A novel monoclonal antibody PC322 inhibits prostate cancer cell autophagy

Xing Kang1, Xu Zhang1, Xuejia Zhu1, Yuhan Yan2, Chong Li1,3,4,5*

1Core Facility for Protein Research, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China
2Department of Management Science and Engineering, School of Business, Renmin University of China, Beijing 100872, China
3Beijing Jianlan Institute of Medicine, Beijing 100190, China
4Beijing Zhongke Jianlan Biotechnology Co., Ltd., Beijing 101400, China
5Guyuzhongke (Beijing) Medical Technology Co., Ltd., Beijing 100061, China

Abstract: Autophagy is an important element in tumor progression. The clinical and commercial success of monoclonal antibodies (MAbs) for cancer has emerged as the fastest growing therapy. Prostate cancer is well appropriately suited for antibody therapy. PC322 is our newly prepared prostate cancer MAbs. In our current study, we described the impacts of PC322 on autophagy and biological behaviors in vitro and explored the possible mechanism of PC322 in prostate cancer cell autophagy. Role of PC322 on autophagy of LNCaP and C4-2 cells was observed with fluorescence microscopy, western blotting, and flow cytometry. Further, the function of PC322 on biological behaviors of prostate cancer cells was detected by Cell Counting Kit-8 kit, Hoechst 33342, wound healing assay, and cell adhesion assay. Finally, we found that autophagy-related genes3 (ATG3) was upregulated with stimulation of rapamycin, while PC322 inhibited ATG3 expression. In summary, in vitro experiments demonstrated that the new MAb PC322 inhibits cell autophagy, proliferation, and metastasis of LNCaP and C4-2 cells, and promote their apoptosis. PC322 inhibits LC3 conversion and suppresses the formation of autophagosomes by reducing ATG3 expression.

Keywords: prostate cancer; monoclonal antibody; autophagy; autophagosomes; autophagy-related genes

1. Introduction

In developed countries and regions, prostate carcinoma is the most frequently diagnosed carcinoma in men[1]. In 2012, it is assessed that 1.1 million worldwide newly recognized prostate carcinoma cases occurred, as a percentage of 15% in diagnosed male cancers, of which about 70% occurred in more developed countries and regions[2]. Prostate carcinoma is the fifth in male cancer mortality, which is higher in underdeveloped areas[2]. Prostate carcinoma diagnosis depends on transrectal ultrasound (TRUS) biopsy, but TRUS cannot be used for staging[3]. Compared to pathological staging, imaging staging still has lower accuracy[3]. The nomograms developed from clinical grading, prostate-specific antigen and Gleason score can be used to estimate extra-capsular invasive risk in radical prostatectomy cases[4]. In terms of treatment, localized prostate cancer patients are under a variety of treatment options[5]. Numerous studies have shown that most low-risk patients can avoid any therapies within 10 years and only 1% of them die for prostate cancer[6,7]. Although the emergence of new drugs in recent years has improved the prognosis of metastatic castration-resistant prostate carcinoma[8], all the latest medicine
for advanced prostate carcinoma is pricey under patent. In addition, controversies over screening and treatment of prostate cancer still remain. Therefore, it is necessary to further study the potential mechanism of the occurrence and progress of prostate cancer and propose new prognoses and treatment measures.

Autophagy is a course in which damaged organelles, long-lived proteins, and wastes in the cell are transported to lysosomes for degrading\[9\]. In general, autophagy maintains a very low level for cell homeostasis but can be strongly induced as a primary protective response under conditions of stress, such as nutritional deprivation, growth factor absent, oxidative stress, anoxia, radiation, and antineoplastic therapies\[8\]. The impact of autophagy in cancer is contradictory and has not been fully elucidated: Whether it is promoting or inhibiting tumor cells survive depends on cell type, genetic context, cancer development stage, and nature of the stressor\[8,9\]. In this study, we presuppose that autophagy promotes the progression of tumors. As a mechanism of promoting survival, transformed cells use autophagy to adapt to tumor microenvironment caused by insufficient blood supply\[7\]. As a mechanism of resistance and survival promotion, anticancer treatment leads to autophagy in some tumor cells; autophagy inhibition gains the efficacy of anticancer therapies in these cases\[12,13\].

The achievements of monoclonal antibodies (MAbs) in clinical and commerce have emerged as one of the fastest growing therapies for many diseases, including cancer. The prostate is a non-essential organ and the mass and section of metastatic and recurrent tumors are apt, so prostate cancer is very suitable for antibody therapy. These attributes increased researchers’ interests in the clinical estimation of established and newly discovered therapeutic MAbs for prostate cancer. MAbs approved to be used for the treatment of other solid tumors include anti-human epidermal growth factor receptor-2 MAbs trastuzumab\[14,15\], anti-epidermal growth factor receptor MAbs cetuximab and panitumumab\[16,17\], and anti-vascular endothelial growth factor MAbs bevacizumab\[18-20\]. The results of genomics have produced a large number of novel clinical targets for prostate cancer. More and more efforts have been undertaken to build up naked antibodies or antibody-drug conjugates against these targets, and many MAb products are currently at various stages of preclinical and clinical research. PC322 is a newly prepared Mab against prostate cancer.

We immunized BALB/c mice with fresh human prostate cancer tissue protein homogenate, then prepared lymphocyte suspension from mice spleen and fused with myeloma cells. Hybridoma cells secreting antibodies were determined by ELISA. The positive hybridoma cells were cloned and supernatants were collected. The MAb PC322 was purified by Protein G affinity chromatography. At present, PC322 is in the stage of in vitro research.

2. Materials and Methods

2.1. MAb PC322 preparation and purification

BALB/c mice were immunized by intraperitoneal injection of 10ug total fresh homogenates of human prostate cancer tissue protein 3 times at intervals of 2 weeks. Cell fusion was carried out 3 days after intensive immunization. 24 hours later, HAT selective medium (Invitrogen) was added for selective culture. Hybridoma cell lines secreting antibodies were identified by ELISA. After 2-3 rounds of cloning culture, the hybridoma cell clones which can stably produce high titer; MAbs were extended and preserved, and the supernatants of cell culture were collected. The MAb was purified by Protein G affinity chromatography column (General Electric Company, USA).

2.2. Observation of autophagic bright spots

LNCaP and C4-2 cells at the logarithmic growth stage were sown on four-well plates containing Thermaxon sterile plastic cover slide (Nalge Nunc International, USA) at 50,000 cells/well and permitted to adhere overnight. GFP-LC3 and mRFP-GFP-LC3 plasmids were transfected into these cells basing on the procedure provided by Lipofectamine 2000 Transfection Reagent (ThermoFisher). 24h after transfection, those cells were dealt with PC322 and 200nM Rapamycin (RAPA) (Gene Operation, USA), respectively, for 24 h, and then fixed with 4% paraformaldehyde (PFA) for 10 min at room temperature, washed with PBS for 10 min. The autophagic spots were observed with the fluorescence microscope.

2.3. Western blotting

Prostate cancer cells were dealt with PC322 or RAPA for 24h. Total protein was extracted, quantified and separated with 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, and then transferred onto a nitrocellulose membrane. Membranes were probed with anti-beta-actin (Sigma-Aldrich, A1978), Anti-LC3B (Sigma-Aldrich, L7543), and HRP- conjugated secondary antibody (Sigma-Aldrich, A1978) for 24 h, and then fixed with 4% paraformaldehyde (PFA) for 10 min at room temperature, washed with PBS for 10 min. The autophagic spots were observed with the fluorescence microscope.

2.4. IC50 determination

LNCaP/C4-2 cells were sown on 96-well plates at 2000/100ul per well and allowed to adhere overnight. The test materials were placed on the plate and cultured for 24, 48, and 72h, respectively. IC50 was counted by Cell Counting Kit (CCK-8) (Dojindo). Absorption at 450nm was measured by a microplate reader (BIO-RAD).

2.5. Apoptosis assay

Prostate cancer cells were sown on four-well plates containing Thermaxon sterile plastic cover slide (Nalge Nunc, USA) with a cell number of 50,000/well and
permitted to adhere overnight. The cells were treated with PC322 or RAPA for 24h and then fixed with Carnoy fixed solution (three parts of ethanol and one part of acetic acid) for 10 min, stained with Hoechst 33342 at the indoor temperature in the dark for 15 min. Apoptotic bodies were observed and analyzed with fluorescence microscopy.

2.6. Cell migration assay

Cell migration was detected by wound healing assay. Prostate cancer cells were sown on six-well plates at 50,000 cells/well and permitted to adhere overnight. The cells were washed with PBS and resuspended in fresh serum-free medium. Cells were treated with different experimental conditions at 0, 6, 12, 24, and 48h. Cell numbers in wound were calculated and the migration ratio was obtained by comparing with the cell numbers in the wound at 0h.

2.7. Cell attachment assay

A 96-well plates were coated with 10μg/mL Laminin I (Trevigen, USA) for 1h (several wells uncoated were left as the negative control) and blocked with 5% FBS (Millipore, USA) and resuspended to 40,000 cells per mL in RPMI-1640 with 10% FBS (Millipore, USA), and incubated for 5h. The plates were shaken at 2000 rpm for 10s and washed with 1mg/mL BSA 3 times. After washing away unattached cells, fixed attached cells with 4% paraformaldehyde for 30 min at the indoor temperature and dyed with 5mg/mL crystal violet (Sigma-Aldrich, Germany). After destaining with ddH2O and solubilization of the dye with 2% SDS, the absorbance at 550nm of every well was recorded with a microplate reader.

2.8. qRT-PCR

Total mRNA from cells was isolated and cDNA was reversely transcribed with a Cell/Tissue to cDNA Kit (Selleck, USA) pursuant to the protocol given by the manufacturer. These cDNA specimens were then amplified by qPCR (Applied Biosystems 7300, USA) with primer sequences of ULK1 (5′-CCAGAGCAACATGATGCGG-3′ and 5′-CCTTTCCGTCTAGTGCTG-3′), PIK3C3 (5′-TAGGAGGAACACGCGTTCG-3′ and 5′-GCTTTCTACATATGCGG-3′), MITF (5′-ACAGAATTTGGCACAC-3′), GCNC (5′-GAATCGAGAATGTGCTG-3′), β-actin (5′-CAGCCGAGGTTGGTCTG-3′ and 5′-TCACGGGTGATGCCTG-3′), b-actin (5′-ACTGCTCCTACAGTCCATC-3′ and 5′-ACCATCGATGCTGAG-3′), and β-actin (5′-ACATGGCAATGGGCTACAGG-3′ and 5′-ACATTCGATCAGAGAG-3′). The quantization of autophagy-related genes (ATG) mRNA level was standardized to β-actin mRNA level with a comparative Ct method.

3. Results

3.1. Effects of PC322 on autophagy of human prostate carcinoma cells

Fluorescence microscopy was utilized to observe the formation of LC3 fusion protein tracing autophagy. Western blotting was used to detect LC3-II/I ratio change to detect autophagosome formation. Flow cytometry served to assess the degree of autophagy. Compared with LC3-I, LC3-II has the character of depositing to lipid and specifically recruiting to autophagosomes, which offers a transformation from diffusion to punctate staining of protein and enhances its electrophoretic mobility on gels so that it could be a marker of autophagy[21]. In addition, green fluorescent protein-LC3 (GFP-LC3) fusion protein can display autophagosomes with fluorescence microscopy[23].

The LC3 fusion protein can be classified into two systems: GFP-LC3 single fluorescent indicator system and mRFP-GFP-LC3 double fluorescent indicator system. The single fluorescence indicator system GFP-LC3 utilizes the principle that LC3 aggregates during autophagy[22], that is, GFP-LC3 fusion protein diffuses in the cytoplasm without autophagy, and GFP-LC3 fusion protein translocates into vesicle during autophagy. Several bright green fluorescent spots were observed by fluorescence microscopy, one of which corresponded to an autophagosome. Autophagy can be stimulated by RAPA[21]. LNCaP and C4-2 cells transfected with GFP-LC3 plasmid were treated with PBS (control), RAPA (200 nM), RAPA (200 nM) combined PC322 (1:100 diluted solution) for 24h after. Autophagic bright spots were observed with fluorescence microscopy. In RAPA-treated group, autophagic bright spots were observed in cells, while in RAPA combined with PC322 treated group, autophagic bright spots were significantly reduced (Figure 1a). mRFP-GFP-LC3 double fluorescent indicator system was used to track changes of LC3 and autophagy flux. GFP is an acid-sensitive protein, while mRFP is a stable fluorescence expression protein. When autolysosomes are formed, GFP will be quenched by the acidic environment and the ratio of GFP and mRFP can be used to evaluate the process of autophagy flux. The yellow spots after red-green fluorescence fusion represent autophagosomes, and the red spots represent
autolysosomes. When autophagosomes fuse lysosomes normally, the number of red fluorescence spots will be greater than yellow fluorescence spots. If downstream of autophagy is blocked, and autophagosomes and lysosomes cannot fuse smoothly, the fluorescence spots will mainly be yellow. LNCaP and C4-2 cells transfected with mRFPGFP-LC3 plasmids were treated with PBS (control), RAPA (200 nM), RAPA (200 nM) combined PC322 (1:100 diluted solution) for 24h. The results were observed by fluorescence microscopy. After red-green fluorescence emergence, obvious red spots were seen in RAPA treated cells, indicating that autophagosomes formation is stimulated, and the fusion of autophagosomes and lysosomes occurs normally (Figure 1d). In RAPA combined PC322 treated group, bright spots are predominantly yellow, but the amount is small, indicating that the formation of autophagosomes and the downstream is blocked (Figure 1d).

During autophagy, a small segment of the polypeptide is enzymatically hydrolyzed by microtubule-associated protein light chain 3 (MAP-LC3) to form LC3-I. LC3-I binds to phosphatidylethanolamine (PE) and converts to liposoluble (LC3-II)\(^{[23]}\). Therefore, the LC3-II/I ratio served to assess autophagy level. The lysates of C4-2 cells collected 24h after treatment with PBS (control), RAPA (200 nM), RAPA (200 nM) combined PC322 (1:100 diluted solution), and RAPA (200 nM) combined 3-methyladenine (3-MA) (2 mM) for 24 h. Inhibitor of phosphatidylinositol 3-kinase VPS34, 3-MA inhibits autophagy\(^{[22]}\). GFP-LC3 transfected cells were set as gates, and green fluorescence intensity was measured to represent the degree of autophagy by flow cytometry (Figure 1c). RAPA treated group shows the highest fluorescence intensity, while RAPA combined PC322 treated group shows the lowest fluorescence intensity.

**Figure 1.** PC322 inhibits autophagy of LNCaP and C4-2 cells. (a) Representative fluorescence microscopic fields of LNCaP and C4-2 cells after transfection with GFP-LC3. Red arrows indicate the formation of autophagosomes. (Scale bar=10μm). (b) Representative results of western-blot analysis of LC3-I and LC3-II in LNCaP and C4-2 cells and the comparative quantification of LC3-II/I ratio in PBS-treated (control), Rapamycin (RAPA)-treated or RAPA combined PC322 treated C4-2 cells. β-Actin was used as an internal reference. (c) Flow cytometry detecting the number of autophagosomes. GFP-LC3 transfected cells were set as gates. The black line represents control group, the red line represents RAPA treated group, the blue line represents the RAPA combined PC322 treated group, and the green line represents the RAPA combined 3-MA treated group. The fluorescence intensity was measured to represent the degree of autophagy. (d) Representative fluorescence microscopic fields of LNCaP and C4-2 cells after transfection with mRFPGFP-LC3. White arrows indicate autophagosome and autolysosomes. Yellow bright spots forming by red-green fluorescent merge indicates autophagosomes, and red bright spots indicate autolysosome. White arrows indicate autophagosome and autolysosomes. (Scale bar=10μm).
3.2. Effects of PC322 on biological behaviors of prostate carcinoma cells

The effects of PC322 on LNCaP and C4-2 cells were detected in terms of proliferation, apoptosis, migration, and adhesion. The impact of PC322 on the proliferation of LNCaP and C4-2 cells was examined by IC50 determination with Cell Counting Kit (CCK-8, Dojindo) (Figure 2a). LNCaP and C4-2 cells were dealt with PBS (control), chloroquine (CQ) (50μM), PC322 (1:100), CQ (50μM) combined PC322 (1:100) for 24, 48, and 72h, and then counted with CCK-8 kit. CQ, an autophagy inhibitor, significantly inhibits the proliferation of LNCaP and C4-2 cells, while PC322 inhibits the proliferation of LNCaP and C4-2 cells on a certain extent.

To explore the function of PC322 on apoptosis of prostate cancer cells, LNCaP and C4-2 cells were stained with DNA-specific dye Hoechst 33342 for 24h after treatment with PBS (control), RAPA (200nM), PC322 (1:100), and RAPA (200nM) combined PC322 (1:100) and observed with fluorescence microscope. Morphological changes of nucleus chromatin during apoptosis can be divided into three stages: Stage I is cracked or corrugated, part of which is concentrated; Stage IIa is highly condensed and marginalized; and Stage IIb is fragmented and apoptotic bodies are produced. Blue nuclei can be viewed under ultraviolet light with a fluorescence microscope (Figure 2b). Three fields of each group were chosen to calculate apoptotic cells and the total cell amount to achieve the proportion of apoptotic cells. The apoptosis of RAPA treated group is inhibited, while PC322 promotes apoptosis. There is an essential difference between RAPA treated group and control group (Figure 2b, P<0.01).

Wound healing assay of C4-2 cells was used to examine cell migration ability (Figure 2c). C4-2 cells were treated with PBS (control), PC322 (1:100), RAPA (200nM), and RAPA (200nM) combined PC322 (1:100) for 48 h followed by scratching after cell adherence, and then observed with...
a microscope. At least three fields were selected and recorded for each well, and the cell numbers in the wound after 48h of treatment were compared and measured at the same location in the same field with which at the beginning of treatment (0h) with ImageJ. The results showed that PC322 inhibited the migration of C4-2 cells, which were quite different from the control group ($P<0.05$). RAPA has a certain role in promoting migration, but there is no statistical significance. Tumor cells migrate through adhesion. Laminin is a mediator of cell-matrix adhesion. The adhesion ability of C4-2 cells to the extracellular matrix was detected with the laminin-1 coated 96-well plate (Figure 2d). PC322 inhibits the adhesion of C4-2 cells, and the difference is statistically significant ($P<0.05$).

### 3.3. Impacts of PC322 on the expression of ATG

From previous experiments, we found that PC322 inhibits the occurrence of LNCaP and C4-2 cell autophagy. To clarify the mechanism by which PC322 inhibits autophagy, we proceeded qRT-PCR to detect ATG expression[22] (ULK1, PIK3C3, MTOR, ATG3, ATG5, ATG7, ATG10, ATG12, ATG16L1, ATG16L2, DRAM1, and DRAM2) in PBS-treated (control), RAPA-treated and RAPA combined PC322-treated LNCaP and C4-2 cells (Figure 3). ATG3 expression is upregulated with RAPA stimulation and inhibited by PC322 with statistical significance ($P<0.01$).

### 3.4. The mechanism of PC322 acting on prostate cancer cell autophagy

Autophagosome formation involves a series of complexes composed of ATG products[21]. ATG1, ATG13, and ATG17 form uncoordinated 51-like kinase 1 (ULK1)[24]. When nutrients are adequate, mammalian RAPA target protein complex 1 (mTORC1) binds to ULK1 to inhibit autophagy. RAPA inhibits the activity of the mTORC1 kinase, then mTORC1 isolates from ULK1 complex, inducing nucleation and elongation[8]. Class III phosphatidylinositol 3-kinase (PI3KCIII) complex takes part in nucleation phagophore[21]. Two ubiquitin-like conjugation systems are associated with the process of vesicle elongation[21]. One is the ATG12-ATG5 conjugation system. The other is the LC3/ATG8 conjugation system, in which PE conjugate to LC3/ATG8 through continuous activities of protease ATG4, E1-like enzyme (ATG7), and E2-like enzyme (ATG3)[21]. Lipid conjugation promotes the transformation LC3-I to LC3-II. LC3-II conjugates to the newly formed vesicle until autophagosomes and lysosomes fuse to autolysosomes. The inner membrane of autophagic vacuoles and its content is degraded in lysosomes. The degradation products are released into the cytoplasm for biosynthesis and metabolism. We found that PC322 could inhibit ATG3, thus presumed that PC322 could inhibit LC3 conversion by inhibiting ATG3 expression and inhibit the formation of autophagosomes (Figure 4).

### 4. Discussion

In our study, when RAPA was used to stimulate autophagy in LNCaP and C4-2 cells, LC3 fusion protein-traced autophagosomes were observed with fluorescence microscopy, LC3-II/I ratio was increased in western blotting, and fluorescence intensity was enhanced in flow cytometry.

While it was found that all the indicators above decreased in RAPA combined PC322 treated group, which shows that PC322 could affect autophagy of LNCaP and C4-2 cells. It is not clear which stage of autophagy PC322 acts on, but we can make the following assumptions. Autophagic spots of GFP-LC3 single fluorescence indicator system in RAPA combined PC322 treated group were significantly reduced, indicating that PC322 inhibits autophagosomes formation or promotes autophagosomes and lysosomes fusion into autolysosomes. While in mRFP-GFP-LC3 double fluorescence indicator system, the yellow spots merged from red and green fluorescence were dominant in RAPA combined PC322 treated group, indicating that the synthesis of autophagosomes was blocked and the downstream of fusing with lysosomes was also hindered. Western blotting results showed that LC3-II/I ratio in RAPA combined PC322 treated group was decreased, and the higher concentration of PC322, the lower LC3-II/I ratio, indicating that PC322 inhibits LC3-I transforming to LC3-II or promotes the degradation of LC3-II. To clarify its specific role, CQ should be conjunctively used to inhibit the degradation of LC3-II before observing the change of LC3-II/I ratio. In addition, when detecting ATGs expression in RAPA treated cells and RAPA combined PC322 treated cells by qRT-PCR, it was found that ATG3 was upregulated in RAPA treated cells, while PC322 inhibited ATG3 expression. ATG3, together with ATG7, connects PE to LC3/ATG8 and then LC3-I converts to LC3-II[21]. Based on the above experimental

![Figure 3](image3.png)  
**Figure 3.** PC322 inhibits the expression of autophagy-related genes3 (ATG3). (a) Relative expression of ATG in PBS-treated (control), Rapamycin (RAPA) treated and RAPA combined PC322 treated LNCaP and C4-2 cells. The expression of ATG 3 was different between the RAPA group and control group, RAPA combined with PC322 group and RAPA group ($P<0.01$). (b) Agarose gel electrophoresis was used to verify the results of qRT-PCR in Figure 3a.
results and analysis, we deduced that PC322 inhibits the transformation of LC3-I to LC3-II by reducing ATG3 expression, thereby suppresses the formation of autophagosomes. The impact of autophagy in cancer is controversial and has not been fully elucidated. As a suppression mechanism, autophagy accelerates tumor cell survival to hypoxia, nutritional restriction, and metabolic stress caused by insufficient blood supply. If the protective mechanism of autophagy for cancer cells is generally recognized, PC322 will have the potential of cancer treatment due to its inhibition of autophagy.

In the study of impacts of PC322 on biological behaviors of LNCaP and C4-2 cells, we detected the proliferation, apoptosis, migration, and adhesion. It was found that PC322 inhibits proliferation, adhesion, and migration, and promotes apoptosis. Autophagy inhibitors improve the sensibility of tumor cells to anticancer treatment and have been applied in many underway clinical experiments as adjuvant or prime therapies for lung carcinoma, pancreatic carcinoma, breast carcinoma, skin carcinoma, and prostate carcinoma. At present, the only drug inhibiting autophagy and authorized by FDA is CQ and hydroxychloroquine, whose derivative. In our experiments, we found that CQ significantly inhibited LNCaP and C4-2 cells proliferation. Autophagy constitutes a stress adaptation mechanism to avoid cell death in a specific environment. However, to maintain intracellular stability, autophagy can also constitute a cell death pathway, so the functional interrelation between apoptosis and autophagy is complicated. In addition to removing the aggregation of potentially harmful (inhibiting apoptotic) protein, autophagy stimulation may have a broad inhibitory effect on apoptosis.

For example, autophagy stimulated by RAPA pretreatment can reduce mitochondrial aggregation by 50% and reduce cell sensitivity to MOMP-dependent apoptotic stimulation. According to our experimental results, we found that PC322 inhibits LNCaP and C4-2 cell autophagy and promotes apoptosis. It is concluded that autophagy inhibits apoptosis as a mechanism of cell protection. Many pieces of evidence have shown the key role of autophagy in migration by especially adjusting cell-matrix adhesion sites transformation. Further study the function of autophagy in migration and adhesion not just provide a better comprehension of adhesion adjustment but help to reveal the mechanisms of autophagy in biochemical and molecular fields. Our study found that cell adhesion and migration ability increase in RAPA treated group, but there is no significant difference, while cell adhesion and migration ability decrease in PC322 treated group, the difference is statistically significant. Focal adhesion undoubtedly plays a significant aspect in the whole biological process, particularly in numerous processes which depending on migration. Looking ahead, the main target is to determine whether autophagy adjusts these actions in vivo.

Some studies have discussed the function of autophagy in embryonic heart development. It has been found that ATGs function is losing result in heart defects in zebrafishand mice, indicating the physiological importance of autophagy-dependent migration.

5. Conclusion

In this study, a novel MAb PC322 was demonstrated
to prohibit autophagy of human prostate carcinoma cells in vitro. It was also found that PC322 could inhibit the proliferation and metastasis of LNCaP and C4-2 cells, and promote their apoptosis. To clarify the effect of PC322 on ATGs expression in prostate carcinoma cells, we detected the expression of ULK1, PIK3C3, MTOR, ATG3, ATG5, ATG7, ATG10, ATG12, ATG16L1, ATG16L2, DRAM1, and DRAM2 in RAPA treated and RAPA-combined PC322 treated LNCaP, C4-2 cells. The conclusion that PC322 inhibits ATG3 expression was obtained. Based on the known mechanism of autophagy, we speculate that PC322 inhibits the transformation of LC3-I to LC3-II by inhibiting ATG3 expression, thus inhibiting the formation of autophagosomes. PC322, the core of our study, is a new MAb against prostate cancer. Although our research has obtained the above preliminary results, there are still many aspects to be explored, and many problems remain to be clarified. The mechanism of PC322 on autophagy of prostate carcinoma cells is still at the level of gene expression, further detection at the protein level is needed, and how PC322 inhibits ATG3 expression is still unknown. Pathologically, further studies are needed to elucidate how autophagy-related migration and adhesion affect the progression of cancer metastasis. More data are required to support the conclusion that PC322 affects the biological behavior (proliferation, apoptosis, migration, and adhesion) of prostate cancer cells. As a MAb, the epitope of PC322 is necessary to be figured out. It’s worth expecting to conduct animal trials. These problems remain to be further studied later.

In a word, the inhibition of PC322 on autophagy of prostate carcinoma cells provides a different idea for prostate cancer therapy.

6. Acknowledgments
The National Natural Science Foundation of China (81672956, 81472413, 81602644) and the Beijing Science and Technology Project (Z181100003818003) supported this work.

7. Author’s Contributions
X. Kang did study design and data analysis, X. Zhang did cell culture, X. Zhu did antibody preparation, C. Li interpreted data, X. Kang and C. Li meditated the study and wrote the manuscript, and Y. Yan proofread the manuscript. All authors viewed and agreed with this final manuscript for submission.

Consent for Publication
All authors have agreed to publish this manuscript.

Conflicts of Interest
No potential conflicts of interest were declared.

References


