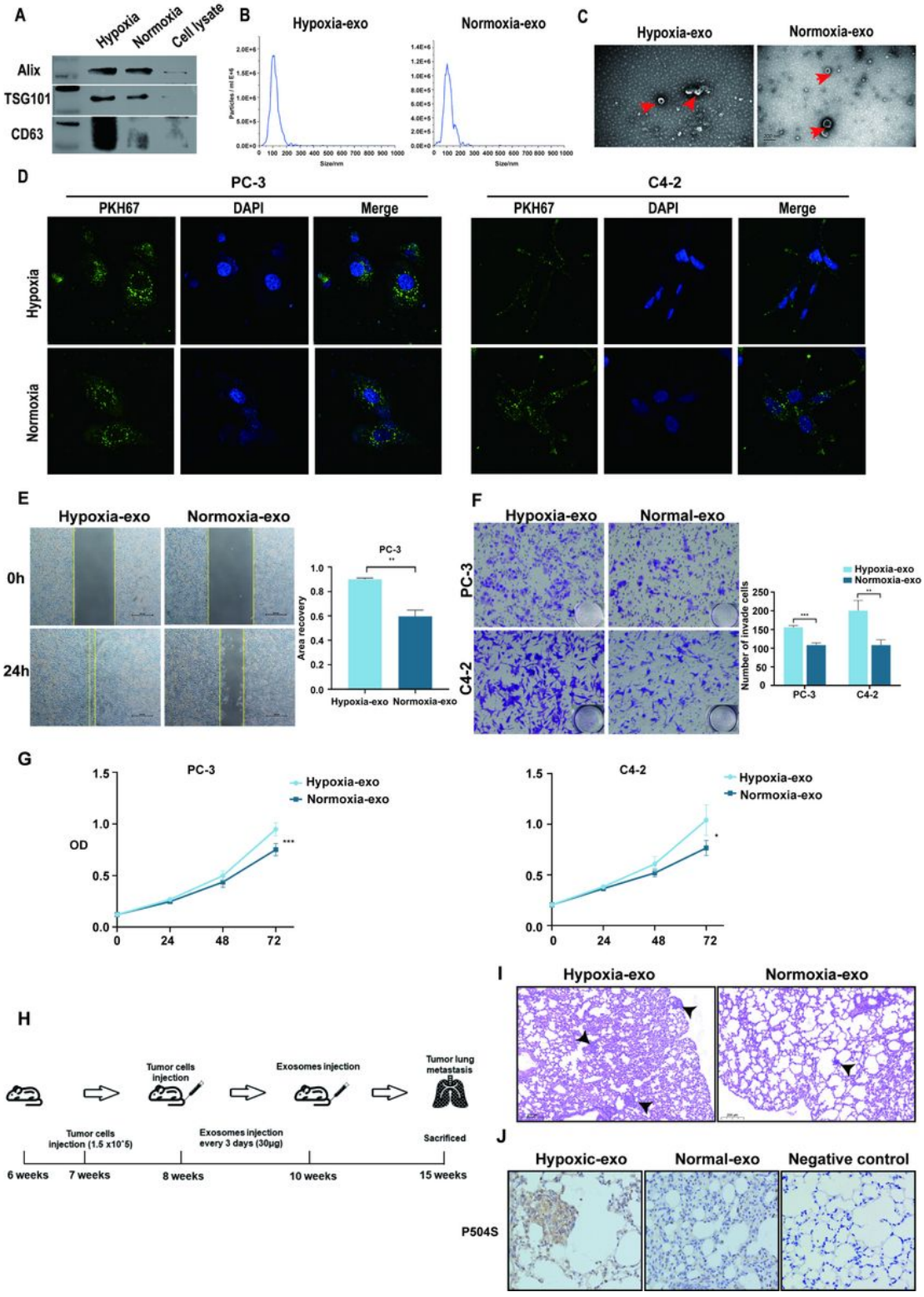

Figure 2

Roles of CAF-derived exosomes in facilitating PCa progression within the hypoxic TME. (A) Characterization of exosomes derived from CAFs under both hypoxic and normoxic conditions using

western blot analysis (Alix, TSG101, CD63). (B) Particle size detection of exosomes through Nanoparticle Tracking Analysis (NTA). (C) Visualization of exosomes using Transmission Electron Microscopy (TEM). Exosomes are highlighted by the red arrow. (D) Internalization of exosomes by PC-3 and C4-2 cells examined by laser scanning confocal microscope (630X). (E) The migration, (F) invasion, (G) and proliferation ability of PC-3 and C4-2 cells treated by CAF-secreted exosomes under hypoxic or normoxic conditions. (H) Schematic diagram depicting the construction of a model of lung metastases in nude mice. Lung tissue were harvested after tail vein injections of exosomes in 8 weeks and tissue sections were stained with HE (I) and p504S (J). I *P 0.05, **P 0.01, ***P 0.001.

Figure 3

Profiling distinctively expressed miRNAs in CAF Hypoxic and Normoxic Exosomes. (A, B) Visual representation of a heatmap and a volcano plot illustrating DE-miRNAs between CAF-derived hypoxic and normoxic exosomes. (C, D) GO and KEGG analysis of target genes regulated by DE-miRNAs. (E) RT-qPCR analysis of DE-miRNAs in PCa cells after CAF hypoxic and normoxic exosomes treatment. (F) RT-qPCR analysis of miR-2682-3p, miR-184, and miR-500a-3p level in PCa cells after CAF hypoxic and normoxia exosomes treatment. *P 0.05,**P 0.01,***P 0.001.

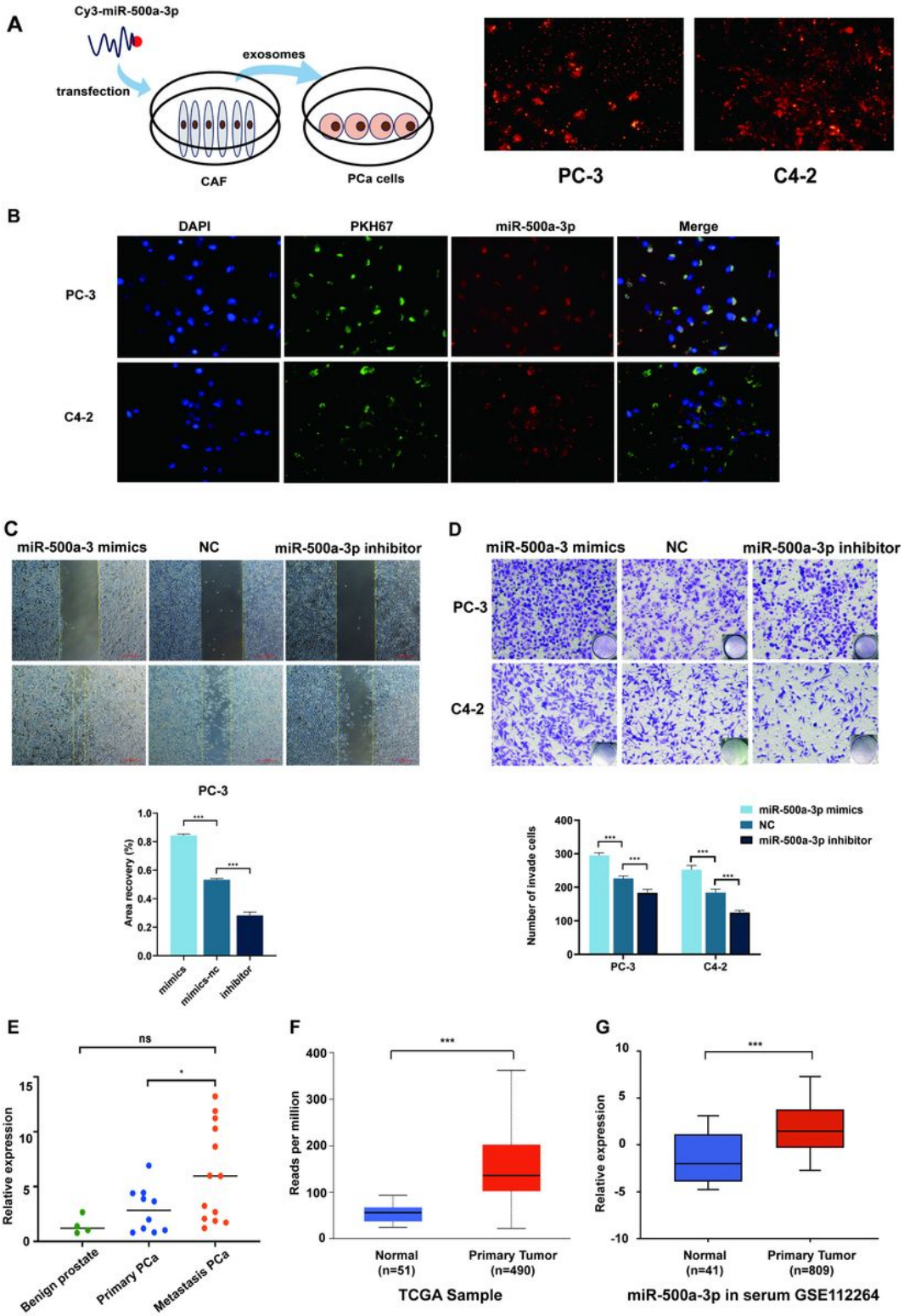

Figure 4

Exosomal miR-500a-3p: A Distinctive Indicator of Prostate Cancer Progression. (A)Cy-3 labelled miR-500a-3p were packaged in exosomes by CAFs, followed by visualization within PCa cells exhibiting a distinctive red fluorescence. (B) Fluorescence microscopy depicting the spatial arrangement of PKH67-labeled exosomes (green) and cy-3 labeled miR-500a-3p (red) subsequent to uptake by PC-3 or C4-2 cells. Effect of exosomes on the migration (C) and invasive (D) ability of prostate cancer cell PC-3 and C4-2. (E)

RT-qPCR analysis of miR-500a-3p expression in serum exosomes from benign BPH, organ confined or metastatic PCa patients. (F) Examination of miR-500a-3p expression in normal and malignant prostatic tissues using the TCGA database. (G) Comparative evaluation of miR-500a-3p expression in serum samples from PCa patients and healthy controls within the GSE112264 dataset. *P 0.05,**P 0.01,***P 0.001.

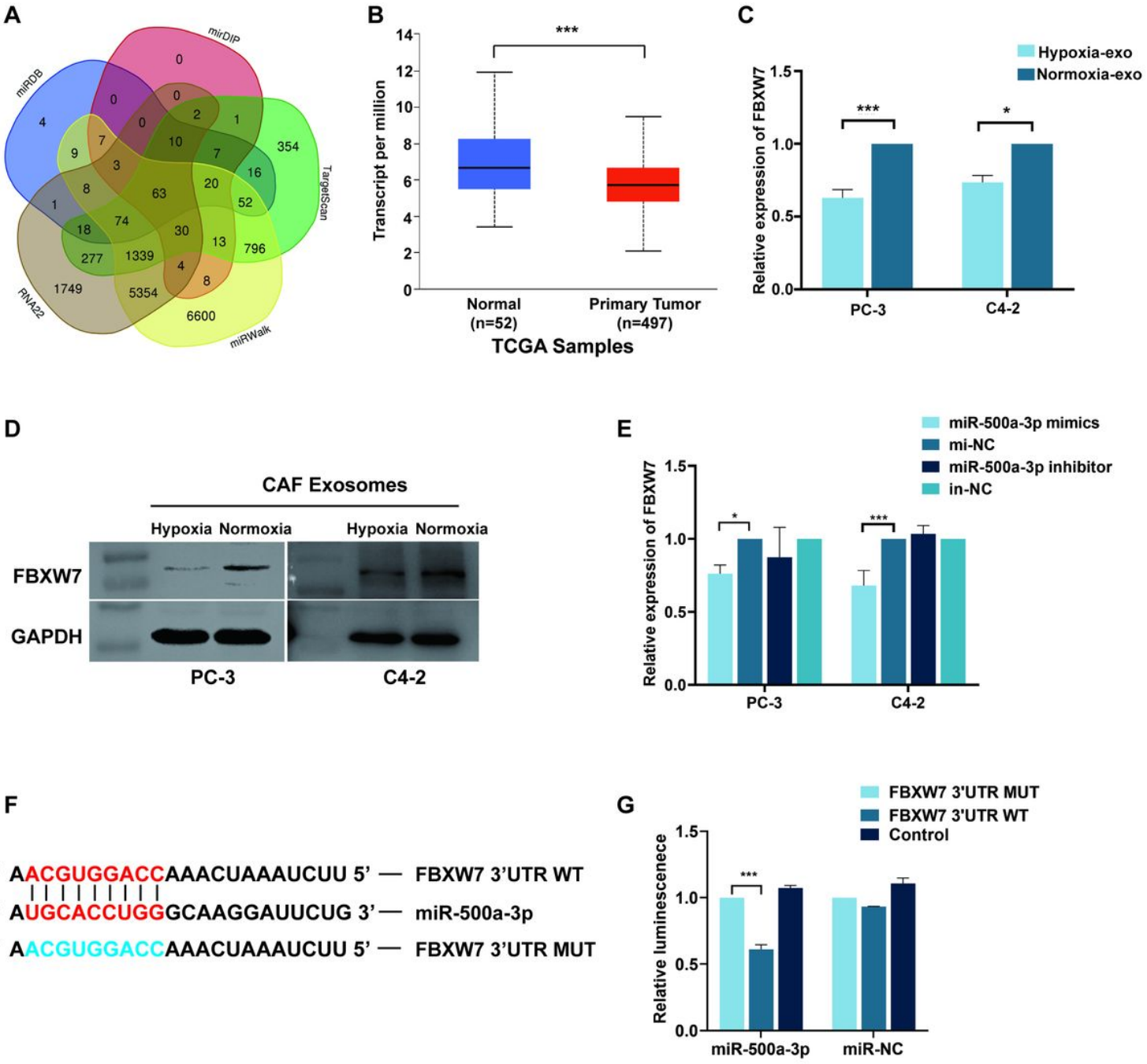

Figure 5

FBXW7 was a target gene of miR-500a-3p. (A) FBXW7 was a target gene of miR-500a-3p. (A) Venn diagram showing the common target genes of miR-500a-3p through five miRNA prediction databases. (B) The TCGA database showing the contrasting expression levels of FBXW7 mRNA between PCa tissues

and normal controls. (C, D) RT-qPCR and western blotting analysis of FBXW7 expression after CAF hypoxic and normoxia exosomes treatment. (E, F) RT-qPCR and western blotting analysis of FBXW7 expression after miR-500a-3p mimics and inhibitor transfection in PCa cells. (G) Nucleotide sequences of miR-500a-3p, along with the WT and MUT 3' untranslated regions (UTR) of FBXW7. (H) Execution of a luciferase assay in 293T cells, validating the direct interaction between miR-500a-3p and the 3'-UTR of FBXW7. Control group was transfected with empty plasmids. *P 0.05, **P 0.01, ***P 0.001.

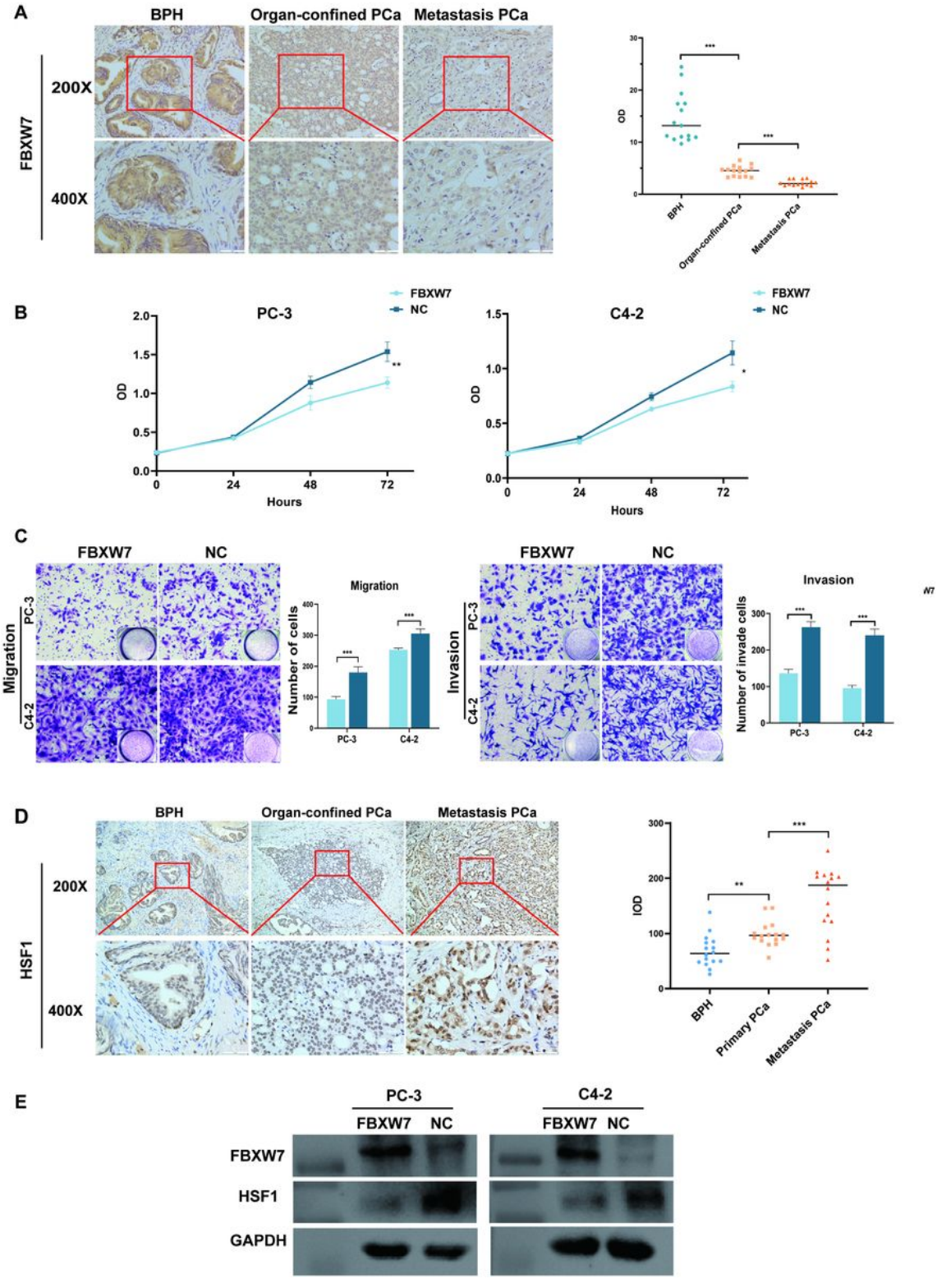

Figure 6

FBXW7 regulate PCa progression through suppressing HSF1 expression. (A) Representative images of IHC for FBXW7 in BPH and PCa tissues with or without metastasis (left). Quantitative assessment of IHC scores is illustrated (right). (B) CCK-8 assay conducted to evaluate cellular proliferation subsequent to FBXW7 plasmid transfection. (C) Migration and invasion assays performed to assess alterations in cellular phenotypes subsequent to transfection with FBXW7 plasmids. (D) Representative images of IHC for HSF1 in BPH and PCa tissues with or without metastasis (upper). Statistical analysis of the IHC scores (down). (E) Western blotting analysis of HSF1 expression after FBXW7 plasmid transfection in PC-3 and C4-2 cells.

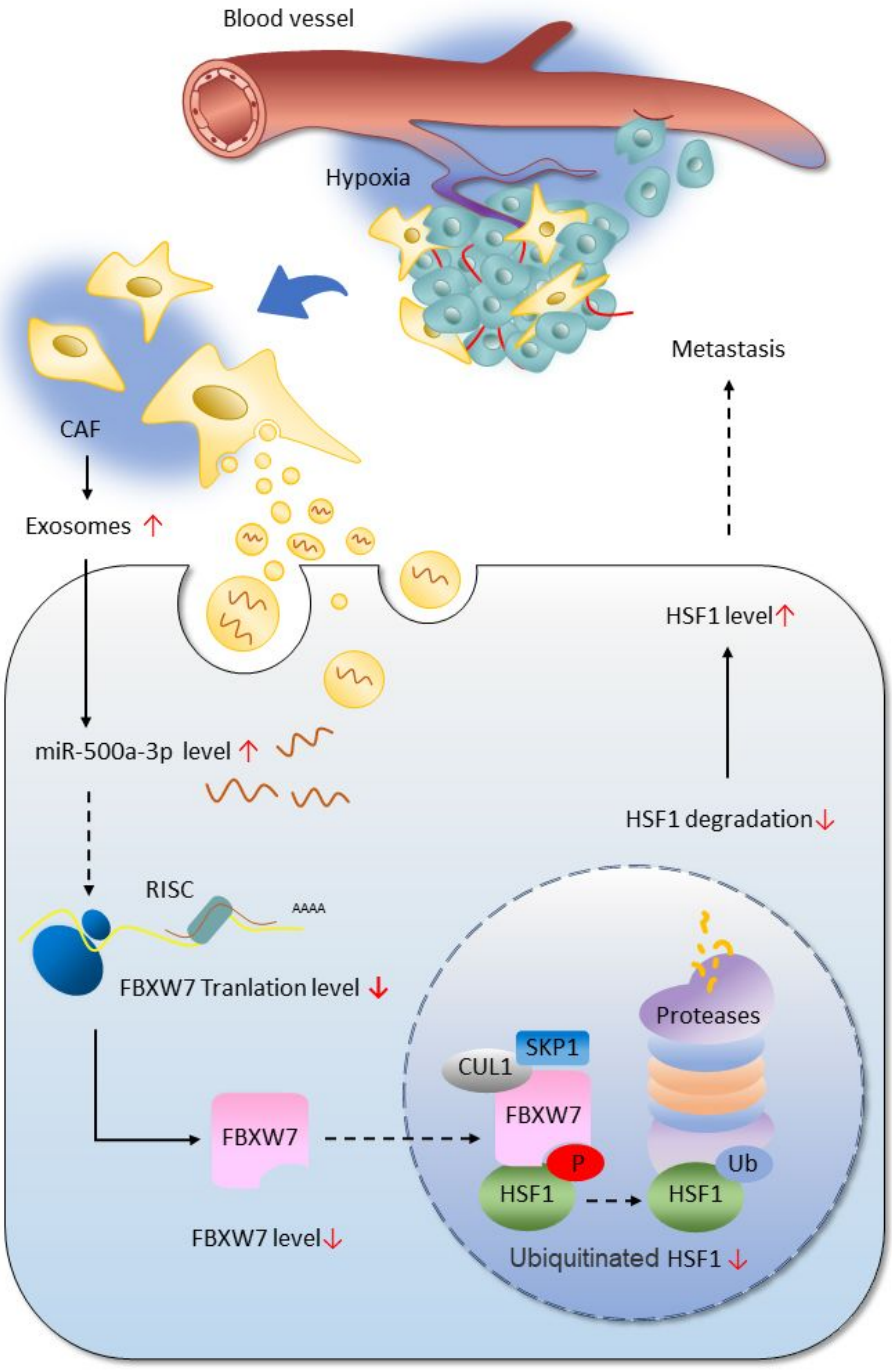

Figure 7

Schematic diagram depicts that CAFs-derived exosomes promote PCa metastasis through miR-500a-3p/FBXW7/HSF1 axis under hypoxic microenvironment.

Supplementary Files

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- Supplementary.pdf