UC Davis

UC Davis Previously Published Works

Title

Autophagy as a modulator and target in prostate cancer

Permalink

<https://escholarship.org/uc/item/7j63v3xm>

Journal

NATURE REVIEWS UROLOGY, 11(9)

ISSN

1759-4812

Authors

Farrow, Jason M

Yang, Joy C

Evans, Christopher P

Publication Date

2014

DOI

10.1038/nrurol.2014.196

Peer reviewed

Autophagy as a modulator and target in prostate cancer

Jason M. Farrow, Joy C. Yang and Christopher P. Evans

Abstract | Autophagy, or ‘self-eating’, is an adaptive process that enables cells to cope with metabolic, toxic, and even infectious stressors. Although the adaptive capability of autophagy is generally considered beneficial, autophagy can also enhance nutrient utilization and improve growth characteristics of cancer cells. Moreover, autophagy can promote greater cellular robustness in the context of therapeutic intervention. In advanced prostate cancer, preclinical data provide evidence that autophagy facilitates both disease progression and therapeutic resistance. Notably, androgen deprivation therapy, taxane-based chemotherapy, targeted kinase inhibition, and nutrient restriction all induce significant cellular distress and, subsequently, autophagy. Understanding the context-dependent role of autophagy in cancer development and treatment resistance has the potential to improve current treatment of advanced prostate cancer. Indeed, preclinical studies have shown that the pharmacological inhibition of autophagy (with agents including chloroquine, hydroxychloroquine, metformin, and desmethylclomipramine) can enhance the cell-killing effect of cancer therapeutics, and a number of these agents are currently under investigation in clinical trials. However, many of these autophagy modulators are relatively nonspecific, and cytotoxicity in noncancerous tissues is still a concern. Moving forward, refinement of autophagy modulation is needed.

Farrow, J. M. *et al.* *Nat. Rev. Urol.* **11**, 508–516 (2014); published online 19 August 2014; doi:10.1038/nrurol.2014.196

Introduction

In good health and in disease, the biomolecular adaptations to ‘stress’ are necessarily dynamic. Complex, integrated biological processes, such as inflammation, immune response, and nutrient depletion require coordinated regulation of cellular machinery and efficient use of available nutrients. Autophagy, or ‘self-eating’ is an essential and highly conserved homeostatic process that can achieve such coordinated regulation. Although ‘self-eating’ has negative connotations, research shows that a basal level of autophagy is critical for normal development and homeostasis.^{1–3} Autophagy maintains cellular homeostasis by metabolizing cytoplasmic protein waste through the lysosomal system. Furthermore, research has shown that autophagy can suppress tumour development.^{4,5} However, autophagy is multifaceted, and not always protective. In established tumours, autophagy can meet the heightened nutrient demand of proliferative cancer cells,⁵ and can enable cancer cell survival during therapeutic intervention by radiotherapy, chemotherapy, and hormone therapy.⁶ Understanding the context-dependent role of autophagy in cancer development, and specifically in treatment resistance, has the potential to improve current treatment paradigms, including treatment of advanced prostate cancer.^{7–9} Current treatments for prostate cancer have limited and transient

effectiveness. The adaptive capabilities of autophagy potentially contribute to this limited effectiveness, and the use of autophagy manipulation concurrently with prostate cancer therapy is under investigation. In this Review, we provide an overview of autophagy and the available data in support of autophagic modulation (with added emphasis on alterations occurring within prostate cancer models). We describe current prostate cancer treatments associated with alterations in autophagy, and the current status of research providing support for the use of adjuvant autophagic modulation alongside current prostate cancer treatments.

Autophagy and tumorigenesis

Autophagy

Autophagy (also known as macroautophagy) is a highly conserved evolutionary process that is involved in a number of cellular homeostatic processes that regulate cytoplasmic biomass, organellar abundance, and organellar distribution, and that remove harmful protein aggregates and intracellular toxins.^{2,10,11} Autophagy also has important immunological roles, and is capable of removing intracellular pathogens and influencing both innate and adaptive immunity.^{11–14} The exact morphological and molecular steps of autophagy have been extensively reviewed,¹⁵ and can be briefly summarized in two essential steps (Figure 1). During autophagy, cytoplasmic material is engulfed by an isolation membrane called a phagophore, producing a double-membrane structure known as an autophagosome.¹⁵ Autophagosomes are

Department of Urology,
School of Medicine,
University of California,
Davis, 4860 Y Street,
Sacramento,
CA 95817, USA (J.M.F.,
J.C.Y., C.P.E.).

Correspondence to:
C.P.E.
cpevans@ucdavis.edu

Competing interests

C.P.E. has a research grant from Medivation/Astellas and is a consultant/advisory board member, clinical trial investigator and shareholder of Medivation. The other authors declare no competing interests.

Key points

- Autophagy is a dynamic metabolic process that facilitates nutrient utilization and toxin removal: too little or too much autophagy can have beneficial or deleterious effects depending on the context
- Understanding the context-dependent role of autophagy in cancer development, and specifically in treatment resistance, has the potential to improve current treatment of advanced prostate cancer
- Preclinical prostate cancer models show significant upregulation of autophagy in response to androgen deprivation therapy, taxane-based chemotherapy, targeted inhibition of kinases, and amino acid restriction
- Adjuvant autophagy inhibition in preclinical prostate cancer models improves cell killing and tumour responsiveness to treatment
- Several autophagic modulators are under active investigation in clinical trials, including chloroquine and hydroxychloroquine
- Currently available autophagy modulators are relatively nonspecific, and cytotoxicity in noncancerous tissues is a concern: moving forward, refinement of autophagy modulation is needed

Figure 1 | Initiation and mechanisms of autophagy. **a** | Starvation stimulates AMPK, blocking mTOR and thus promoting autophagy. Growth-factor deprivation reduces class 1 PI3K-AKT activity, reducing both mTOR stimulation and Beclin 1 inhibition. As such, growth-factor deprivation will also activate autophagy. **b** | The autophagosome matures by enclosure of cellular debris, consisting of organellar waste and toxic metabolites, within an isolation membrane, and is trafficked to lysosomes where it fuses and creates the autolysosome. **c** | The autolysosome digests cellular biomass and eliminates toxins, which provides nutrients and favourable growth conditions. Abbreviations: AKT, protein kinase B; AMPK, adenosine monophosphate-activated protein kinase; ATG13, autophagy-related protein 13; ATG101, autophagy-related protein 101; FIP200, RB1-inducible coiled-coil protein 1; PI3K, phosphoinositide 3-kinase; ULK1, serine/threonine-protein kinase ULK1.

then trafficked to lysosomes, where the contents are destroyed by lysosomal enzymes.¹⁵ The active cycling of cytoplasmic material through the lysosomal system, or autophagic flux, is an essential determinant of autophagic activity in research models.¹⁶

Perhaps not surprisingly, given the diverse metabolic and immunological functions of autophagy, autophagic

dysregulation has been linked to tumorigenesis. Mice that are heterozygous for disruption of the autophagy gene *Becn1* develop multiple spontaneous malignancies.¹⁷ Furthermore, the monoallelic deletion of the human homologue of this gene occurs in several human cancers.² Autophagy is upregulated at the immunological synapse between dendritic cells and CD4+ T cells, influencing T-cell activation and possibly antitumour immunity. The autophagy system is capable of tremendous plasticity, and determination of the consequences of too little or too much autophagy on immune system function requires further research.^{11,12} Nevertheless, although a protective role for autophagy is generally accepted, in established tumours autophagy might actually facilitate carcinogenesis.^{18,19} Studies have shown autophagy to be upregulated in the internal environment of tumours, areas that are characteristically low in nutrient and oxygen levels.^{4,5,20} Experimental models with knockout of essential autophagy genes (*Becn1*, *Atg5*, and *Atg7*) are sensitive to metabolic stress.¹⁸ Moreover, the upregulation of key autophagy markers in tumour tissues has been linked to poor therapeutic outcomes.⁹

The apparently context-dependent role of autophagy, as defined by the presence or absence of disease, is complicated by the cross-talk between the autophagic and apoptotic molecular pathways.^{2,21,22} Interactions have been identified between intermediates from both pathways, such as the apoptosis regulator Bcl-2 and the autophagic Beclin 1, and the autophagic LC3B (microtubule-associated proteins 1A/1B light chain 3B) and the apoptotic tumour necrosis factor receptor superfamily member 6 (also known as fas), and these interactions are largely counter-regulatory, so that upregulation of one pathway downregulates the other.^{15,21} In other words, the autophagic and apoptotic pathways can be considered competitive determinants of cell fate. However, if the homeostatic balance between autophagy and apoptosis is lost, either pathway can potentially drive cellular demise. Cell lines overexpressing Beclin 1, a key autophagy-regulated protein, show hyperactive autophagy and ultimately complete self-digestion.^{23–25} Autophagy-associated cell death (type II programmed cell death), is characteristically caspase-independent and histologically distinct from apoptosis (type I programmed cell death),²³ so autophagy can continue to facilitate cell death even in the context of apoptotic dysfunction.^{23,26} Apoptotic dysfunction is not uncommon in cancer pathogenesis, so upregulation of autophagy, even in established tumours, can have nuanced implications in terms of cell fate.²⁷ In summary, the contextual meaning of autophagic activity is complex, depending not only on the presence of cancer, but on the apoptotic status as well.²²

Autophagic modulation for cancer therapy

Given the dynamic role of autophagy in tumour pathogenesis, and that most cancer treatments induce autophagy, adjuvant autophagy inhibition has become an area of research interest. The results have been encouraging, and studies have shown that the pharmacological

Table 1 | Phase I and phase I/II clinical trials relevant to autophagy and prostate cancer

Interventions	Status	Clinical trial title
Phase I		
Akt inhibitor MK2206; hydroxychloroquine; pharmacological study; laboratory biomarker analysis	R	Akt inhibitor MK2206 and hydroxychloroquine in treating patients with advanced solid tumours or prostate or kidney cancer
Vorinostat; temsirolimus; laboratory biomarker analysis; PET/CT	R	Temsirolimus and vorinostat in treating patients with metastatic prostate cancer
Everolimus; lupron; bicalutamide; radiation	R	Study of everolimus added to combined hormonal and radiation therapy for high risk prostate cancer
Bicalutamide; everolimus; leuprolide acetate; external beam radiation therapy	R	Everolimus, bicalutamide, and leuprolide acetate in treating patients undergoing radiation therapy for high-risk locally advanced prostate cancer
Everolimus; radiation therapy	R	Phase I trial of the mammalian target of rapamycin (mTOR) inhibitor everolimus plus radiation therapy (RT) for salvage treatment of biochemical recurrence in prostate cancer patients following prostatectomy
DEC-205-NY-ESO-1 fusion protein vaccine; sirolimus; laboratory biomarker analysis	R	Vaccine therapy with or without sirolimus in treating patients with NY-ESO-1-expressing solid tumours
Temsirolimus; vinorelbine ditartrate	ANR	Temsirolimus and vinorelbine ditartrate in treating patients with unresectable or metastatic solid tumours
AZD2014	NYR	Investigating the effects of AZD2014 therapy given prior to radical prostatectomy in men with high risk prostate cancer
ADI-PEG 20; docetaxel	R	Phase I trial of ADI-PEG 20 plus docetaxel in solid tumours with emphasis on prostate cancer and non-small cell lung cancer
Phase I/II		
Metformin	ANR	Metformin in castration-resistant prostate cancer
Everolimus; gefitinib	C	Everolimus and gefitinib in treating patients with progressive glioblastoma multiforme or progressive metastatic prostate cancer
Everolimus; docetaxel	C	The use of RAD001 with docetaxel in the treatment of metastatic, androgen independent prostate cancer
Cixutumumab; diagnostic laboratory biomarker analysis; temsirolimus	ANR	Cixutumumab and temsirolimus in treating patients with metastatic prostate cancer
Everolimus; docetaxel; bevacizumab	R	Safety study and effectiveness of docetaxel with RAD001 and bevacizumab in men with advanced prostate cancer
Temsirolimus; bevacizumab; polymorphism analysis; laboratory biomarker analysis	ANR	Temsirolimus and bevacizumab in hormone-resistant metastatic prostate cancer that did not respond to chemotherapy
Docetaxel; temsirolimus	R	CESAR study in prostate cancer with temsirolimus added to standard docetaxel therapy (CEPTAS)
Sirolimus; immunohistochemistry staining method; neoadjuvant therapy	ANR	Sirolimus before surgery in treating patients with advanced localized prostate cancer
Temsirolimus	C	An open-label exploratory study in newly diagnosed prostate cancer patients
Abbreviations: ANR, active, not recruiting; C, completed; NYR, not yet recruiting; R, recruiting.		

inhibition of autophagy can enhance the cell-killing effects of cancer therapeutics in preclinical models.^{28–30} On the basis of the success of preclinical reports demonstrating therapeutic efficacy, concurrent autophagy inhibition is now also being explored in a number of clinical trials for patients with refractory malignancies, including glioblastoma, breast cancer, multiple myeloma, and lung cancer.³¹ Several autophagy inhibitors have been tested in both preclinical and clinical models, and these inhibitors are typically classified by the stage at which they inhibit formation of the mature autophagosome complex. Early stage inhibitors typically interfere with the recruitment of essential autophagy related proteins, whereas late stage inhibitors prevent the binding of lysosomes or the acidification of the autolysosome complex.³² In prostate cancer research, inhibitors of autophagy used in laboratory research include 3-methyladenine, and

small inhibitory RNAs (siRNAs) targeting specific autophagy-related genes (Atgs), although the use of siRNAs has largely been relegated to preclinical studies as a confirmatory tool demonstrating autophagy inhibition. More clinically applicable inhibitors of autophagy include chloroquine, its derivative hydroxychloroquine, and bafilomycin A1.³² Chloroquine and hydroxychloroquine, which are lysosomotropic amines traditionally used to treat malaria and rheumatoid arthritis, have been most-widely studied.^{10,33} They are inexpensive and have well-known pharmacokinetic profiles, facilitating their use in clinical trials (Tables 1 and 2).

Other notable autophagy inhibitors being investigated in the context of prostate cancer treatment include metformin and desmethylclomipramine. Metformin, an oral biguanide that is commonly prescribed to treat non-insulin-dependent diabetes, also inhibits

Table 2 | Phase II clinical trials relevant to autophagy and prostate cancer

Interventions	Status	Clinical trial title
Hydroxychloroquine	ANR	Hydroxychloroquine in treating patients with rising PSA levels after local therapy for prostate cancer
Docetaxel; hydroxychloroquine	THR	Docetaxel and hydroxychloroquine in treating patients with metastatic prostate cancer
Abiraterone; ABT-263; hydroxychloroquine	R	Phase II study of ABT-263/abiraterone or ABT-263/abiraterone/hydroxychloroquine in prostate cancer
Metformin	ANR	Metformin hydrochloride as first-line therapy in treating patients with locally advanced or metastatic prostate cancer
Placebo and castration; metformin and castration	R	Castration compared to castration plus metformin as first-line treatment for patients with advanced prostate cancer
Metformin; placebo; TAXOTERE®	R	Metformin-docetaxel association in metastatic hormone-refractory prostate cancer
Metformin	NYR	Impact of the addition of metformin to abiraterone in metastatic-prostate-cancer patients
Pasireotide; everolimus; laboratory biomarker analysis	R	Pasireotide (SOM230) with or without everolimus in treating patients with hormone-resistant, chemotherapy-naïve prostate cancer
Temsirolimus; diphenhydramine	THR	Impact of temsirolimus therapy on circulating tumour cell biology in men with castration resistant metastatic prostate cancer
Carboplatin; everolimus; prednisone; laboratory biomarker analysis; pharmacological study	ANR	Carboplatin, everolimus, and prednisone in treating patients with metastatic prostate cancer that progressed after docetaxel
Torisel	ANR	Single agent temsirolimus (Torisel®) in chemotherapy-naïve castration-resistant prostate cancer patients
Everolimus	ANR	Everolimus as first-line therapy in treating patients with prostate cancer
Everolimus; conventional surgery	ANR	Everolimus in treating patients with newly diagnosed localized prostate cancer
Everolimus	R	Phase II study of RAD001 in a neoadjuvant setting in men with intermediate-risk or high-risk prostate cancer
Bicalutamide; everolimus	ANR	Bicalutamide with or without everolimus in treating patients with recurrent or metastatic prostate cancer
Everolimus	C	RAD001 in patients with metastatic, hormone-refractory prostate cancer
Temsirolimus; conventional surgery; neoadjuvant therapy	C	Neoadjuvant CCI-779 followed by radical prostatectomy in treating patients with newly diagnosed prostate cancer who have a high risk of relapse
Temsirolimus	C	CCI-779 in treating patients with prostate cancer
Everolimus	C	Molecular, genetic, and genomic assessments from patients treated with RAD001
Ridaforolimus	C	AP23573 in patients with taxane-resistant androgen-independent prostate cancer (AIPC)(8669-017)
Ridaforolimus; placebo	C	Bicalutamide and ridaforolimus in men with prostate cancer (MK-8669-002)
Pantoprazole	R	Pantoprazole and docetaxel for men with metastatic castration-resistant prostate cancer

Abbreviations: ANR, active, not recruiting; C, completed; NYR, not yet recruiting; R, recruiting; THR, terminated, has results.

autophagy,³² and the mechanism of this inhibition is being explored. Metformin is known to activate 5' adenosine monophosphate-activated protein kinase (AMPK), a potent inhibitor of the serine/threonine-protein kinase mTOR (mammalian target of rapamycin),³⁴ but this would suggest that metformin should be a stimulator of autophagy. This apparent contradiction can be explained by the proposed effect of metformin on Beclin 1, a core autophagy regulator. Results of a study published in 2010³⁵ showed that metformin can sequester Beclin 1, inhibiting autophagy despite the inhibition of mTOR. Metformin also has antiproliferative effects in a variety of cancer cell lines (including prostate, ovarian, breast, colorectal, and endometrial carcinoma models),^{36–42} and several studies suggest that patients taking metformin for diabetes mellitus have decreased prostate cancer incidence and severity.^{43,44} Thus, metformin possesses several characteristics that make it a readily

translatable autophagy inhibitor, and suggest specific efficacy in prostate cancer. Desmethylclomipramine, a tricyclic antidepressant, also inhibits autophagy.⁴⁵ Like hydroxychloroquine and metformin, its widespread use improves its value as a clinically viable autophagy inhibitor. Desmethylclomipramine is believed to decrease autophagic flux by inhibiting the fusion of the autophagosome and the lysosome, but more work is needed to fully understand its mechanism of action.⁴⁶

Although we believe that many, if not most, prostate cancer treatments induce a cytoprotective form of autophagy, several treatments, such as everolimus (RAD001), zoledronic acid, atorvastatin, YM155, and (-)-gossypol, can induce toxic, or unsustainably elevated, autophagic activity.⁴⁷ Mechanistically, these cases of cytotoxic autophagy have been attributed to the sustained activity of critical autophagy promoters, namely autophagy associated proteins and Beclin 1,⁴⁷ although

Figure 2 | Prostate cancer treatments and autophagy. Therapeutic interventions for prostate cancer either decrease total androgen (abiraterone, ADT) or impede androgen receptor activity (bicalutamide, enzalutamide). The androgen receptor negatively controls autophagy, so androgen receptor inhibition promotes autophagy through pathways including AMPK activation, which inhibits mTOR. Aberrant protein expression caused by ADT can also upregulate autophagy. Abbreviations: ADT, androgen deprivation therapy; AMPK, 5' adenosine monophosphate-activated protein kinase; mTOR, mammalian target of rapamycin.

a direct link between upregulated autophagy promoters and cell death has remained elusive. Shen *et al.*⁴⁸ screened 1,400 compounds for the ability to promote increased autophagic flux, but found no evidence for associated autophagic death. Some researchers have proposed that autophagy can precede cell death, but does not necessarily cause it. For example, Giampietri *et al.*⁴⁹ demonstrated enhanced tumour necrosis factor α (TNF- α)-dependent apoptosis in PC3 cells (prostate cancer cells without a functioning androgen receptor) when autophagy was induced. Given the controversy currently surrounding cytotoxic autophagy, the term 'autophagy-associated cell death' is commonly used. More research is required to improve our understanding of autophagy-associated cell death and to determine whether or not there is a clinical role for adjuvant autophagy induction in prostate cancer treatment.

Prostate cancer therapies and autophagy Androgen deprivation therapy

Androgen ablation is a common therapeutic intervention for advanced prostate cancer that results in widespread death of the prostate epithelium and dramatic—albeit transient—clinical improvements.⁵⁰ Unfortunately, advanced prostate cancer treated with androgen deprivation therapy (ADT) ultimately progresses to a lethal phenotype known as castration-resistant prostate cancer (CRPC).⁵¹ Research conducted in our laboratory and elsewhere indicates that ADT stimulates autophagic activity, facilitating the development of CRPC.⁴⁶ Androgens have an influential role in autophagy, and the androgen receptor is a key mediator.^{52,53} The androgen receptor negatively controls autophagic activity, and the targeted inhibition of this receptor positively influences autophagy (Figure 2).¹ Bennett *et al.*¹ determined that treatment of LNCaP cells with bicalutamide, a nonsteroidal androgen receptor antagonist, induces autophagy in the absence of any additional stressors. We have further substantiated these findings, showing that targeted inhibition of the androgen receptor with

enzalutamide produces significant autophagic flux in prostate cancer models *in vitro* and *in vivo*.⁴⁶ Taken together, these data suggest that ADT, and perhaps more importantly androgen receptor inhibition, can directly promote the cellular adaptations that help overcome the metabolic stress of hormone withdrawal.

Knowledge of the exact molecular events that link androgen receptor inhibition to autophagy is still somewhat fragmented, but mTOR appears to have a critical role. Xu *et al.*⁵⁴ demonstrated that androgen treatment directly affects mTOR activity and that the androgen receptor promotes *MTOR* mRNA synthesis. This finding has been reinforced by studies showing that androgen deprivation specifically decreases levels of downstream mTOR metabolites.⁵⁴ In our lab, androgen deprivation and prolonged androgen receptor blockade with enzalutamide were associated with AMPK activation and, ultimately, mTOR suppression via phospho-Raptor (regulatory-associated protein of mTOR).⁴⁶ Similarly, others have shown that AMPK directly regulates mTOR activity in response to imbalances in the core energy metabolites, AMP and ATP.^{52,55} An indirect effect of androgen receptor inhibition is hypoxia. Androgen withdrawal impedes angiogenesis, and the resultant nutrient deprivation and hypoxia further stimulate autophagy.⁵⁶ These data substantiate the influence of the androgen receptor on autophagic flux, but cannot completely explain the regulation of autophagy. For example, PC3 cell lines lacking androgen receptor can also upregulate autophagy during ADT.⁵² Potential explanations for this phenomenon include alterations in proto-oncogenes like *PTEN* (phosphatase and tensin homologue), variations in tumour suppressors like cellular tumour antigen p53, and the aberrant expression of downstream androgen receptor targets.

Given that androgens and the androgen receptor are valuable targets in prostate cancer treatment, and that their therapeutic benefit is limited by autophagy, studies are currently underway to determine whether autophagy modulation could improve the therapeutic effect of ADT. Available data provide evidence that concurrent androgen receptor and autophagy inhibition synergistically promote cell death,⁵⁷ and that this inhibition is dependent on both dose and duration.⁵¹ Bennett *et al.*¹ demonstrated that inhibiting autophagy with 3-methyladenine enhanced cell death 1.5-fold in cells treated with bicalutamide. Colquhoun *et al.*³⁹ demonstrated that LNCaP cells treated with bicalutamide and metformin exhibited significant reductions in colony formation rates (compared with no treatment), which also held true for PC3 cell lines, albeit at much higher doses of bicalutamide and metformin. In our laboratory, clomipramine and metformin significantly increased the cytotoxicity associated with enzalutamide *in vitro*, and in mouse models *in vivo*, wherein enzalutamide and clomipramine in combination decreased tumour size by 91%, compared with a 78% decrease with enzalutamide and metformin.⁴⁶ Several ongoing clinical trials are evaluating adjuvant autophagy inhibition in the context of ADT and androgen receptor blockade (Tables 1 and 2). In summary, the androgen

Figure 3 | Src kinase, autophagy and prostate cancer. The Src kinase complex is activated by EGF, and the cytokines IL-6 and IL-8. The Src kinase complex is composed of Src, Etk, and FAK and undergoes crossactivation upon stimulation. The Src kinase complex negatively regulates autophagy and also promotes androgen-independent growth of prostate cancer cells. Abbreviations: EGF, epidermal growth factor; Etk, cytoplasmic tyrosine-protein kinase BMX; FAK, focal adhesion kinase; Src, proto-oncogene tyrosine-protein kinase Src.

receptor has important, but apparently nonessential, roles in autophagy induction. More research is needed to understand the nuanced roles that the androgen receptor has throughout the progression of prostate cancer.

Taxane-based chemotherapy

Microtubules are cytoskeletal structures that rapidly fluctuate through polymerization states to meet a multitude of cellular needs, including mitosis, protein synthesis, and subcellular organization.⁵⁸ These processes are in high demand in rapidly dividing cells, and consequently, targeting microtubules has been a popular strategy in cancer treatment. In prostate cancer, docetaxel (a microtubule-stabilizing agent or taxane) is a current standard-of-care in men with symptomatic CRPC, providing a survival benefit of approximately 2 months.⁵⁹ Finding explanations for this limited therapeutic efficacy has been exceedingly difficult, especially with regards to autophagy. Early studies showed a variable degree of autophagic activity during taxane-based therapies, with some studies showing that taxanes induce autophagy and others showing that they inhibit autophagy.^{60–62} The discrepancies in these findings can in part be explained by the fact that different concentrations of taxanes and different cell lines were used. Published in 2013, the results of a study using breast cancer cells suggest that the autophagic response to taxanes is context-dependent, with the duration of treatment, and the intensity of environmental factors contributing to the degree of autophagic activity.⁶³

Despite the variability in the available data, mechanistic links between taxanes and autophagy have been described. Theoretically, taxanes should inhibit autophagy, as they target microtubules, the core constituents of autophagosome formation and trafficking, but this paradigm has limitations. Evidence shows that autophagy can be upregulated in response to taxanes. Some researchers have proposed that taxanes only target specific elements of the microtubule structure, such as the mitotic spindle, leaving enough remaining function to stimulate the core events of autophagy.⁶⁴ Another

explanation is that structurally damaged microtubules could induce autophagy through signalling cascades mediated by Raf-1.⁶⁵ Added insight was provided by Notte *et al.*,⁶³ who identified two key pathways, the first involving inhibition of mTOR, and the second involving activation of the c-Jun N-terminal kinase (JNK), which phosphorylates Bcl-2 and Bcl-XL and disrupts the binding of Beclin 1, freeing it to activate autophagy.⁶³ Interestingly, although these pathways had similar functions, they were differentially activated according to treatment duration and environmental stress.⁶³

Just as taxanes influence autophagy, autophagy seems to be an important factor in taxane resistance, specifically in prostate cancer cells. LNCaP-AI cells, which are chronically maintained in androgen-depleted conditions, are approximately 2.5 times more resistant to docetaxel than LNCaP cells grown in androgen-replete media.¹ Moreover, restoration of androgens to the LNCaP-AI cells can restore docetaxel sensitivity. Autophagy is believed to be responsible for this resistance, and when autophagy was suppressed with 3-methyladenine, the cytotoxic effect of bicalutamide and docetaxel increased twofold.¹

Kinase inhibitors

Tyrosine kinases are essential mediators of signal transduction, and any aberrant activity in these kinases can result in abnormalities in cellular proliferation, differentiation, apoptosis, and metabolism.^{5,11} Src family kinases (SFKs) are a family of nonreceptor tyrosine kinases, and Src is perhaps the most widely characterized tyrosine kinase, with important roles in tumour progression in several different cancers, including glioblastoma, colon, lung, breast, and prostate cancers.⁶⁶ In prostate cancer specifically, SFK activation has been implicated in disease invasiveness and metastatic potential, specifically to bone.^{67–69} SFK activity has shown oncogenic effects in both androgen-dependent and androgen-independent prostate cancers, suggesting that SFK activity is involved in the progression of prostate cancer to castration resistance.^{67,70} The pathogenesis of SFKs in prostate cancer has largely been attributed to their ability to activate the androgen receptor independent of androgen stimulation. SFK activity can be regulated by a number of growth factors during androgen deprivation (Figure 3), including epidermal growth factor (EGF), IL-6, IL-8, and neurokinins (gastrin-releasing peptide), and these factors activate important proliferative pathways, notably Ras/Raf/Erk and PI3K/Akt.^{5,67,70–73}

The involvement of SFKs in prostate cancer pathogenesis, and the knowledge of prominent molecular elements of their activity, led to the introduction of SFK inhibitors, including dasatinib and saracatinib, into clinical research. Saracatinib can decrease tumour migration and metastasis in prostate cancer cell lines.⁶⁹ However, SFK inhibitors have been decidedly less successful at inducing significant levels of apoptosis in these cell lines, possibly owing to the influence of autophagy.^{70,74} When SFKs are inhibited, the PI3K/Akt/mTOR/S6K signalling cascade is impeded, resulting in upregulation

Figure 4 | Amino acid deprivation, autophagy and prostate cancer. ADI hydrolyses arginine into citrulline. To replenish arginine, cells have a two-step synthetic pathway involving ASS and ASL. Prostate cancers deficient in either ASL or ASS are unable to synthesize the necessary amount of arginine *de novo*. At this point, arginine deficiency can activate several different cellular metabolic pathways. Pathway 1, AMPK is activated and mTOR is subsequently inhibited, promoting autophagy. Pathway 2, citrulline causes dissociation of Bcl-2, freeing Beclin 1 to stimulate autophagy. Pathway 3, citrulline causes dissociation of Bcl-2, freeing Bak/Bax to activate apoptosis. Treatment of cells with ADI can promote cell death, mediated by apoptosis or prolonged autophagy, with longer periods of ADI favouring apoptosis. Abbreviations: ADI, arginine deiminase; AMPK, adenosine monophosphate-activated protein kinase; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; Bak, Bcl-2 homologous antagonist/killer; Bax, apoptosis regulator BAX; Bcl-2, apoptosis regulator Bcl-2; mTOR, mammalian target of rapamycin.

of autophagy.^{5,67,69,70} Additionally, SFKs are believed to regulate glucose transport (for example, by GLUT1 and GLUT4), and once glucose transport is compromised, the cells will be energetically stressed, further promoting autophagy through AMPK activation.^{5,67} Although various models directly implicate aberrant SFK activity in prostate cancer pathogenesis, and targeted inhibition of SFKs has proved somewhat useful, autophagy provides a mechanistic escape that limits the therapeutic potential of this drug class.

Inhibition of autophagy with concurrent SFK inhibition supports the idea that autophagy is associated with this limited efficacy. Inhibition of autophagy by each of three independent approaches—chloroquine, 3-methyladenine, and siRNA-directed knockdown of *Atg7* (an essential autophagy constituent)—enhanced PC3 cell death by means of increased apoptotic activity. In addition, chloroquine enhanced the therapeutic efficacy of saracatinib in PC3 xenographic mouse models: after 14 days of tumour growth, the addition of chloroquine to saracatinib inhibited tumour growth by 64%, compared with 26% inhibition in tumours treated with saracatinib alone.⁶⁹ In summary, SFKs are influential components of the cellular proliferative and adaptive machinery, and their inhibition can affect prostate cancer progression. However, the therapeutic impact of SFK inhibition is limited by the paradoxical molecular overlap that supports both treatment effect and treatment resistance. Fortunately, the data seem to suggest that adjunct autophagy inhibition undermines this resistance.

Arginine deiminase

Amino acid deprivation has long been established as a cancer treatment modality.⁷⁵ A variety of amino acids,

including glutamine, methionine, leucine, and arginine, have been selectively eliminated to challenge the metabolic needs of growing cancers.⁷⁵ In prostate cancer research, accumulating evidence suggests that prostate cancer cells are especially susceptible to arginine restriction. In an analysis of 88 prostate cancer specimens, none expressed argininosuccinate synthetase, suggesting limitations in *de novo* arginine synthesis.⁷⁵ These prostate cancers should be exquisitely sensitive to any arginine depletion, and indeed when CWR22RV1 cells (CRPC cells lacking argininosuccinate synthetase) were treated with arginine deiminase (ADI)—an enzyme that degrades arginine—cell death was accelerated.⁷⁵ These results were replicated in CWR22RV1 xenografts in nude mice.⁷⁵ When this killing effect was further analysed, AMPK was notably activated and the Akt/mTOR/S6K pathway was found to be deactivated (Figure 4), suggesting that not only was autophagy upregulated, but that greater therapeutic efficacy could be achieved with autophagy inhibition. Treatment of prostate cancer cell lines with chloroquine accelerated the apoptosis induced by ADI.⁷⁵ Chloroquine itself had little effect on cellular viability, supporting therapeutic synergy with ADI. The prosurvival function of autophagy was confirmed with the siRNA knockdown of *Beclin 1*, which effectively inhibited autophagy and promoted apoptosis. However, the synergistic effects of ADI and autophagy inhibition were limited to CWR22RV1 cells. In LNCaP cells that express functional argininosuccinate synthetase, combined ADI and chloroquine had little effect. Collectively, these data suggest that autophagy could be a valuable target in prostate cancers that are found to lack argininosuccinate synthetase. Phase I/II clinical trials in hepatoma and melanoma cell lines have already demonstrated some success with this strategy (Table 1), but clinical trials are needed in appropriately selected patients with prostate cancer to fully determine the therapeutic efficacy of ADI with concurrent autophagy inhibition.⁷⁵

Conclusions

Our understanding of prostate cancer progression continues to evolve. The dynamic role of autophagy in prostate cancer progression is a molecularly intriguing and potentially modifiable factor in prostate cancer pathogenesis. Fundamentally, the degree of autophagic activity is important: too little or too much autophagy portends either beneficial or deleterious consequences depending on the state of carcinogenesis and the involvement of therapeutic intervention. The advances in prostate cancer treatments, specifically advances in targeted androgen synthesis and androgen receptor inhibition, microtubule inhibition, cell-signalling inhibition, and amino acid deprivation, stimulate the adaptive response of autophagy in prostate cancer models. Furthermore, autophagy can be modulated in these models to improve therapeutic efficacy. To date, a number of human trials investigating autophagy in cancer have been undertaken, with several in later phases demonstrating therapeutic efficacy. Clinical trials specific to prostate cancer are

ongoing, and will, we hope, further substantiate what has been observed preclinically. Moving forward, refinement of autophagy modulation is needed. Currently available autophagy modulators are relatively non-specific, and cytotoxicity in noncancerous tissues is still a concern.¹⁹ The influence of autophagic manipulation on immunological activity is also uncertain. Moreover, given the context-dependent role of autophagy, appropriate selection of patients and directional modification of autophagy are essential.² Few autophagy-specific biomarkers are currently known, and the development of a greater set of biomarkers will certainly help to characterize prostate cancers that are more dependent on autophagy and to better inform treatment paradigms using autophagic modulation.

Review criteria

A systematic literature review was conducted primarily via electronic database searches of PubMed/MEDLINE. Searches were conducted with the following combinations and iteration of the following terms: “autophagy”, “prostate cancer”, “castration-resistant prostate cancer”, “androgen deprivation therapy”, “abiraterone”, “enzalutamide”, “taxanes”, “docetaxel”, “kinase inhibitors”, “arginine deiminase”, “chloroquine”, “hydroxychloroquine”, “metformin”, “desmethylclomipramine”, “autophagy inhibitors”, and “autophagy modulators”. All selected papers were full-text and printed in English. Preference was for papers located in core clinical journals and/or reviews. The majority of selected papers were published between 2005 and 2014. Reference lists informed ongoing reference searching and selection.

- Bennett, H. L. *et al.* Does androgen-ablation therapy (AAT) associated autophagy have a pro-survival effect in LNCaP human prostate cancer cells? *BJU Int.* **111**, 672–682 (2013).
- Mizushima, N., Levine, B., Cuervo, A. M. & Klionsky, D. J. Autophagy fights disease through cellular self-digestion. *Nature* **451**, 1069–1075 (2008).
- Xie, Z. & Klionsky, D. J. Autophagosome formation: core machinery and adaptations. *Nat. Cell Biol.* **9**, 1102–1109 (2007).
- Degenhardt, K. *et al.* Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. *Cancer Cell* **10**, 51–64 (2006).
- Leone, R. D. & Amaravadi, R. K. Autophagy: a targetable linchpin of cancer cell metabolism. *Trends Endocrinol. Metab.* **24**, 209–217 (2013).
- Chen, N. & Karantza, V. Autophagy as a therapeutic target in cancer. *Cancer Biol. Ther.* **11**, 157–168 (2011).
- Chodak, G. W. & Warren, K. S. Watchful waiting for prostate cancer: a review article. *Prostate Cancer Prostatic Dis.* **9**, 25–29 (2006).
- Patel, A. R. & Klein, E. A. Risk factors for prostate cancer. *Nat. Clin. Pract. Urol.* **6**, 87–95 (2009).
- Cheong, H., Lu, C., Lindsten, T. & Thompson, C. B. Therapeutic targets in cancer cell metabolism and autophagy. *Nat. Biotechnol.* **30**, 671–678 (2012).
- Deretic, V. Autophagosome and phagosome. *Methods Mol. Biol.* **445**, 1–10 (2008).
- Townsend, K. N. *et al.* Autophagy inhibition in cancer therapy: metabolic considerations for antitumor immunity. *Immunol. Rev.* **249**, 176–194 (2012).
- Deretic, V. Autophagy: an emerging immunological paradigm. *J. Immunol.* **189**, 15–20 (2012).
- Levine, B., Mizushima, N. & Virgin, H. W. Autophagy in immunity and inflammation. *Nature* **469**, 323–335 (2011).
- Zhang, Y., Morgan, M. J., Chen, K., Choksi, S. & Liu, Z. G. Induction of autophagy is essential for monocyte-macrophage differentiation. *Blood* **119**, 2895–2905 (2012).
- Choi, A. M., Ryter, S. W. & Levine, B. Autophagy in human health and disease. *N. Engl. J. Med.* **368**, 1845–1846 (2013).
- Klionsky, D. J. *et al.* Guidelines for the use and interpretation of assays for monitoring autophagy. *Autophagy* **8**, 445–544 (2012).
- Qu, X. *et al.* Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *J. Clin. Invest.* **112**, 1809–1820 (2003).
- Mathew, R., Karantza-Wadsworth, V. & White, E. Role of autophagy in cancer. *Nat. Rev. Cancer* **7**, 961–967 (2007).
- White, E. Deconvoluting the context-dependent role for autophagy in cancer. *Nat. Rev. Cancer* **12**, 401–410 (2012).
- Lum, J. J. *et al.* Growth factor regulation of autophagy and cell survival in the absence of apoptosis. *Cell* **120**, 237–248 (2005).
- Yonekawa, T. & Thorburn, A. Autophagy and cell death. *Essays Biochem.* **55**, 105–117 (2013).
- Mariño, G., Niso-Santano, M., Baehrecke, E. H. & Kroemer, G. Self-consumption: the interplay of autophagy and apoptosis. *Nat. Rev. Mol. Cell Biol.* **15**, 81–94 (2014).
- Levine, B. & Yuan, J. Autophagy in cell death: an innocent convict? *J. Clin. Invest.* **115**, 2679–2688 (2005).
- Mehrpour, M., Esclatine, A., Beau, I. & Codogno, P. Overview of macroautophagy regulation in mammalian cells. *Cell Res.* **20**, 748–762 (2010).
- Pattingre, S. *et al.* Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell* **122**, 927–939 (2005).
- Kondo, Y., Kanzawa, T., Sawaya, R. & Kondo, S. The role of autophagy in cancer development and response to therapy. *Nat. Rev. Cancer* **5**, 726–734 (2005).
- Yang, Z. J., Chee, C. E., Huang, S. & Sinicrope, F. Autophagy modulation for cancer therapy. *Cancer Biol. Ther.* **11**, 169–176 (2011).
- Carew, J. S. *et al.* Targeting autophagy augments the anticancer activity of the histone deacetylase inhibitor SAHA to overcome Bcr-Abl-mediated drug resistance. *Blood* **110**, 313–322 (2007).
- Katayama, M., Kawaguchi, T., Berger, M. S. & Pieper, R. O. DNA damaging agent-induced autophagy produces a cytoprotective adenosine triphosphate surge in malignant glioma cells. *Cell Death Differ.* **14**, 548–558 (2007).
- White, E. & DiPaola, R. S. The double-edged sword of autophagy modulation in cancer. *Clin. Cancer Res.* **15**, 5308–5316 (2009).
- Amaravadi, R. K. *et al.* Principles and current strategies for targeting autophagy for cancer treatment. *Clin. Cancer Res.* **17**, 654–666 (2011).
- Yang, Z. J., Chee, C. E., Huang, S. & Sinicrope, F. A. The role of autophagy in cancer: therapeutic implications. *Mol. Cancer Ther.* **10**, 1533–1541 (2011).
- Sotelo, J., Briceño, E. & López-González, M. A. Adding chloroquine to conventional treatment for glioblastoma multiforme: a randomized, double-blind, placebo-controlled trial. *Ann. Intern. Med.* **144**, 337–343 (2006).
- Vakana, E., Altman, J. K. & Platanius, L. C. Targeting AMPK in the treatment of malignancies. *J. Cell. Biochem.* **113**, 404–409 (2012).
- Ben Sahra, I. *et al.* Targeting cancer cell metabolism: the combination of metformin and 2-deoxyglucose induces p53-dependent apoptosis in prostate cancer cells. *Cancer Res.* **70**, 2465–2475 (2010).
- Ben Sahra, I. *et al.* The antidiabetic drug metformin exerts an antitumoral effect *in vitro* and *in vivo* through a decrease of cyclin D1 level. *Oncogene* **27**, 3576–3586 (2008).
- Cantrell, L. A. *et al.* Metformin is a potent inhibitor of endometrial cancer cell proliferation—implications for a novel treatment strategy. *Gynecol. Oncol.* **116**, 92–98 (2010).
- Cazzaniga, M., Bonanni, B., Guerrieri-Gonzaga, A. & Decensi, A. Is it time to test metformin in breast cancer clinical trials? *Cancer Epidemiol. Biomarkers Prev.* **18**, 701–705 (2009).
- Colquhoun, A. J. *et al.* Metformin enhances the antiproliferative and apoptotic effect of bicalutamide in prostate cancer. *Prostate Cancer Prostatic Dis.* **15**, 346–352 (2012).
- Zakikhani, M., Dowling, R., Fantus, I. G., Sonenberg, N. & Pollak, M. Metformin is an AMP kinase-dependent growth inhibitor for breast cancer cells. *Cancer Res.* **66**, 10269–10273 (2006).
- Shank, J. J. *et al.* Metformin targets ovarian cancer stem cells *in vitro* and *in vivo*. *Gynecol. Oncol.* **127**, 390–397 (2012).
- Zhang, Y. *et al.* Effects of metformin on CD133+ colorectal cancer cells in diabetic patients. *PLoS ONE* **8**, e81264 (2013).
- Evans, J. M., Donnelly, L. A., Emslie-Smith, A. M., Alessi, D. R. & Morris, A. D. Metformin and reduced risk of cancer in diabetic patients. *BMJ* **330**, 1304–1305 (2005).
- Spratt, D. E. *et al.* Metformin and prostate cancer: reduced development of castration-resistant disease and prostate cancer mortality. *Eur. Urol.* **63**, 709–716 (2013).
- Rossi, M. *et al.* Desmethylclomipramine induces the accumulation of autophagy markers by blocking autophagic flux. *J. Cell Sci.* **122**, 3330–3339 (2009).
- Nguyen, H. G. *et al.* Targeting autophagy overcomes Enzalutamide resistance in castration-resistant prostate cancer cells and improves therapeutic response in a xenograft model. *Oncogene* <http://dx.doi.org/10.1038/onc.2014.25>.

47. Kung, H.-J. *et al.* in *Prostate Cancer*, (ed. Tindall, D. J.) 497–518 (Springer, 2013).
48. Shen, S. *et al.* Association and dissociation of autophagy, apoptosis and necrosis by systematic chemical study. *Oncogene* **30**, 4544–4556 (2011).
49. Giampietri, C. *et al.* Autophagy modulators sensitize prostate epithelial cancer cell lines to TNF- α -dependent apoptosis. *Apoptosis* **17**, 1210–1222 (2012).
50. Bennett, H. L., Fleming, J. T., O'Prey, J., Ryan, K. M. & Leung, H. Y. Androgens modulate autophagy and cell death via regulation of the endoplasmic reticulum chaperone glucose-regulated protein 78/BiP in prostate cancer cells. *Cell Death Dis.* **1**, e72 (2010).
51. Kaini, R. R. & Hu, C. A. Synergistic killing effect of chloroquine and androgen deprivation in LNCaP cells. *Biochem. Biophys. Res. Commun.* **425**, 150–156 (2012).
52. Li, M. *et al.* Autophagy protects LNCaP cells under androgen deprivation conditions. *Autophagy* **4**, 54–60 (2008).
53. Jiang, Q. *et al.* Targeting androgen receptor leads to suppression of prostate cancer via induction of autophagy. *J. Urol.* **188**, 1361–1368 (2012).
54. Xu, Y., Chen, S. Y., Ross, K. N. & Balk, S. P. Androgens induce prostate cancer cell proliferation through mammalian target of rapamycin activation and post-transcriptional increases in cyclin D proteins. *Cancer Res.* **66**, 7783–7792 (2006).
55. Chhipa, R. R., Wu, Y. & Ip, C. AMPK-mediated autophagy is a survival mechanism in androgen-dependent prostate cancer cells subjected to androgen deprivation and hypoxia. *Cell. Signal.* **23**, 1466–1472 (2011).
56. Lozy, F. & Karantza, V. Autophagy and cancer cell metabolism. *Semin. Cell Dev. Biol.* **23**, 395–401 (2012).
57. Ziparo, E. *et al.* Autophagy in prostate cancer and androgen suppression therapy. *Int. J. Mol. Sci.* **14**, 12090–12106 (2013).
58. Barrett, K. E., Boitano, S., Barman, S. M. & Brooks, H. L. *Garong's Review of Medical Physiology* 23rd edn Ch. 2 (McGraw-Hill, 2012).
59. Tannock, I. F. *et al.* Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N. Engl. J. Med.* **351**, 1502–1512 (2004).
60. Köchl, R., Hu, X. W., Chan, E. Y. & Tooze, S. A. Microtubules facilitate autophagosome formation and fusion of autophagosomes with endosomes. *Traffic* **7**, 129–145 (2006).
61. Viola, G. *et al.* MG-2477, a new tubulin inhibitor, induces autophagy through inhibition of the Akt/mTOR pathway and delayed apoptosis in A549 cells. *Biochem. Pharmacol.* **83**, 16–26 (2012).
62. Veldhoen, R. A. *et al.* The chemotherapeutic agent paclitaxel inhibits autophagy through two distinct mechanisms that regulate apoptosis. *Oncogene* **32**, 736–746 (2013).
63. Notte, A., Ninane, N., Arnould, T. & Michiels, C. Hypoxia counteracts taxol-induced apoptosis in MDA-MB-231 breast cancer cells: role of autophagy and JNK activation. *Cell Death Dis.* **4**, e638 (2013).
64. Long, B. H. & Fairchild, C. R. Paclitaxel inhibits progression of mitotic cells to G1 phase by interference with spindle formation without affecting other microtubule functions during anaphase and telophase. *Cancer Res.* **54**, 4355–4361 (1994).
65. Eum, K. H. & Lee, M. Crosstalk between autophagy and apoptosis in the regulation of paclitaxel-induced cell death in v-Ha-ras-transformed fibroblasts. *Mol. Cell. Biochem.* **348**, 61–68 (2011).
66. Yeatman, T. J. A renaissance for SRC. *Nat. Rev. Cancer* **4**, 470–480 (2004).
67. Kung, H. J. Targeting tyrosine kinases and autophagy in prostate cancer. *Horm. Cancer* **2**, 38–46 (2011).
68. Lee, Y. C. *et al.* Src family kinase/abl inhibitor dasatinib suppresses proliferation and enhances differentiation of osteoblasts. *Oncogene* **29**, 3196–3207 (2010).
69. Wu, Z. *et al.* Autophagy blockade sensitizes prostate cancer cells towards Src family kinase inhibitors. *Genes Cancer* **1**, 40–49 (2010).
70. Lamoureux, F. *et al.* Blocked autophagy using lysosomotropic agents sensitizes resistant prostate tumor cells to the novel Akt inhibitor AZD5363. *Clin. Cancer Res.* **19**, 833–844 (2013).
71. DaSilva, J., Gioeli, D., Weber, M. J. & Parsons, S. J. The neuroendocrine-derived peptide parathyroid hormone-related protein promotes prostate cancer cell growth by stabilizing the androgen receptor. *Cancer Res.* **69**, 7402–7411 (2009).
72. Lee, L. F., Guan, J., Qiu, Y. & Kung, H. J. Neuropeptide-induced androgen independence in prostate cancer cells: roles of nonreceptor tyrosine kinases Etk/Bmx, Src, and focal adhesion kinase. *Mol. Cell. Biol.* **21**, 8385–8397 (2001).
73. Lee, L. F. *et al.* Interleukin-8 confers androgen-independent growth and migration of LNCaP: differential effects of tyrosine kinases Src and FAK. *Oncogene* **23**, 2197–2205 (2004).
74. Yang, J. C. *et al.* Aberrant activation of androgen receptor in a new neuropeptide-autocrine model of androgen-insensitive prostate cancer. *Cancer Res.* **69**, 151–160 (2009).
75. Kim, R. H., Bold, R. J. & Kung, H. J. ADI, autophagy and apoptosis: metabolic stress as a therapeutic option for prostate cancer. *Autophagy* **5**, 567–568 (2009).

Acknowledgements

The work is supported by funding from the following sources: the Department of Defense, the Stand Up To Cancer Prostate Cancer Foundation Prostate Dream Team Translational Cancer Research grant, made possible by the generous support of the Movember Foundation (Stand Up To Cancer is a programme of the Entertainment Industry Foundation administered by the American Association of Cancer Research), and the National Center for Advancing Translational Sciences at the National Institutes of Health, through grant number UL1 TR000002 and the linked TL1 TR000133 award.

Author contributions

All authors researched data for the article, and contributed to discussions of content, and review and editing of the manuscript before submission. J.M.F. wrote the article.