N-Myc Promotes Angiogenesis and Therapeutic Resistance of Prostate Cancer by TEM8

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Research Article
Abstract

Although patients with early localized prostate cancer can achieve a longer survival, castration-resistant prostate cancer (CRPC) has gradually developed with the use of androgen deprivation therapy (ADT). N-Myc and TEM8 play an important role in the progression of several cancer types. However, the underlying mechanism of how N-Myc and TEM8 promote the progression of prostate cancer remains unclear. In this study, the expression of N-Myc and TEM8 were detected in benign prostatic hyperplasia (BPH) and prostate cancer (PCa) tissues by immunohistochemistry (IHC). To find the mechanism of prostate cancer resistance to treatment, LNCaP cell lines were maintained in RPMI 1640 medium supplemented with 10% Charcoal-stripped fetal Bovine Serum. Subsequently, R language software was used to verify our results. Tubule formation assay of human umbilical vein endothelial cell (HUVEC) was conducted to examine the effect of N-Myc and TEM8 overexpression on angiogenesis of prostate cancer cells. IHC results showed a positive correlation between the expression of N-Myc and TEM8 in prostate cancer tissues. Further analysis showed that N-Myc and TEM8 were associated with clinicopathological features and poor prognosis in prostate cancer patients. We found that the overexpression of N-Myc and TEM8 promotes proliferative ability and angiogenesis. Further, N-Myc and TEM8 overexpression was associated with therapeutic resistance. N-Myc promoted angiogenesis and therapeutic resistance in prostate cancer via TEM8. Hence, targeting N-Myc/TEM8 pathway in prostate cancer would be a novel therapeutic strategy to advance the treatment of prostate cancer patients.

1. Introduction

Prostate cancer (PCa) is the most diagnosed malignancy in men and the second leading cause of cancer-related deaths in the US. Although the recent improvement in treatment options has significantly reduced the incidence rate, there has been a steady increase in advanced or metastasized prostate cancers[1]. Generally, the symptoms of prostate cancer in its early stage are not obvious in most cases, which makes the treatment of prostate cancer patients more difficult as some patients have metastasis at the time of diagnosis [2]. The progression of prostate cancer follows a multistep process that includes prostate intraepithelial neoplasia (PIN), local prostate cancer, advanced prostate adenocarcinoma with local invasion and metastatic prostate cancer [3]. In recent times, the advent usage of Androgen deprivation therapy (ADT) has brought some symptomatic relief to prostate cancer patients. However, this also leads to the development of castration-resistant prostate cancer. Therefore, a deeper understanding of the mechanism of the disease process is relevant in our quest to find a novel and specific biomarker for prostate cancer treatment.

N-Myc belongs to the MYC proto-oncogene family and it is known for its classical oncogenic activity and association with human neuroblastoma. Mounting studies have indicated that N-Myc is involved in all facets of prostate cancer progression [4–6] including but not limited to the transformation of castration-resistant prostate cancer to the neuroendocrine direction [7, 8]. Previous study has shown that the expression of TEM8 is up-regulated in the prostate cancer cell lines LNCaP and 22RV1 particularly after the overexpression of N-Myc [9]. However, the detailed mechanism of how N-Myc interacts with TEM8 to
promote prostate cancer growth has scarcely been studied. TEM8, also known as ANTXR1, is a cell-surface transmembrane protein initially identified in colonic tumor vascular endothelial cells [10]. Some studies have shown that the vascular density in high-grade prostate cancer is significantly higher than that in low-grade prostate cancer tumors [11], which implicates angiogenesis as a mechanism of prostate cancer progression. TEM8 is specifically expressed in tumor blood vessels and some tumor cells [12, 13] suggesting its potential advantages as an anti-tumor angiogenesis target [14, 15]. Even though TEM8 is associated with poor prognosis in several solid tumors [16–19], little is known about its role in prostate cancer. This study is therefore designed to elucidate how N-Myc interacts with TEM8 to promote angiogenesis and treatment resistance in prostate cancer.

2. Material And Methods

2.1 Patients and Specimens

Formalin-fixed-paraffin-embedded tissues were collected from 151 patients who underwent surgical operation at the First Affiliated Hospital of Anhui Medical University. The study was approved by the Ethical Committee of Anhui medical university.

2.2 RT-qPCR assays

Total RNA was isolated from cultured cells using Trizol reagent (Invitrogen,) according to the manufacture's protocol. RNA was reversely transcribed into cDNA using the PrimeScript RT Master Mix (Takara, RR036A). Gene-specific primers as follows:

GAPDH: Forward, 5’-CATGAGAAGTATGACAACAGCCT-3’,
reverse, 5’-AGTCCTTCCACGATACCAAAGT-3’;

N-Myc: Forward, 5’-CACGTCCGCTCAAGAGTGTC-3’,
reverse, 5’-GTTTCTGCGACGCTCACTGT-3’;

TEM8: Forward 5’-GATGATGATGGTCTGCCTAAGA-3’,
reverse 5’-TCTTTGCCTTTTCCAACCTTAGC-3’.

2.3 Cell culture and construction

The cell lines were maintained in RPMI 1640 medium (HyClone, SH30809.01), supplemented with 10% FBS (BI, 04-001-01A) or 10% Charcoal-stripped fetal Bovine Serum (MRC, CCS30010.01HT), 1.5 mM L-Glutamine and 1% Penicillin-Streptomycin solution. Lentiviral vector for N-Myc and TEM8 was purchased from GenePharma (Shanghai, China) and stably transduced into both LNCap and C4-2 cell lines. Finally,
stable prostate cancer cells were successfully screened with the appropriate concentration of puromycin. The efficiency of overexpression was analyzed by quantitative real-time PCR (qPCR) and Western blot. Also, cell lines with N-Myc overexpression were transfected with shRNA- TEM8. The sequences of shRNA-TEM8 were designed as follows: GCTGAACCATTCCACCATATGT. A non-targeting shRNA (Genepharma) was used as a control.

2.4 Western Blotting

Western blotting was conducted under standard protocols. Briefly, proteins obtained from prostate cancer cells were lysed using RIPA buffer. Proteins were loaded onto 10% SDS-PAGE gels and then transferred to NC membranes. Nonspecific binding was blocked with 5% dried skim milk. The membrane was incubated overnight at 4°C with the following primary antibodies: anti-GAPDH (dilution 1:1000, ProteinTech, 10494-1-AP), anti-N-Myc (dilution 1:1000, CST, #51705s), anti-TEM8 (dilution 1:400, Abcam, #13798) and anti-AR (dilution 1:2000, CST, 5153s). After incubation with peroxidase-conjugated secondary antibody, the protein bands were observed with ECL. The intensity of the protein bands was normalized with GAPDH.

2.5 Immunohistochemical analysis

Formalin-fixed-paraffin-embedded samples were obtained from benign prostatic hyperplasia and prostate cancer patients. Briefly, sections were deparaffinized with xylene and rehydrated with graded ethanol. Heat-induced antigen retrieval was performed according to the relevant antibody instructions and endogenous peroxidase activities were blocked with hydrogen peroxide for 10 min at room temperature. The sections were incubated with the following antibodies; anti-N-Myc antibody (dilution 1:640, CST, #51705s), anti-TEM8 antibody (dilution 1:20, NOVUS, 200C1339). Positive and negative controls were incubated in parallel with the primary antibody. The positive staining showed diffuse homogeneity pale yellow to brown yellow granules. The immunohistochemistry procedure and scoring of N-Myc, TEM8 expression were conducted as previously described [20].

2.6 Cell Counting Kit-8

100ul of cell suspension was added to each well (96 well plate), and cultured for 1 day, 2 days and 3 days respectively. After incubation with 10 ul of cck8 solution for 4 hours, the absorbance was measured at 450 nm.

2.7 Tubule Formation Assay

Matrigel (# 356234, Corning) was added into precooled 96-well plates (60μL/well) and incubated at 37°C for 30min. 3×10^4 HUVEC cells were inoculated into each well, and the corresponding concentrated supernatant was added and placed into the incubator for further incubation. The tube formation was
observed under an inverted microscope at different time points. Image J was used to measure the total lengths of the tube.

### 2.8 Oncomine and GEO datasets analysis

To validate the prognostic value of N-Myc and TEM8 in prostate cancer patients, Oncomine microarray database (https://www.oncomine.org) was used. In this study, the threshold was defined as p-value = 0.0001, 2 fold change and top 10% gene rank. Then, Taylor Prostate Dataset [21] from Oncomine about Overall Survival Follow-up Time (Months) and gene expression level of N-Myc and TEM8 were plotted using GraphPad Prism software.

In GSE150368 datasets, LIMMA package and Edger package of R software was used to detect and screen differentially expressed genes (DEGs) in prostate tissue before and after ADT treatment. DEGs analysis was performed based on the screening criteria of FDR>0.05 and |log (FC)|>1. KEGG enrichment analysis was used to detect the relationship between function and signaling pathway of DEGs. To study the interaction between DEGs, the String Online database was used to identify the protein-protein interaction (PPI) network (Http://string-db.org) with a confidence score of ≥ 0.15

### 2.9 Statistical Methods

All the experiments had three independent replicates and the data were presented as mean±SD. Statistical testing was done using Pearson chi-squared test or Log-rank test unless otherwise indicated. A p-value of <0.05 was considered statistically significant.

### 3. Results

#### 3.1 N-Myc and TEM8 expression were associated with clinicopathological features of PCa and there was a positive correlation between them.

IHC results showed that the positive rate of N-Myc and TEM8 expression was significantly higher in PCa than BPH samples (Fig. 1A, Table 1). The high levels of N-Myc and TEM8 were associated with a high Gleason score, advanced TNM stage and Osseous metastasis. However, only TEM8 expression and not N-Myc was associated with a high PSA levels (Table 2).

We observed that N-Myc expression was positively correlated to the expression of TEM8 in PCa (R = 0.244, P = 0.02) (Table 3). According to the oncomine database, patients with high expression of N-Myc or TEM8 had a significantly lower overall survival rate compared to those with low N-Myc or TEM8 expression (Fig. 1B). Taken together, N-Myc and TEM8 expressions are closely related to the clinical progression and prognosis of prostate cancer patients.
3.2 N-Myc overexpression upregulated TEM8 expression in PCa cells.

This study found the expression of N-Myc and TEM8 to increase with an increase in the degree of prostate cancer (P < 0.05) (Fig. 2A), suggesting that N-Myc and TEM8 are involved in the progression of prostate cancer. To further study the relationship between N-Myc and TEM8 in prostate cancer progression, stable cell lines with N-Myc and TEM8 overexpression were generated by lentivirus infection, and the results was observed by fluorescence microscopy (Fig. 2B). Subsequently, the overexpression of N-Myc and TEM8 were verified in LNCaP and C4-2 cell lines by western blot and PCR (Fig. 2C).

To validate the results obtained from clinical samples, the mRNA and protein expressions of TEM8 were detected respectively in N-Myc overexpressing stable cell lines. Our study further confirmed that N-Myc regulated the expression of TEM8 in prostate cancer cells (Figure. 2D).

Figure 1. Expression of N-Myc and TEM8 in prostate tissues and their correlation with prognosis. (A) N-Myc was negatively expressed in BPH. (B) N-Myc was positively expressed in PCa (left), and N-Myc was expressed in the nucleus and/or cytoplasm of PCa cells (right). (C) TEM8 was negatively expressed in BPH. (D) TEM8 was negatively expressed in PCa (left), and TEM8 was expressed in the membrane and/or cytoplasm of PCa cells (right). (E) N-Myc and TEM8 was related to the overall survival rate of PCa as shown by Taylor Prostate dataset in the Oncomine database

| Group            | n   | N-Myc       | p-value | TEM8        | p-value  |
|------------------|-----|-------------|---------|-------------|---------|
|                  |     | low,n(%)    | high,n(%) | low,n(%)    | high,n(%) |      |
| Benign Prostate  | 60  | 56(93.33)   | 4(6.67)  | 57(95.00)   | 3(5.00)  | < 0.001*** |
| Adenocarcinoma   | 91  | 67(73.63)   | 24(26.37)| 33(36.26)   | 58(63.74)|         |

* P < 0.05, ***P < 0.001
### Table II

**Clinicopathological parameters in prostate cancer**

| Characteristic          | n   | N-Myc expression | P-value | TEM8 expression | P-value |
|-------------------------|-----|------------------|---------|-----------------|---------|
|                         |     | low,n(%)        |         | high,n(%)       |         |
| Age, years              |     |                  |         |                 |         |
| ≤ 70                    | 45  | 30(66.67)        | 0.136   | 18(40.00)       | 0.463   |
| > 70                    | 46  | 37(80.43)        | 0.016   | 15(32.61)       | 0.679   |
| PSA at initial diagnosis(mg/L) |     |                  |         |                 |         |
| < 20                    | 40  | 32(80.49)        | 0.222   | 20(50.00)       | 0.016   |
| ≥ 20                    | 51  | 35(68.00)        | 0.016   | 13(25.49)       | 0.741   |
| Gleason score           |     |                  |         |                 |         |
| ≤ 7                     | 43  | 38(88.37)        | 0.003   | 21(48.84)       | 0.013   |
| > 7                     | 48  | 29(60.42)        | 0.018   | 12(25.00)       | 0.543   |
| TNM stage               |     |                  |         |                 |         |
| I-II                    | 45  | 40(88.11)        | 0.001   | 22(48.89)       | 0.001   |
| III-II                  | 46  | 27(58.70)        | 0.013   | 11(23.91)       | 0.013   |
| Osseous metastasis      |     |                  |         |                 |         |
| No                      | 71  | 59(83.10)        | < 0.001 | 30(42.25)       | 0.025   |
| Yes                     | 20  | 8(40.00)         | 0.001   | 3(15.00)        | 0.025   |

* P<0.05, ** P<0.01, *** P<0.001

### Table III

**Correlation between expression of N-Myc and TEM8 in prostate cancer tissues**

| N-Myc | TEM8 | n   | rs  | P-value |
|-------|------|-----|-----|---------|
|       |      | high|     |         |
| high  | 20   | 4   | 24  | 0.244   | 0.02*   |
| low   | 38   | 29  | 67  |         |         |
|       | 58   | 33  | 91  |         |         |

* P<0.05
Figure 2. Prostate cancer cell lines were detected after overexpression of N-Myc and TEM8 with lentivirus. (A) The mRNA expression level of N-Myc and TEM8 in LNCaP, C4-2 and PC3 cell lines. (B) N-Myc and TEM8 overexpressing stable cell lines for LNCaP and C4-2 by lentivirus infection as observed under fluorescence microscope (×100). (C) Expression of N-Myc and TEM8 were detected in lentivirus-transfected prostate cell lines at mRNA and protein levels respectively. The mRNA expression level of N-Myc and TEM8 in LNCaP, C4-2 and PC3 cell lines. (D) Expression of TEM8 was detected in N-Myc overexpressing stable cell lines (*P < 0.05, ** P < 0.005, *** P < 0.001).

### 3.3 Overexpression of N-Myc and TEM8 promoted the proliferation and tubule formation of prostate cancer cells.

In N-Myc overexpressing stable cells (LNCaP and C4-2), the expression of TEM8 was verified after TEM8 shRNA knockdown (Fig. 3A). When the proliferative ability of prostate cancer cells was detected at a fixed time point, we found that compared with LNCaP/Vector, the growth rate of cancer cells was significantly faster in LNCaP/N-Myc and LNCaP/TEM8 groups (P < 0.05). However, the proliferation of cancer cells was lower in LNCaP/N-Myc/shTEM8 compared with LNCaP/N-Myc, and the same trend can be observed in the C4-2 group (Fig. 3B). Next, we found from tube formation experiments that overexpression of N-Myc and TEM8 significantly promoted angiogenesis in prostate cancer (Fig. 3C).

### 3.4 N-Myc overexpression confers LNCaP cells resistant to ADT treatment.

CCK8 experiment demonstrated that LNCaP/N-Myc and LNCaP/TEM8 cells could promote the proliferation of prostate cancer cells after ADT treatment. This suggests that N-Myc and TEM8 are likely to have ADT resistance (Fig. 4A). With the extension of ADT treatment time, the expression level of AR protein in LNCaP/Vector cells decreased gradually, however, the opposite trend was observed in LNCaP/N-Myc cells. Although the expression of AR did not increase significantly in LNCaP/TEM8 cells, the expression of TEM8 was increased (Fig. 4B).

To further confirm our experimental results, we used the bioinformatics method to analyze the differently expressed genes of PCa before and after ADT. 1693 differently expressed genes were obtained, including 1227 up-regulated genes and 466 down-regulated genes (Supplementary Table S1). To explore the enrichment of the functions and pathways of the differential gene, KEGG analysis showed that the differential gene was associated with pathways such as Cytokine-cytokine receptor interaction, Staphylococcus aureus infection, Human T-cell leukemia virus 1 infection, Cell adhesion molecules and Chemokine signaling pathway (Fig. 4C). The network core genes were obtained according to the number of gene adjacent nodes, and TEM8 had 75 adjacent nodes (Fig. 4D, Supplementary Table S2). The up-regulated and down-regulated differential genes were summarized (Fig. 4E). Using SRTING online database, we constructed a protein network interacting with TEM8 protein (Fig. 4F).
Discussion

N-Myc, a critical oncoprotein required for neuroendocrine tumor development, is overexpressed and amplified in approximately 5% of PCA and 40% of NEPC [22, 23]. During the progression of prostate cancer, N-Myc overexpression can potentiate the escape of tumors from AR dependence and promote the appearance of CRPC and NEPC [8]. Dardenne et al. found that N-Myc can cooperate with EZH2 to establish a new signaling pathway conducive to tumor cell survival, which can drive the differentiation of prostate cancer to the neuroendocrine direction [7]. AURKA stabilizes the N-Myc gene, and Beltran et al. found that inhibition of AURKA resulted in loss of neuroendocrine differentiation [22]. Other emerging evidence shows that N-Myc amplification is also associated with a higher vascular density in Neuroblastoma [24]. However the detailed underlying mechanism of action of N-Myc in PCa is not fully understood.

TEM8, an integrin-like cell surface transmembrane protein, was originally identified for its high expression in colorectal tumor endothelial cells [14]. Subsequently, TEM8 was found to be a receptor for anthrax toxin, mediating the entry of anthrax toxin into cells [25]. Antibodies against TEM8 extracellular domains have shown a broad range of anti-tumor activity due to their ability to target TEM8 and selectively inhibit pathological angiogenesis without an increase in toxicity [26]. In breast cancer, specific antibodies against TEM8 can target both triple-negative breast cancer stem cells and the tumor-associated vascular system to induce tumor regression [27]. Although this implicates TEM8 to have potential advantages as a tumor therapeutic target, such evidence has rarely been reported in prostate cancer.

We found that N-Myc and TEM8 expression in clinical samples correlated with prostate cancer tissue type, tumor progression, and prognosis. The positive rates of N-Myc and TEM8 were significantly higher in prostate cancer tissues with Gleason score above 7 and TNM stage at II/IV. The expression of N-Myc and TEM8 was significantly higher in patients with bone metastasis than those without bone metastasis. Furthermore, expression of N-Myc and TEM8 was found to be associated with poor prognosis in prostate cancer patients. These results suggest that N-Myc expression is a predictor of advanced stages of prostate cancer and plays a key role in prostate cancer progression. Moreover, we first demonstrated a significant positive correlation between N-Myc and TEM8 expression in PCa samples. Further experiments revealed that N-Myc overexpression upregulated the expression of TEM8 expression. This finding is consistent with the study by Dardenne’s study [9]. To characterize the functional role of N-Myc and TEM8 in prostate cancer, our findings suggest that N-Myc promotes a higher proliferation rate in prostate cancer cells by regulating TEM8. However, the regulatory action of N-Myc on TEM8 in prostate cancer still requires further exploration.

There is increasing evidence that the incidence of lethal PCa might be increased following the increase usage of androgen deprivation therapy, which has brought difficulty in the treatment of advanced prostate cancer. Our tubule formation assays confirm that N-Myc and TEM8 may play a key role in angiogenesis in prostate cancer. Herein, we found that overexpression of N-Myc and TEM8 significantly increased the proliferation of PCa cells after a period of ADT treatment. Meanwhile, compared with the
control group, the expression level of AR protein in N-Myc overexpression group was not completely abrogated but showed a gradual increase. Consistent with our findings, previous studies have demonstrated that N-Myc has an inhibitory action on the expression of AR in PCa cells and thus rendering ADT ineffective. We showed that N-Myc upregulation after ADT treatment was associated with an increase in the expression of TEM8. Interestingly, AR protein levels did not change significantly and remained at a high level in the overexpressed TEM8 group after ADT treatment, suggesting that TEM8 can potentiate the escape of prostate cancers from castration. This is suggestive that N-Myc can potentiate the escape of prostate cancer cells from ADT therapy by upregulating the expression of TEM8.

Changes in protein levels after ADT and tubule formation assays in our study showed that N-Myc may increase therapeutic resistance and angiogenesis in prostate cancer by regulating TEM8.

**Conclusion**

Our study has revealed new insights into the role of N-Myc and TEM8 in promoting angiogenesis and therapeutic resistance in prostate cancer. This study also showed for the first time that N-Myc can regulate the expression of TEM8 in prostate cancer. Our findings showed the expression of TEM8 to be associated with markers of prostate cancer progression. Hence, our finding supports the notion that TEM8 can be used as a marker to indicate treatment response in advanced prostate cancer patients.

**Abbreviations**

CRPC  Castration resistance prostate cancer  
ADT  Androgen deprivation therapy  
BPH  Benign prostatic hyperplasia  
PCa  Prostate cancer  
IHC  immunohistochemistry  
HUVEC  human umbilical vein endothelial cell  
PIN  prostate intraepithelial neoplasia  
shRNA  Short hairpin RNA  
AR  Androgen receptor  
DEGs  differentially expressed genes  
PPI  protein-protein interaction
NEPC  Neuroendocrine prostate cancer

Declarations

Funding

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Conflicts of interest

The authors declare that they have no potential conflicts of interest.

Availability of data and materials

The datasets used in this study are available from the first author upon reasonable request.

Code availability

Not applicable.

Authors' contributions

YY and CL designed the research. WL, LY, YH and SY collected the data. ML, LF, GH cooperated to perform the experiments and drafted the manuscript. YC, WM, HZ, ZT, LZ and Louis BK reviewed and revised the manuscript. All authors have reviewed the article and approved the final manuscript.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication
Not applicable.

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Figure 1

Expression of N-Myc and TEM8 in prostate tissues and their correlation with prognosis. (A) N-Myc was negatively expressed in BPH. (B) N-Myc was positively expressed in PCa (left), and N-Myc was expressed in the nucleus and/or cytoplasm of PCa cells (right). (C) TEM8 was negatively expressed in BPH. (D) TEM8 was negatively expressed in PCa (left), and TEM8 was expressed in the membrane and/or
cytoplasm of PCa cells (right). (E) N-Myc and TEM8 was related to the overall survival rate of PCa as shown by Taylor Prostate dataset in the Oncomine database.

**Figure 2**

Prostate cancer cell lines were detected after overexpression of N-Myc and TEM8 with lentivirus. (A) The mRNA expression level of N-Myc and TEM8 in LNCaP, C4-2 and PC3 cell lines. (B) N-Myc and TEM8 overexpressing stable cell lines for LNCaP and C4-2 by lentivirus infection as observed under
fluorescence microscope (×100). (C) Expression of N-Myc and TEM8 were detected in lentivirus-transfected prostate cell lines at mRNA and protein levels respectively. The mRNA expression level of N-Myc and TEM8 in LNCaP, C4-2 and PC3 cell lines. (D) Expression of TEM8 was detected in N-Myc overexpressing stable cell lines (*P < 0.05, ** P < 0.005, *** P < 0.001).
Effects of N-Myc, TEM8 on the proliferative ability and tubule formation of PCa cells. (A) The mRNA and protein levels expression of TEM8 were verified after LNCaP/N-Myc and C4-2/N-Myc cells were treated with TEM8-shRNA. (B) Compared with the control group, the overexpression of N-Myc and TEM8 affected the proliferative ability of LNCaP and C4-2 cells. (C) HUVEC tube formation assay was determined using the supernatant of LNCaP cells.

Figure 4
Effects of ADT treatment on proliferation and protein expression of stable cell lines. (A) After 0, 1, 4 and 7 days of ADT treatment, the proliferation rates of LNCaP/N-Myc and LNCaP/TEM8 cells were higher than those of the control group (*P < 0.05, ** P < 0.005, *** P < 0.001). (B) ADT treatment of LNCaP/Vector, LNCaP/N-Myc and LNCaP/TEM8 cells could not inhibit the expression of N-Myc and TEM8. (C) KEGG pathway enrichment analysis of the differentially expressed genes after ADT treatment. (D) The Hub genes were identified. (E) A heatmap of ADT-treated differentially expressed genes in the GEO dataset were plotted. (F) Protein-protein interaction (PPI) network related to TEM8 was constructed using the STRING online database.

![Diagram showing the regulation of N-Myc and TEM8](image)

**Legend**
- Castration-resistant prostate cancer cell
- Prostate cancer cell

**Figure 5**

Our study has revealed new insights into the role of N-Myc and TEM8 in promoting angiogenesis and therapeutic resistance in prostate cancer. This study also showed for the first time that N-Myc can regulate the expression of TEM8 in prostate cancer. Our findings showed the expression of TEM8 to be associated with markers of prostate cancer progression. Hence, our finding supports the notion that TEM8 can be used as a marker to indicate treatment response in advanced prostate cancer patients.